# PROCEEDINGS

## Volume 63


# WESTERN SECTION AMERICAN SOCIETY OF ANIMAL SCIENCE

**Phoenix, Arizona**
**July 15–19, 2012**

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## PRODUCTION, MANAGEMENT, AND ENVIRONMENT



## RUMINANT NUTRITION

## SYMPOSIUM

# 2011-2012 WSASAS COMMITTEES

* Denotes Committee Chair

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L. Camacho, Graduate Student Representative (13, NDSU)

**AWARDS**

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A. L. Van Eenennaam, (12, UC-Davis)
S. L. Archibeque (13, CSU)
S. I. Paisley, (13, UW)
D. C. Rule, (13, UW)

**SYMPOSIUM**

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B. H. Kirch (13, CSU)
J. A. Paterson (14, MSU)
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R. Cockrum (13, UW) Graduate Student Representative
L. E. Camacho, (14, NDSU) Graduate Student Representative
R. C. Waterman, (14, USDA-LARRL)
J. G. Berardinelli (14, MSU)
E. J. Scholljegerdes (14, NMSU)

**PAPER COMPETITION**

H. L. Neibergs (12, WSU)*
R. L. Endecott (12, MSU)
S. L. Ivey (12, NMSU)
R. K. Peel (12, CSU)
S. L. Lake (13, UW)
C. J. Mueller (13, OSU)
E. J. Scholljegerdes (13, NMSU)
J. K. Ahola (14, CSU)

**YOUNG SCHOLAR AWARD**

C. K. Larson (2013, Zinpro Corp.)*
J. C. Whittier (13, CSU)
G. E. Moss (2013, UW)
R. L. Ashley (2014, NMSU)
M. MacNeil (2012, USDA, LARRL)
G. P. Lardy (2012, ND)
P.G. Hatfield (2014, MT)

**ACADEMIC QUADRATHLON**

D. C. Rule (UW)*
J. B. Lamb (BYU-Idaho)
S. A. Sotto-Navaro (NMSU)
B. Bowman (USU)
H. Han (CSU)
S. L. Archibeque (CSU)

**EXTENSION**

T. R. Whitney (12, TAMU)*
C. P. Mathis (12, TAMU) was at NMSU
D. R. Zobell (13, USU)
R. F. Cooke (14, OSU)
J. B. Hall (14, UI)
R. L. Endecott (14, MSU)

**NECROLOGY**

D. H. Crews, Jr., Past-President (12, CSU) *

**NOMINATING**

D. H. Crews, Jr., Past-President (14, CSU) *
G. E. Moss, Past-President (13, UW)
R. A. Battaglia (12, UI)

# BUSINESS MEETING MINUTES

Western Section, American Society of Animal Science
June 23, 2011
Miles Community College, Miles City, MT

President Andy Roberts called the meeting to order at 7:30 AM.

## Acceptance of 2010 WSASAS Minutes

After a call for addition or amendments, minutes of the 2010 WSASAS Business Meeting were accepted as printed in the 2011 Western Section ASAS Proceedings.

## 2010 Financial and 2011 Meeting Reports

***Glenn Duff, Secretary-Treasurer, Montana State University***

The WSASAS financial report as of December 31, 2010 was summarized. In the 2010 calendar year, the Section total revenue was $28,128.96 and total expense was $24,844.26, leaving a balance of $58,404.68. The detailed report is included in these minutes as an appendix.

The 2011 Western Section ASAS meeting was held at Miles Community College in Miles City, MT. There were 141 registrants for the WSASAS meeting. There were 94 papers accepted for publication in the Proceeding of the WSASAS; 15 Graduate Student Competition Papers, 37 other oral presentations and 45 posters. There were 109 registered for the awards banquet.

## Necrology Committee Report

***Gary Moss, Past-President, University of Wyoming***

Two WSASAS members passed away during 2010-2011:
Dr. Howard H Stonaker, Fort Collins, Colorado
Dr. Roy Ax, University of Arizona

The report was followed by a moment of silence in memory of these members.

## Nominating Committee Report

**Committee Members**
G.E. Moss, Past President, University of Wyoming
R. Battaglia, University of Idaho
K.C. Olson, South Dakota State University

**Nominees for 2011 WSASAS elections were:**
President Elect: Glenn Duff, Montana State University
Secretary Treasurer: Bret Taylor, USDA-USSES
Graduate Student Representative: Laun Hall, North Dakota State University

**Elections results were:**
President-Elect: Glenn Duff, Montana State University
Secretary Treasurer: Bret Taylor, USDA-USSES
Graduate Student Representative: Leticia (Ely) Camacho, New Mexico State University

## Extension Symposium Committee Report

Not applicable this year

## Beef Symposium Committee Report

The 2011 WSASAS Beef Symposium was held from 8:00 AM to 5:00 PM on June 21, at the Fort Keogh Livestock & Range Research Laboratory. Fort Keogh was especially fitting because the first four annual meetings of Western Section of ASAS (1928 through 1931) took place at the Research Center. The symposium this year included a tour that will gave participants a brief overview of the rich history of Fort Keogh (established August 1876 following Battle of the Little Bighorn in June 1876, where General George A. Custer met his demise) and highlight ground breaking animal and range research that has occurred since Fort Keogh was transferred from the military to the United States Department of Agriculture for establishment of the United States Range Experiment Station in 1924. Featured speakers at the symposium were Drs. Robert E. Short and Robert B. Staigmiller. The “Bobs” shared their memorable experiences and accomplishments over the 94 scientific years of their careers at Fort Keogh. This information will be presented during a tour of the historic horse barn, where many “firsts” in animal research took place. Other presentations included: Natural history of the Northern Great Plains (Dr. Kurt Reinhart), significant range science findings in the last 80 years (Dr. Mark Petersen), significant range nutrition findings in nearly 40 years (Dr. Richard Waterman), significant beef cattle genetic findings in nearly 80 years (Dr. Mike MacNeil), and recent research on factors affecting pregnancy (Dr. Tom Geary) and beef cattle efficiency (Drs. Andy Roberts and Rachel Endecott).

## Academic Quadrathlon Committee Report

***2011 Academic Quadrathlon Report***
***Dan Rule, University of Wyoming, AQ committee chair***

J. B. Lamb (BYU-Idaho)
S. A. Soto-Navarro (NMSU)
R. D. Weidemeier (USU)
H. Han (CSU)

The Western Regional Academic Quadrathlon contest was held in conjunction with the Western Section American Society of Animal Science meetings in Miles City, Montana in June, 2011. The contest categories included a written challenge, an oral presentation on a topic of current relevance to animal agriculture, a laboratory practical challenge with application of applied knowledge in up to 10 disciplines and species, and finished with a double elimination quiz bowl. Participating universities included the University of Wyoming, (placed first in each category resulting in top score for overall winner), New Mexico State University (2nd overall), Colorado State University (3rd overall), Oregon State University, and BYU-Idaho. This year's overall winning team, The University of Wyoming, will compete in the national Academic Quadrathlon beef quiz bowl contest that will be held in Nashville, Tennessee in February, 2012 in conjunction with the annual National Cattleman's Beef Association convention.

University of Wyoming team: Dr. Dan Rule (Advisor), Steph Schroeder, Mandy Thomas, Dr. Doug Hixon (Animal Science Department Head), Colin Yorges, and Brice Macintosh.

Special thank you for the 2011 AQ contest goes out to Dr. Rachel Endecott of Montana State University (stationed at USDA ARS Fort Keogh, Miles City) who organized the contest for this year's regional competition. The many hard working folks who provided assistance with the event also deserved our sincerest thanks.

Subsequent to the contest lengthy discussion on future AQ events and the national AQ quiz bowl occurred during the business meeting. The 2012 Western Section meetings and AQ contest will be held during the national ASAS meetings in Phoenix, Arizona in July. Organization of next year's Western AQ are currently underway and tentatively include lab practicum events at Arizona State University, Tucson. The other events require a few rooms for written challenges, oral presentations, and the quiz bowl. Imperative to the timing of the AQ, the quiz bowl rounds need to be part of the reception for the meetings.

Timing and location of the national AQ beef quiz bowl was also discussed. Major discussion pertained to conducting the national contest during the national meetings in 2012, as well as during future national ASAS meetings. No firm recommendations were decided upon; however, the idea of conducting the 2012 national AQ quiz bowl right after the Western Section AQ so that the national AQ quiz bowl was held during the reception of the 2012 meetings in Phoenix has merit and will be subject to further discussion among Western Section AQ participating schools and the national ASAS office.

## Awards Committee Report

***A. J. Roberts President-Elect (11, USDA, ARS, Ft. Keogh)***

T. E. Engle (11, Colorado State University)
J. M. Thompson (11, Oregon State University)
M. D. MacNeil (11, USDA-ARS-LARRL)
A. L. Van Eenennaam, (13, Univeristy of California-Davis)
A. Ahmadzadeh (13, University of Idaho)

### 2011 Recipients

**DISTINGUISHED SERVICE AWARD**

Recipient: Dr. John Paterson, Montana State University
Sponsor: DSM Nutritional Products, Inc
c/o Scot Williams and Yvonne Towns
Parsippany, NJ 07054-1298
Nominators: Dr. Glenn Duff and Mo Harbac, Montana State University

**DISTINGUISHED TEACHING AWARD**

Recipient: Dr. Daniel C. Rule, University of Wyoming
Sponsor: Elanco Animal Health
c/o Dr. Todd Armstrong
2001 W. Main Street
PO Box 708
Greenfield, IN 46140-2714
Nominators: Drs. Doug Hixon and Paul Ludden, University of Wyoming

**EXTENSION AWARD**

Recipient: Dr. Steven Paisley, University of Wyoming
Sponsor: Western Section ASAS
Nominator: Dr. Doug Hixon, University of Wyoming

**YOUNG SCIENTIST AWARD**

Recipient: Dr. Shawn Archibeque, Colorado State University
Sponsor: Western Section ASAS
Nominator: Dr. Terry Engle, Colorado State University

Dr. Andy Roberts and Denny Crews presented awards at the banquet. Andy Roberts thanked all who submitted nominations and encouraged nominators to get to work early and nominate our colleagues in 2012.

## Applied Paper Awards

***Connie Larson, Chair***

**1st Place** - J. E. Sprinkle[1], D. W. Schafer[1], S. P. Cuneo1, D. Tolleson[1], and R. M. Enns[2]. Effects of a long acting trace mineral rumen bolus upon range cow productivity. [1]University of Arizona, Tucson, AZ 85721; [2]Colorado State University, Fort Collins, CO 80523

**2nd Place** - R. N. Funston, J. A. Musgrave, T. L. Meyer, and D. M. Larson. Effect of calving period on ADG, reproduction, and first calf characteristics of heifer progeny. University of Nebraska West Central Research and Extension Center, North Platte.

**3rd Place -** N.L. Hojer[1], M.B. Hubert[1], D.L. Gay[1], V.N. Owens[2], A.D. Ressett[1], R.H. Pritchard[2], K. Karges[3], and K.C. Olson[1]. Response of beef cows and calves after supplementation with a novel distiller's grain during gestation. [1]South Dakota State University, Rapid City, SD; [2]South Dakota State University, Brookings, SD; [3]POET Nutrition, Sioux Falls, SD

## Graduate Student Competition Committee Report

***Darrin Boss, Montana State University, Northern Agricultural Research Center, Havre, MT Chair***

Members:
D. L. Boss (11, MSU-Havre), Chair
H. L. Neibergs (12, WSU)
S. L. Ivey (12, NMSU)
R. L. Endecott (12, MSU)
R. K. Peel (12, CSU)
S. L. Lake (13, UW)
E. J. Scholljegerdes (13, NMSU)
C. Mueller (13, OSU)

Darrin Boss will complete his three year term this year. The committee has a few names nominated as a replacement and we will forward them upon confirmation and those expressing interest. Holly Niebergs has agreed to assume the responsibilities of Committee Chair.

The WSASAS Graduate Student Competition Committee reviewed fifteen abstracts and all were accepted or accepted with revision for inclusion into the proceedings. All fifteen Graduate students completed their papers and all were allocated time for the oral portion of the competition. As in the past the abstracts were not included in the overall score of the competition and both the oral and written portions were equally weighted (50% each). University student participation was: Colorado State University – 2, Oregon State University – 2, University of Arizona – 2, New Mexico State University – 3, Montana State University – 2, University of Wyoming – 1, Oklahoma State University – 1, and Ohio State University – 1. Three universities were not eligible for the Zinpro Graduate Institution Award (UWy, OK and Ohio). The committee would like to congratulate all the participants and encourage increased participation in the future so all schools that fit the criteria for the Zinpro Graduate Institution Award in the Section to qualify for the award. Again in 2010 - 2011 as in years past the committee had the pleasure of reviewing some wonderful work and research presentations of the Graduate Students of WSASAS.

**3. K. Quinn, New Mexico State University**

CXCL12 and CXCR4 expression in peripheral blood from pregnant and non-pregnant sheep: implications in pregnancy diagnosis. K. Quinn and R. Ashley, *New Mexico State University*, *Las Cruces, NM, USA*.

**2. N. P. Miller, New Mexico State University**

Effects of flaxseed level and processing on site and extent of digestion in beef cows fed native hay. N. P. Miller*[1], S. L. Kronberg[2], and E. J. Scholljegerdes[1], [1]*New Mexico State University*, *Las Cruces, NM, USA*. [2]*USDA-ARS,Northern Great Plains Research Laboratory*, *Mandan, ND, USA*.

**1. F. M. Abreu, Ohio State University**

The effect of follicle age on pregnancy rate in beef cows. F. M. Abreu*[1,2], L. H. Cruppe[1], C. A. Roberts[2], E. M. Jinks[3], K. G. Pohler[3], M. L. Day[1], and T. W. Geary[2], *[1]The Ohio State University, Columbus, Ohio, USA, [2]USDA-ARS Fort Keogh LARRL, Miles City, Montana, USA, [3]University of Missouri, Columbia, Missouri, USA.*

## Zinpro Graduate Student Institution Award

***New Mexico State University***

It would be my recommendation to no longer accept word documents. It is no longer a financial hardship or a very difficult task to convert all documents to a pdf format. By requiring pdf it will remove the competition committee from any disagreement in formatting what is submitted. The paper is in the format exactly as entered and is unaltered on other computers format or versions of word. The paper is evaluated as presented by the author, different Word version and print format still affect presented paper.

## Advising and Coordinating Committee Report

***Prepared by J. B. Taylor, Chair***

Members:
J. K. Ahola
J. E. Bruemmer
K. M. Cammack
R. R. Cockrum (Graduate Student Representative)
J. B. Glaze
D. M. Hallford
B. J. May
M. P. Shipka

For FY2011, the Advising and Coordinating Committee made recommendation to the Executive Committee on one item.

On February 16, 2011, R. R. Cockrum submitted a proposal for a Graduate Student Lunch and Learn Session to be included in the 2011 Western Section American Society of Animal Science annual meeting program. After several modifications, R. R. Cockrum submitted a finalized draft of the proposal titled, "Active involvement in professional societies to assist in the transition form graduate student to animal scientist;" a copy of the proposal is attached. On March 9, 2011, the proposal was forwarded to the Advising and Coordinating Committee members for review and comment. K. M. Cammack, J. B. Glaze, D. M. Hallford, and M. P. Shipka applauded the idea and recommended that the proposal be forwarded to the Executive Committee for consideration. M. P. Shipka suggested that the program should start at 12:30 pm in order to provide time for people

to gather, finish their lunches, and visit. No objections from any committee member were made. On March 17, 2011, the proposal was forwarded to the Executive Committee for consideration.

## WSASAS 2011 Proposal

Graduate Student Lunch and Learn Session 6/21/11 or 6/22/11 12:00 PM – 2:00 PM

Title: "Active involvement in professional societies to assist in the transition from graduate student to animal scientist"

Abstract. National ASAS recently released a survey to gauge what direction and programs graduate student members would like to see provided by their professional society. Western Section ASAS graduate student members were specifically interested in student and employer networking opportunities, more workshops, and sectional graduate student societies. The relationship between students and their chosen professional societies should be a symbiotic one. As the society promotes student involvement and career advancements the student will continue to support their society through membership and participation in activities. Currently, 55% of graduate student members of WSASAS are unsure if they will continue membership with ASAS. After graduation, 50%, 47.7%, 29.5%, and 25% of current WSASAS graduate student members anticipate pursuing a career in academia, industry, extension, and government, respectively. Therefore, I propose to conduct a Lunch and Learn session with graduate students to 1) provide information on the benefits and items available for graduate students that assist in networking and job placement, 2) determine what items are missing or needed to ensure success of students, and 3) provide an open forum discussion using animal scientists representing government, industry, academia, and extension to provide a prospective on how ASAS has impacted their careers and their transition from student to their respective career. Below is a brief itinerary detailing the Graduate Student Lunch and Learn session.

**INTRODUCTION:**

R. R. Cockrum 5 min.
WSASAS Graduate Student Director

**FEEDBACK DISCUSSIONS:**

Rebecca R. Cockrum 20 min.
WSASAS Graduate Student Director

"How WSASAS can enhance your graduate student experience"

Items to discuss:

- Graduate BULLetin
- Webinar
- ASAS website
- ASAS graduate student website
- ASAS Facebook
- e-Career Tool
- ASAS Twitter account

Allison Meyer 20 min.
Past National ASAS Graduate Student Director

"What does WSASAS need to provide to enhance graduate student experience"

**OPEN FORUM PANEL:**

Dr. Jennifer Hernandez Gifford 10 min.
Assistant Professor, Oklahoma State University
jah.hernandez_gifford@okstate.edu

Dr. Doug Hixon 10 min.
Department Head, University of Wyoming
dhixon@uwyo.edu

Dr. Connie Larson 10 min.
Research Nutritionist, Zinpro
CLarson@Zinpro.com

Dr. Bret Taylor 10 min.
Research Animal Scientist, USDA Agricultural Research Service
bret.taylor@ars.usda.gov

Questions 20 min.

Closing: R. R. Cockrum 5 min.
WSASAS Graduate Student Director

## Report from the ASAS President

Dr. Margaret E. Benson, President Elect ASAS, Washington State University

Meghan Wulster-Radcliffe, ASAS Executive Director

## Transfer of the Gavel

Denny Crews transferred the WSASAS Presidency to Andy Roberts and Past-President Denny Crews as presented with the Presidential plaque.

The 2011 Western Section Business Meeting was adjourned by President Roberts at 9:00 AM.

# APPENDIX

## WSASAS Detailed Financial Report: December 31, 2010

Glenn C. Duff, Secretary-Treasurer

| | Actual @ 12/31/10 |
|---|---|
| **Balance as of January 1** | **$55,119.98** |
| **REVENUE AND SUPPORT** | |
| Dues-ASAS | 1,520.00 |
| Registrations | 75.00 |
| Ticketed Events | 2,610.00 |
| Donations-Awards | 4,150.00 |
| Symposium Support-ASAS | 3,000.00 |
| Proceedings | 11,707.91 |
| Investment Earnings Gain(Loss) | 5,066.05 |
| **Total Revenue and Support** | **28,128.96** |
| **EXPENSES** | |
| Awards/Plaques | 6,331.00 |
| Quadrathalon | 3,100.00 |
| Convention Center | 3,047.94 |
| Travel-Staff | 533.67 |
| Proceedings | 447.57 |
| Postage, Shipping & Supplies | 62.79 |
| Miscellaneous | 3,000.00 |
| Insurance | 225.00 |
| Telephone | 48.59 |
| General Printing | 1,641.82 |
| Staff Support | 6,405.88 |
| **Total Expenses** | **24,844.26** |
| **Net Revenue over Expense** | **3,284.70** |
| **Balance as of December 31** | **$58,404.68** |

# DISTINGUISHED SERVICE AWARD

Proceedings, Western Section, American Society of Animal Science

Vol. 63, 2012

## BEFORE YOU CHARGE FORWARD, MAKE SURE YOU LOOK BACK

**J.A. Paterson**

Department of Animal and Range Sciences, Montana State University, Bozeman

### INTRODUCTION

Students of Animal Science history learn to respect those scientists who dedicated their lives to improving animal protein production, dissemination of research information and the training of graduate students. Conducting research is where new facts are discovered and new concepts, methods and techniques are formulated for use in the teaching of students, farmers and ranchers and consumers. As a graduate student matures through the processes of scientific inquiry, communication through both written and oral presentations, and success in finding that first position, this evokes a professor's pride. Among the numerous achievements that students and their major professors have been responsible for in the beef industry include producing more beef with fewer animals. Each pound of beef has been produced with less feed energy, less land, less water, less fossil fuel and has resulted in a significant decrease in total carbon emissions. However, as one reads theses or dissertations, are the efforts of professors and graduate students who published their research more than 50 yr ago which lead to these dramatic improvements appreciated? For example, Dr. George Seidel from Colorado State University (presentation at the 2011 Range Beef Cow Symposium) said that prior to the 1950s there was no frozen semen, no disposable tools for AI, no EPDs, no estrus synchronization, no embryo transfer and minimal vaccination for reproductive diseases. The intent of this presentation is to encourage the appreciation of the research breakthroughs by animal science professors over the past 50 yr.

### THE PHILOSOPHY OF TEACHING FROM THE PAST

People learn from the experiences, successes and failures of others. The student from the 1950s is in many ways vastly different than the student of the 2010s. In the 1960s' more that 65% of animal science students had farm/ranch backgrounds compared with less than 30% by the 1990s (Taylor and Kauffman, 1983) and less than 15% today (Cross, 2010; personal communication). In a "tongue and cheek" description of the incoming freshman at Colorado State in the 1950s, L.E. Wahsburn (1956) described the first semester student as one who arrives screeching on campus in a "souped-up" jalopy equipped with everything but a thermo-nuclear warhead. His first question is "Where are we going to keep the horse because the Ol Man is bringin' him up at Home comin?" His speech was a peculiar and irritating mixture of cowboy slang and bobby-soxer "pig latin". Prior to meeting with his academic advisor, the first semester student has been told that the College consisted of a "bunch of dopes" who didn't know enough to farm or ranch successfully, and consequently could do nothing but waste taxpayers money. However, after four years of classwork and educational experiences, Washburn hoped that a college education provided the student the tools necessary to be a life-long learner. His goal as an educator was to try and make the animal science student, first and foremost, a thinking man, and by doing so produce a competent professional. Dr. Hubert Heitman (1917-1993) from the University of California said that colleges and universities were not trade schools and the professor's job was to train students as thinking, useful citizens and not to train drones. However, Bill Tyznik from Ohio State University wrote (1968) that he was concerned that teaching devices, rigid curricula and testing programs seemed to be detrimental to the molding of individuals and appeared to be leaning toward massive cookie machines that cut out "gingerbread" boys and girls.

Graduate education must include both teaching and research emphasis, working together, not separately. Teaching a graduate student how to develop into a competent scientist involves both a focused academic program as well as tutoring by the major professor. Taylor and Kauffman (1983) reported that an excellent teacher (mentor) was one who 1) motivated students to think and learn, 2) was current in knowledge of the subject, 3) knew how to teach, 4) was concerned about the student and 5) was enthusiastic.

### RESEARCH PHILOSOPHY

Research has always had the noble objectives of prolonging life, the improvement of health and comfort, and the achievement of a greater happiness. Then as now, efforts by animal scientists are focused on improving the efficiency of production, new product development and elimination of waste. One of the earliest Presidents of the Western Section was Dr. Claire Terrill. He defined research as "the serious curiosity satisfied by logical and critical experimentation to discover facts or truth". Dr. M.E. Ensminger who was the Chairman of the Department of Animal Husbandry at Washington State said (1951) that the processes of teaching a student to perform research involved the following progression; 1) selecting a pertinent problem, 2) preparing a project outline and work plan, 3) securing the necessary

funds, 4) conducting the research study, 5) publishing the results and 6) interpreting and applying the results. He believed that most projects did a commendable job carrying out the first five steps but, scientists' lagged behind in the sixth step because they were unable to disseminate research results in a form that extension colleagues or anyone else could use. J.E. Morrison the Director of Extension at Colorado State in a paper presented in 1955 at the Western Section meetings hosted by the University of WY wrote that research was useless if it gained us nothing. We have no gain if no one would accept the results of your research.

In 1964, Dr. Burr Ross, Dean of Agriculture from Oklahoma State University was invited to make a Western Section presentation at Montana State University. He told the attendees that he was concerned about where animal research would be done in the future and what kind of graduate student would be produced. He believed that most federal grants were made available to support basic and fundamental research programs and that applied research for the most part was not too attractive to the granting agencies. It was his opinion that many of our departments were staffed with people who did not have the competency or interest in the kind of research projects which could qualify for many kinds of federal grants. It was also his opinion that many graduates had inadequate training or even the motivation to be competitive in many areas of research. They were better suited to go into extension or commercial jobs. Disagreeing with this assessment, Dr. Glenn Frank, the former President of the University of Wisconsin, believed that the future of America was in the hands of two men—the investigator and the interpreter.America had an ample supply of investigators, but there was a shortage of responsible interpreters, men and women who could effectively be a communicator between specialist and farmer/rancher.

Forty years after Dr. Ross' delivered his critical presentation, Dr. Marty Vavra (2005) from the Eastern Oregon Agricultural Research Center wrote that the decline in base funding for agricultural research and the resultant increased need to capture grant funds has greatly weakened the quality of research at Land Grant Universities. Long-term research requires stable base funding and freedom from the pressures of counting publications for tenure, promotion, or salary increases. If funding is reduced, faculty positions will not be refilled, and this results in reduced teaching opportunities for the graduate student. As Ritchie and Corah (1996) summarized, "Clearly, one of the most sobering challenges we face is the question: "As research dollars continue to dwindle, will the opportunities for graduate training in the field of animal science decline, creating a serious void in competent, properly trained personnel to work in the various phases of the beef cattle industry?"

Even though the challenges from 50 yr ago remain today, the work of graduate students and major professors in the United States are still the envy of the world. Why else would so many students without a farm or ranch background and foreign students seek an education in our Animal Science Departments? Because of this continuing influx of students, Animal Science Departments have the largest enrollments in many agricultural colleges.

## SUCCESSES IN ANIMAL SCIENCE RESEARCH

In a presentation to the National Cattlemen's Association Research and Education Committee in 1996, Harlan Ritchie and Larry Corah summarized many breakthroughs that have impacted the beef industry. These include:

- A method for freezing and storing bull semen that resulted in a significant increase in the use of AI by the seed stock sector.
- The in vitro Tilley and Terry Method and the Van Soest fiber methods gave researchers a rapid and economical method for studying the effects of numerous factors on the digestibility of nutrients.
- Animal breeding research led to the formation of the Beef Improvement Federation.
- An energy system devised by University of California scientists' partitioned net energy into maintenance (**NEm**) and gain (**NEg**) which is the foundation of ration formulation.
- Research in the 1970s led to the release of the ionophores monensin and lasalocid which resulted in feed savings in excess of $250 million/year and even greater improvements in production efficiency with the use of growth promoting implants.
- Results by physiology researchers were successful in discovering numerous hormonal interrelationships that impacted reproduction, growth and environmental adaptability.

An animal scientist cannot have a successful career without a solid scientific foundation of knowledge in their chosen area if study. Becoming a member of the American Society of Animal Science should be mandatory for the serious graduate student who wants to remain current in cutting edge research. Reading the history of beef cattle nutrition research is fascinating. One starting place for the beginning ruminant nutrition student is to read the review of beef cattle nutrition research from 1908 until 1958 by John Riggs from Texas A&M (1958). This paper is interesting because these scientists conducted the majority of their work without many of the modern instruments and methods available to animal scientists today. Conversely, if the student is interested in knowing if the projections about beef cattle nutrition were accurate for the years 1958-2008, the student should read the paper by William Beeson (1958).

Scientists from our Western Section universities and research stations have made outstanding research contributions. One of the excellent overviews of research focused on techniques for cattle grazing rangelands is the paper by Joe D. Wallace (1993) from New Mexico State University. Beginning in the 1950s and ending in the

1990s, this paper outlines the "players" in range nutrition research. Among these professors were Tony Lesperance and V.R. Bohman (NV), Don Clanton (NE), Brent Theurer (AZ), Bob Raleigh (OR), Lorin Harris (UT) C.J. Kercher

(WY), Oscar Thomas and Mark Peterson (MT), John Butcher (UT), Joe Wallace (NM), Jim Oldfield and Marty Vavra (OR), and R.J. Kartchner from USDA-ARS at Ft. Keogh (MT). Animal scientists interested in the foundation of range research methodologies should find and read this paper.

The improvement in the production and sustainability of beef cattle in the US has been remarkable over the past 50 yr. Research has been responsible for producing beef cattle which require 10% less feed energy, 30% less land, 14% less water and produce 18% less green-house gases. Beef produced per cow has more than doubled during the past 50 yr and yet we have the lowest cow inventory since 1952. Thirty years ago it required approximately 600 d to grow calves from birth to slaughter; today it requires 480 d. Research has shown the benefits of crossbreeding, and it effects on weaning weights (25% improvement), genetic correlations, survivability, stayability, performance testing, accurate selection decisions based on EPDs and evaluation of sires based on residual feed intake (RFI). Selection of animals for low RFI can result in 10% lower feed intakes and a 25% reduction in methane production – clearly desirable under drought conditions. Using DNA from a Line 1 Hereford was the basis of a bacterial artificial chromosome library and the bovine genome sequence.

Sixty years ago, ranchers did not have ready access to frozen semen, estrus synchronization, prostaglandins, ultrasound, freezing of embryos and vaccines for reproductive diseases. One of the most widely adapted management strategies has been the use of body condition scoring and it relationship with return to estrus, conception rates, and feeding strategies. Research has shown the consequences of protein restriction of the cow on the depression in the productivity of her calves (fetal programming). In order to improve nutrient utilization, routine usage of ionophores ($250 million dollar savings in feed), implants, grain byproducts and non-protein nitrogen usage has been widely adopted. One of the major accomplishments was the research with P and protein supplementation to increase forage intake, growth and reproductive efficiency.

Laboratories that routinely provide nutrient analyses are using methods based on forage and grain chemistry research with confirmatory trials conducted during the 1970s and 1980s. Consumer concerns over animal care resulted in implementation of beef quality assurance programs starting in the mid 1980s. Beef cattle production in the western United States in the future will continue to depend on the utilization of high forage diets for the cowherd.

## TODAY AND TOMORROW

I believe that Dean Burr Ross would have generally been pleased with our teaching and research productivity since he delivered his 1964 challenge to the Western Section.

However, Dr. Russell Cross from Texas A&M University was asked by the Beef Production and Research Committee of the National Cattlemen's Beef Association (2010) to give an overview of the challenges facing tomorrows Animal Science Departments. Compared with 50 yr ago, Animal Science Departments will continue to shift toward more fundamental research and away from applied production research. There will be a more complex clientele because of the changing view of animals by society. Stakeholder needs will continue to change and students, faculty and facilities will evolve to meet those needs. The demographics of students will also continue to change: 70% female; 80% urban; 85% entering with a preference for horses or companion animals and these students may have a minimal background in food-animal production. There will continue to be a shift in research focus where departments follow the money vs. the public good. Students will be more diverse and learning will occur in multidisciplinary settings. Departments of Animal Science will persist and survive but will look different that they do currently.

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# GRADUATE STUDENT PAPER COMPETITION

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# EFFECT OF SUPPLEMENTING ACTIVATED CHARCOAL ON THE INTAKE OF HONEY MESQUITE BY LAMBS

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**ABSTRACT:** A study was conducted to determine if intake of honey mesquite (*Prosopis glandulosa* Torr.) leaves by sheep could be increased by supplementing four levels of activated charcoal supplemental (0.0, 0.33, 0.67 and 1.00 g/kg of BW). Twenty wether lambs (36.6 ± 0.6 kg) were randomly assigned to the 4 treatment levels. Lambs were fed 2.0% of body weight of low-quality Sudan-grass hay (*Sorghum bicolor*) and 80 g/d of molasses for 7 days, and then for following 16 days lambs were fed 1.9% of body weight of low-quality Sudan-grass hay and 80 g /day of molasses mixed with the assigned level of activated charcoal. After the 7-d acclimation period, lambs were also given ad libitum access to honey mesquite leaves that had been thawed before feeding. Repeated measures analyses were used to determine if level of activated charcoal fed to lambs affected daily intake of mesquite leaves. No differences ($P = 0.52$) in the intake of mesquite leaves were detected. Mean intake of mesquite leaves was 20.7 ± 3.7, 23.8 ± 3.8, 20.2 ± 3.7, and 27.3 ± 3.7 g/day for the 0.0, 0.33, 0.67 and 1.0 levels, respectively. Consumption of mesquite leaves varied greatly among lambs, ranging from 1.24 to 6.25% of the diet. Differences in hay intake ($P = 0.23$) and lamb weight gain ($P = 0.58$) were not detected among supplemental charcoal treatments. Future studies examining the consumption of honey mesquite leaves by sheep should consider the potential variability in intake among individual animals.

**Key words:** activated charcoal, honey mesquite, molasses, Sudan-grass hay, wethers.

## INTRODUCTION

Honey mesquite (*Prosopis glandulosa*) is a highly invasive species found throughout the Southwest. It outcompetes other plant species in search for water, light and nutrients, and reduces amount of desirable foraging species. Control methods such as mechanical and chemical removal are often not cost-effective. Therefore, using mesquite as a forage resource in southwestern rangelands could increase the sustainability of livestock operations (Witmore, 2009).

Potentially, mesquite could be a valuable forage resource, because its crude protein content and fiber levels are similar to moderate quality alfalfa hay. However, phenolic compounds in its leaves can be detrimental for ruminant animals (Lyon et al., 1988). Poage et al. (2000) used activated charcoal to increase intake of bitterweed, which contains sesquiterpenes. Authors suggested activated charcoal reduced toxicosis and ultimately increased bitterweed consumption. Witmore (2009) also indicated supplementation of activated charcoal might enhance intake of mesquite leaves by livestock.

The objective of this study was to evaluate free choice consumption of mesquite leaves by sheep with activated charcoal supplementation. We hypothesized that there would be a non-linear relationship between intake of activated charcoal (medicine) and mesquite leaves (source of toxins).

## MATERIALS AND METHODS

The New Mexico State University Animal and Use Committee approved the research protocol.

***Feeding Trials*.** Our study was conducted during the winter of 2012 (mid-January to early February) and lasted 24 days. Twenty yearling Rambouillet wether lambs (36.6 ± 0.6 kg) were randomly assigned to 4 treatments in a completely randomized design. Treatments consisted of 4 levels of daily supplementation of activated charcoal: 0, 0.33, 0.67 and 1g/kg of BW. Lambs were kept in individual pens and fed daily at 0800 and 1700 h. Lambs were weighed before and after the study. During a 7-d adjustment period, lambs were fed Sudan grass hay (*Sorghum bicolor*) at 2.0% of BW (**DMB**) and 80 g of molasses. Following the acclimation period, a 17-d feeding trial was conducted. Lambs were fed low quality hay at 1.9% of BW (DMB) and a mixture of molasses (80 g) plus activated charcoal each day. The mixture of molasses and activated charcoal was placed on top of the hay in a rubber pan. Mesquite leaves were offered ad libitum in a separate pan. Mesquite leaves were harvested by hand at the Chihuahuan Desert Rangeland Research Center located 35 km north of Las Cruces, NM and placed in a cooler with ice for a maximum of 3 h and then frozen and stored until the trial. About 2/3 of the mesquite leaves were harvested on June 15 2011 and remaining leaves were harvested on June 29 2009. All lambs were fed leaves from each harvest date an equal number of days. Mesquite leaves were thawed in a refrigerator the night before feeding. Based on intake levels observed by Witmore (2009), we initially placed 11 g DM of mesquite leaves in each feeder during the morning when hay and molasses were fed. In the evening (1700 h), feeders were monitored and more mesquite was placed in the feeder if 75% of the mesquite leaves were consumed. During the first 4 days, all lambs received the same amount of leaves unless 75% of the leaves were consumed in the morning meal.

After 4 days, an additional 11 g DM of mesquite leaves were offered per meal if lambs consumed all leaves the previous day. The amount of leaves was reduced if lambs consumed less than 50% of the leaves the previous day. Our approach was to ensure lambs had ad libitum access to mesquite leaves without feeding excessive amounts of harvested mesquite leaves.

Hay and mesquite orts were collected each morning. Orts were weighed daily and stored until the end of the trial. Hay and mesquite orts were dried at 50°C for 48 h and ground in a Wiley mill to pass a 1-mm screen. Hay, mesquite leaves, and orts were composited across days and analyzed for CP, NDF, and ADF (SDK Labs, Hutchison, KS) using standard analytical procedures. All intakes and nutrient concentrations are expressed on a DM basis.

***Statistical Analysis.*** Intake of mesquite leaves was analyzed using the repeated measure of PROC MIXED (SAS Inst. Inc., Cary NC). The model included treatment (level of activated charcoal supplementation), day, and treatment by day interaction. Lamb was used as the subject and covariance between repeated records was modeled using autoregressive order1, compound symmetry, and unstructured covariance structures (Littell et al., 1996). Of the three covariance structures evaluated, the structure resulting in the lowest Akaike's Information Criterion (**AIC**) value was selected. Mean mesquite and hay intake after the acclimation period was evaluated with PROC MIXED using a model that contained treatment.

## RESULTS AND DISCUSSION

***Chemical Analyses.*** The Sudan grass hay used in this study was low quality with a relative feed value of 78. Crude protein concentration was 10.51%. The NDF and ADF values were 63.57% and 45.80%, respectively. In contrast, the relative feed value of mesquite leaves (143) was equivalent of moderate quality alfalfa hay. The crude protein concentration of mesquite leaves was 16.10% and NDF and ADF levels were 40.73 and 33.65%, respectively. Regarding crude protein content, our results differ with those of Lyon et al. (1988), where nutritive values of 6 mesquite species were compared with alfalfa (*Medicago sativa* L); the lowest CP content was for *Prosopis alba* with 15%. Conversely, NDF and ADF values reported by Lyon et al. (1988) were similar to those in our study (35.5 and 43.3% for *P. nigra and P. alba*). Similarly, Witmore (2009) reported similar NDF and ADF values for small (40.9 and 27.2%) and large mesquite leaves (36.4 and 29.2%), but CP concentrations in that study for small and large leaves (21.3 and 20.7%, respectively) were greater than those observed in this study. Crude protein values in this study are similar to those obtained by Riveros (1992) for *Prosopis tamarugo* leaves.

***Mean Intake.*** No differences ($P = 0.23$) in hay intake were detected among treatments (Table 1). Similarly, no differences among treatments ($P = 0.61$) were detected in mean mesquite leaf intake. When expressed as a percentage of the diet, mesquite intake averaged 3.65% and varied from 1.24 to 6.25% of the diet. During the first half of the trial (first 8 d), mesquite intake varied from 0.06 to 7.42% of the diet. During the second half of the study (d 9 to 16), mesquite leaves comprised 1.44 to 7.58% of the diet. Negative post-ingestive feedback has been reported with inclusions of more than 5% of the diet as honey mesquite (Witmore, 2009; Baptista and Launchbaugh, 2001). However, a 6-mo study conducted by Abedelnoor et al. (2009) feeding *P. juliflora* leaves at varying levels (5 to 15%) with silage to sheep revealed that organic matter digestibility was improved when mesquite was added to the diet at 5 and 10%. Moreover, they suggested sheep could tolerate this species with silage at up to 15% of the diet. Abedelnoor et al. (2009) indicate that if appropriately mixed with other forages, mesquite is a viable feeding source during dry seasons.

No differences in lamb weight gain were detected ($P = 0.58$). Overall lambs lost 2.32 ± 0.62 kg during the study.

***Daily Intake.*** No differences in daily intake of mesquite leaves were detected ($P = 0.52$). Intake of mesquite leaves changed ($P < 0.001$) during the course of the study. Lambs consumed more mesquite as the study progressed (Figure 1). Intake of mesquite leaves followed a cubic function of day of study:

$$\text{Intake (g/d)} = -6.62 + 8.87*\text{day} - 0.94*\text{day}^2 + 0.04*\text{day}^3$$

Intake of mesquite leaves varied greatly among lambs during the study (Figure 2). Mesquite intake often followed cyclical patterns. Periods of high intake were often followed by periods where lambs consumed few, if any, mesquite leaves. Witmore (2009) reported a similar trend in which intake of mesquite leaves varied cyclically.

In contrast to this study, intake of forages with plant secondary metabolites has been enhanced by supplementation in other studies. Banner et al. (2000) conducted 3 experiments to determine the effect of activated charcoal and barley on sagebrush intake by lambs. In the first experiment, activated charcoal and barley increased sagebrush intake significantly (304 g) compared with the treatment with just barley (248 g). However, Banner et al. (2000) suggested barley had a pivotal role on sagebrush consumption because it has sulfur-containing amino acids that facilitate detoxification. Witmore (2009) observed increased intake of honey mesquite leaves with supplementation of activated charcoal. That study utilized a crossover design with lambs as their own control and a shorter feeding period (Witmore, 2009).

## IMPLICATIONS

Honey mesquite is a shrub found extensively throughout the Southwest that has a relatively high nutrient content compared with grasses during late spring and early summer. Little research has been conducted to assess its effects on livestock or on mechanisms to increase intake. Further studies are needed to understand how honey mesquite affects livestock and how it can serve as a forage to enhance the sustainability of livestock grazing in the southwestern United States and arid regions around the world.

**Table 1**. Mean intake of Sudan hay and honey mesquite leaves of lambs supplemented with four levels of activated charcoal

| Level of Activated Charcoal Supplementation | Sudan Hay, g/d | | Mesquite Leaves, g/d | | Mesquite Leaves in Diet, % | |
|---|---|---|---|---|---|---|
| Item | Mean | SE | Mean | SE | Mean | SE |
| 0.00 g/kg BW | 612.4 | 33.4 | 20.8 | 4.3 | 3.00 | 0.66 |
| 0.33 g/kg BW | 540.8 | 33.4 | 24.8 | 4.3 | 4.02 | 0.66 |
| 0.67 g/kg BW | 563.0 | 33.4 | 20.2 | 4.3 | 3.18 | 0.66 |
| 1.00 g/ kg BW | 512.0 | 33.4 | 27.3 | 4.3 | 4.41 | 0.66 |

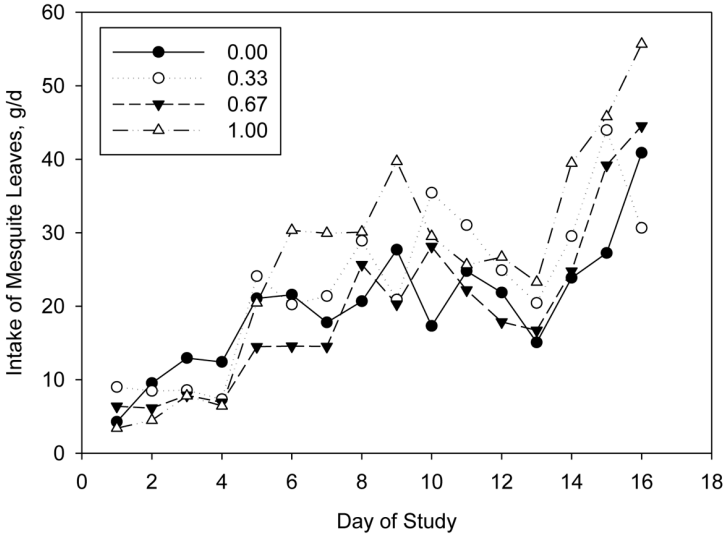


**Figure 1.** Daily intake of honey mesquite leaves by lambs supplemented with activated charcoal at 0.00, 0.33, 0.67, or 1.00 g/kg BW.

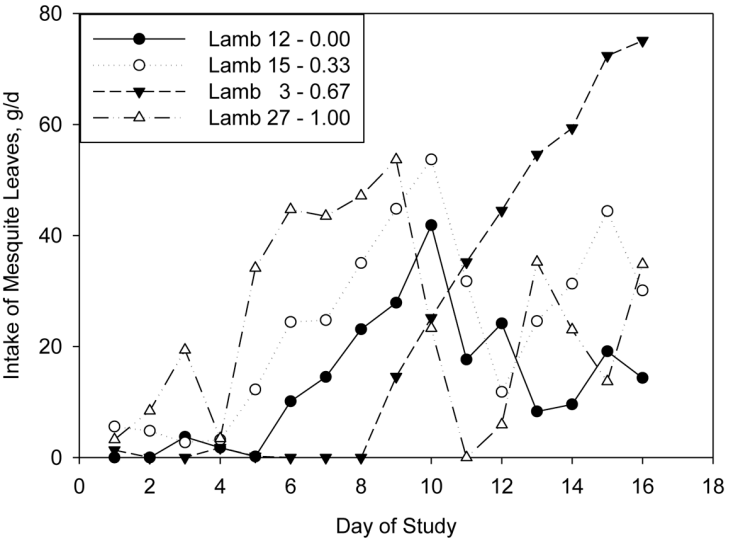


**Figure 2**. Examples of daily intake of mesquite leaves by individual lambs supplemented with 0.00, 0.33, 0.67, and 1.00 g/kg BW of activated charcoal.

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# PRE-BREEDING β-HYDROXYBUTYRATE CONCENTRATION INFLUENCES CONCEPTION DATE IN YOUNG POSTPARTUM RANGE BEEF COWS

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**ABSTRACT:** Cows in a negative energy balance after calving often have reduced reproductive performance, which is suggested to be mediated by metabolic signals. The objective of this 3-yr study was to determine the association of serum metabolites, resumption of estrus, milk production, cow BW change, BCS, and calf performance on conception date in 2- and 3-yr-old beef cows (n = 131) grazing native range at the Corona Range and Livestock Research Center. Cows were classified by conception date in a 60-d breeding season as early conception (EC; conceived in the first 15 d of breeding) or late conception (LC; conceived during the last 45 d of breeding). Date of conception was calculated from the following year calving date. Starting d 30 postpartum, blood samples were collected twice/wk for progesterone analysis to estimate days to resumption of estrus and serum metabolite analysis. As a chute-side measure of nutrient status and glucose sufficiency, whole-blood β-hydroxybutyrate (BHB) concentrations were measured 2 wk prior to breeding. Whole-blood BHB and serum glucose concentrations were lower ($P \leq 0.04$) in EC cows than LC cows. Serum insulin concentrations were greater ($P = 0.03$) in EC cows relative to LC cows. Serum NEFA and urea N concentrations were not different ($P \geq 0.32$) by cows classified by conception date. The initial calving date during the year of the study was not different ($P = 0.19$) between EC and LC cows. The interval required for resumption of estrus after calving was shorter ($P = 0.04$) in EC cows, which is expected due to treatment classification. Milk production was not different ($P = 0.28$) between EC and LC cows. Cow BW and BCS and were not different ($P \geq 0.12$) at any period between EC and LC cows. Calf weaning (205-d) BW was not different ($P = 0.67$) by date of conception. This study indicates that elevated BHB concentrations prior to breeding is coupled with prolonged postpartum anestrus in young beef cows as measured by a delayed time of conception.

**Key words:** beef cows, conception date, β-hydroxybutyrate

## Introduction

Cows grazing primarily dormant range in the semi-arid southwest experience negative energy balance during early lactation. Changes in blood metabolites and metabolic hormones during early lactation, resulting from negative energy balance (**NEB**) after calving, can act as signals to allow or inhibit reproduction (Beam and Butler, 1999). DiCostanzo et al., (1999) demonstrated that intraruminal infusion of acetate for 96 h in ovariectomized heifers experiencing negative energy balance resulted in increased plasma concentrations of acetate, whole-blood β-hydroxybutyrate (**BHB**), and NEFA, which reduced mean concentrations and pulse amplitude of LH. In dairy cows, a decreased serum BHB concentration was associated with increased pregnancy rates after first AI (Walsh et al., 2007) and decreased interval to first ovulation (Reist et al., 2000).

Efficient nutrient utilization or energy metabolism in young cows identified by faster glucose and acetate clearance rates was found to be related to reproductive performance (Waterman et al., 2006 and Mulliniks et al., 2011; respectively). However, the metabolic signals mediating reproduction are not fully understood. The hypothesis of our research was that young beef cows grazing native dormant range that conceive earlier in the breeding season would have improved metabolic responses to nutrient limitations and nutrient demands of lactation shown by decreased whole-blood BHB concentrations. Therefore, the objective of this study was to determine the association of serum metabolites, resumption of estrus, milk production, cow BW and BW change, BCS, and calf performance on conception date in 2- and 3-yr-old beef cows grazing native range at the Corona Range and Livestock Research Center.

## MATERIALS AND METHODS

All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University's Institutional Animal Care and Use Committee.

Studies were conducted over 3 yrs at New Mexico State University's Corona Range and Livestock Research Center (**CRLRC**). Average elevation for CRLRC is 1,900 m with an average rainfall of 401 mm, most of which occurs in July and August (Torell et al., 2008). Forages at this study site were primarily blue grama (*Bouteloua gracilis*), threeawns (*Aristida* spp.), and common wolftail (*Lycurus phleoides*). Pasture was 762 ha and contains approximately 355 kg/ha of standing forage (A. Cibils, New Mexico State University, personal communication). The pasture used in this study was stocked at a rate that was 50% less than the NRCS recommended rate so that forage availability was assumed not to limit cow productivity (USDA-NRCS, 2002). Three

ruminally cannulated cows were used to collect diet extrusa samples for analysis of CP (AOAC, 2000) and NDF (Van Soest et al., 1991). Extrusa samples were collected in April prior to breeding via ruminal evacuation techniques described by Lesperance et al. (1960). Extrusa samples from study pasture averaged (OM basis) 11.3, 5.1, and 8.1% CP and 80.0, 78.6, and 85.9% NDF for yr 1, 2, 3; respectively.

Cows were 2- and 3-yr-old (n = 131) that were primarily of Angus breeding with some Hereford influence. Four cows were not pregnant at the end of the experiment and therefore, were not utilized in the analysis. Management before calving and after calving was similar in all years and among all cows. At least 60 d prior to the initiation of calving, cows were fed 1.6 kg/cow of a 36% CP cube once per week. Cow/calf pairs were moved to a common pasture within 10 d after calving, where a 36% CP supplement was fed 2×/wk at a rate of 908 g/(cow·d$^{-1}$). Date of conception for the study year was estimated from the subsequent years calving date (minus 285 d for gestation). Cows were retrospectively classified as early conception (**EC**) or late conception (**LC**). A 60-d breeding season was utilized in all years and was initiated in mid-May with a bull-to-cow ratio of 1:26. Cows conceiving within the first 15 d of the breeding season were considered as EC cows and the last 45 d for LC cows. Cows were moved to an ungrazed (in previous year) pasture prior to the initiation of breeding in all years. Initiation of breeding occurred on average 67 ± 2 d postpartum across all years.

Serum samples were collected twice weekly on days of supplementation (Monday and Friday) via coccygeal venipuncture (Corvac, Sherwood Medical, St. Louis, MO) beginning approximately 35 d postpartum (by cow) for analysis of progesterone to determine days to first estrus (2 consecutive progesterone concentrations ≥ 1.0 ng/mL). After supplementation, blood samples were collected, cooled, and subsequently centrifuged at 2,000 × *g* at 4°C for 20 min after collection. Serum was harvested and stored at -20ºC in plastic vials for later analysis. Serum was analyzed for progesterone concentration by solid phase RIA (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Schneider and Hallford (1996). Inter- and intra-assay CV were less than 10%. Cows were diagnosed pregnant by rectal palpation at weaning or a few weeks later. Serum samples were also analyzed for insulin, glucose, NEFA, IGF-I, and serum urea N. To evaluate nutrient status of each cow, serum samples were composited by cow for a pre-breeding serum sample. Composited serum samples were analyzed using commercial kits for NEFA (Wako Chemicals, Richmond, VA) and Urea N (Thermo Electron Corp., Waltham, MA). Glucose was analyzed with a commercial kit (enzymatic endpoint, Thermo Electron Corp., Waltham, MA). Insulin was analyzed by solid-phase RIA (Count-A-Coat, Siemens Medical Solutions Diagnostics; Los Angeles, CA) as reported by Reimers et al. (1982). Serum IGF-I samples were quantified by double antibody RIA (Berrie et al., 1995). Inter- and intra-assay CV were less than 10%. As a chute-side measure of nutrient status and glucose sufficiency, whole-blood β-hydroxybutyrate (**BHB**) concentrations were measured (MediSense/Abbott Laboratories, Abingdon, UK, validated by Byrne et al., 2000; Endecott et al., 2004; Voyvoda and Erdogan, 2010) in early-May, 2 weeks prior to breeding.

A subsample of cows was randomly selected to be an equal representation of age were milked by a portable machine (Porta-Milker, Coburn Company, Inc., Whitewater, WI) approximately 57 d postpartum. Milking procedures were a modified weigh-suckle-weigh technique described by Waterman et al. (2006). Milk weights were recorded to calculate 24-hr milk production. Milk samples were analyzed for lactose, butterfat, solids non-fat, and protein by Pioneer Dairy Labs, DHIA (Artesia, NM).

After calving, cows were weighed weekly until termination of the breeding season, and at weaning. Days to BW nadir was calculated from the lowest BW after calving. Body condition scores (1 = emaciated, 9 = obese; Wagner et al., 1988) were assigned to each cow by visual observation and palpation prior to calving, at branding, and weaning by 2 trained technicians. Calves were weighed at birth, branding, and weaning in each year. Branding weights and weaning weights were adjusted for a 55-d branding and 205-d weaning weight with no adjustments for sex of calf or age of dam.

### *Statistical Analysis*

Normality of data distribution and equality of variances of measurements were evaluated using PROC UNIVARIATE, the Levene test, and PROC GPLOT, respectively. Data were analyzed as a completely randomized design with cow as the experimental unit using the Kenward-Roger degrees of freedom method. The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to test all main effects and all possible interactions. The model included fixed effects of conception date groups, cow age, year, sex of calf, and their interactions. All interactions remained in the model regardless of significance. Separation of least squares means was performed by the PDIFF option of SAS when a significant ($P \leq 0.05$) effect of treatment was detected.

## RESULTS AND DISCUSSION

Increased concentrations of BHB are indicative of metabolic dysfunction resulting from poor adaptation to negative energy balance (Herdt, 2000). Cows classified as EC had reduced ($P = 0.04$; Table 1) concentrations of BHB 2 wk prior to breeding relative to cows classified as LC. In dairy cows, a decreased serum BHB concentration pre-breeding was associated with increased pregnancy rates from first service AI (Walsh et al., 2007) and decreased interval to first ovulation (Reist et al., 2000). Iwata et al. (2011) reported elevated BHB concentrations suppressed LH pulse frequency and amplitude and proposed that ketone bodies might function as a negative energy signal to inhibit gonadal functions through suppression of gonadotropin secretion.

Serum glucose concentration was less ($P = 0.02$) in EC cows compared with their counterparts. The decrease in serum glucose and BHB concentrations suggests that serum glucose clearance rate was increased, while increased tissue uptake of glucose improved oxidation of acetate and deterred

the subsequent rise in BHB concentration. Serum insulin concentrations were greater ($P = 0.03$) in the EC cows relative to LC cows, which may have facilitated glucose metabolism. In addition, increased serum insulin concentrations may have had a positive effect on the restoration of LH pulse frequency as reported by Chagas (2003). Insulin, when glucose is available, can stimulate release of GnRH from the hypothalamus (Arias et al., 1992). However, Pushpakumara et al. (2003) reported no differences in serum concentrations of glucose or insulin concentrations between dairy cows conceiving early or late in the breeding season.

Serum NEFA and serum urea N concentrations were not different ($P > 0.32$) between EC and LC cows. In contrast to the current study, Ospina et al. (2010) reported that NEFA concentrations have a stronger association with reproductive performance than BHB in dairy cows. The severity of negative energy balance between a dairy cow and range beef cow, due to a 4 to 5 fold greater milk yield, may explain some of these differences. Pushpakumara et al. (2003) reported that serum BHB concentration prior to breeding was a better indicator of metabolic status than serum NEFA and glucose concentrations.

Insulin-like growth factor-I has been suggested to be a better indicator of rebreeding performance of first calf heifers than BCS or BW change (Roberts, 2008). In this study, serum concentration of IGF-I was not different ($P = 0.28$) between EC and LC cows. Pushpakumara et al. (2003) reported a tendency for decreased IGF-I concentrations prior to breeding in late pregnancy cows relative to early pregnancy cows.

Twenty-four-hour milk production 10 d before onset of breeding did not differ ($P = 0.28$) between EC and LC cows. Concentrations of milk butterfat, protein, lactose, and solids-non-fat also were not different ($P \geq 0.24$) between EC and LC cows. Pushpakumara et al. (2003) reported no difference in 24-h milk production between dairy cows that become pregnant to an early or late service. However, increased acetate utilization as detected by decreased BHB and glucose concentrations indicates alterations in whole blood nutrient availability, which could result in decreased milk production.

Calving date during the initial year of the study was not different ($P = 0.19$) between EC and LC cows. As designed, EC cows conceived early in the breeding season relative to LC cows ($P < 0.01$). Interval from calving to resumption of estrus was shorter ($P = 0.04$) in EC cows compared with LC. Early conception cows returned to estrus 7 days before LC cows. Increasing the number of young cows becoming pregnant early in the breeding season would increase their opportunity to remain in the herd by calving early in the subsequent calving seasons.

Days to BW nadir and interval from BW nadir to estrus were not different ($P \geq 0.18$; Table 3) between EC and LC classification groups. Cow BW and BCS were similar between EC and LC cows at all measurement times ($P \geq 0.12$). However, BW loss from the initiation of the study to the beginning of breeding was greater ($P = 0.01$) in LC cows relative to EC cows. The difference in BW loss could be due to differences in efficiency of energy utilization during early lactation. Late conception cows did gain more ($P = 0.04$) BW from BW nadir to the end of breeding with no differences ($P > 0.14$) in BW change at any other time period. Calf BW at birth, branding, and weaning were not influenced ($P \geq 0.46$) by conception date classification group.

In conclusion, serum metabolite differences during early lactation was an effective means to segregate cows based on classification of young beef cows according to their conception date. This study indicates that elevated β-hydroxybutyrate concentrations prior to breeding are related to or may have a detrimental effect on the interval to resumption of estrus in young beef cows and thereby prolong time of conception. A decreased concentration of β-hydroxybutyrate resulted in an earlier conception date in young lactating beef cows. Therefore, BHB concentrations prior to breeding may be a useful predictive indicator of days to resumption of estrus and conception date. In addition, chute-side measurement of β-hydroxybutyrate concentrations may provide producers opportunity to manage cows differently to improve overall reproductive efficiency.

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**Table 1.** Association of pre-breeding blood ketones, serum metabolites, and milk production with 2- and 3-yr-old cows grazing native range classified as early or late conception

| | Conception date[1,2] | | | |
|---|---|---|---|---|
| Pre-breeding measurement | EC | LC | SEM | P-value |
| n = | 65 | 62 | -- | -- |
| Blood Ketones, μmol/L | | | | |
| Whole-blood β-hydroxybutyrate | 297 | 371 | 20 | 0.04 |
| Serum Metabolites | | | | |
| Glucose, mg/dL | 52.9 | 58.1 | 1.7 | 0.02 |
| Insulin, ng/mL | 0.81 | 0.56 | 0.07 | 0.03 |
| NEFA, mmol/L | 501 | 491 | 19 | 0.69 |
| Urea N, mg/100 mL | 9.07 | 9.74 | 0.48 | 0.32 |
| IGF-I, ng/mL | 45.78 | 42.04 | 3.46 | 0.42 |
| Milk[3], g/d | | | | |
| 24-h milk Production | 5991 | 6510 | 335 | 0.28 |
| Lactose | 298 | 320 | 16 | 0.34 |
| Protein | 159 | 175 | 9 | 0.24 |
| Solids-non-fat | 512 | 553 | 28 | 0.31 |
| Butterfat | 197 | 215 | 15 | 0.41 |

[1]Conception date was estimated from the subsequent years calving date.
[2]EC = Early conception (conceived with the first 15 d of breeding); LC = late conception (conceived during the last 45 d of breeding season).
[3]Milk production was evaluated in a subset of cows approx. d 57 postpartum (10 d before onset of breeding).

**Table 2.** Association of calving date and reproductive measurements with 2- and 3-yr-old cows grazing native range classified as early or late conception

| | Conception date[1,2] | | | |
|---|---|---|---|---|
| Measurement | EC | LC | SEM | *P*-value |
| Calving date[3], Julian d | 62 | 65 | 2 | 0.19 |
| Reproductive | | | | |
| Conception date, Julian d | 142 | 165 | 1 | <0.001 |
| Resumption of estrus[4], d | 71 | 78 | 2 | 0.04 |
| BW nadir, d | 59 | 57 | 4 | 0.71 |
| Nadir to estrus[5], d | 13 | 22 | 5 | 0.18 |

[1]Conception date was estimated from the subsequent years calving date.
[2]EC = Early conception (conceived with the first 15 d of breeding); LC = late conception (conceived during the last 45 d of breeding season).
[3]Calving date of the study year.
[4]Interval from calving to resumption of estrus.
[5]Interval from BW nadir to resumption of estrus.

**Table 3.** Association of cow BW and BW change, BCS, calf weight change with 2- and 3-yr-old cows grazing native range classified as early or late conception

| | Conception date[1,2] | | | |
|---|---|---|---|---|
| Measurement | EC | LC | SEM | *P*-value |
| Body condition score | | | | |
| Initial | 4.5 | 4.6 | 0.04 | 0.81 |
| Branding | 4.4 | 4.2 | 0.05 | 0.12 |
| Weaning | 4.6 | 4.7 | 0.06 | 0.80 |
| Cow BW, kg | | | | |
| Initial | 449 | 454 | 5 | 0.41 |
| Nadir | 368 | 368 | 4 | 0.97 |
| Begin of breeding | 393 | 388 | 5 | 0.39 |
| End of breeding | 400 | 408 | 5 | 0.19 |
| Weaning | 447 | 453 | 5 | 0.37 |
| Cow BW change, kg | | | | |
| Initial to begin of breeding | -56 | -67 | 3 | 0.01 |
| Initial to nadir | -81 | -87 | 4 | 0.24 |
| Initial to end of breeding | -49 | -46 | 4 | 0.59 |
| Nadir to end of breeding | 32 | 40 | 3 | 0.04 |
| Nadir to weaning | 79 | 85 | 3 | 0.14 |
| Initial to weaning | -2 | -1 | 4 | 0.87 |
| Calf BW, kg | | | | |
| Birth | 34 | 33 | 1 | 0.46 |
| Branding[3] | 70 | 68 | 2 | 0.49 |
| Weaning[4] | 203 | 205 | 4 | 0.67 |

[1]Conception date was estimated from the subsequent years calving date.
[2]EC = Early conception (conceived with the first 15 d of breeding); LC = late conception (conceived during the last 45 d of breeding season.
[3]Branding BW adjusted for 55-d BW.
[4]Weaning BW adjusted for 205-d BW.

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# EFFECTS OF ALGAL MEAL SUPPLEMENTATION TO FINISHING WETHERS ON PERFORMANCE AND CARCASS CHARACTERISTICS

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**ABSTRACT:** Crossbred wethers (n = 40; initial BW = 45.3 kg ± 3.5) were used in a randomized complete block design to evaluate the effects of titrated concentrations of algal meal as a protein supplement on live performance, live health status and carcass characteristics. Wethers were blocked by time and randomly assigned to one of the 5 treatments. Treatments included: 1) soybean meal and rice meal as protein supplementation sources (CON); 2) 5% of algae meal on a DM basis as a protein replacement (5%A); 3) 10% of algae meal on a DM basis as a protein replacement (10%A); 4) 15% of algae meal on a DM basis as a protein replacement (15%A); and 5) 20% of algae meal on a DM as a protein replacement (20%A). All diets were isocaloric and isonitrogenous All wethers were fed a high concentrate finishing diet once daily in individual stalls. Wethers were individually weighed on d -1, 0, 21, and 28. On d 22, wethers were transported to metabolic crates for determination of nutrient digestibility and retention. On d 28, animals were transported to a commercial abattoir for harvest. Initial (45.4 kg) and final (44.5 kg) BW, average daily gain (ADG) for adjustment period (0.24 kg/d), ADG for metabolism period (-0.84 kg/d), DMI (1.38 kg/d), and gain-to-feed (0.187) were similar ($P > 0.05$) across treatments. Furthermore, hot carcass weight, subcutaneous adipose depth, *Longissimus* muscle area, calculated YG, marbling score, dressing percentage, muscle percentage, body wall thickness, Leg score, Leg circumference, flank streaking, quality grade, carcass conformation and carcass length were also similar ($P > 0.05$) across treatments. Research results suggest that feeding up to 20% of algae co-product meal as a replacement protein source to finishing wethers is feasible with limited impact on performance and carcass characteristics as compared with the standard protein sources that have been used by the industry. Further research may be necessary to determine the response of different levels of supplementation of algal meal for sheep, effects on animals in a different physiological stage or effects on other ruminants in the finishing diet on performance and carcass merit.

**Key words:** algae, co-product, protein, ruminant nutrition

## INTRODUCTION

Animal nutrition is considered the most costly component in modern animal production (ARS-USDA, 2012). To be biologically, economically and environmentally sustainable, the development of new technologies and alternative feedstuffs are necessary to enhance performance and quality of animal protein products for the future. With grain commodity and fuel prices rising in the past three years (ERS-USDA, 2012), development of alternative sources of fossil fuels such as corn ethanol and biofuels is of the utmost importance.

Some co-products from the ethanol industry have been utilized in animal diets such as wet distillers grains, distillers solubles, corn gluten feed, and their efficiency have been tested over the years among different species (Gill et al., 2008; Klopfenstein et al., 2008; Dib et al., 2010; Schoonmaker et al., 2010; Bremer et al., 2011). The main hypothesis is that algal meal would behave in the rumen like soybean meal not affecting live growth performance, carcass characteristics and health status.

As a result, algae biodiesel industry increases as a potential future fuel resource also producing a high protein and fiber co-product where ruminant production systems could take advantage, utilizing it as a replacement of soybean meal, corn gluten meal, and distillers grains, among others. The use of an algal meal could be a valuable, economic, and environmentally sustainable substitution for these common agricultural crops. Since algal meal has not yet been approved for feeding livestock, there is a paucity of data regarding their use by large ruminant animals.

Therefore, this experiment was designed to determine the effects of varying inclusions of algal meal supplementation on performance, blood chemistry and live health status, and carcass characteristics of finishing wethers.

## MATERIALS AND METHODS

The experiment was conducted to conform to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and was approved by the Institutional Animal Care and Use Committee. Animals were inspected by a veterinarian before the study started and also once a week throughout the experiment.

***Animals and Treatments.*** Forty yearling crossbred wethers were evaluated in a randomized complete block design for DMI, feed efficiency, ADG, blood chemistry and live health status, live final BW and carcass characteristics to address the effects of titrated concentrations of algal meal. The animals were blocked by time with each block consisting of 10 animals starting on treatments one week apart from each other. Animals were randomly assigned to 1 of 5 treatments of the experiments and every block had two animals in each treatment per block.

The experiment consisted of two periods; 1) adjustment period (21 d) and 2) metabolism period (7 d). The treatments (n = 8 wethers/treatment) for the experiment consisted of: 1) soybean meal and rice meal as protein supplementation sources; 2) 5% of algae meal on a DM basis as a protein replacement; 3) 10% of algae meal on a DM basis as a protein replacement (10%A); 4) 15% of algae meal on a DM basis as a protein replacement (15%A); and 5) 20% of algae meal on a DM as a protein replacement (20%A); All diets were isocaloric and isonitrogenous. All wethers were fed a high concentrate finishing diet once daily. Table 1 represents the nutrient analyses for the algal meal and table 2 represents the diets for the 5 treatments. Both treatment diets were formulated to meet or exceed the NRC (1996) requirements for CP, Ca, K and P.

**Table 1.** Analyzed nutrient analysis for algal meal, % DM

| Nutrient content | %, DM |
|---|---|
| Crude Protein, % | 21.12 |
| Acid Detergent Fiber, % | 11.875 |
| Neutral Detergent Fiber, % | 23.16 |
| Fat (EE), % | 1.96 |
| Calcium, % | 6.01 |
| Phosphorous, % | 0.435 |
| Potassium, % | 0.52 |
| Magnesium, % | 0.74 |
| Sodium, % | 5.685 |
| Chloride, % | 4.25 |
| Sulfur, % | 0.85 |
| Copper, mg/kg | 19.95 |
| Iron, mg/kg | 5355 |
| Lead, mg/kg | 3.785 |
| Zinc, mg/kg | 41.6 |

***Experiment Facilities.*** Each individual pen was 1.5 x 2.0 m, concrete floor, wood shavings for animal bedding, allowed for ad libitum access to water, feed bunk and the facility was covered and had a wall on the north side and was open on the south side. When the study began, wethers weighed 45.3 ± 3.5 kg. Feed intake was measured for a 28 d period using individual bunks. Wethers were fed once daily at 0730 h and feed refusals were collected, frozen at - 20° C, composited by animal and week, and dried to calculate DMI. Wethers had ad libitum feed access to one of the five treatment diets CON, 5%A, 10%A, 15%A and 20%A.

***Data Collection.*** Blood samples and body weights were measured on d 0, 21 and 28 of the feeding period. On d 22, wethers were transported to metabolic crates for determination of nutrient digestibility and retention. On d 28, animals were transported to a commercial abattoir for harvest. Blood chemistry data was obtained utilizing VetScan iSTAT (Abaxis, CA) with the EC8+ and CG8+ cartridges that includes: glucose, Na, K, Cl, $PCO_2$, Total $CO_2$, pH, ionized Ca, hematocrit, hemoglobin, $PO_2$, $HCO_3$, Base Excess, soluble $O_2$, blood urea nitrogen and anion gap.

Individual BW gain was calculated by the difference in BW on d 21 and d 0, and d 28 and d 21, on study and reported as adjustment period ADG and metabolism period ADG. During the adjustment period, initial BW, final BW, DMI, and blood samples were collected. Final BW for the adjustment period was utilized as initial BW for the metabolism period. During the balance trial, feed, orts, total feces and total urine were collected and 10% of the daily amount was aliquoted over the 5 d balance trial, frozen and retained for subsequent analysis. Final BW and blood were collected immediately following the completion of the balance trial.

***Statistical Analysis.*** All data were analyzed using the MIXED procedures (SAS Inst. Inc., Cary, NC). The model consisted of fixed effects of treatment and block, and random effect of animal. Live performance data were analyzed not only for the entire adjustment feeding period, but also for the metabolism period separately.

**Table 2.** Finishing diets for yearling wethers using algal meal compared with standard industry soybean meal diet at 0, 5, 10, 15, or 20% of the dietary DM

| | Treatment | | | | |
|---|---|---|---|---|---|
| Ingredient, % | CON | 5% algal meal | 10% algal meal | 15% algal meal | 20% algal meal |
| Corn | 49.25 | 48.90 | 49.25 | 49.60 | 49.90 |
| Soybean Meal | 15.25 | 13.75 | 12.00 | 10.30 | 8.75 |
| Rice meal | 29.00 | 26.25 | 23.00 | 19.70 | 16.25 |
| Algal Meal | 0.00 | 5.00 | 10.00 | 15.00 | 20.00 |
| Limestone | 1.50 | 1.10 | 0.75 | 0.40 | 0.10 |
| Supplement | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |

## RESULTS AND DISCUSSION

Results for live performance are presented on table 3. During the feeding period, initial (45.4 kg) and final (44.5 kg) BW, average daily gain (ADG) for adjustment period (0.24 kg/d), ADG for metabolism period (-0.84 kg/d), DMI (1.38 kg/d), and gain-to-feed (0.187) were similar ($P \geq 0.18$) across treatments.

Results for carcass characteristics are presented on table 4. Furthermore, hot carcass weight, subcutaneous adipose depth, *Longissimus* muscle area, calculated YG, marbling score, dressing percentage, muscle percentage, body wall thickness, Leg score, Leg circumference, flank streaking, quality grade, carcass conformation and carcass length were also similar ($P \geq 0.27$) across treatments. Significant differences were observed on leg score measurements ($P < 0.01$), with greater values presented on treatment 10%A followed by treatments 20%A, CON, 5%A and 15%A respectively. Since no pattern was observed across treatments it is possible to assume that the differences were artifactual.

Blood chemistry results presented all measurements within a normal range from pre-study period until harvest time. No difference was observed across treatments for health status. According to veterinarian inspections, no side effects were observed due to treatment and behavior and vital signs were considered the same across treatments. Additionally, during harvest, there were no apparent abscesses of the internal organs noted upon inspection.

This study was one of the first studies to feed lipid extracted algal meal as a feedstuff for ruminant animals consuming a high concentrate diet. Previous research in our lab evaluated the use of algal meal in the diets of growing rats (Howe et al., 2010) and rabbits (Howe et al., 2011). There was some limitation upon growth parameters and gain:feed in the rats, but not in the rabbits. This was speculated to be due to

**Table 3.** Live growth performance of yearling wethers (n = 8/treatment) consuming finishing rations with 0, 5, 10, 15, or 20% of the dietary DM as algal meal

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | CON[1] | 5% Algal Meal | 10% Algal Meal | 15% Algal Meal | 20% Algal Meal | SEM | *P*-value |
| Initial BW, kg | 46.4 | 45.2 | 45.6 | 44.6 | 44.9 | 0.91 | 0.68 |
| Final BW, kg | 45.3 | 44.6 | 45.9 | 44.8 | 42.0 | 1.15 | 0.18 |
| ADG adjustment, kg | 0.22 | 0.24 | 0.25 | 0.26 | 0.22 | 0.04 | 0.96 |
| ADG metabolism, kg | -0.81 | -0.82 | -0.70 | -0.81 | -1.03 | 0.18 | 0.78 |
| G:F | 0.139 | 0.170 | 0.164 | 0.189 | 0.149 | 0.05 | 0.77 |
| DMI, g | 1,409.5 | 1,258.2 | 1,482.9 | 1,354.9 | 1,376.2 | 90.41 | 0.55 |

**Table 4.** Carcass characteristics of yearling wethers (n=8/treatment) consuming finishing rations with 0, 5, 10, 15, or 20% of the dietary DM as algal meal

| Treatments | CON[1] | 5% Algal Meal | 10% Algal Meal | 15% Algal Meal | 20% Algal Meal | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| HCW, kg | 23.4 | 23.1 | 23.1 | 22.9 | 21.8 | 0.73 | 0.59 |
| Dressing, %[a] | 51.6 | 51.6 | 50.2 | 51.1 | 51.7 | 0.64 | 0.49 |
| 12th rib fat, cm | 0.34 | 0.39 | 0.51 | 0.57 | 0.40 | 0.11 | 0.57 |
| Marbling score[b] | 48.29 | 48.09 | 47.36 | 47.17 | 48.27 | 0.44 | 0.27 |
| LM area, $cm^2$ | 13.54 | 13.50 | 12.95 | 12.54 | 13.26 | 0.61 | 0.76 |
| USDA yield grade[c] | 1.77 | 1.93 | 2.42 | 2.67 | 2.00 | 0.43 | 0.57 |
| Percentage of muscle,% | 48.29 | 48.09 | 47.36 | 47.17 | 48.27 | 0.44 | 0.27 |
| Body Wall Thickness, cm | 1.32 | 1.44 | 1.66 | 1.63 | 1.39 | 0.13 | 0.32 |
| Leg Score | $11.25^{y}$ | $10.75^{yz}$ | $11.45^{y}$ | $10.12^{z}$ | $11.37^{z}$ | 0.18 | <0.01 |
| Leg Circumference, cm | 64.80 | 63.40 | 64.43 | 63.22 | 63.38 | 0.73 | 0.46 |
| Carcass Length, cm | 108.06 | 107.69 | 107.69 | 108.38 | 106.94 | 0.77 | 0.75 |

[a]Dressing percentage = carcass weight / average live weight (4% shrink).
[b]USDA marbling score where 450 = slight50, 500 = small0, and 550 = small50
[c]USDA calculated yield grade = (Fat thickness in inches * 10) + 0.4
[y,z] Least square means within a row without a common superscript differ ($P < 0.05$)

the more advanced symbiosis between animal and digestive microflora in the cecotrophic rabbits, which allowed them to more fully utilize the highly fibrous algal meal. Similarly, the wethers used in the current study similarly were able to utilize the product similar to the diets without algal meal. This further confirms that algal meal may serve as a viable feedstuff for animals that have a symbiotic relationship that allows for use of feeds with larger amounts of fiber, in this case 23.16% NDF. Understanding the nutritional values of *Nannochloropsis* oc. as a potential animal supplement, Archibeque et al. (2009) compared the nutrient profiles of *Nannochloropsis* biomass, *Nannochloropsis* meal (lipid extracted), soybean meal, and steam flaked corn. As a result, the comparison showed that the NDF and ADF fiber composition of *Nannochloropsis* meal was greater than soybean meal, and steam flaked corn, %, 25.12% vs. 11.45% and 9.59%), and 6.64 vs. 5.89, 2.92% respectively.

## IMPLICATIONS

Lipid extracted algal meal might be feasible alternative for protein supplementation for ruminants in the future. Further research may be necessary to determine the response of different levels of supplementation of algal meal for sheep and other ruminants, effects on animals in a different physiological stage or effects on other ruminants in the finishing diet on performance and carcass merit.

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# INFLUENCE OF THE LEVEL OF DRIED DISTILLERS GRAINS WITH SOLUBLES ON FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, SERUM TESTOSTERONE CONCENTRATIONS, AND SEMEN QUALITY OF GROWING RAMS[1]

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**ABSTRACT:** The objective of this study was to evaluate the effects of dried distillers grains with solubles (DDGS) on ram lamb feedlot performance, carcass characteristics, serum testosterone concentration, and semen quality. One hundred twenty ram lambs (40.4 ± 9.1 kg; western whiteface × Suffolk) were used in a completely randomized design to determine the effects of DDGS on feedlot performance and carcass characteristics. Rams were allotted into one of four dietary treatments (n = 4 pens/treatment; 10 rams/pen): 1) 0DDGS: 85% corn and 15% commercial market lamb pellet; 2) 15DDGS: 15% DDGS substituted for corn on a % DM basis, 70% corn, and 15% commercial market lamb pellet; and 3) 30DDGS: 30% DDGS substituted for corn on a % DM basis, 55% corn, and 15% commercial market lamb pellet. Rams were weighed on consecutive days at the beginning (d 0, 1) and end (d 96, 97 and d 116, 117) of the trial. Scrotal circumference was measured on all rams on d 84, 96, and 116. Semen and blood samples were collected on a subset of 48 rams (4 rams/pen; 16 rams/treatment; n = 4). Blood samples were collected every 14 d throughout the study. Semen samples were collected on d 84, 98, and 112. Rams were fed to market weight, shipped to a commercial abattoir, and harvested for carcass data collection. Initial BW, final BW, change in scrotal circumference, days on feed, carcass characteristics, serum testosterone concentrations, and spermatozoa motility score were not different ($P \geq 0.23$) due to dietary treatment. However, DMI increased linearly ($P < 0.001$) as DDGS increased in the ration, resulting in a linear increase ($P = 0.02$) in ADG. Additionally, spermatozoa concentration decreased linearly ($P = 0.05$) as DDGS concentration increased in the ration. Increasing DDGS in the diet did not have a negative impact on ram feedlot performance or carcass characteristics; however, in this preliminary study, sperm production may have been negatively affected, necessitating the need for additional research on the impact of distillers grains on ram development.

**Key words:** dried distillers grains with solubles, rams, semen quality

## INTRODUCTION

Ethanol production has increased from 1.5 million gallons in 2000 to approximately 13 million gallons in 2010 in the United States (Renewable Fuels Association, 2012). With this expansion brings an affordable and viable feed source for ruminants, dried distillers grains with solubles (**DDGS**). Research involving the feeding of DDGS to ruminants has become more prominent in the past few years due to the rising costs of feedstuffs, particularly corn. During the growing and finishing phase, DDGS fed to steers at 30% of the diet did not affect any performance variable or carcass characteristic (Leupp et al., 2009). Similar results were observed by Schauer et al. (2008) and Neville et al. (2010) where finishing lamb performance and carcass characteristics were not negatively impacted when lambs were fed DDGS at 60% of the total diet.

We are not aware of research evaluating the effects of DDGS on male reproductive performance; however, research is available on feeding increased dietary CP to growing males (an artifact of increasing DDGS in rations, as DDGS is relatively high in CP). Rams fed a high energy and protein diet had increased testosterone concentrations at the beginning of the trial, but as the trial duration increased, the differences in testosterone concentrations were reduced (Martin et al., 1994). Hotzel et al. (1998) observed an increase in testosterone concentrations in Merino rams fed a diet above energy requirements.

We hypothesized that feeding increasing levels of DDGS (0, 15, and 30% of DM) would have no deleterious effects on ram feedlot performance or carcass characteristics, but would reduce semen quality and have a negative impact on testosterone concentrations. Therefore, the objectives of this study were to evaluate the effects of DDGS on ram lamb feedlot performance, carcass characteristics, serum testosterone concentration, and semen quality.

## MATERIALS AND METHODS

All procedures were approved by the animal care and use committee of North Dakota State University. This study

[1]Partial support for this research was provided by the North Dakota Corn Council. We thank Poet Nutrition and Kip Karges for the donation of the dried distillers grains with solubles for the project. The authors also thank David Pearson, Don Stecher, and Donald Drolc for their assistance in conducting this trial.

was conducted at the Hettinger Research Extension Center in Hettinger, North Dakota.

***Animals and Diets.*** One hundred twenty crossbred rams (40.4 ± 9.1 kg; western whiteface x Suffolk; approximately 90 d of age) were used in a completely randomized design. Rams were allotted into one of four isocaloric dietary treatments (n = 4 pens/treatment; 10 rams/pen; Table 1): 1) **0DDGS**: 85% corn and 15% commercial market lamb pellet; 2) **15DDGS**: 15% DDGS substituted for corn on a % DM basis, 70% corn, and 15% commercial market lamb pellet; and 3) **30DDGS**: 30% DDGS substituted for corn on a % DM basis, 55% corn, and 15% commercial market lamb pellet. Rams had ad libitum access to the ground ration via self-feeders. Rams had continuous access to water and shade. Rams were weighed on two consecutive days at the beginning (d 0, 1) and end of the trial (d 96, 97 and d 116, 117), and weighed once every 28 d. Scrotal circumference was measured on d 84, 96, and 116 of the trial. Two slaughter dates were utilized for the trial. The first slaughter date included all rams weighing at least 67 kg except those involved with the semen quality and testosterone portions of the trial. The second slaughter date included all remaining rams on trial. Hot carcass weight was recorded on the day of slaughter. All other carcass characteristics were recorded following a 24 h chill. At each slaughter date, rams were shipped to Superior Farms in Denver, CO for carcass data collection. One ram was removed from the trial prior to being shipped for slaughter due to non-treatment related purposes.

***Sampling and Laboratory Analysis.*** Ground ration samples were collected every 14 d and dried at 55°C for 48 h to determine DM and ground to pass a 2-mm screen. Ground ration samples were composited within dietary treatment. Dietary composite samples were shipped to a commercial lab (Midwest Laboratories, Inc., Omaha, Nebraska) for proximate analysis and mineral concentrations. Orts were collected and weighed every 28 d throughout the study to determine DMI.

***Reproductive Performance.*** Forty-eight rams (a sub-sample of the 120 rams in the feedlot study described above; 4 rams/pen; 16 rams/treatment; n = 4) were chosen for semen quality and serum testosterone concentration analysis. Semen from 48 rams was collected on d 84, 98, and 112 of the study via electro-ejaculation. Spermatozoa motility score and concentration of spermatozoa in the ejaculate, via a hemocytometer on the fresh ejaculate sample, were used to determine semen quality. The spermatozoa motility score (rate of forward movement; RFM) was determined on a 1 to 4 scale, with 1: no forward movement, all dead; 2: slow forward movement; 3: moderate forward movement; and 4: fast forward movement. Every 14 days throughout the duration of the trial, a 10 mL blood sample was collected via jugular venipuncture from each of the 48 rams and immediately placed on ice until serum could be harvested post- centrifugation. Blood samples were collected via a 20 gauge × 1 inch vacutainer needles into serum separator 16 × 100 mm tubes. Serum was frozen at -20ºC until serum testosterone analysis (IMMULITE 1000 Total Testosterone; LKTW1; Siemens Diagnostic; Los Angeles, CA). The intra- and inter-assay CVs were 8.1 and 8.5%, respectively.

***Statistical Analysis.*** Ram feedlot performance, carcass characteristics, and scrotal circumference were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen serving as the experimental unit. The fixed effect included in the model was dietary treatment with the random effect of pen nested in treatment. The fixed effect of day was utilized in the repeated measures analysis for testosterone concentrations, and spermatozoa motility score and concentration. The model included the fixed effects of dietary treatment, day, and treatment × day. When a significant *F*-test was observed ($P \leq 0.15$), pre-planned comparisons of linear and quadratic contrasts were utilized to partition treatment effects. Significance was determined at $P \leq 0.05$. All interactions that were not clearly significant ($P \geq 0.20$) were removed from the model. To partition day effects and treatment × day interactions, LS Means was utilized ($P \leq 0.05$).

## RESULTS AND DISCUSSION

***Feedlot and Carcass Characteristics.*** Initial BW was not different ($P = 0.89$; Table 2) between dietary treatments by design. Final BW and days on feed were not affected ($P \geq 0.50$) by dietary treatment. Average daily gain increased ($P = 0.02$) linearly as DDGS in the diet increased. Previous research has suggested that lambs consuming rations containing DDGS have increased ADG compared with those lambs consuming no DDGS (Schauer et al., 2008). Dry matter intake increased linearly ($P <$

0.001) as the amount of DDGS increased in the ration. These results are similar to those observed by Schauer et al. (2008) when DDGS inclusion was increased to 60% of the ration as a replacement for barley. In the current study, the linear increase in DMI led to a linear reduction ($P < 0.001$) in G:F as the inclusion of DDGS in the diet increased. Although G:F was negatively affected, this did not negatively impact days on feed ($P = 0.54$); therefore, as expected, the DDGS did not have any deleterious effects on ram feedlot performance.

No differences in carcass characteristics were detected ($P \geq 0.26$; Table 2) by dietary treatment. These results were similar to Schauer et al. (2008) and Neville et al. (2010) in which there were no deleterious effects on carcass characteristics with DDGS inclusion in the diet of feedlot lambs. Based on our research, DDGS can be safely substituted for corn in ram finishing rations without causing a negative impact on carcass characteristics.

***Reproductive Performance.*** No difference in the change in scrotal circumference was detected ($P = 0.61$; Table 2). Contrary to the results in the current study, Martin et al. (1994) noted an increase in scrotal circumference in rams fed the high protein and energy rations compared with the rams fed the low energy and protein rations. Hötzel et al. (1998) also observed an increase in the change in scrotal

circumference throughout the study in rams fed to have an increased rate of gain. Similar results were also observed in bulls, in which bulls were fed increased energy diets (Coulter and Kozub, 1984) with a resulting increase in testicular size. Although TDN was not different between diets in our trial, the CP of the diets increased with increasing concentrations of DDGS. Therefore, the results in the current study were not expected. There was no treatment × day interaction ($P$ = 0.86; Figure 1) or treatment effect ($P$ = 0.97) on plasma testosterone concentrations. However, there was a day effect ($P$ < 0.001) for testosterone concentrations, with testosterone concentration increasing as the trial progressed. This was expected as rams became more mature throughout the study; therefore, the testosterone concentrations would be expected to increase as the rams reached maturity. Contrary to the current study, testosterone concentrations were decreased in mature Merino rams fed a sub- maintenance diet compared with those fed a supra- maintenance (Hötzel et al., 1998). Martin et al. (1994) observed similar results to Hötzel et al. (1998), in which the high and intermediate energy and protein fed rams had increased testosterone concentrations compared with the low energy and protein fed rams.

There was not a treatment × day interaction ($P$ = 0.29) for spermatozoa concentration. Spermatozoa concentration was reduced linearly ($P$ = 0.05; Table 2) as DDGS in the diet increased. Coulter and Kozub (1984) observed a reduction in epididymal spermatozoa reserves and motility in bulls fed a high energy diet. The current results as well as previous research (Coulter and Kozub, 1984), may suggest increased protein or fat or both in the diet cause a reduction in spermatogenesis. This may be due to increased fat deposits within the scrotum and even the spermatic cord (Senger, 2005). The spermatozoa concentration on d 98 was significantly less ($P$ = 0.04; Figure 2) than on d 112. This reduction on d 98 may have been due to the stage of spermatogenesis the rams were in during the 47 d cycle (Senger, 2005). The spermatozoa may have been mainly in the immature, or proliferation, phase of spermatogenesis and would not have been ejaculated at this time (Senger, 2005). There were no effects of treatment ($P$ = 0.23) or day ($P$ = 0.24) on the spermatozoa motility score. However, there was a treatment × day interaction ($P$ = 0.02; Figure 3). The 15DDGS rams had a reduced ($P$ = 0.006) motility score on d 98 compared with d 112. Although not significant ($P$ = 0.12; Table 2), there was a linear numerical reduction in motility score as DDGS increased in the diet. Rocha et al. (1995) did not observe any deleterious effects on spermatozoa motility or semen volume when utilizing fish meal as a rumen undegradable protein (**RUP**) source. Dried distillers grains with solubles are often used as a source of RUP; however, care must be taken when feeding DDGS due to the increased sulfur content. Therefore, the increased sulfur content of the DDGS may have led to the contradicting results between the current study and Rocha et al. (1995).

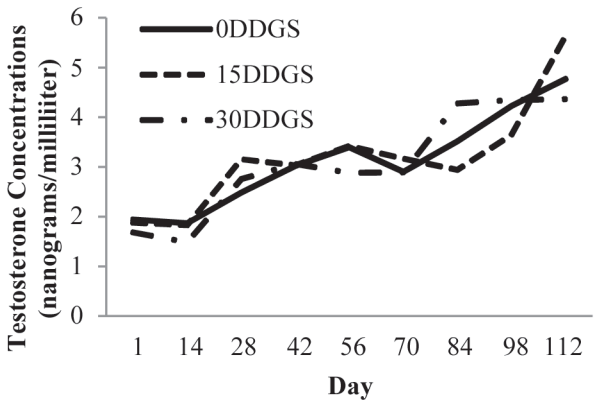


**Figure 1.** The effects of day and dried distillers grains with solubles on testosterone concentrations of growing ram lambs. Treatments were 0DDGS: 85% corn and 15% commercial market lamb pellet; 15DDGS: 15% DDGS substituted for corn on a % DM basis; and 30DDGS: 30% DDGS substituted for corn on a % DM basis. $P$-values: treatment, $P$ = 0.97; day, $P$ < 0.001; treatment × day, $P$ = 0.86.

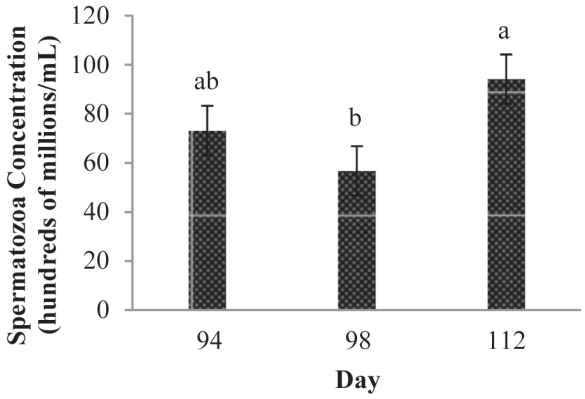


**Figure 2.** The effects of day on spermatozoa concentration. Spermatozoa were counted on a hemocytometer. $P$-values: treatment, $P$ = 0.13; day, $P$ = 0.04; treatment × day, $P$ = 0.29. [a, b] Bars with different superscript are different ($P$ < 0.05).

## IMPLICATIONS

The current research suggests that growing rams can be fed up to 30% (DM basis) of DDGS without causing deleterious effects to feedlot performance and carcass characteristics. However, care must be taken when feeding DDGS to growing rams due to a possible reduction in spermatozoa concentration. Further research is needed to elucidate why semen quality is affected and if actual fertility of rams is compromised by feeding increasing concentrations of DDGS.

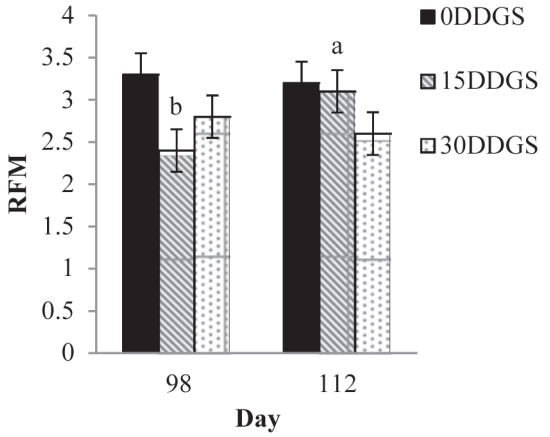


**Figure 3**. The effects of day and dried distillers grains with solubles on spermatozoa motility score. Treatments were 0DDGS: 85% corn and 15% commercial market lamb pellet; 15DDGS: 15% DDGS substituted for corn on a % DM basis; and 30DDGS: 30% DDGS substituted for corn on a % DM basis. Spermatozoa motility score (rate of forward movent; RFM): 1 = no forward movement; 2 = slow forward movement; 3 = moderate forward movement; and 4 = fast forward movement. *P*-values: treatment, $P = 0.23$; day, $P = 0.24$; treatment × day, $P = 0.02$. [a,b] Bars with different superscript are different ($P < 0.05$).

**Table 1.** Ingredient and nutritional composition of diets fed to feedlot ram lambs (DM basis)

| | Dietary Treatment[1] | | |
|---|---|---|---|
| Item | 0DDGS | 15DDGS | 30DDGS |
| Ingredient, % | | | |
| Corn | 85.0 | 70.0 | 55.0 |
| DDGS[2] | — | 15.0 | 30.0 |
| Commercial Market Lamb Pellet[3] | 14.8 | 14.3 | 13.8 |
| Calcium Carbonate[4] | 0.2 | 0.7 | 1.2 |
| Nutritional Composition | | | |
| DM, % | 89.5 | 90.1 | 91.0 |
| TDN, % | 84.6 | 84.6 | 84.3 |
| CP, % | 13.8 | 16.0 | 19.4 |
| Ash, % | 4.7 | 5.5 | 6.4 |
| NDF, % | 18.0 | 22.2 | 26.1 |
| ADF, % | 4.6 | 5.5 | 5.7 |
| Crude fat, % | 2.3 | 3.7 | 4.6 |
| Sulfur, % | 0.2 | 0.4 | 0.5 |
| Phosphorus, % | 0.5 | 0.5 | 0.6 |
| Calcium, % | 1.2 | 1.3 | 1.6 |

[1]Diets (DM basis) were balanced to meet or exceed requirements of growing rams (NRC, 2007). Treatments were 0DDGS: 85% corn and 15% commercial market lamb pellet; 15DDGS: 15% DDGS substituted for corn on a % DM basis; and 30DDGS: 30% DDGS substituted for corn.

[2]Dried distillers grains with solubles.

[3]Commercial Market Lamb Pellet contained: 0.22 g/kg Chlortetracycline; 38.0% CP; 3.75-4.75% Ca; 0.6% P; 3.0-4.0% salt; 1.2 ppm Se; 52,863 IU/kg Vitamin A; 5,286 IU/kg Vitamin D; and 209 IU/kg Vitamin E.

[4]Calcium carbonate was included in the diet to obtain a Ca:P ratio of at least 2:1.

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**Table 2.** Effects of dried distillers grains with solubles on feedlot performance, carcass characteristics, and seminal quality of growing rams

| | Dietary Treatment[1] | | | | | Contrasts[4] | |
|---|---|---|---|---|---|---|---|
| Item | 0DDGS | 15DDGS | 30DDGS | SEM[2] | *P*-value[3] | Linear | Quadratic |
| Initial BW, kg | 41.3 | 40.6 | 40.4 | 1.41 | 0.89 | 0.65 | 0.90 |
| Final BW, kg | 83.6 | 83.9 | 86.3 | 1.77 | 0.50 | 0.28 | 0.64 |
| ADG, kg/d | 0.44 | 0.45 | 0.47 | 0.01 | 0.06 | 0.02 | 0.52 |
| DMI, kg/ram/d | 2.05 | 2.34 | 2.53 | 0.06 | 0.001 | <0.001 | 0.55 |
| Days on Feed, d | 109 | 108 | 107 | 1.6 | 0.54 | 0.27 | 0.90 |
| G:F, kg of gain/kg of DMI | 0.19 | 0.17 | 0.17 | 0.004 | <0.001 | <0.001 | 0.09 |
| HCW, kg | 41.7 | 42.5 | 42.5 | 1.12 | 0.81 | 0.57 | 0.77 |
| Dressing % | 50.0 | 50.4 | 50.1 | 0.37 | 0.67 | 0.72 | 0.41 |
| Ribeye Area, $cm^2$ | 3.05 | 3.09 | 3.08 | 0.07 | 0.90 | 0.74 | 0.74 |
| Fat Depth,[5] cm | 0.22 | 0.22 | 0.21 | 0.01 | 0.88 | 0.69 | 0.76 |
| Body Wall Thickness, cm | 1.05 | 1.11 | 1.13 | 0.04 | 0.26 | 0.11 | 0.70 |
| Leg Score[6] | 12 | 12 | 12 | 0.25 | 0.54 | 0.80 | 0.29 |
| Conformation Score[6] | 12 | 12 | 12 | 0.21 | 0.47 | 0.49 | 0.32 |
| Flank Streaking[7] | 351 | 375 | 357 | 11.57 | 0.30 | 0.70 | 0.14 |
| Quality Grade[6] | 12 | 12 | 12 | 0.16 | 0.29 | 0.24 | 0.30 |
| Yield Grade[8] | 2.6 | 2.6 | 2.5 | 0.14 | 0.88 | 0.69 | 0.76 |
| BCTRC,[9] % | 45.0 | 44.7 | 44.9 | 0.30 | 0.84 | 0.79 | 0.60 |
| Scrotal Circumference Change, cm | 1.50 | 1.25 | 1.73 | 0.34 | 0.61 | 0.65 | 0.39 |
| Spermatozoa Concentration[10] | 91.8 | 69.3 | 63.0 | 10.17 | 0.13 | 0.05 | 0.52 |
| Spermatozoa Motility Score[11] | 3.3 | 2.8 | 2.7 | 0.23 | 0.23 | 0.12 | 0.52 |

[1]Treatments were 0DDGS: 85% corn and 15% commercial market lamb pellet; 15DDGS: 15% DDGS substituted for corn on a % DM basis; and 30DDGS: 30% DDGS substituted for corn on a % DM basis.
[2]n = 4
[3]*P* -value for the *F*-test of the mean.
[4]*P*-value for linear and quadratic effects of increasing dried distiller's grains with solubles.
[5]Adjusted fat depth and yield grades.
[6]Leg score, conformation score, and quality grade: 1 = cull to 15 = High Prime.
[7]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.
[8]Yield grade = 0.4 + (10 × adjusted fat depth).
[9]Percent boneless, closely trimmed, retail cuts (% BCTRC) = [49.936 – (0.0848 × 2.204 × Hot Carcass Weight, kg) – (4.376 × 0.393 × 12th rib fat thickness, cm) – (3.53 × 0.393 × body wall thickness, cm) + (2.456 × 0.155 × LM area, $cm^2$)].
[10]Spermatozoa concentration in the hundreds of millions per milliliter counted on a hemocytometer.
[11]Spermatozoa motility score: 1 = no forward movement; 2 = slow forward movement; 3 = moderate forward movement; and 4 = fast forward movement.

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# EFFECT OF WEANING METHOD ON WELFARE AND PERFORMANCE OF BEEF CALVES DURING RECEIVING

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**ABSTRACT:** We evaluated welfare and performance of beef calves during receiving that had previously been subject to 1 of 3 ranch-of-origin weaning methods 28 d in duration: drylot weaning + dam separation (D), pasture weaning + fence-line contact with dams (PF), and pasture weaning + fence-line contact with dams + supplemental feed delivered in a bunk (PF+S). Calves assigned to D were fed a diet designed to promote a 1-kg ADG at a DMI of 2.5% of BW (17.7% CP and 0.93 Mcal $NE_g$/kg); PF calves had access to native forage only; and PF+S calves had access to native forage and received a ration of the diet fed to D at a rate of 1% of BW 3× weekly. Weaning-phase ADG tended ($P = 0.08$) to be greater for D than for PF or PF+S; however, incidence of undifferentiated fever during weaning was similar ($P = 0.22$) between treatments. At the end of the weaning phase, all calves were transported 4 h to a feedlot, penned according to treatment (n = 6 pens/treatment), and fed a receiving diet (14.9% CP and 0.93 Mcal $NE_g$/kg) ad libitum. Feed intake, growth, and health were monitored during a 60-d receiving period. Observations of calf behavior were made 3× daily for the first 7d of receiving; the proportion of calves in each pen that were eating, resting, or pacing was recorded by 2 trained observers and reported as a pen average. During the first 30 d of receiving, ADG was less ($P < 0.01$) for PF than for D and PF+S; however, ADG of D was greater ($P < 0.01$) than that of PF and PF+S during the entire 60-d receiving phase. Diet DMI and G:F were also greater ($P \leq 0.01$) for D than for PF calves during receiving. Fewer PF calves were observed at the bunk during the first 4 d of receiving (treatment × day; $P < 0.01$) than D or PF+S calves; however, the numbers of calves observed at the bunk were similar (treatment × day; $P = 0.64$) across treatments by d 6. We interpreted these data to suggest that animal performance and welfare during the receiving period were not improved by pasture weaning + fence-line contact with dams compared with drylot weaning + dam separation. Best-management practices for animal welfare may involve initiating diet transitions from forage to grain at the ranch of origin.

**Key words:** animal welfare, health, preconditioning

## INTRODUCTION

Ranch-of-origin preconditioning has been advocated as a means of improving the welfare and performance of beef calves by easing the stresses associated with weaning, transport, diet change, and commingling (Cole, 1985). Preconditioning methods that involve pasture weaning coupled with maternal contact (i.e., fence-line weaning) have been promoted as possible best-management practices for minimizing stress (Smith et al., 2003). Fence-line weaning reduced morbidity compared with drylot weaning (Boyles et al., 2007; Mathis et al., 2008). Additionally, Price et al. (2003) found that maintaining fence-line contact with dams after weaning reduced behavioral distress when compared with abrupt separation from dams. These studies focused on performance and behavior during weaning on the ranch of origin. Little information has been published relating to carryover effects of fence-line weaning compared with conventional drylot weaning on performance and behavior during feedlot receiving. Therefore, our objectives were to measure growth and health during a 28-d ranch-of-origin weaning phase and during a 60-d feedlot receiving phase among beef calves subjected to 1 of 3 ranch-of-origin preconditioning programs: drylot weaning + dam separation, pasture weaning + fence-line contact with dams, and pasture weaning + fence-line contact with dams + supplemental feed delivered in a bunk. In addition, we recorded incidences of behavioral distress among these treatments during first 7 d of feedlot receiving.

## MATERIALS AND METHODS

Animal care practices used in our study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978.1).

Angus × Hereford calves (n = 460; initial BW = 225 ± 35 kg) originating from the Kansas State University commercial cow-calf herds in Manhattan, KS and Hays, KS were used in this experiment. Calves were weaned at approximately 180 d of age. All calves were de-horned and steer calves were castrated before 60 d of age. At weaning, calves were weighed individually and assigned randomly to 1 of 3 ranch-of-origin weaning methods: drylot weaning + dam separation (**D**), pasture weaning + fence-line contact with dams (**PF**), and pasture weaning + fence-line contact with dams + supplemental feed delivered in a bunk (**PF+S**).

All calves were individually weighed at the time of maternal separation and were given initial vaccinations against respiratory pathogens (Bovi-Shield Gold 5, Pfizer Animal

Health, Exton, PA), clostridial pathogens (Ultrabac 7, Pfizer Animal Health), and *H. somnus* (Somubac, Pfizer Animal Health). In addition, all calves were treated for internal and external parasites (Ivomec, Merial Limited, Atlanta, GA). Booster vaccinations were administered 14 d later.

Within location, calves assigned to PF and PF+S were maintained for 28 d in a single native pasture (minimum area = 48 ha). Dams were maintained for the first 7 d of this period in adjacent native pastures that afforded fence-line contact with calves (minimum frontage = 200 m; 4-strand, barbed-wire fence with the bottom 2 wires electrified). Fresh water, salt, and mineral supplements were available continually. Calves assigned to D were transported (< 48 km) immediately after separation from dams and confined within location to a single earth-surfaced pen (minimum area = 20 $m^2$/calf; bunk space = 0.46 m/calf).

Calves assigned to D were fed a diet formulated to promote 1 kg ADG at a DMI of 2.5% of BW during the weaning phase of the study (Table 1). Calves assigned to PF had access to native forage only (Table 2), whereas calves assigned to P+S calves had access to native forage and received a ration of the diet fed to D at a rate of 1% of BW 3× weekly. No adjustments were made to feed delivery rate during the weaning phase. Calves assigned to P+S were sorted into a single pen located adjacent to the fence line shared with dams at 0900 h on Mondays, Wednesdays, and Fridays during the weaning phase. The ration was offered in portable bunks (bunk space = 0.46 m/calf). Pens afforded drinking water in open-topped tanks and consumption of the ration was complete by 1100 h at each feeding episode.

Table 1. Composition of the weaning diet*

| Ingredient composition | DM, % |
|---|---|
| Alfalfa extender pellets | 33.0 |
| Corn gluten feed | 18.2 |
| Wheat middlings | 14.6 |
| Cracked corn | 11.5 |
| Cottonseed hulls | 10.9 |
| Dried distillers grain | 7.8 |
| Supplement | 4.0 |
| **Nutrient composition** | **Amount** |
| CP, % of DM | 14.28 |
| $NE_m$, Mcal/kg | 1.50 |
| $NE_g$, Mcal/kg | 0.93 |

*Diet also contained salt, Zn sulfate, and Rumensin 80 (Elanco, Greenfield, IN).

Table 2. Nutrient composition of native pasture forage available to pasture-weaned beef calves (DM basis)

| Nutrient | Manhattan | Hays |
|---|---|---|
| DM, % | 89.5 | 91.3 |
| CP, % | 3.2 | 4.1 |
| NDF, % | 74.4 | 74.8 |
| ADF, % | 51.8 | 48.6 |

All calves were monitored for symptoms of respiratory disease at 0700 and 1400 h daily during the weaning phase of our study. Calves with clinical signs of BRD, as judged by animal caretakers, were removed from pens or pastures and evaluated. Calves were assigned a clinical score (scale: 1 to 4; 1 = normal, 4 = moribund), they were weighed and assessed for fever. Calves with a clinical illness score > 1 and a rectal temperature > 40.0°C were treated with therapeutic antibiotics according to label directions (1st incidence = Baytril, Bayer Animal Health, Shawnee Mission, KS; 2nd incidence = Nuflor, Merck Animal Health, Summit, NJ). Cattle were evaluated 72 h post-treatment and re-treated based on observed clinical signs.

At the end of the 28-d weaning period, all calves were transported 4 h from their respective ranch of origin to the Western Kansas Agricultural Research Center in Hays, KS and weighed individually upon arrival. At that time, calves were stratified by sex and assigned to 1 of 18 pens by treatment (6 pens / treatment). Animals were fed once daily at 0700 h and bunks were evaluated each morning at 0630 h. If the previous days feed was consumed, total feed delivered was increased by approximately 2% of the previous days feed delivery. Bunks were managed using a slick-bunk management method to minimize feed refusals (Pritchard and Bruns, 2003). Dry matter intake was estimated based on feed delivered to the pen. Calf health was monitored as during the weaning phase of the study.

Beginning on the morning after feedlot arrival, animal behavior was assessed 3× daily for 7 d by 2 trained observers. The numbers of calves performing specific behaviors (eating, pacing, vocalizing, drinking, resting, and ruminating) were recorded for each pen. Observations were taken 1 h before feeding, at the time of feeding, and 6 h post-feeding. In addition, calves were weighed individually on d 30 and d 60 of the receiving phase of the experiment.

Weaning period performance, receiving intake, and receiving performance were analyzed as a completely randomized design (PROC MIXED; SAS Inst. Inc., Cary, NC). Weaning period sickness was analyzed using PROC GLIMMIX (SAS). All models included terms for treatment and location, with pen replacing location in the receiving analyses. When protected by a significant F test ($P < 0.05$),

Table 3. Composition of the receiving diet

| Ingredient composition | DM, % |
|---|---|
| Ground sorghum grain | 47.8 |
| Wet distillers grains | 11.0 |
| Ground sorghum hay | 33.9 |
| Supplement* | 7.3 |
| **Nutrient composition** | **Amount** |
| CP, % of DM | 16.82 |
| $NE_m$, Mcal/kg | 1.50 |
| $NE_g$, Mcal/kg | 0.93 |

* Supplement contained Rumensin 80 (Elanco, Greenfield, IN), Tylan 40 (Elanco), limestone, salt, and trace minerals.

Table 4. Performance of beef calves while subjected to 1 of 3 28-d ranch-of-origin preconditioning regimens

| Item | Drylot | Pasture+Supplement | Pasture | SEM |
|---|---|---|---|---|
| Start weight, kg | 226 | 228 | 228 | 14.4 |
| End weight*, kg | 235 | 220 | 218 | 10.6 |
| ADG, kg/d | 0.31[a] | -0.28[ab] | -0.34[b] | 0.184 |
| Incidence of undifferentiated fever, % | 5.01 | 0.63 | 1.91 | 1.825 |

* BW measured immediately upon feedlot arrival.
[a, b] Means within rows without common superscripts tend to differ ($P = 0.08$).

least squares treatment means were separated using the method of Least Significant Difference. Receiving-period behavioral observations were analyzed using PROC GLIMMIX (SAS). Models included terms for treatment, pen, day, time, and all appropriate interactions. Pre-planned contrasts were used to elucidate treatment differences. Treatment differences in performance and pregnancy data were discussed when $P < 0.05$; tendencies were discussed when $P > 0.05$ and $< 0.10$.

## RESULTS AND DISCUSSION

***Weaning Period.*** Calf ADG during the 28-d weaning period tended ($P = 0.08$) to be greater for drylot-weaned calves (D) than for pasture-weaned calves receiving no supplement (PF; Table 4). Based on the chemical analyses of our pasture forage, these results were expected. In previous research, fence-line weaned calves gained 95% more weight than abruptly-weaned calves during the first 2 wk of preconditioning and maintained that difference for 10 wk post-weaning (Price et al., 2003); however, calves in that study were fed a single diet across treatments.

Our treatments were designed such that calves assigned to D were on a greater plane of nutrition than calves assigned to either PF or PF+S. This condition is typical of drylot- vs. pasture-weaning programs in Kansas. Supplement provided to PF+S in our study was designed to train pasture-weaned calves how to eat out of a bunk rather than to promote BW gains that were competitive with D. One causative feature of poor initial feedlot performance is stress associated with learning to eat from a bunk (Hutcheson and Cole, 1986). Walker et al. (2007) weaned calves either in a drylot or on pasture without supplement for 21 d. All calves were subsequently moved into a feedlot. Drylot-weaned calves in that study exhibited more vigorous feeding behavior during the first 4 days in the feedlot than pasture-weaned calves. In addition, BW gain was greater for drylot- weaned calves than for pasture-weaned calves during a 30-d feedlot receiving period.

Incidence of undifferentiated fever was not different ($P = 0.22$) among treatments during the weaning phase of our study. Step et al. (2008) indicated that preconditioned calves were less susceptible to disease during weaning and receiving than calves sold through auction markets immediately after separation from dams. Preconditioning was applied to both drylot- and pasture-weaned calves in our study. Supporting results were reported by Krebs et al. (2010) who noted that serum acute phase protein concentrations were not different in calves weaned either abruptly or in two-stages. Conversely, Walker et al. (2007) reported increased morbidity in drylot-weaned calves compared with pasture-weaned calves.

***Receiving Period.*** We observed calves at the time of feeding as an indicator of their desire to eat from a bunk during the first 7 d of receiving. A greater (Treatment × day; $P < 0.05$) proportion of D than PF came to the bunk at time of feeding during the first 5-days of receiving (Figure 1). Similarly, a greater proportion (Treatment × day; $P < 0.05$) of D than P+S came to the bunk at time of feeding during the first 4-days of receiving. Walker et al. (2007) also reported that drylot-weaned calves had more favorable feeding activity during the first 4 d in the feedlot than unsupplemented, pasture-weaned calves.

Buhman et al. (2000) recorded feeding behavior of recently-received calves purchased through an auction market in an attempt to determine the number of observations needed for appropriate statistical analyses of behavior in feedlot environments. During the first 10 d of the receiving period, these researchers indicated that the CV for feeding behaviors were large and would have required 50 animals / treatment to detect a 20% change in feeding behavior with 95% confidence coefficient. We were able to detect treatment differences with similar sensitivity by averaging feeding behaviors by pen within treatment (6 pens / treatment with 25-28 head per pen) in our study.

During the receiving period, D calves had greater ($P < 0.01$) ADG from arrival to d 60 and greater BW ($P < 0.01$) on d 60 than either pasture-weaned treatment (Table 5). This increase in performance was driven by greater ($P < 0.01$) DMI by D than by PF or PF+S. In addition, G:F was greater ($P = 0.01$) for D than for PF calves; G:F of PF+S calves was intermediate and similar to D and PF. Significantly, providing calves with supplement in a bunk on pasture did not improve receiving ADG ($P > 0.05$) or DMI ($P > 0.05$) compared with pasture-weaned calves receiving no supplement.

Pasture-weaned calves in our study were supplemented infrequently (3 × weekly for 4 wk) and ate less feed during receiving that drylot-weaned calves. Conversely, Boyles et al. (2007) reported no difference in feed consumption between drylot-weaned calves and pasture-weaned calves that were provided supplement daily. It may be possible to achieve greater performance and feed intake with pasture-weaned calves during receiving when supplementation is provided more frequently than in our study.

Incidence of undifferentiated fever during the receiving period was small (0.9%); therefore, we did not report summary statistics on this data. Step et al. (2008) found that

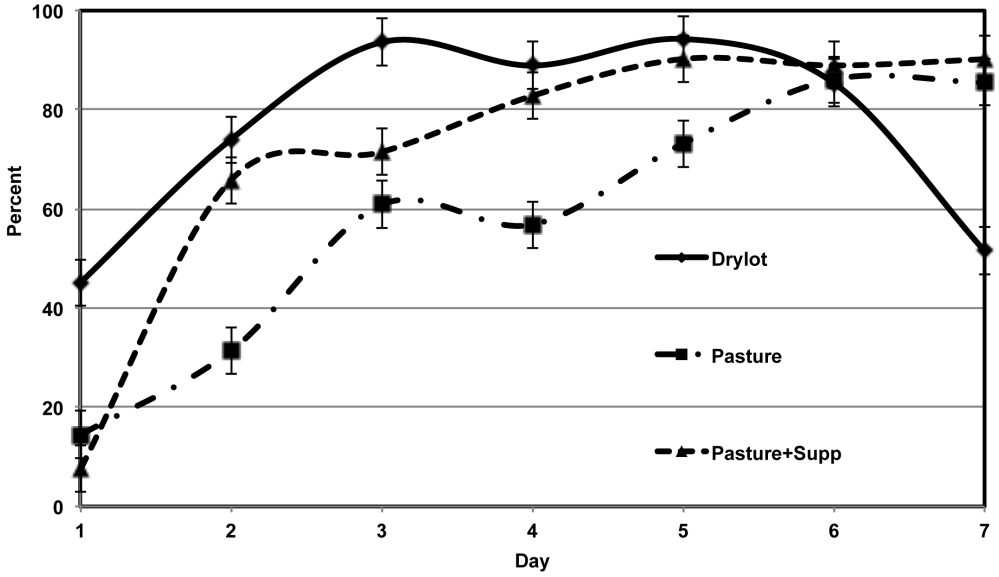


**Figure 1.** Proportion of calves observed at feed bunks immediately after feed delivery (Treatment × time, $P < 0.05$; Maximum SEM = 4.71).

Table 5. Performance of beef calves subjected to 1 of 3 ranch-of-origin preconditioning regimens during a 60-d feedlot receiving period

| Item | Drylot | Pasture+Supplement | Pasture | SEM |
|---|---|---|---|---|
| Arrival BW, kg | 235 | 220 | 218 | 10.6 |
| BW on d 30, kg | 265[a] | 249[b] | 242[b] | 3.9 |
| BW on d 60, kg | 316[a] | 297[b] | 292[b] | 4.3 |
| ADG, kg/d | | | | |
| Arrival to d 30 | 1.12[a] | 1.09[a] | 0.89[b] | 0.040 |
| Arrival to d 60 | 1.42[a] | 1.33[b] | 1.28[b] | 0.027 |
| DMI (Arrival to d 60), kg/d | 7.80[a] | 7.70[b] | 7.72[b] | 0.007 |
| Gain:feed (Arrival to d 60) | 0.182[a] | 0.173[ab] | 0.166[b] | 0.0038 |

[a, b] Means within rows without common superscripts differ ($P < 0.05$).

ranch-of-origin preconditioned calves were less likely to be treated for BRD and had lesser serum acute phase protein concentrations than calves sold through common marketing channels. Previous work (Boyles et al., 2007; Mathis et al, 2008) reported greater incidence of disease during receiving in drylot-weaned calves compared with pasture-weaned calves. In our study, the health of drylot-weaned calves was equivalent to that of pasture-weaned calves.

Preconditioning is thought to add value to all segments of beef industry through decreased calf morbidity, decreased costs associated with morbidity, reduced drug use, increased feed efficiency, greater weight gain, and greater beef quality. In spite of this, adoption of preconditioning management practices by the cow-calf segment of the beef industry has been relatively slow (49.8% of cow-calf producers sold their calves immediately at weaning, NAHMS, 2007). Calf performance during preconditioning on the ranch of origin is variable (Pritchard and Mendez, 1990; Step et al., 2008; Thrift and Thrift, 2011). As a result, economic returns associated with preconditioning are difficult to predict. A majority of the reluctance to adopt preconditioning is related to inconsistent financial rewards (King et al., 2006). Pasture-weaning systems may be a lower-cost alternative to conventional drylot-weaning systems; however, decreased growth performance during pre-shipment weaning and receiving may result.

## IMPLICATIONS

We interpreted these data to suggest that animal performance and welfare during the receiving period were not improved by pasture weaning + fence-line contact with dams compared with drylot weaning + dam separation. Optimal growth during feedlot receiving was achieved when calves were weaned in a drylot and fed a concentrate-based diet during a 28-d ranch-of-origin preconditioning period. The drylot weaned calves in our study were approximately 20 kg heavier at the end of the receiving period than calves weaned in pastures. Weary et al. (2008) indicated that the

most significant stressors associated with weaning were maternal separation and dietary transition from forages to concentrates. To our knowledge, no previous study has attempted to elucidate which of these two factors has greater relative influence calf performance during receiving. Based on our behavior and performance data, it appeared that previous experience consuming a concentrate-based diet from a bunk paid greater dividends during receiving than reducing stress associated with maternal separation through fence-line contact with dams. Best-management practices for animal welfare may involve initiating diet transitions from forage to grain at the ranch of origin.

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# EFFECTS OF TIMING OF VACCINATION (DAY 0 VERSUS DAY 14 OF A RECEIVING PERIOD) WITH A MODIFIED-LIVE RESPIRATORY VIRAL VACCINE ON PERFORMANCE, FEED INTAKE AND FEBRILE RESPONSE OF BEEF HEIFERS

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**ABSTRACT**: The objective of this study was to evaluate the effects of timing of the administration of a modified-live respiratory viral vaccine (IBR-$PI_3$-BRSV-BVD) on d 0 or on d 14 of a receiving period on performance, feed intake and febrile response in beef heifers. Our hypothesis was vaccine timing will alter febrile response and feed intake of feeder cattle. Thirty-six heifers (Angus and Angus crosses; initial BW = 265 ± 20 kg) were ranked by BW and assigned to treatment pens (9 pens total) in a completely randomized design. Treatments (3 pens/treatment with 4 heifers/pen) included no vaccine (CON), vaccination on d 0 (DO), and a delayed vaccination on d 14 (D14) of the receiving period. Heifers were fed in 6 x 12 m pens with GrowSafe feeding systems. Daily intakes were recorded and BW measured on d -1, 0, 14, 27, and 28. Temperature probes were attached to controlled intrauterine drug release devices (CIDR; active compound was removed) and vaginal temperatures were recorded every 5 min for the experiment; vaginal temperatures were then averaged for every h before data analysis. All data were analyzed using pen as the experimental unit. No differences ($P > 0.10$) among treatments were observed for initial BW, final BW, ADG for d 0 to end, or overall G:F. A treatment x day interaction ($P < 0.05$) was observed for feed intake. Daily intake was decreased for D14 versus D0 on d 14 ($P < 0.01$) and 15 ($P < 0.10$) and decreased ($P < 0.05$) on d 15 for the average of vaccinated calves versus CON. Eating rate (grams consumed/eating duration) was decreased ($P < 0.05$) on day 14 for D14 versus D0. A treatment x d interaction ($P < 0.01$) was observed for vaginal temperature. Vaginal temperature was increased ($P < 0.10$) on d 1 for D0 versus D14 heifers and increased for D14 versus D0 on d 14 ($P < 0.01$), 15 ($P < 0.05$) and 16 ($P < 0.05$). Our results suggest that time of administration of a modified-live respiratory viral vaccine can alter feed intake and vaginal temperature in feeder heifers.

**Key words:** beef heifers, febrile response, feed intake, respiratory vaccines

## INTRODUCTION

Bovine Respiratory Disease (**BRD**) is the most common and costly problem in feedlot cattle in North America, having a $750 million annual cost to industry (Chirase et. al., 2001). This disease accounts for approximately 75% of morbidity and over 50% of mortality in feedlot and thus a major contributor to net profit loss (Taylor et al., 2010). Calves treated for BRD once returned $40.64 less than uninfected calves, treated twice returned $58.35 less, three or more times returned $291.93 less (Fulton et al., 2002). Bovine respiratory disease is a secondary infection due to a bacterial/viral infection, such as bovine herpes virus-1, which causes broad immunosuppression in infected cattle and as a result impairs resistance to secondary bacterial and viral infections. Bovine herpes virus-1 is one of most important pathogens in BRD (Nandi et al., 2009). Bovine herpes virus-1 causes the respiratory disease infectious bovine rhinotracheitis (**IBR**), often leading to susceptibility to BRD. Other important viruses include bovine viral diarrhea (**BVD**), parainfluenza-3 (**$PI_3$**), and bovine respiratory syncytial virus (**BRSV**). General health management includes vaccinating against these pathogens in the form of a combined vaccine of IBR, $PI_3$, BRSV and BVD.

Immunological competence is arguably the most important subject in newly received cattle. Cattle encountering stress due to shipping, processing, environmental factors, and commingling may result in cattle that are immune-compromised. This deficient immune state may result in cattle that have reductions in performance as well as being incapable of building an ample immune response to a vaccination. There is a renewed interest in evaluation of vaccination protocols. Chirase et al. (2001) reported decreased BW gain in calves receiving a vaccination on-arrival. Alternatively, Richeson et al. (2009) found no differences in ADG between calves vaccinated on-arrival and calves receiving delayed vaccination. Inflammation is a necessary and normal immune response to injury or infection (Sheldon and Verhulst 1996). In vivo research has shown increased skeletal muscle degradation as a result of inflammation. There is a shortage of data relating febrile response and its relationship between immune response and performance in cattle. Therefore, the focus of this study was to assess the effects of an IBR-$PI_3$-BVD-BRSV vaccination on d 0 versus a delayed vaccination on d 14 on feed intake, febrile response, immune measures, and vaccine titers. Null hypothesis Treatment will cause no alteration in fever response.

## MATERIALS AND METHODS

Procedures were approved by the Montana State University Agricultu re Care and Use Committee, 2012-AA01.

Thirty six crossbred heifer calves (Angus and Angus crosses; average initial BW = 265 ± 20 kg) were used. Twenty-two heifers were purchased from a commercial Montana feedlot and 14 heifers originated at the Montana State University ranch. Purchased calves were in transit for approximately 3 hours and hauled 150 miles to the Montana State University facility. Montana State University calves were weaned approximately 2 mo. prior to study initiation and were fed grass hay. Upon arrival, heifers were weighed unshrunk. Heifer BW was ranked and heifers were assigned random numbers then assigned to treatment pens (3 pens/treatment with 4 heifers/pen). Each pen contained 1 or 2 purchased calves, the remaining being Montana State University ranch calves. Pens were randomly assigned to treatments including: control (**CON**; no vaccination), d 0 vaccination (**D0**) and a 14-d delayed vaccination (**D14**). Pens were concrete-surfaced covered pens (6 x 12 m) with two pens sharing an automatic watering system. Each pen contained one GrowSafe feed bunk.

All heifers were weighed upon arrival (d 0) and d 28. In addition, D0 and D14 heifers were weighed on d 14 of the experiment. A vaginal temperature was constantly recorded by means of an indwelling vaginal temperature probe starting on d 0, ending d 28. Temperature probes were attached to vaginal controlled internal releasing device (CIDR; hormones were removed). Temperatures were recorded every 5 minutes and averaged every hour for the duration of the study.

Calves were fed 70% concentrate diets containing corn/barley grain mix, grass hay, and a pelleted protein supplement. Diets were mixed daily. Daily allotments of diets were fed in GrowSafe feeders starting at 0730. Feed samples were collected weekly and analyzed for DM. Grab samples of the concentrate diet was collected weekly. Diet DM was determined on samples that were dried at 100° C for approximately 24 h.

Heifers were vaccinated subcutaneously on either d 0 (D0) or d 14 (D14) at approximately 1000h with 2 mL of Alpha-7 (Boehringer Ingelheim, Ingelheim Germany) and 2 mL Vision 8 Somnus (Intervet, Summit, NJ ). Control heifers (CON) received no vaccination.

Performance data were analyzed with a model that included treatment and pen using Proc Mixed procedures (SAS Inst. Inc., Cary, NC). Feeding behavior and febrile response were first analyzed using GLM procedures of SAS with a model that included treatment, heifer (treatment), day, and treatment x day. When a treatment x day interaction was observed, the data were analyzed by day using Proc Mixed procedure. Contrasts were used to separate treatment means. Contrasts included 1) control versus the average of vaccinations and 2) on arrival versus delayed vaccination.

## RESULTS AND DISCUSSION

No morbidity or mortality was observed for any of the cattle in this study. No differences ($P > 0.10$) were observed among treatments (D0, D14, and CON) for initial BW, final BW, ADG for d 0 to end, or overall G:F (Table 1). Consequences of building an immune response including catabolic processes could possibly be observed beyond the time of vaccination (Kyriazakis et al., 1998). Consequently, although there were no ADG or BW differences in this receiving period, further observation over time period may elucidate any adverse effects of cattle not mounting a satisfactory immune response related to vaccination or timing of vaccination. Cattle are perpetually exposed to antigenicity through all stages of life, therefore sufficient, consistent immunity is especially important in times of stress, such as shipping and processing (Klasing and Barnes, 1988.). Evolutionarily theory predicts the risk of fighting an infection will outweigh the value of avoiding a reduction in nutrients to support a competent immune system (Lochmiller and Deerenberg, 2000). An animal will allocate more energy into maintaining an immune system, although the risk is low, the cost may be much greater (i.e., death). The cost of failing to respond to an infection (death) is much greater than sacrificing nutrition to immunity (Klurfeld, 1993). Performance may be affected over time, especially if cattle are struggling to build a suitable immune system.

Daily intake (g) was measured using the GrowSafe system. A treatment x d interaction ($P < 0.05$) was observed for feed intake. Daily intake was decreased for D14 versus D0 on d 14 ($P < 0.01$) and 15 ($P < 0.10$) and decreased ($P < 0.05$) on d 15 for the average of vaccinated calves versus CON (Figure 1). Eating rate (g consumed/eating duration) was decreased ($P < 0.05$) on d 14 for D14 versus D0. Reduced feed intake is observed in virtually all species fighting infection. Even mild immune taxation including vaccination has been shown to decrease normal feed intake (Gandra and Scrimshaw, 1961). One proposed mechanism behind reduction in feed intake is that this decrease promotes a more efficient immune system, possibly because more energy can be devoted to fighting an infection (Kyriazakis et al., 1998). An alternative theory is that an animal may be more selective in its diet, reducing the chance of ingesting substances that could be potentially harmful and add to the infection (Kyriazakis et al., 1998; Lochmiller and Deerenberg, 2000).

A treatment x day interaction ($P < 0.01$) was observed for vaginal temperature. Vaginal temperature was increased ($P < 0.10$) on d 1 for D0 versus D14 heifers and increased for D14 versus D0 treatments on d 14 ($P < 0.01$), 15 ($P < 0.05$) and 16 ($P < 0.05$). The increased febrile response could have been a result of these cattle being in a better nutritional state and thus having the ability to build a better immune response. Cattle which have a nutritional advantage may not experience so many physiological tradeoffs to maintain a satisfactory immune system during an infection as-well-as throughout

**Table 1.** Effects of vaccination timing (on arrival versus delayed 14 d) on performance of beef heifers during a 28-d receiving period

| | Treatments[1] | | | | *P*-value contrast[3] | |
|---|---|---|---|---|---|---|
| Item | CON | D0 | D14 | SEM[2] | CON vs. Vacc | D0 vs. D14 |
| Pens (heifers) | 3 (12) | 3 (12) | 3 (12) | - | - | - |
| BW, kg[4] | | | | | | |
| Initial | 262 | 268 | 266 | 5.96 | 0.51 | 0.84 |
| d 14 | - | 286 | 279 | 5.20 | - | 0.32 |
| Final | 299 | 303 | 301 | 5.6 | 0.66 | 0.76 |
| Performance, d 0 to 28 | | | | | | |
| ADG, kg | 1.3 | 1.2 | 1.2 | 0.10 | 0.58 | 0.88 |
| DMI, kg[5] | 9.8 | 8.8 | 9.1 | 0.41 | 0.16 | 0.64 |
| G:F | 0.13 | 0.14 | 0.13 | 0.01 | 0.82 | 0.53 |

[1]Treatments were day of vaccination: CON = Control, no vaccination; D0 = heifers received respiratory (IBR, PI3, BVD,BRSV) vaccination on d 0 of the receiving period; and D14 = heifers received respiratory (IBR, PI3, BVD, BRSV) vaccination on d 14 of the receiving period

[2] Pooled SEM.

[3]Contrasts evaluated were CON versus average of vaccinated heifers and d 0 vaccination vs. d 14 vaccination.

[4] Heifers were weighed two consecutive d at the beginning and end of the experiment.

[5]A treatment x day interaction ($P < 0.05$) was observed for intake; therefore, data were analyzed by day. Data are presented in Figure 2

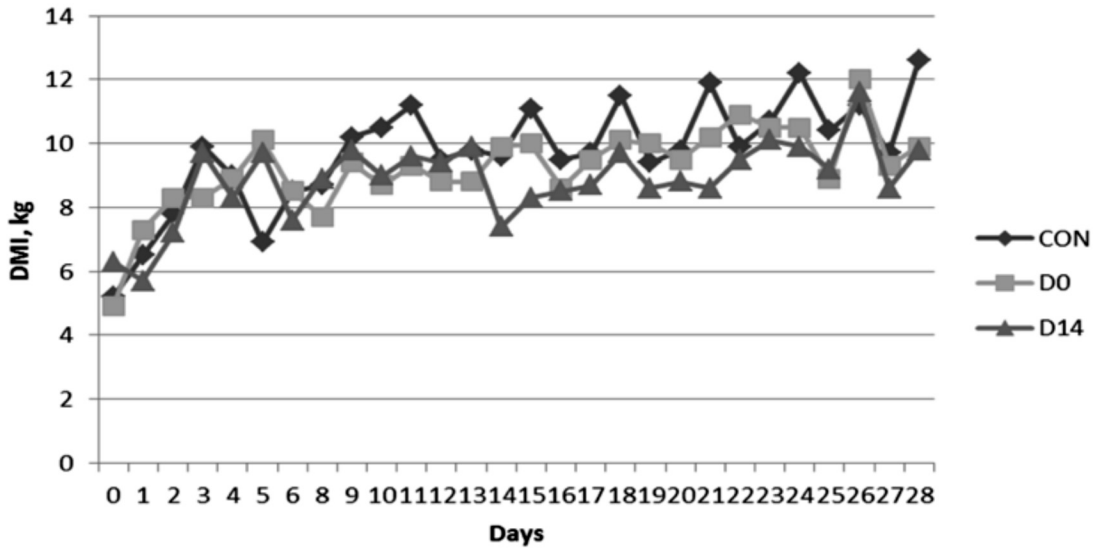


**Figure 1.** Daily DMI response to vaccinations administered on d 0 or d14 of the receiving period. Daily intake was decreased for D14 versus D0 on d 14 ($P < 0.01$) and 15 ($P < 0.10$) and decreased ($P < 0.05$) on d 15 for the average of vaccinated calves versus control (CON).

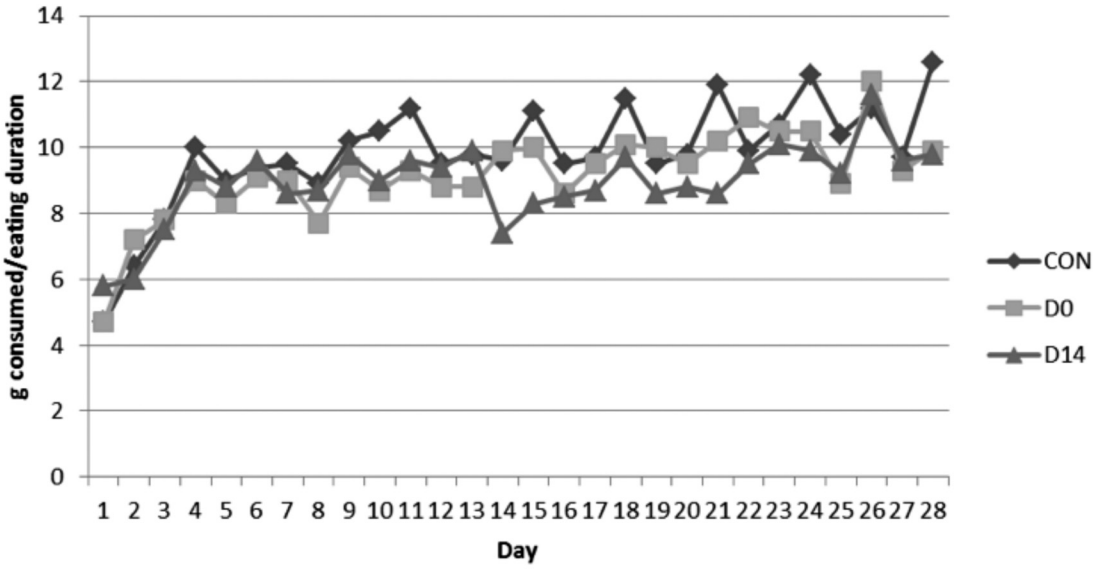


**Figure 2.** Eating rate (g consumed/eating duration) for heifers receiving viral vaccinations on d 0 or d 14 of the receiving period. Eating rate (grams consumed/eating duration) was decreased ($P < 0.05$) on d 14 for D14 versus D0.

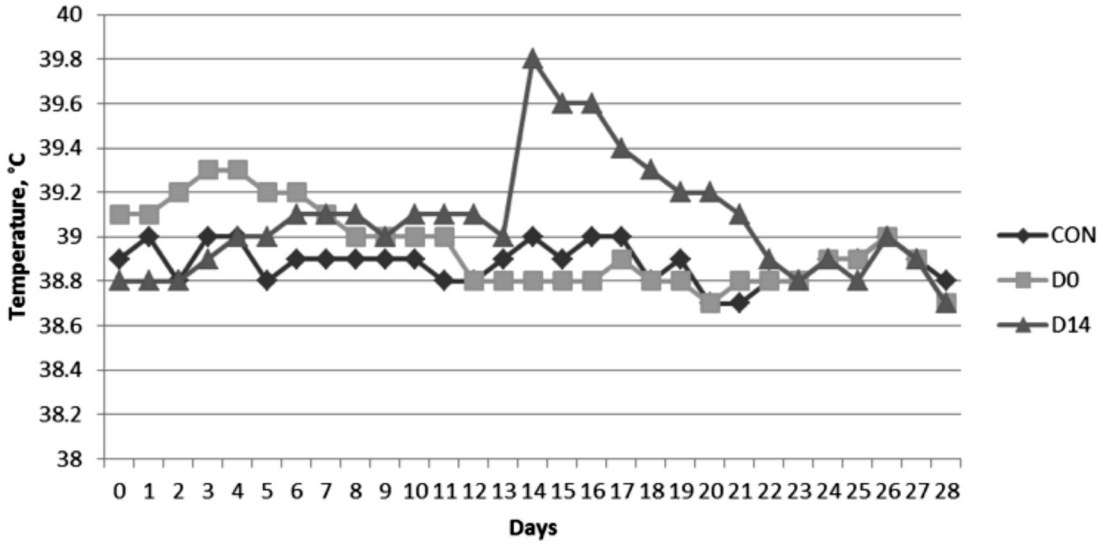


**Figure 3.** Daily vaginal temperature response to vaccinations administered on day 0 or day 14. Control (CON) heifers were not vaccinated. Vaginal temperature was increased ($P < 0.10$) on d 1 for D0 versus D14 heifers and increased for D14 vs. D0 on d 14 ($P < 0.01$), 15 ($P < 0.05$) and 16 ($P < 0.05$).

life. There is extreme nutritional demand for mounting and maintaining immunity. This bodily function may take away nutritional energy from growth and reproduction to generate sufficient immunity (Sheldon and Verhulst, 1996). Fever is a necessary and beneficial response when fighting an infection or responding to an increased immune requirement. Although beneficial, febrile response can be physically and nutritionally demanding. Cooper et al. (1992) reported that even a slight immune challenge, such as vaccination, can increase the metabolic rate of a host 15 to 30%. Nutritional state may be significant in terms of recovery and replenishing energy and body tissue lost due to an immune challenge attributable to febrile response.

## IMPLICATIONS

Vaccinating cattle with a modified live respiratory vaccine will increase body temperature and alter feed intake. Delaying vaccination altered feeding behavior for approximately 3 days versus altered feed intake for 1 day when the vaccine is administered during the start of the receiving period. Managers can use these data when determining vaccination protocols.

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# ASSESSMENT OF CHESTNUT TANNIN EXTRACT SUPPLEMENTATION ON ANIMAL PERFORMANCE AND RUMINAL FERMENTATION PROFILES IN FEEDLOT FINISHING DIETS

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**ABSTRACT:** The objective of this study was to assess animal performance and ruminal fermentation when feedlot finishing beef steers were fed with supplementation of chestnut tannin extract (CTE). Eight *Charolais-cross* steers (average BW = 495 kg) were used in a duplicated 4 × 4 Latin square experiment with a 2 × 2 factorial arrangement of treatments. The 4 animals in one of 2 squares were surgically fitted with a ruminal cannula. Within squares, animals were randomly assigned to a sequence of 4 diets during each of the four 21-d periods (14 d of treatment adaptation and 7 d of data collection and sampling); CTE supplementation (without vs. with CTE) and ionophore (ION; monensin administered as a Rumensin, Elanco Animal Health, Indianapolis, IN) supplementation (without vs. with ION). Animals were fed a finishing diet consisting of 8.0% alfalfa hay, 7.0% corn silage, 77.0% rolled barley grain, 3.0% CTE or wheat straw, and 5.0% feedlot supplement without or with ION. Intake of DM/d was not affected by CTE supplementation or ION, whereas DMI/kg BW tended to increase ($P$ = 0.08) by CTE supplementation. Supplementation of CTE increased ADG ($P$ = 0.01), but ION did not influence ADG. Total VFA concentration did not differ in response to supplementing CTE or ION. Additionally, molar proportions of acetate and propionate were similar between dietary treatments. Molar proportion of butyrate tended to increase ($P$ = 0.10) by CTE supplementation, but not by ION. Mean ruminal pH measured for 48 h averaged 6.39 across dietary treatments, but was not influenced by dietary treatments. In contrast, daily episodes with ruminal pH < 5.80 tended to increase ($P$ = 0.10) by CTE supplementation, while ION increased the daily episodes only with CTE supplementation, resulting in an interaction between CTE and ION ($P$ = 0.03). Supplementation of CTE had minor impacts on ruminal fermentation, except that CTE affected total buffering capacity of finishing beef steers. Further research is needed to investigate positive effects on animal performance by CTE supplementation in feedlot finishing diets.

**Key words:** beef finishing steers, chestnut tannin extract, ruminal fermentation profiles

## INTRODUCTION

In North America, diets consumed by feedlot cattle typically contain mostly grain, and provide little fiber from forages. These high-energy diets are rapidly digested in the rumen, leading to high concentrations of VFA in ruminal fluid (> 100 m*M*), relatively low ruminal pH (Beauchemin et al., 2001), and eventually ruminal acidosis (**RA**; Nagaraja and Titgemeyer, 2007). Ruminal acidosis has been defined as a fermentation disorder in the rumen, characterized by a less than normal rumen pH (less than 5.5). This syndrome results in a number of vague symptoms which often are hard to pinpoint, and subacute RA is believed to result in losses of as much as $15 to 20 per animal in lost efficiency (Schwartzkopf-Genswein et al., 2003). Supplementing antimicrobial compounds (included in the feed additive group) have become an essential management tool to prevent or to control RA to maintain production efficiency in feedlot cattle (Carro and Ranilla, 2003). Ionophores (**ION**) such as monensin are additives included in this group. The most prominent effects of ION are an increased feed efficiency and a decrease in DMI. Because of the latter changes in the eating-behavior, it helps to prevent RA (McGuffey et al., 2001). However, the widespread use of antibiotic feed additives by the North American feedlot cattle industry to maximize production efficiency has prompted an interest in possible alternatives, such as bacterial direct-fed microbials (Ghorbani et al., 2002) and organic acids (malate and fumarate; Castillo et al., 2004). However, overall efficacy of the feed additives is generally poor and varies depending upon diet composition and feeding management.

Nutritional and toxic effects of tannins present in various foodstuffs, feed, and fodder have been actively investigated. A body of evidence has suggested that, in ruminants, tannins can reduce the ruminal digestion of plant proteins, improve the retention of dietary N, and enhance productive parameters (Min et al., 2003). Most of the properties determining the effects of tannins on plant proteins seem to have been ascertained, but there has been little effort to examine the interaction of tannins with protein and starch in cereal grains. In addition to the nutritive effects, antibacterial effects have been reported for plant tannins (Chung et al., 1998; Min et al., 2008). For instance, Min et al. (2008) reported that some plant tannin extract (**TE**) were highly inhibitory to the selected pathogens. Hence, functionally bioactive tannins may provide alternatives to conventional antimicrobial feed additives to effectively reduce risk of RA by controlling RA-causing bacteria. The objective of this study was to test

our overall hypothesis that supplementation of chestnut TE (**CTE**) in finishing beef steer diet would reduce risk of RA and beneficially modify ruminal fermentation. In addition, we were interested if effects of CTE supplementation would differ in response to ION supplementation which is widely used in ruminant diets. Therefore, this study was undertaken to determine the effects of 2 antimicrobial agents, CTE and ION, on growth performance and ruminal fermentation characteristics in finishing beef steers.

## MATERIALS AND METHODS

***Animals, Experimental Design, and Diets.*** Eight Charolais-cross steers (average BW = 495 kg) were used; 4 steers were surgically fitted with ruminal cannulas. The design of the experiment was a double 4 × 4 Latin square with each period lasting 21 d (14 d of treatment adaptation and 7 d of data collection and sampling). The steers were allocated to squares by whether they were surgically cannulated, and the 2 squares were conducted simultaneously. Within squares, steers were randomly assigned to a sequence of 4 diets with a 2 × 2 factorial arrangement. Treatments were CTE supplementation (without: **−CTE** vs. with: **+CTE**) and ION supplementation (without: **−ION** vs. with: **+ION**). The CTE (*Castanea sativa* Mill; containing about 80% hydrolyzable tannins) supplement (Chemtan, Exter, NH) was provided at a rate of 3% DM. Ionophore (monensin administered as a Rumensin premix, Elanco Animal Health, Indianapolis, IN) was incorporated into the diet at the dose of 16 mg/kg of DM (350 mg/d), which corresponds to the dose recommended by the manufacturer for the use in beef cattle rations. Animals were fed a finishing diet consisting of 8.0% alfalfa hay, 7.0% corn silage, 77.0% rolled barley grain, 3.0% CTE or wheat straw, and 5.0% feedlot supplement without or with ION. The ingredient and chemical composition of the TMR used in the experiment is shown in Table 1. Animals were fed for ad libitum intake, housed in individual pens, and had free access to water during the experiment. All animals were individually fed once at 0800 h. Feed offered and refused was recorded daily, and daily samples were collected to determine DMI. Body weight was measured at the beginning and end of each period.

***Sampling, Data Collection, and Chemical Analyses.*** Samples of the TMR fed and orts for individual cows were collected daily during the data collection period, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA), and stored for subsequent analyses. Contents of DM of the samples were used to calculate intakes and digestibilities of DM and nutrients. Analytical DM concentration of samples was determined by oven drying at 135°C for 3 h; OM was determined by ashing, and N content was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company. Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

***Ruminal Fermentation Characteristics.*** To minimize carry-over effects from period to period in a square consisting of cannulated steers, on the last day of Periods 1, 2, and 3 the rumen of each steer was emptied manually and the contents were placed into the rumen of the next steer within the square that was to receive that treatment. Thus, each steer started the period with rumen contents corresponding to the same treatment it was fed. Ruminal pH was measured continuously for 2 d of each period (d 15 to 17) using indwelling electrodes. An electrode (model PHCN-37; Omega Engineering, Stamford, CT) was inserted into the rumen of each steer through the cannula. Ruminal contents were sampled from cannulated steers 0, 3, and 6 h after the a.m. feeding on d 20 and 21. Approximately 1 L of ruminal contents was obtained within the rumen, composited by steer, and strained through a polyester screen (pore size 355 μm; B & S H Thompson, Ville Mont-Royal, QC, Canada). Five milliliters of the filtered ruminal fluid was taken at 3 h after the morning feeding and added to 1 mL of 25% of meta-phosphoric acid, and the samples were retained for VFA determination. The VFA were quantified using a GLC (model 6890 series II) with a capillary column (30 m × 0.32 mm i.d., 1-μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA), and flame-ionization detection. Crotonic acid was used as an internal standard. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium.

**Table 1.** Ingredient and chemical composition of the diets (DM basis)

| | Diet[1] | |
|---|---|---|
| Item | −CTE | +CTE |
| Ingredient | | |
| Corn silage | 7.0 | 7.0 |
| Alfalfa hay, chopped | 8.0 | 8.0 |
| Barley, steam rolled | 77.0 | 77.0 |
| Wheat straw | 3.0 | - |
| CTE | - | 3.0 |
| Feedlot supplement[2] | 5.0 | 5.0 |
| Chemical composition | | |
| DM, % | 84.1 | 84.1 |
| CP | 9.65 | 9.37 |
| NDF | 25.5 | 10.4 |
| ADF | 22.7 | 8.44 |

[1]-CTE= TMR without chestnut tannin supplementation; +CTE = TMR with chestnut tannin supplementation.

[2]Composition: 5.0% NaCl, 0.24% Mg, 0.76% K, 200 ppm Cu, 400 ppm Mn, 650 ppm Zn, 2 ppm Se, 22 ppm I, 9 ppm Co, 121,000 IU/kg Vitamin A, 37,400 IU/kg Vitamin D, 55 IU/kg vitamin E, and 360 ppm Rumensin® (Elanco Animal Health, Indianapolis, IN).

***Statistical Analysis.*** Data were summarized for each animal by measurement period. All data in this study were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) with animal as an experimental unit. Data for intake and BW were analyzed with a model that included the effects of CTE (−CTE vs. +CTE), square (noncannulated vs. cannulated animals), ION supplementation (−ION vs. +ION), and the interaction between CTE and ION. Animal, period, and animal by period by square were the terms of the random statement. Data for pH and VFA profiles were analyzed with a model that included the effects of CTE, ION supplementation, and the interaction between CTE and ION. Significant effects of the treatment were declared if $P < 0.05$, and trends were accepted if $0.05 < P < 0.10$.

## RESULTS

Supplementation of CTE and ION did not affect DMI (Table 2). While CTE supplementation increased ADG, supplementing ION did not influence ADG. Mean ruminal pH was 6.39 across dietary treatments (Table 2), and it was not affected by CTE or ION supplementation. In addition, neither CTE nor ION supplementation influenced minimum and maximum pH. However, daily episodes eliciting ruminal pH < 5.8 tended to increase ($P = 0.10$) by CTE supplementation, and ION supplementation further increased the daily episodes with +CTE diet, resulting in an interaction between CTE and ION. Total VFA concentration did not differ in response to CTE or ION supplementation. Molar proportion of acetate and propionate did not change due to supplementing CTE or ION, resulting in no effect of acetate-to-propionate ratio. In contrast, molar proportion of butyrate tended to increase by CTE supplementation, but not ION supplementation.

**Table 2.** Effect of chestnut tannin extract (CTE) and ionophore (ION) on growth performance and ruminal fermentation characteristics of finishing beef steers

| | −CTE | | +CTE | | | Contrast[2] ($P$ =) | | |
|---|---|---|---|---|---|---|---|---|
| Item[1] | −ION | +ION | −ION | +ION | SEM | CTE | ION | INT |
| DMI, kg/d | 13.2 | 13.4 | 13.6 | 14.0 | 0.59 | 0.17 | 0.44 | 0.79 |
| ADG, kg/d | 1.36 | 1.66 | 2.01 | 2.19 | 0.27 | 0.01 | 0.28 | 0.78 |
| Min. pH | 5.58 | 5.69 | 5.69 | 5.63 | 0.107 | 0.74 | 0.75 | 0.22 |
| Mean pH | 6.45 | 6.36 | 6.37 | 6.37 | 0.115 | 0.71 | 0.65 | 0.70 |
| Max. pH | 7.09 | 6.58 | 6.98 | 7.03 | 0.194 | 0.37 | 0.22 | 0.15 |
| pH < 5.8 | | | | | | | | |
| Daily episodes | 18.0 | 6.27 | 13.8[b] | 30.3[a] | 8.05 | 0.10 | 0.68 | 0.03 |
| Duration, h/d | 1.92 | 0.32 | 3.95 | 5.63 | 2.565 | 0.15 | 0.98 | 0.49 |
| Area, pH × min | 20.3 | 2.87 | 42.3 | 47.6 | 27.64 | 0.23 | 0.82 | 0.67 |
| Total VFA | 103.1 | 95.6 | 95.7 | 93.8 | 6.47 | 0.33 | 0.32 | 0.55 |
| Individual VFA | | | | | | | | |
| Acetate (A) | 51.3 | 50.5 | 50.2 | 51.2 | 2.29 | 0.89 | 0.95 | 0.52 |
| Propionate (P) | 38.1 | 36.2 | 35.6 | 34.6 | 4.24 | 0.53 | 0.64 | 0.88 |
| Butyrate | 6.31 | 7.77 | 9.34 | 9.60 | 2.001 | 0.10 | 0.53 | 0.66 |
| A:P | 1.34 | 1.56 | 1.56 | 1.61 | 0.307 | 0.53 | 0.52 | 0.71 |

[a,b]Means in the same row within −CTE and +CTE subgroups with different superscripts differ based on single degree of freedom contrasts ($P < 0.05$).

[1]Min. pH = minimum pH; Max. pH = maximum pH; Total VFA expressed as m*M*; Individual VFA expressed as mol/100 mol.

[2]*P*-value for factorial contrasts: chestnut tannin extract (−CTE vs. +CTE), ionophore (−ION vs. +ION), and the interaction between CTE and ION (INT).

## DISCUSSION

Supplementation of CTE failed to elicit the beneficial responses on ruminal pH and VFA profiles that we hypothesized. Effects of tannins have been inconsistent depending on both dose and type of tannins (Mueller-Harvey, 2006). In our previous in vitro batch culture study (Eun et al., 2011), we observed that overall growth patterns of 2 ruminal acidosis-causing bacteria, *Selenomonas ruminantium* and *Streptococcus bovis*, differed in response to TE addition; adding TE decreased growth of SR starting at 2 h, and CTE was most effective to decrease growth of *Selenomonas ruminantium* at 12 and 24 h followed by mimosa and quebracho TE. At 24 h, CTE decreased growth of *Selenomonas ruminantium* at 48%. Similarly, growth of *Streptococcus bovis* was inhibited by adding TE beginning at 4 h. At 12 and 24 h, CTE elicited the least growth of *Streptococcus bovis* followed by mimosa and quebracho TE. Addition of CTE decreased growth of *Streptococcus bovis* at 73% at 24 h (Eun et al., 2011). These positive responses on the inhibition of growth toward the ruminal acidosis-causing bacteria motivated us to conduct the current in vivo study. Contrasting results between the two studies may have been resulted from basal diets. In the current study, mean ruminal pH was 6.39, which could not create favorable physiological condition for CTE to act on inhibition of ruminal acidosis-causing bacteria. It has been reported that ruminal microbes have the ability to metabolize tannins, particularly hydrolysable tannins (O'Donovan and Brooker, 2001) or adapt to the presence of tannins through modification of either microbial physiology or metabolism (Frutos et al., 2004).

Although fermentation profile response to CTE supplementation was not observed, ADG was increased for steers receiving the CTE supplement. Several factors could contribute to this finding, with improved protein utilization being among them (Vasta et al 2008). Tannins have the ability to bind and precipitate feed proteins, preventing ruminal degradation and increasing the availability of high quality proteins to the small intestine (Aerts et al., 1999). The positive effect of supplementing CTE on ADG warrants further study to identify the mechanism behind the increase of ADG with CTE supplementation in beef finishing diets.

Also, the lack of effect of ION to improve growth performance is different from what has often been reported in the literature (Felix, et al., 2011). Supplementing ION has also been reported to increase acetate-to-propionate ratio (McGuffey et al., 2001; Jenkins et al., 2003), but we did not observe the effect in the current study. As we suggested with CTE supplementation, the relatively high ruminal pH observed in this study may have also diluted potential effects of ION supplementation on ruminal fermentation. This could also contribute to the general observation of no effects with CTE × ION interactions.

## IMPLICATIONS

Our findings indicate that CTE supplementation may have the potential to increase ADG and growth performance of finishing beef steers. As TE typically increases N utilization efficiency by ruminants, further studies need to be focused on whole animal N utilization to identify the mechanism of the potential effects. In addition, potential value of CTE needs to be further tested if CTE supplementation would have dose response with different diet compositions.


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# EVALUATION OF THE INCIDENCE, CAUSES, AND POTENTIAL SOLUTIONS FOR THE OCCURRENCE OF DISABLED OR NON-AMBULATORY CATTLE WITHIN THE CALIFORNIA BEEF AND DAIRY INDUSTRIES

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**ABSTRACT:** A survey was conducted to evaluate how California beef and dairy operations sell market cows and bulls and identify key contributors to these animals becoming disabled or non-ambulatory (NA). Surveys were mailed to 9,778 California beef and dairy producers using the California Beef Council mailing list. Twenty-nine questions were included to evaluate beef and dairy producer demographics, the incidence of NA cattle on- farm or on-ranch, management and marketing practices utilized for market cows and bulls, and reasons animals become NA. Response rate for the mailed portion of the survey was 3.9%. Completed surveys were received from 446 producers (n = 403 beef, n = 43 dairy). The mean age (± SD) for survey respondents was 62 ± 12.3 yr for beef producers and 55 ± 13.1 yr for dairy producers. Survey responses clearly indicated that most beef (77.5%) and dairy producers (62.5%) market their animals at a livestock auction market, which was more ($P < 0.05$) than any other option. The direct sale of market cows and bulls to a beef packer was ($P < 0.05$) the next most common outlet among both beef (11.7%) and dairy (22.9%) respondents. Rates of culling, euthanasia, and death loss among cows were 10.8, 1.2, and 1.3%, respectively, for beef respondents, and 33.1, 2.1 and 3.3%, respectively, for dairy respondents. However, 35.6% of beef and 95.2% of dairy respondents indicated they had at least 1 non-ambulatory cow in the previous 12 mo. The majority ($P < 0.05$) of beef and dairy survey respondents indicated they would consider on-farm euthanasia (92.1 and 88.1%, respectively), and gunshot was identified as the primary method ($P < 0.05$) of euthanasia among beef (76.2%) and dairy (78.0%) producers. Dystocia was reported to be the primary cause ($P < 0.05$) of NA status in cows among beef (46.5%) and dairy (79.1%) respondents. These data suggest that management of potential causes of NA status continues to be a challenge among beef and dairy cattle producers and solutions to reduce NA incidence are needed.

**Key words:** beef cattle, dairy cattle, non-ambulatory, survey

## INTRODUCTION

According to USDA (2011) data, during the 2010 calendar year 3.6 million beef cows, 2.8 million dairy cows, and 622,000 bulls were harvested in the U.S. When combined, these animals made up 20.9% of all cattle harvested. Cull cows and bulls (commonly referred to as "market" cows and bulls) represent an important part of the total beef supply since they are widely used to supply beef to consumers via retail and food service outlets.

The existence of non-ambulatory (**NA**) cattle in the U.S., or those that have the potential to become NA if subjected to a stressor (i.e. long-distance transport to slaughter) has become an animal welfare concern. During the open interview portion of the National Market Cow and Bull Beef Quality Audit (**NMCBBQA**) 2007, "animal welfare" issues and "condemnation rates of downers prior to slaughter" were both included on the list of "Top 10 Quality Challenges" that faced the market cow and bull beef industry from 1999 to 2007, based on interviews with packing plant management and USDA FSIS personnel (Hale et al., 2007).

Based on United States Animal Health Association survey data collected during 2003 and 2004, the authors estimated that 0.38 to 0.40% of all beef and dairy cattle nationwide (including all cows and calves) were NA for any reason at some point during the year (USAHA, 2006). Based on the July 1, 2011 U.S. total cattle inventory of 100 million head (USDA, 2011), this equates to approximately 380,000 to 400,000 NA cattle occurring annually.

The objective of this project was to survey California beef and dairy cattle operations to: 1) characterize marketing of cull cows and bulls, 2) identify animal traits that influence culling decisions, and 3) document specific characteristics that beef and dairy producers believe contribute to market cattle becoming NA. Ultimately, results of this survey will provide needed baseline information about common production practices that influence the condition of market cows and bulls as they enter commerce.

## MATERIALS AND METHODS

***Survey.*** In early 2011, 9,778 surveys were mailed to California beef and dairy producers using the California Beef Council's database. The survey instrument included 29 questions intended to evaluate beef and dairy producer demographics, incidence of NA cattle on-farm or on-ranch, management and marketing practices utilized for market cows and bulls, and reasons animals probably become NA. Identical surveys were also available on-line

(via www.surveymonkey.com) and printed surveys were also distributed at 2 cattle producer meetings held by the California Cattlemen's Association and California Farm Bureau Federation. All survey responses and answers were anonymous. Producers were asked to fill out the survey to the best of their knowledge, including providing estimates. On-line and mailed survey responses were accepted through July 2011.

Only responses in which the producer indicated they were in the cow/calf (beef), seedstock (beef), or dairy segment of the beef industry were included in the data analysis and results. Respondents who indicated they operated a calf ranch, feedlot, or had no cattle operation were removed from the dataset. Also, producers who operated in multiple industry segments (e.g. cow/calf and dairy) were asked to complete 2 surveys; one on behalf of each entity.

***Statistical Analyses.*** Survey data were compiled, incidence rates were determined using Microsoft Excel, and data were analyzed via chi-square (SAS Inst. Inc., Cary, NC). Raw incidence rates (raw percentages) are reported here, and comparisons were made within beef survey respondents or within dairy survey respondents, but not across producer types.

## RESULTS AND DISCUSSION

Completed surveys were received from 446 producers (n = 403 beef, n = 43 dairy). The overall response rate for the mailed portion of the survey was 3.9%. The primary source of completed surveys was via mailing (86.1%), followed by completion at face-to-face producer meetings (11.9%) and on-line (2.0%).

The mean age (± SD) for survey respondents was 62 ± 12.3 yr for beef producers and 55 ± 13.1 yr for dairy producers. Some education beyond high school, including completion of a bachelor's or advanced degree, was completed by 88.9% of beef producers and 78.6% of dairy producers. Herd size numbers were also requested. The results showed that 86.0% of beef cow operators had less than 500 cows; however, 14% had at least 500 cows. Almost 1% of beef survey respondents had 3,000 or more cows. In contrast, over one-third (37.5%) of dairy operations had 1,000 cows or more. Among these larger producers, 5% had at least 5,000 cows. As would be expected, dairy cow producers had larger cow inventories than beef cow operators among survey respondents.

When asked "What is the primary method of marketing your cull/market cows and bulls," both beef and dairy survey respondents clearly indicated that a livestock auction market (77.5 and 62.5%, respectively) was used more than any other option ($P < 0.05$). The direct sale of market cows and bulls to a beef packer was the second most common outlet ($P < 0.05$) among both beef and dairy survey respondents. Beef operators used order buyers and other methods at a greater rate ($P < 0.05$) than buying stations; however, dairy operators used buying stations, order buyers, and other options at the same level.

Consistent with our results, dairy survey data collected in 2006 and reported by USDA NAHMS (2007) indicated that 66% of dairy cows were sold via livestock auction markets. And, the authors also reported that direct sale to packing plants involved 17.5% of cull dairy cows. Due to the fact that the majority of market cattle are sold via livestock auction markets, these data suggest that future educational efforts aimed at reducing NA animal incidence should be focused primarily on the relationship between cattle producers and livestock market owners.

The survey respondent (person who actually completed the survey) was the person who made the decision to market/cull a mature cow or bull among the majority of beef (80.6%) and dairy (64.8%) operations. Employees, family members, and other personnel made that decision on some operations, but in a fairly small number of cases among beef (3.6%, 13.6%, 2.1%, respectively) and dairy (18.5%, 16.7%, 0.0%, respectively) respondents. It is clear that educating the owner and/or manager of a cattle operation about avoiding NA cattle should be the highest priority.

When asked to estimate the percentage of culls sold by survey respondents that went directly to slaughter (vs. being purchased by another producer), both beef and dairy producers indicated that at about three-quarters or more of their cows (78.2 to 83.1%) and bulls (74.5 to 80.2%) went to slaughter (beef and dairy, respectively). Although estimates of incidences were not compared statistically between sexes or operation types, the largest range between mean respondent estimates was less than 10 percentage points overall (83.1% for beef cows vs. 74.5% for dairy bulls).

Beef and dairy producers were asked to prioritize factors they consider when deciding to market a cull cow or bull by ranking a list of criteria provided to them (Table 1). Pregnancy status (i.e. open or late bred) was a high priority among 75.3% of beef producers, and greater ($P < 0.05$) priority than all other factors. Two-thirds (66.5%) of producers said age (including the lack of adequate teeth) and about one-half (52.5%) said injury or illness were high priorities. Breed type (or hide color) and loss of production were indicated as high priorities by only about one-third (37.5 and 35.3%, respectively) of respondents. Traits generally considered of low priority among beef cow producers included inadequate feed availability (85.5% said "low"), calf performance or size (62.8% low), and current market price (59.3% low). In contrast, the vast majority of dairy respondents indicated that loss of production (88.4%), pregnancy status (88.4%), and injury/illness (74.4%) were all of high priority, and greater ($P < 0.05$) than other factors. In comparison, Hadley et al. (2006) reported causes for culling dairy cows to be primarily health reasons (including 79.5% of cows), which generally included health-related factors such as mastitis, injury, disease, and reproduction. In the current study it is clear that a number of traits were of minimal (i.e. "low") priority among dairy producers, likely due to the fact that they are not economically-relevant to dairy production (or are not considered problematic for dairy producers). These

traits include breed type, feed availability, disposition, calf performance, and age.

These results clearly indicate the traits of most economic importance to beef and dairy cattle operations. Further, they provide insight into how producers make culling decisions. It should be noted that several traits are not related, or do not contribute, to the incidence of an animal becoming NA (i.e. breed type, calf performance, disposition, pregnancy status, etc.). However, several traits (i.e. age, injury/illness, etc.) are directly related, and indicate the need for producers to consider them when making culling decisions.

In addition to evaluating how producers prioritize general culling criteria, the survey also documented if producers considered certain additional traits prior to determining if an animal has the potential to even enter the marketplace in the first place (e.g., cleared drug withdrawal, strong enough to tolerate pre-slaughter long-distance transport). The 2 most common criteria ($P < 0.05$) considered by both beef and dairy operations when determining to market a cull cow or bull were "drug withdrawal clearance" and "soundness for transport" (Table 2). However, 18.0% of beef producers and 9.3% of dairy producers did not consider an animal's drug withdrawal status when determining if it can be marketed, suggesting the potential for food safety and drug residue problems. Further, 22.5% of beef respondents and 16.3% of dairy respondents did not indicate that soundness for transport was considered prior to sending a cull animal into the marketplace. Granted, every item on the list of criteria provided in the survey was considered by at least 50% of respondents. However, numerous criteria that are directly related to an animal's well-being, and possibly its likelihood of becoming NA, were not considered by a large number of survey respondents, including body condition and cancer eye, which were not considered by many beef (26.5 and 32.5%, respectively) and dairy (37.2 and 46.5%, respectively) respondents.

A fairly large number of survey respondents indicated that in the past, factors were present on their operations which may have made an animal unfit to enter the marketplace. At least one-third of beef respondents indicated the presence of cows or bulls that were ill or injured (46.0%), had severe cancer eye (42.0%), or were severely lame (35.3%). About one-quarter (23.0%) of producers indicated the presence of animals that had not cleared drug withdrawal. Only 25.0% of beef producers indicated that they did not have any problems present previously. Numerically greater incidence rates were reported among dairy respondents, in which over two- thirds of producers had cows or bulls that were ill or injured (72.1%), did not clear drug withdrawal time (69.8%), or were severely lame (67.4%). Only 11.6% of dairy respondents had no problems present. These data show that factors contributing to the incidence of NA status or food safety issues (i.e. violative drug withdrawal residues) are potentially occurring on beef and dairy operations.

Surveyed producers were asked to indicate the rate of euthanasia on their operation (i.e., number of cows and bulls euthanized annually on-farm or on-ranch as a percent of inventory), which ranged from 0.2 (dairy) to 0.7% (beef) for bulls and 1.2 (beef) to 2.1% (dairy) for cows. The primary method of euthanasia was stated to be gunshot ($P < 0.05$) for both beef (76.2%) and dairy (78.0%) industries. The existence of euthanasia, albeit at a low rate according to our survey, suggests that some producers are actively working to avoid NA problems in the marketplace by euthanizing cattle instead of selling them into the marketplace. Compared with natural deaths, numerically more cows died of natural causes (1.3 and 3.3%, beef and dairy, respectively) than from euthanasia. Due to the relatively high rate of culling (Table 3), on-farm euthanasia, and on-farm deaths within the dairy industry, it is logical to assume that there is a greater likelihood for NA cows to come from dairy operations rather than beef operations. Therefore, focusing BQA educational materials and efforts toward dairy producers to highlight factors contributing to NA status would have the largest amount of impact on this issue.

Survey respondents were also asked about their willingness to consider on-farm or on-ranch euthanasia, rather than sending an animal into the marketplace, if one of the previous characteristics were present in one of their animals. In response, the vast majority (92.1 and 88.1% of beef and dairy respondents, respectively) indicated their willingness to use euthanasia. Conversely, 1 in 13 beef producers and 1 in 8 dairy producers responded that they would not consider euthanasia as an alternative to marketing an animal.

Among respondents who indicated that they would not consider on-farm or on-ranch euthanasia, the primary factors contributing to this decision were the expense of rendering services (48.4%) and the potential for lost revenue (45.2%) among beef producers (Table 4). Many (19.4%) beef respondents also listed limitations for on-farm burial as a major factor, but the lack of available rendering services or ability to euthanize was only listed by 9.7% of producers. In contrast, the dairy industry listed rendering service expense (40.0%), limitations for on-farm burial (40.0%), and lost revenue (20.0%) as reasons for not using on-farm euthanasia.

Based on these data, it is clear that producers need access to reasonably-priced rendering services in order for euthanasia use to become more widespread on cattle operations. Further, educational programs are needed to convey the importance of euthanasia to both beef and dairy producers on NA incidence in the marketplace and its influence on animal welfare.

When asked about the incidence of NA animals on their operations, the majority of beef respondents indicated that they had not had an NA cow (65.4%) in the past 12 mo. However, 18.1 and 10.4% of operations had one NA cow or bull, respectively, while 2 or more NA cows occurred on 16.5% of operations and 2 or more NA bulls were on 4.9% of operations. However, in contrast 88.1% of dairy operations had more than 3 NA cows in the past 12 mo. Granted, this value is related to the larger mean herd size of dairy respondents in this survey (vs. beef respondents);

**Table 1.** Survey respondents' prioritization (high, medium, or low) of criteria considered when deciding to market a cull cow or bull, by operation type

| | Operation type (%) | | | | | |
|---|---|---|---|---|---|---|
| | Beef | | | Dairy | | |
| Variable[1] | High | Med | Low | High | Med | Low |
| Loss of production (i.e., milk) | 35.3$^{d}$ | 20.5$^{cd}$ | 44.3$^{c}$ | 88.4$^{a}$ | 7.0$^{d}$ | 4.7$^{f}$ |
| Not pregnant or late bred | 75.3$^{a}$ | 9.8$^{e}$ | 15.0$^{f}$ | 88.4$^{a}$ | 7.0$^{d}$ | 4.7$^{f}$ |
| Age and/or inadequate teeth | 66.5$^{b}$ | 16.5$^{d}$ | 17.0$^{f}$ | 7.0$^{bc}$ | 39.5$^{ab}$ | 53.5$^{d}$ |
| Injury or illness | 52.5$^{c}$ | 19.0$^{cd}$ | 28.5$^{e}$ | 74.4$^{a}$ | 11.6$^{cd}$ | 14.0$^{f}$ |
| Current market price | 20.4$^{f}$ | 20.4$^{cd}$ | 59.3$^{b}$ | 16.3$^{b}$ | 51.2$^{a}$ | 32.6$^{e}$ |
| Calf performance or size | 15.0$^{f}$ | 22.3$^{bc}$ | 62.8$^{b}$ | 4.7$^{bc}$ | 20.9$^{bc}$ | 74.4$^{bc}$ |
| Disposition | 26.3$^{e}$ | 37.3$^{a}$ | 36.5$^{d}$ | 4.7$^{bc}$ | 34.9$^{b}$ | 60.5$^{cd}$ |
| Breed type or hide color | 37.5$^{d}$ | 27.0$^{b}$ | 35.5$^{d}$ | 2.3$^{c}$ | 4.7$^{d}$ | 93.0$^{a}$ |
| Inadequate feed available | 5.5$^{g}$ | 8.8$^{e}$ | 85.5$^{a}$ | 2.3$^{c}$ | 11.6$^{cd}$ | 86.0$^{ab}$ |
| Other | 18.3$^{f}$ | 19.3$^{cd}$ | 62.5$^{b}$ | 7.0$^{bc}$ | 0.0$^{d}$ | 93.0$^{a}$ |

[1]Survey respondents were allowed to mark more than one variable as high, medium, or low priority.
[a-f] For beef or dairy, means in the same column without a common superscript differ ($P < 0.05$).

**Table 2.** Criteria considered by survey respondents when determining if a cull cow or bull has the potential to enter the marketplace, by operation type

| | Operation type (%) | |
|---|---|---|
| Criteria[1] | Beef | Dairy |
| Cleared drug withdrawal period | 82.0$^{a}$ | 90.7$^{a}$ |
| Soundness for transport | 78.5$^{ab}$ | 83.7$^{a}$ |
| Market price | 59.0$^{d}$ | 55.8$^{b}$ |
| Body condition | 73.5$^{bc}$ | 62.8$^{b}$ |
| Cancer eye presence/severity | 67.5$^{c}$ | 53.5$^{b}$ |
| Other | 8.3$^{e}$ | 0.0$^{c}$ |

[1]Survey respondents were allowed to mark more than one variable in their answer.
[a-e] Within beef or dairy, means without a common superscript differ ($P < 0.05$).

**Table 3.** Percentage of total cow and bull inventories that were culled in the past 12 mo among survey respondents, by operation type

| | Operation type (%) | |
|---|---|---|
| Variable | Beef | Dairy |
| Cows, % | 10.8 | 33.1 |
| Bulls, % | 19.0 | 37.2 |

Percentages were not compared statistically between sexes or operation types.

**Table 4.** Among survey respondents not willing to consider on-farm or on-ranch euthanasia, factors that contributed to that position, by operation type.

| | Operation type (%) | |
|---|---|---|
| Factors[1] | Beef | Dairy |
| Lost revenue | 45.2$^{ab}$ | 20.0 |
| Limitations for on-farm burial | 19.4$^{b}$ | 40.0 |
| Rendering services too expensive | 48.4$^{a}$ | 40.0 |
| Rendering services unavailable | 9.7$^{c}$ | 0.0 |
| No means to euthanize an animal | 9.7$^{c}$ | 0.0 |
| Other | 0.0$^{c}$ | 0.0 |

[1]Survey respondents were allowed to mark more than one factor in their answer.
[a-d]Within beef or dairy, means without a common superscript differ ($P < 0.05$).

however, it is clear that a very large percentage of dairies in California are dealing with NA cows on a regular basis.

One of the most important aspects of this survey included the documentation of factors that beef and dairy producers felt contributed to a cow or bull becoming NA. Our intention was to identify critical control points that producers could focus their energy on in order to ultimately reduce NA incidence in their cows and bulls. When provided with a list of possible contributing factors to NA status, beef and dairy respondents both indicated that calving difficulty was clearly the primary cause of the incidence of NA cows (46.5% beef, 79.1% dairy), and greater ($P < 0.05$) in importance than all other factors. Beef producers indicated that a broken leg (with no known cause) was the second-largest contributor ($P < 0.05$) to NA status. Stifle injury (21.0%) and general weakness (19.5%) were also noted by many beef producers. In contrast, over half (58.1%) of dairy respondents indicated that metabolic diseases (including milk fever, ketosis, etc.), often associated with transition cows (those cows in the physiologically intense phase transitioning from the third trimester of pregnancy to parturition and early lactation), contributed to NA incidence. As well, factors including mastitis, general weakness, broken leg (of an unknown cause), hoof problem, and stifle injury were noted by more than 20% of respondents as contributing to NA status.

Limited data are available discussing the causes of the NA condition in cattle; however, a review was published by Stull et al. (2007). The authors indicated that dairy cows become NA typically around parturition, with hypocalcemia and dystocia being the primary risk factors, in addition to injuries caused by falling in some cases. Stull et al. (2007) further indicated that the primary reason for beef cows to become NA was due to calving paralysis, which is consistent with results of the current study. And, the authors indicated that most NA cattle are of dairy origin.

Methods and systems used by beef and dairy producers to avoid marketing cull animals that have not met a drug withdrawal period are varied. As expected, most beef (54.0%) and dairy (69.8%) respondents indicated that hand-written records were used more ($P < 0.05$) than any other system. However, a considerable number of beef (26.8%) and dairy (16.3%) operators depended on their memory to ensure an animal isn't marketed too early. Further, 11.0 and 11.6% of beef and dairy respondents, respectively, indicated that they did not use drugs that have withdrawal periods. While possible, this is highly unlikely due to the existence of drug withdrawal regulations associated with cattle pharmaceuticals including vaccines and anthelmintics, which are widely used in the cattle industry. It's more likely that producers are unaware of drug withdrawals in some products they are using. Of most concern, are the 2.0 (beef) and 2.3% (dairy) of respondents who indicated that they do not track drug withdrawal information.

In the current study, there was a significant percentage of producers who do not keep track of withdrawal times. Those who do not have a system in- place to avoid the marketing of animals that have not met drug withdrawal time jeopardize the entire beef production industry. Although limited in number, it is clear that additional education focused on these producers is warranted.

## IMPLICATIONS

Producers have an opportunity to use management tools to keep NA cattle from entering in to the market and ultimately the food chain. These data show a need for focused efforts on creating procedures for tracking drug withdrawal times to prevent cattle from entering the market before they have met drug withdrawal times. There is also a need to create protocols for determining the appropriate time to euthanize NA cattle. Additionally, there is a need for a cost effective disposal of dead animals to prevent them from entering into the marketplace.

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# EFFECT OF 2, 4, AND 6-HOUR INTERVALS BETWEEN 2 PROSTAGLANDIN $F_{2\alpha}$ INJECTIONS ADMINISTERED WITH 5-DAY CO-SYNCH + CIDR PROTOCOL ON PREGNANCY RATE IN BEEF COWS

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**ABSTRACT:** The objective of this study was to compare fixed-time AI (TAI) pregnancy rates resulting from intervals of 2, 4, or 6-h between two prostaglandin (PG) $F_{2\alpha}$ injections administered in the 5-d CO-Synch + controlled internal drug-release device (CIDR) estrous synchronization protocol. Angus and Angus-crossbred cows (n = 901) maintained on native pasture at 3 locations were randomly assigned to 3 treatments after blocking for BCS and postpartum interval (PPI). All cows received gonadotropin-releasing hormone (GnRH) and CIDR on d -5, PG with CIDR removal on d 0, and GnRH with TAI at 72 h. Cows received a second injection of PG at 2, 4, or 6 h after the first injection of PG. Pregnancy rates were determined by ultrasonography 40 ± 2 d following TAI. Body condition score and PPI did not differ ($P > 0.74$) between estrous synchronization protocols averaging 4.3 ± 0.03 and 75 ± 1.03 d, respectively. Fixed-time AI pregnancy rates were greater ($P < 0.05$) in cows receiving two PG injections separated by a 6-h interval (51.4%) compared with cows receiving two PG injections separated by 2 and 4-h intervals (40.6 and 41.2%, respectively). Reducing the interval between PG injections from 6 to 2 h resulted in a 10-percentage point reduction in TAI pregnancy rates for cows synchronized with the 5-d CO-Synch + CIDR protocol.

**Key words**: beef cow, controlled internal drug-release device, fixed-time AI, gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$

## INTRODUCTION

A multitude of estrous synchronization protocols are available to facilitate AI in the beef industry; however, less than 10% of cow-calf producers utilize AI technology (USDA, 2009). The most readily adopted estrous synchronization and AI protocols are those that yield high pregnancy rates while minimizing the frequency of processing events, labor requirements, and pharmaceutical costs. Protocols which utilize a progestin controlled internal drug-release device (**CIDR**) in conjunction with injections of gonadotropin releasing-hormone (**GnRH**) and prostaglandin (**PG**) $F_{2\alpha}$ followed by fixed-time AI (**TAI;** i.e. CO-Synch + CIDR) have gained popularity because they eliminate the need for estrus detection and result in pregnancy rates of > 40% (Lauderdale et al., 2009). These CO-Synch + CIDR protocols involve administration of 1 injection of PG; however, modifications to this protocol to a 5-d CIDR have included a second injection of PG. Two injections of PG increase labor requirements and pharmaceutical costs; however, TAI pregnancy rates have ranged from 46 to 67% when 1 injection of PG has been administered (Peel et al., 2010b; Wilson et al., 2010) and 50 to 80% when two injections of PG were administered 6 to 12 h apart (Bridges et al., 2008; Kasimanickam et al., 2009; Peel et al., 2010b; Wilson et al., 2010). Improved pregnancy rates have been observed with the additional PG injection (Kasimanickam et al., 2009); however, the time and stress associated with holding cattle for 6 to 12 h with calf separation following the first injection of PG may be undesirable and difficult without proper holding facilities. Shortening the interval between PG injections would allow administration of both injections of PG in one day and could simplify implementation of the protocol. Therefore, the objective of this study was to compare the pregnancy rates of lactating beef cows submitted to the 5-d CO-Synch + CIDR estrous synchronization protocol with 2 doses of PG administered at intervals of 2, 4, or 6 h.

## MATERIALS AND METHODS

Nine hundred-one multiparous cows at 3 locations were used in this experiment and were managed in accordance with an approved Colorado State University Animal Care and Use Committee protocol. The experiment utilized 234 Angus-crossbred beef cows from the Colorado State University (**CSU**) Eastern Colorado Research Center (**ECRC**) in Akron, CO; 414 Angus seedstock cows from the CSU Beef Improvement Center (**BIC**) in Encampment, WY; and 253 Angus crossbred cows from a privately operated cooperating ranch (Rabbit Creek; **RC**) in Livermore, CO. Cows were maintained on native pasture at all locations and were not supplemented with protein or forage prior to or during the experiment; however, standard mineral supplements were provided ad libitum.

Cows at each location were assigned to one of three estrous synchronization treatments after blocking for BCS (1 to 9 scale; 1 = emaciated, 9 = obese; Richards et al., 1986) and post-partum interval (**PPI**). The 3 treatments evaluated in this experiment included a 5-d Co-Synch + CIDR protocol with two injections of PG administered at 2, 4, or 6 h intervals

(Figure 1). All cows received 100 µg gonadorelin diacetate tetrahydrate (GnRH, 2 mL Cystorelin, Merial Limited, Duluth, GA) and were fitted with a CIDR [EAZI-Breed CIDR (1.38 g progesterone) cattle insert, Pfizer Animal Health, New York, NY) on d -5. On d 0, cows were separated from their calves, CIDR removed, and injected with two 25 mg of dinoprost tromethamine [prostaglandin $F_{2\alpha}$ (PG), 5 mL Lutalyse, Pfizer Animal Health]. Cows were returned to their calves after receiving the second dose of PG. All cows received a second injection of GnRH and were TAI at 72 ± 3 h following the first PG injection.

A single evaluator scored all cows for body condition at all locations in this experiment. Artificial insemination technicians (n = 5 at BIC, 5 at ECRC, and 3 at RC) and sires (n = 16 at BIC, 4 at ECRC, and 17 at RC) were randomly distributed among treatments. Pregnancy rates to TAI were diagnosed via transrectal ultrasonography (Aloka SSD 500 with a 5 MHz linear probe, Aloka Co., Ltd. Wallingford, CT) at 40 ± 2 d post-TAI. Bulls were withheld for a minimum of 10 d following TAI so that experimental pregnancy rates could be distinguished from those resulting from natural breeding. Bulls remained with cows for 40 d.

Cows with inaccurate records or missing CIDR at time of CIDR removal were excluded from the data analysis (n = 9). Pregnancy rate was analyzed using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC) with protocol, location, location x protocol, AI technician(location), sire(location), BCS, and PPI in the model. Nonsignificant terms ($P > 0.10$) were removed from the model by backward elimination and the analysis was repeated. The final model contained protocol, location, AI technician(location), and PPI. Least squares means were calculated in GLIMMIX and separated using least significant differences of the logits ($P < 0.05$). Body condition score and PPI were analyzed using the GLM procedure (SAS) with protocol, location, and their interaction in the model. Least squares means for BCS and PPI were separated using least significant differences when $P < 0.05$.

## RESULTS AND DISCUSSION

To reduce the labor required and stress on calves from sorting calves from their dams twice, Peel et al. (2010a) evaluated the effect of calf separation during a 12 h period between two PG injections using the 5-d CO-Synch + CIDR protocol. No impact on AI pregnancy rates were reported

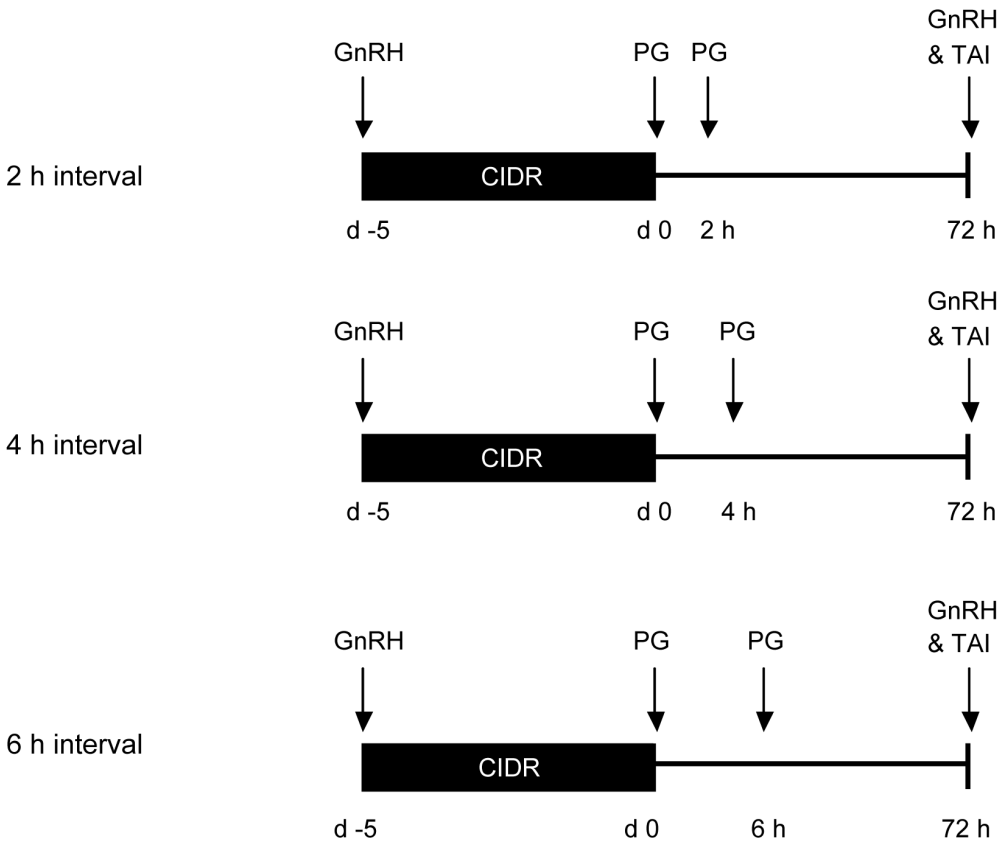


**Figure 1.** Diagram of protocols used to synchronize estrus and ovulation in beef cows. All cows were treated with gonadotropin-releasing hormone (GnRH) and controlled internal drug-release insert (CIDR) on d -5, removal of CIDR and two injections of prostaglandin (PG) $F_{2\alpha}$ at 0 and 2, 4 or 6 h following removal of CIDR on d 0, and GnRH with fixed-time AI (TAI) at 72 h.

and the authors concluded that keeping the calves separate did not incur any negative effects and would simplify the application of this protocol. Therefore, in the current study calves remained separated from cows until after the second PG injection was administered.

Cow BCS and PPI did not differ ($P = 0.84$ and 0.75, respectively) between estrous synchronization protocols averaging 4.3 ± 0.03 and 75 ± 1.03 d, respectively. Body condition score was least ($P < 0.001$) for cows at RC (4.0 ± 0.03), intermediate for cows at BIC (4.4 ± 0.02), and greatest for cows at ECRC (4.7 ± 0.03). Postpartum interval from calving to the start of the study was greater ($P < 0.001$) in cows at ECRC (79 ± 1.2 d) and BIC (78 ± 0.9 d) compared with cows at RC (68 ± 1.1 d). There was no protocol by location interaction for BCS ($P = 0.90$) or PPI ($P = 0.96$). Fixed-time AI pregnancy rates were greater ($P < 0.05$) in cows given PG 6 h apart (51.4%) than in cows given PG 2 or 4 h apart (40.6 and 41.2%, respectively). Fixed-time AI pregnancy rates were greater ($P < 0.01$) in cows at RC (54.6%) than BIC (36.0%) with TAI pregnancy rates at ECRC being intermediate (42.9%) and similar ($P > 0.20$) to both other locations.

Timed-AI pregnancy rates following administration of two injections of PG in 5-d CO-Synch + CIDR protocols with TAI at 60 to 72 h have ranged from 50 to 80% (Bridges et al., 2008; Kasimanickam et al., 2009; Peel et al., 2010a,b; Whittier et al., 2010; Wilson et al., 2010; Bridges et al., 2011). The interval between two PG injections in these studies ranged from 2 to 12 h; however, only Whittier et al. (2010) and Peel et al. (2010b) directly compared TAI pregnancy rates among different intervals between two PG injections in the 5-d CO-Synch + CIDR protocol. In agreement with our results, TAI pregnancy rates were greater in beef cows synchronized with two injections of PG administered 6.5 versus 2.3 h apart (57 versus 53%, respectively) in the 5-d CO-Synch + CIDR protocol (Whittier et al., 2010). In contrast to these and our results, Peel et al. (2010b) reported similar TAI pregnancy rates between two PG injections administered 6 and 12 h apart in the 5-d CO-Synch + CIDR protocol (50 and 51%, respectively). We and Whittier et al. (2010) observed a 4 to 10 percentage unit decrease in TAI pregnancy rates when PG was administered < 6 h apart suggesting that the interval between PG injections with the 5-d CO-Synch + CIDR protocol should be at least 6 h.

Bridges et al. (2008) recommended administering two injections of PG in the 5-d CO-Synch + CIDR protocol in order to ensure complete luteolysis of the newly formed corpus luteum. Luteolysis was numerically, but not statistically, greater in beef cows receiving two injections of PG 8 h apart compared with one injection of PG in the 5-d CO-Synch + CIDR protocol (96 and 88%; $P = 0.13$) and greater TAI pregnancy rates were observed in cows receiving two versus one PG injection (55 versus 48%; Bridges et al., 2011). Bridges et al. (2011) also reported that two injections of PG administered concurrently at CIDR removal (0 h) resulted in luteolysis and TAI pregnancy rates (93 and 51%, respectively) that were intermediate and similar to those rates in cows who received one injection of PG at 0 h or two injections of PG separated by 8 h. We could find no published reports of studies comparing luteolysis in cows receiving two injections of PG administered at different intervals in the 5-d CO-Synch + CIDR protocol.

Prostaglandin $F_{2\alpha}$ is released in a series of pulses every 6 to 12 h (Peterson et al., 1975; Ginther et al., 2007). Kindahl et al. (1976) reported that PG was released over a 2 to 3-d time period and that the duration of PG pulses ranged from 1 to 5 h in length. Mann and Lamming (2006) reported that PG release episodes averaged 4 h in length and suggested that 5 release episodes of this duration were required for luteolysis. Ginther et al. (2009) reported that a 2-h intrauterine infusion of 0.5 mg PG best simulated a natural PG pulse. Prostaglandin $F_{2\alpha}$ appears to have an immediate luteolytic affect (Kindahl et al., 1976; Silvia et al., 1991; Mann and Lamming, 2006; Ginther et al., 2007); however, continued release of PG or additional sequential pulses may be required to complete the process (Kindahl et al., 1976; Ginther et al., 2009). These data suggest that two injections of PG administered 2 or 4 h apart could be mimicking 1 naturally occurring pulse of PG and that multiple injections of PG administered at an interval of > 6 h might be more representative of conditions occurring naturally in the cow and result in greater TAI pregnancy rates following estrous synchronization.

## IMPLICATIONS

Shortening the interval between PG injections from 6 to 2 or 4 h reduced TAI pregnancy rates by 10 percentage points in lactating beef cows synchronized with the 5-d CO-Synch + CIDR protocol. These results suggest that the interval between two PG injections with the 5-d CO-Synch + CIDR protocol should be at least 6 h.


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# EFFECTS OF PAIN MITIGATION AND METHOD OF CASTRATION ON BEHAVIOR AND FEEDLOT PERFORMANCE IN CULL BEEF BULLS

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**ABSTRACT:** The objectives of this study were to evaluate the effects of castration method (band vs. surgical) and use of analgesia on behavior and feedlot performance in cull bulls. Angus, Hereford, and Angus crossbred bulls (n = 20; initial BW 384 ± 59.3 kg; 336 ± 20.0 d old) were housed in feedlot pens equipped with the ability to measure individual daily feed intake. A balanced randomized block design using a 2 x 2 factorial arrangement of treatments was utilized. Factors included: 1) castration method, and 2) analgesia presence. A multimodal analgesia protocol (MMA) was used and consisted of subcutaneous ketamine-stun containing butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg), ketamine (0.04 mg/kg), and a local 2% lidocaine hydrochloride anesthetic block of the spermatic cords (10 mL per cord) and scrotum (10 mL) on d 0. Flunixin meglumine (1.2 mg/kg) intravenously (iv) on d 0, 1, 2 and 3. Cattle were stratified to treatments based on breed, BW, age and a temperament score. Treatments included: 1) band castration without analgesia (BAND), 2) band castration with analgesia (BAND-MMA), 3) surgical castration without analgesia (SURG), and 4) surgical castration with analgesia (SURG-MMA). Chute exit velocity (EV) and time in chute (TIC) were collected on d -9, 0, 1, 2 and 13. Willingness-to-enter-chute (WTE) score, rectal temperature (TEMP), heart rate (HR), and respiration (RESP) were collected on d 0, 1, 2, 3 and 13. Cattle were weighed on d -9 and 13 while feeding behaviors were collected continuously for 57 d pre-castration and 28 d post-castration. There was a tendency ($P < 0.09$) for ADG to be greater in cattle receiving analgesia. Both SURG treatments exhibited greater ($P < 0.001$) TEMP on d 1 and on d 2 ($P < 0.05$) when compared with BAND treatments. Mean DMI was greater ($P = 0.02$) in MMA treatments when compared with non-medicated treatments. The SURG treatment, when compared with SURG-MMA and BAND, exhibiting greater ($P = 0.04$, $P = 0.04$, respectively) meal size. Results suggest that pain mitigation reduces the impact of castration on ADG and DMI.

**Key words:** behavior, bulls, castration, ketamine-stun, pain

## INTRODUCTION

Castration of male cattle is a common practice in the beef cattle industry. Castrating bulls has been shown to reduce DMI and ADG, and increase serum cortisol and haptoglobin concentrations when compared with intact bulls (Faulkner et al., 1992), indicating that there is both a physiological stress and inflammatory response to castration. When surveyed in 2011, veterinarians associated some level of pain with castration of beef calves (Fajt et al., 2011). Despite the reduction in animal performance and pain caused by castration, roughly 15 million livestock castration procedures occur yearly (USDA-NASS, 2009). The benefits of castration, including reduced aggression, reduced sexual activity, reduced incidence of dark-cutting carcasses, improved carcass quality grade, and fewer unwanted pregnancies (Worrell et al., 1987; Faulkner et al., 1992), are considered to outweigh the disadvantages.

There are 2 commonly used methods of castration; banding and surgical castration. Reduced acute pain response occurs with banding, but it is associated with decreased ADG in 14-mo-old beef bulls (Fisher et al., 2001). Surgical castration with a scalpel is noted as the most common castration method in bulls among veterinarians surveyed in the U.S. (Coetzee et al., 2010). Local anesthetics at castration have been shown to reduce cortisol response (Thüer et al., 2007) and improve ADG in 5.5-mo-old bull calves (Fisher et al., 1996). Non-steroidal anti-inflammatory drugs (**NSAID**), specifically flunixin meglumine, have been shown to visibly reduce pain response up to 8 h post-castration (Currah et al., 2009). The objectives of this study were to evaluate the effects of castration method (band vs. surgical) and use of a multimodal analgesia approach on behavior and feedlot performance in yearling cull bulls.

## MATERIALS AND METHODS

This project was approved by the Institutional Animal Care and Use Committee at Colorado State University (project #10-2285A).

***Animals.*** Angus (n = 5), Hereford (n = 4), and Angus, Hereford and Simmental crossbred (n = 11) cull bulls weighing 384 ± 59.3 kg and 336 ± 20.1 d old, were castrated via 4 treatments in a 2 × 2 factorial arrangement with factors being: castration method, and analgesia presence.

***Housing and Feeding.*** All animals were housed in one 30-head feedlot pen (30 m × 60 m). Ad libitum feed was provided to the pen, though only 4 animals could access feed at one time. The diet was composed of corn silage, ground alfalfa hay, and a vitamin and mineral supplement and

delivered via 4 radio frequency identification (**RFID**)-linked individual feedbunks (Growsafe Systems Ltd., Airdrie, AB, Canada) that enabled collection of daily individual animal feed intake in a group feeding environment. Animals were acclimated to this feeding system for 14 d, which began 80 d prior to castration. The bulls were vaccinated with Clostridium Perfringens, Types C & D Tetanus Toxoid (Bar Vac CD/T, Boehringer Ingelheim, St. Joseph, MO) 14 d prior to castration.

***Behavioral Measurements.*** As animals entered the chute on d 0, 1, 2, 3, and 13, a willingness-to-enter-the-chute (**WTE**) score was assigned using a 9-point scale with 1 defined as "entered chute without pressure on the animal's flight zone," and 9 as "the handler was unable to drive the animal into the squeeze chute." All handlers were trained to evaluate animals using the WTE scoring system.

On d -9, 0, 1, 2, and 13, objective time in chute (**TIC**) and exit velocity (**EV**) values were collected using an infrared sensor timing system (FarmTek Inc., North Wylie, TX). The EV (m/s) was collected beginning 1.892 m from the head catch and ending 1.892 m beyond that point.

At the time of castration (d 0), bulls received a procedure response score (**RS**) on a 4-point scale which was determined by the technician observing the procedure and indicated the degree an animal demonstrated pain responses to the procedure. The score system included: 0 = standing on all 4 feet during the castration procedure, 1 = treading of front or rear feet during the castration procedure, 2 = 1 to 3 kicks with rear feet during the castration procedure, 3 = more than 3 instances of kicking or hopping on rear feet during the castration procedure.

***Treatments.*** Bulls were stratified by breed, BW, age, and a combined temperament score collected on d -9 into 1 of 4 treatments: band castration without analgesia (**BAND**), band castration with analgesia (**BAND-MMA**), surgical knife castration without analgesia (**SURG**), and surgical knife castration with analgesia (**SURG-MMA**).

***Procedures.*** Surgical knife castrations (SURG and SURG-MMA) were prepared by scrubbing the scrotum with pieces of role cotton soaked in dilute betadine solution. The distal aspect of the scrotum was excised using a sterile scalpel blade. The external spermatic fascia was stripped away from the testes using 4 × 4 gauze pads. Once exposed, the spermatic cord was twisted using a Henderson Castration Tool (Stone Manufacturing, Kansas City, MO) attached to a 14 volt cordless electric drill (Dewalt Industrial Tool Co., Baltimore, MD). The same experienced veterinarian completed all surgical castrations to ensure consistency. Oxytetracycline (LA-200, Pfizer Animal Health, New York, NY) was administered subcutaneously (4 mg/kg) to both SURG and SURG-MMA treatments.

Band castrations (BAND and BAND-MMA) were completed by securing a latex band around the neck of the scrotum using a Callicrate Bander (NO-BULL Enterprises, St. Francis, KS). The same experienced technician completed all band castrations throughout the study.

Analgesia treatment groups (BAND-MMA and SURG-MMA) received an injection containing butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg), and ketamine (0.04 mg/kg) administered subcutaneously upon restraint in the chute (Abrahamsen, 2008). In addition, 2% lidocaine was used as a local anesthetic injected at 10 ml per spermatic cord and a 10 ml subcutaneous infiltration of the scrotum. Upon restraint, all animals were haltered and flunixin meglumine (Flunixin Injectable, Norbrook Laboratories, Ltd., Newry, Ireland) was administered iv (1.2 mg/kg) to MMA treatments. These animals also received flunixin meglumine (1.2 mg/kg iv) on d 0, 1, 2, and 3.

Dry matter intake was averaged for 57 d before castration to establish baseline feeding behaviors for each individual animal. Post-castration feeding behavior data were collected for each animal d 0 to 28.

***Statistical Analyses.*** The experimental unit for the current study was individual animal. Exit velocity, TIC, HR, TEMP, RESP and ADG were analyzed using a generalized linear mixed model and least square means with a fixed effect statement to analyze differences in treatment and d. Rectal temperature, WTE, HR, and RESP were analyzed using a generalized liner mixed model with d 0 as a covariate (SAS Inst. Inc., Cary, NC). Dry matter intake was analyzed with generalized liner mixed model which included radial smoother within the random statement. Given the nature of the acute response elicited from ketamine stun, EV and RS on d 0 were analyzed independent of other d of collection.

## RESULTS AND DISCUSSION

Exit velocity on d 0 was similar ($P > 0.10$) between treatments or main effects of castration method or presence of analgesia. When EV was analyzed independent of d 0 with d -9 as a covariate, mean EV across all other days in MMA treatments was 0.37 ± 0.163 m/s greater ($P = 0.04$) than treatments that did not receive pain mitigation.

Willingness-to-enter-chute did not differ ($P > 0.10$) across main effects of castration method and presence of analgesia. Similarities across treatments within WTE indicate that neither method of castration or MMA influence WTE.

There was no method × MMA interaction ($P = 0.46$) Response score was affected by the main effect of MMA, as seen by SURG animals exhibiting a greater ($P < 0.01$) RS than BAND, and animals not receiving analgesia exhibiting a greater ($P < 0.01$) RS than MMA treatments (Figure 1). A study examining the effects of intravenous ketamine and xylazine administration at a dose of 0.1 mg/kg and 0.05 mg/kg, respectively, found that castration without any sedation resulted in a greater number of animals that exhibited violent escape behavior at castration (Coetzee et al., 2010). The findings from the current study support the contention that multimodal administration of analgesia decreases acute pain responses when castrating yearling bulls.

Time in chute was greater ($P < 0.001$; Table 1) in SURG than BAND treatments by 318.0 ± 48.75 seconds. In cattle that received pain mitigation, TIC was greater

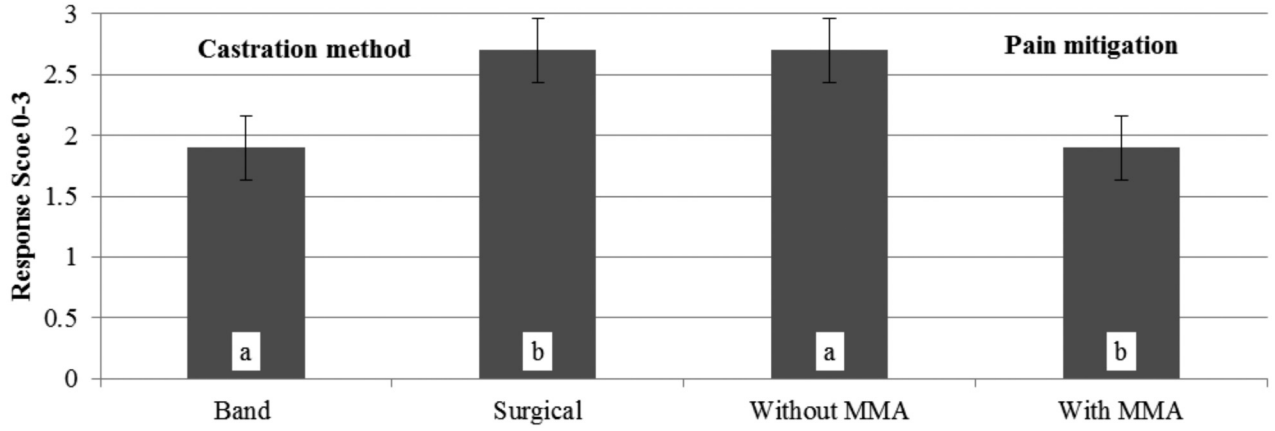


**Figure 1.** Mean response score at time of castration by main effect of method of castration and pain mitigation presence. BAND = band castration and BAND-MMA = band castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, 1, 2 and 3, SURG = surgical castration, SURG-MMA= surgical castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, d 1, d 2 and d 3. Response score was determined as: 0 = standing on all 4 feet during the castration procedure, 1 = treading of front or rear feet during the castration procedure, 2 = 1 to 3 kicks with rear feet during the castration procedure, 3 = more than 3 instances of kicking or hopping on rear feet during the castration procedure. There was no method × pain mitigation interaction. Pain mitigation was provided via a multimodal analgesia approach (MMA). [a,b]Within main effect of method of castration or presence of analgesia, means without common superscript differ ($P < 0.05$).

**Table 1.** Least square means for time in chute (TIC) across method of castration and pain mitigation[1]

| | Main effects | | | |
|---|---|---|---|---|
| | Method | | Pain mitigation[2] | |
| Item | Band castration | Surgical castration | Without MMA | With MMA |
| TIC (s) | 560.1 [a] ± 32.50 | 878.1 [b] ± 36.34 | 661.5 [a] ± 34.47 | 776.8 [b] ± 34.47 |

[1]BAND = band castration, BAND-MMA = band castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, 1, 2 and 3, SURG = surgical castration and SURG-MMA= surgical castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, 1, 2 and 3.
[2]Pain mitigation was provided via a multimodal analgesia approach (MMA).
[ab]Within main effect of method of castration or presence of analgesia means without common superscript differ ($P < 0.05$).

($P = 0.03$) by 115.4 ± 48.75 seconds. These results indicate that both surgical castration and administration of analgesia are more time consuming that band castration and omission of analgesia. While examining the application of these results, time has the potential to play a role in industry acceptability.

There was a method × day interaction ($P = 0.03$) for TEMP. Mean TEMP in the SURG treatments was 0.77 ± 0.03°C greater ($P < 0.001$) than BAND treatments on d 1. On d 2, TEMP for SURG was 0.38 ± 0.03°C greater ($P = 0.03$) than BAND treatments. Rectal temperature among surgical castrates in the present study may be accredited to an inflammatory response to the soft tissue injury at the surgical site.

There was a method × d interaction ($P = 0.005$) for HR. Heart rate for SURG was 25.84 ± 7.3 beats/min greater ($P = 0.001$) than BAND treatments on d 1. There were no other differences ($P > 0.10$) for HR between methods across day.

There was a tendency ($P = 0.07$) for a method × d interaction for RESP. A potential reason for the RESP differences seen in SURG castrates may be due to increased epinephrine or inflammatory mediators in circulation in response to the surgery. Increased inflammatory mediators would be a consistent connection between the elevated HR and TEMP observed in SURG castrates (Cahn and Line, 2005).

**Table 2.** Average daily gain (kg/d) and DMI (kg/d) across castration method and pain mitigation treatments [1, 2, 3, 4]

| Treatment | ADG | SEM | DMI | SEM |
|---|---|---|---|---|
| BAND | 0.68 | 0.212 | 9.51 [a] | 0.277 |
| BAND-MMA | 1.13 | 0.212 | 10.41 [b] | 0.225 |
| SURG | 0.99 | 0.212 | 9.73 [a] | 0.395 |
| SURG-MMA | 1.28 | 0.212 | 10.39 [b] | 0.175 |

[1]BAND =band castration, BAND-MMA= band castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, 1, 2 and 3, SURG = surgical castration, SURG-MMA= surgical castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, 1, 2 and 3.
[2]There were no method or method × MMA interactions ($P > 0.10$) in ADG.
[3]There was a tendency ($P = 0.09$) for ADG to greater in MMA animals.
[4]Average daily gain was calculated from d -9 and 13.
[a,b] Means without common superscript differ ($P = 0.02$) across main effect of MMA.

There were no differences ($P > 0.05$) in ADG across main effect of method of castration, presence of analgesia or method of castration × presence of analgesia. There was a tendency ($P = 0.09$) for an interaction in ADG across MMA main effects (Table 2), with MMA treatments gaining 0.37 kg/d greater than those that did not receive analgesia. An experiment that evaluated the effects of a xylazine epidural and flunxin meglumine administration at band castration found no differences in ADG between medicated and non-medicated groups (Gonzalez et al., 2010). In a previous study, growth was greater in surgical castrates than banded castrates, and both groups had reduced growth when compared with intact bulls (Fisher et al., 2001). Similarly, in the current study there were no differences ($P = 0.30$) in ADG across the main effect of method.

Mean DMI throughout the trial was greater ($P = 0.02$) in MMA treatments (Table 2). Across the main effect of method, BAND treatments exhibited 2.62 ± 0.716 kg greater ($P = 0.003$) DMI on d 1. And though there was no d × MMA interaction, MMA treatments exhibited 1.57 ± 0.713 kg greater ($P = 0.03$) DMI, 2.83 ± 0.713 kg greater ($P < 0.001$) DMI on d 2 and 1.44 ± 0.713 kg greater ($P = 0.04$) DMI on d 3. Our results differed from the reported in the literature (Gonzalez et al., 2010), in which pain mitigation decreased DMI. This difference could be attributed to sedation strategy (subcutaneous ketamine stun vs. xylazine epidural) or administration of flunixin meglumine (d 0, 1, 2 and 3 vs. d 0).

The data demonstrate increased DMI over the 28 d among MMA cattle. There was also a tendency ($P = 0.09$) for increased ADG in MMA treatments. The current study only examined the 28 d period after castration and it is not known if these effects would be compensated for by the time of slaughter. Ketamine and butorphanol controlled substances and must be administered by a Drug Enforcement Administration licensed veterinarian, which could inhibit widespread use in the beef industry. Hence, further investigation into pain mitigation strategies that are more accessible to the industry is needed.

As used in the present study, MMA combines multiple analgesics to target an array of pain pathways. Previous studies have shown that the MMA principle allows for a synergistic effect of drugs by using a lower dose of each drug in combination to minimizes the detrimental effect of each (Lamont, 2008).

The MMA protocol used in the current study incorporated an $\alpha_2$-adrenergic agonist (xylazine), an opioid (butorphanol), a N-methyl-D-asparate receptor antagonist (ketamine), an NSAID (flunixin meglumine) and a local anesthetic (lidocaine hydrochloride; Abrahamsen, 2010). The results of the current study suggest that it is this multimodal approach that allowed for production and welfare benefits in regards to ADG, DMI and RS. It could be hypothesized that the absence of negative side-effects associated with the products used in the current study can be attributed to this MMA approach.

There is a relatively limited understanding of pain response in the bovine making it difficult to determine what behaviors to evaluate and how to score these behaviors during potentially painful procedures such as castration in order to evaluate methods of pain mitigation. This study showed differences (or tendencies) in ADG, DMI, HR, TEMP, RS and EV, but not WTE or VOC. Further investigation into behaviors related to pain associated with castration will be vital for the approval of more effective pain mitigation strategies for use with castration in food animals.

## IMPLICATIONS

Results of this study indicate benefits associated with analgesia at castration, supporting the necessity of further research in analgesia at castration. Continuing research in painful behaviors will be essential in quantifying the effects of these products.

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# FETAL AND MATERNAL INDUCTION OF ANGIOGENIC FACTORS DURING EARLY PREGNANCY

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**ABSTRACT:** Early pregnancy, when most embryonic losses occur, is a critical period in which vital placental vascularization is established. Adequate vascular development supports embryonic survival and subsequent fetal growth. Vascular endothelial growth factor (VEGF) is the most potent inducer of angiogenesis, and factors regulating VEGF may ultimately affect vascularization. Activation of chemokine receptor 4 (CXCR4) by CXCL12 increases VEGF synthesis and secretion, which in turn stimulates CXCL12 and CXCR4 production. This synergistic regulation may influence placental vascularization. Our laboratory reported elevated CXCR4 in endometrium during early pregnancy in sheep, but the relationship between the CXCL12/CXCR4 signaling pathway and angiogenic factors such as VEGF, fibroblast growth factor (FGF2), and angiopoietin 1 (ANG1) is lacking in ruminants. We hypothesized CXCL12, CXCR4, and select angiogenic factors and their receptors would increase in placental tissue during early pregnancy. To test this hypothesis, caruncle and fetal extraembryonic membrane tissues were collected on d 20, 25 and, 30 of pregnancy, with d 10 of the estrous cycle as a control. Real time PCR was used to assess relative mRNA levels. Expression levels were normalized by standard methods, and subjected to ANOVA with Newman-Keuls post hoc test to determine significant differences ($P < 0.05$). In caruncles, CXCL12 and CXCR4 increased on d 20 in pregnant ewes. Also, FGF2 increased during early to mid-placentation. In fetal extraembryonic membranes, CXCL12, CXCR4, ANG1, and VEGF were induced with advancing pregnancy, whereas FGF2 and VEGFR2 peaked on d 25. The increase of angiogenic factors in fetal placenta during implantation and placentation highlights the concept that the fetus regulates its vascularization in synergy with the maternal placenta. The relationship between VEGF and CXCL12/CXCR4 underscores the potential role for this chemokine system in placentation. These results provide strong support for enhanced signaling between chemokines and angiogenic factors within the fetal-maternal interface.

**Key words:** chemokine, pregnancy, vascularization

## INTRODUCTION

Improving livestock fertility is paramount for efficient agricultural productivity and sustainability of food supplies. It is evident that proper vascular development of the placenta is extremely important for fetal growth and survival, as aberrant vascular development is linked to a number of serious pregnancy-related complications including intrauterine growth restriction, preeclampsia or early pregnancy loss (Meekins et al., 1994; Macara et al., 1996). Most placental growth occurs during early gestation, with limited growth taking place during the last half of gestation. Numerous signaling molecules such as chemokines and angiogenic factors regulate vascularization of the placenta, thus affecting the overall health and development of the fetus throughout pregnancy (Igwebuike, 2009; Grazul-Bilska et al., 2010).

Chemokines are multifaceted proteins, which serve as chemoattractants, triggering leukocyte migration, and performing non-immune cell functions. Specifically, chemokine receptor four (**CXCR4**) has one defined ligand, CXCL12 and disruption of this chemokine or its receptor in mice results in serious vascular abnormalities (Nagasawa, 2001). As this pathway promotes vascular growth in other tissues, it is possible that this pathway is promoting placental vascularization. Furthermore, because pro-angiogenic factors such as vascular endothelial growth factor (**VEGF**), fibroblast growth factor two (**FGF2**), and angiopoietin one (**ANG1**) primarily mediate vascular formation, this chemokine system may stimulate these important angiogenic factors during early placental progression as well. Interestingly, CXCL12 increases synthesis and secretion of VEGF, which stimulates expression of CXCL12 and CXCR4 (Rosenkilde and Schwartz, 2004). This synergistic regulation could be affecting vascularization of the placenta and the increase in angiogenesis needed for fetal survival. Also, FGF2 and VEGF stimulate CXCR4 expression, but not other chemokine receptors (Salcedo and Oppenheim, 2003). As VEGF is the most potent inducer of vascularization, and FGF2 and ANG1 promote angiogenesis, it is important to determine what signaling mechanisms could be stimulating these angiogenic factors. We previously demonstrated that CXCR4 increases in endometrium on d 35 of pregnancy in sheep, concurrent with increased CXCL12 in the blood (Ashley et al., 2011). Due to this increase on d 35, it is plausible that this unique ligand–receptor pair promotes proper vascularization of the placenta; however, a more defined timeframe of this increase needs to be elucidated. In addition, the CXCL12/CXCR4 pathway elicits angiogenesis and may also promote angiogenic factor expression during early placental development, but the connection between these pathways is lacking in ruminants.

As such, we hypothesized that CXCL12, CXCR4, and select angiogenic factors and their receptors would increase in placental tissue during early pregnancy. The objective of this study was to determine if mRNA for CXCL12, CXCR4, and select angiogenic factors and their receptors, is differentially expressed using real-time PCR (**qPCR**) on d 20, 25 and 30 of pregnancy and if differences exist between pregnant and non-pregnant ewes.

## MATERIALS AND METHODS

New Mexico State University Animal Care and Use Committee reviewed and approved all experimental procedures using animals.

***Animals and Tissue Collection***. Estrus was synchronized in Rambouillet-cross ewes during the mid to late luteal phase with two injections of dinoprost tromethamine (5 mg i.m.; Lutalyse; Pfizer, New York, NY) administered 4 h apart. Upon detection of estrus (d 0) by a vasectomized ram, ewes were placed into experimental groups. Ewes (n = 5/d) were anesthetized with sodium pentobarbital (20 mg/kg, i.v.) on either d 20, 25, or 30 of gestation and also from mid-luteal, nonpregnant (d 10 of the estrous cycle) control ewes. The reproductive tract was removed using a mid-ventral laparotomy, tissues were collected and snap frozen in liquid nitrogen, and stored at -80°C for subsequent RNA isolation. Ewes were then euthanized by exsanguination.

***RNA Isolation.*** Total RNA was extracted from caruncle and fetal membrane tissue using Tri Reagent BD (Molecular Research Center Inc., Cincinnati, OH) according to manufacturer's directions. RNA was eluted in RNAse-free water and treated with TURBO DNA-free kit (Ambion, Foster City, CA).

***Real-time Polymerase Chain Reaction.*** Analysis was completed using qPCR as previously published (Ashley et al., 2011). Briefly, cDNA was synthesized and qPCR was performed using a CFX96 Touch Real-Time PCR Detection System. Primer sequences are listed on Table 1. The GAPDH amplicon did not change across days or pregnancy status and was used to normalize each target via the ΔCq method (Schmittgen and Livak, 2008). Data are represented by graphing $2^{-\Delta Cq}$ values calculated for each gene of interest.

***Statistical Analysis.*** Data were subjected to ANOVA analysis appropriate for a completely randomized design. When a significant effect ($P < 0.05$) was detected, day means were separated using Newman-Keuls test on normalized Cq values using Prism (Version 5 from GraphPad Software, Inc.).

**Table 1.** Reverse and forward primer sequences for each gene of interest

| Gene | Reverse Primer Sequence | Forward Primer Sequence |
|---|---|---|
| GAPDH | 5-CGTTCTCTG CCTTGACTGTG-3 | 5-TGACCCCTTCA TTGACCTTC-3 |
| CXCL12 | 5-GGTCAATG CACACTTGCCTA-3 | 5-CCTTGCCG ATTCTTTGAGAG-3 |
| CXCR4 | 5-GAGTCGA TGCTGATCCCAAT-3 | 5-AAGGCTAT CAGAAGCGCAAG-3 |
| VEGF | 5-AAATGCTTTCT CCGCTCTGA-3 | 5-TCACCAAAG CCAGCACATAG-3 |
| VEGFR1 | 5-TCCACAAA TCTTGGCCTTTC-3 | 5-GTGCAGAT GGACGAGGACTT-3 |
| VEGFR2 | 5-GCTCCAGC TCTGAAAAC-3 | 5-TGAGAGCCC CTGATTACACC-3 |
| ANG1 | 5-AGGAGGC TGGTGCCTATCTC-3 | 5-TCTGGAGC ATGTGATGGAAA-3 |
| FGF2 | 5-AGTGCCA CATACCAACTGGA-3 | 5-GTGCAAACCG TTACCTTGCT-3 |

## RESULTS

The expression of mRNA for CXCL12, CXCR4 and select angiogenic factors was evaluated using qPCR. In caruncle tissue, all targets were detected at all time points. Expression of CXCL12 increased on d 20 and CXCR4 increased on d 20 and 25 compared with non-pregnant ewes (Figure 1). No differences were noted ($P > 0.05$) between pregnant and non-pregnant ewes for the angiogenic factors. When comparing days of gestation, however, FGF2 increased on d 20 compared with d 30, with CXCL12 rising similarly on d 20 compared with d 25 and 30 (Figure 2).

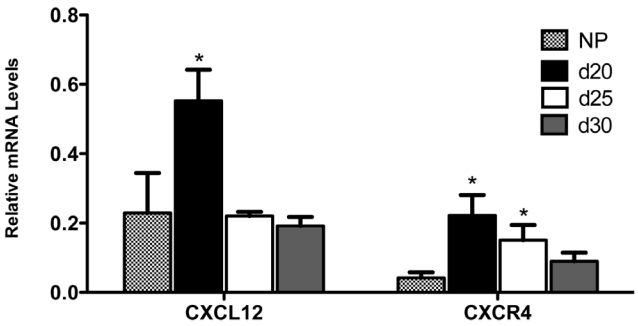


**Figure 1.** Expression of CXCL12 and CXCR4 mRNA is up regulated in caruncle tissues in pregnant ewes (d 20, 25 and 30) compared with non-pregnant ewes (NP). Expression of mRNA for CXCL12 was elevated (*$P < 0.05$) on d 20 of pregnancy compared with non-pregnant ewes and CXCR4 was elevated (*$P < 0.05$) on d 20 and 25 of gestation compared with non-pregnant ewes.

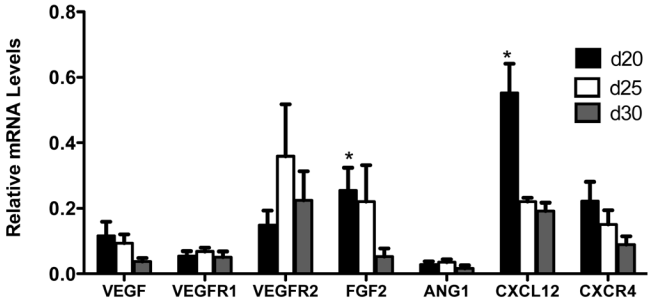


**Figure 2.** Expression of FGF2 and CXCL12 mRNA in caruncle samples was elevated (*$P < 0.05$) on d 20 of pregnancy compared with d 30 and, d 25 and 30 respectively. Expression of mRNA for CXCR4 and other angiogenic factors remained constant across days tested.

Expressions of all transcripts were detected during early gestation within fetal membrane tissues. An increase of CXCL12 and VEGF mRNA occurred on d 25 and 30 compared with d 20 of pregnancy ($P < 0.05$). Receptors for CXCL12 and VEGF followed a similar pattern, with CXCR4 and VEGFR1 increasing on d 25 and 30 compared with d 20, and VEGFR2 peaking specifically on d 25 compared with d 20 and 30. Also, ANG1 displayed the most robust expression, peaking on d 30 compared with d 20 of pregnancy (Figure 3).

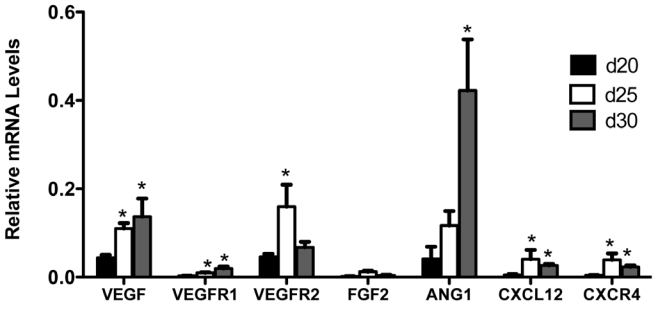


**Figure 3.** Expression of mRNA for VEGF, VEGFR1 and CXCL12, CXCR4 was elevated (*$P < 0.05$) on d 25 and 30 of gestation compared with d 20 in fetal membrane samples. Elevated mRNA expression of VEGFR2 occurred on d 25 compared with d 20 and 30 (*$P < 0.05$). ANG1 was elevated (*$P < 0.05$) on d 30 compared with d 20 of pregnancy.

## DISCUSSION

Early gestation is characterized by a number of crucial events including maternal recognition of pregnancy, implantation, and placentation with the latter playing a paramount role in exchange of nutrients between the maternal and fetal interface. Proper placental development is characterized by extensive angiogenesis, which must occur early for subsequent development and growth of the fetus throughout gestation (Reynolds and Redmer, 2001). Interestingly, CXCL12 and CXCR4 gene knockout (**KO**) mice have severe vascular abnormalities not observed in any other chemokine or chemokine receptor KO mice, suggestive of a unique role for this chemokine signaling system in vascularization (Tachibana et al., 1998). Also, angiogenic factors such as VEGF, FGF2, and ANG1 are critical growth inducers, with VEGF being the most potent inducer of vascularization (Grazul-Bilska et al., 2010). The importance of VEGF inducing angiogenesis is evident in several gene KO mice studies. In VEGF KO mice, embryonic lethality occurs during early gestation, with prominent cardiovascular defects and placenta abnormalities. Furthermore, KO of the VEGF receptors results in poor placental vascularization and embryonic lethality (Carmeliet et al., 1996; Ferrara et al., 1996). Angiogenic factors FGF2 and ANG1 also play important roles during early pregnancy. Expression of FGF2 and its receptors are present within the uterus and placenta of several mammalian species including cows, pigs, and humans (Ornitz et al., 1996; Edwards et al., 2011). In mice, FGF2 stimulates trophoblast cell migration leading to proper implantation of the blastocyst, and in human trophoblast cells, FGF2 promotes angiogenesis (Hamai et al., 1998; Taniguchi et al., 1998). The ANG1 protein primarily plays a role in vascular stabilization and has 2 specific receptors Tie1 and Tie2. When ANG1 binds to Tie2, it elicits microvascular organization and endothelial survival. Also, Tie2 or ANG1 deficiency in mice leads to embryonic lethality causing an increase in ANG2, which is known to destabilize blood vessels (Suri et al., 1996; Holash et al., 1999; Kwak et al., 1999; Papapetropoulos et al., 1999). Due to CXCL12/CXCR4 inducing vascularization, and angiogenic factors playing an important role in the regulation of angiogenesis, these signaling mechanisms could potentially be working together to regulate growth and vascularization of the placenta. This association is plausible because CXCL12 increases VEGF synthesis and secretion, which further increases expression of CXCL12 and CXCR4 (Rosenkilde and Schwartz, 2004). Because VEGF is a potent inducer of vascularization it is important to understand upstream signaling events that regulate VEGF expression. In caruncle tissue, we saw an increase in CXCL12/CXCR4 in pregnant compared with non-pregnant ewes; however there was no changes in angiogenic factor expression. As this chemokine system is associated with stimulating expression of angiogenic factors, the increase we observed in pregnant ewes could mean it is establishing and regulating expression of these angiogenic factors during early pregnancy in sheep leading to vascularization of the placenta and fetal survival. The increase of CXCL12 and FGF2 on d 20 of pregnancy in caruncle tissues also represents the prominence of vascular development during early gestation. This development may be regulated by a unique communication between CXCL12 and FGF2. In human endothelial cells, FGF2 drives expression of CXCL12 and CXCR4 and activation of CXCR4 further induces FGF2 (Salcedo, et al., 1999). This similar expression pattern is seen within caruncle tissue on d 20 during early vascular development.

Changes in mRNA expression of CXCL12/CXCR4 and select angiogenic factors, in fetal membrane tissue further emphasize the potential stimulation pattern between these signaling mechanisms. Placental vascularization occurs very early in gestation (Igwebuike, 2009; Grazul-Bilska et al., 2010). We observed an increase of VEGF, its 2 receptors and CXCL12, and CXCR4 on d 25 and/or d 30 of pregnancy compared with d 20. This increase of these angiogenic factors further confirms that these signaling pathways are important for placental vascularization and growth of the fetus during early gestation. An intriguing increased expression of ANG1 occurred later on in gestation, demonstrating that this angiogenic factor may play a role in vascular stabilization within the placenta. Due to increases of these signaling mechanisms in early gestation within fetal membrane tissue, the fetus likely regulates its own vascularization in an autocrine/paracrine fashion. Historically, many studies have focused specifically on regulation of angiogenesis within the maternal side. However, based on these results, further study

should be directed toward signaling mechanisms occurring within the fetus during early gestation. From this, a better understanding of early gestation within ruminants can be gained, thus reducing early fetal growth restriction and embryonic loss.

## IMPLICATIONS

Proper development of the placenta is key to a successful pregnancy, and if it is compromised, intrauterine growth restrictions can occur leading to poor pregnancy outcomes including embryonic mortality. Angiogenic factors including the chemokine CXCL12 are regulated during early pregnancy, and may play a role in angiogenesis, a crucial factor for placental formation (Reynolds et al., 1992; Reynolds and Redmer, 1995, 2001; Ashley et al., 2011). The symbiotic expression of CXCL12, CXCR4 and VEGF, VEGFR1 and VEGFR2 in the fetal membrane could mean the fetus regulates its own vascular growth, thus leading to its survival. Based on these data, it is important to not only look at the regulation patterns on the maternal side, but also from the fetal side as well. More research should be initiated to study the relationship of CXCL12, CXCR4 and angiogenic factors during early gestation with an emphasis on the fetal role in placental development.

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# EFFECT OF SWATH GRAZING ON FORAGE INTAKE AND WASTAGE BY EWES

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**ABSTRACT**: Sixty non-pregnant, non-lactating mature white faced ewes (Targhee 65.4 ± 5.84 kg BW in 2010 and Rambouillet 61.9 ± 6.28 kg BW in 2011) were used in a 2 year study to evaluate wastage, intake, and nutrient composition of a pea/barley forage fed either as baled hay in confinement (CONFINEMENT) or swathed and left to graze (GRAZE). Forage DMI was estimated using IVDMD and chromic oxide as a marker for estimating fecal output. Forage wastage was estimated by sampling and weighing the initial swath, standing, and baled forage, weighing the forage again after a 7-d collection period, and subtracting the estimated forage DMI. Samples of baled, swathed, and standing forage were collected in August and October and analyzed for CP and in situ dry matter disappearance (ISDMD). There was no treatment by year interaction ($P = 0.56$) for BW change and no difference ($P = 0.33$) between treatments. There was a treatment by year interaction ($P = 0.04$) for DMI. In 2010, DMI was greater ($P = 0.06$) by CONFINEMENT ewes compared with GRAZE ewes (2.4 vs 1.7 kg•ewe$^{-1}$•d$^{-1}$); however, in 2011, DMI did not differ ($P = 0.25$) between treatments. There was no treatment by year interaction ($P > 0.22$) for forage wastage either as a percent of beginning available forage or as kilograms of wastage. Although percentage wastage did not differ between treatments ($P = 0.23$), kilograms of wastage was greater ($P = 0.03$) for GRAZE than CONFINEMENT. There was no difference ($P = 0.24$) for beginning CP among the forage treatments, but there was a treatment difference ($P \leq 0.01$) for ending CP (standing least amount of CP compared with swath and bale) and for CP change in swath, standing, and bale (1.65, -1.71, -0.15%, respectively) in 2010. There was a difference ($P < 0.08$) for beginning CP and ending CP between treatments with bale greater than swath, but no difference ($P = 0.52$) for CP change in 2011. There was no difference ($P > 0.32$) for beginning ISDMD, ending ISDMD, and ISDMD change among the treatments in 2010. There was no difference ($P = 0.28$) for beginning ISDMD between the treatments, but there was a difference ($P < 0.01$) for ending ISDMD and ISDMD change in 2011 as ISDMD decreased in swaths and increased in bales. This research provides a sound biological basis for an economic assessment of using swath grazing in commercial sheep operations.

**Key words:** confinement, ewes, grazing, intake, swath, wastage

## INTRODUCTION

Swath grazing is a feeding strategy used as an alternative to feeding baled forage. Swath grazing reduces costs associated with harvesting forages (Surber et al., 2001); however, animal performance with swath grazing has been variable (Volesky et al., 2002; Karn et al., 2005; Nayigiugu et al., 2007), forage wastage greater (Volesky et al., 2002), and forage quality lower compared with feeding baled forage (Munson et al., 1999; Volesky et al., 2002; Nayigihugu et al., 2007). Swath grazing has been compared with confined feeding in four peer-reviewed articles; however, all of these articles used cattle. The objective of this study was to evaluate ewe BW change, forage wastage, DMI, and nutrient composition of a pea/barley forage either swath grazed or baled and fed in confinement.

## MATERIALS AND METHODS

Activities involving live animals were approved by the Agricultural Animal Care and Use Committee at Montana State University (2009-AA04).

***Ewe Management and Treatments.*** This 2-yr study used sixty non-pregnant, non-lactating mature, white-faced ewes (Targhee 65.4 ± 5.84 kg BW in 2010 and Rambouillet 61.9 ± 6.28 kg BW in 2011). Research was conducted at Montana State University's Fort Ellis Experiment Station near Bozeman.

Sheep grazed pea/barley forage swaths in paddocks (GRAZE) or consumed mechanically harvested and baled pea/barley forage in confinement (CONFINEMENT). The pea/barley forage used in the grazing and feeding treatments was grown on six different plots (3 plots for grazing and 3 plots in which forage was harvested for CONFINEMENT) each year. All sheep had ad libitum access to forage, water, and a salt/mineral supplement. In the GRAZE treatment, three paddocks that measured 91 m in length and 15 m in width were divided into two equal sections measuring 697 m$^2$ each. One section was used for the adaptation period and the second section was used for the data collection period. In the CONFINEMENT treatment, there were three pens measuring 465 m$^2$ each. Each pen contained a combination hay rack with grain trough sheep feeder measuring 3.0 m x 0.6 m x 1 m. Ewes were allowed a 7 d adaptation period to become accustomed to their surroundings and treatments (October 2 to October 8, 2010 and September 6 to September

12, 2011). Following the adaptation period there was a 7 d data collection period. Ewes were weighed on September 25 and October 23, 2010 and September 6 and on September 20, 2011. Ewes were weighed 16 h after being removed from food and water.

***Forage Wastage.*** Forage availability was estimated in order to calculate wastage. Forage was weighed before and after the data collection period. In the GRAZE treatment, three 1-m section of the swath were removed, weighed, and replaced using methods similar to those described by Volesky et al. (2002). The three recorded weights were averaged and multiplied by the swath length to determine total swath weight. Regrowth or standing forage remaining in the paddock was also sampled by clipping and weighing all forage within ten 0.1-$m^2$ ring samples randomly collected throughout the paddock. This 1.0-$m^2$ weight of forage was multiplied by the area of the paddock to determine total kilograms of forage. In the CONFINEMENT treatment, all bales were weighed prior to feeding. Sheep were fed daily an amount of hay sufficient to ensure a level of refusal indicative of ad libitum consumption (not less than 10%). The refused forage left in the feeder was weighed at the end of the data collection period. Samples were collected from the swath, standing forage, bales, and forage remaining in the feeders for analysis of DM in order to calculate forage availability and wastage on a DM basis.

Wastage (DM basis) was calculated as follows:

$$W = B - E - I$$

where W is wastage, B is the forage available at the beginning of the data collection period, E is the edible forage still available to the ewes at the end of the data collection period, and I is intake for the 7-d data collection period.

***Intake.*** Individual forage DMI by ewes was estimated using the following equation:

$$I = F/1\text{-}D$$

where I is forage intake, F is fecal output, and D is forage digestibility. The equation for fecal output is as follows:

$$F = c/f$$

where F is fecal output, c is g of chromium dosed to each ewe per day, and f is the concentration of chromium in the feces expressed in g of chromium per g of feces. Fecal output was determined by dosing the ewes with 2 g of chromic oxide in gelatin capsules using a plastic balling gun and fecal grab samples were collected once per d at 1000 h similar to Hatfield et al. (1991). Ewes were dosed for 7 d to establish equilibrium and then dosed and fecal sampled for an additional 7 d. Fecal samples were dried at 60ºC, composited by sheep, and analyzed for chromium concentration by atomic absorption spectrometry (Williams et al., 1962). Forage indigestibility was estimated using the in vitro technique from a modified Tilley and Terry (1963) method.

***Nutrient Composition.*** In 2010, samples of swath, standing forage, and bales were collected after baling on August 11, and from the bales and fenced-off section of swath on October 7. In 2011, samples of swath and bales were collected after baling on August 22, and from the bales and fenced-off section of swath on October 27.

All forage samples were dried at 60ºC and ground in a Wiley mill through a 1-mm screen. Two 1-g samples of the 1 mm forage were weighed, dried at 100ºC for 24 h, and reweighed to determine DM content. All forage samples were analyzed for N (AOAC, 2000) using a LECO machine (LECO Corp., St. Joseph, MI). Dry matter disappearance was determined using in situ techniques as described by Bowman and Firkins (1993).

***Statistical Analysis.*** The study was a completely randomized design with pen or paddock as the experimental unit. Each treatment had 3 replications with 10 ewes/pen or paddock. Data for ewe BW, wastage, DMI, and nutrient composition were analyzed using PROC GLM (SAS Inst. Inc., Cary, NC). The statistical model included the effects of year, treatment, and year x treatment for ewe BW, wastage, and DMI, and effects of treatment for nutrient composition. Means were separated using the LSD procedure when a significant F value was found ($P \leq 0.10$).

## RESULTS AND DISCUSSION

***BW Change.*** There was no treatment by year interaction ($P = 0.56$) for BW change and no difference ($P = 0.33$) between treatments (Table 1). Ewe BW change was greater ($P < 0.01$) for ewes in 2010 (6.08 kg) than 2011 (1.6 kg). These results are similar to Volesky et al. (2002) who reported no difference in BW change of calves in the second year of a 2-yr study comparing swath grazing to baled forage. In contrast to these and our results, calves gained 10 kg more (Volesky et al., 2002) and cows had greater ADG (Nayigihugu et al., 2007) on swath treatments compared with baled treatments in one year of these two year studies. Both authors attributed this increased gain to access of the animals to high quality regrowth in the swathed treatment. Differences between their results and ours could be due to differences in animal species used in the respective studies or because both these studies lasted over a month, whereas ewes in our study grazed or were fed ≤ 4 wk. Also in contrast to our results, Karn et al. (2005) and Nayigihugu et al. (2007) reported that cows had less ADG on the swath treatment compared with the baled or feedlot treatment; however, these studies were conducted in the winter and ADG decreased when the swaths were covered with ice and temperatures were below normal.

***Wastage.*** There was no treatment by year interaction ($P > 0.23$) for wastage expressed as either kilograms or as a percent of beginning available forage (Table 2). Kilograms of wastage was greater ($P = 0.03$) in GRAZE compared with CONFINEMENT (78.8 vs. 7.0 kg) and wastage was greater ($P = 0.007$) in 2010 compared with 2011 (87.1 vs. 24.2 kg). However, when wastage was calculated as a percent of beginning available forage, there was no difference between treatments ($P = 0.23$) or year ($P = 0.15$). Voleskey et al. (2002) reported that calves consuming baled forages wasted less forage than calves grazing windrows (12.5 vs. 29%). These authors limited access of the animals to swaths in an attempt to reduce wastage and calves fed bales had access

**Table 1**. Least squares means, SE, and *P*-values for beginning BW, ending BW, BW change, total DMI, DMI (kg•ewe$^{-1}$•d$^{-1}$), and DMI (% BW) for ewes with ad libitum access to pea/barley forage either fed as a baled hay in confinement or swathed and left to graze in 0.2 ha paddocks

| | 2010 | | 2011 | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Confinement | Graze | Confinement | Graze | SE | Treatment | Year | Treatment x Year |
| Beginning BW, kg[1] | 64.73 | 66.29 | 60.76 | 63.14 | 1.16 | 0.13 | 0.02 | 0.73 |
| Ending BW, kg[2] | 71.30 | 71.88 | 62.48 | 64.60 | 1.02 | 0.23 | <0.01 | 0.47 |
| BW change, kg | 6.57 | 5.58 | 1.73 | 1.47 | 0.59 | 0.33 | <0.01 | 0.56 |
| DMI, kg·ewe$^{-1}$·d$^{-1}$ | 2.38$^{b}$ | 1.68$^{a}$ | 1.54$^{a}$ | 1.93$^{a,b}$ | 0.22 | 0.52 | 0.23 | 0.04 |
| DMI, % | 3.54$^{b}$ | 2.45$^{a}$ | 2.52$^{a}$ | 3.05$^{a,b}$ | 0.36 | 0.46 | 0.59 | 0.06 |

$^{a,b}$Within a row, means without a common superscript differ ($P < 0.10$)

[1]Beginning BW was taken when ewes arrived at study site after a 16 hr shrink September 25, 2010 and September 6, 2011

[2]Ending BW was taken 16 hr after sheep were removed from food and water on October 23, 2010 and September 20, 2011

**Table 2**. Least squares means, SE, and *P*-values of beginning forage availability, ending forage availability, wastage, and wastage as a percentage of beginning forage available for ewes with ad libitum access to pea/barley forage either fed as a baled hay in confinement or swathed and left to graze in 0.2 ha paddocks

| | 2010 | | 2011 | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Confinement | Graze | Confinement | Graze | SE | Treatment | Year | Treatment x Year |
| Beginning forage, kg[1] | 246.30 | 378.15 | 122.99 | 185.63 | 19.84 | <0.01 | <0.01 | 0.12 |
| Ending forage, kg[2] | 27.44$^{b}$ | 138.95$^{c}$ | 2.46$^{a}$ | 14.53$^{a,b}$ | 9.80 | <0.01 | <0.01 | <0.01 |
| Wastage, kg[3] | 52.50 | 121.64 | 12.43 | 35.91 | 17.41 | 0.03 | 0.01 | 0.23 |
| Wastage, %[4] | 19.71 | 31.96 | 10.22 | 17.44 | 7.51 | 0.23 | 0.15 | 0.75 |

$^{a-c}$Within a row, means without a common superscript differ ($P < 0.10$)

[1]DM forage available to ewes at beginning of study period October 2, 2010 and September 6, 2011

[2]DM forage available to ewes at end of study period October 8, 2010 and September 12, 2011

[3]Wastage = beginning forage availability – ending forage availability – total DMI

[4]Percent wastage = (wastage / beginning forage availability) * 100

**Table 3**. Least squares means, SE, and *P*-values of beginning CP, ending CP, CP change, beginning in situ dry matter disappearance (ISDMD), ending ISDMD, and ISDMD change for swath, standing forage (stubble and regrowth), and baled pea/barley forage

| | 2010 | | | | *P*-value | 2011 | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| Item | Swath | Standing | Bale | SE | Treatment | Swath | Bale | SE | Treatment |
| Beginning CP, %[1] | 10.80 | 10.00 | 11.83 | 0.68 | 0.24 | 5.30$^{a}$ | 6.93$^{b}$ | 0.47 | 0.07 |
| Ending CP, %[2] | 12.43$^{b}$ | 8.3$^{a}$ | 11.67$^{b}$ | 0.58 | 0.01 | 5.30$^{a}$ | 6.57$^{b}$ | 0.32 | 0.05 |
| CP change, % | 1.65$^{c}$ | -1.71$^{a}$ | -0.15$^{b}$ | 0.28 | < 0.01 | < 0.01 | -0.35 | 0.36 | 0.52 |
| Beginning ISDMD, %[1] | 72.70 | 62.97 | 62.00 | 5.15 | 0.33 | 36.80 | 40.10 | 1.85 | 0.28 |
| Ending ISDMD, %[2] | 69.00 | 65.17 | 67.40 | 2.40 | 0.56 | 29.77$^{a}$ | 43.73$^{b}$ | 1.62 | < 0.01 |
| ISDMD change, % | -3.70 | 2.17 | 5.40 | 5.24 | 0.50 | -7.03$^{a}$ | 3.63$^{b}$ | 2.48 | 0.04 |

$^{a-c}$Within year and row, means without a common superscript differ ($P < 0.10$)

[1]Sampled August 11, 2010 and August 22, 2011

[2]Sampled October 7, 2010 and October 27, 2011

to hay in a circular ring-type feeder. In contrast, Munson et al. (1999) concluded that wastage was greater in the baled treatment compared with the swath treatment based on the amount fed, but wastage was estimated visually and not directly measured.

***Intake.*** There was a treatment by year interaction ($P \leq 0.06$) for DMI (kg•ewe$^{-1}$•d$^{-1}$ and % BW; Table 1). In 2010, both measures of DMI were greater ($P = 0.06$) by CONFINEMENT ewes compared with GRAZE ewes (2.4 vs. 1.7 kg•ewe$^{-1}$•d$^{-1}$); however, in 2011, DMI did not differ ($P = 0.25$) between treatments. Volesky et al. (2002) estimated intake using chromium as an external marker and reported no differences in intake by cattle grazing swaths or fed bales in one year and greater intake by cattle grazing swaths the second year.

***Nutrient Composition.*** There was no difference ($P = 0.24$) for beginning CP among the forage treatments, but there was a treatment difference ($P \leq 0.01$) for ending CP and CP change in 2010 (Table 3). The CP in the swathed forage increased (1.65%), remained relatively constant (-0.15%) in the baled forage, and decreased (-1.71%) in the standing forage. There was a difference ($P < 0.08$) for beginning CP and ending CP between treatments with baled forage being greater than swathed forage (6.93% vs. 5.30% and 6.57% vs. 5.30%, respectively) in 2011. There was no difference ($P = 0.52$) for CP change. Volesky et al. (2002) reported that CP did not differ between swath and bales over time. Likewise, Nayigihugu et al. (2007) reported that CP did not differ over time in both years; in the second year CP was greater for swaths compared with baled forage, but bales were stored uncovered and outside. In contrast, Munson et al. (1999) reported CP decreased over time in both the swath and bales. They also stored bales uncovered and outside

There was no difference ($P > 0.32$) for beginning ISDMD, ending ISDMD, and ISDMD change among the treatments in 2010 (Table 3). There was no difference ($P = 0.28$) for beginning ISDMD between the treatments, but there was a difference ($P < 0.01$) for ending ISDMD in swath and bale (29.77 vs. 43.73%, respectively) and ISDMD change in 2011. The ISDMD decreased (-7.03%) in the swathed forage and increased (3.63%) in the baled forage. Nayigihugu et al. (2007) reported that for the first year IVDMD did not differ over time or between the swath and baled treatments, but in the second year it declined over time in both treatments.

## IMPLICATIONS

This research provides a sound biological basis for an economic assessment of using swath grazing in commercial sheep operations.

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# MATERNAL DIET RESTRICTION IN BEEF COWS ALTERS FETAL CARDIOVASCULAR HEMODYNAMICS AND FETAL AND PLACENTAL DEVELOPMENT DURING EARLY PREGNANCY

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**ABSTRACT:** Objectives were to examine the effects of maternal nutrient restriction on fetal cardiovascular hemodynamics, and fetal and placental development during early gestation. On d 30 of pregnancy, multiparous beef cows were randomly assigned to dietary treatments of 100% (CON; n = 6) or 60% of NRC recommendations (RES; n = 6). Cows were individually-fed once daily using Calan gates at 1000 h. On d 85 of gestation, fetal blood flow was determined via Doppler ultrasonography followed by cow slaughter. Ultrasound measurements included fetal heart rate (HR), umbilical blood flow (uBF), pulsatility index (PI), and resistance index (RI). Fetal PI and RI were not affected ($P \geq 0.19$) by maternal restriction, while fetal HR was decreased ($P = 0.006$) in RES vs. CON fetuses. Umbilical BF, uBF relative to placental weight, and uBF relative to cotyledon weight were not affected by maternal nutrient restriction, while uBF relative to fetal weight was decreased ($P = 0.03$) in RES vs. CON. Fetal weight tended to be greater ($P = 0.07$) in RES vs. CON fetuses. Total placentome weight was greater ($P = 0.002$) in RES vs. CON cows; however, cotyledon weight, caruncle weight, fetal membranes, and chorioallantoic/amniotic fluid volume were not different ($P > 0.27$) between treatments. Placental efficiency (fetal weight: placentome weight ratio) tended to be greater ($P = 0.10$) in CON vs. RES cows. Heart girth and ponderal index were increased ($P = 0.004$) in RES vs. CON fetuses, whereas biparietal distance and crown rump length were not different ($P \geq 0.28$) between treatments. Maternal nutrient restriction during early gestation increased fetal growth and placentome weight, whereas uBF was decreased relative to fetal weight. Therefore, it would appear that maternal nutrient restriction leads to compensatory fetal and placental development during early gestation.

**Keywords**: maternal restriction, pregnancy, umbilical blood flow

## INTRODUCTION

Beef cows are commonly managed in grazing systems where quality of forage varies according to the regional conditions. Often poor quality forage can affect nutritional and physiological status of the dam, as well as the calf (Wu et al., 2006). Intrauterine growth restriction is associated with altered fetal organ development and subsequent performance of offspring (Godfrey and Barker, 2000; Wu et al., 2006). As calf growth post-natal is largely dependent upon fetal growth and development in utero, it is important to determine how management decisions could impact the growth trajectory of the bovine fetus. Even during the time of early embryonic development, when nutrient requirements appear trivial for conceptus growth, maternal nutrition can alter organogenesis and establishment of the placenta, which are imperative for proper prenatal growth and development (Robinson et al., 1999).

The placenta plays a major role in the regulation of fetal growth. Placental nutrient transport efficiency is directly related to utero-placenta blood flow (Reynolds and Redmer, 1995). Gases, nutrients, and metabolic end products are exchanged between maternal and fetal circulation via the placenta (Reynolds and Redmer, 1995, 2001; Bleul et al., 2007). Increases in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on growth of the placenta during early pregnancy followed by dramatic development and reorganization of the uteroplacental vasculature during the last half of gestation (Meschia, 1983; Reynolds and Redmer, 1995). If an insult during early gestation impairs placental development, calf development during later pregnancy could be altered. Therefore, we hypothesized that nutrient restriction during early gestation in beef cows would impair conceptus development. The specific objective was to examine the effect of maternal nutrient restriction during early gestation on umbilical hemodynamics and conceptus development.

## MATERIALS AND METHODS

All procedures involving animals were approved by the North Dakota State University (**NDSU**) Animal Care and Use Committee.

***Animals and Management.*** Twelve gestating, non-lactating, multiparous beef cows (initial BW = 623.1 ± 16.6 kg) of similar genetic background were transported from the NDSU Beef Research and Teaching Unit to the Animal Nutrition and Physiology Center (**ANPC**) located on NDSU campus within 3 d post artificial insemination. Prior to initiation of the experiment, cows were trained to use the Calan gate system. Cows were grouped with 3 to 4 head per pen. All cows were fed chopped grass hay [8.02% CP and 57.93%

TDN (DM basis)] a mineral and vitamin supplement from their arrival at ANPC until d 30 of pregnancy (100% of NRC recommendation for NE for maintenance and fetal growth and to meet or exceed the recommendations for MP, minerals, and vitamins; NRC, 2000). Pregnancy was confirmed on d 25 or 26 post-insemination via transrectal ultrasonography. On d 30 of pregnancy, cows were assigned randomly to receive the same grass hay and mineral and vitamin supplement at either 100% NRC recommendations for NE for maintenance and fetal growth and to meet or exceed the recommendations of MP, minerals, and vitamins; (CON; n = 6); or 60% of the NE recommendations (RES; n = 6) from d 30 to 85 of gestation.

Cows were individually fed once daily in a Calan gate system at 1000 h. The mineral and vitamin supplement was top-dressed 3 times a week at a rate of 0.18 % of hay DMI to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC 2000). Cows were weighed weekly throughout the experiment and dietary intake adjusted relative to BW.

***Ultrasonography Evaluation.*** Umbilical hemodynamics were obtained via color-Doppler ultrasonography (Aloka SSD-3500; Aloka America, Wallingford, CT, USA) at 0700 h on the day prior to slaughter (d 84 of gestation). Measurements included fetal heart rate (**HR**), umbilical blood flow (**uBF**), pulsatility index (**PI**), and resistance index (**RI**). Briefly, a transrectal-finger probe (~5 x 2 cm; Aloka UST-672; 5.0 MHz) was inserted into the rectum and the umbilical cord was located. In B-mode using the linear transducer, a longitudinal section of the umbilical cord was visualized by manually turning the transducer of the probe. The probe was aligned with the umbilical cord at an average angle of insonation of 58 ± 3 degrees. Once an adequate portion of umbilical cord was identified, the sample gate cursor was placed over the umbilical artery whereas at the same time pulsatile waves in D mode (Doppler spectrum) were recorded. Three similar waveforms from 3 separate ultrasonography measurements (average time of ultrasonography examination was 12.0 ± 0.7 min) were obtained and averaged for every ultrasonography examination (a total of 9 measurements per animal). Fetal HR, umbilical PI and RI, and uBF, were calculated by pre-programmed Doppler software where PI = [peak systolic velocity (**PSV**) - end diastolic velocity (**EDV**)] / mean velocity (**MnV**); RI = (PSV-EDV)/PSV; BF (mL/min) = MnV (cm/s) x ($\pi$/4) x diameter$^2$ (cm$^2$) x 60 s.

***Slaughters.*** On d 85 of pregnancy, cows where transported from ANPC to the NDSU Meat Laboratory. Briefly, cows were stunned with a captive-bolt gun and exsanguinated. The gravid uterus was immediately collected and weighed. The fetus was immediately removed from the placenta at the umbilicus and weighed. Biparietal distance, heart girth, and crown rump length were measured for each fetus. Fetal ponderal index was calculated using the following equation: ponderal index = fetal weight (kg)/crown rump length (m$^3$). Chorioallantoic and amniotic fluids were combined and volume was recorded. After each individual placentome was weighed, placentome dimensions [length **(l)**, width **(w)**, depth **(d)**] were measured using digital calipers and were separated manually into cotyledonary and caruncular portions. The mass of total cotyledonary tissue and total caruncular tissue was recorded. Average placentome density (g/cm$^3$) was calculated as placentome weight divided by placentome volume (l*w*d). Placental efficiency was calculated as fetal weight divided by total placentome weight. After all placentomes and fetal membranes were removed, the uterus was reweighed to obtain an empty uterine weight.

***Statistical Analysis.*** Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Class statement included treatment and fetal sex. Model statement included treatment and fetal sex. When a significant treatment effect was detected ($P \leq 0.05$), treatment differences were separated using the PDIFF option of the LSMEANS statement.

## RESULTS

Umbilical PI and RI were not affected ($P \geq 0.25$) by maternal nutrient restriction. Fetal HR was decreased ($P$ = 0.006) in RES compared with CON fetuses (185 vs. 173 ± 2 bpm, respectively). Absolute uBF (46.3 ± 3.4 mL/min), uBF relative to total placentome weight (170 ± 10 mL·min$^{-1}$·kg$^{-1}$), and uBF relative to total cotyledon weight (1.7 ± 0.3 mL·min$^{-1}$·g$^{-1}$) was not affected ($P \geq 0.13$) by maternal nutrient restriction. However, uBF relative to fetal weight was decreased ($P$ = 0.03) in RES compared with CON cows (300 vs. 440 ± 40 mL·min$^{-1}$·kg$^{-1}$, respectively). Fetal weight tended to be greater ($P$ = 0.07) in RES compared with CON, where fetuses from RES had a 14% weight increase compared with fetuses from CON cows (140 vs. 120 ± 10 g). Heart girth and ponderal index were greater ($P$ = 0.004) in fetuses from RES vs. CON cows (10.8 vs. 10.3 ± 0.2 cm; 27.3 vs. 21.1 ± 1.2 kg/m$^3$), whereas fetal biparietal distance (2.5 ± 0.09 cm) and crown rump length (17.0 ± 0.01 cm) were not different ($P \geq 0.28$) between treatments. Gravid (2.6 ± 0.2 kg) and empty uterine weight (1.1 ± 0.1 kg) were not different ($P > 0.20$) between treatments. There were more ($P$ = 0.02) placentomes in RES compared with CON cows (68.4 and 45.2 ± 6.2 placentomes, respectively), and therefore the total mass of the placentomes was greater ($P$ = 0.002) in RES vs. CON cows (118.8 vs. 84.8 ± 5.9 g, respectively). Average placentome weight was not affected ($P$ = 0.51) by maternal treatment. Moreover, average placentome volume (1.3 ± 0.2 cm$^3$) and density (1.9 ± 0.2 g/cm$^3$) were not affected ($P \geq 0.65$) by maternal nutrient restriction. In addition, total cotyledon weight (41.3 ± 6.2 g), total caruncle weight (48.5 ± 6.6 g), fetal membrane weight (142 ± 29 g), and chorioallantoic and amniotic fluid volume (865 ± 83 mL) were not different ($P >$ 0.27) between treatments. Placental efficiency (fetal weight: placentome weight ratio) tended to be greater ($P$ = 0.10) in CON vs. RES cows (1.4 vs. 1.2 ± 0.1, respectively).

## DISCUSSION

We reject our initial hypothesis that maternal nutrient restriction during early gestation in beef cows would impair

fetal hemodynamics and conceptus development. It appears that fetal growth was spared due to increased placental growth in restricted cows. Previous research in beef cows, with a nutrient restriction to 50% of the requirements from d 30 to d 125 of gestation reduced caruncular and cotyledonary weight compared with control cows at the end of the restriction. However, when cows were realimented caruncular weight was similar between restricted and control cows at d 250 (Zhu et al., 2006). Vonnahme et al. (2007) demonstrated that restriction from d 30 to 125 did not affect placenta vascularity. Conversely, upon realimentation, placental vascularity was altered near term, indicating that the placenta compensated after restriction. In this study, cows were slaughtered at d 85 of gestation following 55 d of maternal nutrient restriction (from d 30 to d 85 gestation). We observed differences in placentome weight and number where RES cows had greater mass and greater number of placentomes than CON cows by d 85 of gestation. Therefore, 55 d of maternal nutrient restriction during early gestation appears to lead to increased placental growth and development in RES compared with CON cows. Vonnahme et al. (2007) observed dramatic differences in capillary vascularity after the realimentation period (from d 125 to 250), suggesting an alteration of placental development and function by early nutrient restriction. In our study, Reyaz et al. (2012) showed that cotyledonary arteries from RES cows were more sensitive to bradykinin-induced relaxation compared with cotyledonary arteries from CON cows. Reynolds et al. (2006) summarized several studies that used sheep as a model of compromised pregnancies during late gestation (i.e. overfed and underfed dams, heat and hypoxia stress, multiple pregnancies) where most of the studies showed a decrease in uBF and a decrease in fetal and placental weight. More specifically, nutrient restriction in sheep during early gestation (from d 28 to 78) resulted in intrauterine growth restriction in RES vs. CON fed ewes (Vonnahme et al., 2003). In human fetuses, abnormal umbilical artery blood flow during late gestation indicates intrauterine growth restriction, suggesting high risk for perinatal death (Kingdom et al., 1997). Currently, a paucity of research exists on umbilical hemodynamics in cattle; however, if we are able to determine abnormal umbilical wave forms during early gestation in cows we might be able to elucidate strategies to improve neonatal health. In this study we observed that d 85 fetuses from RES cows tended to be heavier compared with fetuses from CON cows suggesting an increased nutrient extraction from the nutrient restricted dams. As uBF was similar between treatments we hypothesize that nutrient uptake by the placenta in nutrient restricted animals must be enhanced. Studies are ongoing to determine how realimentation would impact fetal growth and placental function.

## IMPLICATIONS

Pregnancy success and offspring health are dependent on nutrient intake and conceptus growth and development. Optimization of growth and development after birth is important for the profitability of the livestock industry. In our study, nutrient restriction during early gestation appears to alter placental function. More research is necessary to further understand the early changes in placental and fetal development and how these changes would affect later fetal development and neonatal health.

## ACKNOWLEDGMENTS

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# EFFECTS OF NATURAL SERVICE AND ARTIFICIAL INSEMINATION BREEDING SYSTEMS ON PREGNANCY RATES AND DAYS TO CONCEPTION

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**ABSTRACT:** Four hundred eighty crossbred beef cows and 86 crossbred beef heifers were used to compare the effects of two breeding systems on pregnancy rates and days to conception. Cattle were stratified by age and BCS, and assigned randomly to one of two treatments: 1) Females exposed to natural service bulls for the duration of the breeding season (NS; n = 284), or 2) females exposed to estrous synchronization and a fixed-time AI (d 0; 7-d Co-Synch + CIDR), followed by exposure to natural service bulls for the duration of the breeding season (TAI, n = 282). Bulls were introduced on d 1 and both treatments were managed as a cohort in the same pastures. Blood samples were collected on d -20 and -10 to determine cyclic status. On d 49 and again at least 40 d after bull removal from pastures, transrectal ultrasonography was used to determine pregnancy status and fetal age. Overall, 42.8% of cattle were cyclic at the beginning of the breeding season. Treatment × cyclic status interactions ($P < 0.01$) were present for the proportion of cows detected pregnant on d 49, the proportion of cows pregnant at the end of the breeding season, and days from the beginning of the breeding season to conception. A greater proportion ($P < 0.05$) of cyclic cattle in the TAI treatment (88%, 104 of 118) had a viable fetus detected on d 49 of the breeding season compared with cyclic cattle in the NS treatment (74%, 88 of 119) and non-cyclic cattle in the TAI (75%, 122 of 163) and NS treatments (77%, 120 of 156). A greater percentage ($P < 0.05$) of cyclic cattle in the TAI treatment (94%, 111 of 118) were pregnant at the end of the breeding season compared with non-cyclic cattle in the TAI treatment (84%, 136 of 162) whereas cyclic (88%, 105 of 119) and non-cyclic (89%, 140 of 157) cattle in the NS treatment were intermediate. Both cyclic (11.6 ± 1.4 d) and non-cyclic (14.5 ± 1.4 d) cattle in the TAI treatment became pregnant earlier in the breeding season ($P < 0.05$) compared with cyclic (19.9 ± 1.4 d) and non-cyclic (17.9 ± 1.4 d) cattle in the NS treatment. Breeding systems for beef cattle that incorporated TAI altered pregnancy rates and days to conception compared with natural service breeding systems.

**Key words:** artificial insemination, estrous synchronization, natural service

## INTRODUCTION

The area of production most critical in terms of profit potential in beef cow-calf operations is the ability of a cow to give birth and raise a healthy calf until weaning (Dickerson, 1970). Reproductive performance is variable among herds (Larson et al., 2006; Dahlen et al., 2010) and estimates indicate the beef industry loses $2.8 billion in revenue as a result of infertility (Lamb et al., 2011). Incorporating estrous synchronization (**ES**) and AI into beef operations may result in improved reproductive performance, weaning weight, carcass quality, and genetic value, along with reduced calving difficulty (Sprott, 2000). Modern ES protocols allow cows an increased opportunity to become pregnant on the first day of the breeding season. The implementation of fixed-time AI protocols has resulted in similar pregnancy rates to protocols that require heat detection (Lemaster et al., 2001).

Experiments have used clean-up bulls after the use of ES and AI (Stevenson et al., 1997; Geary et al., 2001), but lack the use of a traditional breeding system as a control. Natural service with no ES protocol needs to be used as a control to determine the overall effect of an ES and AI breeding system. One example, Sa Filho et al. (2009) reported significantly greater pregnancy rates when AI and ES were used compared with natural service in *Bos indicus* cattle.

The objective of this study was to determine if the use of an estrous synchronization protocol with fixed-time artificial insemination and use of a clean-up bull would increase pregnancy rates and reduce days to conception compared with a traditional natural bull breeding system. Our hypothesis was that cattle receiving estrous synchronization and artificial insemination would have greater pregnancy rates and decreased days to conception compared with cattle bred via natural service mating without estrous synchronization.

## MATERIALS AND METHODS

This project was approved by the Institutional Animal Care and Use Committee of North Dakota State University.

***Animals and Treatments.*** A combination of 480 crossbred Angus cows and 86 crossbred Angus heifers (n = 566) were used in two locations: 1) Central Grasslands

Research Extension Center (n = 86 heifers and n = 405 cows) and 2) Hettinger Research Extension Center (n = 81 cows). All animals were stratified by age, BCS, and days postpartum (cows only), then assigned to one of two treatments in a completely randomized design: 1) natural service (**NS**, n = 284), exposed to natural service bulls for the duration of the breeding season, or 2) artificial insemination (**TAI**, n = 282), exposed to ES [7-d Co-Synch + CIDR (Larson et al., 2006)] and a fixed-time AI (d 0) followed by exposure to natural service bulls (clean-up bulls) for the duration of the breeding season. Females in the TAI treatment received a gonadotropin-releasing hormone (**GnRH**; as 100 µg as 2 mL of Factrel i.m.; Fort Dodge Animal Health, Fort Dodge, IA) injection as well as a controlled internal drug releasing device (**CIDR**; Pfizer Animal Health, Madison, NJ) on d -10. On d -3, the CIDR inserts were removed and a prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**; Lutalyse, 25 mg i.m.; Pfizer Animal Health, Madison, NJ) injection was administered. On d 0 a second injection of GnRH was administered coincident with fixed-time artificial insemination. Bulls were introduced to the herd on d 1 and both treatments were managed as a cohort in the same pastures. Bulls passed a breeding soundness exam (American Society for Theriogenology) and were stocked at a rate of 30 cows/bull and 15 heifers/bull for the cow and heifer groups, respectively.

***Blood Sample and Analysis.*** Blood samples for all females were taken on d -20 and -10 via coccygeal veinipuncture into 10 mL Vacutainer tubes containing sodium heparin (BD, Franklin Lakes, NJ). Samples were immediately placed on ice for 2 h then centrifuged at 1,200 × *g* for 20 min and plasma was collected and stored at -20°C. Concentrations of plasma progesterone (**$P_4$**) were analyzed in duplicate by RIA using progesterone kits (Coat-A-Count; Diagnostic Products Corp. Los Angeles, CA). The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 µL. Assay tubes for the standard curve contained 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 ng/tube. Assay sensitivity for a 100 µL sample was 0.1 ng/mL. The intra and inter-assay CV were 5.5% and 6.3%, respectively. Cows were considered to be cyclic at the initiation of treatments if at least 1 of 2 blood samples (collected on d -20 and d -10) had concentrations of $P_4 \geq 1$ ng/mL (Perry et al., 1991).

***Pregnancy Determination.*** Transrectal ultrasonography was used to determine presence and age of a viable fetus, using an Aloka 500 with a 5 MHz linear probe on d 49 and again at least 40 d after the bulls were removed from breeding pastures.

***Statistical Analysis.*** Day 49 and final pregnancy rates were analyzed using the GLM and GENMOD procedures (SAS Inst. Inc., Cary, NC), whereas days to conception were analyzed using the GLM procedure. The statistical model included the effects of treatment, cyclic status, location and the respective interactions. Significance was determined with an alpha of $P < 0.05$.

## RESULTS AND DISCUSSION

At the initiation of the breeding season, 42.8% of all cattle were cyclic. The mean BCS of all cattle was 5.2 (range of 4 to 8) and mean days postpartum was 65.6 d (range of 21 to 99 d) for suckled cows on d 0. Treatment × cyclic status interactions ($P < 0.01$) were present for the proportion of cows detected pregnant on d 49, the proportion of cows pregnant at the end of the breeding season, and days from the beginning of the breeding season to conception (days to conception). A greater proportion ($P < 0.05$) of cyclic cattle in the TAI treatment (88%, 104 of 118) had a viable fetus detected on d 49 of the breeding season compared with cyclic cattle in the NS treatment (74%, 88 of 119) and non-cyclic cattle in the TAI (75%, 122 of 163) and NS treatments (77%, 120 of 156; Figure 1). Similarly, Geary et al. (2001) reported that there was no difference in TAI pregnancy rates between cyclic and non-cyclic cattle receiving an OvSynch or CO-Synch ES protocol. In contrast, Stevenson et al. (1997) stated cyclic cattle that receive ES and AI had greater pregnancy rates to AI than non-cyclic cattle.

Overall AI pregnancy rates were 55%. The use of the ES and AI allowed more cattle to become pregnant on the first day of the breeding season. This reduction in the number of non-pregnant cows at the start of the breeding season would allow bull stocking rate to be reduced. The bulls needed for an operation that utilizes ES and AI may be reduced by half, recouping most if not all the expenses needed for ES and AI (Johnson and Jones, 2008). Depending on bull purchase price, maintenance and health costs, and interest on purchases, operating costs for herds that incorporate AI may be less compared with herds that use only a natural breeding system (Johnson and Jones, 2008).

Both cyclic (11.6 ± 1.4 d) and non-cyclic (14.5 ± 1.4 d) cattle in the TAI treatment became pregnant earlier in the breeding season ($P < 0.05$) compared with cyclic (19.9 ± 1.4 d) and non-cyclic (17.9 ± 1.4 d) cattle in the NS treatment (Figure 2). The decreased days to conception is due primarily to the greater proportion of cattle bred to AI on the first day of the breeding season. The reduction in days to conception could potentially reduce the calving season length and labor needed with a more concentrated calving season (Sprott, 1999). However, the length of the calving season is dictated by the length of the breeding season. Rodgers et al. (2012) reported the mean calving date was altered by ES and AI, but the length of the breeding season was not different compared with that of the natural service treatment. If days to conception is a true indication of date of calving, cattle in the TAI treatment would have calves earlier in the calving season with the potential to be heavier at weaning.

A greater proportion ($P < 0.05$) of cyclic cattle in the TAI treatment (94%, 111 of 118) were pregnant at the end of the breeding season compared with non-cyclic cattle in the TAI treatment (84%, 136 of 162) whereas cyclic (88%, 105 of 119) and non-cyclic (89%, 140 of 157) cattle in the NS treatment were intermediate (Figure 3). Interestingly, fewer

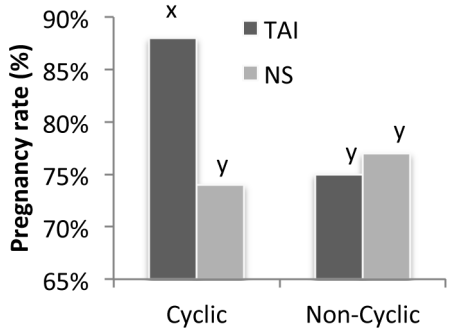


**Figure 1.** Pregnancy rates on d 49 of breeding season. Treatment × cyclic status $P < 0.01$. Cyclic cattle on the fixed-time AI (TAI) treatment had significantly ($P < 0.05$) greater pregnancy than non-cycling TAI and cycling and non-cycling natural service (NS). $^{x,y}$ Means lacking common superscript differ ($P < 0.05$).

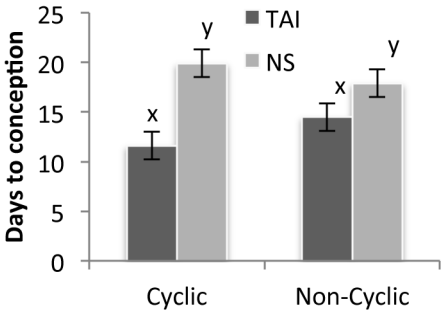


**Figure 2.** Mean days to conception from start of breeding season. Treatment × cyclic status $P < 0.01$ Both cyclic and non-cyclic fixed-time AI (TAI) treatment cattle had significantly ($P < 0.05$) decreased days to conception compared with cattle in the natural service (NS) treatment. $^{x,y}$ Means lacking common superscript differ ($P < 0.05$).

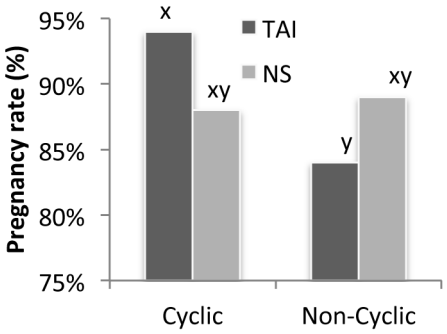


**Figure 3.** Pregnancy rates at least 40 d after bull removal. Treatment × cyclic status $P < 0.01$. Cyclic cattle in the fixed-time AI (TAI) treatment had significantly ($P < 0.05$) greater pregnancy rates than non-cyclic TAI treatment cattle and natural service (NS) were intermediate. $^{x,y}$ Means lacking common superscript differ ($P < 0.05$).

non-cyclic cattle in the TAI treatment were pregnant at the final pregnancy check compared with cyclic cattle in the TAI treatment. This goes against a common theory that states ES protocols will initiate cyclicity and result in a greater proportion of non-cyclic cattle pregnant at the end of a breeding season compared with systems that do not incorporated AI. In contrast, no differences in final pregnancy rates were observed among cyclic and non-cyclic of both cows and heifers that received an ES protocol with an injection of GnRH (Stevenson et al. (1997). The discrepancy between our season ending pregnancy rates and stated theory require further verification to substantiate common industry claims.

## IMPLICATIONS

Breeding systems for beef cattle that incorporated artificial insemination and estrous synchronization altered pregnancy rates and days to conception compared with natural service breeding systems. Cattle bred via artificial insemination became pregnant earlier in the breeding season and have the potential to be born earlier in the calving season and be heavier at weaning. Subsequent efforts will evaluate whether current indicators of potential increased weaning weight for calves resulting from the AI treatment translate into benefits realized at the producer level.

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# DIFFERENCES IN ALLELE FREQUENCY DISTRIBUTION OF BOVINE HIGH-DENSITY GENOTYPING PLATFORMS IN HOLSTEINS AND JERSEYS

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**ABSTRACT**: Two single nucleotide polymorphism (SNP) genotyping arrays, the Illumina High-Density Bovine BeadChip Array (BovineHD; 777,962 SNP) and the Affymetrix Axiom Genome-Wide BOS 1 Array (BOS1; 648,874 SNP), are available for bovine genomics analyses, such as quantitative trait loci (QTL) fine mapping and genomic selection. These genotyping arrays are of interest to researchers for their high marker density relative to other genotyping platforms. Differences in allele frequency distribution between arrays contributes to their efficacy for association studies as QTL may have rare alleles (low minor allele frequency, MAF), limiting the extent of linkage disequilibrium (LD) possible with intermediate MAF SNP. To evaluate MAF distribution differences between arrays in Holstein (HO) and Jersey (JE) breeds, we genotyped 16 DNA samples (10 HO, 6 JE) from UC Davis cows and their sires with both BovineHD and BOS1, and MAF distribution was determined within breed. A greater proportion of SNP had MAF equal to zero in BOS1 relative to BovineHD (HO: BOS1 45% vs. BovineHD 28%; JE: BOS1 55% vs. BovineHD 39%), which given the fewer number of total SNP in BOS1 resulted in fewer BOS1 SNP for all MAF. However, of polymorphic SNP, low to intermediate MAF SNP (MAF $\leq$ 0.20) were proportionally more highly represented in BOS1 (HO 45%; JE 42%) relative to BovineHD (HO 38%; JE 39%). An important issue in genotyping arrays is marker redundancy. SNP in complete LD have collinear effects, reducing the accuracy and stability of marker effect estimates when jointly analyzed. To evaluate if removal of redundant SNP altered the MAF distribution in BovineHD and BOS1, LD pruning was performed using SVS7. Using the LD threshold $r^2 \geq 0.99$, a greater proportion of polymorphic SNP were removed from BovineHD relative to BOS1 (HO: BovineHD 75% vs. BOS1 57%; JE: BovineHD 86% vs. BOS1 79%), yielding more informative SNP at nearly all MAF in BOS1 for HO (+14,079 SNP). The proportion of low MAF SNP in BOS1 (HO 37%; JE 32%) became similar to BovineHD (HO 34%; JE 31%), and the correlation between BovineHD and BOS1 MAF distributions rose in HO (0.58 to 0.95) but did not change in JE.

**KEYWORDS:** cattle, genotyping, single nucleotide polymorphism

## INTRODUCTION

As of August 2011, 1,144 U.S. Holstein animals have been genotyped using the Illumina High-Density Bovine BeadChip Array (**BovineHD**), and >70,000 have been genotyped using the Illumina Bovine SNP50 BeadChip assay (Cole et al., 2012). In the past year, another high density genotyping platform was introduced for use in cattle, the Affymetrix Axiom Genome-Wide BOS 1 Array (**BOS1**). Both platforms were designed to improve genomic coverage relative to lower density arrays such as the Bovine SNP50 and be informative across a wider variety of breeds (Illumina, 2010; Affymetrix, 2011). Greater marker density and thus reduced gap size should increase the average linkage disequilibrium (**LD**) between adjacent markers (and QTL) and the accuracy of genomic analyses such as genomic selection (de Roos et al., 2008; Harris and Johnson, 2010b). However, greater density may result in many non-informative loci due to complete LD between markers. Increased emphasis on coverage across breeds can imply reduced representation of SNP that are polymorphic within any particular breed or small population or both, leading a large proportion of array SNP to be monomorphic. Another consideration is the allele frequency of those loci that are in LD with QTL. As described by Goddard (2009), if QTL follow a U-shaped distribution, a genotyping array in which markers have intermediate frequency or perhaps follow a uniform distribution would provide relatively few SNP which could be in complete LD with QTL. Therefore, low minor allele frequency (**MAF**) SNP may be of particular interest in QTL mapping studies. In that context, power and sample size depend on the marker allele frequencies (Chapman and Wijsman, 1998; Xiong and Jin, 1999).

It was the objective of this study to determine the MAF distribution of the BovineHD and BOS1 arrays in small populations of two highly used dairy breeds and determine whether there was a greater proportion of low MAF SNP present in one array relative to the other. Knowledge of these two parameters could substantially impact decisions as to which array is more appropriate for specific applications.

## MATERIALS AND METHODS

Genetic analysis was performed on a population of 16 purebred dairy cattle derived from five 3-generation

families of the maternal grandsire, the dam, and one or more daughters. In these five families, three were of the Holstein breed (N = 10), and two were of the Jersey breed (N=6). DNA extraction, genotyping, and quality control were described in Rincon et al. (2011). Briefly, DNA was extracted from blood samples and submitted for genotyping using the BovineHD BeadChip array (Illumina Inc., San Diego, CA) and the Axiom Genome-Wide BOS 1 array (Affymetrix Inc., Santa Clara, CA) by GeneSeek Inc. (a Neogen Co., Lincoln, NE). Genotype scoring was conducted using Genome Studio (Illumina Inc., San Diego, CA) or Genotyping Console (Affymetrix Inc., Santa Clara, CA) software for Illumina and Affymetrix genotype data, respectively.

SNP Variation Suite v.7 (SVS7; Golden Helix Inc., Bozeman, MT) was used for data analysis. Genotype statistics (specifically, MAF) were computed within each breed both before and after genotype filtering using the LD Pruning tool. LD Pruning involved the use of an Expectation Maximization (EM) algorithm to complete the following: 1) linkage disequilibrium $r^2$ estimation for each pair of markers within a window of 50 markers moving in increments of five markers and 2) pruning the first marker of any pair for which the $r^2$ was greater than a threshold value. For this analysis, the threshold value for $r^2$ was chosen to be 0.99. This differs from the criteria used by Rincon et al. (2011) for the same dataset, in which LD pruning was used across breeds, and an $r^2$ threshold of 0.9 was used.

With the exception of sporadically missing data due to genotyping error, the limited sample size in this data set allowed MAF to be considered categorically, with increments between 0 and 0.5 of 1/2n where n was the number of individuals genotyped per breed. For this reason, trends in minor allele frequency were considered in increments of 0.05 for Holstein animals and 0.083 for Jersey animals. Low MAF was defined as MAF $\leq$ 0.20.

## RESULTS

The numbers of SNP remaining after data trimming is presented in Table 1. Greater than 99% of SNP present on the BovineHD and BOS1 arrays were genotyped in each breed. A large proportion of SNP were monomorphic (MAF = 0) in each breed, and a greater proportion of BOS1 SNP were monomorphic relative to BovineHD. In these populations, there were 180-200K more polymorphic (MAF > 0) SNP present in BovineHD relative to BOS1. Using the LD Pruning tool on the remaining SNP removed a large number of loci, ~400K from BovineHD and ~200K from BOS1. Using these combined exclusion criteria; the number of informative loci in Holsteins was approximately one-fifth of the total SNP present on each array and in Jerseys, less than one-tenth.

Tables 2 and 3 show the MAF distribution in BovineHD and BOS1 before and after applying the SVS7 LD Pruning tool in Holstein and Jersey samples, respectively. For both breeds, before LD Pruning, BOS1 contained a larger proportion of low MAF SNP (MAF $\leq$ 0.20), which was consistent within each constituent MAF increment. However, relatively more low MAF SNP in BOS1 were in linkage disequilibrium in this population such that performing LD pruning generated a reduced SNP set that was more similar in SNP density and MAF distribution to that generated from BovineHD.

In Holsteins, the BOS1 set after LD pruning is larger. Therefore, while the distribution of MAF became more similar to that of BovineHD, there are more informative SNP at nearly all MAF increments in BOS1, though the total number of additional SNP is <20K. In Holsteins, the reduced representation of low MAF SNP after LD Pruning in BOS1 results in a higher correlation in MAF between arrays, 0.58 before LD Pruning and 0.95 after. In Jerseys, while LD Pruning did remove a larger proportion of low MAF SNP as in Holsteins, the correlation in MAF between arrays did not improve (0.95 to 0.84).

**Table 1.** Single nucleotide polymorphism (SNP) markers on each array after each step in data trimming for Holstein (HO) and Jersey (JE) animals

| Breed | HO | | JE | |
|---|---|---|---|---|
| Array | BovineHD | BOS1 | BovineHD | BOS1 |
| Total SNP (%) | 777,962 (100%) | 648,874 (100%) | 777,962 (100%) | 648,874 (100%) |
| Missing genotypes | 402 (<<1%) | 10 (<<1%) | 449 (<<1%) | 22 (<<1%) |
| SNP with MAF = 0 (%) | 219,098 (28%) | 291,145 (45%) | 305,512 (39%) | 355,225 (55%) |
| Polymorphic SNP (MAF > 0; %) | 558,462 (72%) | 357,719 (55%) | 472,001 (61%) | 293,627 (45%) |
| Polymorphic SNP removed by linkage disequilibrium (LD) pruning (%) | 419,837 (75%) | 205,015 (57%) | 405,809 (86%) | 231,508 (79%) |
| Polymorphic SNP with LD $r^2$<0.99 (%) | 138,625 (25%) | 152,704 (43%) | 66,192 (14%) | 62,119 (21%) |

**Table 2.** Minor allele frequency (MAF; %) distribution for polymorphic SNP (MAF>0) present in the Holstein genotypes before and after linkage disequilibrium (LD) pruning

| Polymorphic SNP (%) | Bovine HD | BOS1 | Bovine HD LD $r^2$< 0.99 | BOS1 LD $r^2$< 0.99 |
|---|---|---|---|---|
| Low MAF (≤0.20) | 38.0 | 45.3 | 34.3 | 36.5 |
| 0.00 < MAF ≤ 0.05 | 8.1 | 10.3 | 6.2 | 7.0 |
| 0.05 < MAF ≤ 0.10 | 9.3 | 11.9 | 8.6 | 8.9 |
| 0.10 < MAF ≤ 0.15 | 10.2 | 12.1 | 8.8 | 9.9 |
| 0.15 < MAF ≤ 0.20 | 10.5 | 11.0 | 10.7 | 10.6 |
| 0.20 < MAF ≤ 0.25 | 10.7 | 10.7 | 10.6 | 11.2 |
| 0.25 < MAF ≤ 0.30 | 11.2 | 9.8 | 12.0 | 11.0 |
| 0.30 < MAF ≤ 0.35 | 11.1 | 9.8 | 11.5 | 11.5 |
| 0.35 < MAF ≤ 0.40 | 11.7 | 9.8 | 12.8 | 11.8 |
| 0.40 < MAF ≤ 0.45 | 11.3 | 9.7 | 12.0 | 11.8 |
| 0.45 < MAF ≤ 0.50 | 6.1 | 4.9 | 6.8 | 6.1 |
| Correlation | 0.58 | | 0.95 | |

**Table 3.** Minor allele frequency (MAF; %) distribution for polymorphic SNP (MAF>0) present in the Jersey genotypes before and after linkage disequilibrium (LD) pruning

| Polymorphic SNP (%) | Bovine HD | BOS1 | Bovine HD LD $r^2$< 0.99 | BOS1 LD $r^2$< 0.99 |
|---|---|---|---|---|
| Low MAF (≤0.20) | 38.5 | 41.8 | 30.7 | 31.9 |
| 0.00 < MAF ≤ 0.083 | 19.7 | 22.4 | 12.7 | 14.8 |
| 0.083 < MAF ≤ 0.167 | 18.8 | 19.4 | 18.0 | 17.1 |
| 0.167 < MAF ≤ 0.250 | 17.4 | 16.0 | 16.7 | 16.5 |
| 0.250 < MAF ≤ 0.333 | 18.3 | 17.2 | 20.1 | 18.8 |
| 0.333 < MAF ≤ 0.417 | 17.4 | 16.8 | 18.0 | 19.5 |
| 0.417 < MAF ≤ 0.500 | 8.4 | 8.1 | 14.5 | 13.3 |
| Correlation | 0.95 | | 0.84 | |

## DISCUSSION

There are two major reasons to remove uninformative SNP from genomic analyses: 1) linear functions of uninformative SNP may predict random error in the reference data, and 2) increased collinearity between informative and uninformative SNP can distribute the effect of a QTL across many SNP (Harris and Johnson, 2010b). Therefore, the effect of including uninformative SNP is to reduce the accuracy and stability of marker effect estimates, and this problem would be exacerbated for traits that follow an infinitesimal model and with increasing numbers of uninformative SNP in the dataset.

Most genomic prediction analyses using the Bovine SNP50 have used quality control criteria that included trimming monomorphic, very low MAF (a variety of thresholds but generally MAF<1-5%), and collinear SNP. As of October 2008, quality control of SNP for U.S. and Canadian genomic prediction analyses in Holsteins (N=14,720) excluded 6,572 monomorphic SNP, 3,649 SNP with MAF<0.02, and 2,628 SNP due to collinearity with at least one other SNP (Wiggans et al., 2009). The determination of collinearity was by comparing genotypes for every pair of SNP in MAF increments of 0.025 with the highest quality locus retained in any highly correlated pair. Unlike most genomic prediction studies in cattle, the MAF distribution in that dataset was reported. Above the exclusion threshold of MAF<0.02, a relatively uniform distribution of SNP by MAF increment was observed. Although MAF distribution was not reported, other

studies in Holsteins have reported similar proportions of SNP excluded from genomic analysis using the Bovine SNP50. Using a population of 2,066 Italian Holsteins, the application of the procedure described by Wiggans et al. (2009) flagged 3,523 monomorphic SNP, 9,286 low MAF SNP, and 9,390 total SNP for collinearity including 1,276 which were flagged only for this criteria (Van Kaam et al., 2010). In New Zealand dairy genomic evaluations, 1,844 SNP were excluded due to high linkage disequilibrium ($r^2 > 0.975$) in a combined population of 5,212 Holstein-Friesian, Jersey, and Friesian-Jersey crossbred bulls (Harris and Johnson, 2010).

In contrast to studies using the Bovine SNP50, substantially larger proportions of monomorphic and collinear SNP were present in the BovineHD and BOS1 arrays in this dataset. However, distribution of allele frequencies of the BovineHD was very similar to the Bovine SNP50. The MAF distribution of BovineHD SNP was relatively uniform for intermediate MAF increments, with reduced representation of low MAF SNP. This trend was exacerbated somewhat by applying LD Pruning. In contrast, the BOS1 included a markedly greater proportion of low MAF SNP, although the total number of polymorphic SNP was reduced relative to BovineHD. The application of LD Pruning removed more low MAF relative to intermediate MAF SNP from BOS1, generating a similar MAF distribution to that observed in BovineHD (i.e., uniform for intermediate MAF and somewhat reduced for low MAF SNP). Therefore, it is not expected that BOS1 would be more useful for QTL mapping studies due to a better representation of rare alleles.

An important issue to consider is the small sample size used to estimate allele frequencies and linkage disequilibrium. Estimating LD using small sample sizes would be expected to be less accurate and more biased. Khatkar et al. (2008), using a population of 1,546 Holstein-Friesian bulls genotyped for 15,036 SNP, demonstrated that for a sample size of 25, $r^2$ estimates were inflated relative to those estimated from a sample of 1000 individuals, with only a moderate correlation between estimates (0.66). The authors concluded that $r^2$ accuracy was compromised for samples of less than 75 individuals. However, this effect became noticeable at longer inter-marker distances (>40 kb) than those represented in this study. In a multinational population of 1,214 Holstein, Brown Swiss, and Fleckvieh cattle, the proportional degree of inflation in LD estimates was twice as large at >50 cM than at <5 cM inter-marker distances when using 40 individuals rather than 100 (Lipkin et al., 2009). To reduce the extent of inflation in $r^2$ estimates, sliding windows of relatively few markers were used in this study, such that markers in long-range and/or nonsyntenic LD were not pruned. This method was compromised only to the extent that errors in annotation incorrectly position SNP. For example, Wiggans et al. (2009) reported nine highly correlated SNP assigned to different chromosomes in the Bovine SNP50. In this respect, it was expected that more recent, greater density chips had some advantage over the Bovine SNP50 due to improvement in genome annotation. Fadista and Bendixen (2012) reported that while the Bovine SNP50 versions 1 and 2 contained 99 and 449 SNP, respectively, which did not map to their reported genomic positions, the BovineHD contained only 14. Though BOS1 was not compared with BovineHD in that study, we did not expect annotation errors to be appreciably greater. Therefore, it was unlikely that any large differences in the extent of SNP pruning for either HD chip were caused by annotation error.

Substantially more SNP were pruned in this study due to high LD within breed than those reported in multi-breed datasets. Harris et al. (2011) reported 363,269 SNP pruned from BovineHD data due to $r^2 > 0.99$ in a combined reference population of 4,211 Holstein Friesian, Jersey, and Holstein Friesian-Jersey crossbred cattle. This was similar in magnitude to that reported by Rincon et al. (2011) where 384,907 SNP were pruned from the BovineHD for $r^2 > 0.9$ using the combined data of the Holstein and Jersey animals analyzed in this study. Approximately 20K fewer SNP were pruned by Harris et al. (2011), likely due to the increased diversity present in a larger reference set.

It might be reasonable to expect that more SNP would be polymorphic across breeds than within breeds, but another consideration is whether the extent of SNP variability present in two to three families is representative of the breed. Rincon et al. (2011) reported that based on 64 BovineHD reference genotypes derived from Holstein and Jersey cattle, the 16 samples analyzed in this study are representative of 94.4% of Holstein and Jersey SNP variation. However, considering each breed separately, LD Pruning removed a much larger proportion of SNP from Jersey animals than from Holstein animals, reducing SNP density for Jerseys nearly to that of the Bovine SNP50. This reflected the failure of six animals to be representative of haplotype diversity in the Jersey breed, and suggests that the application of LD Pruning in larger reference sets will generate larger datasets of informative SNP for that breed.

This study aimed to characterize allele frequency distribution of HD genotyping platforms in a small but representative sample of Holstein and Jersey dairy cattle. While the findings of this study did not suggest a definitive advantage of one platform over the other, the small scale of this study and its focus on only two cattle breeds did not preclude differences in MAF distribution between arrays in other breeds.

## IMPLICATIONS

This study aimed to answer whether there are differences in minor allele frequency distribution between the HD genotyping platforms currently available for use in cattle using data from two highly used dairy breeds. While BOS1 has a larger proportion of low MAF SNP, a greater proportion of these SNP are in linkage disequilibrium within breed in this dataset, and thus the exclusion of SNP that are uninformative due to near perfect LD reduced the proportion of low MAF SNP in BOS1 to be similar to that observed with the BovineHD. As such, based on these data, there is not a substantial difference between arrays in terms of the MAF distribution of informative SNP for genomic analyses.

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# THE LIFETIME PRODUCTIVITY OF BEEF FEMALES INITIALLY CONCEIVING TO, OR SIRED BY, ARTIFICIAL INSEMINATION OR NATURAL SERVICE

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**ABSTRACT:** Artificial insemination and estrous synchronization can be valuable tools for the beef cattle industry due to the ability to increase productivity and reproductive efficiency. Therefore, the objectives of this study were to: 1) compare lifetime productivity between heifers that conceived to AI with those that conceived to natural service (NS) as a yearling, and 2) compare lifetime productivity between females that were the result of an AI mating with those that were the result of an NS mating. Calving and breeding records (n = 6,693) were utilized from 1,173 Angus females collected at 1 location from 1991 to 2010. The first objective classified heifers into 2 groups: conceived by AI or NS. The second objective categorized females into 4 dam groups, depending on whether they were conceived by an AI bred heifer (H-AI), an NS bred heifer (H-NS), an AI bred cow (C-AI), or an NS bred cow (C-NS). Cutoff dates were formulated to distinguish between AI and NS born calves by documenting the AI date and summing a 290-d gestation length. Economic significance was analyzed using weaning weights from all calves produced by each female while in the herd, along with price data collected from the nearest marketing center. Yearling heifers that conceived to AI were older and heavier (P = 0.02) at breeding than heifers that conceived by NS. When compared with heifers that were conceived by NS, females that conceived through AI had a greater ($P < 0.001$) average weaning weight, weaned more ($P < 0.001$) weight, and produced more ($P < 0.001$) total calves. Consequently, females that conceived to AI as yearlings yielded greater ($P < 0.001$) revenue throughout their lifetime in the cow herd than heifers that conceived to NS. There was a positive correlation between heifer age at breeding and lifetime productivity. These data suggest that if the majority of a beef cowherd calves within the first 30 d of the breeding season, producers can maximize the productivity and efficiency of each female, and as a result, increase total revenue per cow.

**Key words:** artificial insemination, beef cattle, lifetime productivity, natural service

## INTRODUCTION

Optimizing productivity of each beef cow is essential to all beef cow herds. Productivity of the beef cow is highly dependent on reproductive efficiency, and is commonly measured by the number of calves the female produces throughout her lifetime (Dziuk et al., 1983). Artificial insemination and estrous synchronization are underutilized by the beef industry, which allow for reproductive efficiency and overall productivity to be increased. Artificial insemination allows for the utilization of elite genetics for rapid progress within the herd. Unfortunately, only 7.2% of beef cows receive AI each year (NAHMS, 2007). By conceiving to a synchronized estrus, females weaned calves 13 d older and 9.5 kg heavier on average than heifers who did not conceive via estrous synchronization (Schafer et al., 1990). Heifers that calve early in the calving season tend to be more productive over their lifetime than those who calve late in the season, producing calves that grew significantly faster and were heavier at weaning while having a greater average calf production when compared with females who were born late in the calving period (Lesmeister et al., 1973). Several studies have been conducted analyzing the immediate effects of females who calve at the beginning of the calving season; the proportion of females who breed early in the breeding season produce more uniform calf crops and the calving season is shortened (Dziuk and Bellows, 1983).

To stimulate the utilization of these techniques, long term-data documenting lifetime productivity are required. Limited literature has been published explaining the difference between lifetime productivity of heifers who conceive to AI and those that conceive to NS as yearlings. The objectives of this study were 1) compare lifetime productivity between heifers that conceived to AI and those that conceived to NS as a yearling, and 2) compare lifetime productivity between females that were a result of an AI mating vs. a NS mating.

## MATERIALS AND METHODS

***Cattle Records.*** Breeding and calving records were acquired from the John E. Rouse Colorado State University Beef Improvement Center from 1991 to 2010, resulting in 6,693 records from 1,173 purebred Angus females. In addition, weaning weight records from all calves produced from each dam were maintained until she left the herd. These records were utilized to classify heifers as conceiving to either AI or natural service (**NS**) as yearlings. To determine if

a heifer conceived to AI, a 290-d gestation period was added to the AI date, creating a specific cutoff date. All heifers that calved before the cutoff date were considered conceived to AI, while females who calved after this date were considered to have conceived to NS. By using this same model, it was determined if a heifer was the product of an AI or NS mating. Each yr. the ranch initiated AI in heifers 3 to 4 wk prior to the cows, which allowed for further classification of the females, creating 4 different dam groups. Utilizing the recorded calving date, a female could be the product of a heifer that conceived to AI (**H-AI**), a heifer that conceived to NS (**H-NS**), a cow that conceived to AI (**C-AI**), or a cow who conceived to NS (**C-NS**).

***Economic Analyses.*** This ranch typically markets their calves in October at weaning. Therefore average price data was collected for the month of October from the local livestock auction (Torrington Livestock Markets, LLC, Torrington, WY) from 1991 to 2010. Prices were gathered for both steers and heifers in the following weight divisions: 136 to 159 kg, 160 to 181 kg, 182 to 204 kg, 205 to 227 kg, 228 to 250 kg, 251 to 272 kg, and 273 to 295 kg. Prices obtained were then multiplied by the weaning weight of each calf in order to produce a value for each calf that a female produced. Actual prices for each year a calf was produced were utilized to determine the lifetime revenue for each female. Lifetime revenue using the average price scenario took the average price from 1991 to 2010 for each weight division, and was not adjusted for inflation.

Prices were then adjusted in order to reflect the current market conditions with a maximum price difference, where significant price differences existed between weight divisions and a minimum price difference where slight price differences existed among weight divisions. The average price for the 182 to 204 kg weight division from 1991 to 2010 was used as the base price for the study. This base price did not change under maximum and minimum price difference conditions. Maximum prices were determined by the difference between each of the weight divisions, and the 182 to 204 kg base price. The difference was then multiplied by a factor of 2 and added to the set base price. Minimum prices were formulated in the same fashion, except the difference was multiplied by a factor of 0.25.

***Statistical Analyses.*** Dependent variables in this study included the yearling weight of the female, age at her first AI, average calf weaning weight, lifetime weight weaned, lifetime number of calves weaned, postpartum interval, and lifetime revenue. These dependent variables were analyzed using a generalized linear model utilizing the GLM procedure (SAS Inst. Inc., Cary, NC). Fixed effects included heifer age at first breeding, conception treatment (if the heifer conceived to AI or NS), and the yearling weight of the heifer as a covariate.

## DISCUSSION

Females that conceived to AI as a yearling were older and heavier ($P = 0.02$) than females who conceived to NS. Females that conceived to AI also had a greater ($P = 0.04$) average weaning weight, weaned more ($P < 0.0001$) total kg, and more ($P < 0.0001$) total calves than females who conceived to NS as yearlings. The average weaning weight for a heifer who initially conceived to AI was 5 kg heavier than those who conceived to NS. In a previous study, calves born earlier in the calving season weighed more than those that calved late due faster rate of pre weaning gain and older age (Lesmeister et al., 1973).

By conceiving to AI rather than NS, an additional 438 kg ± 23.8 kg and 2 ± .11 calves were produced over a female's lifetime (Table 1). This can be attributed to an increased postpartum recovery time for heifers calving as 2 yr olds that conceived earlier in the breeding season. Postpartum interval is associated with age and weight of the heifer at breeding, heifers that were heavier and older at breeding had a greater postpartum interval (Patterson et al., 1999) Consequently, heifers who conceived early in the breeding season had a longer postpartum interval. Females that conceived to AI averaged 5 d longer for postpartum recovery than heifers who conceived to NS, which was not different ($P = 0.67$). This can be attributed to the female conceiving at a younger age, consequently increasing postpartum recovery time as 2-yr olds.

Females that conceived to AI as a yearling had greater ($P < 0.0001$) lifetime revenue than females that conceived to NS (Table 2). The most notable revenue difference between female groups was under the minimum price difference scenario. Under this scenario, heifers who conceived to AI had a revenue that was $974 greater ($P < 0.0001$) than heifers conceiving to NS. The smallest revenue difference occurred under the actual price difference, where heifers that conceived to AI had a revenue of $922 greater ($P < 0.0001$) than heifers that conceived to NS. Due to the fact that heifers that conceived to AI weaned an average of 438 kg more, we can conclude that increased lifetime revenue of each female across all 4 price scenarios between the 2 heifer groups can be directly attributed to differences in weaning weight and are not dependent upon market conditions.

Heifers from cows that were conceived to NS had the lowest ($P < 0.0001$) average yearling weight and average age at their first breeding compared with the other 3 dam groups. However, no difference ($P > 0.10$) was found between average weaning weight, lifetime weight weaned, lifetime calves weaned, or postpartum interval between the 4 dam groups (Table 3). Heifers that conceived to AI had the highest ($P > 0.10$) average weaning weight and heifers from C-NS had the lowest ($P > 0.10$) average weaning weight, compared with other dam groups. However, no difference ($P = 0.24$) was found in average weaning weight between H-AI and C-NS.

Given the data, we expect that H-AI females would have a greater weight weaned and number of calves weaned over her lifetime, and on the other end of the spectrum, C-NS females would have the lowest. However, there were no differences ($P > 0.10$) in weight weaned or number of calves produced from a female over her lifetime between dam groups in any of the 4 price scenarios (Table 4).

**Table 1.** LS Means ± SE for weaning weight, lifetime weight weaned, calves weaned, and postpartum interval for females that conceived to AI or natural service (NS)

| Item | n | Average yearling weight (kg) | Average age at 1st AI (d) | Average weaning weight (kg) | Lifetime weight weaned (kg) | Lifetime calves weaned | Average postpartum interval[1] (d) |
|---|---|---|---|---|---|---|---|
| Conceived to AI | 871 | $309^{b} \pm 1.8$ | $429^{b} \pm 1.6$ | $210^{b} \pm 1.0$ | $1,072^{f} \pm 23.8$ | $5.2^{f} \pm 0.11$ | $92 \pm 5.1$ |
| Conceived to NS | 302 | $300^{a} \pm 3.2$ | $418^{a} \pm 2.7$ | $205^{a} \pm 1.8$ | $634^{e} \pm 43.1$ | $3.0^{e} \pm 0.20$ | $87 \pm 10.2$ |

[a,b] Means within a column without a common superscript differ ($P < 0.05$)
[e,f] Means within a column without a common superscript differ ($P < 0.0001$)
[1] Postpartum interval was defined as the number of days between calving date of a female, and when AI occurred

**Table 2.** LS Means ± SE for lifetime revenue produced from females that conceived to AI or natural service (NS)

| Item | n = | Lifetime revenue produced ($) per female | | | |
|---|---|---|---|---|---|
| | | Actual price | Average Price[1] | Maximum Price[2] | Minimum Price[3] |
| Conceived to AI | 871 | $2,483^{a} \pm 56.6$ | $2,334^{a} \pm 51.3$ | $2,302^{a} \pm 50.4$ | $2,359^{a} \pm 52.0$ |
| Conceived to NS | 302 | $1,561^{b} \pm 96.9$ | $1,376^{b} \pm 92.8$ | $1,364^{b} \pm 91.2$ | $1,385^{b} \pm 94.3$ |

[ab] Means within a column without a common superscript differ ($P < 0.0001$)
[1] Difference under the average price scenario
[2] Difference under the maximum price scenario
[3] Difference under the minimum price scenario

**Table 3**. LS Means ± SE for weaning weight, lifetime weight weaned, calves weaned, and postpartum interval for heifers that were sired by AI or natural service (NS)

| Dam group[1] | n = | Average yearling weight (kg) | Average age at 1st AI (d) | Average weaning weight (kg) | Lifetime weight Weaned (kg) | Lifetime calves weaned | Average postpartum interval (d) |
|---|---|---|---|---|---|---|---|
| H-AI | 195 | $308^{a} \pm 3.9$ | $450^{a} \pm 3.2$ | $210 \pm 2.4$ | $974 \pm 57.3$ | $4.6 \pm 0.26$ | $88 \pm 10.2$ |
| H-NS | 40 | $299^{ab} \pm 8.1$ | $421^{b} \pm 7.1$ | $209 \pm 4.6$ | $870 \pm 111.7$ | $4.2 \pm 0.54$ | $88 \pm 22.9$ |
| C-AI | 618 | $314^{a} \pm 1.0$ | $427^{b} \pm 1.8$ | $209 \pm 1.2$ | $966 \pm 29.7$ | $4.7 \pm 0.14$ | $87 \pm 5.8$ |
| C-NS | 320 | $293^{b} \pm 2.9$ | $403^{c} \pm 2.5$ | $207 \pm 1.8$ | $989 \pm 43.0$ | $4.7 \pm 0.20$ | $84 \pm 8.3$ |

[1] H-AI = females produced by a heifer who conceived to AI, H-NS = females produced by a heifer who conceived to NS, C-AI = females produced by a cow that conceived to AI, C-NS = females produced by a cow who conceived to NS
[a-c] Means within a column without a common superscript differ ($P < 0.0001$)

**Table 4**. LS Means ± SE for lifetime revenue produced for females that were sired by AI or natural service (NS)

| Item | | Lifetime revenue produced ($) per female | | | |
|---|---|---|---|---|---|
| Dam group[1] | n = | Actual price | Average Price[2] | Maximum Price[3] | Minimum Price[4] |
| H-AI | 195 | 2,223 ± 136.5 | 2,124 ± 124.0 | 2,083 ± 121.8 | 2,155 ± 125.9 |
| H-NS | 40 | 1,949 ± 265.5 | 1,901 ± 240.6 | 1,878 ± 236.3 | 1,917 ± 244.3 |
| C-AI | 618 | 2,253 ± 70.9 | 2,092 ± 64.4 | 2,068 ± 63.2 | 2,110 ± 65.4 |
| C-NS | 320 | 2,313 ± 102.2 | 2,168 ± 92.5 | 2,139 ± 90.9 | 2,188 ± 93.9 |

[1] H-AI – females out of a heifer who conceived to AI, H-NS – females out of a heifer who conceived to NS, C-AI – females out of a cow that conceived to AI, C-NS – females out of a cow who conceived to NS No differences ($P < 0.05$)

[2] Difference under the average price scenario

[3] Difference under the maximum price scenario

[4] Difference under the minimum price scenario

## IMPLICATIONS

The utilization of AI and estrous synchronization optimize important economic traits. Estrus synchronization in combination with AI can minimize the postpartum interval, allowing the majority of the cow herd to calve toward the beginning of the calving season. Thus, reducing calf loss, labor at calving can be minimized, allowing heavier and more consistent calf crops at weaning. AI and estrous synchronization prove to be valuable tools, allowing for a female to wean more total pounds and produce more calves optimizing her lifetime productivity

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# IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH FEED EFFICIENCY IN RAMS

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**ABSTRACT:** Residual feed intake (RFI) is a measure of efficiency that is time consuming, expensive, and labor intensive to obtain, making it an ideal trait for marker-assisted selection. The objectives of this research were to 1) estimate heritability and identify single nucleotide polymorphisms (SNP) associated with feed efficiency in sheep and 2) trace identified SNP to the corresponding gene or genomic region. We hypothesized that regions of the genome corresponding to feed efficiency and heritability could be determined using the Ovine SNP50 BeadChip. Individual intake measurements were collected on rams from two separate performance tests (Dual Purpose Ram Test and Blackface Ram Test) at the University of Wyoming (n = 330) from 2009 to 2011 using the GrowSafe System. Individual RFI (actual feed intake – predicted feed intake) values were generated. Blood was collected via the jugular and DNA was isolated. Single nucleotide polymorphisms in ram DNA were genotyped using the Ovine SNP50 BeadChip on the Illumina Infinium HD BeadChip Assay. Percentage loci scored per animal and locus, Hardy Weinberg deviations, consistency with recorded animal gender, pedigree and breed, replicate sample reproducibility, and unusual allelic ratio analyses were conducted through Genome Studio and R for quality control analysis. In total, 50,896 SNP passed quality control tests. A genome-wide association study (GWAS) analysis was conducted in R using the GenABEL package to identify SNP and estimate heritability using a polygenic model, $Y = \mu + G + e$ whereby μ is the overall mean, $G$ is the random polygenic effects, and $e$ is the random residual. Heritability for RFI was estimated as 0.14, which is similar to reports in cattle. A nominal genome-wide threshold ($P < 3.02^{-04}$) was obtained for four SNP. Corresponding genes to identified SNP were determined using the UCSC Genome Browser. Corresponding genes included: *Zinc finger 1* (*GLIS1*), *interleukin 1 receptor accessory protein-like 1* (*IL1RAPL1*), and *sex-determining region y – box 5* and *6* (*SOX5* and *SOX6*). Though four potential markers for RFI were identified, sample size must increase and markers must be independently validated prior to their use for marker-assisted selection.

**Key words:** residual feed intake, sheep, single nucleotide polymorphism

## INTRODUCTION

For livestock production to remain sustainable and economically efficient, it is imperative that researchers provide genomic tools that target polygenic traits of economic importance. This can be achieved through the use of genome-wide association studies (**GWAS**) and high-density single nucleotide polymorphism (**SNP**) chips. Residual feed intake (**RFI**), the variation around basal metabolic maintenance for a given growth rate, has been suggested as an alternative measure for feed efficiency as it is independent of growth and carcass traits (Koch et al., 1963; Herd and Bishop, 2000; Arthur et al., 2001). As sheep producers incur feed costs of 50 to 70% of their total inputs (Nash, 1991), feed efficiency parameters should be considered when constructing breeding programs. Archer et al. (2004) realized 8 to 38% increases in genetic gains and 9 to 33% increases in profitability in beef cattle when incorporating feed intake into breeding schemes for the selection of RFI. However, obtaining feed intake measurements for RFI is time consuming, expensive, and labor intensive. In sheep and cattle, approximately 60-70 d are needed to accurately determine individual RFI values (Sainz and Paulino, 2004; Cockrum et al., 2011). The costs associated with measuring individual feed intake to determine RFI ranges from $150 to $450 (Archer et al., 2004). The combination of the time, labor and costs associated with collecting individual feed intake measurements and the moderate to high heritability ($h^2$ = 0.16 to 0.43) of RFI makes it an ideal trait for marker-assisted selection (Herd et al., 2003). We hypothesized that the Ovine SNP50 BeadChip can be used to estimate heritability and identify regions in the sheep genome associated with feed efficiency. The objectives of this research were to 1) identify SNP and the genomic heritability associated with feed efficiency in sheep, and 2) determine the genomic region within the sheep genome associated with identified SNP.

## MATERIALS AND METHODS

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

***Animal Procedures.*** Feed intake measurements were collected from 2009 to 2011 from the University of Wyoming ram dual purpose (**DP**) and blackface (**BF**) breed performance tests (n = 330) using the GrowSafe System. Performance

tests were composed of 5 contemporary groups (Fall 2009 = 1; Summer 2010 = 2; Fall 2010 = 3; Summer 2011 = 4; and Fall 2011 = 5). The DP tests were conducted for 140 d for contemporary groups 1 and 3 and 84 d for contemporary group 5. The BF test was conducted for 76 d for contemporary group 2 and 75 d for contemporary group 4. Dual-purpose rams (n = 205) were primarily composed of Rambouillet and BF rams (n = 125) were composed of Suffolk and Hampshire breeds. Rams were fed medicated forage-based pelleted rations (13.5 to 15% CP). Preliminary research in our laboratory has shown that regardless of pellet composition, feed efficiency status does not differ. Body weights were collected weekly for the DP rams and every 25 d for the BF rams. Upon cessation of the performance tests, blood was collected for DNA isolation via the jugular using EDTA$^-$ lined vacutainer tubes (Tyco Healthcare Group LP, Mansfield, MA) to prevent clotting.

***RFI evaluation.*** Individual feed intake data was collected using the GrowSafe System. The GLM procedure (SAS Inst. Inc., Cary, NC) was used to predict estimated feed intake using the equation: $y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \varepsilon$, where $\beta_x$ = coefficient estimate, $X_1$ = ADG, $X_2$ = $MMWT^{0.75}$ (metabolic mid-weight), and ε = residuals. Individual RFI values were calculated by the difference between actual feed intake and estimated feed intake (Koch et al., 1963). Body composition measures were not incorporated into the predicted feed intake model as no added benefit to RFI values was expected (Arthur et al., 2003).

***Laboratory Procedures.*** A modified protocol to extract DNA from white blood cells was used (Montgomery and Sise, 1990). After the mixture of Digestion Buffer, Proteinase K, Sodium Dodecyl Sulfate, and white blood cells incubated over night at 65 °C, 10 M $NH_4Ac$ (1 mL) was added and mixed vigorously. Samples were then centrifuged and supernatant was combined with 100% Absolute Ethanol (20 mL). Using a Pasteur pipette, DNA was spooled out of ethanol and dried. A Tris-EDTA$^-$ (TE) buffer was then added to the isolated DNA and eluted. Based on Nanodrop readings (Thermo Scientific, NanoDrop Products, Wilmington, DE), DNA was diluted using TE buffer to approximately 100 ng/µL for SNP detection. The Illumina Infinium HD Ultra BeadChip Protocol (Illumina, Inc., San Diego, CA) was used to analyze isolated DNA on the Ovine SNP50 BeadChip at AgResearch, Limited (Invermay, New Zealand).

***Quality Control Analysis.*** Genome Studio and R were used to evaluate genotypes according to Dodds et al. (2009). In total, 50,896 markers and 328 rams had an average call rate > 0.95% and a minor allele frequency > 0.76%. Due to missing values or poor call rates, 49 animals were eliminated from the GWAS analysis. A principle component (**PC**) analysis was performed to convert genotype observations on individuals into linear uncorrelated variables. As expected, the majority of the variation was captured in the PC1 analysis (75%) suggesting that the genetic distances of the DP rams and BF rams differ widely. In addition to lambda ($0.86 \pm 6.91^{-05}$), the gene *RXFP2* was used to confirm accuracy of the polygenic model as it has been implicated in regulating horn size in sheep (Johnston et al., 2011). The SNP associated with *RXFP2* was confirmed to be in high association ($P = 9.283^{-04}$) with the horn phenotype within these populations.

***Genome-Wide Association Analysis.*** The GenABEL package in R was used to identify the genomic kinship inbreeding coefficient ($f_g$) from the relationship matrix (**G**) for contemporary groups by generating identical-by-state statistics. A GWAS analysis was conducted in R using the GenABEL package to identify SNP and estimate genomic $h^2$ using a polygenic model: $Y = \mu + G + e$, whereby µ is the overall mean, $G$ is the vector of random polygenic effects, and $e$ is the random residuals. Fixed effects included birth type (single, twin, triplet), birth year (2008 to 2011), flock (1 to 33), and contemporary group (1 to 5). Data were fitted to a fixed effect and polygenic model whereby G was derived from the SNP. Each SNP was tested individually as an additive effect using a simple fixed model that incorporated errors. Identified genotypes were aligned to the ovine genome using the UCSC Genome Browser (ISGC Ovis Aries 1.0/oviAri1). As observation numbers were minimal, a nominal genome-wide association threshold was established as ($P < 3.02^{-04}$) and candidate genes/regions were subsequently identified as being associated with RFI in sheep.

## RESULTS

The genomic kinship was used to generate inbreeding coefficients to obtain greater accuracy than using pedigree information. Genomic inbreeding coefficients of 9.7% and 9.5% were observed for DP and BF rams, respectively (Table 1).

Table 2 visually depicts the 1,781 genotypes that were associated with RFI ($P < 0.05$). There were 90 genotypes

**Table 1.** Genomic inbreeding coefficients of performance tested rams for feed efficiency

| Contemporary Group | Year | Breed Type | n | Mean[1] $f_g$ % |
|---|---|---|---|---|
| 1 | Fall 2009 | Dual Purpose | 60 | 10.7% |
| 2 | Summer 2010 | Blackface | 77 | 9.4% |
| 3 | Fall 2010 | Dual Purpose | 75 | 10.6% |
| 4 | Summer 2011 | Blackface | 70 | 9.6% |
| 5 | Fall 2011 | Dual Purpose | 48 | 7.7% |
| Total | | | 330 | |

[1]Genomic kinship inbreeding coefficient percentage.

**Table 2.** Genotypes corresponding to each chromosome for feed efficiency in sheep

| Chromosome | ($P < 0.05$)[1] | ($P \leq 3.22^{-03}$)[2] | Peak SNP | Position | Pc1df[3] |
|---|---|---|---|---|---|
| 0 | 13 | 1 | s51002.1 | 0 | 0.00093 |
| 1 | 210 | 10 | s47478.1* | 28.5 | 0.00019 |
| 2 | 152 | 7 | OAR2_169305598.1 | 169.3 | 0.00094 |
| 3 | 165 | 8 | OAR3_70730693.1 | 70.7 | 0.00044 |
| 4 | 80 | 1 | OAR4_95780657.1 | 95.8 | 0.00070 |
| 5 | 111 | 6 | OAR5_43271631.1 | 43.3 | 0.00030 |
| 6 | 63 | 1 | OAR6_77867959.1 | 77.9 | 0.00132 |
| 7 | 63 | 6 | OAR7_29044289_X.1 | 29.0 | 0.00056 |
| 8 | 68 | 4 | OAR8_67633238.1 | 67.6 | 0.00087 |
| 9 | 69 | 5 | OAR9_31965185.1 | 32.0 | 0.00111 |
| 10 | 76 | 8 | OAR10_4440936.1 | 4.4 | 0.00063 |
| 11 | 26 | 0 | | | |
| 12 | 57 | 2 | OAR12_40003464.1 | 40.0 | 0.00098 |
| 13 | 52 | 2 | OAR13_82500579.1* | 82.5 | 0.00014 |
| 14 | 53 | 5 | OAR14_45342785.1 | 45.3 | 0.00033 |
| 15 | 46 | 3 | OAR15_37760673.1* | 37.8 | 0.00025 |
| 16 | 56 | 1 | OAR16_76611508.1 | 76.6 | 0.00108 |
| 17 | 51 | 1 | s20107.1 | 2.3 | 0.00188 |
| 18 | 74 | 7 | s68358.1 | 25.3 | 0.00146 |
| 19 | 44 | 0 | | | |
| 20 | 29 | 1 | OAR20_41400757.1 | 41.4 | 0.00302 |
| 21 | 30 | 1 | s20463.1 | 32.8 | 0.00282 |
| 22 | 25 | 0 | | | |
| 23 | 24 | 0 | | | |
| 24 | 40 | 2 | s37249.1 | 35.8 | 0.00102 |
| 25 | 29 | 2 | s35968.1 | 29.3 | 0.00081 |
| 26 | 36 | 2 | s23193.1 | 45.1 | 0.00137 |
| OA | 3 | 0 | | | |
| X | 46 | 4 | DU450886_629.1* | 33.3 | 0.00025 |
| Total | 1,781 | 90 | | | |

[1]SNP that reached a nominal threshold ($P < 0.05$) of the total 50,896 markers (top 3.5%).
[2]SNP that reached a nominal threshold ($P \leq 3.22^{-03}$) of the total 50,896 markers (top 0.2%).
[3] *P*-value corrected for λ inflation.
*Candidate markers for feed efficiency that achieved nominal threshold ($P < 3.02^{-04}$) of the total 50,896 SNP.

associated with feed efficiency that reached a nominal threshold of ($P \leq 3.2^{-3}$). Both the genotype data (Table 2) and the Manhattan plot (Figure 1) indicated that chromosomes 1, 2, 3, 10, and 18 contained the majority of identified genotypes ($n \geq 7$) that achieved threshold. Heritability for RFI was low ($h^2 = 0.14$) in this study.

Markers that reached a nominal threshold of $P < 3.02^{-04}$ (n = 4) were aligned to the oviAri1 assembly (**Table 3**). Identified genes included: *GLIS family zinc finger 1* (***GLIS1***), *interleukin 1 receptor accessory protein-like 1* (***IL1RAPL1***), and ***SRY*** *sex determining region box-5* and *-6* (***SOX5*** and ***SOX6***). The SNP (chromosome 13, 82.5 Mb) that was most associated ($P = 1.40^{-4}$) with RFI did not annotate to a specific gene.

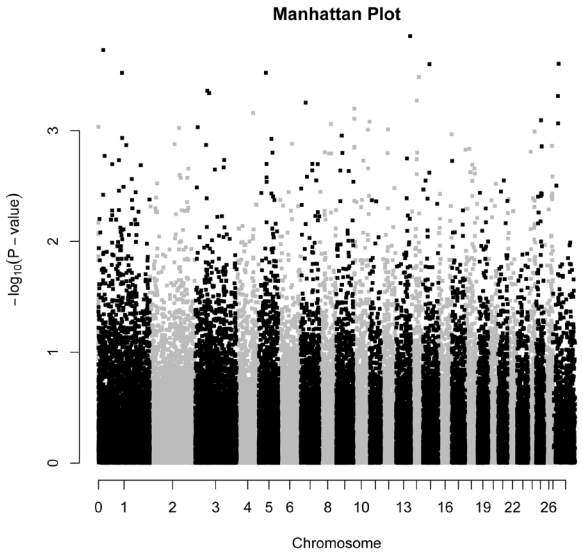


**Figure 1.** Manhattan plot showing whole-genome analysis after quality control analyses of rams (n = 281) measured for RFI. Each dot (n = 50,896) represents a genotype from the Ovine SNP50 BeadChip.

## DISCUSSION

***Genomic Kinship Inbreeding Coefficient.*** The majority of DP and BF sheep producers incorporate linebreeding in their selection schemes to maintain purebred lines, which explains the average $f_g$ of 9.6%. In comparison, Cammack et al. (2005) found an average inbreeding coefficient of 3.2%

**Table 3.** Candidate markers for RFI in rams

| Chr | Position (Mb) | Pc1df[1] | Candidate Gene[2] | RefSeq |
|---|---|---|---|---|
| 13 | 82.5 | 0.00014 | ----- | ----- |
| 1 | 28.4 | 0.00019 | *GLIS1* | NM_147221 |
| X | 33.3 | 0.00025 | *IL1RAPL1* | NM_014271 |
| 15 | 37.8 | 0.00025 | *SOX6* | NM_001191418 |
| | | | *SOX5* | NM_001083471 |

[1]*P*-value corrected for λ inflation.
[2]Candidate genes for RFI in rams that nominal threshold ($P < 3.02^{-04}$) of total 50,896 SNP.

in an experimental composite population of sheep. Pedigree-based *f* do not account for recombination cross-over events during meiosis that can affect which alleles that are passed to progeny. Rams used in this GWAS are likely more representative of purebred production populations.

***Associated Markers*** Chromosomes 1, 2, 3, 10, and 18 had the highest number of markers ($n \geq 7$) associated with feed efficiency within the highest ranked (0.2%) SNP of total genotypes. Nkrumah et al. (2007) found quantitative trait loci (**QTL**) associated with feed efficiency on chromosomes 1, 5, 7, 8, 12, 16, 17, and 26 with the most significant QTL occurring on chromosome 5. However, Sherman et al. (2008) identified associations with RFI QTL on chromosomes 2, 5, 10, 20, and 29, with the largest effect on chromosome 2, in beef cattle. The SNP50 chip was designed to target SNP randomly placed every ~40 kb across the genome, allowing for an unbiased approach. With sparse information available on the sheep genome, previous research in cattle must be drawn upon as a comparison to results found in sheep for feed efficiency. Experimental differences including species, breed, feed intake measurement method, and observation numbers can also contribute to observed genotypic differences.

***Genomic Heritability.*** Genomic $h^2$ (0.14) was much less than estimates from pedigree analyses in our study. Though lower than originally estimated (Arthur et al., 2001), results confirm genomic $h^2$ for this trait in cattle (Rolf et al., 2010).

***Candidate Genes.*** Barendse et al. (2007) hypothesized that energy conserved by metabolic efficiency may be shunted towards reproduction or tissue construction. Candidate genes *SOX5* and *SOX6* are involved in the regulation of embryonic development and cellular fate. Furthermore, *GLIS1* may play a role in the regulation of cellular processes during embryonic development (Kim et al., 2003). Candidate gene *IL1RAPL1* is involved in controlling inhibitory networks during cerebral development and has been implicated in X-linked cognitive impairment (Gambino et al., 2007, 2009). It is unknown how *IL1RAPL1* may contribute to feed efficiency in sheep or cattle.

## IMPLICATIONS

The impact of using genomic kinship $h^2$ as opposed to pedigree-based $h^2$ is unknown; however, future research is needed to decipher the accuracy and correlation associated with genomic kinship $h^2$. Several markers within chromosomes 1, 2, 10, and 18 were identified that may contain regions that are involved in the regulation of feed efficiency. Though four potential markers for RFI were identified, sample size must increase and markers must be validated in independent populations prior to use for marker-assisted selection. As technology continues to develop, the use of high-density SNP chips will become more economically feasible for livestock producers to use as a breeding management tool.

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# OUT-OF-SEASON REPRODUCTIVE PERFORMANCE OF EWES SYNCHRONIZED TO ESTRUS WITH VARIOUS 5 DAY PROTOCOLS

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**ABSTRACT:** The objective of this experiment was to evaluate the reproductive performance of ewes after synchronization to estrus with progesterone ($P_4$) impregnated controlled internal drug release (CIDR) inserts in combination with GnRH and PG. Dorset and Katahdin ewes (n = 61 and 17, respectively) were assigned randomly during the anestrous period (April) to 1 of 4 treatments: 1) untreated (U; n = 16); 2) CIDR (0.3 g $P_4$) inserts for 5 d (C; n = 21); 3) CIDR insert for 5 d and PG (dinoprost, 10 mg i.m.) at CIDR removal (P; n = 20); and 4) GnRH (gonadorelin, 0.02 mg i.m.) at CIDR insertion and PG at CIDR removal (G; n = 21). Rams equipped with marking harnesses were introduced at CIDR removal (d 0) and ewes were observed at 0800 h and 1700 h daily for breeding marks. Blood samples were collected via jugular venipuncture on d -12, -5, 0, 1, 2, 3, 4, 5, 8, 11, 14, 17, and 20 relative to CIDR removal and analyzed for serum concentrations of $P_4$ via RIA. Reproductive performance data were collected which included: days to estrus, days to lambing, percentage of ewes exhibiting estrus, pregnancy rate, lambing rate, and prolificacy. There was a treatment × time interaction ($P \leq 0.05$) for concentrations of $P_4$. Concentrations of $P_4$ were decreased ($P \leq 0.04$) in G compared with U on d 2. On d 14, C and P had greater ($P \leq 0.03$) concentrations of $P_4$ and G tended ($P = 0.06$) to have greater $P_4$ concentrations compared with U. In contrast, concentrations of $P_4$ on d 20 were greater ($P \leq 0.03$) in U compared with C. Days to estrus after CIDR removal, as indicated by breeding marks, were greater ($P \leq 0.02$) in U (6.5 ± 1.05) and P (5.9 ± 0.88) compared with G (3.0 ± 0.88). Pregnancy rate within 7 d post CIDR removal were similar ($P = 0.57$) for U, C, P, and G (30 ± 12.27, 30 ± 11.12, 35 ± 10.96, and 51 ± 10.78%, respectively). Similarly, no differences ($P \geq 0.32$) were detected for all remaining reproductive performance data. It appeared the 5 d CIDR coupled with GnRH and PG improved estrus synchronization; however, reproductive performance was not improved by either of the 5 d CIDR protocols. These results warrant further research to determine the efficacy of industry-wide application of the 5 d CIDR in anestrous ewes.

**Key words:** controlled internal drug release, ewe, synchronization

## INTRODUCTION

Controlled internal drug releasing (**CIDR**) inserts were approved for use in sheep by the U.S. Food and Drug Administration (**FDA**) in 2009. Prior to approval, common industry practice was to insert a CIDR for 12 to 14 d with or without an injection of gonadotropin at removal. The current label recommendation is to insert one CIDR per ewe for 5 d with the intention of inducing estrus in ewes during the anestrous season (FDA, 2009). Use of the CIDR, as a synchronization tool, could benefit sheep producers by condensing the lambing season and reducing labor, feed, and facility costs. Producers strategically selling lamb during ethnic holidays and 'out-of-season' can take advantage of increased lamb prices at these times. In addition, uniform lamb crops are easier to manage and market (Carlson et al., 1989).

In two studies by Knights et al. (2000, 2001), a 5 d CIDR treatment was found to stimulate an effective estrus response in seasonally anestrous ewes compared with untreated ewes. Addition of a PG injection at CIDR removal elicited a greater percentage of ewes observed in estrus and a greater lambing rate to the first service period compared with ewes treated with PG alone (Dixon et al., 2006). Moreover, in a recent study, Titi et al. (2010) reported improvements in estrus synchronization and prolificacy with the inclusion of an injection of GnRH prior to insertion and PG at removal of intravaginal progesterone (**$P_4$**) sponges for 5 d during the breeding season. Limited research has been conducted utilizing the 5 d CIDR in combination with GnRH and PG in seasonally anestrous ewes. Therefore, we hypothesized ewes receiving a CIDR coupled with GnRH and PG treatment would display a more synchronized estrus when compared with untreated ewes. The objective of this study was to determine the efficacy and reproductive performance of ewes during the anestrous season after synchronization to estrus with $P_4$ impregnated CIDR inserts in conjunction with GnRH and PG.

## MATERIALS AND METHODS

All procedures involving animals used in this research were approved by the North Dakota State University Institutional Animal Care and Use Committee.

***Animals and Treatments.*** Multiparous and nulliparous Dorset and Katahdin (n = 61 and n = 17, respectively) ewes from the North Dakota State University Fargo Sheep Unit were randomly assigned during the anestrous period (April) to 1 of 4 treatments: 1) untreated (**U**, n = 16); 2) $P_4$ impregnated CIDR insert (EAZI-BREED CIDR Sheep Insert, 0.3 g $P_4$,

Pfizer Animal Health, New York, NY) for 5 d (**C**, n = 21); 3) 5 d CIDR and PG (Lutalyse, 10 mg/mL dinoprost tromethamine i.m., Pfizer Animal Health, New York, NY) at CIDR removal (**P**, n = 20); and 4) GnRH (Factrel, 0.02 mg/mL gonadorelin hydrochloride i.m., Pfizer Animal Health, New York, NY) given at CIDR insertion (d -5) and PG at CIDR removal (**G**, n = 21). Prior to treatment application, ewes were managed as a common group in a dry lot and isolated from all males. Once treatments were initiated, ewes had ad-libitum access to alfalfa hay and had continuous access to fresh water. Intact rams were equipped with marking harnesses and introduced to ewes immediately post CIDR removal with a ram to ewe ratio not exceeding 25:1. Ewes were observed for breeding marks (detected estrus) twice daily at 0800 h and 1700 h and ewes remained with rams for 42 d.

***Blood Collection and Analysis.*** Blood samples were collected via jugular venipuncture into 10 mL serum tubes (BD Vacutainer Serum, Becton, Dickinson and Company, Franklin Lakes, NJ) on d -12, -5, 0, 1, 2, 3, 4, 5, 8, 11, 14, 17, and 20 relative to CIDR removal (d 0). Samples were immediately placed on ice for 2 h prior to centrifugation, centrifuged at 4˚C for 30 min at 1,500 × *g* and serum was collected in plastic 2.0 mL microcentrifuge tubes and frozen at -20 ˚C until assayed. Serum samples were assayed for circulating $P_4$ using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA; Stevenson et al., 2011). The assay kit was validated for ovine serum (Hamra et al., 1986; Schneider and Hallford, 1996) using an assay volume of 100 µl. Assay tubes for the standard curve contained 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, and 20 ng/ul. Assay sensitivity for a 100-µl sample was 0.1 ng/mL. Ewes were determined to be cycling if serum $P_4$ concentrations were greater than 1 ng/mL in either or both the d -12 or -5 samples. All samples with ewe as experimental unit were ran within the same assay and treatments were run in a random order. The intra- and inter-assay CVs were 2.28 and 2.21%, respectively.

***Statistical Analysis.*** Repeated measures of the MIXED procedure (SAS Inst. Inc., Cary, NC) were used to analyze serum concentrations of $P_4$. The model included the main effects of treatment, time, cycling status, and treatment × time interaction. Days to detected estrus and days to lambing were examined using MIXED procedures of SAS. Models included effects of treatment, breed, age, cycling status, and all possible interactions. Reproductive performance data included: percent ewes exhibiting estrus, pregnancy rate, lambing rate, and prolificacy. The number of ewes marked by a ram within 7 d of ram introduction as a percentage of all ewes on study was represented by the percent of ewes exhibiting estrus. Pregnancy rate was defined as the number of ewes lambing as a percentage of ewes in all treatments and the number of lambs born per ewe exposed to rams is represented by lambing rate. Lastly, prolificacy was the number of lambs born per ewe lambing. These components of reproductive performance data were classified as either to the first service period which was 7 d post CIDR removal or overall beginning from d 0 until rams were removed at d 42. These data were analyzed using the GLIMMIX procedures of SAS. The model included the main effects of treatment, breed, age, cycling status, and all possible interactions. Treatment differences were considered significant at $P \leq 0.05$. Interactions that were clearly not significant ($P > 0.20$) were removed from the model.

## RESULTS AND DISCUSSION

***Serum Concentrations of Progesterone.*** In the present study, there was a treatment × time interaction ($P \leq 0.05$, Figure 1) for concentrations of $P_4$. As expected, all treatments receiving a 5 d CIDR insert showed numerically elevated concentrations of $P_4$ at CIDR removal compared with U ewes. It appears, the administration of GnRH 5 d prior to CIDR insertion and PG at removal successfully induced ovulation or caused luteinization or both of the dominant follicle. Concentrations of $P_4$ were decreased ($P \leq 0.04$) in G compared with U on d 2 thus indicating more ewes were in estrus. Even though, prior to treatment initiation, a majority (62%) of ewes were classified as cycling as indicated by a $P_4$ serum concentration sample greater than 1 ng/mL on either or both d -12 or -5. This is not uncommon for the Dorset and Katahdin breed; however, since the overall pregnancy rate (42%) for U ewes was numerically less than the cycling rate (62%), we feel that the ewes were not fully cyclic. It has been documented that a semi-cyclic pattern in ovarian activity is evident during early and late anestrous ewes (Cole and Miller, 1935). Although no differences were detected ($P = 0.64$) among treatments in $P_4$ concentrations, the U treated ewes, had a mean $P_4$ concentration of 3.35 ng/mL on d 0 followed by decreased $P_4$ concentrations on d 4 to 1.40 ng/mL (Figure 1). The decrease in $P_4$ concentrations may have been a result of a short cycle, where the corpus luteum (**CL**) develops a shorter than normal lifespan or the follicular phase induced by the first exposure to the ram was too short (Martin et al., 1986). We feel ram introduction stimulated cyclicity in the U group and they developed functional CL to produce similar quantities of $P_4$ compared with the other treatments. These results are consistent with Martin et al. (1986), who reported that after 6 d of the short cycle, a second ovulation is induced followed by a fertile cycle of normal length. This apparent effect of ram introduction provides justification that a majority of the ewes were either non-cyclic or semi-cyclic. After d 14, serum $P_4$ concentrations of ewes in the U treatment increased to levels similar to all other treatments ($P > 0.10$). On d 14, C and P had greater ($P \leq 0.03$) concentrations of $P_4$ and G tended ($P = 0.06$) to have greater $P_4$ concentrations compared with U. In contrast, concentrations of $P_4$ on d 20 were greater ($P \leq 0.03$) in U compared with C. In the current study, the reduction in $P_4$ concentrations at d 2 suggests decreased luteal activity, which may lead to a more uniform synchronization to estrus.

***Reproductive Performance Data.*** Days to estrus after CIDR removal, as indicated by breeding marks, were greater ($P \leq 0.02$, Table 1) in U (6.5 ± 1.05 d) and P (5.9 ± 0.88 d) compared with G (3.0 ± 0.88 d). This data suggests G treated ewes displayed a tighter synchrony to estrus. Wheaton et

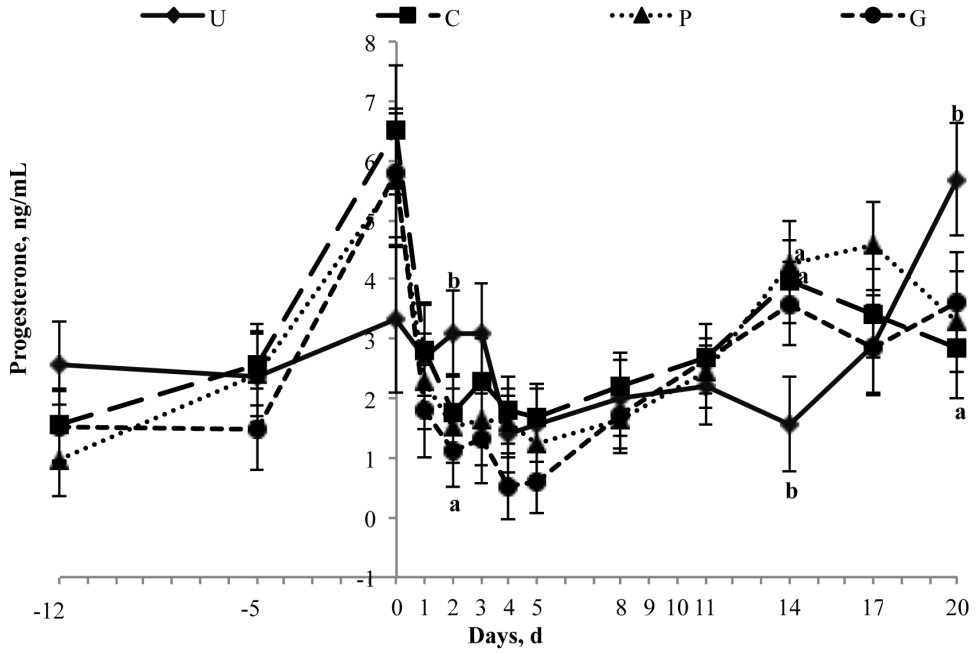


**Figure 1**. Concentrations of progesterone ($P_4$) before and after 5 d controlled internal drug release (CIDR) synchronization treatment protocols. Treatments: U = untreated; C = CIDR inserted on d -5 and removed on d 0; P = 5 d CIDR and PG given at removal; G = GnRH given at CIDR insertion and PG given at removal. A treatment × time interaction was detected ($P \leq 0.05$). Means with different superscripts within day differ ($P < 0.05$).

**Table 1**. Reproductive performance of ewes after 5 d controlled internal drug release (CIDR) synchronization treatment protocols

| | Treatment[1] | | | | | |
|---|---|---|---|---|---|---|
| Variable | U | C | P | G | SEM[2] | *P*-value[3] |
| Days to estrus[4] | 6.5[a] | 3.4[bc] | 5.9[ac] | 3.0[b] | 1.05 | 0.02 |
| Days to lambing[5] | 152 | 152 | 154 | 150 | 2.99 | 0.55 |
| Ewes exhibiting estrus, %[6] | | | | | | |
| First service period[7] | 63 | 70 | 65 | 92 | 0.11 | 0.32 |
| Overall | 94 | 82 | 100 | 96 | 0.07 | 0.34 |
| Pregnancy rate, %[8] | | | | | | |
| First service period | 30 | 30 | 35 | 51 | 0.17 | 0.57 |
| Overall | 42 | 39 | 55 | 58 | 0.12 | 0.59 |
| Lambing rate[9] | | | | | | |
| First service period | 0.36 | 0.42 | 0.55 | 0.64 | 0.18 | 0.65 |
| Overall | 0.47 | 0.51 | 0.85 | 0.71 | 0.18 | 0.47 |
| Prolificacy[10] | | | | | | |
| First service period | 1.22 | 1.45 | 1.38 | 1.19 | 0.22 | 0.70 |
| Overall | 1.15 | 1.31 | 1.55 | 1.24 | 0.19 | 0.44 |

[a-c]Values within the same row with different superscripts differ ($P \leq 0.05$).
[1]Treatments: U = untreated; C = CIDR inserted on d -5 and removed on d 0; P = 5 d CIDR and PG given at removal; G = GnRH given at CIDR insertion and PG given at removal with ram exposure starting on d 0.
[2]U: n = 16; C: n = 21; P: n = 20; G: n = 21.
[3]*P*-value for F-tests of the mean.
[4]Days to estrus post ram introduction as indicated by marks from rams equipped with marking harnesses.
[5]Days to lambing post ram introduction.
[6]The number of ewes marked by a ram within 7 d of ram introduction as a percentage of all ewes treated.
[7]First service period is defined as the first 7 d post CIDR removal.
[8]The number of ewes lambing as a percentage of ewes in all treatments.
[9]The number of lambs born per ewe exposed to rams.
[10]The number of lambs born per ewe lambing.

al. (1992) also observed ewes treated with a CIDR having a reduced interval to estrus compared with untreated ewes. No differences were detected ($P \geq 0.32$) among treatments for percentage of ewes exhibiting estrus. However, in a study by Wheaton et al. (1992), the percentage of untreated ewes (95%) exhibiting estrus and those receiving a CIDR insert for 12 d (100%) was similar to the percentage of U ewes exhibiting estrus (94%) in the current study. In two separate reports, Knights et al. (2000, 2001) reported a greater proportion of $P_4$ treated ewes were marked by rams compared with untreated ewes. Pregnancy rate within 7 d post CIDR removal were similar ($P = 0.57$) in the current study for U, C, P, and G (30 ± 12.27, 30 ± 11.12, 35 ± 10.96, and 51 ± 10.78%, respectively). In contrast, Knights et al. (2000, 2001) documented an increase in pregnancy rate to the first service period and an increase in lambing rate (Knights, 2000) in $P_4$ treated ewes than in untreated ewes. No differences ($P \geq 0.44$) were observed among treatments for lambing rate and prolificacy. In contrast to the current study, Titi et al. (2010) reported an increase in fecundity in ewes treated with exogenous $P_4$ when coupled with the use of GnRH and PG. Although no differences were detected among reproductive performance data this study suggests that G treatment was more effective at concentrating estrus shortly after CIDR removal compared with U and P treatments.

## IMPLICATIONS

The recently approved 5 d CIDR for use in sheep during the anestrous period when used alone was not effective in generating synchronization results desired by a producer. However, when the CIDR was coupled with GnRH and PG estrus synchronization was improved in ewes when compared with untreated ewes during the anestrous season, but did not influence reproductive performance. The efficacy of CIDR protocols to synchronize pregnancy and improve fertility during the anestrous period was not clearly demonstrated and more research is needed prior to recommendation for the utilization of the 5 d CIDR in the commercial sheep industry.

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# EFFECTS OF MATERNAL FLUOXETINE DOSAGE ON LAMB SERUM HORMONE CONCENTRATIONS AND REPRODUCTIVE TRAITS

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**ABSTRACT:** Fluoxetine (human anti-depressant; selective serotonin reuptake inhibitor; FLX) depresses lactation in women and research has been conducted to evaluate sheep as a human lactation model. Fluoxetine has been shown to appear in the milk, yet little is known on how this drug will influence progeny. Our objective was to evaluate the effect of maternal FLX exposure on lamb growth and development. We utilized a completely randomized design with 18 mature Suffolk ewes (BW = 91 ± 12 kg; BCS = 2.0 ± 0.5). Treatments consisted of 0 (control) and 80 mg FLX; ewes were individually fed FLX using ground corn as the carrier at 0700 beginning on approximately d 126 of gestation and continued until 3 wk postpartum. The resulting 31 Suffolk-cross lambs were weighed at birth (birth weight = 4.6 ± 1.0 kg) and weaning (BW = 24.0 ± 3.2 kg; approximately 9 wk of age) and ADG was calculated. Blood samples were taken from lambs on d 1, 3, 5, 7, 14, 21, 28, 42, 56, and 66 and serum was analyzed for serum prolactin (PRL), IGF-I, and triiodothyronine ($T_3$) concentrations. Following weaning, ewe lambs were kept for subsequent evaluation. A total of 21 Suffolk-cross ewe lambs (BW = 44.7 ± 4.32 kg) were evaluated for age at puberty and pregnancy rate. No additional treatment was administered, as we only wanted to evaluate the effect of maternal FLX. Average daily gain from birth to weaning was similar ($P = 0.42$) between control and FLX-exposed lambs. Lambs exposed to maternal FLX had depressed PRL ($P = 0.005$), IGF-I ($P < 0.0001$), and $T_3$ ($P = 0.03$) compared with controls. Age at first cycle was similar ($P = 0.53$) between FLX-exposed and control ewe lambs. Similarly, pregnancy rates were comparable ($P = 0.37$) between both control and FLX-exposed ewe lambs. Maternal treatment with FLX reduced PRL, IGF-I, and $T_3$ serum concentrations in lambs; however this effect did not appear to have a negative impact on growth or reproductive performance.

**Keywords**: growth, fluoxetine, sheep

## INTRODUCTION

Depression is common in women during pregnancy and lactation and typically it is recommended to treat the depression with antidepressants. Selective serotonin reuptake inhibitors (**SSRI**) are a common choice during pregnancy and lactation due to their safety, effectiveness, and lower occurrence of maternal side effects (Nonacs and Cohen, 2002). Specifically, a SSRI that is commonly used is fluoxetine (**FLX**; Prozac, Eli Lilly & Co., Indianapolis, IN).

Selective serotonin reuptake inhibitors such as FLX act to increase the bioavailability of serotonin (**5-HT**) by preventing its reuptake into the cell, and subsequent degradation into its metabolite. The use of FLX during pregnancy has also been shown to cause negative fetal outcomes, such as depressed growth and fetal abnormalities (Chambers et al., 1996). In general, FLX depresses birth weight and gestation length, but the depression was greater in the group where the fetus was exposed to FLX the entire pregnancy compared with the group that was only exposed in the first trimester, suggesting that late pregnancy exposure to FLX is more severe (Chambers et al., 1996). Chambers et al. (1996) also reported that pre-natal exposure to FLX increased the risk of minor malformations compared with controls.

Animals have become a useful model to determine effects of antidepressants on pregnancy and lactation due to limited human experimental studies. Furthermore, comparisons between the fetal lamb and humans have shown favorable results and similarities in physiological functions (Rurak et al., 1991). Several human health studies used sheep in experiments with FLX (Morrison et al., 2001; Kim et al., 2004). We hypothesized that FLX exposure pre- and postnatally would depress lamb growth and growth related hormone concentrations; while delaying puberty and decreasing fertility. Thus, our objective was to determine if sheep could be a suitable model to study the effects of FLX on neonatal growth and development.

## MATERIALS AND METHODS

Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. All experimental procedures were completed at New Mexico State University, Las Cruces, NM (32º 19' 11'' N, 106º 45' 55'' W; elevation 1,219 m).

***General.*** Animals exposed to FLX remained on New Mexico State University's Sheep Unit for at least 5 months post-exposure. Kim et al. (2004) noted that in pregnant sheep the elimination half-life of FLX and norfluoxetine (**NFLX**; metabolite of FLX) is 6.7 and 23 h, respectively. In the fetal lamb, the elimination half-life of FLX and NFLX is 0.6 and 0.8 h, respectively (Kim et al., 2004). We believe there to be no residual FLX or NFLX remaining in the animal if or when they sold.

***Animal, Facilities, and Diet.*** Eighteen Suffolk cross ewes (BW = 91 ± 12 kg; BCS = 2.0 ± 0.5) in late gestation were used in this experiment. Prior to the experiment

estrus was synchronized using a progesterone-impregnated intravaginal insert (EAZI-BREED CIDR, 0.3 progesterone; Pharmacia and Upjohn Co., Hamilton, New Zealand) and rams were penned with the ewes on Sept. 13, 2011. Mature Suffolk and Rambouillet rams were used for breeding and were fitted with marking harnesses. The date of breeding was recorded and rams remained with ewes for approximately 2 estrous cycles. Following mating, rams were removed from pens and ewes were fed maintenance diets until approximately the third trimester of pregnancy.

Ewes were fed once daily 2.7 kg chopped alfalfa hay and 454 g of ground corn during the last trimester of pregnancy and through parturition. Feeding time was 0700 and minerals and fresh water was available free choice via automatic waterers. Ewes were penned in 2 dry lot pens with fence-line feed wood bunks where they received the alfalfa hay. Corn was fed individually as ewes were sorted into 1.5 x 1.5 m pens at 0700 in order to facilitate the administration of treatment until parturition. Approximately 4 wk prior to parturition ewes were vaccinated with *Clostridium perfringens* types C & D – Tetanus Toxoid (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO).

At birth, lambs were individually identified by unique premise ear tag and scrapie tag; their navels dipped with iodine. Lambs were weighed at birth (birth weight = 4.6 ± 1.0 kg) and weaning (BW = 24.0 ± 3.2 kg; approximately 9 wk of age) and ADG was calculated. Lambs had access to feed and water before and after weighing. Lambs were docked and male lambs were castrated using elastrator bands at 1-2 wk of age and all lambs were vaccinated with *Clostridium perfringens* types C & D – Tetanus Toxoid (Boehringer Ingelheim Vetmedica, Inc.) and given an injection of a selenium and vitamin E (Bo-Se; Intervet/Schering-Plough Animal Health, Summit, NJ). A mix of 3:1 ground corn to soybean meal with added Clovite (Pfizer Animal Health, New York, NY) and sodium chloride was provided ad libitum via a creep feeder for the lambs at approximately 30 d of age. Weaning occurred at approximately 65 d of age and lambs were penned separately from their dams.

Following weaning, ewe lambs were kept for subsequent evaluation. A total of 21 Suffolk-cross ewe lambs (BW = 44.7 ± 4.32 kg) were evaluated for age at puberty and pregnancy rate. No additional treatment was administered, as we only wanted to evaluate the effect of maternal FLX. At approximately 7 mo of age, ewe lambs from the initial lactation study as well as other replacement ewe lambs (not on study previously) were weighed and blood collection began. A total of 23 ewe lambs (BW = 51.0 ± 8.1 kg) were weighed August 15, 2011 and 2 ewes were removed from study due to illness not related to treatment. Ewes received 1.4 kg of chopped alfalfa hay and 0.9 kg of ground corn and were provided water ad libitum. Ewe lambs were exposed to a fertile mature Dorper ram, fitted with a marking harness, for approximately 2 estrous cycles beginning on October 31, 2011. At approximately d 70 of gestation, external flank ultrasound (3.5 MHz probe; SSD-500V, Aloka Co., Tokyo, Japan) was conducted to determine pregnancy.

***Design and Treatments.*** The experiment was a completely randomized design with ewe as the experimental unit. Ewes carrying twins [determined by external flank ultrasound (3.5 MHz probe; SSD-500V, Aloka Co., Tokyo, Japan) at approximately d 70 of gestation] were selected and stratified by breeding date to treatments. Ewes were separated into 2 pens, with 5 ewes from each treatment randomly assigned to each pen on approximately d 126 of gestation. Treatment dosing also began on this day and continued until 3 wk after lambing. Treatments consisted of no FLX (control) or 80 mg FLX. Ewes were orally dosed daily at 0700 h by top dressing 454 g of ground corn with 0 or 80 mg FLX. Treatments were administered while ewes were individually penned at 0700 daily. Two fluoxetine capsules, 40 mg each, were opened and contents were dispensed on moistened corn (5 mL water) and were rinsed with 5 mL water. Corn fed to control ewes was also top dressed with 10 mL water. Only ewes had access to the treated ground corn. Two ewes were removed from study, 1 ewe died due to unknown causes and the other lambed late due to being rebred. Lambs remained on the same treatment as their dam (ie. lambs from control ewes served as controls, whereas, lambs from FLX-treated ewes were considered FLX).

***Serum Collection and Analyses.*** On d 1, 3, 5, 7, 14, 21, 28, 42, 56, and 66 after lambing, blood serum was collected via jugular venipuncture from lambs using sterile serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO). Blood samples were centrifuged (1,500 × g at 4 ˚C for 15 min) and serum was harvested and stored frozen (-20º C) until assayed. Serum samples from d 1, 3, 5, 7, 14, and 21 were assayed for prolactin (**PRL**) concentration using procedures outlined by Spoon and Hallford (1989; intra-assay CV = 8.5%). Serum samples from d 7, 14, 21, 28, 42, 56, and 66 were evaluated for concentration of IGF-I (Berrie et al., 1995, as modified by Camacho et al., 2012; intra-assay CV = 13.4%) and triiodothyronine ($\mathbf{T_3}$; Wells et al., 2003; intra-assay CV = 4.05%; inter-assay CV = 0.2%). For the second half of the experiment evaluating reproductive traits of ewe lambs, blood serum was collected as described previously twice weekly starting on August 19, 2011, approximately 7 mo of age, for a total of 21 samples as the ewe lambs began cycling. The final serum samples were collected on October 28, 2011. Serum progesterone concentrations were determined by RIA as described by Schneider and Hallford (1996; mean intra-assay CV = 4.8%; inter-assay CV = 3.4%).

***Statistical Analyses.*** The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to analyze lamb birth weight and ADG, PRL, IGF-I, $T_3$, and $P_4$ values. Lamb birth weight and ADG were single measures with diagonal covariant structure and main effect of treatment. Serum PRL, IGF-I, $T_3$, and $P_4$ values were analyzed as a repeated measure over days where lamb was in the whole plot and the sub-plot was day and day × treatment interaction. The covariant structure for serum PRL, IGF-I, $T_3$, and $P_4$ values was autoregressive as it was the best fit for our data. For pregnancy data, the FREQ procedure of SAS was used. Individual lamb was the experimental unit for all variables.

## RESULTS AND DISCUSSION

Birth weights and ending weights (d 112) were similar for control and FLX-treated lambs ($P = 0.35$; Table 1). Each lamb ADG was calculated by subtracting the birth weight from the BW and dividing by number of days postpartum. We observed no treatment × day interactions, so only main effects of treatment are reported. Lamb ADG from BW taken on d 7, 14, 21, 28, 42, 56, 70, 84, and 112 post-lambing were similar between control and FLX lambs ($P > 0.22$; Table 1), but only the ADG at weaning (d 56) is reported. While the direct effects of SSRI treatments cause an increase in the amount of extracellular serotonin, further side effects are possible. Weight loss is a common side effect when adults take FLX; researchers suggested that FLX may directly decrease weight gain in infants who receive FLX through breast milk (Chambers et al., 1999). In humans, reduced birth weights and postnatal weight gain were observed when women were exposed to FLX during their third trimester (Nordeng et al., 2001). A study conducted with rats, showed that pregnant rats receiving FLX had lower BW gain and delivered smaller pups (Vorhees et al., 1994). Ewes treated with FLX had shortened gestation lengths in comparison to the controls (145.6 vs. 147.9 d; $P = 0.02$; SEM = 0.6; P. Black, unpublished data) which may have impacted lamb growth values. Similar lamb ADG may be due to the lack of differences in birth weights.

No treatment × time interactions were observed for IGF-I, PRL, or $T_3$, thus only main effects of treatment are reported in Table 2. Serum IGF-I, PRL, and $T_3$ concentrations were lower ($P < 0.05$) in FLX-exposed lambs compared with controls (Table 2). Ewe lambs from control and FLX-treated ewes had similar ages at puberty ($P = 0.53$) and comparable pregnancy rates ($P = 0.37$; Table 3).

**Table 1.** Birth weights and periodic ADG of lambs from multiparous Suffolk-cross ewes treated with 0 (control) or 80 mg fluoxetine (FLX) beginning on d 126 of gestation and continued until 3 wk after lambing

| Item | Control | FLX | SEM[2] | *P*-value |
|---|---|---|---|---|
| Lambs, no. | 15 | 15 | | |
| Birth weight, kg | 4.7 | 4.3 | 0.2 | 0.35 |
| End weight (d 112), kg | 32.9 | 32.9 | 0.96 | 0.99 |
| ADG[1] | | | | |
| d 1-56 | 0.25 | 0.23 | 0.011 | 0.23 |

[1]Gain was calculated by taking the BW at d 56 minus birth weight and dividing it by 56.
[2]Most conservative standard error (n = 15).

**Table 2.** Serum hormone concentrations of lambs from multiparous Suffolk-cross ewes treated with 0 (control) or 80 mg fluoxetine (FLX) beginning on d 126 of gestation and continued until 3 wk after lambing. Samples were taken beginning on 1 wk of age through weaning[1]

| Item | Control | FLX | SEM[2] | *P*-value |
|---|---|---|---|---|
| Lambs, no. | 15 | 15 | | |
| IGF-I, ng/mL | 230.7 | 175.7 | 6.5 | < 0.0001 |
| Prolactin, ng/mL | 50.4 | 30.8 | 4.5 | 0.005 |
| Triiodothyronine, ng/mL | 2.26 | 1.99 | 0.08 | 0.03 |

[1]No treatment × day interaction was observed ($P > 0.10$); thus only main effects of treatment are reported.
[2]Most conservative standard error (n = 15).

**Table 3.** Age at puberty as determined by 2 consecutive serum progesterone concentrations over 2 ng/mL and pregnancy rates in ewe lambs from Suffolk-cross ewes treated with 0 (control) or 80 mg fluoxetine (FLX) beginning on d 126 of gestation and continued until 3 wk after lambing

| Item | Control | FLX | SEM[1] | *P*-value |
|---|---|---|---|---|
| Lambs, no. | 12 | 9 | | |
| Age at puberty, d | 234 | 237 | 3.6 | 0.53 |
| Pregnancy rate[2] | | | | |
| No, % | 4.76 | 9.52 | | |
| Yes, % | 52.38 | 33.33 | | |

[1]Most conservative standard error (n = 9).
[2]Percentage of total calculated within chi-sq program (Chi Square = 0.81, $P = 0.37$).

Insulin-like growth factor-I is similar to insulin and its primary role is regulating growth. During pregnancy, fetal tissues have been found that express IGF-I early in pregnancy and concentrations of IGF-I in fetal and cord serum are related to fetal size (Giudice et al., 1995). Following parturition, IGF-I has a prevalent role in regulation of growth (LeRoith, 1997). In a human study, SSRI (including FLX) administered throughout pregnancy resulted in increased numbers of IGF-I receptors in placental tissue of infants exposed to FLX when compared with controls (Davidson et al., 2009). They suggested that the increase in IGF-I receptors in FLX-treated infants could be a compensatory mechanism for depressed IGF-I and cortisol concentrations in an attempt to improve growth (Davidson et al., 2009). Hilli et al. (2009) observed that fetal SSRI exposure decreased IGF-I concentration in cord blood. No research has evaluated postnatal serum FLX concentrations of IGF-I in infants, hence in utero exposure to FLX could have lingering effects on growth.

Thyroid hormones play an active role in normal brain development during pre- and post-natal development (Morreale de Escobar, 2001). Early in gestation, the fetus is reliant on maternal $T_4$ and lack of $T_4$ for the fetus could result in irreversible mental and psychomotor impairments (Morreale de Escobar, 2001). Maternal thyroid hormones are necessary for normal fetal growth and development. Selective serotonin reuptake inhibitors in general have been found to decrease $T_3$ and $T_4$ in adults, but no work has been conducted in infants (Gitlin et al., 2004). We found that lambs born to FLX-treated ewes had depressed concentrations of $T_3$. This could be the result of the potential depression of $T_3$ and $T_4$ in the FLX-treated dams as seen by Gitlin et al. (2004). More research is needed to fully understand the mechanism of the depression of $T_3$ in offspring.

Prolactin is inhibited by dopamine (**DP**) and its release can be signaled by 5-HT (Muller and Nistico, 1989). Depression in PRL concentrations in the current experiment could be the result of increased concentrations of DP. In a rat study, FLX caused increased extracellular concentrations of 5-HT, DP, and norepinephrine (Bymaster et al., 2002). When FLX was administered to pregnant ewes, maternal PRL concentrations were not affected (Morrison et al., 2005). We also observed in previous work that maternal PRL concentrations were unchanged in the first 3 d following parturition in ewes (P. Black, unpublished data). We measured lamb serum PRL concentrations over the first month post-natal and observed a depression in PRL. In a previous study, we observed that lambs exposed to FLX prenatally had depressed PRL concentrations in the first 3 d following birth (P. Black, unpublished data). No other studies have evaluated serum PRL concentrations in progeny exposed to maternal FLX; however, we believe that FLX depresses PRL due to its ability to increase DP concentrations.

## IMPLICATIONS

Fluoxetine when dosed orally to pregnant and lactating ewes resulted in depressed concentrations of insulin-like growth factor-I, prolactin, and triiodothyronine concentrations in their offspring. However, no significant effect of fluoxetine was observed on lamb average daily gain or reproductive soundness as age at puberty and pregnancy rates were similar between control and fluoxetine-exposed ewe lambs. Thus, based on current and previous studies, further research is needed to study the potential negative outcomes of exposing progeny to fluoxetine during pregnancy and lactation.

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# DIGESTIBILITY OF ALGAL BIOFUEL CO-PRODUCT IN A FORAGE DIET

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**ABSTRACT:** Co-product produced from extraction of oil from microalgae grown for biofuel production represents a novel feedstuff for ruminants. The objective of this study was to determine the influence of lipid extracted algae (LEA) on feed intake and diet digestibility when included in a forage diet. We hypothesized that an isonitrogenous addition of LEA to a forage diet would yield results similar to soybean meal (SBM). Fifteen crossbred wethers (43 ± 1.4 kg BW) fitted with ruminal and duodenal cannulas were used in a completely randomized design. Lambs were fed twice per day at 110% of previous 3 d DMI. Experimental diets included: 1) sorghum-sudan hay (CP 8.3%; NDF 52.79%, DM basis; HAY), 2) sorghum-sudan hay plus LEA (CP 13.6%; NDF 44.19% DM basis; ALGAE), and 3) sorghum-sudan hay plus SBM (CP 10.6%; NDF 51.24%, DM basis; SOY). Animals were adapted to treatments for 10 d followed by 5 d sample collection. Treatment did not influence OM intake ($P = 0.99$) or total tract OM and NDF digestibility ($P > 0.10$). Total tract CP digestibility was lowest for ALGAE while SOY and HAY did not differ (75.86 vs 84.88 and 82.12 ± 1.177%, respectively; $P < 0.01$). Ruminal pH, liquid dilution rate, and volume did not differ ($P > 0.10$) by treatment. Soybean meal increased ($P < .01$) ruminal ammonia by 57.4% and 56.0% compared with ALGAE and HAY, respectively. Total VFA production and molar proportion of butyrate did not differ ($P > 0.14$) by treatment. Acetate was highest for HAY, lowest for SOY, and ALGAE did not differ from HAY and SOY (74.11, 71.57, and 69.07 ± 1.245 mol/100 mol for HAY, ALGAE, and SOY, respectively; $P = 0.04$). Propionate was greatest for ALGAE which differed from HAY, while SOY was similar to ALGAE and HAY (14.80, 19.89, and 17.85 ± 1.233 mol/100 mol HAY, ALGAE, and SOY, respectively; $P = 0.04$). Acetate:Propionate ratio was lowest ($P = 0.03$) for ALGAE. Adding ALGAE to a forage diet resulted in increases in propionate production while OM and NDF digestibility was comparable to SOY. However, ALGAE resulted in the lowest ruminal ammonia and total tract CP digestibility. These data imply that CP in LEA may not be as soluble as CP in SBM, therefore we reject our hypothesis.

**Key words:** biofuels, digestibility, sheep

## INTRODUCTION

The Energy Independence and Security Act of 2007 and the Renewable Fuels Standard, have mandated that by 2022, 36 billion gallons of renewable fuels must be blended per year, of which, at least 21 billion gallons must be supplied by advanced biofuels. Advanced biofuels are derived from renewable biomass including cellulosic ethanol and microalgae, that create no more than half of the greenhouse gas emissions of the fuel they replace (Cortes-Caminero, 2010).

Microalgae are photosynthetic cell factories that convert sunlight and carbon dioxide to biomass which includes lipids, proteins and carbohydrates (Chisti, 2007). There are many proposed benefits to utilizing microalgae for biofuel production such as: high oil productivity per acre compared to oilseed crops, ability to grow in many water sources including brackish, fresh, and marine, and little competition from the production agricultural industry for land used in algal cultivation. It is common for strains of microalgae to contain 20-50% of their dry weight in lipid (Chisti, 2007). In the biofuel production process, lipid is separated from the algal cell by various extraction methods. The lipid is refined to produce transportation and aviation biofuels while the lipid extracted algal biomass (**LEA**) could represent a potential revenue stream in a variety of industries. The use of LEA in livestock diets could be a potential high volume market. Our laboratory nutrient analysis of LEA, from *Nannochloropsis sp.*, reveals that LEA ranges in crude protein from 17 to 35% (unpublished data). We hypothesized that an isonitrogenous addition of LEA to a forage diet would yield similar feed intake and diet digestibility when compared to soybean meal (**SBM**). The objective of this study was to determine the influence of LEA on feed intake and diet digestibility when included in a forage diet.

## MATERIALS AND METHODS

All procedures for the following experiment were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Fifteen crossbred wether lambs (43 ± 1.4 kg BW) were fitted with a J-style duodenal (manufactured by New Mexico

State University Department of Animal and Range Sciences) and ruminal cannula (Bar Diamond, Inc., Parma, ID). Lambs were individually housed in a temperature controlled room within 1.2 m x 2.3 m pens with concrete floors. Pens were bedded with wood shavings. Lambs were randomly assigned to pen and treatments were randomly assigned to individual pens.

***Diets.*** Lambs were assigned to 1 of 3 dietary treatments in a complete randomized design (n = 5). Animals were allowed ad libitum access to water and treatments were delivered twice daily at 0630 and 1800 h at 110% of previous 3 d DMI. Treatments were: 1) sorghum-sudan hay (6.4% CP and 62.3% NDF; DM basis) with no supplemental protein (**HAY**), 2) HAY plus LEA (**ALGAE**), 3) HAY plus SBM (**SOY**). Treatments ALGAE and SOY were formulated to contain 12.0% CP (DM basis). The *Nannochloropsis* sp. LEA used in this study was purchased as a solvent extracted, dried, granular ingredient and was analyzed for CP, NDF, and ether extract (25.9%, 24.7%, 15.3%, respectively, DM basis) through a commercial laboratory (SDK Laboratories, Hutchison, KS). Diet composition and actual nutrient analysis are presented in Table 1.

***Sampling.*** The experimental period was 15-d, with 10-d allotted for adaptation to ensure adequate adjustment of the gastrointestinal tract to the dietary treatment and 5-d of intensive sampling. Beginning on d 11 to d 15 of the experimental period, feed, orts, and fecal samples were collected for analysis. Samples of each treatment were collected at feeding and composited by treatment. Orts were collected daily at 1800, weighed for DMI calculation, and stored for nutrient analysis. Lambs were fitted with canvas fecal bags on d 8 for adaptation (Hastings Canvas, Hastings, NE). Total fecal collection was conducted from d 11 to 15 at 1800. Feces were weighed, sub-sampled, and composited (10% of output; wet basis) by animal. Fecal samples were stored frozen for later analysis of CP, NDF, DM and OM. Immediately prior to the 0630 feeding on d 15, 50 mL of Co-EDTA was dosed intraruminally for fluid passage rate determination. Ruminal fluid was collected at 2, 4, 6, 8, 10, and 12 h post- dosing via suction strainer (Bar Diamond, Parma ID). Immediately after collection, ruminal pH was measured using a portable pH meter (Accumet AP72, Fisher Scientific, Pittsburgh, PA). Aliquots of ruminal fluid were divided and stored in 15-mL conical tubes for VFA, Co- EDTA, and acidified with 2.5 mL of 5% HCl for $NH_3$ analysis. All ruminal fluid samples were immediately placed on ice and later frozen and stored at -20°C.

***Laboratory analysis.*** Feed, orts, and fecal samples were dried for 48 h in a 55°C forced-air oven and ground (Wiley mill; 2-mm screen). All feed, orts, and fecal samples were analyzed for DM and ash (AOAC, 1990). Orts and fecal samples were analyzed for NDF and CP and feed samples for CP, NDF, ether extract, and OM at a commercial laboratory (SDK Laboratories, Hutchison, KS). Ammonia nitrogen (**$NH_3$**) in ruminal fluid was analyzed using the procedures of Broderick and Kang (1980), adapted to a microtiter plate (BioTek Instruments, Winooski, VT) and measured at 630 nm. Gas chromatography was used to measure VFA concentration in ruminal fluid (Goestch and Galyean, 1983). Cobalt concentration of ruminal fluid was determined with an air- plus-acetylene flame using

**Table 1.** Ingredient composition and chemical analysis of diets fed to wethers

| | Treatments[1] | | |
|---|---|---|---|
| Item | HAY | ALGAE | SOY |
| Ingredient composition, % of DM | | | |
| Sorghum-sudan hay | 66.58 | 47.80 | 56.91 |
| Lipid extracted algae | - | 21.46 | - |
| Soybean meal | - | - | 10.74 |
| Corn | 12.21 | 13.69 | 13.69 |
| Molasses | 6.17 | 6.74 | 6.70 |
| Dicalcium phosphate | 1.46 | 0.23 | - |
| Limestone | 2.43 | - | 0.28 |
| Vitamin ADE Mixture[2] | 0.40 | 0.40 | 0.39 |
| NaCl | 0.72 | 0.72 | 0.71 |
| Actual nutrient analysis, % DM[3] | | | |
| CP | 8.27 | 14.06 | 10.20 |
| NDF | 51.66 | 43.37 | 50.05 |
| Ether extract | 1.55 | 2.22 | 1.43 |
| OM | 86.71 | 90.47 | 90.48 |

[1]Treatments: HAY = sorghum-sudan hay (6.4% CP and 62.3% NDF; DM basis) with no supplemental protein; ALGAE = HAY plus lipid extracted algae; SOY = HAY plus soybean meal.
[2]Vitamin ADE Mixture: mixture containing vitamin A at 30,000 IU/g of mix, vitamin D at 176,000 IU/g of mix, and vitamin E at 275 IU/g of mix.
[3]Nutrient analysis performed by SDK Laboratories (Hutchison, KS)

atomic absorption spectroscopy (PerkinElmer Model 3110, Waltham, MA) as described by Uden et al. (1980).

***Calculations and Statistical Analysis.*** Passage rate and digestibility were calculated as described in Scholljegerdes et al. (2004). All data were analyzed as a completely random design using the MIXED model (SAS Inst. Inc., Cary, NC) with repeated measures for VFA and $NH_3$. Animal was the experimental unit and animal × treatment was the random variable. The model included treatment, day, and treatment × day. Using Akaike's information criterion, compound symmetry was determined to be the most desirable covariance structure. Means were calculated using LSMEANS. Treatment effects were considered significant at a $P \leq 0.05$ and as a tendency if $P > 0.05$ and $\leq 0.10$. When *F*-tests were significant, mean separations were performed using PDIFF. There were no treatment x day interactions ($P \geq 0.24$) observed, therefore only main effects are presented.

## RESULTS AND DISCUSSION

***Intake and Digestibility.*** The addition of LEA or SBM to a forage diet did not affect OM, CP or NDF ($P \geq 0.13$) intake. Total tract digestibility of OM and NDF ($P \geq 0.12$; Table 2) were also not affected. These data differ from Guthrie and Wagner (1988) who demonstrated that increasing levels of SBM (0, 121, 241, 362, 603 g/d) added to a forage diet increased DMI in beef heifers. Bodine et al. (2000) evaluated the effects of supplementing corn and SBM to beef steers consuming prairie hay and reported that with increasing level of SBM supplementation (0, 33, 66, 100% of DIP requirement) there was an increase in total OM intake. Total tract digestibility of OM in this same study also increased linearly with SBM inclusion level. One possible explanation for this is that the CP in the hay used by Guthrie and Wagner (1988) was 5% CP and in Bodine et al. (2000) was 6.1%. According to Mathis et al. (2000), when basal forage is above 7% CP, protein supplementation does not improve digestibility. The CP of forage used in this experiment was 6.43% and may account for the lack of increase in digestibility of OM and NDF with protein supplementation. Our results are similar to that of Bohnert et al. (2002), who reported no effect of CP supplementation on OM intake of forage that was 5.2% CP.

Total tract digestibility of CP was similar for HAY and SOY treatments, while ALGAE reduced ($P < 0.01$) CP digestibility by 7.8% and 11.2% compared to HAY and SOY, respectively. These data suggest that CP in LEA, may not be as digestible as CP in SBM or HAY. These observations are further supported by ruminal $NH_3$ values observed in this experiment. Specifically, ruminal $NH_3$was greatest for SOY, while ALGAE and HAY did not differ (6.61 vs. 3.66 and 3.72 ± 0.56 mM, respectively; ($P < 0.01$). Soybean meal is considered a good source of RDP (NRC, 2000). A linear increase in ruminal $NH_3$ was seen by Bodine et al. (2000), when increasing levels of SBM were included with a diet of prairie hay and supplemental corn. According to Satter and Slyter (1974) ruminal $NH_3$ levels should range 1.2 to 2.9 mM to support ruminal bacterial growth. Ruminal $NH_3$ cocnentrations observed in our study were well within

**Table 2.** Influence of adding lipid extracted algae or soybean meal to sorghum-sudan hay

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Item | HAY | ALGAE | SOY | SEM[2] | *P*-value |
| OM Intake, g/Kg BW | 12.5 | 12.6 | 12.9 | 2.167 | 0.99 |
| CP Intake, g/Kg BW | 1.0 | 1.7 | 1.2 | 0.207 | 0.12 |
| NDF Intake, g/Kg BW | 7.3 | 5.6 | 6.9 | 1.077 | 0.53 |
| Digestibility | | | | | |
| OM | 62.5 | 55.4 | 66.4 | 3.524 | 0.12 |
| CP | 82.1[a] | 75.9[b] | 84.9[a] | 1.177 | < 0.01 |
| NDF | 81.6 | 79.6 | 81.0 | 2.771 | 0.88 |
| Ruminal pH | 6.8 | 6.8 | 6.8 | 0.065 | .096 |
| Ruminal $NH_3$, mM | 3.7[a] | 3.7[a] | 6.6[b] | 0.56 | <0.01 |
| Ruminal Volume, L | 8.6 | 5.8 | 7.5 | 1.017 | 0.19 |
| Ruminal Outflow, L/h | 0.5[a] | 0.3[b] | 0.3[b] | 0.061 | < 0.05 |
| Ruminal Dilution, %/h | 6.2 | 5.5 | 3.9 | 0.798 | 0.16 |
| Ruminal total VFA, mM | 58.8 | 66.6 | 67.8 | 7.603 | 0.67 |
| Ruminal VFA, mol/100 mol | | | | | |
| Acetate | 74.1[a] | 71.6[ab] | 69.1[b] | 1.245 | 0.04 |
| Propionate | 14.8[a] | 19.9[b] | 17.8[ab] | 1.233 | 0.04 |
| Butyrate | 9.2 | 6.7 | 8.2 | 0.837 | 0.14 |
| Acetate:propionate | 5.0[a] | 3.7[b] | 4.1[ab] | 0.307 | 0.03 |

[1]Treatments: HAY = sorghum-sudan hay (6.4% CP and 62.3% NDF; DM basis) with no supplemental protein; ALGAE = HAY plus lipid extracted algae; SOY = HAY plus soybean meal.
[2]n = 5
[a,b]values within rows with differing superscripts differ $P \leq 0.05$ between treatments.

this range; therefore, N was not limiting in the rumen and likely was not the reason for a reduction in total tract CP digestibility.

No differences ($P \geq 0.16$) were observed for ruminal volume or liquid dilution rate, however, ruminal outflow did differ ($P = 0.05$). The ruminal fluid outflow rates for HAY were nearly double that for SOY and ALGAE. This is counterintuitive due to the fact that total OM intake and OM digestibility did not differ across treatments. Intake and digestibility will influence ruminal passage rate (Galyean and Owens, 1991). It is not clear as to why ruminal outflow differed to this extent, however, one could speculate that because HAY had a greater proportion of the diet as Sorghum-sudan hay (66.5% of DM), salivary production was increased, which in turn increased fluid entering the rumen and ultimately fluid outflow (Jacques et al., 1989).

***Ruminal Fermentation Characteristics.*** Effect of treatment on ruminal environment is presented in Table 2. Ruminal pH did not differ by treatment ($P = 0.96$). Total VFA and the molar proportion of butyrate were similar across treatments ($P \geq 0.14$). Acetate was least ($P = 0.04$) for SOY, while HAY and ALGAE were similar (69.1 vs. 74.1, and 71.8 ±1.24 mol/100 mol, respectively). Propionate was highest (P = 0.04) for ALGAE, however SOY was similar to both ALGAE and HAY (19.89 vs. 14.80, and 17.85 ±1.23 mol/100 mol, respectively). This resulted in a treatment difference ($P = 0.03$) for the acetate to propionate ratio which was lowest for ALGAE versus HAY and SOY was similar to both other treatments (3.68 vs 5.03 and 4.12 ± 0.31 mol/100 mol, respectively). Because VFA represent the main supply of metabolizable energy for ruminants (Van Soest, 1982), a reduction in total VFA production would be energetically unfavorable for the nutrition of the animal (Busquet et al., 2006). These data indicate that the inclusion of LEA, while not increasing total VFA production could cause a shift in the molar proportion of individual VFA, particularly propionate. Similar results have been seen in studies supplementing sources of RDP to forage based diets (Hannah et al., 1991; Koster et al., 1996), which Hannah et al. (1991) attributed to a greater level of starch in their supplement. This may suggest that the LEA in the ALGAE treatment has a higher fraction of soluble carbohydrate than originally expected, and would account for the shift in individual VFA.

Overall, the isonitrogenous inclusion of LEA in a forage diet resulted in increased propionate production, decreased acetate production and a lowered acetate:propionate ratio. The ALGAE had the least ruminal $NH_3$ concentrations, which suggests that LEA might be a poor source of RDP and its total tract digestibility of CP was not similar to that of SBM as we hypothesized. Therefore, we reject our hypothesis that an isonitrogenous addition of LEA to a forage diet would result in similar results to that of SBM. Further work on the inclusion of LEA in a diet high in RDP would give data necessary to formulate guidelines on the inclusion of LEA in the diet of ruminant animals, as would the inclusion of data related to site and extent of digestion by use of duodenal flow data.

## IMPLICATIONS

These results imply that the addition of lipid extracted algae to a forage diet will increase propionate, an important glucogenic precursor. Further, similar organic matter intake indicates that lipid extracted algae may be as palatable as soybean meal. Based on the results of this study we believe that the inclusion of lipid extracted algae in sheep diets is feasible. Therefore, making its use in livestock feed a viable outlet for this co-product of the algal cultivation industry.

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# EFFECTS OF PREOVULATORY ESTRADIOL CONCENTRATION ON EMBRYO SURVIVAL AND PREGNANCY ESTABLISHMENT IN BEEF COWS

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**ABSTRACT:** The role of estradiol during the preovulatory period on embryo survival and pregnancy establishment has not been characterized in beef cows. We hypothesized that preovulatory estradiol is important for embryo survival and pregnancy establishment in beef cows. In order to establish the importance of estradiol during the preovulatory period on embryo survival, ovariectomized multiparous cows (n = 24) received estradiol cypionate (ECP), estradiol benzoate (EB) or no treatment (CON) to mimic a preovulatory period. Prior to treatment, all cows received a progesterone- releasing device (CIDR) for 7 d, 25 mg injection of prostaglandin-$F_{2\alpha}$ (PGF) at CIDR removal (d -2), and an injection of GnRH (100 µg; d 0) 2 d later to mimic a follicular phase. Utilizing a 3x3 Latin Square design, cows received either ECP 36 h before GnRH injection, EB 12 h before GnRH injection, or no estradiol (CON). Luteal phase progesterone was mimicked with 2x daily increasing progesterone injections from d 3 to 6 and use of CIDRs from d 7 to 29. On d 7 after GnRH injection, each cow received one embryo and a a progesterone-releasing device (CIDR). Another CIDR was added 24 h following embryo transfer and every 6 d, the older of the two CIDR was replaced with a new CIDR. Blood was collected every 4 h between d -2 and d 0 for characterization of serum estradiol profiles. Blood was collected on d -2, -1, 0, 3 to 7, 14, and 17 to 29 to characterize progesterone profiles. Serum estradiol profiles were different ($P < 0.001$) between treatments. Mean serum progesterone concentrations were decreased ($P = 0.05$) for EB and ECP treated cows compared with CON. Transrectal ultrasonography on d 29 indicated that 4% of CON, 29% of EB, and 21% of ECP treated cows were pregnant. Expression of interferon stimulated genes ISG15, Mx2, and Oas1 on d 19 indicated 66.0% of CON, 69.3% of EB, and 71.0% of ECP treated cows were pregnant. Thus, 62, 39.7, and 50% of pregnancies in CON, EB, and ECP treated cows, respectively were lost from d 19 to 29. Overall, results indicate greater embryonic survival and pregnancy establishment in cows exposed to estradiol in the preovulatory period.

**Key words:** embryo survival, estradiol, pregnancy establishment

## INTRODUCTION

The role of estradiol during the preovulatory period on embryo survival and pregnancy establishment has not been well characterized in beef cows. The mechanism by which preovulatory estradiol concentrations affect pregnancy success may be related to several factors. Serum estradiol concentrations have been positively correlated with ovulatory follicle size (Perry et al., 2005; Atkins et al., 2010) and cows ovulating smaller follicles following GnRH injection experienced greater reproductive failure (Perry et al., 2005). Bridges et al. (2010) reported greater serum estradiol concentrations and pregnancy rates among cows induced to ovulate a follicle of similar size but allowed a longer period of proestrus. Decreased levels of estradiol in the preovulatory period have also been associated with premature luteolysis (Mann and Lamming, 2000). None of these earlier studies allowed separation of potentially beneficial effects of estradiol from other traits.

Miller et al. (1977) evaluated the effects of estradiol in ovariectomized ewes by treatment with low estradiol (single injection of 25 µg) or high estradiol (35 µg distributed across 5 injections) to mimic the preovulatory period. Following embryo transfer on d 4, ewes that did not receive the increased estradiol supplementation were less likely to develop a normal embryo, had a reduced uterine weight at d 21, and had reduced amounts of uterine luminal protein on d 21 (Miller et al., 1977). The above body of work suggests a direct effect of estradiol on embryo development and survival. Several estrous and ovulation synchronization studies have been conducted using estradiol cypionate (**ECP**) or estradiol benzoate (**EB**) treatments during proestrus with reported benefits in pregnancy rate (Ahmadzadeh et al., 2003; Colazo et al., 2004). Souza et al., (2005) demonstrated differential estradiol profiles in cattle using estradiol benzoate or estradiol cypionate. We hypothesized that cows exposed to estradiol during the preovulatory period would have increased embryonic survival and pregnancy establishment when all other factors were held constant. In order to test the effects of estradiol exposure during the preovulatory period on embryo survival, two forms of estradiol were utilized: ECP and EB. The objective of this study was to examine the role

of preovulatory estradiol concentration on embryo survival and pregnancy establishment without the effects of follicle size, proestrous interval, subsequent progesterone exposure, or endogenous estradiol secretion.

## MATERIAL AND METHODS

All procedures involving animals used in this research were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee and the South Dakota State University Animal Care and Use Committee.

***Animals and Treatments.*** Crossbred multiparous beef cows (n = 24; 579 ± 58 kg BW) were ovariectomized by lateral lumbar incision and randomly assigned to a 3x3 Latin Square experimental design. After surgery, cows received a progesterone-releasing device (**CIDR**; Eazi-Breed CIDR containing 1.38 g of progesterone, Pfizer Animal Health, Madison, NJ) for 1 mo to mimic a luteal phase. The experiment was performed in 3 phases with the first 2 phases at Fort Keogh and the final phase at the South Dakota State University Beef Breeding Unit. Nine days before simulation of ovulation, d 0; cows received a CIDR. On d -2, CIDRs were removed and cows were given a single injection of prostaglandin-$F_{2\alpha}$ (**PGF;** 25 mg, i.m.). According to the Latin Square assignment, cows were assigned to one of three treatments for each phase of the experiment: ECP [2.5 mg, (1mg/mL in sesame oil carrier, i.m.) 36 h prior to GnRH], EB [1.2 mg, (1mg/mL in sesame oil carrier, i.m.) 12 h prior to GnRH], or **CON** (no exogenous estradiol). On d 3, 4, 5, and 6, all cows received 2 progesterone injections (10mg/mL in sesame oil carrier, i.m.) 12 h apart at the following doses: 40, 80, 160, and 240 mg, respectively. On d 7, all cows received a single embryo (stage 4, 5, or 6; quality grade 1 or 2; all stages and grades were equally assigned across treatments), a single 500 mg i.m. injection of flunixin meglumine (Merck Animal Health, Summit, NJ), and a CIDR. The embryo transfer technician was the same throughout. On d 8, cows received an additional CIDR, and every 6 d thereafter, the older of the two CIDR was removed and replaced with a new CIDR.

***Ultrasonography.*** Pregnancy status for each animal was determined by transrectal ultrasonography using an Aloka SSD-3500 Ultrasound with a 10 MHz convex probe or an Aloka 500 with a 7.5 MHz linear probe (Aloka, Wallingford, CT) at d 29 and confirmed at d 32. Viability of an embryo was confirmed by presence of a heartbeat.

***Blood Collection and RIA.*** Blood was collected by coccygeal or jugular venipuncture into 10mL vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ) at the following times relative to GnRH: d -9, d -2, every 4 h from -36 h to 0 h, d 3, 4, 5, 6, 7, and d 14. Samples were placed at 4°C for approximately 24 h. Samples were centrifuged at 1,200 x *g* for 25 min at 4°C and serum was collected and stored at -20°C until radioimmunoassay was performed. Radioimmunoassay (RIA) was performed on all serum samples to measure progesterone concentrations using the methods described by Engel et al. (2008). Inter- and intraassay CV were 13.67% and 4.52%, respectively and assay sensitivity was 0.4 ng/mL. In order to characterize circulating estradiol profiles from -48 h until GnRH administration, RIA was performed using the methods described by Perry and Perry (2008). Inter- and intraassay CV were 18.1% and 5.7%, respectively and assay sensitivity was 0.4 pg/mL.

***Collection of Blood Leukocytes and RT-PCR.*** Plasma and blood leukocytes were collected by coccygeal or jugular venipuncture into 10 mL vacutainer tubes containing EDTA (BD Vacutainer) on d 17 through 28 relative to GnRH. The blood was placed on ice immediately after collection and then centrifuged at 1,200 x g for 25 min at 4°C within 1 h of collection. Plasma was collected and stored at -20°C until used for RIA. Blood leukocytes were collected, mixed in a 1:1 volumetric ratio with Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH), and stored at -80°C until RNA isolation. Isolation of RNA was performed using an SV Total RNA Isolation System (Promega, Madison, WI) according to manufacturer's instructions. Pure RNA was dissolved in nuclease free water and RNA concentration was determined using a spectrophotometer (NanoDrop Technologies, Wilmington, DE). Isolated RNA samples were stored at -80°C. RNA from d 17, d 19, and d 21 were diluted to 12 ng/µL and RT-PCR was performed in triplicate using iScript One-Step RT-PCR Kit With SYBR Green (BioRad, Hercules, CA). Expression of ISG15, Mx2, OAS1, and GAPDH was measured using the primers listed in table 1. Amplification occurred using a Stratagene MX3000P (d 19 samples) or an ABI Prism 7000 (d 17 and d 21 samples). GAPDH was used as an internal control housekeeping gene and each plate contained negative controls to detect any background contamination. The SYBR Green reaction was performed for genes with the reverse transcription at 42°C for 30 min and 95°C for 10 min to inactivate reverse transcription. For all of the genes of interest, transcription was followed by 40 cycles of 30 sec at 95°C for melting; 1 min at 60°C for annealing; and 1 min at 72°C for extension. Primers (Table 1) for were previously published for GAPDH (Han et al., 2006), ISG15, Mx2, and OAS1 (Green et al., 2010). Amplicons were confirmed for product size on 2% agarose gels and were verified for identity by sequencing (Iowa State University Genomics Core).

***Statistical Analysis.*** The experiment was designed as a series of nine 3x3 Latin squares. Rows of the squares corresponded to cows. Columns of the squares represented the 3 phases of the experiment. One square was eliminated from the analysis due to removal of a cow with uterine adhesions to the abdominal wall that was detected during the first phase of study.

Analyses of hormone and expression profiles employed mixed linear models methodology, and when the binary variate pregnancy (0 = not pregnant, 1 = pregnant) was the dependent variable, used procedures for logistic regression. Hormone profiles and expression profiles were analyzed as repeated measures using split-plot in time models. The whole plot was

**Table 1.** Genes, primer sequences, and primer locations for genes amplified during RT-PCR

| Gene | Primer | Primer Sequence | Primer Location | Reference |
|---|---|---|---|---|
| Isg15 | Forward | 5'-CAGCCAACCAGTGTCTGCAGAGA-3' | 14-36 | Green et al., 2010 |
| | Reverse | 5'-CCAGGATGGAGATGCAGTTCTGC-3' | 284-306 | |
| Mx2a | Forward | 5'-CTTCAGAGACGCCTCAGTCG-3' | 2071-2090 | Green et al., 2010 |
| | Reverse | 5'-TGAAGCAGCCAGGAATAGTG-3' | 2283-2302 | |
| Oas1 | Forward | 5'-ACCCTCTCCAGGAATCCAGT-3' | 1157-1176 | Green et al., 2010 |
| | Reverse | 5'-GATTCTGGTCCCAGGTCTGA-3' | 1336-1355 | |
| GAPDH | Forward | 5'-GATTGTCAGCAATGCCTCCT-3' | 543-562 | Han et al., 2006 |
| | Reverse | 5'-GGTCATAAGTCCCTCCACGA-3' | 617-636 | |

a randomized complete block design with squares as blocks and treatments were factors included in the Latin squares. In the analyses of expression profiles, a linear covariate was also included in the model to adjust for expression of the house-keeping gene (GAP). Whole plot error was a composite of the cow x period x treatment interaction mean square (the usual error for a single Latin square) pooled over squares and pooled interactions of square with the factors included in the Latin squares (the usual error for a randomized complete block design). Note, when the pooled Latin square error means square was tested against the randomized complete block error mean square, it did not approach significance ($P > 0.20$). The sub-plot consisted of hours relative to GnRH and two-factor interactions of time with each factor included in the Latin squares. Residual variance was considered error for effects in the subplot. The analysis of pregnancy did not include the subplot, but was otherwise similar to the analyses described above. Effects of gene expression (adjusted for expression of GAP) on pregnancy rate were also assessed using logistic regression.

## RESULTS AND DISCUSSION

Serum estradiol concentrations during the simulated preovulatory period were different ($P < 0.01$) between treatments (Figure 1). Based on the study by Souza et al. (2005) using similar treatments, the circulating estradiol profiles followed the expected trends, with the EB treatment peaking earlier and with greater magnitude than ECP. Serum progesterone concentrations were decreased ($P = 0.05$) in EB and ECP cows compared with the CON cows (Figure 2). This difference was not expected but may be related to changes in steroid hormone metabolism due to estradiol exposure. Based on the concentrations of progesterone reported by Fields et al., (2009) in our lab, the levels achieved in the current study should have been sufficient to maintain pregnancies.

Transrectal ultrasonography on d 29 revealed pregnancy rates of 21%, 29%, and 4% for the ECP, EB, and CON treatments, respectively. This indicated substantial pregnancy loss between d 7 and d 29. In order to characterize this loss, expression of 3 genes known to be stimulated by interferon-τ (interferon-stimulated genes) was utilized. Peripheral blood leukocytes have been reported to have increased expression of ISG15, Mx2, and OAS1 on d 15 and 18 of pregnancy in ewes (Yankey et al., 2001) and in cows on d 16, 18, and 20 (Gifford, et al., 2007; Green, et al., 2010). Ovariectomy eliminated the deleterious effects of luteolysis on pregnancy, therefore, with adequate progesterone supplementation, we expected that most cows would retain a pregnancy to d 29 if preovulatory estradiol were not beneficial. Since maternal recognition of pregnancy must occur before luteolysis (Roberts, et al., 1996), interferon stimulated gene expression on d 17 is of particular interest for identifying those cows that did not receive adequate trophoblastic signaling to maintain the pregnancy. For the 59 cows that did not remain pregnant until d 29, changes in expression of interferon stimulated genes may be useful in determining the timing of embryo loss. Table 2 illustrates the projected timing of embryo loss based upon ranking the combined results of ISG15, Mx2, and OAS1 expression. For d 17 and 21, cows were ranked by probability of pregnancy for each gene based on logistic regression. The threshold for pregnancy prediction on d 17 and 21 was based on ranking higher than the mean rank of cows confirmed pregnant on d 29 via ultrasound. These conservative estimates were used to calculate the predicted pregnancy rates in table 2. These pregnancy rates clearly demonstrate the importance of preovulatory estradiol in embryo survival and pregnancy establishment. Exposure to estradiol in the preovulatory period, whether in the ECP or EB treatment increased the odds of pregnancy at d 29 ($P < 0.05$). While a similar percentage of cows in each treatment were likely pregnant on d 17 and 21, the uterine environment of cows that were not exposed to increased estradiol prior to progesterone treatment were less capable of supporting a pregnancy.

Expression of ISG15, Mx2, and OAS1 were not different between treatments ($P > 0.10$). As anticipated, the expression of these three genes on each of the days measured was greater ($P < 0.01$) in cows that were confirmed to be

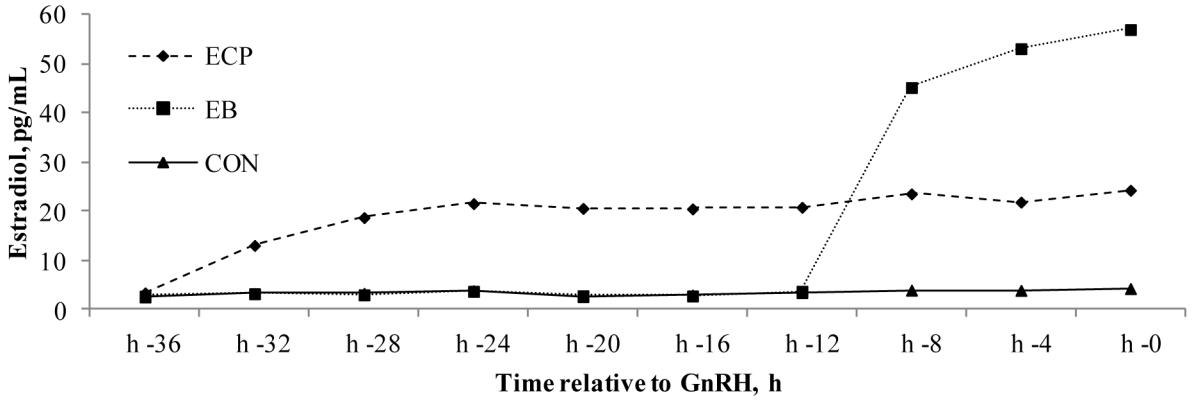


**Figure 1.** Serum estradiol concentration of ovariectomized cows that received estradiol cypionate (ECP), estradiol benzoate (EB), or control (CON) treatment during the simulated preovulatory period. Cows treated with ECP received 2.5 mg 36 h prior to GnRH, cows treated with EB received 1.2 mg 12 h prior to GnRH, CON cows received no exogenous estradiol.

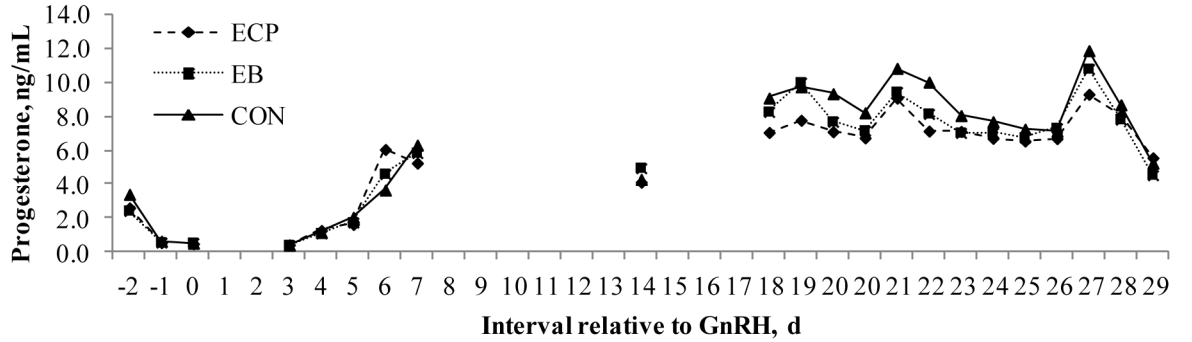


**Figure 2.** Serum progesterone concentration of ovariectomized cows that received estradiol cypionate (ECP), estradiol benzoate (EB), or control (CON) treatment during the simulated preovulatory period. Cows treated with ECP received 2.5 mg 36 h prior to GnRH, cows treated with EB received 1.2 mg 12 h prior to GnRH, CON cows received no exogenous estradiol.

**Table 2.** Pregnancy rates by treatments on d 7, 17, 21, and 29.

| Day of study | ECP[1] | EB[2] | CON[3] |
|---|---|---|---|
| 7 | 100% | 100% | 100% |
| 17 | 50% | 42% | 33% |
| 21 | 42% | 29% | 25% |
| 29 | 21%$^{y}$ | 29%$^{y}$ | 4%$^{z}$ |

[1]2.5 mg estradiol cypionate 36 h prior to GnRH on d 0
[2]1.2 mg estradiol benzoate 12 h prior to GnRH on d 0
[3]no exogenous estradiol
$^{yz}$ means within a row having different superscripts are different ($P < 0.05$)

pregnant when compared with cows that were not. This is consistent with other studies (Han, et al., 2006; Green et al., 2010) which reported increased interferon gene expression within pregnant cows on d 17, 18, 19, and 20 compared with non-pregnant cows. Expression of Mx2, was also increased in pregnant cows when compared with non-pregnant cows on d 16, 18, and 20 after insemination (Gifford et al., 2007).

Expression of ISG15 and Mx2 differed in the present study ($P < 0.01$) due to day, but expression of OAS1 did not differ ($P > 0.10$) by day. The increased expression of these two genes in pregnant cows on d 21 when compared with d 17 is consistent with the results determined by Green, et al. (2010) in which an increase in ISG15, Mx2, and OAS1 was observed on d 20 relative to d 18. It is not clear why OAS1 expression did not change during this time in the present study. Correlations between the interferon stimulated genes were similar to those found by Green et al. (2010).

## IMPLICATIONS

The use of ovariectomized cows in the present study allowed us to demonstrate the importance of estradiol during the preovulatory period on embryo survival and pregnancy establishment. Cows that received either estradiol benzoate or estradiol cypionate during a simulated estrus had greater pregnancy rates on d 29 than cows that did not receive estradiol. Thus, at least a portion of the beneficial effect of increased proestrous interval and ovulatory follicle diameter on pregnancy success is due to the effects of a concurrent increase in estradiol on uterine environment.

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# INDIVIDUAL MINERAL SUPPLEMENT INTAKE BY EWES SWATH GRAZING OR CONFINEMENT FED PEA-BARLEY FORAGE

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**ABSTRACT:** Sixty mature ewes (non-pregnant, non-lactating) were used in a completely randomized design to determine if feeding method of pea-barley forage (swath grazing or hay in confinement) had an effect on individual ewe mineral consumption. Thirty ewes were randomly allocated to 3 confinement pens and 30 ewes were randomly allocated to 3 grazing plots. The study was conducted during Sept. 25 to Oct. 15, 2010 and repeated Sept. 6 to 19, 2011. Targhee ewes (65.4 ± 5.84 kg BW) were used in 2010. Rambouillet ewes (61.9 ± 6.28 kg BW) were used in 2011. Ewes had ad libitum access to food, water, and a mineral supplement containing 12% salt with 2% titanium dioxide ($TiO_2$) added as an external marker to estimate individual mineral intake. On d 1 of the study, mineral was weighed and placed in covered feeders in pens and plots. Mineral was weighed and added as needed and at the end of the collection period, remaining mineral was weighed to provide an estimate of total mineral intake via disappearance. Forage DMI was calculated using estimates of fecal output, obtained by dosing gelatin capsules containing 2 g chromic oxide ($Cr_2O_3$) every day for 14 d, and in vitro 48 h forage DM digestibility. Fecal grab samples were collected from each individual ewe for 7 d and composited by ewe. Forage and mineral intakes were analyzed using individual ewe as the experimental unit, while plot or pen was used as the experimental unit for intake CV. A year x treatment interaction ($P < 0.01$) existed for forage and mineral DMI. Ewes in confinement pens consumed more forage than grazing ewes in 2010 (2.60 vs. 1.86 kg•ewe$^{-1}$•d$^{-1}$, respectively), but less than grazing ewes in 2011 (1.99 vs. 2.49 kg•ewe$^{-1}$•d$^{-1}$, respectively). Mean mineral intake was highest ($P < 0.01$) by grazing ewes in 2011 and 2010 (average 69 g•ewe$^{-1}$•d$^{-1}$), intermediate by ewes in pens in 2010 (57 g•ewe$^{-1}$•d$^{-1}$), and lowest by ewes in pens in 2011 (31 g•ewe$^{-1}$•d$^{-1}$). A year x treatment interaction ($P = 0.05$) existed for mineral DMI CV which was greater ($P = 0.04$) for the pen treatment in 2011 (67 vs. 34%) but not different ($P > 0.05$) between treatments in 2010. In this study, both swath grazing ewes and ewes in confinement consumed more mineral than recommended by the mineral manufacturer and the NRC indicating that more research is needed to develop a better understanding of the factors that regulate and impact mineral intake.

**Key words:** confinement, mineral, supplement, swath

## INTRODUCTION

In Montana, ewes typically consume 14 to 28 g of mineral-supplement per day, which results in an annual cost per ewe of $4.49 (Kott, 2005). Since feed and supplement costs can be more than 50% of total operational costs for range livestock producers (USDA, 1994), monitoring supplement intake, and making sure animals receive the appropriate amount of nutrients, could reduce production costs. While researchers have looked at individual intake of protein and energy supplements (Curtis et al., 1994; Bowman and Sowell, 1997; Sowell et al., 2003), few studies have attempted to evaluate variation in individual consumption of mineral. There is a lack of information on the variation in mineral intake, especially in sheep, and there is also an absence of information comparing the forage and mineral intakes of swath-grazing sheep to the intakes of sheep fed hay in confinement.

The objective of this study was to determine if feeding method of pea-barley forage (swath grazing or hay fed in confinement) had an effect on individual ewe mineral consumption. Our null hypothesis was that individual mineral supplement intake would not differ by ewes grazing or confinement-fed pea-barley forage.

## MATERIALS AND METHODS

All animal procedures were approved by the Montana State University Agricultural Animal Care and Use Committee (Protocol #2009-AA04).

***Sheep Selection and Management.*** Sixty mature ewes were selected from the Bair Ranch in Martinsdale, MT to be used in the first year of data collection. These ewes were chosen from a band of approximately 2,000 Targhee ewes. The Targhee ewes (65.4 ± 5.84 kg BW) chosen were non-pregnant, non-lactating. For the second year of data collection, 60 mature Rambouillet ewes (61.9 ± 6.28 kg BW, non-pregnant, non-lactating) were selected from the Red Bluff Research Ranch near Norris in Madison County, MT. In 2010, ewes were transported from the Bair Ranch on September 25th to Montana State University's Fort Ellis Research Station in Bozeman, MT. The ewes arrived at approximately 1230 h after being held off of feed and water overnight. They were immediately paint branded for identification purposes and their shrunk weights were recorded. Beginning on September 25, 2010, ewes were randomly assigned to 3 grazing plots and 3 confinement pens.

In 2011, ewes were transported from Red Bluff Research Ranch on September 6 to Montana State University's Fort Ellis Research Station in Bozeman, MT. The ewes arrived at approximately 1100 h after being held off of feed and water overnight. Similarly to 2010, ewes were immediately paint branded for identification purposes and their shrunk weights were recorded. Beginning on September 6, 2011, ewes were randomly assigned to 3 grazing plots and 3 confinement pens. During 2010 and 2011 there were 10 ewes in each pen and plot and all ewes had ad libitum access to food, water and a mineral supplement with 2% titanium dioxide ($TiO_2$) added as an external marker to estimate mineral intake.

Mineral feeders were placed in confinement pens and grazed plots. There was 1 mineral feeder/10 ewes. The feeders were raised off the ground and protected from rain and wind so the mineral was not lost or wasted. On d 1 of the data collection period, all mineral was removed from the feeder, weighed, and placed back in the feeder, along with an additional amount, to insure ad libitum consumption. At the end of the collection period, the remaining mineral was removed from the feeder, weighed, and mineral disappearance was recorded.

For 7 d (September 25 to October 1, 2010 and September 6 to September 12, 2011) ewes in the grazing plots were restricted to half of a plot (0.07 ha) for an adaptation period, and ewes in confinement pens were fed their respective hay. Throughout this week, the sheep in each plot and pen were moved into handling facilities and dosed with $Cr_2O_3$ as an external marker to estimate fecal output. Gelatin capsules filled with 2 g of $Cr_2O_3$ were administered with a balling gun between 1000 h and 1100 h every day as to avoid disturbing their major grazing periods. The $Cr_2O_3$ reached equilibrium rate during the first week of dosing before fecal collection (Smith and Reid, 1955). Following the adaptation period, ewes were moved into the remaining half of a plot with fresh forage for the data collection period of 7 d (October 2 to October 8, 2010 and September 13 to 19, 2011). During this data collection period, both sheep in plots and pens were gathered, dosed with $Cr_2O_3$, and fecal grab samples were collected from each individual animal between 1000 h and 1100 h. Sheep in the confinement pens were moved into an adjacent handling facility for $Cr_2O_3$ dosing and fecal grab samples.

***Forage Digestibility and Intake.*** Forage indigestibility was estimated using the in vitro technique from a modified Tilley and Terry (1963) method, where 20 mL of a pre-heated buffer solution were added to the samples and the incubation was carried out for 48 h instead of 72 h.

Chromium (Cr) concentration was determined in rectal grab samples (Ellis et al., 1982). Fecal output (**FO**) was estimated using the following equation:

FO, g/d = Cr daily dose, g/d / Fecal Cr concentration, g/g

Forage dry matter intake was estimated using the following equation:

Forage DMI, kg/d = FO, kg/d / (forage indigestibility)

Titanium dioxide was added to the mineral supplement as an external marker to estimate supplement intake. Fecal samples were analyzed for titanium (Ti) content (Myers et al., 2004). Individual ewe supplement intake was estimated using the following equation:

Supplement intake = (fecal Ti, g/g x FO, g/d) / supplement Ti concentration, g/g

A technique described by Myers et al. (2004) was used to determine the Ti content of the fecal samples. Samples were read on a UV/Vis spectrophotometer (2010) and an ICP machine (2011) at 410 nanometers.

***Mineral.*** The mineral fed in this study (Payback- Sheep Range Mineral 16-8) was purchased from Cenex Harvest States Inc. located in Montana (Table 1). Titanium dioxide was mechanically mixed into the mineral supplement at 2% of the total product to be used as an indigestible external marker. A 22.7 kg bag of mineral had 226.8 g of $TiO_2$ mixed in. The target mineral intake was 7.09 to 14.17 g•ewe$^{-1}$•d$^{-1}$ based on manufacturer recommendations. The mineral/$TiO_2$ mixture was available to the sheep throughout the duration of the study. Glindemann et al. (2009) reported that the equilibrium of intake and excretion of $TiO_2$ was reached 5 days after initial $TiO_2$ administration.

***Statistical Analyses.*** Data were analyzed as a completely randomized design using the Proc GLM procedure (SAS Inst., Inc., Cary, NC). For all data, the experimental unit was individual ewe. Means were separated using the LSD procedure when a significant $F$ value was found ($P \leq 0.05$).

## RESULTS

***Forage Quality and Production.*** Estimated forage production in 2011 was 19% greater (1743 kg/ha) than forage

**Table 1.** Composition of mineral supplement with a target intake of 7 g•ewe$^{-1}$•d$^{-1}$ (Values provided by manufacturer)

| Item | Amount |
|---|---|
| Calcium, min. | 12.0% |
| Calcium, max. | 14.0% |
| Phosphorus, min. | 12.0% |
| Salt, min. | 11.0% |
| Salt, max. | 12.5% |
| Magnesium, min. | 3.0% |
| Cobalt, min. | 4 ppm |
| Copper, min. | 7 ppm |
| Iodine, min. | 100 ppm |
| Manganese, min. | 1,800 ppm |
| Selenium, min. | 19.0 ppm |
| Zinc, min. | 2,000 ppm |
| Vit. A, min. | 250,000 IU/lb |
| Vit. D, min. | 25,000 IU/lb |
| Vit. E, min. | 500 IU/lb |

production in 2010 (Table 2). Crude protein requirements for ewes at maintenance range from 104 g for 60 kg BW to 113 g for 70 kg BW ewes, respectively (NRC, 2007). Based on estimated DMI values, and CP concentration of the forage, all ewes met their CP protein requirements.

***Forage Intake and Ewe Performance.*** Treatment did not affect ($P > 0.07$) ewe weight or ADG, however, ewes were heavier ($P < 0.01$) and gained more weight ($P < 0.01$) in 2010 compared with 2011 (Table 3). There was a year x treatment interaction for forage DMI ($P < 0.01$; Table 3). In 2010, ewes in pens consumed more forage DM than grazing ewes (2.60 vs. 1.86 kg•ewe$^{-1}$•d$^{-1}$), while grazing ewes consumed more forage DM than ewes in pens (2.49 vs. 1.99 kg•ewe$^{-1}$•d$^{-1}$) in 2011.

***Mineral-Supplement Intake.*** All individual fecal samples contained some level of $TiO_2$, indicating that all ewes consumed the mineral. Fecal $TiO_2$ concentrations from ewe samples overestimated individual mineral intake so that total intake for each treatment was more than what was actually fed based on disappearance measurements. Therefore, individual mineral intakes were adjusted for the actual amount of supplement fed.

There was a year x treatment interaction ($P = 0.01$; Table 3) for mean mineral DMI. Mean mineral DMI was highest by grazing ewes in 2011 (73 g•ewe$^{-1}$•d$^{-1}$), and lowest by ewes in pens in 2011 (31 g•ewe$^{-1}$•d$^{-1}$). Ewes grazing or in pens in 2010 were intermediate and not different in mineral DMI (average 61 g•ewe$^{-1}$•d$^{-1}$).

***Distribution of Mineral-Supplement Intake.*** There was a year x treatment interaction for mineral DMI CV ($P = 0.05$; Table 4). Mineral supplement DMI CV was highest by ewes in pens in 2011 (67%), and lowest by the grazing ewes in 2011 (34%). The mean mineral DMI CV in 2010 was intermediate and not different between grazing ewes and ewes in pens (average 51%).

A visual presentation of the variation in individual ewe mineral intake is given in Figure 1 and 2.

**Table 2.** Production and composition of pea-barley forage consumed byewes fed in confinement pens or grazing plots (based on clipped samples)

| | 2010 | | 2011 | |
|---|---|---|---|---|
| Item | Pen | Plot | Pen | Plot |
| Production, kg/ha | 3710 | 3491 | 4364 | 4580 |
| Composition, % | | | | |
| DM | 93.2 | 93.6 | 93.4 | 91.3 |
| OM | 92.7 | 91.9 | 92.1 | 92.1 |
| CP | 10.5 | 11.3 | 7.1 | 7.3 |
| NDF | 50.8 | 55.0 | 48.7 | 50.6 |
| ADF | 28.7 | 29.5 | 26.6 | 27.1 |

## DISCUSSION

Substantial variation in individual animal intake of protein and energy supplements in loose, liquid, and block form has been reported (Bowman and Sowell, 1997, Sowell et al., 2003). The type of supplement offered to cattle and sheep, the conditions under which it is fed, previous experience with supplements, social interactions, and forage quality and availability has been shown to influence the amount of supplement consumed by individual animals (Bowman and Sowell, 1997). Curtis et al. (1994) found that grazing Merino wethers, offered a self-fed lupine seed supplement (wheaten chaff plus lupins), had an extremely wide range of intakes. Thirty-three percent consumed less than 150 g/d, and 8% consumed over 1,200 g/d. Sixty-one percent of the wethers did not meet the target supplement consumption, and

**Table 3.** Performance, forage dry matter intake, and mineral-supplement intake by ewes consuming pea-barley forage in confinement pens or swath grazing plots

| | 2010 | | 2011 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Treatment | | | | | *P*-value | | |
| Item | Pen | Plot | Pen | Plot | SE | Year | Trt | Year*Trt |
| No. of ewes | 28 | 28 | 29 | 28 | - | - | - | - |
| Weight, kg | | | | | | | | |
| Initial | 64.7 | 66.2 | 60.8 | 63.2 | 1.09 | 0.02 | 0.07 | 0.66 |
| Ending | 71.3 | 71.8 | 62.5 | 64.7 | 1.08 | <0.01 | 0.21 | 0.44 |
| Wt change | 6.6 | 5.6 | 1.7 | 1.5 | 0.46 | <0.01 | 0.20 | 0.43 |
| ADG, kg | 0.24 | 0.21 | 0.12 | 0.11 | 0.02 | <0.01 | 0.26 | 0.69 |
| Forage intake | | | | | | | | |
| DM, kg | 2.6$^{b}$ | 1.9$^{a}$ | 2.0$^{a}$ | 2.5$^{b}$ | 0.14 | 0.96 | 0.40 | <0.01 |
| DM, g/kg BW | 3.9$^{b}$ | 2.7$^{a}$ | 3.3$^{a}$ | 3.9$^{b}$ | 0.23 | 0.21 | 0.30 | 0.01 |
| Mineral-supplement intake | | | | | | | | |
| Mean DMI g/d | 57$^{b}$ | 64$^{bc}$ | 31$^{a}$ | 73$^{c}$ | 5.2 | 0.10 | <0.01 | 0.01 |

$^{a–c}$Means within a row with different superscripts differ ($P < 0.05$)

**Table 4.** Mineral-supplement intake distribution by ewes consuming pea-barley forage in confinement pens or swath grazing plots

| | 2010 | | 2011 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Treatment[1] | | | | | *P*-value | | |
| Item | Pen | Plot | Pen | Plot | SE | Year | Trt | Year*Trt |
| Supplement DMI, g | | | | | | | | |
| Min. | 10 | 24 | 10 | 41 | 4.0 | 0.08 | 0.05 | 0.07 |
| Max. | 118 | 118 | 83 | 122 | 14.4 | 0.31 | 0.21 | 0.22 |
| Mean | 57[b] | 64[b] | 31[a] | 73[b] | 6.4 | 0.22 | 0.05 | 0.02 |
| Supplement DMI CV, % | 55[bc] | 47[ab] | 67[c] | 34[a] | 5.3 | 0.92 | 0.04 | 0.05 |

[1]Experimental unit was either confinement pen or grazed plot
[a-c]Means within a row with different superscripts differ ($P < 0.05$)

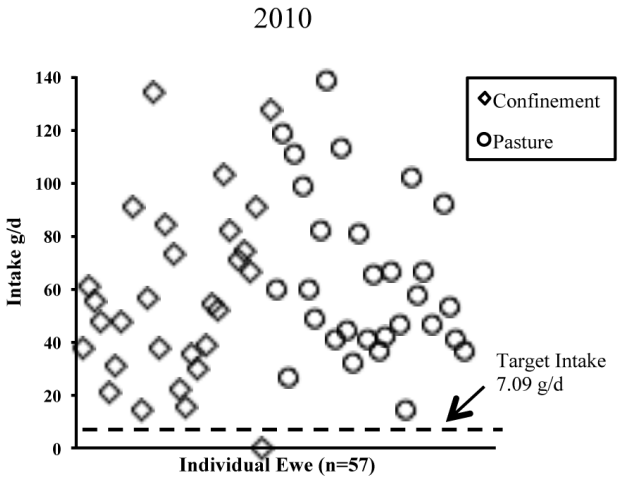


**Figure 1.** 2010 individual ewe mineral intake g/d. Target intake was 7.09 g/d.

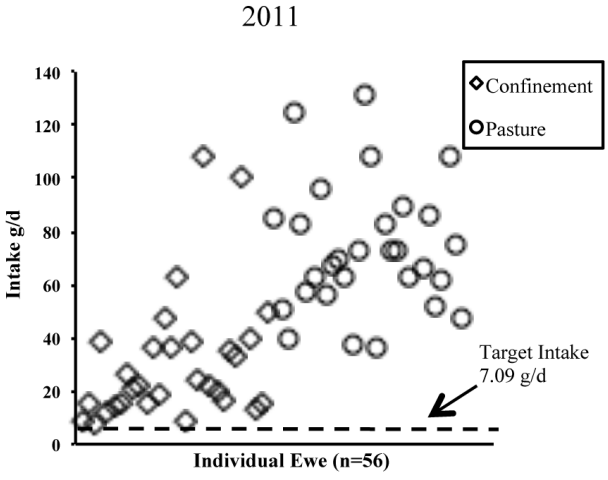


**Figure 2.** 2011 individual ewe mineral intake g/d. Target intake was 7.09 g/d.

the CV of individual supplement intake was 83% (Curtis et al., 1994).

In this study, both swath grazing ewes and ewes in confinement consumed more mineral than recommended by the mineral manufacturer and the NRC. This indicates that more research is needed to develop a better understanding of the factors that regulate and impact mineral intake.

## IMPLICATIONS

There is a lack of literature on individual mineral intake in sheep and little literature on the variation in sheep mineral intake. This research adds to the literature and the body of knowledge. It is also biological research that becomes the foundation for economic evaluation and will aid feed companies in formulating mineral rations that meet sheep requirements. In this study, both swath grazing ewes and ewes in confinement consumed more mineral than recommended by the mineral manufacturer and the NRC. More research is needed to develop a better understanding of the factors that regulate and impact mineral intake so the appetite of over-eaters can be curbed reducing mineral costs for producers.

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# EFFECTS OF WEANING AGE AND WINTER DEVELOPMENT ENVIRONMENT ON HEIFER GRAZING DISTRIBUTION[1]

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**ABSTRACT:** The objective of this experiment was to determine if early weaning (approximately 125 d) vs. normal weaning (approximately 250 d) and wintering replacement heifers in drylot vs. rangeland affected heifer grazing distribution during the subsequent summer. Heifer calves from the 2009 and 2010 calf crop (n = 104 and 73, respectively) were allocated to the 2 weaning treatments and then stratified by age into the 2 winter development treatments. During the summer of yr 1 heifers were allocated to 2 pastures by winter treatment, and in yr 2 all 4 treatment combinations were allocated to separate pastures. A subset of heifers from each group were selected to wear global positioning system (GPS) collars (n = 2 and 5 in yr 1 and 2, respectively). Readings were taken from the GPS every 15 min in yr 1 and every 65 s in yr 2. The GPS coordinates were then analyzed relative to ecological sites, water locations, fence locations, and temperature using Arc GIS (Esri, Redlands, CA). Winter treatment affected ($P < 0.05$) preference index (PI) for claypan and loamy sites in 2010, and distance from water in 2011. Day of sampling affected ($P < 0.05$) claypan and loamy site PI in 2010 and thin claypan site PI in 2011. Day of sampling interacted with winter treatment ($P < 0.05$) for distance from water in 2010, sand and thin claypan site PI in 2010 and thin claypan site PI in 2011, while day of sampling interacted with weaning treatment for distance from water in 2011. A winter by weaning treatment interaction affected ($P < 0.05$) thin claypan site PI in 2011. Temperature had an effect on distance to fenclines in 2010 ($P < 0.001$). There was a temperature interaction with wintered treatment effect on distance to water in 2011 ($P < 0.001$). There was a three-way interaction ($P < 0.05$) between weaning treatment, winter treatment and ambient temperate on the distance from water and between weaning treatment, winter treatment and day of sampling on claypan and sand site PI in 2011. In conclusion, winter development influenced patterns of range utilization. Day-of-sampling interactions indicated that range heifers did not adjust preferences and thus were already adapted to the range environment, whereas drylot heifers adjusted preferences over time suggesting they re-learned how to utilize the range environment.

**Key words:** grazing distribution, heifer development, weaning

## INTRODUCTION

There have been multiple research projects on different heifer development treatments to evaluate effectiveness of alternative programs (Olson et al., 1992; Arthington and Kalmbacher, 2003; Salverson et al., 2005). Past research has suggested that rangeland may be an effective resource to develop heifers that are destined to become range cows (Olson et al., 1992; Salverson et al., 2005). Factors that affect grazing behavior include, but are not exclusive to, early life experiences, presence of peers, physiological or nutritional state of the animal, inherited senses, and spatial memory (Launchbaugh and Howery, 2005). Some specific factors that have been found to influence cattle grazing habits are vegetation biomass available in the area and water location (Gillen et al., 1984; Owens et al., 1991; Pinchak et al., 1991; Cibils et al., 2008).

The objective of this study was to determine how weaning treatment and winter development environment affects grazing distribution and pasture utilization throughout the following summer months. We hypothesized that range heifers would have a more broad pasture distribution and better utilization. This was based on the range heifers grazing all winter and retaining grazing skills and cues learned from dams while suckling. Drylot heifers would have a re-learning period before they would be able to fully utilize the pasture. We also hypothesized that normal-weaned heifers would have better grazing habits than early-weaned heifers because they spent more time grazing with their dams so they learned and retained different cues.

## MATERIALS AND METHODS

All animal procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

***Design and Treatments.*** Heifer calves from the 2009 and 2010 calf crop (n = 104 and 73, respectively) were split into two different groups to either be early weaned (**EW**- about 125 d) or normal weaned (**NW**- about 250 d). These groups were based on cow assignments to weaning treatments for another study that was currently ongoing. In that study, cows were stratified into two groups and then each group was randomly assigned to either be early- or normal-weaned when they entered the study, then the sex of the calf

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determined which post-weaning experiment the calves could go into. Within the two weaning-date groups, heifers were stratified by age into two groups and each group was randomly assigned to a winter development treatment. These groups were either wintered in the drylot (**D**) or wintered on rangeland (**R**) from weaning to breeding. This created the following four treatments: 1) early weaned and wintered in drylot (**ED**) 2) early weaned and wintered on rangeland (**ER**) 3) normal weaned and wintered in drylot (**ND**) 4) normal weaned and wintered on rangeland (**NR**). Heifers wintered in drylot received mixed grass and alfalfa hay (yr 1: 87.1% DM, 11.6% CP, 52.5% TDN; yr 2: 87.3% DM, 12.3% CP, 53.4% TDN) ad libitum plus 1.8 kg of a dried distiller's grain (**DDGS**)-based supplement/hd/d (yr 1: 93.4% DM, 22.7% CP, 75.8% TDN; yr 2: 91.8% DM, 25.4% CP, 76.7% TDN). Heifers wintered on rangeland also received 1.8 kg/hd/d of the same supplement. During the winter when the ground was snow covered to a depth that precluded grazing, range heifers received the same hay as the drylot heifers. Heifers in the ER treatment combination consumed 226 kg/hd of hay in yr 1 and 305 kg/hd of hay in yr 2. Heifers in the NR treatment combination consumed 219 kg/hd of hay in yr 1 and 294 kg/hd of hay in yr 2. More hay was fed to both treatment combinations in year 2 because of a greater number of days with great enough snow depth to warrant hay feeding. Over the winter, each treatment combination was allocated to a separate pen or pasture. After estrus synchronization and timed AI (June 19, 2010 and June 9, 2011), all heifers were placed on rangeland to graze through the summer. During the summer of yr 1, heifers were allocated by winter treatment to 2 pastures, and all 4 treatment combinations were allocated to separate pastures during the summer of yr 2.

***Year 1 GPS Locations.*** Two heifers from each treatment combination were selected based on being near the mean treatment group 365 d BW to represent their group's grazing habits. The mean 365 d BW was calculated for each group and the two heifers that were closest to the mean and also average in phenotype were selected. These heifers wore a global positioning system (GPS) – very high frequency (VHF) collar (GPS-Log-V2, Kedziora Innovation Group Mannsville, NY) that recorded location at 15 minute intervals throughout the summer grazing season.

***Year 2 GPS Locations.*** Five heifers were randomly selected from each group to wear a GPS collar using the same selection criteria as year 1. These collars contained a Garmin GPS unit (eTrex Legend H, Garmin, Olathe, Kansas) that recorded location every 65 seconds.

***GPS Analysis.*** Preference indices (**PI**) by each heifer for common ecological sites in each pasture were calculated by intersecting an ecological site layer (NRCS Soil Survey Staff, 2010) with the GPS coordinates in Arc Map (Rock Wars, Golden, CO). The PI was then calculated by dividing the number of GPS points on each ecological site per day by the total GPS points that given day. That gave the percentage of time spent each day on a given ecological site. That number was then multiplied by the percentage of the total area occupied by that ecological site to calculate the preference index.

Fencelines and water sources were mapped using a GeoExplorer GeoXH (Trimble GPS Navigation; Sunnyvale, CA). With the use of Arc Map, the distance between every GPS data point for each heifer and the nearest water source and fenceline was calculated. Mean distances from the fencelines and water sources for each heifer were found for each hour of each day over the entire summer grazing period. Hourly ambient temperature readings were collected from an onsite weather station.

***Statistical Analysis.*** Preference indexes for ecological sites were analyzed by repeated measures using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included the independent variables of weaning treatment, wintering treatment, and day of the grazing season, along with their interactions. Day of the grazing season was considered a continuous variable and therefore treated as a covariate. Fence and water distances were also analyzed by repeated measures using the MIXED procedure of SAS using the same model.

The temperature effect on distance from water and fenceline was also analyzed as described above, with the model consisting of the independent variables of weaning treatment, wintering treatment, temperature and their interactions.

## RESULTS AND DISCUSSION

***Ecological Site PI.*** In yr 1 R heifers preferred ($P$ = 0.01) claypan ecological sites more than D heifers (PI of 6.46 ± 0.661 and 1.14 ± 0.410, respectively). There was a tendency ($P$ = 0.10) for R heifers to increase their PI by 0.07 ± 0.028 each day of the grazing season. However, over the summer all groups had an increase of 0.04 ± 0.018 units of PI for claypan soil sites ($P$ = 0.02). In yr 2, there was a three way interaction between day, weaning treatment, and winter treatment on claypan PI (Figure 1). The following regression equations were generated:

ED claypan PI = 33.0 ± 2.39 + (-0.500 ± 0.56 × day)

ER claypan PI = -0.04 ± 4.56 + (0.19 ± 0.111 × day)

ND claypan PI = 0.09 ± 3.93 + (0.01 ± 0.101 × day

NR claypan PI = 2.5 ± 1.86 + (-0.03 ± 0.042 × day)

The ER and ND did not have a preference for claypan sites and NR had a slight preference. None of these preferences changed over the summer. In contrast, ED heifers had an extremely high initial PI for claypan soil sites, which then decreased through the grazing season ($P$ < 0.001)

Drylot heifers had a greater initial preference ($P$ < 0.001) for loam ecological sites than R heifers (1.76 ± 0.54 and 0.31 ± 0.080, respectively) and all groups had an increase ($P$ < 0.001) of 0.01 ± 0.002 preference units each day in yr 1. There were no differences in PI for loam sites in yr 2.

In yr 1, R heifers had a greater initial preference ($P$ = 0.02) for sand soil sites than D heifers and increased preference throughout the summer ($P$ = 0.001), whereas D heifers had no change in preference as the summer progressed (Figure 2). The following regression equations were generated:

D sand PI = 0.57 ± 0.311 + (0.004 ± 0.008 × day)

R sand PI = 2.40 ± 0.327 + (0.03 ± 0.010 × day)

In yr 2, there was a period, weaning treatment and winter treatment interaction ($P$ = 0.007) for sand site PI (Figure 3). The following regression equations were generated:

ED sand PI = 0.3 ± 1.36 + (0.03 ± 0.023 × day) ER

sand PI = 0.59 ± 0.764 + (-0.004 ± 0.016 × day) ND

sand PI = 1.43 ± 0.699 + (-0.01 ± 0.016 × day) NR

sand PI = 1.96 ± 0.513 + (0.05 ± 0.011 × day)

Normal-weaned range heifers initially had a greater PI than other treatments that increased (P < 0.001) each day. All other groups had a smaller initial preference that did not change during the summer.

Thin clay pan site preference in yr 1 was initially greater in D heifers than R heifers (Figure 4). The following regression equations were generated:

D TCP PI = 1.04 ± 0.061 + (-0.006 ± 0.002 × day)

R TCP PI = 0.84 ± 0.064 + (0.001 ± 0.002 × day)

However, over the summer D heifers decreased ($P$ < 0.001) preference and R heifers had no change. In yr 2, there was a weaning treatment by winter treatment interaction on thin clay pan site preference. Normal range heifers had a PI of 0.553 ± 0.087, which was similar to ED with a PI of 0.73 ± 0.082 and different from ER (0.95 ± 0.081) and ND (1.06 ± 0.087). ED was also similar to ER, but different from ND. All groups had a 0.005 ± 0.002 increase in PI over the summer ($P$ < 0.001).

***Distance from Fences.*** In yr 1, the range heifers' average distance from the fenceline was greater ($P$ = 0.03) than the drylot heifers (140.0 ± 5.03 m and 96.4 ± 4.09 m, respectively). However, in yr 2 there was a tendency ($P$ = 0.09) for a weaning by winter treatment interaction that indicated ED heifers mean distance was further from the fence than the NR heifers (137 ± 12.7 m and 80 ± 15.1 m, respectively). Heifers mean distance to the fence increased as temperature increased at 1.63 ± 0.383 m ($P$ < 0.001) per degree C in yr 1. However, in yr 2 there was only a tendency ($P$ = 0.09) for a 1.26 ± 0.739 m increase in distance from the fenceline per degree C as temperature increased.

***Distance from Water.*** Past research has found that the location of water influences pasture distribution (Gillen et al., 1984; Owens et al., 1991; Pinchak et al., 1991; Cibils et al., 2008) and our finding support this. In yr 1, there was an interaction between day and winter treatment ($P$ = 0.02). The R heifers started the grazing season at a closer mean distance to water than the D heifers (118 ± 37.5 m and 503 ± 34.2 m, respectively), and the average distance for range heifers did not change ($P$ > 0.05) over the summer but the

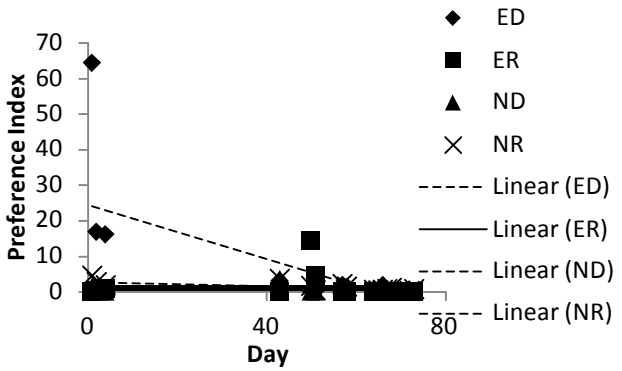


**Figure 1.** Influence of the interaction of weaning and winter environment treatments over the subsequent summer on preference index for claypan ecological sites in yr 2. Treatments were early-weaned and developed in drylot (ED), normal-weaned and developed in drylot (ND), early-weaned and developed on range (ER), and normal-weaned and developed on range (NR).

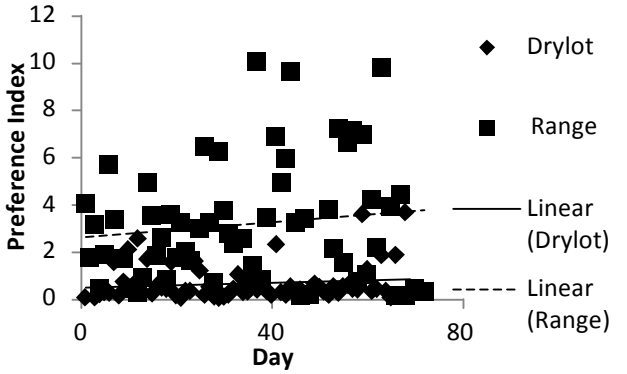


**Figure 2.** Effect of winter development environment on preference index for sand ecological sites in yr 1. Drylot and range were the environment of development from weaning to summer grazing as yearlings.

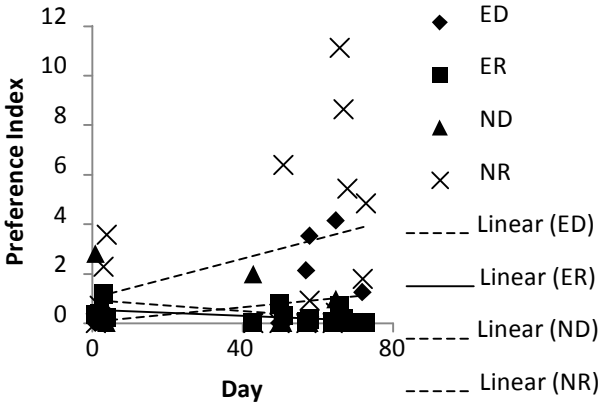


**Figure 3.** Weaning and winter treatment interaction effect on preference indexover the subsequent summer for sand ecological sites in yr 2. Treatments were early-weaned and developed in drylot (ED), normal-weaned and developed in drylot (ND), early-weaned and developed on range (ER), and normal-weaned and developed on range (NR).

D heifers moved closer at a rate of 2.97 ± 0.969 m/day ($P$ = 0.002). This suggests that once the D heifers became more familiar with where the water source was in relation to other resources, they did not venture as far away. In yr 2, there was no change over time in relation to the winter treatment, but the average distance to water was greater ($P$ < 0.001) for the R heifers than the D heifers (441 ± 18.3 m and 172 ± 17.6 m, respectively). There was also a day by weaning treatment interaction ($P$ = 0.002; Figure 5). The following regression equations were generated:

EW distance to water = 316 ± 33.8 + (0.75 ± 0.801 × day)

NW distance to water = 420 ± 31.3 + (-2.58 ± 0.736 × day)

The EW heifers started the grazing season closer to waterthan NW, and the EW mean distance to water did not change ($P$ > 0.05) over the season while the NW heifers reduced distance over time ($P$ < 0.001). Early-weaned drylot heifers had a tendency ($P$ = 0.07) to be closer to water than ER heifers in yr 2 (98.8 ± 21.02 m and 541 ± 26.9 m, respectively). There was also a tendency ($P$ = 0.09) in yr 2 for the mean distance from water to decrease by 0.92 ± 0.544 m/day. This suggested that the ER heifers had improved grazing distribution relative to the other treatments combinations because they had spent the greatest amount of post-weaning time in the grazing setting on rangeland.

There was an interaction between temperature and winter treatment for influence of temperature on distance from water in yr 1 ($P$ < 0.001; Fig. 6). The following regression equations were generated:

D distance to water = 1352 ± 26.1 + (-35.5 ± 1.03 ×ºC)

R distance to water = 202 ± 29.2 + (-4.9 ± 1.11 × ºC)

Drylot-developed heifers were further from water than range-developed heifers at cooler temperatures, but ventured closer to water as temperature increased. In yr 2 there was a three way interaction between temperature, weaning, and winter treatments ($P$ = 0.007; Fig. 7). The following regression equations were generated:

ED distance to water = 127 ± 41.2 + (-1.8 ± 2.16 ×ºC)

ER distance to water = 412 ± 54.0 + (7.2 ± 2.53 ×ºC)

ND distance to water = 270 ± 41.2 + (-2.82 ± 0.278 × ºC)

NR distance to water = 497 ± 41.2 + (-7.56 ± 0.284 × ºC)

Both ED and ND heifers started closer to the water and did not change distance with temperature. However, ER heifers increased distance and NR heifers reduced distance as temperature increased. This suggested that other factors besides water location affected grazing distribution.

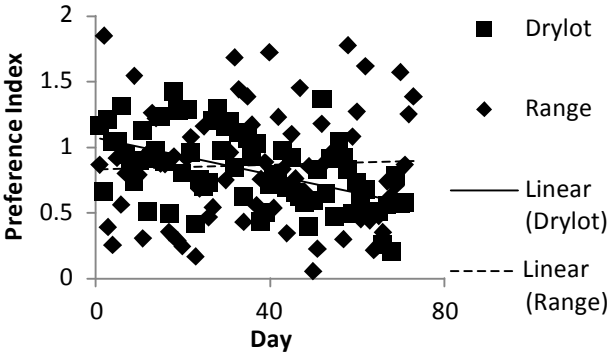


**Figure 4.** Effect of winter development environment on preference index for thin clay pan ecological sites in yr 1. Drylot and range were the environment of development from weaning to summer grazing as yearlings.

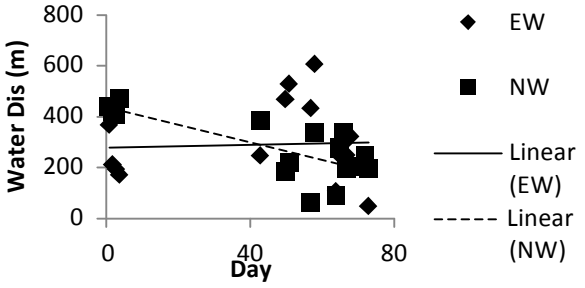


**Figure 5**. Change over time in mean distances from water by weaning treatment during the summer in yr 2. EW and NW represent early- (125 d) vs. normal-weaned (250 d), respectively.

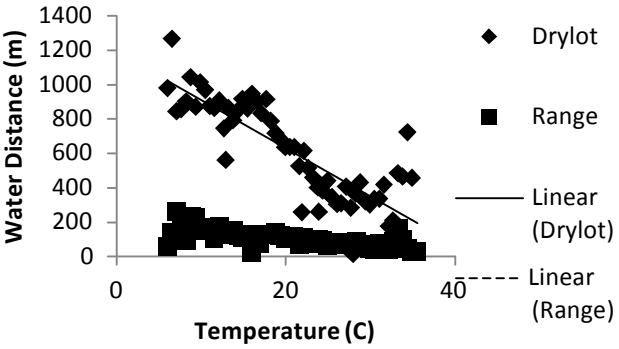


**Figure 6**. Winter treatment effect on mean distances from water during summer in relation to ambient temperature in yr 1. Drylot and range were the environment of development from weaning to summer grazing as yearlings.

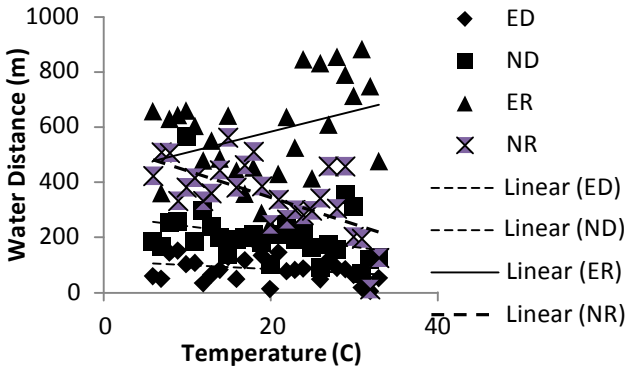


**Figure 7.** Weaning and winter treatment interaction effect on mean distance from water in relation to temperature in yr 2. Treatments were early-weaned and developed in drylot (ED), normal-weaned and developed in drylot (ND), early-weaned and developed on range (ER), and normal-weaned and developed on range (NR).

## IMPLICATIONS

Heifer grazing distribution is based on learned experiences, social cues and pasture environment. In this study, heifers that were wintered on rangeland initially utilized the pasture more evenly because the drylot heifers apparently needed to have a learning period. Past research has shown that water and vegetation location influences cattle pasture distribution and our results support that. By wintering heifers on rangeland, a producer will have heifers that do not require a learning period during the summer and will be able to better utilize their pastures.

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# EFFECTS OF DISTILLERS DRIED GRAINS WITH SOLUBLES SUPPLEMENTATION ON HEIFERS GRAZING NORTHERN GREAT PLAINS RANGELAND AND SUBSEQUENT FEELOT PERFORMANCE

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**ABSTRACT:** The objective of this study was to evaluate the effects of distillers dried grains with solubles (DDGS) supplementation on animal performance while grazing northern Great Plains rangeland, as well as the effects of supplementation on subsequent feedlot performance and carcass characteristics. Eighty-two yearling heifers (319.5 ± 4.0 kg) were utilized in a completely random design to examine the outlined objectives. Heifers were stratified by BW and randomly assigned to one of six groups, with each group randomly assigned to one of two treatments treatment: 1) no supplement (CON) or 2) DDGS supplemented at 0.6% BW (SUP). Distillers dried grains with solubles were delivered daily and placed in plastic lined feed bunks. Stocking rates were 1AU/1.6 ha. This project consisted of two portions: 70 d grazing study and a 109 d finishing study. Feedlot pens coincided with grazing pastures. All heifers received a common corn-based finishing ration daily for the duration of the 109 d. At the start of the grazing study, initial BW was not statistically different ($P > 0.09$). Final BW and ADG were greater for heifers supplemented DDGS ($P \leq 0.03$) during the grazing portion of this project. Heifers supplemented DDGS gained an additional 0.21 kg/d and came off of pasture 11.2 kg heavier than heifers not receiving DDGS. No statistical differences in animal performance were observed ($P \geq 0.13$) during the finishing period. No differences in carcass characteristics were observed ($P \geq 0.23$). Although not statistically different, it is noted that heifers supplemented DDGS had greater marbling (Modest-514) compared with unsupplemented heifers (Small-470). Producers could benefit from this small increase in marbling score; allowing the carcasses to qualify for certified programs thus resulting in premium returns. Distillers dried grains with solubles improved average daily gain of yearling heifers grazing northern Great Plains rangeland with no adverse effects on feedlot performance or carcass characteristics.

**Key words**: distillers dried grains with solubles, feedlot performance, yearling cattle

## INTRODUCTION

At the start of grazing season, actively growing forage contains protein that is highly degradable in the rumen, which can cause a MP deficiency in cattle (Klopfenstein et al., 2001). More so as grazing continues, the quality of forage declines as it matures, which leads to a decrease in digestibility (Greenquist et al., 2009). Since this decline in digestibility can lead to decreased animal performance; supplementation is critical to offset the nutritional deficiencies and maintain production demands (Caton and Dhuyvetter, 1997). Distillers dried grains with solubles (**DDGS)** has often been used as a viable supplement due to the amount of energy and undegradable intake protein (**UIP**). MacDonald et al. (2007) found that the added UIP provided by DDGS supplementation increased ADG by meeting the MP requirements in heifers grazing smooth bromegrass pastures.

Distillers dried grains with solubles has been shown to increase animal performance when added to beef finishing rations. However, research is limited when considering the effects of supplementation on animal performance after grazing. Morris et al. (2006) found that after supplementing DDGS during summer grazing in the Nebraska Sandhills there were no negative effects on feedlot ADG, DMI, and G:F. Therefore, the objective of the present study was to evaluate the effects of DDGS supplementation on animal performance while grazing northern Great Plains rangeland, as well as the effects of supplementation on subsequent feedlot performance and carcass characteristics. Our hypothesis was supplementation of distillers dried grains with solubles to cattle grazing northern Great Plains rangeland would increase average daily gain, without negatively affecting performance during finishing or carcass quality.

## MATERIALS AND METHODS

Procedures were approved by the North Dakota State University Animal Care and Use Committee prior to initiation of study.

***Grazing.*** This study was conducted at Central Grasslands Research and Extension Center (**CGREC**) located in south central North Dakota, approximately 14 km NW of Streeter, ND. Kentucky bluegrass (*Poa pratensis)* was the major plant species on study site (Neville and Patton, unpublished data). Other important forage species in the area include, blue grama (*Bouteloua gracilis*), needle and thread (*Stipa comate*), sunsedge *(Carex heliohilia*), and western snowberry (*Symphoricarpos occidentalis*; Hirschfeld et al., 1996). Eighty-two heifers (319 ± 4.0 kg) were utilized in a

completely random design. Heifers were stratified by BW and then randomly assigned to one of six groups for a 70 d grazing study starting on June 6th. Groups were assigned randomly to one of two treatments: 1) no supplement (**CON**) or 2) 0.6% of BW DDGS supplementation (**SUP).** Pastures served as the experimental unit (n = 3 pastures per treatment). Stocking rates were 1AU/1.6 ha. Heifers were allowed continuous access to water, trace-mineralized salt blocks (American Stockman Hi-Salt with EDDI; North American Salt Company, Overland Park, KS), and mineral blocks (Purina Mineral Block 12:12 HI-SE; Purina Mills, LLC, St. Louis, MO). Supplemental DDGS were offered in feeders, one placed in each pasture according to treatment. Total amount of DDGS supplemented per pasture for 7 d was calculated and divided by 5 to determine amount of DDGS to be pail fed 5 d per week. Refused feed was removed and weighed before each feeding at 0800 h. Initial and final BW were the average of 2 BW taken on consecutive days, with intermediate BW taken every two weeks to keep supplementation consistent with increasing BW.

Sample forage clippings were taken from pastures at 3 time intervals, starting at the beginning of the experiment, and continuing every 28 d until the end of the grazing study. At each sampling date five 0.25 $m^2$ plots were clipped per pasture, or 15 total plots per treatment.

***Feedlot and Carcass.*** At the end of summer grazing, August 16th, heifers were transported to the CGREC headquarters to begin the 109 d finishing period. Each feedlot pen coincided to a grazing pasture with heifers maintaining the same groups as during grazing. Heifers started on a medium concentrate diet, and were transitioned to a high concentrate diet over 28 d. All heifers received the same corn-based finishing ration, and finishing ration was formulated to meet or exceed dietary NRC requirements. The finishing diet (DM) included 54.8% dry rolled corn, 25% barley, 10% sanfoin hay, 5% corn silage, 5% liquid supplement (Sup-R-Lix NC Feedlot 40 R400; Purina Mills, LLC, St. Louis, MO), and 0.2% limestone. Liquid supplement (DM) included 400 g/T monensin (Elanco, Greenfield, IN), 40% CP, 3% crude fat, 5.5% calcium, 0.3% phosphorus, 2.5% salt, 1.5% potassium, 40,000 IU/lb vitamin A. Refused feed was removed and weighed weekly prior to feeding at 0800 h. Heifers were implanted with Synovex Choice (Fort Dodge Animal Health, Fort Dodge, IA) on d 1 in feedlot. Initial and final BW were the average of 2 BW taken on consecutive days, with continual BW taken every 28 d.

Heifers were harvested at a commercial abattoir and hot carcass weight, 12th rib back fat, percent kidney pelvic heart fat, ribeye area, marbling score, USDA yield grade and quality grade were evaluated by trained personnel.

***Sample Analysis.*** Sample forage clippings were dried using a forced-air oven (65°C; The Grieve Corporation, Round Lake, IL). Dried samples were ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2 mm screen. Forage samples were analyzed for DM, ash, CP, phosphorus, calcium, (methods 934.01, 942.05, 2001.11, 965.17, and 968.08, respectively; AOAC, 2010), IVDMD, and IVOMD. Concentrations of NDF (Van Soest et al., 1991; as modified by Ankom Technology, Fairport, NY) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology).

Feed refusals from grazing and finishing period were collected and dried using a forced-air oven (65°C; The Grieve Corporation, Round Lake, IL) for a minimum of 48 h. Dried feed refusals were analyzed for DM content. Dried samples from finishing period were used to calculate DMI, ground to pass a 2 mm screen and stored for further analysis.

***Statistical Analysis.*** Heifer performance data was analyzed as a completely random design using Mixed procedures (SAS Inst. Inc., Cary, NC). The experimental unit was pasture, and treatment was DDGS supplementation. Treatment differences were considered significant at an alpha of $P < 0.05$.

## RESULTS AND DISCUSSION

***Grazing.*** Initial BW did not differ ($P = 0.09$) between treatments (Table 1). Final BW and ADG were greater ($P \leq 0.03$) for supplemented treatment when compared with unsupplemented treatment. Heifers supplemented DDGS gained an additional 0.21 kg/d and were 11.21 kg heavier at the conclusion of the grazing period than heifers not receiving DDGS. Previous research has shown the DDGS supplementation increased ADG (Morris et al., 2006). Results from forage samples collected during the current study indicated CP was highest in June and decreased in July and August (10.17, 8.88, and 8.67, respectively). Lardy et al. (2004) found similar results for decreasing CP in the native Sandhills range. The results of the present research indicate that supplementing DDGS to heifers will increase performance while grazing. While it is unclear if heifers in the present study were nutrient deficient, the protein and energy provided by DDGS may improve animal performance regardless of nutritional status.

***Feedlot and Carcass.*** Final BW, ADG, DMI, and G:F did not differ ($P \geq 0.13$) among treatments (Table 2). Morris et al (2006) supplemented DDGS to steers grazing summer sandhill range and found no negative effect in DMI, ADG, and G:F. The present study agrees with Morris et al (2006) and suggests that the additional gain attained from the DDGS during grazing does not affect the animal performance during finishing. Average daily gain of heifers in the current study was similar among treatments ($P = 0.78$) which indicates that no compensatory response from the CON heifers was exhibited during finishing. Similarly, Greenquist et al. (2009) found no compensatory response was exhibited by steers supplemented DDGS while grazing during finishing.

Hot carcass weight, and 12th rib fat were similar ($P \geq 0.47$) among treatments (Table 3). Percent kidney pelvic heart fat did not differ among treatments ($P = 0.99$). Ribeye area was similar ($P = 0.50$) and averaged 81.29 $cm^2$ for all treatments. Average USDA yield grades were similar ($P = 0.30$) for SUP

**Table 1:** Effect of distillers dried grains with solubles supplementation to heifers grazing northern Great Plains rangeland on animal performance[1]

| | Treatment[2] | | | |
|---|---|---|---|---|
| Item | CON | SUP | SEM[3] | *P*- value |
| Initial BW, kg | 320.2 | 317.0 | 1.05 | 0.09 |
| Final BW, kg | 358.3 | 369.5 | 2.5 | 0.03 |
| ADG, kg/d | 0.54 | 0.75 | 0.02 | 0.002 |

[1]Means presented are least squares means
[2]CON = no supplement provided, SUP = distillers dried grains with solubles supplemented at 0.6% BW
[3]n = 3

**Table 2:** Effect of distillers dried with solubles supplementation to heifers grazing northern Great Plains rangeland on subsequent feedlot performance[1]

| | Treatment[2] | | | |
|---|---|---|---|---|
| Item | CON | SUP | SEM[3] | *P*- value |
| Final BW, kg | 568.6 | 578.2 | 7.46 | 0.42 |
| ADG, kg | 1.9 | 1.9 | 0.05 | 0.83 |
| DMI, kg | 12.9 | 12.4 | 0.20 | 0.13 |
| G:F, kg | 0.15 | 0.15 | 0.001 | 0.28 |

[1]Means presented are least squares means
[2]CON = no supplement provided, SUP = distillers dried grains with solubles supplemented at 0.6% BW
[3]n = 3

**Table 3:** Effect of distillers dried with solubles supplementation to heifers grazing northern Great Plains rangeland on subsequent carcass characteristics[1]

| | Treatment[2] | | | |
|---|---|---|---|---|
| Item | CON | SUP | SEM[3] | *P*- value |
| HCW, kg | 333.6 | 339.1 | 4.41 | 0.47 |
| Ribeye area, $cm^2$ | 81.94 | 80.65 | 1.23 | 0.50 |
| $12^{th}$ Rib Back fat, cm | 1.2 | 1.3 | 0.1 | 0.56 |
| Marbling score[4] | 470 | 514 | 22.3 | 0.24 |
| KPH, % | 1.85 | 1.85 | 0.08 | 0.99 |
| Quality grade[5] | 10.2 | 10.6 | 0.25 | 0.28 |
| Yield grade | 2.8 | 3.0 | 0.10 | 0.30 |
| Dress, % | 58.7 | 58.3 | 0.33 | 0.52 |

[1]Means presented are least squares means
[2]CON = no supplement provided, SUP = distillers dried grains with solubles supplemented at 0.6% BW
[3]n = 3
[4]Marbling score based on 400 = $Small^{00}$
[5]Quality grade based on Low Choice ($Ch^-$) = 10, High Prime ($Pr^+$) = 15

and CON (2.97, and 2.80 respectively). Marbling scores (where 400 = $small^{00}$, 500 = $modest^{00}$) were not different (*P* = 0.24) across treatments. Although not statistically different, it is noted that heifers receiving DDGS had greater marbling (Modest-514) compared with CON heifers (Small-470). The current research agrees with previous research (Morris et al., 2006) in that supplementation did not negatively affect carcass characteristics.

## IMPLICATIONS

Supplementing distillers dried grains with solubles during grazing is beneficial to producers. Supplementation increased animal performance while grazing without negatively effecting feedlot performance or carcass quality. Producers could benefit from the small increase in marbling scores; allowing the carcasses to qualify for certified programs thus resulting in premium returns.

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# EFFECTS OF POST-AI NUTRITION ON GROWTH PERFORMANCE AND FERTILITY OF YEARLING BEEF HEIFERS

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**ABSTRACT:** The objective of this study was to determine the effects of a change in nutritional plane during the first 21 d post-AI breeding on growth performance, and conception rates of yearling heifers. This experiment was conducted in replicate by the University of Wyoming (Loc. A) in collaboration with Purdue University (Loc. B) using a total of 151, 14 mo old (n = 98 and 53 at Loc. A and B respectively) Angus-cross beef heifers (initial BW = 380 ± 37 kg, and initial BCS = 5.19 ± 0.31 for Loc. A; and, initial BW = 429 ± 29 kg, and initial BCS = 5.24 ± 0.26 for Loc. B). Prior to trial initiation, heifers were developed a BW of approximately 65% of their mature wt by the beginning of the breeding season. Heifers were blocked by breed type and age and stratified by BCS and BW immediately following routine estrous synchronization and AI, and assigned to one of 3 nutritional treatments for a 21 d nutritional study: 1) formulated to meet NRC (2000) energy requirements for heifers to gain at a rate identical to that prior to initiation of trial (GAIN); 2) formulated to meet NRC (2000) energy requirements for maintenance only (MAINTAIN), and 3) formulated to provide 80% of the NRC, 2000 energy requirement for maintenance (LOSE). Data were analyzed using the MIXED and GLIMMIX procedures of SAS for continuous and categorical variables, respectively. Two pre-planned contrasts were used to compare effects of GAIN vs. others and MAINTAIN vs. LOSE. No treatment × location interactions ($P > 0.10$) were detected. As expected, ADG were greatest ($P < 0.01$) for heifers fed the GAIN diet (0.79 kg/d); intermediate for MAINTAIN (0.06 kg/d) and negative for LOSS heifers (-0.37 kg/d). Final BW and BCS followed the same pattern as ADG. Heifers fed the GAIN treatment had greater ($P = 0.04$) first-service conception rates (76.5%) when compared with the other 2 treatments overall. Interestingly, no differences were detected between MAINTAIN (56.2%) and LOSE (60.8%). These results suggest that failure to maintain pre-breeding nutritional plane during the 21 d immediately after AI can reduce conception rates.

**Key words:** beef, heifers, nutrition, post-artificial insemination

## INTRODUCTION

Approximately 80% of the West and Midwest U.S. cow herds are spring calving (USDA, 2010), which means that producers are often breeding their replacement heifers early in the grazing season. Many of these cow herds develop these heifers under a controlled nutritional environment to reach 60 to 65% of their mature weight and then inseminated to maximize productivity (Patterson et al., 1992; Buskirk et al., 1995) and then typically moved to pasture without supplementation (Perry et al, 2009) following AI. It is known that transporting females after insemination can compromise conception rates. Maternal recognition of pregnancy takes place around d 15 to 17 post-insemination (Thatcher et al., 1986) and transporting animals near this time compromises conception rates (Harrington et al., 1995). However, moving heifers within the first 5 days post-insemination does not appear to cause this reduction (Perry et al., 2010) in pregnancy success to AI. Nonetheless, research suggests that conception rates are compromised when heifers are moved from a controlled nutritional environment to a pasture without supplementation (Ferrell, 1982; Perry et al., 2009). These observed low AI conception rates on heifers may be due to early embryonic losses, which are defined as those losses that occur from fertilization until day 28 of pregnancy when differentiation and implantation has occurred (Geary, 2006). A decrease in progesterone concentrations after AI may be responsible for this increase in embryonic mortality (Folman et al., 1973). In herds were post-AI movement occurs within the first several days after insemination, but coincides with early spring forage growth which is high in water content and low in nutrient profile, producers have still experienced a reduction in AI conception rates. Our hypothesis is that when heifers are fed under a controlled nutritional environment, synchronized and artificially inseminated, and then moved within several days to early spring pasture, conception rates are compromised due to a sudden drop in energy intake during the first 21d post-insemination. The objective of this study was to determine the effect that nutritional plane during the first 21 d post-breeding on BW, BCS, AI conception rates, and ultimate reproductive efficiency of yearling beef heifers.

## MATERIALS AND METHODS

All animal procedures were approved by the Animal Care and Use Committee at both institutions.

***Location.*** This experiment was conducted in replicate at facilities of the University of Wyoming, Laramie (**Loc. A**) and Purdue University (**Loc. B**).

***Animals.*** A total of 151, 14 mo old (n = 98 and 53 at Loc. A and B respectively) crossbred beef heifers (initial BW = 380 ± 37 kg, and initial BCS = 5.19 ± 0.31 for Loc. A; and, initial BW = 429 ± 29 kg, and initial BCS = 5.24 ± 0.26 for Loc. B) were utilized immediately following routine estrous synchronization and AI. Heifers were randomly blocked by breed type and age, and stratified by BCS and BW to one of 3 nutritional treatments for a 21 d study. Prior to the initiation of the study, heifers were developed a BW of approximately 65% of their mature wt by the beginning of the breeding season.

***Design and Treatments.*** Equivalent dietary treatments (Table 1) were used in both locations and consisted of: 1) formulated to meet NRC (2000) requirements for heifers to gain at a rate identical to that prior to initiation of trial (**GAIN**); 2) maintenance diet formulated to meet NRC, 2000 nutrient requirements for maintenance (**MAINTAIN**), and 3) diet formulated to provide 80% of the NRC, 2000 energy requirement for maintenance (**LOSE**). Estrous synchronization was accomplished using the industry standard 7-day Co-Synch+CIDR and the MGA/$PGF_{2\alpha}$ protocols at Loc. A and B, respectively. Heifers in Loc. B were bred 12 hr after standing heat until 72 hr after the last PGF2α at which point the remaining heifers were timed-AI. In Loc. A, all heifers were bred on a timed scheme. Immediately following AI, heifers were returned to dry lots and placed on dietary treatment for the 21 d experimental period. Estrus detection was conducted and heifers noted in estrus after the initial AI were rebred by AI during the treatment period. Following the 21 d treatment period, all heifers were commingled and placed on pasture with free-choice mineral for the remainder of the grazing season. Clean-up bulls were utilized for 45 d following AI. Artificial insemination pregnancy rates were determined 35 and 30 d after timed-AI via ultrasonography in Loc A and B respectively. Overall breeding season pregnancy rates were determined 30 d after the conclusion of the breeding season.

***Statistical Analysis.*** Continuous dependent variables including BW, BCS, and ADG were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included the fixed main effects of treatment and location as well as the appropriate interaction, which was not significant ($P > 0.10$) and removed from the model. Categorical variables including AI pregnancy, 2nd service conception, AI+2nd service conception, and overall breeding season pregnancy rates were analyzed using the GLIMMIX procedure of SAS for binary variables. There were no significant treatment × location interactions for any of the reproductive variables measured ($P > 0.10$); therefore data were pooled across locations. Effects of AI sire and AI technician were confounded with herd or location, therefore, the effects of AI sire, AI technician, treatment, and the appropriate interactions on pregnancy and conception rates were evaluated first within herd. For dependent variables of interest, the final model included the fixed main effects of treatment and location. Contrast analyses were conducted using the MIXED and GLIMMIX procedures of SAS. Contrasts included: 1) GAIN vs. the average of all other treatments, and 2) MAINTAIN vs. LOSE. Differences were considered to be significant when $P \leq 0.05$ and a tendency when $P > 0.05$ but $P \leq 0.10$.

## RESULTS

As was expected, ADG (Table 2) was greatest ($P < 0.01$) for heifers fed the GAIN (0.79 kg/d) diet compared with the other 2 diets, and MAINTAIN (0.06 kg/d) was greater ($P < 0.01$) than LOSE (-0.37 kg/d), which had a negative ADG. Final BW, change in BW, final BCS and change in BCS were also analyzed and followed the same pattern as ADG with a $P < 0.01$ for all treatment comparisons, except for final BCS which showed no difference when MAINTAIN was compared with LOSE ($P = 0.62$).

First service conception rate (AI pregnancy rate) was greatest ($P = 0.02$) for heifers on the GAIN diet compared with the other two treatments. Interestingly, no differences ($P = 0.68$) were found between the MAINTAIN and LOSE treatments when compared with each other. In a similar fashion, 2nd service conception rate tended ($P = 0.06$) to be greater for GAIN than MAINTAIN and LOSE, with no difference ($P > 0.05$) between MAINTAIN and LOSE. Therefore, combined conception rate of AI + 2nd service was greater ($P = 0.01$) for GAIN compared with MAINTAIN and LOSE, which were not different from each other ($P = 0.46$). Finally, overall season pregnancy rate also tended to be greater ($P = 0.06$) for GAIN (96.1%), than MAINTAIN (85.7%) and LOSE (84.3%), which did not differ from each other.

## DISCUSSION

Most research on the effects of nutrition in reproduction performance of beef heifers focuses either in the weaning to breeding, or the post-partum stages of production. Thus, limited peer reviewed research can be found on post-AI nutrition. However, there is significant evidence that any kind of stress (nutritional stress included) in the female during early stages of pregnancy can negatively affect embryo development (Ashworth et al., 2009).

As exposed by reductions in ADG, and consequently BW and BCS, heifers in the MAINTAIN and LOSE diet were under nutritional stress, which was reflected on AI and overall conception rates. Results observed in this study are supported by other research evaluating post-AI nutritional management (Perry et al. (2009) Three independent studies compared 1st service conception rates between heifers developed in feedlots and moved to spring pastures with

**Table 1.** Formulated ingredient and chemical composition of diets (DM basis) fed to yearling heifers

| | Treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| | Loc. A[2] | | | Loc. B[2] | | |
| Item | GAIN | MAINTAIN | LOSE | GAIN | MAINTAIN | LOSE |
| DMI, kg | 8.4 | 7.0 | 5.0 | 10.3 | 6.6 | 5.2 |
| Ingredient, % | | | | | | |
| Grass hay | 94.2 | 99.5 | 90.3 | - | - | - |
| Grass-legume hay | - | - | - | 86.2 | 98.3 | 98.3 |
| Distill grain | 5.4 | - | 9.0 | - | - | - |
| Cracked corn | - | - | - | 12.8 | - | - |
| Mineral supplement | 0.4 | 0.5 | 0.6 | 1.1 | 1.7 | 1.7 |
| Chemical composition | | | | | | |
| DM, % | 78.9 | 89.9 | 72.9 | 86.7 | 82.7 | 82.7 |
| NEm Mcal/kg | 1.29 | 1.23 | 1.32 | 1.23 | 1.21 | 1.21 |
| NEg Mcal/kg | 0.72 | 0.67 | 0.75 | 0.68 | 0.66 | 0.66 |
| CP, % | 9.4 | 8.3 | 10.2 | 11.7 | 16.8 | 16.8 |
| TDN, % | 59.5 | 57.4 | 60.6 | 58.2 | 59.9 | 59.9 |

[1]GAIN: diet formulated to meet NRC, 2000 requirements for heifers to gain wt; MAINTAIN: maintenance diet formulated to meet NRC, 2000 nutrient requirements for maintenance; LOSE: diet formulated to provide 80% of the NRC, 2000 energy requirement for maintenance.
[2]Loc. A: Laramie, WY (University of Wyoming); Loc. B: West Lafayette, IN (Purdue University).

**Table 2.** Effect of Post-AI nutrition on growth changes in yearling beef heifers

| | Treatment[1] | | | | Contrast | |
|---|---|---|---|---|---|---|
| Item[2] | GAIN | MAINTAIN | LOSE | SEM[3] | GAIN vs. others | MAINTAIN vs. LOSE |
| ADG[4], kg | | | | | | |
| Loc. A | 0.65 | 0.06 | -0.37 | 0.08 | | |
| Loc. B | 1.04 | 0.08 | -0.37 | 0.10 | | |
| Overall | 0.79 | 0.06 | -0.37 | 0.06 | <0.001 | <0.001 |
| Final BW[4], kg | | | | | | |
| Loc. A | 424 | 376 | 348 | 5.4 | | |
| Loc. B | 449 | 433 | 421 | 7.6 | | |
| Overall | 433 | 396 | 374 | 4.6 | <0.001 | <0.01 |
| Change in BW[4], kg | | | | | | |
| Loc. A | 13.72 | 1.25 | -7.75 | 0.73 | | |
| Loc. B | 21.78 | 1.56 | -7.72 | 1.02 | | |
| Overall | 16.67 | 1.36 | -7.74 | 0.56 | <0.001 | <0.001 |
| Final BCS[4] | | | | | | |
| Loc. A | 5.55 | 5.32 | 5.36 | 0.04 | | |
| Loc. B | 5.40 | 5.33 | 5.24 | 0.06 | | |
| Overall | 5.49 | 5.33 | 5.32 | 0.04 | <0.001 | 0.62 |
| Change in BCS | | | | | | |
| Loc. A | 0.38 | 0.03 | -0.22 | 0.02 | | |
| Loc. B | 0.16 | 0.06 | 0.02 | 0.03 | | |
| Overall | 0.30 | 0.04 | -0.13 | 0.02 | <0.001 | <0.001 |

[1] GAIN: diet formulated to meet NRC, 2000 requirements for heifers to gain wt; MAINTAIN: maintenance diet formulated to meet NRC, 2000 nutrient requirements for maintenance; LOSE: diet formulated to provide 80% of the NRC, 2000 energy requirement for maintenance.
[2]Loc. A: Laramie, WY (University of Wyoming); Loc. B: West Lafayette, IN (Purdue University).
[3]The greatest SEM is presented (n = 98 for Loc. 1; n = 53 for Loc. 2; n = 151 overall).
[4]Location effect significant ($P < 0.05$).

**Table 3.** Effect of Post-AI nutrition on reproductive performance in yearling beef heifers

| Item[2] | Treatment[1] | | | Contrast | |
|---|---|---|---|---|---|
| | GAIN | MAINTAIN | LOSE | GAIN vs. others | MAINTAIN vs. LOSE |
| 1st service conception (AI)[3], % | | | | | |
| Loc.[3] A | 65.6 (21/32) | 46.9 (15/32) | 51.5 (17/33) | | |
| Loc. B | 94.7 (18/19) | 75.0 (12/16) | 77.8 (14/18) | | |
| Overall | 76.5 (39/51) | 56.2 (27/48) | 60.8 (31/51) | 0.02 | 0.68 |
| 2nd service conception, % | | | | | |
| Loc. A | 67.6 (6/11) | 46.9 (4/17) | 31.2 (5/16) | | |
| Loc. B | 100 (1/1) | 25.0 (1/4) | 50.0 (2/4) | | |
| Overall | 58.3 (7/12) | 23.8 (5/21) | 35.0 (7/20) | 0.06 | 0.44 |
| AI+2nd service conception[3], % | | | | | |
| Loc. A | 84.4 (27/32) | 59.3 (19/32) | 66.7 (22/33) | | |
| Loc. B | 100 (19/19) | 81.3 (13/16) | 88.9 (16/18) | | |
| Overall | 90.2 (46/51) | 67.3 (33/49) | 74.5 (38/51) | 0.01 | 0.41 |
| Season pregnancy, % | | | | | |
| Loc. A | 96.9 (31/32) | 81.8 (27/33) | 78.8 (26/33) | | |
| Loc. B | 94.7 (18/19) | 93.8 (15/16) | 94.4 (17/18) | | |
| Overall | 96.1 (49/51) | 85.7 (42/49) | 84.3 (43/51) | 0.06 | 0.59 |

[1]GAIN: diet formulated to meet NRC, 2000 requirements for heifers to gain wt; MAINTAIN: maintenance diet formulated to meet NRC, 2000 nutrient requirements for maintenance; LOSE: diet formulated to provide 80% of the NRC, 2000 energy requirement for maintenance.
[2]Loc. A: Laramie, WY (University of Wyoming); Loc. B: West Lafayette, IN (Purdue University).
[3]Location effect significant ($P < 0.05$).

and without supplementation; and pasture developed heifers moved to feedlots, or maintained in pasture with or without supplementation immediately following AI. In every case, heifers moved from feedlot to pasture with no supplementation resulted in decreased pregnancy rates than those kept in the feedlot or moved to a pasture and supplemented. On the other hand, pasture developed heifers that returned to pasture (with or without supplementation) and heifers moved to a feedlot resulted in similar pregnancy rates. They concluded that pregnancy success of heifers developed in a feedlot tended to be influenced by weight gain after moving heifers to grass. These results suggest that heifers developed in a pasture setting are less responsive to changes in post-AI nutrition. One of the factors influencing this phenomenon may be that those heifers do not experience suppression in nutritional plane during the transition.

Research conducted on the effects of post-AI nutrition in heifers is scarce, and thus more research is necessary to elucidate mechanisms by which post-AI nutrition drives pregnancy success to AI. However, there is evidence suggests that reducing feed intake from a high to a low level immediately following AI severely reduces embryo survival rate in heifers (Dunne et al., 1997; Ashworth et al., 2009), which is perhaps due to a reduction in progesterone concentrations after breeding (Folman et al., 1973).

## IMPLICATIONS

Although the mechanisms involved are not well understood, we can conclude that suppression in nutritional plane of heifers during the 21 d following AI has a negative effect on heifer performance, and ultimately AI conception rates. Therefore, it is biologically beneficial to ensure that heifers maintain their pre-breeding plane of nutrition during the 21 d following AI.

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# ANIMAL HEALTH AND WELL BEING

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# DELAYING A BOVINE VIRAL DIARRHEA VACCINE AND GROWTH IMPLANT WITH METAPHYLAXIS AFFECTS PERFORMANCE, BUT NOT HEALTH OF FEEDLOT HEIFERS[1]

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**ABSTRACT:** Stress and low nutrient intake of newly received feedlot calves may limit efficacy of both a bovine viral diarrhea (BVD) vaccine and growth implant at initial processing. Use of chlortetracycline (CTC) when delaying an initial vaccine and implant may be beneficial. This 56-d study evaluated effects of timing (TIME) of a BVD vaccine and growth implant with or without CTC on health and performance of 312 heifers (184 ± 0.7 kg BW). Initial processing of heifers included treatment with tulathromycin and vaccination for bovine respiratory disease. Heifers were sorted into 24 pens, which were randomly assigned to 4 treatments. Treatments (2 × 2 factorial) were growth implant (Synovex-C; Fort Dodge Animal Health) and BVD vaccination (Bovi-Shield Gold BVD; Pfizer Animal Health) of calves on d 1 (INITIAL) or d 28 (DELAYED) after arrival with (+CTC) or without (-CTC) three 5-d pulse-doses of CTC (Aureomycin; Alpharma Animal Health) in their diets. Diets were based on a complete feed (RAMP; Cargill) and ground corn delivered twice daily. Performance and health were analyzed using MIXED and GLIMMIX procedures of SAS, respectively. A TIME × CTC interaction ($P = 0.06$) occurred for DMI; DMI was lower for DELAYED than INITIAL when heifers received -CTC, but DMI was not different between DELAYED and INITIAL when heifers received +CTC. No TIME × CTC interactions ($P > 0.21$) occurred for BW, ADG, or G:F. Timing of BVD vaccine and implant did not affect ($P = 0.18$) BW of heifers on d 28, but BW were lower ($P = 0.04$) for DELAYED than INITIAL heifers on d 56. From d 29 to 56, ADG was less ($P = 0.02$) and G:F tended to be less ($P = 0.07$) for DELAYED than INITIAL heifers. Delaying the BVD vaccine and implant also tended to lower ($P = 0.09$) ADG from d 1 to 56, but did not affect ($P = 0.27$) G:F. On d 28, BW of +CTC heifers tended to be lower ($P = 0.06$) than -CTC heifers, but CTC did not affect ($P > 0.21$) d-56 BW, ADG, or G:F. Morbidity and mortality during the 56 d were not affected ($P > 0.17$) by treatments. Delaying the initial BVD vaccine and growth implant with or without CTC may not improve animal health, and may have negative implications on performance of newly received feedlot heifers.

**Key words:** implant, heifer, vaccine

## INTRODUCTION

The feedlot receiving period can bring numerous challenges that heighten the need for superior management of cattle nutrition and health. The stress of comingling and transportation can increase the risk of disease, particularly after arrival to the feedlot (Smith, 2004). Nutrients are repartitioned towards support of the immune system in response to disease exposure (Waggoner et al., 2009). Additively, newly received cattle with low nutrient intakes are less capable of supporting metabolic needs of an immune response.

Current practices include vaccinating and implanting calves shortly after arrival to the feedlot. Vaccination is important in reducing the risk of disease by increasing immunity. If cattle are stressed, sick, or both, their ability to illicit an adequate immune response to the vaccine may be impaired (Downey et al., 2011). Growth implants increase feed efficiency and BW gain, reduce the cost of BW gain, and are thereby economically important to the beef industry. If cattle are stressed, sick, or both, growth response to an implant may be limited by low dietary intake and subsequent nutrition restriction. Once newly received calves adapt to the feedlot environment, recover from disease, and attain an adequate nutrient intake, they should be more prepared to meet the growth demands of implants, as well as the immune responses to vaccination. Antibiotic therapy of calves before vaccination and implanting may improve the efficacy of these programs.

Our hypothesis is that delaying the vaccine and growth implant in stressed calves receiving antibiotic therapy will improve the efficacy of the vaccination and implant programs. The objectives of the current study are to evaluate cattle health and performance in response to the effects of timing (**TIME**) of a bovine viral diarrhea (**BVD**) vaccine and growth implant with or without chlortetracycline (**CTC**) and a metaphylaxic treatment with tulathromycin.

## MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee at New Mexico State University.

***Cattle and Initial Processing.*** This experiment was conducted at the Clayton Livestock Research Center and

[1]Authors acknowledge Cargill, Pfizer Animal Health, and Lextron for their support for this experiment.

used 312 crossbred heifers (184 ± 0.7 kg average BW) of Southeast Texas sale barn origin. Initial processing (d 0) included: 1) recording of individually taken BW using a single animal squeeze chute (Silencer; Moly Manufacturing, Inc., Lorraine, KS) suspended from two load cells; 2) unique identification with an ear tag; 3) application of the antiparacitics, doramectin and albendazole (Dectomax and Valbazen; Pfizer Animal Health, Exton, PA); 4) administration of tulathromycin (Draxxin; Pfizer Animal Health) according to label directions; and 5) tipping of horns as needed. Approximately 24 h after initial processing (d 1), individual BW were recorded, and cattle were vaccinated against viral diseases using an intranasal vaccine (Inforce 3; Pfizer Animal Health).

***Experimental Design and Treatments.*** On d 1, heifers were randomly assigned to one of 24 soil- surfaced pens (12 × 35 m) equipped with 10.4 m of bunk line and continuous flow water tanks. There were 13 heifers per pen, and pens were randomly assigned to 1 of 4 treatments. The treatments (Table 1), in a 2 × 2 factorial arrangement, were vaccination against BVD (Bovi-Shield Gold BVD; Pfizer Animal Health) and a growth implant (100 mg progesterone + 10 mg estradiol; Synovex-C; Fort Dodge Animal Health, Fort Dodge, IA) on d 1 (**INITIAL**) or d 28 (**DELAYED**) after initial processing in combination with (+CTC) or without (-CTC) three 5-d pulse-doses of CTC (Aureomycin; Alpharma Animal Health, Bridgewater, NJ). All +CTC rations were formulated to provide 1 g of CTC per 45.45 kg of BW on d 7 to 11, 13 to 17, and 19 to 23. All heifers received an additional colored ear tag on d 1 based on treatment assignment.

***Management, Diets, and Feeding.*** Cattle were fed a diet based on a commercially available complete feed (RAMP; Cargill, Inc., Wayzata, MN) with 20% inclusion of ground corn for the first 28 d, and then the commercial feed with 30% inclusion of ground corn from d 29 to 56 of the receiving period (Table 1). A coccidiostat (Deccox; Alpharma Animal Health, Bridgewater, NJ) was used during the first 28 d on feed, and lasalocid (Bovatec; Alpharma Animal Health) was fed from d 29 to 56. During the receiving period, feed intake of pens of cattle was managed to minimize build-up of orts in feed bunks. Feed delivery and feed bunk evaluation occurred twice daily (morning and evening) to determine the quantity of feed to offer each pen. Diet samples were obtained weekly from randomly selected feed bunks to calculate dietary DM and for nutrient analyses by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

***Collections.*** Performance measurements were taken on d 28 and 56, while health measurements were taken on a daily basis throughout the course of the study. These measurements included DMI, ADG, G:F, morbidity, and mortality. Initial BW were the average of weights which were taken on an individual basis at initial processing and approximately 24 h later (d 1). On d 28 and 56, animals were weighed using a pen scale.

**Table 1.**Composition of receiving diets

| | d 1 to 28[1] | | | | d 29 to 56 |
|---|---|---|---|---|---|
| | INITIAL | | DELAYED | | |
| Ingredient, % of DM | -CTC | +CTC | -CTC | +CTC | All pens |
| RAMP[2] | 79.1 | 79.1 | 79.1 | 79.1 | 69.1 |
| Ground corn | 20.0 | 20.0 | 20.0 | 20.0 | 30.0 |
| Supplement A[3] | 0.53 | - | 0.53 | - | - |
| Supplement B[4] | - | 0.53 | - | 0.53 | - |
| Supplement C[5] | - | - | - | - | 0.53 |
| Mineral supplement[6] | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Nutrient[7], % DM | | | | | |
| CP | 17.46 | 17.56 | 17.46 | 17.56 | 16.49 |
| NDF | 27.42 | 26.75 | 27.42 | 26.75 | 25.85 |
| ADF | 13.51 | 14.03 | 13.51 | 14.03 | 13.18 |
| K | 1.36 | 1.30 | 1.36 | 1.30 | 1.07 |
| Ca | 1.39 | 1.37 | 1.39 | 1.37 | 1.12 |
| P | 0.84 | 0.81 | 0.84 | 0.81 | 0.72 |
| S | 0.36 | 0.33 | 0.36 | 0.33 | 0.33 |

[1]Heifers were fed diets with treatments from d 1 to 28. Treatments, in a 2 × 2 factorial arrangement, were BVD vaccination and a growth implant on d 1 (INITIAL) or d 28 (DELAYED) after initial processing in combination with (+CTC) or without (-CTC) three 5-d pulse-doses (d 7 to 11, 13 to 17, and 19 to 23) of chlortetracycline. From d 29 to 56, calves were fed a common diet.
[2]A commercially available complete feed (Cargill, Inc., Wayzata, MN)
[3]Supplied 22.7 mg decoquinate (Deccox) per 45.45 kg of BW.
[4]Supplied 22.7 mg decoquinate (Deccox) and 1 g chlortetracycline (Aureomycin) per 45.45 kg of BW, respectively.
[5]Contained 42.8 mg lasalocid (Bovatec) per kg of diet DM.
[6]Mineral supplement composition: 90.36% limestone, 5.12% salt, 4.52% trace mineral and vitamin premix (supplied 9% zinc, 5.75% manganese, 1.8% copper, 1600 mg/kg iodine, 500 mg/kg cobalt, 360 mg/kg selenium, 5500 IU/kg vitamin A, 660 IU/kg vitamin D, and 88 IU/kg vitamin E).
[7]Analyzed by SDK Laboratories (Hutchinson, KS).

Clinical monitoring was performed by trained personnel at the same time daily for the 56-d experiment. Animals were scored (0 to 3 scale, with 0 = normal) based on visual appraisal. Animals were observed as feed was delivered to the pen for signs of inappetence, anorexia, decreased activity, abnormal posture, abnormal gait, dyspnea, and depression. Calves with a combined score > 1 were removed from the pen and taken to the processing facility for further evaluation. At the processing facility, the pulled calves were weighed, and a rectal temperature was obtained. If the rectal temperature was > 40°C and clinical assessment (combined score > 1) warranted treatment, then the calf was treated according to the Clayton Livestock Research Center's standard protocol.

***Statistical Analysis.*** Performance data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatments included a 2 × 2 factorial arrangement with pen as the experimental unit. The effects of TIME, CTC, and TIME × CTC interaction were included in the model, and the random effect was pen. Mortality and morbidity data were analyzed as binomial proportions using the GLIMMIX procedure of SAS. Treatment proportions and SE were calculated using the ILINK option. Differences were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

***Effects of Treatment Interactions.*** Treatment interactions occurred for DMI (Table 2). From d 1 to 56, DMI tended to be lower for DELAYED than INITIAL when heifers received -CTC, but DMI was not different between DELAYED and INITIAL when heifers received +CTC (treatment interaction, $P = 0.06$). Therefore, the effects of delaying the BVD vaccine and implant on intake may depend upon the choice to feed CTC. Because the use of growth implants can have an impact on feed intake (Anderson and Botts, 1995), it is likely that the observed differences in DMI were driven by the growth implant, and not the vaccine. Treatment interactions were not significant ($P > 0.21$) for heifer BW on d 28 and 56, nor for ADG or G:F (Table 2). Similarly, no treatment interaction ($P > 0.39$) occurred for morbidity or mortality (Table 3).

***Effects of Delaying the BVD Vaccine and Growth Implant.*** Newly arrived calves in the feedlot are often naïve and do not necessarily know how to eat from a bunk. Low feed intake combined with the additive effects of disease and stress results in poor performance of newly received calves (Galyean et al., 1999). The objective of delaying the BVD vaccination and growth implant for 28 d was to allow calves to recover from stress and disease, and to establish adequate feed intake so that they are more prepared to meet the growth demands of implants, as well as the immune responses to vaccination. In the current study, BW were not different ($P = 0.18$) on d 28, but were lighter ($P = 0.04$) on d 56 for DELAYED than INITIAL heifers (Table 2). From d 1 to 28, ADG was not different ($P = 0.41$) between DELAYED and INITIAL heifers. From d 29 to 56, ADG of DELAYED heifers was less ($P = 0.02$) than INITIAL heifers. From d 1 to 56, ADG tended to be less ($P = 0.09$) in DELAYED versus INITIAL heifers. From d 29 to 56, G:F tended to be less ($P = 0.07$) in DELAYED heifers compared with INITIAL. The tendency for a decreased ADG and significantly less DMI caused by delaying the BVD vaccination and growth implant resulted in an overall G:F that was not different ($P = 0.26$) between DELAYED and INITIAL heifers.

The results of the current study are different to the results of our previous study (McDaniel et al., 2011), which evaluated the effects of delaying a growth implant from d 1 to d 21 on performance of feedlot heifers. McDaniel et al. (2011) reported that delaying an implant did not affect DMI, but decreased calf BW, ADG and G:F during the first 21 d of the study. However, by d 42 of the study, BW, ADG, and G:F were not different between calves receiving the implant on d 21 versus d 1. Therefore, calves were able to compensate from d 21 to 42 for loss of performance during d 1 to 21 in the study by McDaniel et al. (2011). Different results between the current study and the study of McDaniel et al. (2011) could be due to differences in implant strength and chemistry (estrogenic versus androgenic), as well as differences in length (28 d versus 21 d) of the delay in implant. In the current study, performance was not monitored beyond d 56, and therefore it could not be determined whether the compromised performance of the DELAYED heifers could recover later during the feeding period.

Delaying the BVD vaccine and growth implant did not affect ($P > 0.17$) the percentage of calves treated for sickness or the percentage of death loss (Table 3). Although differences between DELAYED and INITIAL were not statistically significant ($P = 0.17$), it is interesting that none of the DELAYED heifers received 3 treatments. Nevertheless, delaying the BVD vaccine and growth implant to allow the cattle to recover from stress and disease, and to establish adequate feed intake was not beneficial.

***Effects of Feeding Chlortetracycline.*** In theory, healthier cattle have improved performance because of increased feed intake, and less demand for nutrient allocation to support immune function. Chlortetracycline is used to reduce the incidence of subclinical disease, and potentially the negative impact of disease on performance. Negating the impact of disease before onset of clinical signs could reduce performance losses. In "high risk" cattle, an effective means of controlling the incidence of sickness may be antibiotic therapy (Duff and Galyean, 2007). According to Cole (1993), feed-based antibiotic therapy may also be an effective method to reduce morbidity in cattle that are not expected to have a high prevalence of disease. The idea behind the concurrent use of CTC with tulathromycin was that cattle which were fed +CTC would have effective blood concentrations of CTC prior to the loss of efficacy of tulathromycin. In this case, the additional protection provided by the use of CTC could reduce losses of performance associated with health issues. However, the use of CTC did not affect ($P > 0.21$) DMI, ADG, or G:F during this experiment (Table 2). On d 28, BW tended ($P = 0.06$) to be heavier in heifers receiving

**Table 2.** The effects of a delayed BVD vaccination and growth implant with or without chlortetracycline on the performance of newly received feedlot heifers[1]

| | Treatment[2] | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | INITIAL | | DELAYED | | | | | TIME × |
| Item | -CTC | +CTC | -CTC | +CTC | SEM | TIME[3] | CTC[4] | CTC[5] |
| BW, kg | | | | | | | | |
| d 1 | 184.8 | 184.1 | 186.7 | 183.9 | 1.36 | 0.52 | 0.21 | 0.45 |
| d 28 | 223.3 | 217.5 | 218.7 | 216.3 | 2.04 | 0.18 | 0.06 | 0.41 |
| d 56 | 269.3 | 266.9 | 262.1 | 264.3 | 2.26 | 0.04 | 0.98 | 0.32 |
| DMI, kg/d | | | | | | | | |
| d 1 to 28 | 4.79 | 4.67 | 4.39 | 4.57 | 0.08 | <0.01 | 0.68 | 0.07 |
| d 29 to 56 | 7.51 | 7.16 | 6.96 | 7.08 | 0.13 | 0.03 | 0.39 | 0.09 |
| d 1 to 56 | 6.15 | 5.91 | 5.67 | 5.81 | 0.10 | <0.01 | 0.65 | 0.06 |
| ADG, kg/d | | | | | | | | |
| d 1 to 28 | 0.18 | 0.90 | 0.84 | 0.97 | 0.160 | 0.41 | 0.64 | 0.21 |
| d 29 to 56 | 1.77 | 1.77 | 1.55 | 1.60 | 0.074 | 0.02 | 0.75 | 0.74 |
| d 1 to 56 | 1.47 | 1.33 | 1.19 | 1.28 | 0.093 | 0.09 | 0.76 | 0.22 |
| G:F | | | | | | | | |
| d 1 to 28 | 0.247 | 0.192 | 0.188 | 0.213 | 0.035 | 0.59 | 0.68 | 0.27 |
| d 29 to 56 | 0.235 | 0.247 | 0.222 | 0.226 | 0.009 | 0.07 | 0.41 | 0.68 |
| d 1 to 56 | 0.240 | 0.225 | 0.209 | 0.221 | 0.015 | 0.26 | 0.90 | 0.39 |

[1]Cattle that died were not excluded from the statistical analyses of performance data. If a calf died, the BW and DMI were still included in the data set. ADG = pen BW at the end of a period minus pen BW at the beginning of a period divided by total head days; head day = number of animals in a pen for a day.
[2]Heifers were fed diets with treatments from d 1 to 28. Treatments, in a 2 × 2 factorial arrangement, were BVD vaccination and a growth implant on d 1 (INITIAL) or d 28 (DELAYED) after initial processing in combination with (+CTC) or without (-CTC) three 5-d pulse-doses (d 7 to 11, 13 to 17, and 19 to 23) of chlortetracycline. From d 29 to 56, calves were fed a common diet.
[3]TIME = *P*-value for main effect of timing of BVD vaccine and growth implant.
[4]CTC = *P*-value for the main effect of CTC.
[5]TIME × CTC = *P*-value for a TIME × CTC interaction.

**Table 3.** The effects of a delayed BVD vaccination and growth implant with metaphylaxis on the health of newly received feedlot heifers

| | Treatment[1] | | | | | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | INITIAL | | DELAYED | | | | | TIME × |
| Item | -CTC | +CTC | -CTC | +CTC | SEM | TIME[2] | CTC[3] | CTC[4] |
| Morbidity, % | | | | | | | | |
| Treated 1 time | 16.67 | 12.82 | 15.38 | 16.66 | 3.64 | 0.72 | 0.72 | 0.49 |
| Treated 2 times | 3.84 | 2.56 | 5.12 | 3.84 | 2.18 | 0.56 | 0.56 | 0.99 |
| Treated 3 times | 1.28 | 1.28 | 0 | 0 | 0.90 | 0.17 | 0.99 | 0.99 |
| Mortality, % | 1.28 | 2.56 | 2.56 | 1.28 | 1.46 | 0.99 | 0.99 | 0.39 |

[1]Heifers were fed diets with treatments from d 1 to 28. Treatments, in a 2 × 2 factorial arrangement, were BVD vaccination and a growth implant on d 1 (INITIAL) or d 28 (DELAYED) after initial processing in combination with (+CTC) or without (-CTC) three 5-d pulse-doses (d 7 to 11, 13 to 17, and 19 to 23) of chlortetracycline. From d 29 to 56, calves were fed a common diet.
[2]TIME = P-value for main effect of timing of BVD vaccine and growth implant.
[3]CTC = P-value for the main effect of CTC.
[4]TIME × CTC = P-value for a TIME × CTC interaction.

-CTC compared with +CTC, but d 56 BW were not different ($P$ = 0.98) between treatments. Chlortetracycline is used to reduce the number of cattle which are pulled for treatment in a feedlot. A reduction in pulls represents an improvement in the profitability of feeding cattle. However, the addition of CTC did not affect ($P$ 2 0.56) morbidity or mortality in this study (Table 3). Feedlot morbidity and mortality of heifers were low, and it is possible that CTC could be more effective when fed to cattle with greater stress and sickness.

***Conclusions.*** Delaying a BVD vaccination and initial growth implant for 28 d tended to decrease heifer performance during the first 56 d on feed in the feedlot. Therefore, delaying a BVD vaccination and initial growth implant did not improve efficacy of the vaccination and implant program in stressed heifers receiving antibiotic therapy. Feeding multiple pulse doses of chlortetracycline to heifers during the first 28 d did not improve animal performance and health. Therefore, use of chlortetracycline did not improve the efficacy of a delayed vaccination and implant program. The managerial decision to delay the initial growth implant may be dependent upon the strength and chemistry of the implant intended for use at initial processing, and managerial decisions to feed multiple pulse-doses of chlortetracycline may be dependent upon the health risk of the newly received calves.

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# EFFECTS OF 24-H TRANSPORT OR 24-H NUTRIENT RESTRICTION ON ACUTE-PHASE AND PERFORMANCE RESPONSES OF FEEDER CATTLE

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**ABSTRACT:** The objective was to compare acute-phase and performance responses of weaned beef cattle exposed to transport or nutrient restriction. Angus × Hereford steers (n = 30) and heifers (n = 15) were balanced by sex and BW, and randomly assigned to 15 pens on d -12 of the experiment. On d 0, pens were randomly assigned to 1 of 3 treatments: 1) transport for 24 h in a livestock trailer (TRANS); 2) no transport, but feed and water deprivation for 24 h (REST); or 3) no transport and full access to feed and water (CON). Treatments were concurrently applied from d 0 to d 1. Total DMI was evaluated daily from d 1 to d 28. Full BW was recorded prior to treatment application and at the end of experiment. Blood samples were collected on d 0, 1, 4, 7, 10, 14, 21, and 28. Mean ADG was greater ($P < 0.01$) in CON vs. TRANS and REST cattle, but similar ($P = 0.46$) between TRANS and REST cattle. No treatment effects were detected on DMI, but CON had greater G:F vs. TRANS ($P < 0.01$) and REST cattle ($P = 0.08$), whereas G:F was similar ($P = 0.21$) between TRANS and REST cattle. Plasma cortisol concentrations were greater ($P \leq 0.05$) in REST vs. CON and TRANS cattle on d 1, 4, 7, 14, 21, and 28, and tended to be greater ($P = 0.10$) in TRANS vs. CON cattle on d 1. Serum NEFA was greater ($P < 0.01$) in REST and TRANS vs. CON cattle on d 1, but also greater ($P < 0.01$) in REST vs. TRANS cattle on d 1. Plasma ceruloplasmin peaked on d 4 for TRANS and REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.58$) for CON cattle. Hence, CON cattle had reduced mean plasma ceruloplasmin concentration vs. TRANS ($P = 0.07$) and REST ($P = 0.01$) cattle. Plasma haptoglobin peaked on d 1 for TRANS and increased from d 1 to 14 in REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.65$) for CON cattle. Hence, TRANS cattle had greater plasma haptoglobin vs. CON and REST cattle on d 1 ($P < 0.01$), whereas REST cattle had greater ($P \leq 0.05$) plasma haptoglobin vs. TRANS and CON cattle on d 7. In conclusion, 24-h transport and 24-h nutrient restriction elicited acute-phase protein reactions, and similarly reduced performance of feeder cattle.

## INTRODUCTION

Cattle are inevitably exposed to stress during their productive life (Carroll and Forsberg, 2007), including psychologic, physiologic, and physical stressors associated with management procedures currently practiced within beef and dairy production systems. An example is road transport, one of the most stressful events in the productive life of a feeder calf. Upon long transportation periods, feeder cattle experience inflammatory and acute-phase responses that often lead to impaired health and productivity during feedlot receiving (Berry et al., 2004; Araujo et al., 2010; Cooke et al., 2011). These stress- induced immune responses may be elicited by several stressors that cattle are exposed to during road transport, including feed and water restriction. In fact, preliminary data from our research group indicated that water and feed deprivation for 24 h increased circulating concentrations of acute-phase proteins in overtly healthy beef steers (Cappellozza et al., 2011).

Therefore, we hypothesized that feed and water restriction are major stimulants of the acute-phase response elicited by road transport. Based on our hypothesis, the objective of this experiment was to compare the effects of 24-h road transport or 24-h water and feed restriction on acute-phase and feedlot receiving performance responses of feeder cattle.

## MATERIALS AND METHODS

This experiment was conducted at the Eastern Oregon Agricultural Research Center, Burns in accordance with an approved Oregon State University Animal Care and Use protocol. Forty-five Angus x Hereford steers (n = 30) and heifers (n = 15) weaned at 7 mo of age were ranked by sex and initial BW (217 ± 3 kg) on d -12 of the study, and randomly allocated to 15 dry lot pens (3 animals/pen; 2 steers and 1 heifer). From d -12 to 0, all pens received alfalfa-mixed hay for ad libitum consumption and 2.3 kg/hd daily (DM basis) of a supplement containing (as-fed basis) 84% corn, 14% soybean meal, and 2% mineral mix. On d 0, pens were assigned to 1 of 3 treatments: 1) transport for 24 h in a commercial livestock trailer for approximately 1,200 km (**TRANS**), 2) no transport, but feed and water deprivation for 24 h (**REST**), or 3) no transport and full access to feed and water (**CON**). Treatments were concurrently applied from d 0 to d 1. On d 1, TRANS and REST cattle returned to their original pens, and all pens received the same diet offered prior to treatment application.

Total and forage DMI were evaluated daily from d1 to 28. Full BW was recorded prior to (d -1 and 0) treatment application and at the end of experiment (d 28 and 29) for ADG calculation. Total BW gain and DMI from d 1 to 28 were used for G:F calculation. Blood samples were collected on d 0 (prior to treatment application), 1 (immediately at the end of treatments), 4, 7, 10, 14, 21, and 28, via jugular

venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing or not sodium heparin for serum and plasma collection, respectively. Plasma samples were analyzed for concentrations of cortisol (Endocrine Technologies Inc., Newark, CA), haptoglobin (Cooke and Arthington, 2012), and ceruloplasmin (Demetriou et al., 1974). Serum samples were analyzed for concentrations of NEFA (Wako Chemicals: Dallas, TX).

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for BW shrink from d 0 to d 1 and ADG contained the effects of treatment, sex, and the interaction. Data were analyzed using calf(treatment × pen) as random variable. The model statement used for DMI and G:F contained the effects of treatment, as well as day and the resultant interaction for DMI only. Data were analyzed using calf( treatment × pen) as the random variable. The specified term for repeated statements wasday, pen(treatment) or calf(treatment × pen) as subject for DMI or hormones and metabolites, respectively, and the covariance structure utilized was based on the Akaike information criterion. Results are reported as least square means and were separated using PDIFF. Significance was set at $P \leq 0.05$. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

## RESULTS AND DISCUSSION

Body weight shrink from d 0 to d 1 was similar ($P = 0.16$) between TRANS and REST, and greater ($P < 0.01$) for both treatments vs. CON (Table 1). Mean ADG was greater ($P < 0.01$) in CON vs. TRANS and REST cattle, and similar ($P = 0.46$) between TRANS and REST cattle (Table 1). No treatment ($P \geq 0.25$) effects were detected on forage, concentrate, and total DMI (Table 1). However, CON had greater G:F vs. TRANS ($P < 0.01$) and tended to have greater G:F vs. REST cattle ($P = 0.08$), whereas G:F was similar ($P = 0.21$) between TRANS and REST cattle (Table 1). Similar to previous research, road transport reduced ADG and G:F during feedlot receiving (Cole et al., 1988). Further, REST cattle experienced similar feedlot receiving performance compared with TRANS cohorts, suggesting that feed and water deprivation are major causes for the reduced performance of transported cattle.

Treatment × day interactions were detected ($P < 0.05$) for cortisol, NEFA, haptoglobin, and ceruloplasmin. Plasma cortisol concentrations were greater ($P < 0.05$) in REST compared with CON and TRANS cattle on d 1, 4, 7, 14, 21, and 28, and tended to be greater ($P = 0.10$) in TRANS compared to CON cattle on d 1 (Figure 1). Serum NEFA concentrations were greater ($P < 0.01$) in REST and TRANS compared with CON cattle on d 1, but also greater ($P < 0.01$) in REST compared with TRANS cattle on d 1 (Figure 1). Plasma ceruloplasmin concentrations peaked on d 4 for TRANS and REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.58$) for CON cattle (Figure 2). Hence, CON cattle had reduced mean plasma ceruloplasmin concentration compared to TRANS ($P = 0.07$) and REST ($P = 0.01$) cattle.

Table 1. Feedlot receiving performance of cattle submitted to transport for 24 h for approximately 1,200 km (TRANS), no transport but feed and water deprivation for 24 h (REST), or no transport and full access to feed and water (CON). [1]

| Item | CON | REST | TRANS | SEM | *P* = |
|---|---|---|---|---|---|
| DMI, kg/d | | | | | |
| Forage | 5.5 | 4.9 | 5.4 | 0.3 | 0.32 |
| Concentrate | 2.3 | 2.3 | 2.3 | 0.1 | 0.52 |
| Total | 7.9 | 7.2 | 7.8 | 0.4 | 0.25 |
| ADG, [2] kg/d | 1.27[a] | 0.97[b] | 0.91[b] | 0.05 | < 0.01 |
| G:F, [3] g/kg | 163[a] | 143[ab] | 127[b] | 7 | 0.03 |
| Shrink, [4] % | 0.07[a] | 8.1[b] | 9.6[b] | 0.7 | < 0.01 |

[1] Within rows, values with different superscripts differ ($P < 0.05$).
[2] Calculated using full BW values obtained prior to (d -1 and 0) treatment application and at the end of experiment (d 28 and 29).
[3] Calculated using total DMI and BW gain from d 0 to d 28.
[4] Based on BW loss from d 1 relative to d 0.

Plasma haptoglobin peaked on d 1 for TRANS and increased from d 1 to 14 in REST cattle (day effects; $P < 0.01$) but did not change ($P = 0.65$) for CON cattle (Figure 2). Hence, TRANS cattle had greater plasma haptoglobin compared to CON and REST cattle on d 1 ($P < 0.01$), whereas REST cattle had greater ($P \leq 0.05$) plasma haptoglobin compared with TRANS and CON cattle on d 7 (Figure 2).

These results suggest that TRANS and REST stimulated mobilization of body reserves, elicited a neuroendocrine stress response, and induced an acute-phase protein reaction that impaired feedlot receiving ADG and G:F (Ellenberger et al., 1989; Sapolsky, 2000; Carroll and Forsberg, 2007). Previous research also reported increased circulating cortisol, ceruloplasmin, and haptoglobin in feeder cattle following road transport, and attributed these outcomes to impaired feedlot receiving performance (Crookshank et al., 1979; Araujo et al., 2010; Cooke et al., 2011). Conversely, the specific effects of feed and water restriction on neuroendocrine and acute-phase parameters have not yet been determined. Supporting these outcomes, recent research from our group demonstrated that neuroendocrine stress reactions can stimulate breakdown of body reserves and activate acute-phase and inflammatory processes in bovine (Cooke et al., 2012). In addition, feed and water deprivation may result in death of rumen microbes and subsequent release of endotoxins (Meiske et al., 1958), which may be absorbed by the ruminal wall and small intestine, incorporated into the circulation (Chin et al., 2006), and elicit neuroendocrine and acute-phase reactions (Carroll et al., 2009). Hence, the acute-phase protein reaction detected in TRANS and REST cattle can be attributed to the increase in circulating cortisol, NEFA, and altered ruminal flora following treatment application.

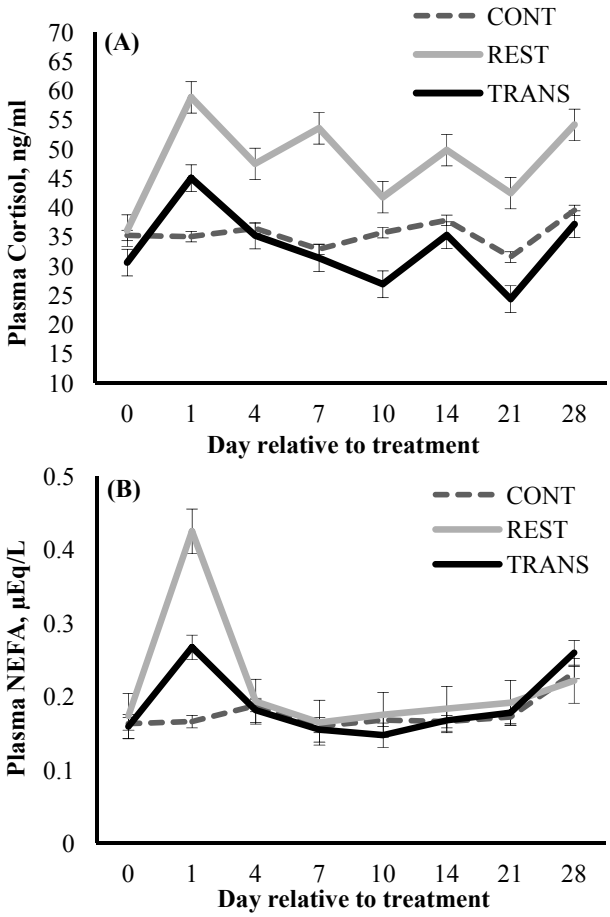


**Figure 1.** Plasma cortisol (Panel A) and serum NEFA (Panel B) in cattle submitted to transport for 24 h for approximately 1,200 km (TRANS), no transport but feed and water deprivation for 24 h (REST), or no transport and full access to feed and water (CON). Treatment × day interactions were detected ($P < 0.05$).

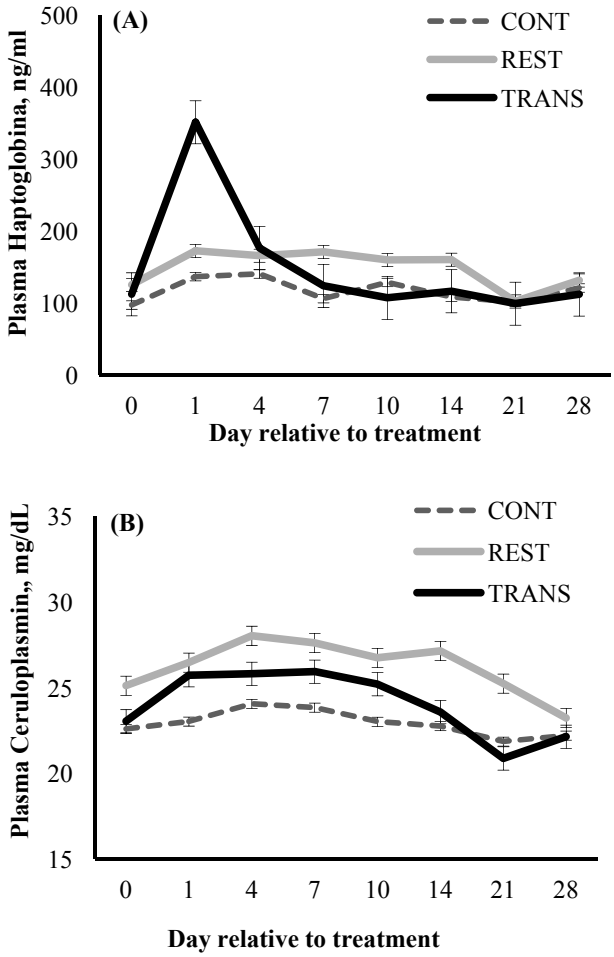


**Figure 2.** Plasma haptoglobin (Panel A) and ceruloplasmin (Panel B) in cattle submitted to transport for 24 h for approximately 1,200 km (TRANS), no transport but feed and water deprivation for 24 h (REST), or no transport and full access to feed and water (CON). Treatment × day interactions were detected ($P < 0.05$).

It is also important to note that the increase in circulating NEFA, cortisol, and ceruloplasmin concentrations was more severe in REST vs. TRANS cattle. Similarly, circulating haptoglobin remained elevated for a longer period in REST vs. TRANS cattle. These results suggest that neuroendocrine stress response was more severe in REST cattle, which caused or was caused by the greater mobilization of body tissues, and resulted in the greater acute-phase reaction compared with that observed in TRANS cohorts. The reasons for this outcome are unknown and deserve further investigation, particularly because TRANS steers also experienced a 24-h feed and water restriction during transport.

## IMPLICATIONS

In conclusion, 24-h transport and 24-h nutrient restriction elicited acute-phase protein responses and similarly reduced performance of feeder cattle. Therefore, feed and water restriction are major causes for the acutephase reaction and reduced feedlot receiving performance typically detected in transported feeder cattle.

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# EFFECTS OF ACCLIMATION TO HUMAN HANDLING ON TEMPERAMENT, PHYSIOLOGICAL RESPONSES, AND PERFORMANCE OF BEEF STEERS DURING FEEDLOT RECEIVING

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**ABSTRACT:** The objective was to compare temperament, plasma concentrations of cortisol and acute-phase proteins, and performance during feedlot receiving of Angus × Hereford steers acclimated or not to human handling. Sixty steers were initially evaluated, within 30 d after weaning, for BW and temperament score (average chute score and exit velocity score; d -30). On d -28, steers were ranked BW and temperament score, and randomly assigned to receive or not (control) the acclimation treatment. During the acclimation phase (d -28 to 0), steers were maintained in 2 pastures according to treatment, and acclimated steers were exposed to a handling process twice weekly (Tuesdays and Thursdays). The acclimation treatment was applied individually to steers by processing them through a handling facility, whereas control steers remained undisturbed on pasture. On d 0, all steers were loaded into a commercial livestock trailer, transported for 24 h, and returned to the research facility (d 1). Upon arrival, steers were ranked by BW within treatment, and randomly assigned to 20 feedlot pens. Total DMI was evaluated daily from d 1 to d 28, and shrunk BW was collected on d -31, 1, and 29 for ADG calculation. Blood samples were collected on d -28, 0 (prior to loading), 1 (immediately upon arrival), 4, 7, 10, 14, 21, and 28 for determination of cortisol, ceruloplasmin, and haptoglobin. Steer temperament was assessed again on d 0. During the acclimation phase (d -28 to 0), no treatment effects were detected ($P = 0.14$) on steer ADG. Acclimated steers had reduced chute score compared with control on d 0 ($P = 0.01$). During feedlot receiving (d 1 to 28), acclimated steers had reduced ADG ($P < 0.01$), DMI ($P = 0.07$), and G:F ($P = 0.03$) compared with control. Acclimated steers had greater plasma cortisol on d 1 ($P = 0.06$), greater haptoglobin on d 4 ($P = 0.04$), and greater ceruloplasmin from d 0 to 10 ($P \leq 0.04$) compared with control. In conclusion, steers exposed to the acclimation process had greater stress-induced cortisol and acute-phase protein responses, resulting in decreased performance during feedlot receiving.

## INTRODUCTION

Temperament is defined as the behavioral responses of cattle when exposed to human handling (Burrow, 1997; Burrow and Corbert, 2000; Curley et al., 2006). Animals with aggressive temperament display nervous or agitated responses during human contact or any other handling procedures. Besides personnel security and animal welfare, temperament has significant implications on beef cattle performance. Our research group was the first to report that beef cows with aggressive temperament have impaired reproductive performance compared with cows with adequate temperament (Cooke et al., 2009a, 2012). In addition, our group recently reported that aggressive beef calves are lighter and consequently less valuable if sold at weaning, and also have decreased growth rates during the feedlot, resulting in reduced carcass marbling, carcass weight, and final carcass value if marketed upon slaughter (Cooke et al., 2011). Therefore, cattle temperament should be used as a management decision criterion to enhance overall productivity and safety of beef operations.

Temperament of feeder calves can be improved by two main strategies. The first is to select the cowherd for calm temperament, which should also benefit the calf crop given that temperament is a heritable trait (Fordyce et al., 1988). Second, recent studies from our group demonstrated that acclimation of young cattle to human handling improved their temperament and enhanced their productivity (Cooke et al., 2009b, 2012). However, this method was only tested with replacement heifers by evaluating their reproductive development. Based on this information, we hypothesized that acclimation to human interaction after weaning will also improve temperament and feedlot productivity of feeder steers. Therefore, the objective of this study was to compare temperament, plasma concentrations of cortisol and acute-phase proteins, and performance during feedlot receiving of steers acclimated or not to human interaction after weaning.

## MATERIALS AND METHODS

The study was conducted at the Eastern Oregon Agricultural Research Center, Burns. Animals utilized were cared for according to an approved Oregon State University Animal Care and Use protocol.

Sixty Angus x Hereford steers were initially evaluated, within 30 d after weaning, for BW and temperament score (average chute score and exit velocity score). Chute score was assessed based on a 5- point scale according to the method described by Arthington et al. (2008). Exit velocity was assessed by determining the speed of the steer exiting the squeeze chute by measuring rate of travel over a 1.8-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Further, steers were divided in quintiles and assigned an exit velocity score on a 5-point scale (1 = slowest quintile; 5 = cows within the fastest quintile). On d -28, steers were

ranked BW and temperament score and randomly assigned to receive or not (control) the acclimation treatment. Steers were maintained on separate meadow foxtail (*Alopecurus pratensis* L.) pastures (30 steers/pasture) according to treatment, and received supplemental alfalfa hay 3 times weekly to sustain a growth rate of approximately 0.5 kg/d. The acclimation treatment was applied individually to steers by processing them through a handling facility, twice week (Tuesdays and Thursdays) for 4 wk, while control steers remained undisturbed on pasture. In addition, during feeding procedures, the technician walked among steers assigned to the acclimation treatment for 15 min to further expose them to human interaction, whereas the same procedure was not applied to control steers.

On d 0, all steers were loaded into a commercial livestock trailer, transported for 24 h for a total of 1,200 km, and returned to the research facility on 1. Upon arrival, steers were ranked by BW within treatment, and randomly assigned to 20 feedlot pens (10 pens/treatment; 3 steers/pen). All pens received 2.5 kg/steer daily of a concentrate (86% corn; 14% soybean meal), whereas meadow foxtail hay was offered in amounts to ensure ad libitum access. Total DMI was evaluated daily from d 1 to d 28, and shrunk BW was collected on d -31, 1, and 29 for ADG calculation. Total DMI and BW gain from d 1 to 28 were used to calculate feedlot receiving G:F.

Blood samples were collected on d -28, 0 (prior to loading), 1 (immediately upon arrival), 4, 7, 10, 14, 21, and 28 via jugular venipuncture into commercial blood collection tubes containing sodium heparin (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ). Steer rectal temperature (**RT**) was measured by digital thermometer (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA) concurrently with each blood collection. All blood samples were harvested for plasma and stored at −80°C until assayed for concentrations of cortisol (Endocrine Technologies Inc., Newark, CA), haptoglobin (Cooke and Arthington, 2012) and ceruloplasmin (Demetriou et al., 1974).

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for ADG contained the effects of treatment. Data were analyzed using steer(treatment × pen) as random variable. The model statement used for DMI and G:F contained the effects of treatment, as well as day and the resultant interaction for DMI only. Data were analyzed using pen(treatment) as the random variable. The model statement used for temperament and physiological measurements contained the effects of treatment, day, and the resultant interactions. Data were analyzed using steer(treatment × pen) as the random variable. The specified term for repeated statements was day, pen(treatment) or steer(treatment × pen) as subject for DMI or temperament and physiological variables, respectively, and the covariance structure utilized was based on the Akaike information criterion. Results are reported as least square means and were separated using LSD. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

## RESULTS AND DISCUSSION

During the acclimation phase (d -28 to 0), no treatment effects were detected ($P = 0.14$) on steer ADG (Table 1). On d 0, acclimated steers had reduced ($P = 0.01$) chute score, and tended ($P = 0.08$) to have reduced temperament score compared with control cohorts (Table 1). However, during feedlot receiving (d 1 to d 28), acclimated steers tended ($P = 0.07$) to have reduced DMI, and had reduced ($P \leq 0.03$) ADG and G:F compared with control cohorts (Table 1). Similarly to our previous work (Cooke et al., 2009b, 2012), acclimation to handling improved temperament of growing cattle. However, steers exposed to the acclimation process experienced reduced feedlot receiving performance compared with control cohorts. This performance outcome was unexpected given that a similar acclimation process enhanced reproductive and overall performance of replacement heifers (Cooke et al., 2009b, 2012).

No treatment effects were detected ($P > 0.24$; data not shown) for RT (38.84 vs. 39.03ºC for ACC and CON steers, respectively; SEM = 0.07). Treatment x day interactions were detected for cortisol, haptoglobin, and ceruloplasmin ($P \leq 0.05$). Acclimated steers had greater plasma cortisol on d 1 ($P = 0.05$), greater haptoglobin on d 4 ($P = 0.04$), and greater ceruloplasmin from d 0 to 10 ($P \leq 0.04$) compared with control

**Table 1.** Temperament and feedlot receiving performance of beef steers exposed (ACC) or not (CON) to handling acclimation procedures[1]

| Item | ACC | CON | SEM | *P* = |
|---|---|---|---|---|
| Temperament variables[2] | | | | |
| Chute score | 1.63 | 2.07 | 0.12 | 0.01 |
| Exit velocity, m/s | 1.97 | 2.28 | 0.16 | 0.18 |
| Temperament score | 2.21 | 2.63 | 0.16 | 0.08 |
| Performance variables | | | | |
| Total DMI, kg/d | 7.09 | 7.40 | 0.11 | 0.07 |
| Acclimation ADG, [3] kg/d | 0.27 | 0.38 | 0.06 | 0.14 |
| Receiving ADG, [4] kg/d | 1.13 | 1.32 | 0.05 | < 0.01 |
| G:F, [5] kg/kg | 166 | 185 | 6 | 0.03 |

[1] Acclimated steers were exposed to a handling process twice week for 4 wk (d -28 to 0), which was applied individually to steers by processing them through a handling facility. Control steers remained undisturbed on pasture.
[2] Obtained on d 0. Chute score (1 to 5 scale), exit velocity, and temperament score were calculated according to the techniques described by Cooke et al. (2011).
[3] Calculated using shrunk values obtained on d -31 and d 1.
[4] Calculated using shrunk values obtained on d 1 and d 29.
[5] Calculating using total DMI and BW gain from d 1 to d 29.

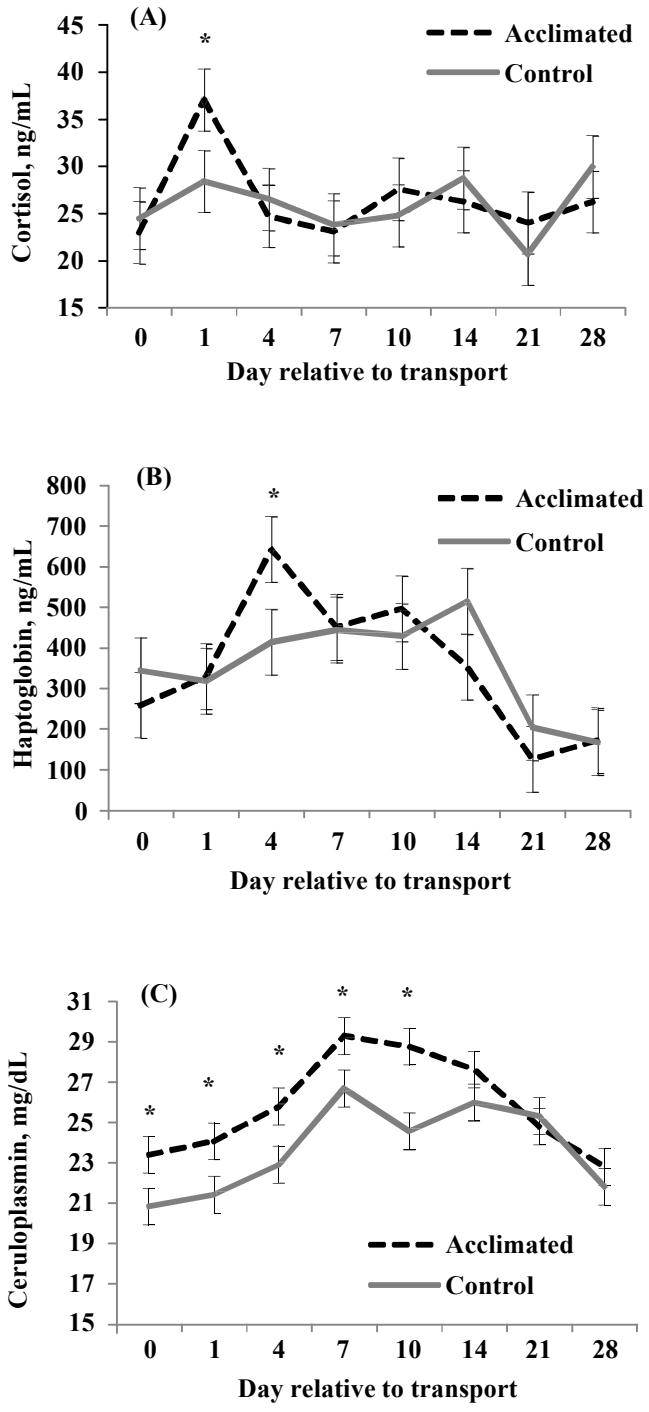


**Figure 1.** Plasma concentrations of cortisol (Panel A), haptoglobin (Panel B), and ceruloplasmin (Panel C) during feedlot receiving (d 1 to d 28) of beef steers exposed (acclimated) or not (control) to handling acclimation procedures (d -28 to 0) and transported for 24 h (d 0 to d 1). A treatment × day interaction was detected ($P \leq 0.05$) for all variables. Treatment comparison within day; * $P < 0.05$.

steers (Figure 1). Contrary to these outcomes, replacement heifers assigned to a similar acclimation process had reduced cortisol (Cooke et al., 2009b) and haptoglobin (Cooke et al., 2012). The exact reasons for the different outcomes to the acclimation process reported herein and by our previous work are unknown and deserve further investigation. Nevertheless, steers assigned to the acclimation process had a more severe neuroendocrine stress and acute-phase protein response upon transportation and feedlot entry compared with control cohorts, which likely contributed to their reduced DMI, G:F, and ADG during feedlot receiving (Arthington et al., 2003; Qiu et al., 2007; Araujo et al., 2010).

## IMPLICATIONS

Acclimation of feeder steers to human handling after weaning improved cattle temperament but increased the neuroendocrine stress and acute-phase responses following transport and feedlot entry, resulting in decreased performance during feedlot receiving.

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# EFFICACY OF NOVEL FEED PRODUCTS TO REDUCE LOCOWEED TOXICITY IN WETHER LAMBS[1]

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**ABSTRACT:** Locoweed may result in impaired performance and possibly death when consumed by livestock. Novel products are needed that increase the tolerance of livestock to swainsonine, the toxicant in locoweed. The objective was to determine the efficacy of proprietary feed products to reduce locoweed toxicity in sheep. Wether lambs (n = 40; 39 ± 0.4 kg BW) were housed individually and fed 620 g/d of alfalfa hay and 100 g/d of corn-based feed in equal portions twice daily for 20 d. Lambs were equally divided into 4 BW blocks, and within block were randomly assigned to 1 of 5 treatments (randomized complete block design). Treatments were: no locoweed or feed products (CON); 20 g/d of locoweed (LOCO); 20 g/d of locoweed and 50 g/d of feed product 1 (AK1); 20 g/d of locoweed and 50 g/d of feed product 2 (AK2); 20 g/d of locoweed and 50 g/d of feed product 3 (AK3). Locoweed and feed products replaced alfalfa hay in the basal diet. Serum from venous blood was collected on d 0, 3, 6, 9, 12, 15, 18, and 20, and rumen fluid was collected on d 9 and 20 of treatment. Swainsonine was detected in serum and rumen fluid of lambs fed LOCO, AK1, AK2, and AK3, but was not detected in lambs fed CON. Serum swainsonine of lambs fed LOCO, AK1, AK2, and AK3 increased ($P < 0.05$) from d 0 to d 3, and remained elevated for the remainder of the study. Serum alkaline phosphatase was greater ($P < 0.05$) in lambs fed treatments with locoweed than CON, and was less ($P < 0.05$) in lambs fed AK3 than LOCO. Serum thyroid hormones (T3 and T4), serum total iron, and serum transferrin saturation were less ($P < 0.05$) in lambs fed treatments with locoweed than CON. Serum thyroid hormones (T3 and T4) were also lower in lambs fed AK1 than LOCO. Serum insulin was lower ($P < 0.05$) in lambs fed AK2 than LOCO. Serum urea N, and rumen fluid pH, ammonia, and total VFA were not different ($P > 0.10$) among treatments. In locoweed-fed treatments, rumen fluid swainsonine was not different ($P > 0.10$) for lambs fed AK1, AK2, or AK3 than LOCO. The results suggest that the novel feed products evaluated in the current study did not reduce serum or rumen swainsonine and had minimal effects on serum chemistry of lambs consuming locoweed.

**Key words:** locoweed, serum, sheep

## INTRODUCTION

Locoweeds (*Astragalus* and *Oxytropis* spp.) are poisonous plants responsible for great economic losses in the livestock industry (Nielsen and James, 1992). Locoweeds contain a toxic alkaloid, swainsonine (Molyneux and James, 1982), which is produced by a fungal endophyte. Adverse effects of locoweed toxicity in animals include neurological abnormalities, emaciation, reproductive disorders, decreased performance, and death (Molyneux et al., 1985). Therefore, treatments or novel products are needed that could be supplemented to animals to decrease the toxic effects of locoweed by either decreasing gastrointestinal swainsonine availability or increasing the tolerance of livestock to swainsonine.

Previous researchers (Bachman et al., 1992; Stavanja et al., 1993; Pulsipher et al., 1994; Dugart-Stavanja et al., 1997) studied the potential for mineral supplements, zeolite clays, activated charcoal, anionic resin, and bentonite clays to bind with swainsonine in the gastrointestinal tract to alleviate locoweed toxicity in cattle, sheep, and rats. Additionally, Greenberg (1994) evaluated the possible means for ammonium chloride and diuretic compounds to increase urinary swainsonine excretion in rats and sheep. However, these studies reported minimal improvements in animals receiving treatments while exposed to locoweed. In contrast, results from preliminary research (unpublished) demonstrated that novel products containing a combination of bacterial cell walls, yeast, and enzymes decreased subclinical symptoms associated with swainsonine toxicity in sheep. Therefore, we hypothesized that supplementation of these novel products may increase livestock tolerance to swainsonine. The objective was to evaluate the effects of three proprietary feed products (Agri-King Inc., Fulton, IL) on serum concentrations of swainsonine, alkaline phosphatase, hormones, and metabolites, as well as rumen characteristics of wether lambs exposed to locoweed (Astragalus allochrous).

## MATERIALS AND METHODS

Experimental procedures were approved by the New Mexico State Institutional Animal Care and Use Committee.

[1]Authors acknowledge A. Temple and Agri-King, Inc. for supply of feed products and support with sample analysis.

***Animals, Design and Treatments.*** The experiment was conducted in the Physiology and Nutrition Building at New Mexico State University in Las Cruces, NM.

Forty wether lambs (39 ± 0.4 kg initial BW) were equally divided into 4 BW blocks, and within each block were randomly assigned to 1 of 5 dietary treatments in a randomized complete block design. The experimental period for each block was 20 d; animals were housed in individual feeding pens from d 1 to 14 for adaptation to dietary treatments, and in metabolism crates from d 15 to 20 for urine and fecal collections (data not presented). Lambs were individually fed a basal diet of 620 g/d of alfalfa hay and 100 g/d of corn-based feed in equal portions twice daily (0730 and 1930 h) for 20 d. Treatments (Table 1) were: no locoweed or feed products (**CON**); 20 g/d of locoweed (**LOCO**); 20 g/d of locoweed and 50 g/d of feed product 1 (**AK1**); 20 g/d of locoweed and 50 g/d of feed product 2 (**AK2**); 20 g/d of locoweed and 50 g/d of feed product 3 (**AK3**). Locoweed and feed products replaced alfalfa hay in the basal diet. Locoweed (*Astragalus allochrous*) was collected in April in southeast New Mexico, allowed to air dry, and passed through a forage chopper (The Western Bear Cat No 5A, by Western Land Roller Co., Hastings, NE) to reduce particle size. The amount of locoweed fed was calculated to supply approximately 2 mg swainsonine per kilogram of live BW daily.

***Sample Collections.*** Blood samples were collected via the jugular vein into 10 mL vacuum tubes (Corvac serum, Kendall, Ontario, CA) at 4 h after the morning feeding on d 0, 3, 6, 9, 12, 15, 18, and 20. Blood samples were stored at room temperature for 30 min to allow clotting before centrifugation (Sorvall RT600B, Thermo Electron Corp., NC) at 1,500 × g for 20 min at 5ºC. Serum was transferred 7-mL polypropylene vials and stored at -20ºC. Rumen fluid (± 100 mL) was collected via oral lavage using a suction strainer (Lodge-Ivey et al., 2009) from each animal 4 h after the morning feeding on d 9 and 20. Rumen fluid pH was measured (portable pH meter; Accumet AP72; Fisher Scientific, Pittsburg, PA) immediately, and then separated into 2 samples. For the first sample, 8 mL of rumen fluid was added to 2 mL of 25% metaphosphoric acid in polypropylene vials and stored at -20ºC for later analysis of VFA. The other rumen fluid sample was stored at -20ºC in polypropylene vials for analysis of swainsonine and ammonia.

***Sample Analysis.*** All blood serum and rumen fluid samples were analyzed for swainsonine concentrations using the modified a-mannosidase inhibition assay as described by Taylor and Strickland (2002). Serum alkaline phosphatase was determined with a commercial kit (Amplite Calorimetric Alkaline Phosphatase Assay Kit #11950, AAT Bioquest, Inc., Sunnyvale, CA) and read on a 96-well microtiter plate reader (MRX HD, Dynex laboratories, Chantilly, VA) at 400 nm. Serum urea N concentrations were determined colorimetrically using a commercial kit (QuantiChrom Urea Assay Kit DIUR-500; Bioassay systems, Haward, CA), and serum NEFA concentrations were determined using a commercial kit (Wako NEFA HR2; Wako Chemicals USA, Inc., Richmond, VA) modified for a microplate reader at 550 nm. Serum insulin (Camacho et al., 2012), triiodothyronine (**T3**; Wells et al., 2003) and thyroxine (**T4**; Richards et al., 1999) were quantified by solid-phase RIA using commercial kits (Siemens Diagnostic, Los Angeles, CA). Serum total iron, unsaturated iron binding capacity, total iron binding capacity, and percent transferrin saturation were all analyzed using a commercial kit (Stanbio Iron and Total Iron Binding Capacity, Procedure No. 0370; Boerne, TX), and serum Fe and Cu were analyzed according to AOAC method 985.01 (AOAC, 1995). Ruminal ammonia concentrations were determined using a colorimetric assay as described by Chaney and Marbach (1962). Rumen VFA concentrations were determined in acidified rumen fluid using gas chromatography (Star 3400, Varian, Walnut Creek, CA) as described by May and Galyean (1996).

***Statistical Analysis.*** The experiment was a randomized complete block design, and all data were analyzed as repeated measures using mixed models (SAS Inst. Inc., Cary, NC). The experimental unit was lamb. Due to a limited number (10) of metabolism crates, lambs were equally divided into 4 complete blocks based on BW and date in metabolism crates. The statistical model included treatment, day, and treatment × day interaction as fixed effects, and block was the random effect. An auto regressive order(1) covariance structure was specified. When treatment × day interactions were not significant ($P > 0.10$), single degree of freedom contrasts were used to compare CON with the average for all other treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3. Because serum and rumen swainsonine concentrations were not detectable for CON, comparisons of locoweed treatments to CON were not evaluated statistically for swainsonine. Differences among treatments were considered significant when $P < 0.05$.

## RESULTS

Lambs fed treatments containing locoweed (Astragalus allochrous) received an average of 2.4 mg swainsonine per kg of BW daily. The locoweed used in this study contained approximately 0.47% swainsonine (verified by the USDA-ARS Poisonous Plant Research Laboratory, Logan, UT).

Swainsonine was detected in serum and rumen fluid of lambs fed LOCO, AK1, AK2, and AK3, but not in lambs fed CON. Serum swainsonine of lambs fed LOCO, AK1, AK2, and AK3 increased ($P < 0.05$) from d 0 to d 3, and remained elevated for the remainder of the study (data not shown). No treatment × day interactions ($P > 0.10$) were detected for all response variables that were measured in serum and rumen fluid. Therefore, all serum and rumen fluid data in Table 2 are least squares means (± SE) for treatment main effects. Lambs fed treatments containing locoweed (LOCO, AK1, AK2, and AK3) had greater ($P < 0.05$) serum alkaline phosphatase and unsaturated iron binding capacity, and had lower ($P < 0.05$) serum T3 and

**Table 1.** Dietary treatments fed to lambs

| Item | Treatments | | | | |
|---|---|---|---|---|---|
| | CON | LOCO | AK1 | AK2 | AK3 |
| Ingredient, g/d | | | | | |
| Alfalfa hay | 620 | 600 | 550 | 550 | 550 |
| Corn grain | 95 | 95 | 95 | 95 | 95 |
| Feed product[1] | 0 | 0 | 50 | 50 | 50 |
| Locoweed[2] | 0 | 20 | 20 | 20 | 20 |
| Molasses | 5 | 5 | 5 | 5 | 5 |
| Nutrient, % DM | | | | | |
| OM | 88.8 | 88.5 | 88.5 | 88.5 | 88.8 |
| NDF | 48.4 | 48.7 | 47.7 | 49.9 | 48.1 |
| ADF | 35.8 | 36.2 | 35.8 | 37.6 | 36.2 |
| CP | 18.3 | 18.4 | 17.3 | 17.5 | 17.7 |
| Swainsonine[3] | | | | | |
| mg/kg DM | 0 | 148.8 | 145.1 | 150.3 | 122.9 |

[1]Novel feed products containing rice hulls (carrier) and a combination of bacterial cell walls, yeast, and enzymes.
[2]Astragalus allochrous (half moon locoweed).
[3]Analyzed using the modified a-mannosidase inhibition assay as described by Taylor and Strickland (2002).

**Table 2.** Serum concentrations of swainsonine, alkaline phosphatase (ALP), hormones, and metabolites, and rumen concentrations of swainsonine, ammonia ($NH_3$), total VFA and pH of lambs exposed to locoweed toxicity and supplemented with novel feed products

| | Treatments[1] | | | | | | Contrasts[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CON | LOCO | AK1 | AK2 | AK3 | SEM | CON vs other | LOCO vs AK1 | LOCO vs AK2 | LOCO vs AK3 |
| Serum[3] | | | | | | | | | | |
| Swainsonine, µg/mL | - | 0.47 | 0.41 | 0.46 | 0.43 | 0.04 | - | 0.06 | 0.79 | 0.17 |
| ALP, mU/mL | 106 | 529 | 455 | 652 | 257 | 57.8 | <0.01 | 0.34 | 0.11 | <0.01 |
| Urea N, mg/dL | 45.4 | 47.0 | 50.6 | 46.0 | 48.1 | 3.29 | 0.14 | 0.09 | 0.65 | 0.59 |
| NEFA, mEq/L | 0.15 | 0.24 | 0.20 | 0.17 | 0.21 | 0.04 | 0.06 | 0.16 | 0.04 | 0.31 |
| Insulin, ng/mL | 0.24 | 0.24 | 0.23 | 0.17 | 0.21 | 0.03 | 0.14 | 0.67 | <0.01 | 0.21 |
| T3, ng/mL | 0.93 | 0.69 | 0.58 | 0.62 | 0.63 | 0.05 | <0.01 | 0.01 | 0.10 | 0.14 |
| T4, ng/mL | 63.5 | 43.3 | 38.5 | 41.9 | 38.1 | 2.49 | <0.01 | 0.01 | 0.49 | <0.01 |
| Total Iron, µg/dL | 166 | 138 | 145 | 135 | 143 | 22.9 | <0.01 | 0.54 | 0.74 | 0.69 |
| UIBC, µg/dL | 110 | 127 | 148 | 146 | 139 | 14.2 | <0.01 | 0.01 | 0.03 | 0.17 |
| TIBC, µg/dL | 271 | 264 | 293 | 282 | 283 | 36.2 | 0.40 | 0.05 | 0.24 | 0.21 |
| TF saturation, % | 59.1 | 50.8 | 48.2 | 47.0 | 49.5 | 2.12 | <0.01 | 0.18 | 0.05 | 0.49 |
| Fe, ppm | 1.46 | 1.12 | 1.13 | 1.10 | 1.06 | 0.18 | <0.01 | 0.94 | 0.87 | 0.59 |
| Cu, ppm | 0.61 | 0.62 | 0.63 | 0.61 | 0.61 | 0.08 | 0.83 | 0.82 | 0.88 | 0.80 |
| Rumen[4] | | | | | | | | | | |
| Swainsonine, µg/mL | - | 7.19 | 6.97 | 7.39 | 7.65 | 0.49 | - | 0.71 | 0.74 | 0.47 |
| pH | 6.72 | 6.79 | 6.79 | 6.73 | 6.78 | 0.06 | 0.39 | 0.97 | 0.47 | 0.91 |
| $NH_3$, mM | 11.1 | 10.0 | 11.3 | 10.0 | 11.5 | 1.04 | 0.68 | 0.29 | 0.97 | 0.20 |
| Total VFA, mM | 111 | 113 | 110 | 109 | 112 | 5.31 | 0.93 | 0.67 | 0.56 | 0.87 |

[1]CON = basal diet of 620 g/d of alfalfa hay and 100 g/d of corn-based feed fed to lambs in equal portions twice daily (0730 and 1930) for 20 d; LOCO = 20 g/d of locoweed replaced alfalfa hay in basal diet; AK1 = 20 g/d of locoweed plus 50 g/d of feed product 1 replaced alfalfa hay in basal diet; AK2 = 20 g/d of locoweed plus 50 g/d of feed product 2 replaced alfalfa hay in basal diet; AK3 = 20 g/d of locoweed plus 50 g/d of feed product 3 replaced alfalfa hay in basal diet.
[2]Fixed effects of treatment × day interaction were not significant ($P > 0.10$) for all response variables, and single degree of freedom contrasts were used to compare CON with the average for all other treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3.
[3]Blood samples were collected from the jugular vein of each animal at 4 h after the morning feeding on d 0, 3, 6, 9, 12, 15, 18, and 20.
T3 = triiodothyronine; T4 = thyroxine; UIBC = unsaturated iron binding capacity; TIBC = total iron binding capacity; TF saturation = transferrin saturation.
[4]Rumen fluid samples were collected from each animal at 4 h after morning feeding on d 9 and 20 via oral lavage with a strainer attached to a manual suction pump (Lodge-Ivey et al., 2009).

T4, serum total iron, serum transferrin saturation, and serum Fe compared with lambs fed CON (Table 2). Lambs fed AK1 had decreased ($P < 0.05$) serum T3 and T4, and greater ($P < 0.05$) unsaturated iron binding capacity and total iron binding capacity than lambs fed LOCO. Lambs fed AK2 had lower ($P < 0.05$) serum NEFA, serum insulin, and transferrin saturation, and had greater ($P < 0.05$) unsaturated iron binding capacity than lambs fed LOCO. Lambs fed AK3 had lower ($P < 0.05$) serum alkaline phosphatase and T4 than lambs fed LOCO. Serum urea N, and rumen fluid pH, ammonia, and total VFA were not different ($P > 0.10$) among treatments. In locoweed-fed treatments, rumen fluid swainsonine was not different ($P > 0.10$) for lambs fed AK1, AK2, or AK3 than LOCO.

## DISCUSSION

***Effects of Feeding Locoweed.*** Lambs fed diets containing locoweed had peak serum concentrations of swainsonine by the first blood collection (d 3) after locoweed exposure. Taylor and Strickland (2002) reported that swainsonine can be detected in blood within hours after animals have consumed locoweed. In the current study, serum swainsonine concentrations were similar to that reported by Stegelmeyer et al. (1995) for sheep fed alfalfa with locoweed at 1.5 mg swainsonine per kg BW.

Serum alkaline phosphatase of lambs fed locoweed was approximately 5-fold greater than CON lambs, which is similar to that reported by Ortiz et al. (1997). Elevated serum alkaline phosphatase indicated acute swainsonine intoxication of lambs, which is likely due to defective glycoprotein processing (Reed, 2004). Lower concentrations of T3 and T4 in lambs fed locoweed than CON are consistent with previous research (Pulsipher et al., 1994; Ortiz et al., 1997; Obeidat et al., 2005b). The effects of swainsonine on cytoplasmic vaculation and tissue death (Molyneux and James, 1982) may cause a thyroid gland abnormality leading to decreased T3 and T4 production.

Decreased serum total iron in locoweed-fed lambs indicates the possible effect of swainsonine on Fe metabolism, mobilization, and transport from its storage in the liver (Reed, 2004) or an indication of renal damage or anemia (Bachman et al., 1992). Serum transferrin saturation, which is the percent of Fe saturation on the α1- glycoprotein transferrin (Reed, 2004) was less in lambs fed treatments with locoweed than CON, perhaps due to altered glycoproteins from swainsonine toxicity. Serum unsaturated iron binding capacity was greater in lambs fed locoweed-containing diets than CON, which indicates less Fe was being bound to transferring sites. Blood urea N was not affected by feeding locoweed to lambs, which is consistent with previous research (Pulsipher et al., 1994; Taylor et al., 2000). A tendency for greater serum NEFA concentrations in lambs fed locoweed compared with CON is an indication of altered energy metabolism, and is consistent with Obeidat et al. (2004). Rumen fluid pH, ammonia, and total VFA concentrations were not different among treatments, and demonstrate that swainsonine has minimal impact on anaerobic microbial fermentation in the rumen. These results are consistent with the results of Reed (2004) and Obeidat et al. (2005a).

***Effects of Feeding Novel Products.*** Although the mechanism of action is not clear, results from preliminary research (unpublished) demonstrated that novel products containing a combination of bacterial cell walls, yeast, and enzymes decreased some of the liver enzymes associated with swainsonine toxicity in sheep. Therefore, our hypothesis was that supplementation of these novel products will increase the tolerance of livestock to swainsonine.

The tendency for serum swainsonine concentrations to be lower for lambs fed AK1 than LOCO could be an indication of decreased swainsonine absorption from the gastrointestinal tract or greater swainsonine removal from the blood. However, excretion of swainsonine in feces and urine was not greater for lambs fed AK1 than LOCO. Also, reduced thyroid hormones (T3 and T4) and greater unsaturated iron binding capacity and total iron binding capacity in lambs fed AK1 than LOCO suggest greater level of toxicity, which indicates that AK1 did not increase the tolerance of lambs to swainsonine.

Serum concentrations of swainsonine and alkaline phosphatase were not different between lambs fed AK2 and LOCO, which is an indication that AK2 did not increase the tolerance of lambs to swainsonine. Lower concentrations of NEFA and insulin indicate improved energy metabolism in lambs fed AK2 than LOCO, but greater unsaturated iron binding capacity and lower transferrin saturation indicates decreased Fe transportation which may lead to anemia.

Serum concentrations of swainsonine were not different, but lower serum alkaline phosphatase in lambs fed AK3 versus LOCO indicate less damage from swainsonine consumption. However, lower serum T4 in lambs fed AK3 compared with LOCO does not support increased tolerance to swainsonine.

## CONCLUSIONS

Greater concentration of serum swainsonine and alkaline phosphatase, and lower serum thyroid hormones, serum total iron, and serum transferrin saturation in lambs fed treatments with locoweed compared with control lambs are indicative of swainsonine toxicity. No differences in rumen fermentation characteristics indicated that locoweed consumption had little impact on anaerobic microbial fermentation. Limited positive changes in serum swainsonine, alkaline phosphatase, hormones, and metabolites indicated that the novel feed products evaluated in the current study had minimal effects on improving serum chemistry of wether lambs with locoweed poisoning.

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# BREEDING AND GENETICS

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# 50 YEARS OF THE WYOMING RAM TEST: HOW HAVE SHEEP CHANGED?

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**ABSTRACT:** Production characteristics of white-faced rams have been systematically evaluated over a 140 d test for gain and wool characteristics in the Wyoming white-faced ram test since 1961. Records from this test are predominantly from Rambouillet rams but records from other breeds such as Columbia and Targhee are included. Individual production traits from test records (n = 3941) from 1961 through 2010 were analyzed to determine how sheep have changed over the last 50 years. Although age of rams on test has remained stable at 354 ± 0.6 d, both weight on and off test have increased linearly ($P < 0.001$) since 1961 with weight off test increasing 22.7 kg over this time frame. This larger body size likely contributed to a linear increase ($P < 0.001$) in clean fleece weight. Rate of gain also increased linearly ($P < 0.001$) with rams gaining approximately 0.23 ± 0.01 kg/d in the early years to a current rate of gain of 0.39± 0.01 kg/d. Although wool characteristics remain an important component to the test index, spinning count, a measure of wool diameter, did not change in a linear manner ($P > 0.05$), and has remained stable at a 62 spinning count. Market forces may influence breed characteristics over time, and thus correlates to market prices were determined with a 0, 2 and 5 yr time lag. Average daily gain was strongly correlated (r > 0.6) with feeder lamb price, and had the strongest correlation with a two year (r = 0.76) time lag. Sheep inventory negatively correlated (r = 0.87) with average daily gain but correlated similarly with a 0, 2 or 5 year time lag. Wool price did not correlate (r < 0.1; $P \geq 0.5$) with spinning count at any of the time lags. Rambouillet rams have increased in size over the last fifty years with an increase in efficiency of production. Although clean fleece weight has increased proportionally to ram size, fiber diameter has remained largely unchanged and did not correlate to market wool price. This suggests that market influences on white-faced ram selection have largely impacted growth traits while avoiding any negative impact on wool quality.

**Key words:** historical trend production, sheep

## INTRODUCTION

Numbers of sheep in Wyoming have declined steadily since the early 1940s. Although there are only 10% as many sheep in Wyoming as there were in 1940 (NASS, 2012), the Wyoming sheep industry is alive and well. Range sheep operations predominate in Wyoming, and for many producers Rambouillet or Rambouillet-cross sheep are preferred. For the past fifty years, production characteristics of white-faced rams have been systematically evaluated in the Wyoming White-faced Ram Test with these records representing ideal breed characteristics of the time. Although some changes in the industry over the past fifty years are obvious even to the casual observer, the magnitude of change as well as the production characteristics that remained stagnant within the Rambouillet breed is intriguing.

## MATERIALS AND METHODS

Performance records (n = 3941) from the Wyoming Ram test from 1961 to 2010 were analyzed to determine how ram size, rate of gain, and fleece characteristics have changed over the past 50 years. This data set is particularly suited for this analysis since the production test has remained relatively stable with predominantly Rambouillet rams from top producers in the region. Although the diet has changed over the years, rams have always been provided an ad libitum diet. These rams would be representative of the best of the breed from the Rocky Mountain Region in each respective year. Historical market prices were used to determine correlations of production characteristics with potential market forces with a time lag of 0, 2 or 5 yr.

## RESULTS AND DISCUSSION

To anyone who has even peripherally been involved with sheep, it is of no surprise that sheep have increased in size over the past fifty years. With age of rams on test remaining relatively stable, ram weights at the end of the test increased ($P < 0.001$) approximately 23 kg from the early 1960's from 87 to 111 kg (Figure 1). This increase in weight is a reflection of an in increase ($P < 0.001$) in growth efficiency with rams almost doubling their average daily gain from approximately 0.23 to 0.39 kg per day (Figure 2).

Clean fleece weight increased linearly ($P < 0.001$) over this period and is likely a reflection of increased ram size with both characteristics increasing about 25%. Even though a clear drive for increased meat production has influenced breed characteristics and production over the past 50 yr, spinning count (a measure of wool fiber diameter) has remained relatively stable with an average spinning count of 62. Rambouillet rams have had a decrease in the amount of wool cover of the face as reflected by a decrease ($P < 0.001$) in face wool score, but changes in the presence of body wrinkles did not change linearly over the 50 year period. However, wrinkle score, as well as face score, is a subjective measure, and is most likely a relative measure of rams present at any

given test such that rams presenting with the most wrinkles on any given test would always score a 3 even though total wrinkling has steadily declined.

To determine if market forces influenced changes in breed characteristics, correlation with lamb market price, sheep inventory and wool prices were determined. Since sheep production is not as elastic as market price, a lag of 0, 2 and 5 years was utilized. Average daily gain strongly correlated ($r > 0.6$) with feeder lamb price, and had the strongest correlation with a 2-yr ($r = 0.76$) lag time. Sheep inventory negatively correlated ($r = 0.87$) with average daily gain but correlated similarly with a 0, 2 or 5 year lag time. A negative correlation indicates an increase in efficiency occurred simultaneously with a decline in total sheep numbers, but a cause and effect is not implied. Wool price was not correlated to spinning count at any of the time lags investigated.

Rambouillet rams have increased in size over the last fifty years with an increase in efficiency of production. Although clean fleece weight has increased proportionally to ram size, fiber diameter has remained unchanged and is not correlated with changes in wool price. This suggests that market influences on white-faced ram selection have largely impacted growth traits while avoiding any negative impact on wool quality.

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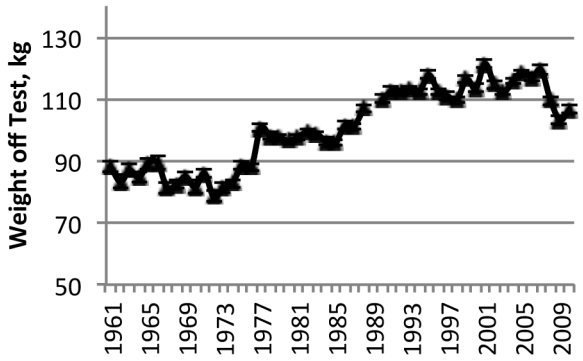


**Figure 1.** Ram weight off (kg) test from rams on the Wyoming White-faced Ram Test from 1961 to 2010.

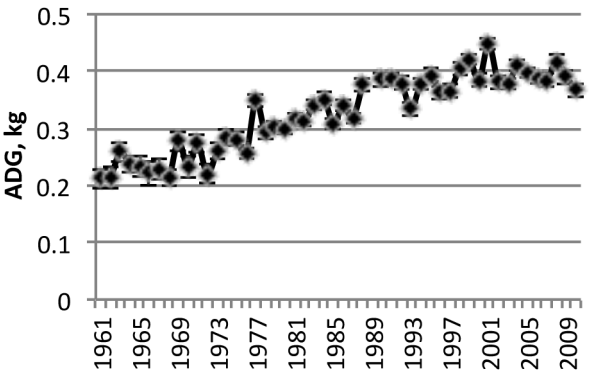


**Figure 2**. Average daily gain (kg) from rams on the Wyoming White-faced Ram Test from 1961 through 2010.

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# GENETIC TRENDS FOR GROWTH-RELATED TRAITS AND CALVING EASE OF SIMMENTAL BEEF CATTLE

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**ABSTRACT:** Genetic change in a trait, over time, is primarily driven by the genetic variability, accuracy of selection, generation interval, and selection intensity and, in multiple trait selection programs, by genetic correlations with other traits of interest. Given modern genetic evaluation tools, and reproductive tools such as artificial insemination, selection intensity is a primary driver of genetic improvement. The main goal of this study was to quantify the selection pressure placed on growth and calving ease in the American Simmental Association (ASA) registry as part of a larger project to evaluate birth weight or calving ease EPD in selection programs. Data consisted of 2,540,928 records, from 1980 to 2011, from ASA. Data included maternal calving ease EPD (CEM) and direct EPD and accuracies for birth weight (BW), weaning weight (WW), yearling weight (YW), and calving ease (CED). To compare genetic trends on an equal scale, mean EPD by year were converted to standard deviation units (sdu). Based on preliminary analysis indicating a change in selection pressure since the importation of Simmental genetics, genetic trend for all studied traits can be divided into two parts, that occurring from 1980 to 1991 (period 1) and that from 1992 to 2011 (period 2). Results show that mean EPD for BW in period 1 increased by 0.017 ± 0.0004 sdu/year, which indicates that breeders put more emphasis on growth and correspondingly experienced increasing birth weight. During this period, mean EPD for CED showed little change (0.0017 ± 0.0003 sdu/year), while mean EPD for CEM increased by 0.023 ± 0.001 sdu/year. During period 2, the rate of genetic change of mean EPD for BW dropped dramatically (-0.024 ± 0.001 sdu/year) while mean EPD for CED increased by 0.019 ± 0.0007 sdu/year. As result, the rate of increase in mean EPD for YW slowed (0.007 ± 0.0006 sdu/year) while that for WW did not show any change (0.00016 ± 0.0007 sdu/year). These results suggest selection emphasis differed between periods, with breeders selecting against BW in period 2 which resulted in a slower genetic change in growth-related traits compared with had they selected directly for calving ease.

**Key words:** calving ease, genetic trend, Simmental

## INTRODUCTION

Large-scale genetic evaluation provides more accurate tools for genetic improvement of a large population. The use of multivariate BLUP procedures allowed breeders to account for genetic correlation between traits and relationships among relatives which increases selection response accordingly (Bennett, 2008). Historically, growth-related traits were the main focus in most selection programs in the beef industry; however, they have a negative genetic correlation with calving ease (Bennett and Gregory, 2001; Koots, 1994; Price and Wiltbank, 1978). Many researchers documented that birth weight is the major factor that causing calving difficulty (Arthur et al., 2000; Bellows et al., 1971; Rice, 1994) albeit not the only factor. Selection for low birth weight results in improvement in ease of calving, but animals with low birth weight genotype often have undesirable genotypes for growth traits. This genetic antagonism between calving ease and birth and postnatal weights might be reduced by selecting directly for calving ease instead of selecting against birth weight as an indicator trait (Burfening et al., 1978; MacNeil et al., 1998). Genetic improvement of calving ease may be increased without sacrificing growth. Over the years, the American Simmental has undergone changes in selection strategies and selection goals. The main objective of this study was to quantify the selection pressure placed on growth and calving ease in the American Simmental Association (**ASA**) registry as part of a larger project to evaluate birth weight or calving ease EPD in selection programs.

## MATERIALS AND METHODS

Data consisted of 2,540,928 records, from 1980 to 2011, from ASA. Data included maternal calving ease EPD (**CEM**) and direct EPD and accuracies for birth weight (**BW**), weaning weight (**WW**), yearling weight (**YW**), and calving ease direct (**CED**).

Table 1 summarizes estimates of mean EPD and accuracy for all studied traits over the years 1980 to 2011, period 1 (1980 to 1991), and period 2 (1992 to 2011).

To compare genetic trends on an equal scale, mean EPD by year for all studied traits were converted to standard deviation units (**sdu**). Genetic (co)variances for all traits are presented in Table 2. Genetic variance of YW was calculated by adding genetic variances of WW and postweaning gain (**GN**) and twice the genetic covariance. Mean EPD by year for studied traits were regressed on year of birth. Based on preliminary visual analysis, a change in selection pressure since the importation of Simmental genetics was observed.

**Table 1.** Mean EPD[1] and accuracy[2] for birth weight (BW), weaning weight (WW), yearling weight (YW), direct calving ease (CED) and maternal calving ease (CEM) in American Simmental cattle

| Years | n | BW | WW | YW | CED | CEM |
|---|---|---|---|---|---|---|
| All data | 2,540,928 | 0.42 | 0.88 | 0.94 | 0.11 | 0.43 |
| 1980-2011 | | (0.34) | (0.31) | (0.30) | (0.29) | (0.24) |
| Period 1 | 1,067,309 | 0.47 | 0.75 | 0.76 | -0.01 | 0.31 |
| 1980-1991 | | (0.34) | (0.32) | (0.32) | (0.29) | (0.24) |
| Period 2 | 1,473,619 | 0.39 | 0.96 | 1.04 | 0.18 | 0.50 |
| 1992-2011 | | (0.34) | (0.30) | (0.30) | (0.29) | (0.23) |

[1]Mean EPD are in standard deviation units.
[2]Accuracy in parenthesis.

**Table 2.** Estimates[1] of genetic (co)variances for birth weight (BW), weaning weight (WW), postweaning gain (GN), yearling weight (YW), direct calving ease (CED) and maternal calving ease (CEM) in American Simmental cattle

| | BW | WW | GN | YW | CED | CEM |
|---|---|---|---|---|---|---|
| BW | **36.62** | 96.55 | 51.99 | - | 172.85 | -19.38 |
| WW | | **1060.22** | 445.84 | - | - | - |
| GN | | | **723.08** | - | - | - |
| YW | | | | **2674.95** | - | - |
| CED | | | | | **402.93** | -99.28 |
| CEM | | | | | | **319.55** |

[1]Estimates of genetic variance are on the diagonal (indicated by boldface) and genetic covariance are above the diagonal.

PROC AUTOREG of SAS software (2011) and chow test were used to determine the structural break point in the data. Year of birth (1991) was chosen as the breaking point ($P < 0.0001$) of the regression line. Therefore, genetic trends for all studied traits were divided into two major time spans, that occurring from 1980 to 1991 (period 1) and that from 1992 to 2011 (period 2).

For each period, mean EPD for BW, WW, YW, CED, and CEM were regressed on year of birth. All regression analyses were performed using R statistical software (v.2.14.1; R Development Core Team, 2011).

## RESULTS AND DISCUSSION

***All Data (1980 to 2011).*** Rate of change of BW decreased ($P < 0.001$) by -0.0087 ± 0.002 sdu/year. Other traits were changed accordingly, especially, CED which showed an opposite trend (Fig. 1) with increasing rate ($P < 0.001$) of 0.013 ± 0.0008 sdu/year during the whole 32-yr time span. Mean EPD for CEM (0.01 ± 0.0008 sdu/year) showed a slow increase ($P < 0.001$). In early years, WW had a steep and upward trend which followed similar patterns to the genetic trend of YW (Figure 1). The linear regression associated with WW and YW were significant ($P < 0.001$) with estimates of 0.01 ± 0.001 and 0.016 ± 0.001 sdu/year, respectively. Rates of 0.22 and 0.24 sdu/year in WW were reported by Baker et al. (1991). Koch et al. (1994) reported genetic change rates for weaning and yearling weights in different selected Hereford lines which ranged 0.174 to 0.239 and 0.210 to 0.256 sdu/year, respectively.

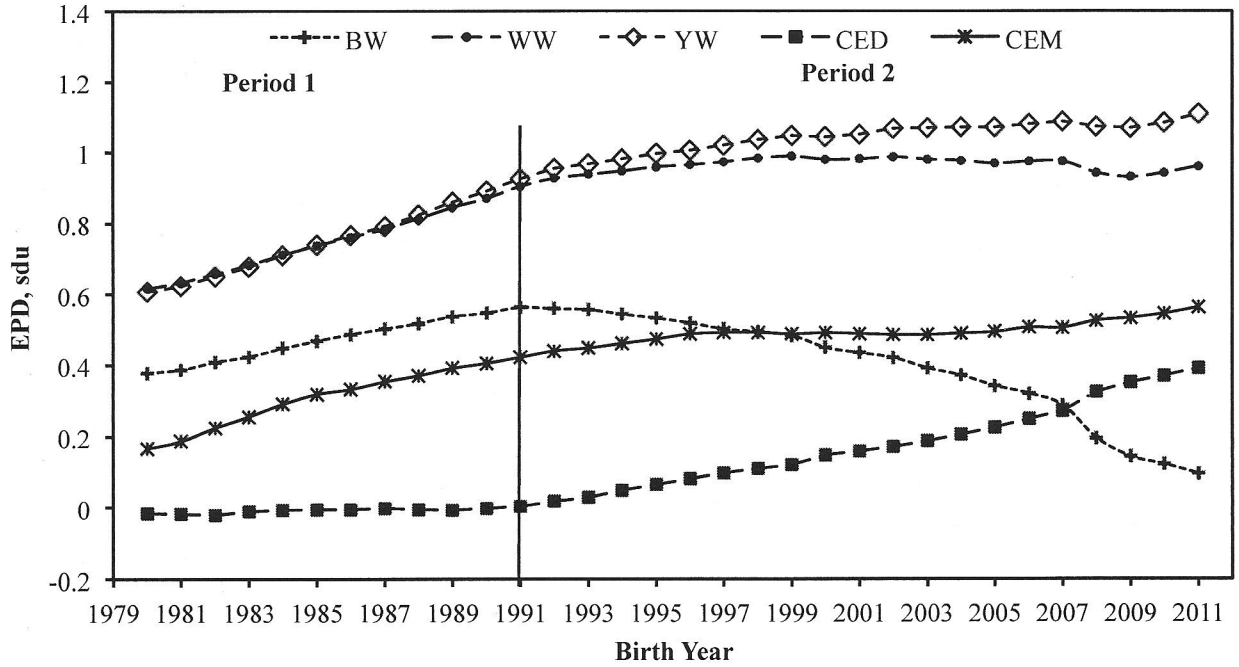


**Figure 1.** Genetic trends in maternal EPD (in standard deviation units; sdu) for calving ease (CEM) and direct EPD (in sdu) for birth weight (BW), weaning weight (WW), yearling weight (YW), and calving ease (CED).

**Table 3.** Genetic trends (sdu/year ± SE) for birth weight (BW), weaning weight (WW), yearling weight (YW), direct calving ease (CED) and maternal calving ease (CEM) in American Simmental cattle.

| Years | BW | WW | YW | CED | CEM |
|---|---|---|---|---|---|
| All data (1980-2011) | -0.0087 ± 0.002*** | 0.01 ± 0.001*** | 0.016 ± 0.001*** | 0.013 ± 0.0008*** | 0.01 ± 0.0008*** |
| Period 1 (1980-1991) | 0.017 ± 0.0004*** | 0.026 ± 0.0004*** | 0.029 ± 0.0005*** | 0.0017 ± 0.0003*** | 0.023 ± 0.001*** |
| Period 2 (1992-2011) | -0.024 ± 0.001*** | 0.00016 ± 0.0007NS | 0.007 ± 0.0006*** | 0.019 ± 0.0007*** | 0.004 ± 0.0005*** |

*** Rate of genetic change was significantly different from zero ($P < 0.001$).
NS Rate of genetic change was not significantly different from zero ($P > 0.05$).

Data showed two major patterns of genetic trends. Sullivan et al. (1999) in their study on 5 breeds, they reported similar patterns which they attributed to the increase of genetic rates of yearling weight or decrease in rates of birth weight.

Estimated rates of genetic change (slopes; sdu/year ± SE) for all studied traits for all data, period 1, and period 2 are represented in table 3.

Figure 1 depicts genetic trends in standardized mean EPD for growth-related traits and direct and maternal calving ease.

***Period 1 (1980 to 1991).*** Table 3 shows that mean EPD for BW, WW, and YW were increased ($P < 0.001$) by 0.017 ± 0.0004, 0.026 ± 0.0004, and 0.029 ± 0.0005 sdu/year, respectively, which indicates that breeders put more emphasis on growth and correspondingly experienced increasing in mean EPD for growth-related traits. These results are consisted with those reported by Elzo et al. (1987) in their study on Simmental, who suggested that, after 1980, breeders selected Simmental sires for their genetic potential for weaning and yearling weights. Baker et al. (1991) reported rates of 0.12, 0.22 and 0.33 sdu/year in birth, weaning, and yearling weights, respectively. Rates of 0.12, 0.26, and 0.27 sdu/year for birth, weaning, and yearling weights, respectively, were reported by Frahm et al. (1985), and 0.25 sdu/year by Aaron et al. (1986). An estimate of 0.17 sdu/year for WW was reported by Irgang et al. (1985). During this period, mean EPD for CED showed ($P < 0.001$) little change (0.0017 ± 0.0003 sdu/year), while mean EPD for CEM had a faster rate of increase ($P < 0.001$) by 0.023 ± 0.001 sdu/year.

***Period 2 (1992 to 2011).*** The rate of genetic change of mean EPD for BW ($P < 0.001$) declined dramatically (-0.024 ± 0.001 sdu/year) while mean EPD for CED increased ($P < 0.001$) by 0.019 ± 0.0007 sdu/year. As result, the rate of increase in mean EPD for YW (0.007 ± 0.0006 sdu/year) slowed ($P < 0.001$) while that for WW (0.00016 ± 0.0007 sdu/year) did not show any change ($P > 0.05$). Rates of genetic change in selected Hereford lines ranged 0.12 to 0.15, 0.17 to 0.24, and 0.20 to 0.24 sdu/year for BW, WW, and YW, respectively (Buchanan et al., 1982). Aaron et al. (1986) reported rates of 0.12, 0.21, and 0.25 sdu/year for BW, WW, and YW, respectively. The linear regression associated with CEM changed very little with estimate of 0.004 ± 0.0005 sdu/year. During this period, apparent changes in selection goals were made. Producers applied new selection strategies resulted in reversing genetic trends for both BW and CED, diminishing the rate of genetic progress for YW, and causing mean EPD for WW to remain constant. These results suggest that producers used below average birth weight sires to improve calving ease which reduced the rates of genetic change in WW and YW.

## IMPLICATIONS

These results suggest selection emphasis differed between periods. Breeders, in period 1, selected animals for faster growth rates. However, during period 2, selection goals were changed and selection against birth weight was applied which resulted in a slower genetic change in growth-related traits compared with earlier selection. Collecting calving ease data in a regular basis as a part of performance data and incorporating these data into genetic evaluations for direct selection of calving ease could be more effective than selection against birth weight.

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# SINGLE NUCLEOTIDE POLYMORPHISMS IDENTIFIED IN POLYGENIC TRAITS THROUGH THE USE OF THE OVINE SNP50 BEADCHIP

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**ABSTRACT:** High-density single nucleotide polymorphism (SNP) chips have shown promise in Genome Wide Association Studies (GWAS) to target genomic regions that play key roles in traits of economic interest. The objectives of this study were to use GWAS to identify SNPs affecting polygenic traits including backfat (BF), loin eye area (LEA), ADG, scrotal circumference (SC), and birth type (BT) in sheep and determine if identified genotypes or regions are associated with multiple traits. Phenotypic measurements were collected on rams from two separate performance tests (Dual Purpose Ram Test and Blackface Ram Test) at the University of Wyoming (n = 330) from 2009 to 2011. Blood was collected via the jugular and DNA was isolated and diluted. Single nucleotide polymorphisms in ram DNA were genotyped using the Ovine SNP50 BeadChip on the Illumina Infinium HD BeadChip Assay. A GWAS analysis was conducted in R using the GenABEL package using a polygenic model, $Y = \mu + G + e$ whereby μ is the overall mean, $G$ is the vector of random polygenic effects, and $e$ is the random residual, was used to identify SNPs. Nominal significance for reported genotypic association with phenotypic traits was ($P < 1.0^{-03}$) based on the pc1df test statistic. There were 11, 4, 5, 19, and 10 SNP that reached genome-wide significance ($P \leq 9.69^{-04}$) for BF, LEA, ADG, SC, and BT, respectively. Candidate genes for BF included: *EPHB* and *A* family, *ANK* family, *FRMPD4, XDH* and *REL*. Loin eye area had four candidate genes, *AAK1, CCDC73, MAGI1* and *MAGI2*. Candidate genes for ADG included: *RGD1310773, RGS2,* and *KLF12*. Candidate gene for SC included: *RBFOXF1, 2, and 3, CUX2, TMEM57, C15H11, CHD1,* and *SLC30A7*. Finally, genes *ODZ1* and *ODZ3, LTBP3,* and *DSCAM* were associated with BT. Results indicate that SNPs can be identified for polygenic traits using GWAS. None of the genotypes that met nominal genome-wide threshold limits were associated with more than one trait. However, further research and validation studies will be required before implementation of marker-assisted selection strategies.

**Key words:** polygenic, sheep, single nucleotide polymorphism

## INTRODUCTION

Genome-wide association studies (**GWAS**) are a useful tool for the analysis of both binary and quantitative traits. Through the incorporation of high-density single nucleotide polymorphism (**SNP**) chips, genotypes associated with diseases and traits of economic importance can be elucidated. When identifying markers associated with traits of economic importance, it is important to investigate other traits that could be unfavorably impacted as identified markers can potentially affect more than one trait (Moore et al., 2009). Traits such as backfat (**BF**), loin eye area (LEA), ADG, scrotal circumference (**SC**), and birth type (**BT**) are all important traits in the sheep and cattle industries. The majority of genome-wide studies have been conducted in cattle and swine, but very little information is available on SNP associations with traits in sheep. The sparse information available has indicated 31 regions within the sheep genome are under genomic selection for pigmentation, skeletal structure, body size, growth and reproduction. Furthermore, sheep are more heterogeneous than cattle as their effective population size is greater than 300 in 75% of breeds (Kijas et al., 2012). Identification of markers associated with traits of economic importance can be incorporated in selection schemes through marker-assisted selection and provide insights into the genomic network of polymorphic traits. We hypothesized that by using the Ovine SNP50 BeadChip to conduct a GWAS, markers associated with variation in polygenic traits could be identified, and that some of these genotypes would be associated with more than a single trait. The objectives of this study were to use GWAS to 1) identify SNP affecting polygenic traits including BF, LEA, ADG, SC, and BT in rams of differing breeds, and 2) determine if identified markers were shared among traits.

## MATERIALS AND METHODS

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

***Animal Procedures.*** Phenotypic measurements were collected from 2009-2011 from the University of Wyoming ram dual purpose (DP) and blackface (BF) breed performance tests (n = 330). Performance tests were composed of 5 contemporary groups (Fall 2009 = 1; Summer 2010 = 2; Fall 2010 = 3; Summer 2011 = 4; and Fall 2011 = 5). Dual-purpose rams (n = 205) consisted of contemporary groups 1 (n = 60), 3 (n = 75), and 5 (n = 48) and were primarily Rambouillet. Blackface rams (n = 125) consisted of contemporary groups 2 (n = 77) and 4 (n = 70) and were composed of Suffolk and Hampshire breeds. Rams were provided a medicated

forage-based pellet (13.5-15% CP) for the duration of the test. Preliminary research in our laboratory has shown that regardless of pellet composition, feed efficiency status does not differ. Weekly weights were collected for the DP rams and every 25 d for the BF rams. Upon cessation of the performance tests, blood was collected for DNA isolation via the jugular using EDTA-lined vacutainer tubes to prevent clotting (Tyco Healthcare Group LP, Mansfield, MA).

***Laboratory Procedures.*** A modified procedure of Montgomery and Sise (1990) was used to extract DNA from white blood cells. After a mixture of Digestion Buffer, Proteinase K, Sodium Dodecyl Sulfate, and white blood cells were incubated over night at 65 °C, 10 M NH4Ac (1 mL) was added and mixed vigorously. Samples were then centrifuged and supernatant was combined with 100% Absolute Ethanol (20 mL). Using a Pasteur pipette, DNA was spooled out of ethanol and dried. A Tris-EDTA- (**TE**) buffer was then added to isolated DNA and eluted over one week. Based on Nanodrop readings (Thermo Scientific, NanoDrop, Wilmington, DE), DNA was diluted using TE buffer to approximately 100 ng/µL for SNP detection. The Illumina Infinium HD Ultra BeadChip Protocol (Illumina, Inc., San Diego, CA) was used to analyze isolated DNA on the Ovine SNP50 BeadChip at AgResearch (Invermay, New Zealand).

***Phenotypic Trait Measurements.*** Carcass traits BF and LEA were measured by ultrasound upon cessation of the performance test. Additionally, ADG and SC were used as indicators for growth. Birth type records (rams born as single = 1, twin = 2, or triplet = 3) were provided by owners.

***Quality Control Analysis.*** Genome Studio and R were used to evaluate genotype results according to Dodds et al. (2009). Of the 52,625 SNPs and 330 animals, 50,896 markers and 328 rams had an average call rate > 0.95% and a minor allele frequency > 0.76%. Due to missing values or poor call rates, 18 to 94 animals were eliminated from the GWAS analysis depending upon the trait. Lambda was generated for BF ($0.79 \pm 4.51^{-05}$), LEA ($0.73 \pm 2.89^{-05}$), ADG ($0.79 \pm 6.14^{-05}$), SC ($0.79 \pm 8.75^{-05}$), and BT ($0.81 \pm 3.40^{-05}$) to confirm fitness of the polygenic model. The gene *RXFP2* was also used to confirm accuracy of the polygenic model as it has been implicated in regulating horn size in sheep (Johnston et al., 2011). The SNP associated with *RXFP2* was confirmed to be in high association ($P = 9.283^{-04}$) with the horn phenotype within our population.

***Genome-Wide Association Study Analysis.*** A GWAS analysis was conducted in R using the GenABEL package to identify SNP using a polygenic model, $Y = \mu + G + e$, whereby µ is the overall mean, $G$ is the vector of random polygenic effects, and $e$ is the vector of random residuals, and estimate a genomic kinship $h^2$ for each trait. Fixed effects included birth year (2008 to 2011), flock (1 to 33), and contemporary group (1 to 5). Data were fitted to the fixed effects and polygenic model where the G (relationship matrix) was derived from the SNP. Each SNP genotype was then tested individually as an additive effect in a simple fixed model that incorporated the errors. Identified SNP were aligned to the ovine genome using the UCSC Genome Browser (ISGC Ovis Aries 1.0/oviAri1). Nominal genome-wide association threshold significance for reporting results was at $P < 1.00^{-03}$ based on the pc1df test statistic generated.

**Table 1.** Genomic heritability and raw means of phenotypic traits.

| Trait[2] | n | $h^2_g$ | Mean ± SE |
|---|---|---|---|
| BF (cm) | 280 | 0.56 | 0.58 ± 0.01 |
| LEA ($cm^2$) | 278 | 0.90 | 22.90 ± 0.23 |
| ADG (kg) | 282 | 0.67 | 0.54 ± 0.01 |
| SC (cm) | 275 | 0.60 | 35.23 ± 0.15 |
| BT[2] | 236 | 0.34 | 1.63 ± 0.04 |

[1]BF (backfat), LEA (loin eye area), ADG (average daily gain), SC (scrotal circumference), BT (birth type).

## RESULTS

***Genetic Kinship $h^2$ of Traits.*** Table 1 shows the $h^2$ and mean for each polygenic trait. Heritabilities for BF, LEA, ADG, SC, and BT were calculated from the polygenic model and were moderate to high (0.34 – 0.90).

***Carcass Traits.*** There were 11 and 4 markers that reached the nominal threshold significance ($P \leq 9.36^{-04}$) for carcass traits BF and LEA, respectively (Table 2). Candidate genes associated with BF included: *Ephrin type B receptor 1, 2,* and *3* (***EPHB1***, ***EPHB2***, ***EPHB3***), *Eprhin type A receptor 2, 3,* and *7* (***EPHA2***, ***EPHA3***, *and* ***EPHA7***), *Ankrin 1, 2,* and *3 erythrocytic* (***ANK1***, ***ANK2***, ***ANK3***), *FERM and PDZ containing 1* (***FRMPD4***), *xanthine dehydrogenase* (***XDH***), and *reticuloendotheliosis viral oncogene homolog* (***REL***). *Adaptor-associated protein kinase 1* (***AAK1***), *coiled-coil domain containing protein 73* (***CCDC73***), *membrane-associated guanylate kinase, WW and PDZ domain-containing protein 1* and *2* (***MAGI1*** and ***MAGI2***) were associated with the SNP that reached threshold for LEA.

***Growth Traits.*** Table 3 shows that growth traits ADG and SC had 5 and 19 SNP, respectively, that reached nominal genome-wide association threshold ($P \leq 9.69^{-04}$). The candidate genes for ADG that were associated with identified SNP included: *uncharacterized protein LOC313156 precursor* (***RGD1310773***), *regulator of G-protein signaling 2* (***RGS2***), *and krueppel-like factor 12* (***KLF12***). Scrotal circumference had eight candidate genes that were associated with SNP that reached genome-wide threshold levels. Candidate genes for SC included: *RNA binding protein fox-1, 2, and 3* (***RBFOX1***, ***RBFOX2***, ***RBFOX3***), *homeobox protein cut-like 2* (***CUX2***), *transmembrane protein 57, chromosome 11 open reading frame 70 ortholog* (***C15H11orf70***), *chromodomain-helicase-DNA-binding protein 1* (***CHD1***), and *zinc transporter 7* (***SLC30A7***).

***Reproductive Efficiency Traits.*** There were 10 SNP that reached the nominal genome-wide reported threshold ($P \leq 9.09^{-04}$) for BT (Table 4); however only four candidate genes were identified. Candidate genes for BT included: *teneurin-1 and 3* (***ODZ1*** and ***ODZ3***), *latent-transforming growth factor beta-binding protein 3* (***LTBP3***), and *down syndrome cell adhesion molecule precursor* (***DSCAM***).

**Table 2.** Genome-wide association analysis of carcass traits to generate corresponding genotypes and candidate genes[1]

| Trait | SNP | Chr | Position (Mb) | Pc1df[2] | Candidate Gene(s) | Ref. Seq. |
|---|---|---|---|---|---|---|
| Back Fat | s31004.1 | 5 | 49.6 | $6.09^{-05}$ | ---- | ---- |
| | OAR1_272791087.1 | 1 | 272.8 | $2.56^{-04}$ | *EPHB1* | NM_001192829 |
| | | | | | *EPHB3* | NM_001192796 |
| | | | | | *EPHB2* | NM_001191498 |
| | | | | | *EPHA7* | NM_001192726 |
| | | | | | *EPHA2* | NM_001205731 |
| | OAR3_119895458.1 | 3 | 119.9 | $3.40^{-04}$ | ---- | ---- |
| | OARX_16106124.1 | X | 16.1 | $4.66^{-04}$ | *FRMPD4* | NM_001033330 |
| | s69692.1 | 11 | 26.8 | $5.34^{-04}$ | *RPAIN* | NM_001076500 |
| | OAR6_15051305.1 | 6 | 15.1 | $5.55^{-04}$ | *ANK1* | NM_001142446 |
| | | | | | *ANK2* | NM_001148 |
| | | | | | *ANK3* | NM_001204403 |
| | OAR2_213755101.1 | 2 | 213.8 | $6.08^{-04}$ | *XDH* | NM_173972 |
| | s73781.1 | 13 | 60.0 | $6.36^{-04}$ | ---- | ---- |
| | OAR3_66627157.1 | 3 | 66.6 | $6.97^{-04}$ | *REL* | NM_002908 |
| | OAR3_101148443.1 | 3 | 101.1 | $8.07^{-04}$ | | |
| | OAR1_167674223.1 | 1 | 167.7 | $9.36^{-04}$ | *EPHA3* | NM_001206113 |
| Loin Eye Area | s15103.1 | 3 | 41.4 | $2.78^{-04}$ | *AAK1* | NM_014911 |
| | OAR15_66971392.1 | 15 | 70.0 | $6.47^{-04}$ | *CCDC73* | NM_001192547 |
| | OAR19_37748290.1 | 19 | 37.7 | $6.78^{-04}$ | *MAGI1* | NM_001033057 |
| | | | | | *MAGI2* | NM_012301 |
| | s44195.1 | 9 | 43.4 | $7.12^{-04}$ | ---- | ---- |

[1]Nominal threshold significance $P < 1.0^{-03}$.
[2]Corrected for inflation factor, λ, for the test statistics.

**Table 3.** Genome-wide association analysis of growth traits to generate corresponding genotypes and candidate genes[1]

| Trait | SNP | Chr | Position (Mb) | Pc1df[2] | Candidate Gene(s) | Ref. Seq. |
|---|---|---|---|---|---|---|
| ADG | s27970.1 | 5 | 21.5 | $9.78^{-05}$ | ---- | ---- |
| | OAR12_13475606.1 | 12 | 13.5 | $4.14^{-04}$ | *RGD1310773* | NM_001107926 |
| | OAR10_49873581.1 | 10 | 49.9 | $5.45^{-04}$ | *RGS2* | NM_001095045 |
| | OAR4_56364872.1 | 4 | 56.4 | $5.93^{-04}$ | *KLF12* | NM_001191270 |
| | OAR4_55899343.1 | 4 | 55.9 | $8.35^{-04}$ | | |
| Scrotal Circumference | s53492.1 | 3 | 143.3 | $1.15^{-04}$ | ---- | ---- |
| | s41631.1 | 18 | 13.3 | $1.80^{-04}$ | ---- | ---- |
| | s14022.1 | 5 | 47.9 | $2.66^{-04}$ | ---- | ---- |
| | OAR23_32932392_X.1 | 23 | 32.9 | $3.21^{-04}$ | ---- | ---- |
| | OAR24_7543424.1 | 24 | 7.5 | $3.46^{-04}$ | *RBFOX1* | NM_001075818 |
| | | | | | *RBFOX2* | NM_001205372 |
| | | | | | *RBFOX3* | NM_001075537 |
| | s62356.1 | 7 | 16.3 | $3.94^{-04}$ | ---- | ---- |
| | OAR2_82367767.1 | 2 | 82.4 | $4.00^{-04}$ | ---- | ---- |
| | s02450.1 | 17 | 59.4 | $4.13^{-04}$ | *CUX2* | NM_001192597 |
| | s12754.1 | 0 | 0 | $4.16^{-04}$ | ---- | ---- |
| | OAR2_254253557.1 | 2 | 254.3 | $4.38^{-04}$ | *TMEM57* | NM_001038647 |
| | OAR15_5763494.1 | 15 | 5.8 | $4.59^{-04}$ | *C15H11orf70* | NM_001046598 |
| | OAR6_103074951.1 | 6 | 103.1 | $5.31^{-04}$ | ---- | ---- |
| | s49197.1 | 10 | 22.0 | $5.68^{-04}$ | ---- | ---- |
| | OAR24_7509611.1 | 24 | 7.5 | $5.92^{-04}$ | *RBFOX1* | NM_001075818 |
| | OAR15_76051176.1 | 15 | 76.1 | $6.16^{-04}$ | ---- | ---- |
| | s60279.1 | 25 | 45.8 | $8.13^{-04}$ | ---- | ---- |
| | OAR5_103915371.1 | 5 | 103.9 | $8.26^{-04}$ | *CHD1* | NM_001192048 |
| | OAR5_103935962.1 | 5 | 103.9 | $8.26^{-04}$ | *CHD1* | NM_001192048 |
| | OAR1_82726417.1 | 1 | 82.7 | $9.69^{-04}$ | *SLC30A7* | NM_001163598 |

[1]Nominal threshold significance $P < 1.0^{-03}$.
[2]Corrected for inflation factor, λ, for the test statistics.

**Table 4.** Genome-wide association analysis of a reproductive efficiency trait to generate corresponding genotypes and candidate genes[1]

| Trait | SNP | Chr | Position (Mb) | Pc1df[2] | Candidate Gene(s) | Ref. Seq. |
|---|---|---|---|---|---|---|
| Birth Type | OAR2_600110660.1 | 2 | 60.0 | $5.52^{-05}$ | ---- | ---- |
| | s63756.1 | 11 | 40.5 | $2.15^{-04}$ | ---- | ---- |
| | s06747.1 | 5 | 85.2 | $2.27^{-04}$ | *ODZ3* | NM_001205307 |
| | | | | | *ODZ1* | NM_001256555 |
| | s63980.1 | 8 | 94.6 | $4.32^{-04}$ | ---- | ---- |
| | s42358.1 | 21 | 47.6 | $4.57^{-04}$ | *LTBP3* | NM_001192738 |
| | OAR4_107022428.1 | 4 | 107.0 | $5.78^{-04}$ | ---- | ---- |
| | OAR1_279962640.1 | 1 | 280.0 | $5.85^{-04}$ | *DSCAM* | NM_001389 |
| | OAR8_59572572.1 | 8 | 59.6 | $6.47^{-04}$ | ---- | ---- |
| | s59135.1 | 9 | 18.5 | $7.96^{-04}$ | ---- | ---- |
| | OAR18_65864849.1 | 18 | 65.9 | $9.09^{-04}$ | ---- | ---- |

[1]Nominal threshold significance $P < 1.0^{-03}$.
[2]Corrected for inflation factor, λ, for the test statistics.

***Genotype Relationships.*** There were multiple gene families that were repeated within traits such as *EPH* and *ANK* families for BF, the *MAGI* family for LEA, the *RBFOX* and *CHD* families for SC, and the *ODZ* family for BT. However, no identified candidate genes reached genome-wide association threshold for more than one trait.

## DISCUSSION

**Genomic *$h^2$ of Traits.*** Known estimates of $h^2$ for BF have averaged 0.36 to 0.72 (Utrera and Van Vleck, 2004; Suzuki et al., 2005). Phenotypic $h^2$ estimates for loin mass area and LEA have spanned from 0.45 - 0.57 in cattle and swine (Suzuki et al., 2005; Cai et al., 2008). Daily gain for 120 to 365 d and post-weaning ADG phenotypic $h^2$ for sheep was estimated as 0.20 and 0.21, respectively (Mousa et al., 1999; Safari and Fogarty, 2003). In cattle, $h^2$ was estimated as 0.34 to 0.41 (Arthur et al., 2001). The $h^2$ for a 90 d SC measurement has been estimated as 0.25 to 0.39 in sheep (Matos et al., 1992; al-Shorepy and Notter, 1996; Safari and Fogarty, 2003). Finally, reports for litter size and BT $h^2$ have ranged from 0.05 to 0.16 in sheep (al-Shorepy and Notter, 1996; Matos et al., 1997; Safari and Fogarty, 2003). Estimated genomic $h^2$ generated from the polygenic model were larger than literature estimates for LEA, ADG, SC, and BT, and BF was within the estimated range. Previous research indicates that phenotypic $h^2$ estimates are lower than those generated from a polygenic model that use genomic estimates. Estimates for this study were corrected for breed and flock effects, which may contribute to the inflation. Estimate differences between phenotype and genomic $h^2$ may be attributed to a proportion of phenotypic variance that incorporates additive genetic variance (Yang et al., 2010).

***Carcass Traits.*** Chromosome 3 had three regions (66.6 Mb, 101.1 Mb, and 119.9 Mb) and chromosome 1 had two regions (167.7 Mb and 272.8 Mb) associated with BF. Chromosome 3 (position 41.4 Mb) may correlate LEA with BF as BF has multiple regions within this chromosome. More research will be required to elucidate genotypes in linkage disequilibrium (LD). Moore et al. (2003) suggested that candidate genes *DGAT1* and *TG* on chromosome 14 are associated with BF. Additionally, Li et al. (2004) determined that there were regions within chromosomes 5, 6, 19, 21, and 28 that were associated with BF in cattle. Two candidate genes, *SLC2A12* and *JAK2* associated with chromosome 1, were suggested to be associated with LEA in swine (Grapes and Rothschild, 2006). Within the nominal genome-wide association threshold used, presence of these candidate genes or regions could not be verified.

***Growth Traits.*** Genotypes for ADG that met genome-wide association threshold had two regions (55.9 Mb and 56.4 Mb) within chromosome 4. Kneeland et al. (2004) identified regions on chromosomes 6, 14, 19, and 21 that were associated with both pre-weaning ADG and post-weaning ADG. Chromosome 2 had two regions (82.4 Mb and 254.3 Mb), 5 had two regions (47.9 Mb and 103.9 Mb), 15 had two regions (5.8 Mb and 76.1 Mb), and 24 had two separate genotypes align to 7.5 Mb for SC. Aside from this research no known genotypes or candidate genes have been identified as associated with SC.

***Reproductive Efficiency Trait.*** Birth type or litter size was associated with regions 59.6 Mb and 94.6 Mb on chromosome 8, but neither region aligned to a candidate gene. Candidate genes *ESR2, BMPR-IB, BMP-15,* and *FECB* have all been suggested to be associated with litter size (Buske et al., 2006; Guan et al., 2006; Chu et al., 2007).

***Relationship of Candidate Genes to Multiple Traits.*** Interestingly, *SLC30A7* was associated with SC and a member of the same *SLC* family was suggested as a candidate gene for LEA in swine (Grapes and Rothschild, 2006). Though no regions or candidate genes were identified in multiple traits, it is possible that the nominal threshold significance was too stringent in this study. To further investigate genotypic relationships among traits of economic importance, an LD analysis must be performed on a greater population of genotyped individuals with denser SNP chip. Furthermore, an independent validation of these results must be conducted. Bolormaa et al. (2011) suggested that associations between traits and genotypes are not consistent among breeds, leading to the difficulty in identifying genotypes among differing populations. This indicates that genotypes associated with economically relevant traits may only be applicable to the populations tested and validated in. Significant research efforts will be required to identify SNP that are applicable in several breeds and species.

## IMPLICATIONS

Identified regions and candidate genes for BF, LEA, ADG, SC, and BT can be used to further elucidate genome networks and pathways to dissect regulatory mechanisms and potentially establish markers for marker-assisted selection. Further research will be required to decipher genotypic differences among species and interrelationships of identified SNP. Before their consideration as potential markers, additional observations must be incorporated in the analyses and validation must be performed on these SNP.

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# THE EFFECT OF DIET ON FEED INTAKE TRAITS AND RELATIONSHIPS WITH CARCASS TRAITS IN SHEEP

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**ABSTRACT:** The objectives of this study were to 1) determine the effects of diet on feed intake traits, and 2) determine the relationship of residual feed intake (RFI) with carcass characteristics in lambs fed either a concentrate or forage-based diet. Growing wethers (initial BW = 51.3 ± 1.2 kg; n = 82) of Rambouillet, Hampshire, and Suffolk breed types were randomly allocated to receive a concentrate (CONC; 50% corn, 31% wheat middlings, 91.6% DM; 12.1% CP, 17.6% NDF, 2.98 Mcal/kg ME; n = 40) or forage-based (FOR; 67.7% alfalfa, 27.5% wheat middlings; 92.3% DM, 16.2% CP, 36.3% NDF, 2.31 Mcal/kg ME; n = 42) pelleted diet. Individual feed intake was measured by the GrowSafe System for 49 d, and BW was recorded weekly. The 20% most (n = 8) and the 20% least (n = 8) efficient wethers from each diet (n = 32 total) were slaughtered, and carcass data were recorded. The MIXED procedure of SAS was used to determine the effect of diet on feed intake, ADG, and G:F using data from all wethers (n = 77), and the effects of diet, RFI class (most or least efficient), and their interaction on carcass traits using data from selected wethers (n = 32); breed and pen were included as random effects. The CORR procedure was used to determine relationships between RFI and carcass characteristics. Overall, feed intake of CONC wethers varied less ($\sigma^2$ = 0.13) than intake of FOR wethers ($\sigma^2$ = 0.25); variation in ADG and RFI did not differ across diets. Feed intake and ADG were greater ($P \leq 0.001$) in FOR fed compared with CONC fed wethers, although G:F was not affected by diet type ($P = 0.23$). Boneless cut percentage tended ($P = 0.10$) to be greater in FOR versus CONC wethers; there were no other effects ($P > 0.13$) on carcass measures. Residual feed intake and USDA quality grade tended ($P = 0.10$) to be positively correlated, but no other relationships ($P > 0.17$) between RFI and carcass measures were found. These data suggest that feed intake and ADG are affected by diet type, and that greater variation in feed intake is associated with a forage-based pelleted diet. Furthermore, selection for RFI should not unfavorably affect carcass traits in sheep.

**Key words:** carcass, residual feed intake, sheep

## INTRODUCTION

Feed costs for livestock are a substantial portion of production costs, and can be included in up to 50 to 70% of total production costs in sheep (Neary, 1997). Similarly, in 2010 feed accounted for approximately 68% of total production cost in cow/calf operations in the U.S. (USDA-ERS, 2010). It is unlikely that the costs of feed will decrease in the near future due to increased competition for feed resources by energy industries and a growing human population. Improving feed efficiency becomes more important as feed costs continue to increase, which can improve performance of animals while decreasing the necessary inputs.

Residual feed intake (**RFI**) is a measurement of feed efficiency that is defined as the difference between the actual and predicted feed intake as it relates to observed ADG (Koch et al., 1963). There is a great deal of research related to RFI in cattle; however, little work has been reported on RFI in sheep. Relationships between RFI and meat quality may be of some concern to producers who select animals based on efficiency. Therefore, it is important to understand whether selection for improved efficiency, particularly RFI, will affect carcass quality. Additionally, little is known about how diet composition affects animal performance and intake during RFI testing, especially in sheep. The objectives of this study were to 1) determine the effects of a concentrate versus a forage diet on feed intake traits and RFI, and 2) determine the relationship of RFI with carcass characteristics in lambs fed either a concentrate or forage-based diet. We hypothesized that intake traits would differ between diets and that neither RFI nor diet would have an effect on carcass measurements.

## MATERIALS AND METHODS

***Animals and Diet.*** Growing wethers (n = 82; initial BW = 51.3 ± 1.2 kg) of Rambouillet, Hampshire, and Suffolk breed types were randomly allocated by BW to receive either a concentrate (**CONC**; n = 40) or forage-based (**FOR**; n = 42) pelleted diet (Table 1). Lambs were acclimated to diets using a 20% increase in proportion of new feed to old feed every 4-5 d until the diet consisted of 100% new pelleted diet ad libitum. Individual feed intake was measured using the GrowSafe System for a 49-d trial period.

Two-day average initial and final BW were obtained to calculate ADG. From this data, RFI was calculated as the deviation of true feed intake from expected feed intake. Expected feed intake was determined by regressing ADG and metablic midweight on actual feed intake (Cammack et al., 2005). Residual feed intake calculations were used to rank wether efficiency. Lambs were removed from the study if they had intact testicles, became injured, or had health complications, leaving 77 wethers for data collection.

**Table 1.** Composition of pelleted diets

| Item | FOR[1] | CONC[2] |
|---|---|---|
| Ingredient, % DM | | |
| Alfalfa pellets | 67.7 | -- |
| Corn | -- | 50.2 |
| Wheat middlings | 27.5 | 31.0 |
| Corn gluten | -- | 10.0 |
| Cane molasses | 2.50 | 2.50 |
| Salt | 1.34 | 1.76 |
| Calcium carbonate | 0.60 | 2.30 |
| Dried distillers grains with solubles | -- | 1.0 |
| Calcium sulfate | -- | 0.75 |
| Potassium chloride | -- | 0.19 |
| Trace minerals and vitamins[3] | 0.34 | 0.36 |
| Analyzed nutrient composition | | |
| DM, % as fed | 92.3 | 91.6 |
| CP, % DM | 16.2 | 12.1 |
| NDF, % DM | 36.3 | 17.6 |
| ADF, % DM | 25.1 | 6.6 |
| ME, Mcal/kg[4] | 2.31 | 2.98 |
| Ca, % DM[4] | 1.2 | 1.3 |
| P, % DM[4] | 0.37 | 0.47 |

[1]FOR = pelleted forage diet
[2]CONC = pelleted concentrate diet
[3]Includes Selenium 1600, Sheep TM ORG-Zn, Flavor APF-168, Vit E 20,000 IU/lb., and CHS/PN VT-FDLT
[4]Calculated from NRC (2007) values.

***Carcass Data Collection.*** The 20% most (n = 8, low RFI) and the 20% least (n = 8, high RFI) efficient wethers from each diet (n = 32 total) based on RFI ranking were slaughtered at the University of Wyoming Meat Laboratory, and carcass data were recorded by a trained technician. Hot carcass weight (**HCW**) was collected on the kill floor after slaughter. Dressing percentage was calculated by dividing HCW by shrunk live weight taken immediately prior to slaughter. Carcasses were chilled for 24 h and ribbed between the 12th and 13th rib. Body wall thickness (**BWT**) was measured approximately 13 cm from midline LM area was measured by taking the average of the left and right LM areas. Muscle conformation and USDA quality grade were estimated according to USDA guidelines. USDA yield grade was calculated by adding 0.4 to the 12th rib fat (inches) and multiplying by 10. Percent boneless retail cuts (**BRC**) were calculated using the following equation:

$$BRC = 49.936 - [0.0848*HCW(lbs)] - [4.376*12^{th}\ rib\ fat(in)] - [3.530*BWT(in)] + [2.456*LM\ area(in^2)]$$

***Statistical Analysis.*** The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to determine the effect of diet on feed intake, ADG, and G:F using data from all wethers (n = 77). The MIXED procedure was also used to determine the effects of diet and RFI selection group (low or high RFI) and their interaction on carcass traits using data from selected wethers (n = 32). Breed and pen were included as random effects for both MIXED procedure analyses. The CORR procedure was used to determine relationships between RFI and carcass characteristics. Alpha was determined to be 0.05.

## RESULTS AND DISCUSSION

***Performance.*** Wethers in this study had an average daily intake of 2.18 ± 0.06 kg for CONC and 3.14 ± 0.08 kg for FOR diets. Feed intake of wethers on the CONC diet varied less ($\sigma^2 = 0.13$) than the intake of wethers on the FOR diet ($\sigma^2 = 0.25$). Feed intake was greater ($P \leq 0.001$) in FOR fed compared with CONC fed wethers. ADG was greater ($P \leq 0.001$) in FOR (0.27 ± 0.01 kg/d) fed compared with CONC (0.20 ± 0.01 kg/d) fed wethers. G:F was not affected by diet type ($P = 0.23$; 0.09 ± 0.003 overall). Residual feed intake ranged from -0.47 to 0.69 for wethers fed the CONC diet, and from -0.70 to 0.80 for wethers fed the FOR diet.

These data suggest that greater variation in feed intake is associated with a forage-based pelleted diet. In contrast to these results, Borton et al. (2005) reported that ADG was greater for lambs fed a concentrate-based diet than lambs fed

**Table 2.** Effects of diet and residual feed intake (RFI) selection group on lamb carcass traits

| Item | Diet[1] | | | RFI Selection Group[2] | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|---|
| | CONC | FOR | SEM | Low | High | SEM | Diet | RFI | Diet * RFI |
| Hot carcass weight, kg | 39.0 | 35.9 | 3.2 | 37.2 | 37.7 | 3.0 | 0.31 | 0.77 | 0.71 |
| Dressing % | 56.5 | 54.4 | 1.7 | 56.5 | 54.8 | 1.7 | 0.39 | 0.58 | 0.39 |
| 12th rib fat, cm | 0.58 | 0.56 | 0.10 | 0.58 | 0.53 | 0.10 | 0.89 | 0.47 | 0.84 |
| Body wall thickness, cm | 2.24 | 2.03 | 0.23 | 2.13 | 2.13 | 0.25 | 0.16 | 0.93 | 0.90 |
| Ribeye area, $cm^2$ | 17.7 | 17.1 | 1.7 | 17.2 | 17.5 | 1.6 | 0.73 | 0.81 | 0.13 |
| Muscle conformation | 12.1 | 11.1 | 1.0 | 11.5 | 11.7 | 0.9 | 0.23 | 0.81 | 0.88 |
| USDA quality grade[3] | 12.1 | 11.6 | 0.9 | 12.1 | 11.6 | 0.9 | 0.47 | 0.39 | 0.94 |
| USDA yield grade | 2.62 | 2.60 | 0.40 | 2.72 | 2.49 | 0.41 | 0.90 | 0.48 | 0.82 |
| Boneless retail cuts, % | 45.3 | 46.0 | 0.6 | 45.6 | 45.7 | 0.6 | 0.09 | 0.71 | 0.24 |

[1] CONC = pelleted concentrate diet; FOR = pelleted forage diet.
[2] Low = 20% lowest RFI lambs of each diet (most efficient); High = 20% highest RFI lambs of each diet (least efficient).
[3] 11 = average Choice; 12 = high Choice.

a forage-based diet in a study where the concentrate diet was 77% shelled corn.

***Carcass Traits.*** Boneless retail cut percentage tended ($P = 0.10$) to be greater for carcasses from FOR versus CONC wethers (Table 2). There were no other effects ($P > 0.13$) of diet, RFI selection group, or their interaction on carcass measures. However, Borton et al. (2005) reported an effect of diet type on HCW, dressing percent, 12th rib fat thickness, BWT, LM area, USDA quality grade, and USDA yield grade, all of which were greater in lambs fed a concentrate-based diet. Differences in results could be attributed to breed, nutrient content differences of diets, or duration of the study.

Residual feed intake and USDA quality grade tended ($P = 0.10$; $r = 0.30$) to be positively correlated. No other relationships ($P > 0.17$) between RFI and carcass measures were found. These results were similar to Cruz et al. (2010), which reported that RFI classification did not affect carcass quality in Angus-Hereford steers. Baker et al. (2006) also reported no differences in carcass quality, except for cooking loss, due to RFI in purebred Angus steers. This data suggests that selection for RFI should not unfavorably affect carcass traits in sheep.

## IMPLICATIONS

Selecting for RFI in sheep and other livestock could decrease feed costs for producers. Residual feed intake having no negative effect on carcass quality allows producers to more comfortably select for RFI in their flocks or herds. If diet does not have an effect on RFI in these animals, there is an even larger possibility of saving on feed costs. Future applications of RFI selection, in turn, could save producers money by decreasing feed costs while not negatively effecting carcass qualities.

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# EXTENSION

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# A SURVEY OF THE PRESENCE, STRUCTURE, AND EFFECTIVENESS OF BEEF QUALITY ASSURANCE (BQA) OR BQA-TYPE PROGRAMS ACROSS THE UNITED STATES

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**ABSTRACT:** In an effort to improve the effectiveness and impact of Beef Quality Assurance (BQA) Programs in the U.S., a nationwide survey of state and regional BQA Program coordinators was conducted. In early 2011, coordinators were asked to complete a 37-question on-line survey about the BQA Program that they oversee. Survey questions were intended to summarize basic information about each program, identify unique and successful approaches to educating or certifying producers or both, and estimate the impact of BQA efforts nationwide. Representatives from 45 of the 50 states completed the survey on behalf of their BQA Program or cattle industry, if no program existed. In 62.2% of states, university extension personnel coordinated the BQA Program. State beef councils provided funding for BQA Programs in 60.0% of states, followed by university extension (53.3%), pilot project grants from the National BQA Program (35.6%), and state cattlemen′s associations (31.1%). User fees were used in 22.2% of states. Formal BQA Certification was offered by 91.0% of states, and 71.1% of states required at least 2 hr of face-to-face training in order for an attendee to become certified. Only 13.3% of states offered multiple levels of BQA Certification, and just 11.1% included an on- farm assessment or audit associated with BQA Certification. In terms of additional BQA-related components offered relatively few states offered dairy BQA Certification (20.8%) or feedyard BQA Certification (35.4%). Youth BQA Certification was available in 43.8% of states, but only 27.1% required youth to be BQA Certified in order to show and sell an animal at the county fair level. On average, there were 28 trainers per state BQA Program. However, most programs (57.1%) had 1 to 9 trainers, while 22.8% had 10 to 49 trainers. At least 50 trainers were present in 20.0% of programs. Overall, 709 trainers were available to BQA Certify producers across the U.S. In summary, these data suggest that the BQA Program includes a large infrastructure of personnel across the U.S.; however, many states offer little beyond basic BQA Certification. Further, the large amount of variation among programs may make development of a uniform nationwide program challenging.

**Key words:** Beef Quality Assurance, cattle, survey

## INTRODUCTION

The U.S. cattle industry's BQA program was originally created as a voluntary and grassroots-driven process control system to better meet consumer demands related to concerns about the safety, quality, and wholesomeness of beef (NCBA, 2010). The core structure of BQA revolves around the "BQA Certification" of cattle producers, which involves training producers about core BQA guidelines as they relate to the end-product that is produced.

On a limited basis, the National BQA Program has been evaluated as to its effectiveness at influencing producer behaviors related to influencing beef quality, as well as the level of participation among cattle producers in this voluntary program. One of the first evaluations of beef cattle producer knowledge about BQA and BQA-related issues was conducted by the USDA National Animal Health Monitoring Service (**NAHMS**). In that study it was reported that when asked about their knowledge of BQA, 51.3% of beef cow/calf respondents indicated that they had heard of BQA (NAHMS, 2008). However, only 22.2% of those that had heard of BQA had attended a BQA meeting or training session. Finally, 57.2% of producers who attended a BQA meeting were officially BQA Certified. Ultimately, this research indicated that less than 5% of cow/calf producers were BQA Certified – a necessity to ensure that producers are aware of BQA guidelines and recommendations, and that they are followed when cattle-related management practices are performed.

Further evaluation of the BQA Program is needed with the goal of improving the effectiveness of BQA and determining if there is an opportunity to use its existence as a marketing tool. Therefore, the objectives of this study were to: 1) document information about each state/regional BQA program, 2) describe unique state BQA program materials, methods, and activities that maximize participation, and 3) promote sharing of BQA resources and strategies among states.

## MATERIALS AND METHODS

An evaluation of state/regional BQA Programs was undertaken via development of a survey instrument. Each state's/region's BQA Program coordinator(s) was contacted and asked to complete the survey, using contact information provided by National BQA Program staff (which is also available via the National BQA Program website at www.bqa.org). States without a known or active BQA Coordinator, beef cattle faculty members in departments of animal sciences at land-grant universities were contacted.

The main focus of this survey was to collect: 1) basic information on each BQA program (e.g., organization in charge, funding source), 2) approaches to educating/ certifying producers (organization that does it, expiration time of BQA Certification, cost, how it is done, BQA Certification levels, audits, etc.), and 3) estimated impact of BQA efforts nationwide (e.g., estimated number of producers certified/educated, number of trainers available).

An initial set of survey questions was developed in concert with National BQA Program staff and further refined based on the targeted survey length. To "test" the survey, 4 BQA experts were asked to complete the draft survey prior to its full release.

Beef Quality Assurance Coordinators were initially contacted by National BQA Program staff in January 2011 and notified that they would be asked to complete a survey about their BQA Program, and that the effort was a National Cattlemen's Beef Association (**NCBA**)-sanctioned survey of each state BQA Program. In February 2011, BQA Coordinators were contacted via email and asked to complete the 37-question survey instrument on-line (via www.surveygizmo.com).

Follow-up emails were sent in March and June of 2011 to BQA Coordinators that had not yet completed the survey. Prior to conclusion of the project in September 2011, personal phone calls were made, and follow-up emails were sent, to every remaining non-respondent.

## RESULTS

Representatives from 45 of the 50 states completed the survey on behalf of their BQA Program or cattle industry, if no program existed. Based on survey results, 45 active state BQA Programs existed in the U.S. In 62.2% of states, university extension personnel coordinated the BQA Program (Figure 1), while the organization that oversaw the "rules" of the program was highly varied and included the state BQA advisory committee (33.3% of states), university extension (24.4%), state cattlemen's associations (17.8%), and state beef councils (8.9%). Further, 51.1% of states did not use a formal BQA Advisory Committee to provide additional oversight for the program's direction.

State beef councils provided funding for BQA Programs in 60.0% of states, followed by university extension (53.3%), National BQA Program pilot project grants (35.6%), and state cattlemen's associations (31.1%), while user fees were used in 22.2% of states (Figure 2). Among states that charged a user fee to become BQA Certified, the mean fee was $19.52 (range = $5 to 50/ person).

Two-thirds (66.7%) of states used face-to-face meetings to keep producers updated about BQA, and over half distributed information via mailed BQA newsletters (55.6%) or websites (53.3%) or both, and email correspondence was used by 26.7% of states. However, about 1 in 10 states did not provide regular updates about their program and few state BQA Programs used Internet- based social media, based on the limited use of blogs (8.9% of states), Facebook (8.9%) or Twitter (4.4%) or both. This appears to be a huge opportunity for state BQA Programs to improve communications with producers about BQA.

Over 91% of states offered a formal BQA Certification option for producers. However, based on pooled survey responses of state BQA Coordinators, 69.4% of people who took the test received a grade of 95% or greater (95 out of 100), with 30.5% receiving a grade of 85% or greater. It was clear that almost no one given the test failed. Recertification was offered by 84.4% of states; however, the recertification test was different from the original test in only 33.1% of states.

The minimum number of hours of training required for BQA Certification was inconsistent across states that offered BQA Certification. Some 28.9% of states required a 2 h or less of face-to-face training in order to become BQA certified, compared with 33.4% of states that required at least 3 h (Figure 3).

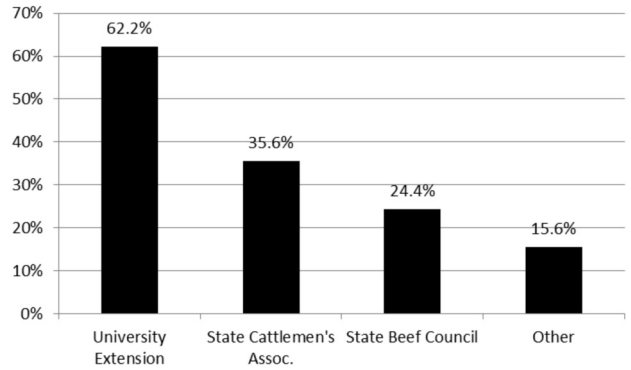


**Figure 1.** Responses to the on-line survey question "In your state, what organization coordinates the beef cattle Beef Quality Assurance Program?" Respondents could mark more than one option in their response (i.e., when combined, percentage total can be greater than 100%).

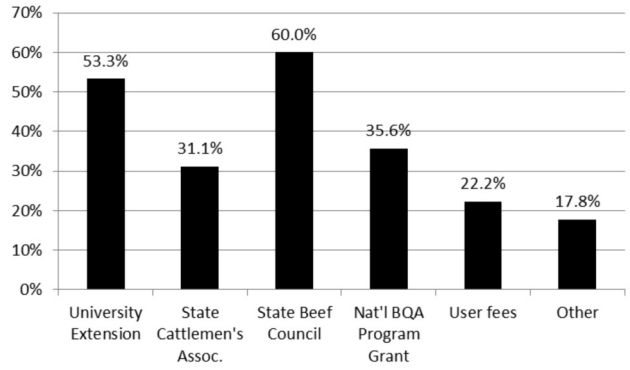


**Figure 2.** Sources of funding for state Beef Quality Assurance Programs indicated by survey respondents Respondents could mark more than one option in their response (i.e., when combined, percentage total can be greater than 100%).

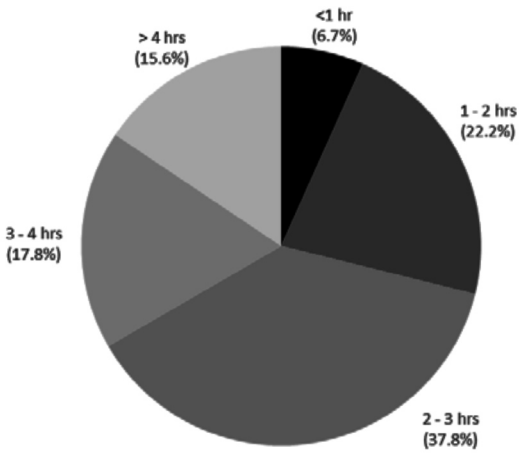


**Figure 3.** Number of hours that a producer must spend in a face-to-face meeting in order to become Beef Quality Assurance (BQA) Certified, based on survey responses.

Only 13.3% of states offered multiple levels of BQA Certification (e.g., level 1, level 2, etc.), and just 11.1% included an on-farm assessment or audit associated with BQA Certification. As seen in Table 1, relatively few states offered additional BQA-related components such as dairy BQA Certification (20.8%) or feedyard BQA Certification (35.4%) or both. Youth BQA Certification was available in 43.8% of states, but only 27.1% of the states required youth to be BQA Certified in order to show and sell at the county fair level.

The number of trainers within each state available to conduct BQA Certifications varied (range = 1 to 200 trainers/state). On average, there were 28 trainers per state BQA Program. However, most programs (57.1%) had 1 to 9 trainers, while 22.8% had 10 to 49 trainers. There were an impressive number of programs (20.0%) that had at least 50 trainers. Overall, 709 trainers were available to BQA Certify producers across the U.S.

Based solely on estimates provided by survey respondents, there were an estimated 50,832 producers BQA Certified in the U.S. at the time the survey was completed. Also based on estimates provided by survey respondents that were pooled, an estimated 72,539 cattle producers had been BQA Certified in the previous 10 yr.

A series of 3 open-ended questions were asked of survey respondents, including reasons for their program's success, current challenges, and anticipated future challenges. Responses were subjectively compiled to provide candid feedback about the status of BQA Programs across the U.S.

"In your opinion, what are the reasons for the success of your state's BQA program?"

- *Program structure* – strength of the National BQA Program and sharing of materials among states, with trainings provided locally.
- *Industry support* – desire by cattlemen and organizations to support the program.
- *Role of university extension* – including motivated trainers funded by extension
- *Partnerships* – with industry organizations, cattle associations, and beef councils.
- *Training methods* – several BQA Certification options with many hands-on in nature.
- *Market access* – there are some cases where BQA Certification is required to participate in a special pooled, preconditioned, and/or commingled sale.

"What are 3 current challenges that your state's BQA program faces that hinders its success?"

- *Funding and support* – including the uncertain future of extension funding and lack of a secure, ongoing source of revenue to allow the BQA program to stand on its own.
- *Program structure* – trainers are spread too thin and unable to make BQA a priority and sole focus, and inadequate new and online materials.
- *Participation lacking* – There is inadequate producer buy-in, making it hard to attract enough producers (especially "non-joiners") to meetings to make organizational efforts worthwhile; dairy producers and veterinarians are not participating.
- *Inadequate financial incentives* – there is no financial benefit of BQA when marketing calves, and buy-in from livestock auction markets is lacking.

**Table 1.** Responses to Beef Quality Assurance (BQA) Program-related questions about BQA Certification and education, summarized from survey responses from state BQA Program Coordinators

| | Response (%) | |
|---|---|---|
| Survey question[1] | Yes | No |
| Is dairy BQA Certification offered in your program? | 20.8 | 79.2 |
| Is feedyard BQA Certification offered in your program? | 35.4 | 64.6 |
| Is youth BQA Certification offered in your program? | 43.8 | 56.3 |
| Is youth BQA Certification required to be able to show/sell? | 27.1 | 72.9 |
| Does your state have a youth BQA manual? | 28.9 | 71.1 |
| Does your state have any BQA materials in Spanish? | 18.8 | 81.2 |

[1]Questions only allowed respondents to provide binomial data ("yes" or "no").

"What are the challenges that you foresee, in the future, concerning BQA? List the top three in priority order?"

- *Declining funding* – from all sources including university extension programming, which will result in dwindling of BQA Program infrastructure.
- *Program structure* – continued inconsistency across states, difficulty in making a one-size-fits-all program, and the challenge of maintaining local control.
- *Maintaining interest* – the BQA Program is becoming stagnant, there is a lack of producer interest since it's not clear what's in it for them, and there is no appeal for producers to get re- certified.
- *Lack of financial incentive* – since BQA is not required, there is little financial benefit of BQA Certification when selling cattle and livestock auction markets and packers do not incentivize cattle being sold from BQA certified producers.

## DISCUSSION

In 2006, the Joint Evaluation Advisory Committee (of the Cattlemen's Beef Board and NCBA) requested an external review of the beef checkoff-funded U.S. BQA Program. As a result, 2 white papers were generated after a series of independently conducted interviews with industry leaders (Dunn, 2006; Odde, 2006). A further final review of these papers was conducted and summarized (Genho, 2006). In these documents, the BQA Program was commended for its extensive use of collaboration among diverse groups, a substantial reduction in injection-site lesions from 1991 to 2001, existence of BQA Programs locally in nearly every state, the BQA Certification of approximately 65,000 producers at the time, and the "ability to educate a large number of producers about BQA in a relatively short time" (Genho, 2006). However, the author also indicated the BQA Program could not continue to rely on past accomplishments and redundant training materials, and that more standardization in core materials was needed. Ultimately, outlining a future strategy for BQA was needed.

The National BQA Program has made substantial accomplishments since its origins in the early 1980s, including the creation of a nationwide, grassroots-driven infrastructure of training and certifying producers in BQA on a limited budget (Genho, 2006). However, less-than desirable participation in BQA by cow/calf producers (NAHMS, 2008) and a lack of information about BQA among consumers – which ironically is one of the major aspects of the BQA mission – indicate that the BQA Program would benefit from strategic updates to several of its components.

Groups that oversee state BQA Programs are highly variable, as are funding sources and the presence of BQA Advisory Committees, which collectively contribute to inconsistencies across state programs. Some of these aspects are unlikely to change, but requiring state programs to institute advisory groups to provide direction may improve industry buy-in.

Communication with producers about BQA is widely done, but there appears to be limited use of internet-based media to accomplish this. The use of BQA Certification is widespread, and several options for Certification are offered. However, BQA Certification tests are not challenging – almost all test-takers pass easily. Recertification in BQA is offered is most states, unfortunately the same test is almost always used to evaluate attendees. Based on the fact that the number of hours required for BQA Certification is highly variable across the U.S., this further contributes to a lack of uniformity.

The vast majority of states offer little beyond basic BQA Certification (e.g., additional levels, audits). Further, the availability of additional aspects of BQA (dairy BQA, feedyard BQA) is limited. Several states offer youth BQA Programs, but this includes less than half. The total number of trainers available across the U.S. is encouraging, which includes 709 trainers, and 20% of states have at least 50 trainers.

Open-ended feedback from state BQA Coordinators indicate that reasons for success of state BQA Programs included local trainings, extension involvement, industry support including partnerships with organizations, success of training methods, and some limited opportunities for BQA required special sales. Challenges hindering success of state BQA Programs included limited and declining funding, trainers having other priorities, inadequate producer participation and buy-in, and absent financial incentives to financially benefit BQA participants. Finally, future challenges of BQA included declining funding (including universities), inconsistent programs across states, getting producers interested in BQA including getting recertified, and absent financial incentives for BQA participation.

In summary, these data suggest that the BQA Program includes a large infrastructure of personnel across the U.S.; however, many states offer little beyond basic BQA Certification. Further, the large amount of variation among programs may make development of a uniform nationwide program challenging.

## IMPLICATIONS

Considerable state-to-state variation exists in BQA Programs. Large opportunities exist to offer more BQA-related aspects including the incorporation of an auditing component, as well as expansion of dairy-, feedyard-, and youth-oriented BQA Programs. The large number of BQA trainers available nationwide has contributed to the BQA Certification of over 50,000 producers in the U.S. However, challenges related to funding, competing priorities among trainers, lack of producer participation and buy-in, absent financial incentives, and inconsistent programs across states will continue to hinder the success of BQA.

## ACKNOWLEDGEMENTS

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# FORAGES AND PASTURES

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# EFFECTS OF CO-GRAZING ON HERBIVORY PATTERNS AND PERFORMANCE BY CATTLE AND GOATS GRAZING NATIVE TALLGRASS RANGELAND INFESTED BY SERICEA LESPEDEZA (*LESPEDEZA CUNEATA*)

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**ABSTRACT:** Lactating crossbred cows with calves (n = 145; initial BW = 579 ± 91 kg) and non-pregnant, non-lactating Boer-cross nannies (n = 200; initial BW = 42 ± 1.9 kg) were used to evaluate the effects of co-grazing on herbivory patterns and animal performance while grazing native tallgrass rangeland infested heavily by sericea lespedeza (SL; average SL biomass in October = 2,061 kg/ha). Nine pastures were assigned randomly to 1 of 2 grazing systems: 5 pastures (65 ha) were grazed by cows + calves only (single-species; 0.8 ha/AUM) and 4 pastures (32 ha) were grazed by cows + calves (0.8 ha/AUM) and goats (multispecies; 0.8 ha/AUE/month). Cows + calves and goats were assigned randomly to pastures. Animal BW was measured at 28-d intervals from June 1 to October 1; BCS were assigned to cows also at those times. Two permanent 100-m transects were marked at the onset of the trial (June 15) within each pasture to estimate botanical composition and SL herbivory. Seasonlong cow BW change, seasonlong calf ADG, and cow pregnancy rates were not different ($P \geq 0.08$) between multispecies and single-species pastures. Conversely, seasonlong cow BCS change was greater ($P < 0.01$) on multispecies pastures than on single-species pastures (0.04 vs. -0.38, respectively). Biomass of SL was not different ($P = 0.97$) between pastures at the outset of the study. The percentage of individual SL plants that had been grazed at the end of the trial was greater ($P < 0.01$) on multispecies pastures than on single-species pastures (94.2 vs. 77.5%, respectively). Final SL biomass in multispecies pastures averaged 1,692 kg/ha, whereas final SL biomass in single-species pastures averaged 2,230 kg/ha (SE = 739.4 kg; $P = 0.37$). Residual forage biomass at the end of the trial was not different ($P = 0.54$) between treatments and averaged 3,622 kg/ha, indicating that forage availability did not limit forage intake during our trial. Our results were interpreted to suggest that grazing cows and goats in combination increased grazing pressure on SL without negatively affecting beef cow or beef calf performance or residual forage biomass.

**Key Words**: beef cows, goats, *Lespedeza cuneata*, multispecies grazing

## INTRODUCTION

Sericea lespedeza (*Lespedeza cuneata;* **SL**), a perennial legume, was first introduced into the United States in the 1890s from Japan. Agronomists quickly learned that it was an adaptable plant, tolerant of shallow, acidic or low-fertility soils. Furthermore, SL was relatively resistant to insects and disease. These factors made SL a popular choice for reseeding strip-mined areas, highway right-of-ways, dams, and waterways in the US for nearly a century.

Sericea lespedeza, a highly competitive and prolific seed producer, is capable of producing 481 to 950 kg of seed/ha annually (Vermeire et al., 2007). Vigorous seed production allows SL to rapidly infiltrate native grasslands that are adjacent to reseeding projects. In Kansas alone, this plant infests approximately 600,000 acres of pasture, reducing native grass production by up to 92% (Eddy et al., 2003).

*Sericea lespedeza*, when mature, contains increased amounts of condensed tannins (Eckerle et al., 2010). Condensed tannins reduce protein digestion by ruminants and are a strong deterrent to consumption (Jones and Mangan, 1977; Eckerle et al., 2011a,b,c). Poor intake of SL translates to negligible grazing pressure by beef cattle, which ensures that it will be able to produce seed and continue to proliferate.

Increasing grazing pressure on SL may slow its advance and allow a measure of biological control. Goats voluntarily consume forages and browse high in tannins. Furthermore, they are commonly used for removal of undesirable plants that are avoided by larger domestic herbivores. Therefore, the objective of our study was to evaluate the effects of co-grazing on herbivory patterns and performance by cattle and goats grazing native tallgrass rangeland infested by sericea lespedeza.

## MATERIALS AND METHODS

All procedures were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978).

Nine pastures that were heavily infested with SL were randomly assigned to 1 of 2 grazing systems: 5 pastures (65 ha) were grazed by cows + calves only (single-species; 0.8 ha/AUM) and 4 pastures (32 ha) were grazed by cows + calves (0.8 ha/AUM) and goats (multispecies; 0.8 ha/AUE/month).

Lactating crossbred cows with calves (n = 145; initial BW = 579 ± 91 kg) were blocked by age and calving date and randomly assigned to 1 of 2 grazing systems: single-species

(**SS**) or multispecies (**MS**). Non-pregnant, non-lactating Boer-cross nannies (n = 200; initial BW = 42 ± 1.9 kg) were randomly assigned to 1 of 4 MS pastures. Animal BW was measured at 28-d intervals from June 1 to October 1; body condition scores (**BCS**; 1 = emaciated, 9 = obese; Wagner et al., 1988) were assigned to cows also at those times. Cattle were allowed to graze pastures freely through the duration of the trial. Pregnancy rate was determined via rectal palpation at the end of the season. Goats were allowed to graze in their respective pastures each day from 0700 until 1400 h. During the evening and nighttime hours, they were confined to a pen to prevent predation.

Two permanent 100-m transects were marked at the onset of the trial (June 15) within each pasture to estimate above-ground forage biomass, botanical composition, and SL herbivory. Total forage biomass was estimated by clipping forage biomass at a height of 1 cm from within randomly-placed sampling frames (0.25 $m^2$; n = 10/pasture). Average total biomass of SL ranged from a low of 206 kg/ha to a high of 2,024 kg/ha during the grazing season. Plant-species composition was estimated using a modified step-point technique (Owensby, 1973). Herbivory of individual SL plants was estimated visually at the end of the study (October 15).

***Statistics.*** Cow and calf performance were analyzed as a completely randomized design (PROC MIXED; SAS Inst. Inc., Cary, NC). The model included terms for treatment, period, and treatment × period. Treatment × period effects were not detected; therefore, main effects of treatment were reported as Least Squares means.

Reproductive responses were analyzed via logistic regression (PROC CATMOD; SAS). Sericea lespedeza herbivory as analyzed via the Chi-square procedure. Means were considered to be different when $P \leq 0.05$. Tendencies were discussed when $P > 0.05$ and $< 0.10$).

## RESULTS AND DISCUSSION

***Cow Performance.*** Cow BW change from d 0 to d 56 was greater ($P = 0.01$) for cows in SS pastures than in MS pastures; SS cows gained 19.5 ± 11.55 kg more BW than MS cows (Table 1). Cow BW change from d 56 to d 112 was not different ($P = 0.32$) between treatments; however, SS cows tended ($P = 0.08$) to have greater BW change from d 0 to d 112 than MS cows. In contrast, Webb et al. (2008) reported no difference in ADG between steers grazing in single-species pastures compared with steers grazing with goats. Greater BW change was not related to a change in pregnancy percentage. Pregnancy rate was not different ($P = 0.40$) between treatments (Table 1).

The pattern in cow BCS change was opposite that of BW change (Table 1). Cow BCS change from d 0 to 56 was greater ($P = 0.01$) for MS pastures than for SS pastures (0.42 vs. -0.05, respectively), whereas, cow BCS change from day 56 to day 112 was not different ($P = 0.52$) between SS and

**Table 1.** Effects of co-grazing on performance of beef cows, calves, and goats grazing native tallgrass pastures heavily infested with sericea lespedeza during a summer grazing season (June 15 to October 15)

| Item | Multispecies Pastures | Single-species Pastures | SE | *P* |
|---|---|---|---|---|
| Cow BW change (d 0 to 56), kg | 19.0 | 38.5 | 5.30 | 0.01 |
| Cow BW change (d 56 to 112), kg | 14.9 | 8.6 | 4.70 | 0.32 |
| Cow BW change (d 0 to 112), kg | 33.6 | 47.6 | 6.90 | 0.08 |
| Cow BCS change (d 0 to 56) | 0.42 | -0.05 | 0.097 | 0.01 |
| Cow BCS change (d 56 to 112) | -0.38 | -0.32 | 0.085 | 0.52 |
| Cow BCS change (d 0 to 112) | 0.04 | -0.38 | 0.092 | < 0.01 |
| Cow pregnancy rate, % | 77.5 | 83.8 | 6.60 | 0.40 |
| Calf ADG (d 0 to 56), kg | 1.18 | 1.26 | 0.044 | 0.14 |
| Calf ADG (d 56 to 112), kg | 0.53 | 0.44 | 0.031 | 0.01 |
| Calf ADG (d 0 to 112), kg | 0.89 | 0.90 | 0.027 | 0.86 |
| Goat ADG (d 0 to 112), kg | 0.17 | - | - | - |

**Table 2.** Effects of co-grazing on herbivory patterns by beef cows, beef calves, and goats grazing native tallgrass pastures heavily infested with sericea lespedeza during a summer grazing season (June 15 to October 15)

| Item | Multispecies Pastures | Single-species Pastures | SE | *P* |
|---|---|---|---|---|
| Total SL plants grazed, % | 94.2 | 77.5 | - | 0.01 |
| Initial SL biomass, kg/ha | 253 | 278 | 739.4 | 0.97 |
| Final SL biomass, kg/ha | 1,692 | 2,230 | 739.5 | 0.37 |
| Initial total forage biomass, kg/ha | 1,451 | 2,306 | 794.9 | 0.43 |
| Final total forage biomass, kg/ha | 3,253 | 3,918 | 794.9 | 0.54 |

MS. In contrast, cow BCS change from d 0 to 112 was greater ($P < 0.01$) for MS pastures than on SS pastures (0.04 vs. -0.38, respectively). We speculated that the conflicting trends in cow BW and cow BCS were driven by differences in gut fill. Cows grazing SS pastures may have had poorer quality diets (particularly from a protein perspective) than cows grazing MS pastures. This condition may have caused greater gut fill and greater BW change among cows on SS pastures that did not translate to greater BCS. Calf performance provided evidence to support this speculation (Table 1). Calf ADG was similar ($P \geq 0.14$) between treatments from d 0 to d 56 and from d 0 to 112; however, calf ADG in MS pastures was greater ($P = 0.01$) in SS pastures from d 56 to d 112, at a time when forages were most mature and, typically, of low relative quality quality.

***Herbivory.*** Biomass of SL was not different ($P = 0.97$) between SS and MS pastures at the outset of the study (Table 2). The percentage of individual SL plants that had been grazed at the end of the trial was greater ($P < 0.01$) on MS pastures than on SS pastures (94.2 vs. 77.5%, respectively). Other researchers reported similar findings (Webb et al., 2008; Abaye et al., 2009). Abaye et al. (2009) suggested that greater grazing pressure on SL in MS pastures caused SL plants to remain in a vegetative state (i.e., with lesser concentrations of condensed tannins; Eckerle et al., 2010) than SL in SS pastures. Furthermore, they reported that cattle maintained on MS pastures continued to graze SL later into the season than cattle maintained on SS pastures.

Final SL biomass in MS pastures averaged 1,692 kg/ha, whereas final SL biomass in SS pastures averaged 2,230 kg/ha (SE = 739.4 kg; $P = 0.37$). Residual forage biomass at the end of the trial was not different ($P = 0.54$) between treatments and averaged 3,622 kg/ha. Based on this figure, we concluded that forage availability did not limit animal intake during our study. Webb et al. (2008) reported that season-ending forage biomass was less for MS pastures than for SS pastures; however, stocking rates in that study were typical of improved pastures and were 12 to 250% greater than those employed in our study.

## IMPLICATIONS

Our results were interpreted to suggest that grazing cows and goats in combination may increase grazing pressure on SL without negatively affecting beef cow or beef calf performance or residual forage biomass. Sericea lespedeza was grazed more frequently in pastures with a full stocking-rate complement of cattle and goats (i.e, 0.8 AUM or AUE/ha for both species) than in pastures stocked with cattle alone (i.e., 0.8 AUM for cattle only).

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# INFLUENCE OF PRE-COLLECTION DIET AND SQUEEZING ON CRUDE PROTEIN CONTENT OF MASTICATE COLLECTED FROM FISTULATED CATTLE

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**ABSTRACT:** In study 1, 12 esophageally fistulated cattle were maintained on two pre-collection diets: HI (24% CP, n = 6) or LO (7.7% CP, n = 6) for 8 d. On d 9, the esophageal plug was removed, screen bottom bags were attached and each cow was presented with 428 g (DM) vegetative grass (VEG; 24% CP, 40% NDF) which had been harvested from subirrigated meadow immediately before presentation. Following VEG collection, each cow was presented with 1032 g (DM) hay (HAY; 7.7% CP, 66% NDF) harvested from subirrigated meadow the previous summer. Blood samples were collected via coccygeal venipuncture and analyzed for BUN content. In study 2, three esophageally fistulated cows sampled Sandhills upland range 12 times from May 21 to August 18. In study 3, five ruminally fistulated steers were fed vegetative smooth bromegrass harvested immediately before presentation. In all three studies, masticate samples were divided and each was either squeezed by hand until no more saliva could be removed (SQZ), or un-squeezed (UNSQZ). All masticate samples were immediately frozen and stored until lyophilized and analyzed for CP content. In study 1, pre-collection diet did not affect ($P$ = 0.49) CP content of masticate. Type of forage offered (VEG vs. HAY) interacted ($P$ = 0.01) with preparation technique, where CP was lost when VEG samples were squeezed (20.0 vs. 21.5% CP for SQZ vs. UNSQZ, respectively; $P < 0.05$) but not when HAY samples were squeezed (7.6 vs. 7.6% CP for SQZ vs. UNSQZ, respectively; $P > 0.05$). BUN levels tended to be higher for HI cows (27.6 ± 4.0 vs. 23.5 ± 3.2 ml/dl; HI vs. LO, respectively; $P$ = 0.08). In this case, total amount of salivary contamination may not have been enough to influence CP content of the masticate samples. In study 2, there was no difference in CP between SQZ and UNSQZ (9.5 and 9.6%; $P$ = 0.66) samples. In study 3, the difference in CP between SQZ and UNSQZ (18.6 and 20.1%) samples was not statistically separable ($P$ = 0.16). Previous diet did not impact CP level of masticate and squeezing impacted CP levels of high quality forage but had little effect on lower quality forage.

**Key words:** diet collection, grazed diets, sample preparation

## INTRODUCTION

Fistulated animals have been used extensively to quantify nutrient intake of grazing animals. This method accounts for the grazing animal's selectivity which is not accounted for in clipped samples. Several factors inherent to using fistulated cattle may affect the degree to which forage masticate samples actually represent grazed animal diets. Salivary contamination and sample preparation technique could influence both the organic and inorganic components of grazed grass samples (Hoehne et al., 1967). Salivary N concentration depends on N content of the pre-collection diet of fistulated cattle and may therefore impact N values of masticate samples. The sample preparation technique of squeezing the sample to remove excess saliva could result in a loss of cell solubles and influence the measurement of forage quality. Therefore, the objectives of these studies were to determine the influence of pre-collection diet and the effect of squeezing on crude protein values of masticate collected from fistulated cattle.

## MATERIALS AND METHODS

In study 1, 12 esophageally fistulated cattle were maintained on two pre-collection diets: 1) grazed vegetative subirrigated meadow (**HI**, 24% CP, n = 6) or 2) meadow hay fed in a dry lot (**LO**, 7.7% CP, n = 6) for 8 d. On d 9, cattle were held off feed for 12 h, then the esophageal plug was removed, screen bottom bags were attached and each cow was presented with 428 g (DM) vegetative grass (**VEG**; 24% CP, 40% NDF) which had been harvested from subirrigated meadow immediately before presentation. Masticate samples were divided and each was either squeezed by hand until no more saliva could be removed (**SQZ**), or unsqueezed (**UNSQZ**). Following vegetative grass masticate collection, each cow was presented with 1032 g (DM) hay (**HAY**; 7.7% CP, 66% NDF) harvested from subirrigated meadow the previous summer. Hay masticate samples were divided and hand squeezed or not in the same manner as vegetative grass masticate samples. Blood samples were collected via coccygeal venipuncture and analyzed for urea nitrogen content (Broderick and Kang, 1980). All masticate samples were frozen, lyophilized, and analyzed for nitrogen content using a Leco, FP 2000 combustion nitrogen analyzer (Leco Corp, St. Joseph, MO) which was converted to CP by multiplying by 6.25. Data were analyzed as a 2 x 2 x 2 factorial arrangement of treatments in a completely randomized design.

In study 2, three esophageally fistulated cows sampled Sandhills upland range 12 times from May 21 to August 18. Masticate samples were divided and each was either squeezed

Table 1. Crude protein, NDF and Ash values of squeezed and unsqueezed hay and vegetative grass masticate samples collected from esophageally fistulated cows fed diets high or low levels in CP pre-collection.

| | High | | | | Low | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hay | | Veg | | Hay | | Veg | | | P values | | | |
| | SQZ | UNSQZ | SQZ | UNSQZ | SQZ | UNSQZ | SQZ | UNSQZ | SE | Previous | Forage | Process | F x P |
| CP | 7.5$^{d}$ | 7.5$^{d}$ | 20.2$^{bc}$ | 21.9$^{a}$ | 7.6$^{d}$ | 7.6$^{d}$ | 19.7$^{c}$ | 21.0$^{ab}$ | 0.5 | 0.49 | < 0.001 | 0.01 | 0.01 |
| NDF | 69.2$^{ab}$ | 66.9$^{b}$ | 53.5$^{c}$ | 45.4$^{d}$ | 72.8$^{a}$ | 67.7$^{b}$ | 50.8$^{c}$ | 42.7$^{d}$ | 2.4 | 0.89 | < 0.001 | < 0.001 | 0.001 |
| Ash | 10.8$^{c}$ | 11.9$^{bc}$ | 18.3$^{a}$ | 17.2$^{a}$ | 12.1$^{bc}$ | 14.2$^{b}$ | 17.2$^{a}$ | 17.5$^{a}$ | 0.7 | 0.39 | < 0.001 | 0.27 | 0.07 |

$^{abc}$Means lacking a common superscript letter differ ($P < 0.05$)

by hand until no more saliva could be removed (SQZ), or un-squeezed (UNSQZ). All masticate samples were frozen, lyophilized, and analyzed for CP content.

In study 3, five ruminally fistulated steers were fed mid-May vegetative smooth bromegrass harvested immediately before presentation. Masticate samples were divided and each was either squeezed by hand until no more saliva could be removed (SQZ), or un-squeezed (UNSQZ). All masticate samples were immediately frozen and stored until lyophilized and analyzed for CP content.

## RESULTS

In experiment 1, pre-collection diet did not affect ($P$ = 0.49, Table 2) CP content of masticate samples. Serum urea nitrogen levels tended to be higher for HI cows (27.6 ± 4.0 vs. 23.5 ± 3.2 ml/dl; HI vs. LO, respectively; $P$ = 0.08). Although a small amount of N is contained in the saliva, earlier tests (Bath et al., 1956) indicate this has very little, if any effect on the protein content of the ingested feed. Weir and Torrell (1959) reported that the added N attributed to salivary contamination of masticate samples collected from esophageally fistulated sheep would only raise the protein content by less than 0.01%. Regardless of whether there was a higher amount of nitrogen present in the saliva of HI cows, the total amount of salivary contamination was too small to influence the total nitrogen content of the sample in this instance.

Type of forage offered (VEG vs. HAY) interacted ($P$ = 0.01) with preparation technique for CP, where CP was lost when vegetative grass masticate samples were squeezed (20.0 vs. 21.5% CP for SQZ vs. UNSQZ, respectively; $P <$ 0.05) but there was no difference between squeezed and un-squeezed hay masticate samples (7.6 vs. 7.6% CP for SQZ vs. UNSQZ, respectively; $P > 0.05$). The pre-ingestion CP value for vegetative grass was 24% and 7.7% for the hay. Type of forage offered (VEG vs. HAY) also interacted ($P$ = 0.001) with preparation technique for NDF. Squeezing masticate samples increased the NDF content of both forage types but to a greater extent for vegetative grass than for hay (52.2 vs. 44.1% NDF for VEG and 71.0 vs. 67.3 for HAY; $P < 0.05$). The pre-ingestion NDF value for vegetative grass was 40% and 66% for the hay. Cell solubles from fresh vegetative grass may go into solution more rapidly than those of the dry hay, possibly accounting for some of the difference observed. Squeezing did not affect Ash content ($P$ = 0.27) of either forage type.

In experiment 2, there was no difference in CP between SQZ and UNSQZ (9.5 and 9.6%; $P$ = 0.66, Table 1) samples. In experiment 3, there was a numerical difference in CP between SQZ and UNSQZ (18.6 and 20.1%; $P$ = 0.16) samples. Previous research investigating the effects of squeezing masticate samples to prepare them for laboratory analysis demonstrated similar CP content between squeezed and un-squeezed samples (Hoehne et al., 1967). However, the forages used in that research were collected on or after July 28, meaning the forage was not vegetative, and was similar in CP content to the hay used in experiments 1 and 2 of the present study. The results of the present study suggest squeezing masticate samples had a larger effect on high quality vegetative grass than lower quality grass or hay. A lower CP level would mean a lower level of soluble CP present to be impacted by preparation technique. This is the

**Table 2.** Crude protein values of squeezed (SQZ) and unsqueezed (UNSQZ) masticate samples collected from esophageally fistulated cows grazing Sandhills upland range from May to August

| | SQZ | UNSQZ | SE | *P*-value |
|---|---|---|---|---|
| CP, % | | | | |
| May 20-21 | 10.6 | 10.6 | 0.6 | 0.96 |
| June 1-2 | 9.9 | 10.3 | 0.6 | 0.50 |
| June 13-14 | 9.9 | 10.3 | 0.6 | 0.50 |
| July 21-22 | 8.1 | 8.4 | 0.6 | 0.64 |
| Aug 18-19 | 8.8 | 8.7 | 0.6 | 0.86 |

first research to test the effects of squeezing high quality, vegetative masticate samples and further work is warranted in this area.

Previous diet did not impact N level of samples collected from esophageally fistulated animals in this study. Squeezing the samples impacts CP levels of high quality forage but has little effect on lower quality forage.

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# MEATS AND MUSCLE

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# FATTY ACID COMPOSITION OF CATTLE FATTENED WITH TROPICAL FORAGE IN RAINY AND DROUGHT SEASON

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**ABSTRACT:** The objective of this study was to quantify intramuscular fat (IMF) and fatty acid composition of meat from cattle fattened on tropical forages (*L. leucocephala*, *P. maximum* and *C. plectostachyus*) under the Intensive Silvopastoril System (SSPi) in two seasons (rainy n = 12 and drought n = 10). Intramuscular fat of *L. dorsi* (Ld) and *Semimembranosus* (Sm) muscles was extracted and fatty acids (Myristic, Myristoleic, Palmitic, Palmitoleic, Etearic, Oleic, Linoleic, Eicosanoic, Arachidonic (ARA) and Docosahexaenoic (DHA) acids were quantified. The effect of season was analyzed with a linear model. The IMF was greater ($P < 0.05$) in rainy (3.53 ± 0.22%) *vs* drought (2.47 ± 0.21%) season and Myristic, Myristoleic, Palmitic, Palmitoleic and Etearic acids were not different ($P > 0.05$) between seasons. Oleic acid had a greater concentration ($P < 0.05$) in rainy season (40.69 ± 3.15%) vs. drought (27.52 ± 3.46%), while Linoleic, Linolenic and Eicosanoic acid concentrations were lower ($P < 0.05$) in rainy season. For the essential fatty acids, ARA concentration was less ($P < 0.05$) in rainy (0.08 ± 0.10%) vs. drought (0.48 ± 0.11%) season and DHA had a similar concentration ($P > 0.05$) at two seasons. It is concluded that beef fattened with tropical forages yield leaner meat in both seasons and its fatty acid composition is maintained during the year. The SSPi can be another alternative towards more sustainable meat production and a promising source of dietary fat for human nutrition.

**Key words:** bovine, meat, intramuscular fat, fatty acids, Silvopastoral

## INTRODUCTION

Overweight and obesity are diseases that are caused by abnormal accumulation of body fat (Soriano, 2006; Valenzuela and Sanhueza, 2009). In Mexico these diseases have increased dramatically in the population (Olaiz et al., 2006). Subsequently, the consumer changes their habits and lifestyles and search healthy and nutritious food (Hodges, 2003). In Mexico some cattlemen return to use Silvo Pastoral Intensive System (**SSPi)** to feed and produce beef in natural conditions without supplementation, veterinary products and agrochemicals. The objective of this study was to quantify intramuscular fat (**IMF**) and fatty acid composition of meat from cattle fattened on tropical forages (*L. leucocephala*, *P. maximum* and *C. plectostachyus*) in two season of the year drought and rainy.

## MATERIALS AND METHODS

***Animal and Feed Characteristics.*** Twenty-two zebu crossbred bullocks were fed under an SSPi based on tropical pastures composed by (*L. leucocephala*, *P. maximum* and *C. plectostachyus*) at the experimental location from Michoacán, México whose geographical coordinates are 19° 04' 22" N and 102º 26' 14" W and 255 msnm (Flores et al., 2009). When the animals target the slaughter weight 427 ± 10.3 kg in drought and 443 ± 9.17 kg in rainy season, they were harvested in a commercial abattoir.

***Meat Samples and Analyses.*** They were removed at 72 h postmortem from the Ld and Sm muscles from each carcass, samples were stored at -20°C until IMF were extracted in duplicate using chloroform-methanol-tridistilled wather (2:1:1) as described by Bligh and Dyer, (1959); AOAC, (1997). To identify and quantify the fatty acids was used a gas chromatograph (Clauruss 400, Perkin Helmer Instruments Inc.) equipped with a Supelco 2380 x 30m capillary column and the standard Supelco 37 FAME mix (Huerta-Leidenz et al., 1993). Identification and quantification were made according with Castillo et al., (2011).

***Statistical.*** The data were conducted using the General Linear procedure (SAS Inst. Inc., Cary, NC) considering the effect of the season of the year in IMF and fatty acids composition.

## RESULTS AND DISCUSSION

The IMF and fatty acid composition in bullocks fattened under SSPi are presented in Table 1. The IMF was less ($P < 0.05$) in drought (2.47 ± 0.21 %) vs. rainy season (3.53 ± 0.22%), according with the USDA standards (Texas Beef Council, 2009) the amount of IMF found in the bullocks match with in the lean beef category. This study was comparable with the IMF from native Thailandese cattle 3.35% (Jaturasitha et al., 2009) with the Braford cattle fattened in Argentinean grasslands 2.73% (Orellana et al., 2009). And also with IMF quantified in bulls 1.71% and steers 3.38% fattened in pastoral system from Brazil (Padre et al., 2006). Well then, results of these study were compared with beef evaluated in Mexico from three livestock regions, were the IMF was 3.0% for the Northern, 2.7% Central and 3.6% South (Rubio et al., 2005), and they were almost similar to the cattle of this study. The fatty acid profile quantified are shown in Table 1, no difference was found ($P > 0.05$)

**Table 1.** Least squares means ± SE of intramuscular fat (IMF) and composition (% of fatty acids) of cattle fattened with tropical forages SSPi at two season

| Variables | Rainy | Drought |
|---|---|---|
| IMF | $3.53 \pm 0.22^{a}$ | $2.47 \pm 0.21^{b}$ |
| Oleic | $40.69 \pm 3.15^{a}$ | $27.52 \pm 3.46^{b}$ |
| Linoleic | $0.11 \pm 0.88^{b}$ | $3.11 \pm 0.97^{a}$ |
| Linolenic | $1.67 \pm 0.51^{b}$ | $6.10 \pm 0.56^{a}$ |
| Eicosaenoic | $0.11 \pm 0.18^{b}$ | $0.93 \pm 0.20^{a}$ |
| Arachidonic acid | $0.08 \pm 0.10^{b}$ | $0.49 \pm 0.11^{a}$ |

[a,b] Different letterss between columns within a row denote significant difference between season ($P < 0.05$).

for Myristic (C14:0), Palmitic (C16:0), Stearic (C18:0), Myristoleic (C14:1) and Palmitoleic (C16:1) in beef for each season. Similar concentrations of these acids are reported in different studies (Jaturasitha et al., 2009; Padre et al., 2006; Orellana et al., 2009) and those values are common between ruminants, probably because they are not affected by nutritional management. An important difference ($P < 0.05$) were detected in Oleic acid (C18:1n-9) between seasons, the concentration was lower in drought (27.52 ± 3.46%) vs. rainy (40.69 ± 3.15%) this results could be due a forage consumption during each season because the disponibility of *Leucaena* was less in rainy. However, when the value found in the rainy season was compared with native Thailandese cattle 39.36% (Jaturasitha et al., 2009) these value were similar. Whereas in the study of Padre et al. (2006) Oleic acid concentration in bulls 28.53% and steers 33.73%, those values were similar to Oleic acid found in meat from bullocks harvested in the drought season. As well as, the 30.53 mg/g of Oleic acid in meat from Braford cattle reported by Orellana et al. (2009). The amount of Oleic acid observed in this study is an advantages for SSPi cattle, because, given information from Padre et al., (2006) mentioned that high consumption of Oleic acid, has a positive impact on human health, this is because it helps to minimize the concentrations of low density cholesterol (**LDL**) and increased high density cholesterol (**HDL**). According with, polyunsaturated fatty acids such as Linoleic (C18:2 n-6) were different ($P < 0.05$) between seasons, drought (3.11 ± 0.97%) vs rainy (0.10 ± 0.88%) those values, were greater than bulls 1.68% and steers 1.27% fed with *P. maximum* in Brazil (Padre et al., 2006). As well as, similar to Linoleic acid 2.70% reported by Jaturasitha et al. (2009) in native Thailandese cattle, when those values were compared against bullocks evaluated at drought season of the present study. Linolenic acid (C18:3 n-3) had a greater ($P < 0.05$) percentage in drought (6.10 ± 0.56%) vs. rainy (1.67 ± 0.51%), which differs from bulls (0.85%) and steers (0.53%) reported by Padre et al., (2006) and native Thailandese cattle which value was 0.18% (Jaturasitha et al., 2009). Eicosanoic acid (C20:5n-3) showed difference ($P < 0.05$) in drought (0.93 ± 0.20%) vs. rainy (0.11 ± 0.18%), in bulls (0.33%) and steers (0.25%; Padre et al., 2006) compared with drought, but the concentration in rainy was greater. The Thailandese bulls had decreased concentrations (0.35%) compared with this study. For ARA (C20:4 n-6), it were high in percentage ($P < 0.05$) in drought (0.49 ± 0.11%) vs rainy (0.08 ± 0.10%). But less than bulls (0.53%) and steers (0.38%) studied by Padre et al., (2006). And equal than 0.44% for the drought season evaluated by Jaturasitha et al. (2009). DHA acid (C22:6 n-3) there was no difference ($P > 0.05$) their concentration was 0.45% in drought vs. 0.29% rainy, these values differ with Padre et al., (2006) who had decreased percentages in bulls (0.02%) and steers (0.03%).

## CONCLUSIONS

The cattle fattened under SSPi produce lean beef during rainy and drought seasons, is an important source of Oleic, Linoleic, and Linolenic acids. This system could be another alternative for sustaintable beef production in tropical areas in Mexico and also is a promising source of dietary fat for human nutrition.

## IMPLICATIONS

The quantification of monounsaturated and polyunsaturated fatty acids requires further investigation in the fatty acid composition of SSPi forage, because there are effects of season of year in the fat composition.

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# PHYSIOLOGY AND ENDOCRINOLOGY

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# ASSESSMENT OF SERUM IGF-I AND β-HYDROXYBUTYRATE CONCENTRATIONS ON REPRODUCTIVE PERFORMANCE PRIOR TO CALVING AND BREEDING IN YOUNG BEEF COWS GRAZING NATIVE RANGE

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**ABSTRACT:** Metabolites involved in the metabolic adaptation to negative energy balance may have the potential to regulate timing of reproductive success. Therefore, the objective of this 4-yr study was to determine the association of serum metabolites, cow BW, BCS, and calf performance on conception date in 2- and 3-yr-old beef cows (n = 381) grazing native range at the Fort Keogh Livestock and Range Research Laboratory. Cows were classified by conception date in a 55 ± 2 d breeding season as early conception (EC; conceived in first 15 d of breeding) or late conception (LC; conceived during the remaining breeding season). Date of conception was calculated from the following year calving date. Blood samples were collected 30 ± 1 d prior to calving and 14 ± 1 d prior to breeding for analysis of serum IGF-I and β-hydroxybutyrate (BHB) concentrations. Conception date for EC cows were 33 d earlier ($P < 0.01$) than LC cows. Sampling time did not interact ($P > 0.10$) with conception date classification group for either serum metabolite. Cow age × conception date interaction ($P = 0.04$) occurred for serum BHB concentrations. Serum BHB concentrations were similar ($P > 0.10$) for 2-yr-old cows relative to their conception date classification. However, serum BHB concentrations were greater ($P = 0.05$) for LC rather than EC in 3-yr-old cows. Serum IGF-I concentrations were greater ($P < 0.01$) for EC cows relative to LC cows. Body condition score and cow BW were not different ($P \geq 0.43$) at calving and breeding between EC and LC cows. Calf birth BW was not different ($P = 0.25$) for EC and LC cows. However, calf weaning (205-d) BW was greater ($P < 0.01$) for LC cows relative to EC cows, indicating a difference in milk production. This study indicates that serum BHB and IGF-I concentrations may be practical indicators of conception date prior to breeding in young beef cows grazing native range.

**Key words:** beef cows, conception date, serum metabolites

## INTRODUCTION

Metabolic adaptation to negative energy balance has the potential to influence timing of reproductive success in beef cows. In addition, there has been increasing evidence that changes in serum metabolites and hormones during periods of negative energy balance are associated with either reduced (Beam and Butler, 1999; Ospina et al. 2009) or enhanced fertility (Roberts et al., 2008). In dairy cows, an increase in serum β-hydroxybutyrate (**BHB**) concentration pre-breeding was associated with decreased pregnancy rates from first service AI (Walsh et al., 2007) and increased interval to first ovulation (Reist et al., 2000). On the other hand, IGF-I has been suggested to be a better indicator of rebreeding performance of first calf heifers than BCS or BW change (Roberts, 2008). Furthermore, circulating IGF-I concentration is associated with nutrient intake (McGuire et al., 1992) and is an indicator of nutrient status in dairy (Spicer et al., 1990) and beef (Roberts et al., 1997) cattle. The hypothesis of our research was that young beef cows grazing native dormant range that conceive earlier in the breeding season would have improved adaptive mechanisms to negative energy balance (from the metabolic load of lactation) shown by decreased circulating β-hydroxybutyrate concentrations. Therefore, the objective of this 4-yr study was to determine the association of serum metabolites, cow BW, BCS, and calf performance on conception date in 2- and 3-yr-old beef cows grazing native range at the Fort Keogh Livestock and Range Research Laboratory.

## MATERIALS AND METHODS

Procedures were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee. The experiment was conducted at the USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, located approximately 1.6 km west of Miles City, MT (46°22´N 105°5´W), at an average elevation of 730 m. Native vegetation on the 22,500-ha research laboratory are primarily cool-season grasses consisting of needlegrass-wheatgrass (*Stipa-Agropyron*) mix. The long-term average precipitation is 343 mm with about 60-70% occurring during the mid-April through mid-June growing season. The average annual forage standing crop at the study site is 870 ± 14 kg/ha (Grings et al., 2005).

Cows used in this study were 2- and 3-yr-old (n = 381) and were from a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise). Management before calving and after calving was similar in all years and among all cows. Date of conception for the study year was estimated from the subsequent years calving date (minus 285 d for gestation). Cows were retrospectively classified as early

conception (**EC**) or late conception (**LC**). A 55 ± 2 breeding season was utilized in all years and was initiated in mid-June. Cows conceiving within the first 15 d of the breeding season were considered as EC cows and cows conceiving in the remainder of the breeding season as LC cows.

Blood samples were collected via coccygeal venipuncture (Corvac, Sherwood Medical, St. Louis, MO) at 2 time periods in relation to calving or breeding; 30 ± 1 d before the start of calving and 14 ± 1 d before the start of breeding. Blood samples were collected, cooled, and subsequently centrifuged at 1,200 × *g* at 4°C for 20 min after collection. Serum was harvested and stored at -20ºC for later analysis. Serum samples were analyzed for IGF-1 and BHB. Serum IGF-I samples were quantified by double antibody RIA (Roberts et al. 2008). Inter-assay and intra-assay CV for IGF-I were 12 and 15%, respectively. As a measure of nutrient status and glucose sufficiency, BHB concentrations were measured utilizing a commercially available diabetic monitor system (MediSense; Precision Xtra; Abbott Laboratories, Abbott Park, IL, validated by Byrne et al., 2000; Endecott et al., 2004; Voyvoda and Erdogan, 2010).

Cows were weighed and BCS (1 = emaciated, 9 = obese; Whitman, 1975) were assigned to each cow by visual observation and palpation at 2 time periods in relation to calving or breeding; 30 ± 1 d before the start of calving and 14 ± 1 d before the start of breeding. Calves were weighed at birth and weaning in each year. Weaning weights were adjusted for 205-d weaning weight with no adjustments for sex of calf or age of dam.

***Statistical Analysis.*** Normality of data distribution and equality of variances of measurements were evaluated using PROC UNIVARIATE, the Levene test, and PROC GPLOT, respectively. Data were analyzed as a completely randomized design with cow as the experimental unit using the Kenward-Roger degrees of freedom method. The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to test all main effects and all possible interactions. The model included fixed effects of conception date group, cow age, year, sex of calf, and their interactions. All interactions remained in the model regardless of significance. Separation of least squares means was performed by the PDIFF option of SAS when a significant ($P \leq 0.05$) effect of treatment was detected.

## RESULTS AND DISCUSSION

Average conception date for EC cows was 33 d earlier ($P < 0.01$; Table 1) than LC cows. This difference is expected due to classification of conception date groups. Body condition score and cow BW were not different ($P \geq 0.43$) at calving and breeding between EC and LC cows. Calf BW at birth was not different ($P = 0.25$) for EC and LC cows. However, calf BW at weaning (205-d) was greater ($P < 0.01$) for LC cows relative to EC cows, suggesting differences in milk production.

A cow age × conception date × sample time interaction ($P < 0.01$; Table 2) occurred for serum BHB concentrations. Serum BHB concentrations were similar ($P > 0.10$) for 2-yr-old cows regardless of conception date classification and sampling time. However, pre-calving serum BHB concentrations were greater ($P = 0.05$) for LC rather than EC in 3-yr-old cows; whereas, pre-breeding serum BHB concentrations were not different ($P > 0.10$) due to calving period for 3-yr-old cows. Circulating concentrations of BHB

**Table 1.** Cow BW, BCS, calf BW, and circulating serum IGF-I concentrations for 2- and 3-yr-old cows classified as early or late conception

| | Conception date[1,2] | | | |
|---|---|---|---|---|
| Measurement | EC | LC | SEM | *P*-value |
| Conception date, Julian d | 169 | 202 | 1 | < 0.01 |
| Cow BW, kg | | | | |
| Pre-calving | 451 | 448 | 7 | 0.80 |
| Pre-breeding | 426 | 426 | 7 | 0.97 |
| Cow BCS | | | | |
| Pre-calving | 5.0 | 5.0 | 0.1 | 0.83 |
| Pre-breeding | 4.5 | 4.4 | 0.1 | 0.43 |
| Calf BW, kg | | | | |
| Birth | 33 | 34 | 1 | 0.25 |
| 205-d adj. weaning | 198 | 224 | 3 | < 0.01 |
| IGF-I, ng/mL | 144 | 130 | 5 | < 0.01 |

[1]Conception date was estimated from the subsequent years calving date: EC = conceived with the first 15 d of breeding; LC = conceived during the remaining of the breeding season.

**Table 2.** Concentrations of β-hydroxybutyrate (BHB) in pre-calving and pre-breeding serum samples from 2- and 3-yr-old cows classified as early or late conception[1]

| | | Sample time | | | | |
|---|---|---|---|---|---|---|
| | Cow | Pre-calving | | Pre-breeding | | |
| Measurement | Age | EC | LC | EC | LC | SEM |
| BHB, μmol/L | 2 | 567 | 518 | 382 | 379 | 5 |
| | 3 | 595 | 710 | 387 | 363 | 6 |

[1]Conception date was estimated from the subsequent years calving date: EC = conceived with the first 15 d of breeding; LC = conceived during the remaining of the breeding season.

will increase when the rate of acetate oxidation is inhibited. A condition that can contribute to reduced oxidation of acetate is an inadequate supply of cellular oxaloacetate derived from serum glucose (Kaneko, 1989). Therefore, increased circulating BHB concentrations are indicative of metabolic dysfunction occurring from poor adaptation to increased energy demands and negative energy balance (Herdt, 2000).

Insulin-like growth factor-I has been suggested to be a better indicator of rebreeding performance of first calf heifers than BCS or BW change (Roberts, 2008). In this study, serum IGF-I concentrations were greater ($P < 0.01$; Table 1) for EC cows relative to LC cows. In agreement, Pushpakumara et al. (2003) reported a tendency for decreased IGF-I concentrations prior to breeding in late pregnancy cows relative to early pregnancy cows. Furthermore, Roberts et al. (1997) reported a relationship between IGF-I concentrations and the time of resumption of ovarian cyclicity in postpartum beef cows.

In this study, cow BW and BCS prior to calving and breeding were not different between animals that conceived early or later in the ensuing breeding season. Body weight and BCS change are considered functional indicators of energy status and reproductive performance after calving (Randel, 1990). Prepartum body energy reserves can be influential in determining days to resumption of estrus and subsequent pregnancy rates in young cows (Short et al., 1990; Spitzer et al., 1995). Results of the present study are in agreement with previous work where no association was observed between BCS at calving and reproductive performance in young range cows when evaluated within a nutritional environment rather than across nutritional treatments (Mulliniks et al., 2012). In the current study, neither BCS nor BW alone were indicative of subsequent reproductive performance. However, measuring precalving BHB concentrations with a hand-held ketone meter were indicative of timing of conception date in 3 year old cows. Therefore, further research may be needed to identify a relative threshold concentration of BHB for young cows without additional management (e.g., feed, estrus induction) input.

This study indicates that serum BHB and IGF-I concentrations may be practical indicators of conception date prior to calving in young beef cows grazing cool-season native range. Rapid chute-side measurements of BHB may be a potential tool to provide producers an opportunity to manage potentially late conception cows differently to improve overall reproductive efficiency and tighten their subsequent calving dates.

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# CARRYOVER EFFECTS ON PROGESTERONE CONCENTRATIONS AND FETAL NUMBERS IN EWES GIVEN HUMAN CHORIONIC GONADOTROPIN

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**ABSTRACT:** Administration of hCG will increase serum progesterone (P4) and potentially increase number of lambs born. The objective of this study was to determine if a carryover effect of previous hCG administration would alter serum P4 concentrations and increase fetal number in ewes in subsequent years. A single dose of hCG was administered on d 4 post mating with the purpose to increase serum P4 concentrations in ewes and increase number of lambs born. Mixed aged Suffolk ewes (n = 40) received an intravaginal P4-containing insert (CIDR; 0.3 g P4) for 10 d to synchronize estrus. Ewes were mated with fertile rams on the first estrus after CIDR removal and were assigned to 1 of 3 treatments. Ewes that were treated with hCG (600 IU; i.m.) 1 yr prior were divided into 2 groups; hCG/hCG and hCG/NO hCG. The hCG/hCG group received hCG (600 IU- i.m.) on d 4 post mating. Ewes that received hCG the year prior and were in the hCG/NO hCG group did not receive hCG and were administered a saline i.m. injection. Control ewes received saline both years. Jugular blood samples were taken from 7 ewes of each treatment group starting on d 1 through 21 d post mating to monitor serum P4. Ovulation rates were determined via laparoscopy on d 44. On approximately d 70, flank ultrasound was used to establish fetal numbers. A treatment x day interaction was observed for serum P4. Ewes in hCG/hCG group had greater ($P < 0.05$) P4 concentrations beginning on d 9 through d 15 than hCG/NO hCG and control treated ewes; whereas, treatments hCG/ NO hCG and controls had similar ($P > 0.05$) serum P4 concentrations. Ovulation rates, CL number ($P > 0.33$), and fetal numbers did not differ ($P > 0.62$) among treatment groups. In conclusion, administration of hCG in subsequent years does not produce carryover effects allowing multiple year administrations of hCG.

**Key words**: corpus luteum, fetal count, human chorionic gonadotropin, progesterone

## INTRODUCTION

Use of exogenous hormones to alter the natural function of the endocrine system has become a common management practice in livestock production. Use of human chorionic gonadotropin (**hCG**) has been shown to increase circulating progesterone (**P4**) concentrations with a tendency to increase fetal numbers (Willard et al., 2003; Yates et al., 2009, 2010; Lankford et al., 2010). Embryonic survival and growth can be increased by exogenous P4 supplementation from d 4 to d 14 post mating (Kleemann et al 1991, 1994). Similar to LH, hCG has been shown to stimulate differentiation of thecal and granulosal cells into small and large luteal cells (Farin et al., 1988). An extended half life and high affinity for hCG to bind to receptors on luteal cells can increase the number of LH receptors and could reduce the effectiveness of endogenous LH pulses (Schmitt et al., 1996). With hCG binding to the luteal cells increasing the amount of LH receptors to which hCG could potentially bind, hCG increases P4 production from the luteal cells. Various doses and timing of administration of hCG have been investigated to determine the most effective protocol for hCG (Breuel et al, 1989; Schmitt et al., 1996; Zamiri et al., 1998; Yates et al 2009; Lankford et al 2010; Richardson et al., 2011). Progesterone concentrations are greatest from treatments where hCG was administered on d 4 of the estrous cycle (Breuel et al., 1989; Redden et al., 2006; Richardson et al 2011). Contradictory reports of no difference in progesterone concentrations, (Gomez-Brunet et al., 2006), corpus luteum (**CL**) count, and fetal numbers (Richardson et al., 2011) cause concern for potential carry over effects of hCG among treated individuals. Therefore, we hypothesize that prior treatment with hCG would reduce the reproductive response to subsequent hCG treatments. The objective of this study was to determine if ewes treated with hCG in prior years would display a carryover effect on serum P4 concentrations and fetal numbers when treated with hCG in the succeeding year.

## MATERIALS AND METHODS

All procedures involving animals were approved by the New Mexico State University International Animal Care and Use Committee.

***Animals and Treatment.*** Forty mixed aged Suffolk ewes received a P4 containing insert (EAZI-BREED CIDR, 0.3 g P4; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. Ten days later CIDR were removed and ewes were randomly sorted into 1of 3 breeding groups. A fertile ram fitted with a marking harness was placed into each breeding group. The initial day of estrus (d 0) was determined by the ram initially marking the ewe and estrus was monitored and recorded daily. Ewes were assigned to 1 of 3 treatment groups. Ewes treated (i.m.) with 600 IU hCG (ProSpec-Tanny TechnoGene, LTD, Rehovot, Israel, CAS: HOR-250) 1 yr prior (Richardson et al., 2011) were divided into 2 groups: hCG/hCG and hCG/NO hCG. The hCG/hCG group (n = 14) received hCG (600 IU, i.m.) on d 4 post mating. Ewes that

received hCG the year prior and were in the hCG/NO hCG group (n = 13) did not receive hCG and were administered a 4-mL i.m. saline injection 4 d post mating. Ewes in the control group (n = 13) received a 4-mL saline injection on d 4 of the estrous cycle during both years.

***Blood Collection and Progesterone Assay.*** The first 7 ewes in each treatment group that were marked by the ram were selected for intensive sampling. Beginning on d 1 of estrus, blood was collected daily by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and continued for 21 d after treatment. Tubes were stored at room temperature for a minimum of 30 min before centrifugation (1,500 x *g*) at 4°C for 20 min. Serum was then transferred to plastic vials and stored frozen until assayed.

Serum P4 concentrations were determined using RIA procedures as described by Schneider and Hallford (1996; Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA). The inter- and intra-assay CV were 3.1 and 2.1%, respectively.

***Laparoscopies.*** On d 44 postmating, 5 ewes from each treatment group were selected for laparoscopy. The number of CL present on each ovary verified ovulation rates. Ewes were held off feed for 24 h and water for 12 h before the procedure. Operation procedure followed protocol described in Richardson et al. (2011). Animals were returned to pens with feed and water and closely monitored for 48 h.

***Pregnancy Determination and Postpartum Measurements.*** *P*regnancy and fetal number was determined by external flank ultrasound (3.5.MHz probe; Aloka, SSD-500V, Japan) on approximately d 70. At parturition, lambs born to a ewe was recorded, as well as gender and weight of lambs.

***Statistical Analysis.*** All data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Corpora lutea number, fetal counts, and number of lambs born per ewe were analyzed using PROC FREQ with Chi-Square. Progesterone concentration was analyzed by PROC MIXED with compound symmetry as the covariance structure. Treatment and ewe was in the whole plot and day and day by treatment was in the subplot.

## RESULTS

A treatment x day interaction ($P < 0.05$) was observed for P4 concentrations (Figure 1). Serum concentrations were similar ($P > 0.05$) among treatments through d 8. Beginning on d 9, serum P4 was greater ($P < 0.05$) in ewes in the hCG/hCG group and remained elevated through d 15. Serum P4 concentrations were similar ($P > 0.05$) among ewes in hCG/NO hCG and control groups.

Number of CL did not differ ($P > 0.33$) among treatments. Of ewes receiving hCG, 20% had ≥ 3 CL whereas 75% of hCG/NO hCG and control ewes displayed ≥ 3 CL (data not shown). The percentage of total ewes carrying multiple fetuses was similar ($P > 0.62$) among treatments on d 70 with 38, 38, and 15 % of ewes in control, hCG/hCG group, and hCG/NO hCG group, respectively having multiple fetuses (Table 1). Of ewes receiving hCG/hCG on d 4 and successfully mated on the first estrus, there was a tendency ($P < 0.08$) for greater birth rates where 83% of ewes gave birth to multiple lambs compared with hCG/NO hCG, 66%, and control ewes, 50% (Table 2). Lambing rates were similar ($P > 0.56$) for ewes bred in the second estrus for control, hCG/hCG, and hCG/NO

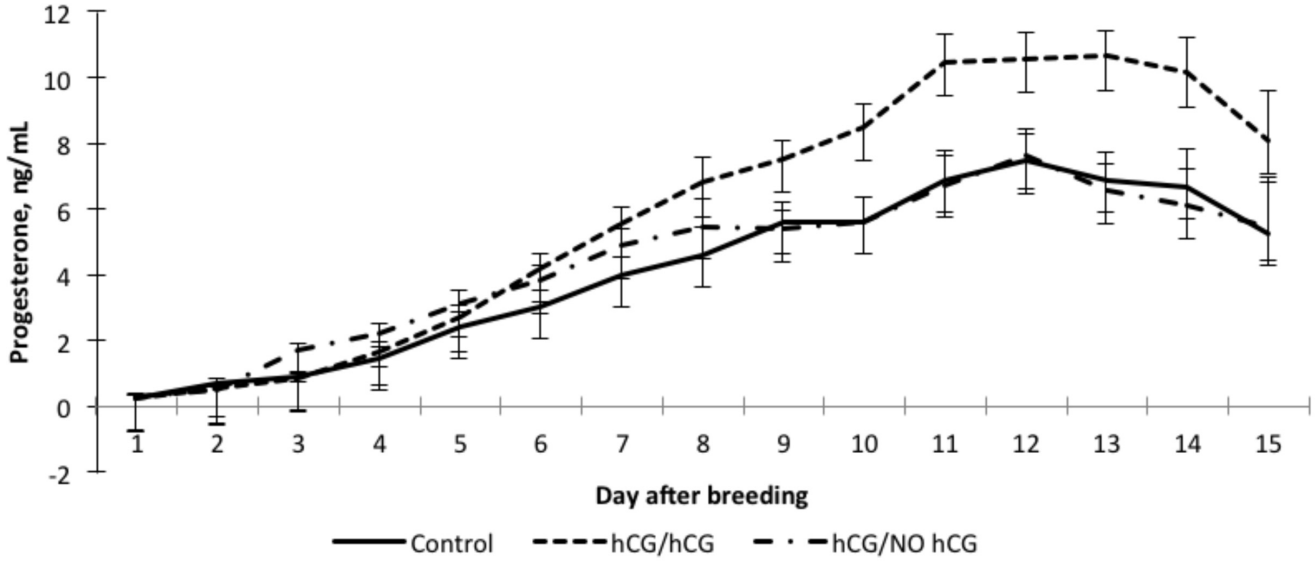


**Figure 1.** Serum progesterone concentrations in response to administration of 600 IU human chorionic gonadotropin (hCG) on d 4 post-mating. Estrus was synchronized using intravaginal P4 containing pessary (CIDR, 0.3 g P4; Pharmacia and Upjohn, Co., Hamilton, New Zealand) for 10 d and ewes were mated with fertile rams. Treatment groups consisted of hCG/hCG, hCG/NO hCG and control. A day by treatment interaction was detected ($P < 0.05$); therefore, data were analyzed within day. Progesterone concentrations were similar ($P > 0.05$) through d 8. Beginning on d 9 through d 15, ewes treated with hCG/hCG on d 4 were different ($P < 0.05$) than for hCG/NO hCG and control ewes. Progesterone concentrations for hCG/NO hCG ewes and controls were similar on all days ($P > 0.05$).

**Table 1.** Percentage of ewes treated with 600 IU human chorionic gonadotropin (hCG) on d 4 post-mating[1] and having 0, 1 or 2 fetuses at 70 d of gestation

| | Treatment[2] | | |
|---|---|---|---|
| Fetal numbers [3] | Control | hCG/hCG | hCG/NO hCG |
| 0 | 15 | 15 | 31 |
| 1 | 46 | 46 | 54 |
| 2 | 38 | 38 | 15 |

[1]Estrus was synchronized using intravaginal progesterone containing pessary (CIDR, 0.3 g P4) for 10 d and were mated with fertile rams on the first estrus after CIDR removal. Ewes were assigned to one of three treatments, hCG/hCG, hCG/ no hCG and control. Chi-Square ($P = 0.62$)

[2]Treatments consisted of 600 IU (4 mL) hCG i.m. on d 4 of the estrous cycle. Control ewes received 4 mL saline i.m. on d 4 of the estrus cycle.

[3]Fetal numbers were determined via external flank ultrasound on d 70 post breeding.

**Table 2.** Percentage of ewes treated with 600 IU human chorionic gonadotropin (hCG) on d 4[1] post-mating that produced single or multiple lambs from the first or second estrus after treatment.

| | Treatment[2] | | |
|---|---|---|---|
| Type of Birth [3] | Control | hCG/hCG | hCG/NO hCG |
| 1st Estrus[4] | | | |
| Single | 50 | 17 | 33 |
| Multiples | 50 | 83 | 66 |
| 2nd Estrus[5] | | | |
| Single | 66 | 20 | 66 |
| Multiples | 33 | 80 | 33 |

[1]Estrus was synchronized using intravaginal progesterone containing pessary (CIDR, 0.3 g P4) for 10 d and were mated with fertile rams on the first estrus after CIDR removal. Ewes were assigned to one of three treatments, hCG/hCG, hCG/ no hCG and control.

[2]Treatments consisted of 600 IU (4.8 mL) hCG i.m. on d 4 of the estrous. Control ewes received 4.8 mL saline i.m. on d 4 of the estrus cycle.

[3]Type of birth was determined at parturition.

[4] Chi-Square ($P < 0.08$)

[5] Chi-Square ($P > 0.56$)

hCG ewes resulting in 33%, 80%, and 33% of ewes carrying multiple fetuses, respectively.

## DISCUSSION

Results from this study support the hypothesis that supplemental hCG during early pregnancy can increase serum P4 concentrations in ewes. This timing of treatment during estrus tends to show a positive relation with serum P4 concentrations and lambing percentages compared with control and hCG/NO hCG ewes. Although serum P4 was different in hCG/hCG ewes on d 9, Lankford et al. (2010) and Richardson et al. (2011) observed a difference in treatments on d 6 of the estrous cycle, or 2 d after hCG administration. The half-life of hCG is approximately 22 h (Schmitt et al., 1996). Repeated use of hCG has been suggested to neutralize the hCG molecule and decrease the responsiveness to the luteal receptors (Sundby and Torjesen, 1978). Siddiqui et al. (2008) detected hCG antibodies in mares that may have prevented hCG from increasing P4 in follicular fluid and raise plasma LH concentrations and decreased blood flow to the follicle inhibiting the maturation and quality of oocyte. In our study, ewes treated with hCG 1 yr prior and saline the following year, showed no statistically detectable increase in serum P4 concentration during the seasonal breeding cycle compared with hCG/hCG treated ewes.

Ovulation rates did not differ ($P > 0.33$) among treatment groups. Greater ovulation frequencies were seen 2 d post hCG injection in ewes (Radford et al., 1984). In our study, 80% of ewes that received hCG on d 4 and were bred at the first estrus had multiples. These data suggest that hCG may increase multiple births. Zamiri and Hosseini (1998) found that multiple births were increased with a 250 IU or 500 IU injection of hCG, yet fertility and lambing rate were not increased due to breeding during the second estrus. The increase in multiple lambs born can be attributed to the increase in blastocyst growth and uterine secretions after hCG administration (Nephew et al., 1994). Wilmut et al. (1985) suggested that when exogenous P4 is supplied, conceptus development is expedited. No effect of hCG on multiple lamb rate was observed for ewes at the second estrus which supports the findings of Zamiri and Hosseini (1998).

## IMPLICATIONS

Progesterone concentrations were increased as well as a tendency to increase multiple births in ewes administered hCG 4 d post mating. Single dose administration as well as timing should be further explored to determine an effective protocol for sheep producers. Carryover effects were not observed in ewes receiving hCG 1 yr prior yet effectiveness of hCG treatment in ewes previously treated with hCG was detected in serum P4 concentrations and multiple births.


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# CORRELATION OF FEED INTAKE AND EFFICIENCY WITH SMALL INTESTINAL ANGIOGENIC FACTOR AND RECEPTOR EXPRESSION IN FINISHING CATTLE BORN TO DAMS FED VARYING LEVELS OF NUTRIENTS DURING EARLY TO MID-GESTATION

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**ABSTRACT:** We hypothesized that gestational nutrition would affect calf feed intake and efficiency as well as small intestinal development, and that feed intake and efficiency would be correlated with small intestinal measures. Multiparous beef cows (n = 36) were fed 1 of 3 diets from d 45 to 185 of gestation: a control (CON) diet of grass hay and supplement to meet NRC recommendations, a nutrient restricted (NR) diet providing 70% of CON $NE_m$, or an NR diet with a ruminally undegradable protein (NRP) supplement to provide similar essential AA as CON. Individual feed intake of calves was measured with the GrowSafe System during finishing. At slaughter (552 ± 10.2 kg BW), the jejunum was sampled for real time RT-PCR analysis of angiogenic factors [vascular endothelial growth factor (*VEGF*), VEGF receptor-1 (*FLT1*), VEGF receptor-2 (*KDR*), endothelial nitric oxide synthase 3 (*NOS3*) and soluble guanylate cyclase (*GUCY1B3*; nitric oxide receptor)]. Data were analyzed as a mixed model with calf sex as block. It was previously reported that maternal nutrition affected calf small intestinal length, but not other measures of intestinal growth, intake, or feed efficiency. Jejunal *GUCY1B3* mRNA expression was affected by maternal nutrition ($P = 0.03$), where calves born to NRP dams had greater ($P < 0.03$) *GUCY1B3* than CON and NR (4.59 vs. 2.85 and 2.56 ± 0.54 relative mRNA expression). There was no effect ($P \geq 0.34$) of maternal nutrition on *VEGF*, *FLT1*, *KDR*, or *NOS3* expression. Feed intake was positively correlated with jejunal mRNA expression of *KDR* (r = 0.37; $P = 0.05$) and *NOS3* (r = 0.35; $P = 0.06$) and tended to be negatively correlated with *VEGF* (r = -0.30; $P = 0.11$). Residual feed intake and G:F were not correlated ($P \geq 0.20$) with angiogenic factor mRNA expression. Results indicate that offspring intestinal gene expression may be affected by gestational nutrition even when apparent tissue growth is unchanged. Additionally, changes in intestinal expression of *VEGF* and *NOS3* systems are associated with feed intake and may alter intestinal vasculature.

**Key Words**: feed efficiency, gestation, small intestine

## INTRODUCTION

Beef cows are often undernourished during gestation due to limiting forage quality and quantity. In many species maternal undernutrition during gestation can alter fetal growth, resulting in impaired development, low birth weight offspring, and potential long-term consequences (Wu et al., 2006; Caton and Hess, 2010). Offspring intestinal tissues are responsive to maternal nutrition during gestation, and ruminant offspring have been shown to have altered small intestinal mass (Reed et al., 2007), cellularity (Reed et al., 2007), proliferation (Meyer et al., 2010c), maltase activity (Caton et al., unpublished data), vascularity (Meyer et al., 2010c; Neville et al., 2010), and angiogenic factor mRNA expression (Meyer et al., 2009; Neville et al., 2010) due to poor gestational nutrition. The gastrointestinal tract serves as the main site for nutrient absorption while also being a major energy and nutrient sink due to its high metabolic activity and rapid turnover. Effects of maternal nutrition on this system, and especially the small intestine, may therefore impact later growth and performance, including feed efficiency.

Feed inputs are the greatest annual cost for cow-calf producers (USDA-ERS, 2010); thus supplementation of poor quality forages may not be practical throughout gestation. Despite this, little is known of how targeted supplementation during specific periods of gestation impacts fetal development and subsequent offspring performance. We hypothesized that gestational nutrition would affect calf feed intake and efficiency as well as small intestinal development, and that feed intake and efficiency would be correlated with small intestinal measures. Our specific objectives were to investigate the effects of maternal nutrient restriction with or without RUP supplementation during early to mid-gestation on offspring feedlot intake, feed efficiency, intestinal angiogenic factor mRNA expression, and their relationships.

## MATERIALS AND METHODS

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

***Cow Management and Dietary Treatments.*** Beef cows used in this study were selected from the 3- and 4-yr old Angus x Gelbvieh cows in the University of Wyoming (**UW**) beef herd, estrous synchronized, and artificially inseminated to a single sire. Cows were managed as one group post-breeding and grazed native range at the UW McGuire Ranch. Pregnancy was diagnosed via transrectal ultrasonography on d 33 of gestation, at which time calves were weaned from 42 of the most uniform cows. These cows were then pen-fed (6 cows/pen) native grass hay (6.2% CP, DM basis) with a protein supplement to provide 10% dietary CP at the UW Livestock Center in Laramie.

On d 40 of gestation, pregnancy was again confirmed, and 36 cows (24 diparous 3-yr old cows and 12 triparous 4-yr old cows) were blocked by parity and randomly allocated by BW to receive 1 of 3 dietary treatments from d 45 to 185 of gestation: a control (**CON**) diet consisting of native grass hay (6.2% CP, DM basis) and a soybean meal-based supplement to meet NRC requirements for mid-gestation, a nutrient restricted (**NR**) diet providing 70% of the $NE_m$ of CON, or an NR diet fed with a RUP supplement (**NRP**) to provide similar essential AA flow to the duodenum as CON. The CON diet was formulated to allow for 0.51 kg/d of BW gain in the non-lactating pregnant cows, based upon the NRC (2000) requirements for pregnant primiparous heifers to gain 0.43 kg/d BW. Minerals and vitamins were added to the CON and NR diets to provide similar intake to the NRP treatment. The RUP supplement (68.7% menhaden fish meal, 24.5% hydrolyzed feather meal, and 6.8% porcine blood meal, DM basis), designed by Scholljegerdes et al. (2005b), was provided to match the duodenal AA flow of CON using the equation: total essential AA flow to the small intestine, g/d = [0.055 x g of OM intake] + 1.546 (Scholljegerdes et al., 2004). This was also adjusted to account for increased RUP supplement degradation in feed-restricted cattle (Scholljegerdes et al., 2005a).

Cows were individually fed one-half of the daily supplement allotment at 0600 and 1600 h daily. After supplement consumption within 20 min, one half of the daily hay was offered to each cow for the remainder of the 2-h feeding period (Whitney et al., 2000). Cows seldom had hay refusals greater than 1 kg. Hay and supplements offered daily were adjusted biweekly for increasing $NE_m$ requirements of gestation and BW change.

***Post-treatment Management.*** After treatment conclusion at d 185 of gestation, cows were managed together through calving and weaning. Calves were weaned (approximately 200 d of age), transported to the UW Sustainable Agriculture Research and Extension Center in Lingle, WY, and backgrounded for 14 d before being placed in the feedlot and penned by sex and dam's dietary treatment. During the growing period (98 d), all animals were fed a diet containing 43% hay, 34% corn silage, 20% corn, and 3% supplement, which was gradually transitioned to a finishing diet containing 82% corn, 7.5% corn silage, 6.5% hay, and 4% supplement.

The GrowSafe feed intake system (model 4000E, GrowSafe Systems Ltd., Airdrie, AB, Canada) was used to record individual daily feed intakes during the finishing period for 84 d. Two-consecutive day BW were taken at the initiation and conclusion of the finishing period to determine ADG. From these data, expected feed intake was determined by regressing ADG and metabolic midweight on actual feed intake. Residual feed intake (**RFI**) was then calculated as the expected feed intake subtracted from the actual feed intake (Cammack et al., 2005).

***Intestinal Tissue Collection and Analysis.*** At the conclusion of the feeding period, steers and heifers were transported to the UW Meat Laboratory for slaughter (steers: n =17, 448 ± 1 d of age, 572.0 ± 14.8 kg BW; heifers: n = 14, 466 ± 1 d of age; 528.6 ± 11.4 kg BW). Steers and heifers were slaughtered using standard commercial methods, and visceral organs were removed for dissection following inspection.

During dissection, a 15-cm section of the jejunum was collected for flash-freezing, as described by Soto-Navarro et al. (2004). The section began at a point on the jejunum adjacent to the mesenteric vein, 15 cm caudal from its junction with the ileocecal vein. This tissue was opened to expose the luminal surface and rinsed with PBS to remove digesta before being diced and flash-frozen by immersion in liquid $N_2$. Frozen tissues were then was stored at -80°C for angiogenic factor mRNA analysis.

***Angiogenic Factor mRNA Expression.*** Jejunal mucosal scrapes were analyzed using quantitative real-time RT-PCR for mRNA expression of vascular endothelial growth factor (***VEGF***), VEGF receptor-1 (***FLT1***), VEGF receptor-2 (***KDR***), endothelial nitric oxide synthase 3 (***NOS3***; produces nitric oxide) and soluble guanylate cyclase (***GUCY1B3***; nitric oxide receptor). See Table 1 for primer sequences used. Total cellular RNA was extracted from frozen tissues using TriReagent (Molecular Research Center, Cincinnati, OH). The RNA pellet was resuspended in 100 µL RNase free water and further purified using the RNeasy kit (Qiagen, Santa Clarita, CA) before purified RNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO).

Two µg of RNA were mixed with 4 µL reverse transcription buffer (5X) and 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA). The mixture was placed in a thermocycler for 5 min at 25ºC, 30 min at 42°C, 5 min at 85°C, and held at 4ºC. The cDNA was diluted with 100 µL nuclease-free water and stored at -20°C until semi-quantitative real-time PCR was performed.

Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) such that amplicons were 100 bp in size. Real-time PCR was performed by mixing 10 µL of diluted cDNA with 12.5µL of SYBR green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), 1 µL/ 500 pmol each forward and reverse primer, and 0.5 µL nuclease free water in each well of a 96 well plate. Amplification was performed using the IQ5 and 40 cycles of 95°C for 30 sec and 60°C for 30 sec. Melting curve analysis was performed post-amplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95°C for 3 min, cooled to 55°C, then the temperature increased by 0.5°C /sec up to 95°C. Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) was used as the reference gene, and all gene expression levels were quantified and reported relative to GAPDH expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Real- time RT-PCR was performed in duplicate for each sample and each gene.

***Statistical Analysis.*** Two cows aborted after treatment initiation, 2 calves died immediately after birth, and 1 calf died in the feedlot, which resulted in 31 total calves at slaughter (CON: n = 11, NR: n = 9, NRP: n = 11).

Data were analyzed in PROC MIXED (SAS Inst. Inc., Cary, NC) with maternal treatment and calf sex (slaughter date) as fixed effects in the model. Means were separated using LSD and were considered significant when $P \leq 0.10$ or were considered tendencies when $P < 0.15$. Data were also analyzed using the CORR procedure of SAS to determine the relationship of RFI, G:F, and feed intake with jejunal angiogenic factor mRNA expression.

## RESULTS

It was previously reported that maternal nutrition affected market weight calf small intestinal length, but not other measures of intestinal growth, feed intake, or feed efficiency (Meyer et al., 2010a, 2012a). Although calf jejunal mRNA expression of *VEGF*, *FLT1*, *KDR*, and *NOS3* were unaffected ($P \geq 0.34$) by maternal treatment, *GUCY1B3* expression was impacted ($P = 0.03$) by maternal nutrition during gestation (Table 2). Calves born to cows fed the NRP diet during early to mid-gestation had greater ($P < 0.03$) jejunal *GUCY1B3* mRNA expression than calves from both CON and NR dams.

Partial correlation coefficients between feed efficiency and intake traits during finishing and jejunal angiogenic factor and receptor mRNA expression at market weight are shown in Table 3. Finishing period RFI was negatively correlated with G:F ($r = -0.58$; $P = 0.001$) and positively correlated with feed intake ($r = 0.46$; $P = 0.01$). Gain:feed was not correlated with feed intake ($P = 0.19$), however. Residual feed intake and G:F during finishing were not correlated ($P \geq 0.20$) with jejunal mRNA expression of *VEGF*, *FLT1*, *KDR*, *NOS3*, or *GUCY1B3*. Despite this, feed intake was positively correlated with both jejunal *KDR* ($P = 0.05$) and *NOS3* ($P = 0.06$) mRNA expression, and tended ($P = 0.11$) to be negatively correlated with *VEGF* expression.

## DISCUSSION

Angiogenesis is the formation of new blood vessels from present vasculature, which is highly regulated by many angiogenic growth factors (Aron and Anthony, 2004). Vascular endothelial growth factor is perhaps the most potent of these and acts through its receptors to promote vascular endothelial cell survival, proliferation, migration, and permeability (Ferrara, 2004). Nitric oxide, produced by NOS3 from the substrate arginine, increases blood flow through vasodilation and stimulation of VEGF production, in addition to increasing vascular permeability, due in part to GUCY1B3 binding of nitric oxide (Martin et al., 2001; Roy et al., 2006). To our knowledge, this is the first report of angiogenic factor mRNA expression in the small intestine of cattle, although it has previously been reported in sheep (Holmes et al., 2008; O'Neil et al., 2008).

Maternal nutrition during gestation has been demonstrated to affect jejunal *GUCY1B3* mRNA expression in both fetal (Neville et al., 2010) and 20-d old (Meyer et al., 2009) lambs. These data from the current and previous studies suggest that alteration of *GUCY1B3* expression is a mechanism by which gestational nutrition may impact ruminant offspring gut development and function long after a maternal nutritional insult. In this study, cows fed NRP had greater circulating arginine than both CON and NR (Meyer et al., 2010a). Nitric oxide was likely elevated in these dams because arginine is its substrate; thus, elevated nitric oxide available to the developing fetus may have stimulated *GUCY1B3* upregulation which may have lasted into postnatal life. Because of the role of GUCY1B3 in producing the many effects of nitric oxide, GUCY1B3 expression may lead to greater vasodilation, angiogenesis, vascular permeability, and blood flow to the intestine to lessen other negative impacts on intestinal development and/or growth. Although market weight calves in this study did not have altered intestinal mass, cellularity, or proliferation due to gestational nutrition, it is possible that increased expression of GUCY1B3 in calves born to NRP dams mediated the lack of differences in intestinal growth. These data also suggest that gene expression in the intestine, which may lead to functional alterations in the tissue, may result from the maternal nutritional environment even when apparent intestinal growth is unchanged.

The current data are also the first attempt to our knowledge to better understand the relationship of intestinal vascularity, blood flow, and angiogenesis with metabolic efficiency. Although these data did not correlate *VEGF* and *NOS3* system expression at the mRNA level with whole animal feed efficiency, there does appear to be relationships between both systems and feed intake. Previous data in ewes support this concept, as nutritional plane during gestation has impacted jejunal mRNA expression of *VEGF*, *FLT1*, *KDR*, and *NOS3* (Neville et al., 2010; Meyer et al., 2012b). Additional data from the current study suggest that more efficient cattle have less small intestinal mass, but more dense intestinal mucosa (Meyer et al., 2012a). Blood flow to and from the intestine is important for tissue growth and nutrient flux; thus, it is probable that vascularization of the tissue plays a role in metabolic efficiency.

In summary, results of this study indicate that offspring intestinal gene expression may be affected by gestational nutrition even when apparent tissue growth is unchanged. These data support *GUCY1B3* as an important gene in small intestinal development that appears to be sensitive to maternal nutrition during gestation. Additionally, changes in intestinal expression of VEGF and NOS3 systems are associated with feed intake and may alter intestinal vasculature. More research is necessary to determine possible associations of intestinal vascularity and metabolic efficiency.

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**Table 1.** Sequence of primers used for bovine angiogenic factors and receptors

| Gene of interest | Description | Forward primer | Reverse primer |
|---|---|---|---|
| *VEGF* | Vascular endothelial growth factor | TCACCAAAGCCAGCACATAG | GCGAGTCTGTGTTTTTGCAG |
| *FLT1* | VEGF receptor 1 | GTATCACTGCAAAGCCAGCA | AGCGTTAACAGGAGCCAGAA |
| *KDR* | VEGF receptor 2 | CCCTTCTTTGAAGCATCAGC | CGTGCTGTTCTTCTTGGTCA |
| *NOS3* | Endothelial nitric oxide (NO) synthase | GTGGAGATCAACCTGGCTGT | CCCTTCTTTGAAGCATCAGC |
| *GUCY1B3* | Soluble guanylate cyclase, binds NO | GAGGATGCCTCGCTACTGTC | CTGCTCCGTTTCCTCTGTTC |

**Table 2.** Effects of maternal nutritional treatment from d 45 to 185 of gestation on offspring jejunal angiogenic factor and receptor mRNA relative expression at market weight

| | Maternal treatment[2] | | | | |
|---|---|---|---|---|---|
| Gene of interest[1] | CON | NR | NRP | SEM | *P*-value |
| *VEGF* | 0.612 | 0.695 | 0.658 | 0.053 | 0.51 |
| *FLT1* | 2.32 | 2.26 | 1.91 | 0.21 | 0.34 |
| *KDR* | 2.58 | 2.19 | 2.19 | 0.26 | 0.43 |
| *NOS3* | 3.44 | 3.73 | 3.56 | 0.56 | 0.93 |
| *GUCY1B3* | 2.85[a] | 2.56[a] | 4.59[b] | 0.54 | 0.03 |

[a,b] Within a row, means with different superscripts differ ($P < 0.05$)

[1]*VEGF* = vascular endothelial growth factor, *FLT1* = VEGF receptor-1, *KDR* = VEGF receptor-2, *NOS3* = endothelial nitric oxide synthase 3, and *GUCY1B3* = soluble guanylate cyclase

[2]Beef cows were fed a control (CON) diet of grass hay and supplement to meet NRC recommendations, a nutrient restricted (NR) diet providing 70% of CON $NE_m$, or an NR diet with a ruminally undegradable protein (NRP) supplement to provide similar essential AA as CON from d 45 to 185 of gestation.

**Table 3.** Partial correlation coefficients between residual feed intake (RFI), G:F, and feed intake during finishing and jejunal angiogenic factor and receptor mRNA relative expression at market weight

| | Jejunal relative mRNA expression[1] | | | | |
|---|---|---|---|---|---|
| Trait | *VEGF* | *FLT1* | *KDR* | *NOS3* | *GUCY1B3* |
| RFI | -0.21 ($P = 0.27$) | 0.01 ($P = 0.98$) | 0.10 ($P = 0.60$) | 0.25 ($P = 0.20$) | 0.18 ($P = 0.35$) |
| G:F | -0.11 ($P = 0.58$) | -0.10 ($P = 0.62$) | 0.02 ($P = 0.92$) | -0.06 ($P = 0.76$) | -0.10 ($P = 0.62$) |
| Feed intake | -0.30 ($P = 0.11$) | 0.02 ($P = 0.92$) | *0.37 ($P = 0.05$)* | *0.35 ($P = 0.06$)* | -0.01 ($P = 0.96$) |

[1]*VEGF* = vascular endothelial growth factor, *FLT1* = VEGF receptor-1, *KDR* = VEGF receptor-2, *NOS3* = endothelial nitric oxide synthase 3, and *GUCY1B3* = soluble guanylate cyclase

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# CORRELATION OF IGF-1, GROWTH HORMONE, AND LEPTIN TO BREEDING BEEF HEIFER PRODUCTIVITY

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**ABSTRACT:** The use of metabolic parameters associated with growth and tissue development may aid in predicting productivity of beef heifers, thus reducing potential production inefficiencies. Dam and offspring production variables were collected on 84 Angus-based crossbred heifers retained from the 2006 (n = 40 heifers; 3.42 parities/ heifer) and 2007 (n = 44 heifers; 2.64 parities/ heifer) calf crops. Blood samples were obtained at approximately 1 yr of age and analyzed for IGF-1, GH, and Leptin concentrations. Data within each hormone category were grouped (GROUP) into upper 25% (HIGH), middle 50% (MID), and lower 25% (LOW) of hormone concentrations using univariate analysis. Production variables within hormone category were analyzed as a one- way ANOVA with GROUP as the main effects and heifer birth year as a covariate. Experimental unit was heifer and the error term was residual error. Pearson correlation coefficients were determined between production variables and hormone concentrations. Pregnancy interval was correlated (r = -0.273; $P$ = 0.020) with IGF-1, with greater concentrations associated with shorter intervals (-5 d/yr). Pregnancy interval was 5.9 d less ($P$ = 0.045) for HIGH vs. MID IGF-1, 9.9 d less ($P$ = 0.013) for HIGH vs. LOW GH, and tended ($P$ = 0.082) to be 4.2 d greater for MID vs. LOW leptin. Number of parity was not associated ($P$ > 0.10) with hormone concentrations or GROUP within hormone categories. Percentage of calves weaned/born tended ($P$ = 0.086) to be less for HIGH (94.7%) vs. MID leptin (99.4%). No correlations ($P$ > 0.10) between hormone concentrations and percent calves weaned/born were observed. Offspring carcass weights (HCW), ratio of ribeye area-to-HCW, and marbling scores were not affected ($P$ > 0.10) by heifer hormone concentrations within any hormone category. Yield grade (YG) of offspring was correlated (r = 0.256, $P$ = 0.057) with heifer IGF-1 concentrations; with MID (2.74) tending ($P$ = 0.073) to have lower YG vs. HIGH (3.01). These data indicate that concentrations of IGF-1, GH, and Leptin at time of replacement heifer selection have limited value as prediction tools of female productivity.

**Key words**: beef heifers, hormones, productivity

## INTRODUCTION

The retention and development of beef heifers allows cow/calf operations to control genetic input and refine their overall breeding program, but can also be costly to the overall operation. Breeding heifers will accumulate 2 to 2.5 years of expenses (e.g., feed, labor, veterinary care) prior to potentially producing their first saleable offspring.

Meek et al. (1999) demonstrated that yearlings and 2-yr old heifers had the least Net Present Value (**NPV**) compared with mature cows up to 8-yr of age primarily due to increased reproductive risk. Due to the combination of added expenses and increased reproductive risk during early heifer development, improving the ability to predict potential future performance of heifers at an early age would be advantageous. The current study is a preliminary evaluation of the potential use of certain hormones associated with tissue growth and composition determined at time of heifer selection as predictors of heifer longevity and progeny performance.

## MATERIALS AND METHODS

Production records on 84 Angus-based, spring- calving, crossbred heifers retained from the 2006 (n = 40 heifers; 3.42 parities/heifer) and 2007 (n = 44 heifers; 2.64 parities/ heifer) calf crops were compiled and analyzed. Heifer selection and culling criteria were similar across years, along with nutrition and breeding management of retained heifers. In general, retained breeding heifers are managed separately from the mature cow herd until 4 yr of age, at which time they are commingled with the mature cow herd. Production and carcass records were maintained using a commercial cattle management software program (CowSense; Midwest MicroSystems L.L.C., Lincoln, NE).

Blood samples were collected on all heifers at approximately 1 yr of age. At time of blood collection the heifers had been grazing late-growth, fall pastures and were not receiving any energy or protein supplementation. Blood was collected via jugular venipuncture into 10 mL non-additive evacuated tubes (Vacutainer, BD, Franklin Lakes, NJ) and allowed to clot for 18 h at 4°C. Tubes were then centrifuged at 2000 x *g* for 30 min with serum harvested and frozen (-20°C) until analysis. Serum samples were sent to University of Missouri for analysis of IGF-1, GH, and leptin concentrations. Serum IGF-1 and GH concentrations were quantified using methods described by Lalman et al. (2000). Serum leptin concentrations were quantified using methods described by Geary et al. (2003). Serum concentrations of each hormone category were grouped (**GROUP**) into the upper 25% (**HIGH**), middle 50% (**MID**), and lower 25% (**LOW**) strata using univariate analysis (Table 1).

**Table 1.** Grouping categories of serum IGF-1, GH, and leptin concentrations from yearling beef heifers.

| Item | HIGH[1] | MID[1] | LOW[1] |
|---|---|---|---|
| | | IGF-1, ng/mL | |
| Range | > 103.82 | 67.35 to 103.82 | < 67.35 |
| Mean | 132.70 | 84.32 | 52.32 |
| n | 21 | 42 | 21 |
| | | GH, ng/mL | |
| Range | > 4.61 | 2.15 to 4.61 | < 2.15 |
| Mean | 8.28 | 3.10 | 1.59 |
| n | 21 | 42 | 21 |
| | | Leptin, ng/mL | |
| Range | > 1.45 | 0.96 to 1.45 | < 0.96 |
| Mean | 2.24 | 1.16 | 0.70 |
| n | 21 | 45 | 18 |

[1]Groups based on univariate analysis of serum hormone concentrations. Upper 25% = HIGH, middle 50% = MID, and lower 25% = LOW.

***Statistical Analysis.*** Production variables within hormone category were analyzed as a one-way ANOVA with GROUP as the main effect and heifer birth year as a covariate. Experimental unit was heifer and residual error was considered the error term. Pearson correlation coefficients were determined between production variables and hormone concentrations.

## RESULTS AND DISCUSSION

***IGF-1 Data.*** Means and correlation coefficients for heifer serum IGF-1 concentrations are presented in Table 2. Regardless of GROUP, there were no differences in overall parities, or number of female or male parities ($P > 0.10$). Pregnancy interval was negatively correlated (r = -0.273; $P = 0.020$) with heifer IGF-1 concentration, but no differences ($P = 0.53$) were detected between MID and LOW heifer groups. Progeny birth weight was negatively correlated (r = -0.264; $P = 0.024$) with heifer IGF-1 concentration, with calves from the HIGH group weighing less at birth versus LOW calves ($P = 0.050$). Progeny carcass backfat thickness was greater in HIGH calves versus either MID or LOW calves, and was positively correlated with heifer IGF-1 concentrations (r = 0.352; $P = 0.007$). Heifer IGF-1 concentrations tended ($P = 0.057$) to be positively correlated with YG, and were negatively correlated (r = -0.275; $P = 0.036$) with retail yield. The current dataset indicates that IGF-1 concentrations may aid in prediction of a heifer's ability to rebreed in a timely manner, along with the potential marketability of her progeny.

***Growth Hormone Data.*** Means and correlation coefficients for heifer serum GH concentrations are presented in table 2. No differences ($P > 0.10$) were detected for number of female or male parities across GH GROUP. Though the correlation coefficient was not significant ($P = 0.17$) for pregnancy interval, the HIGH group tended ($P = 0.096$) to have a longer interval versus MID heifers, and were 10 d longer ($P = 0.013$) than the LOW heifers. Percentage of weaned calves were negatively correlated (r = -0.331) with GH GROUP. Progeny characteristics were similar ($P > 0.10$) across GROUP within all measures except birth weight and KPH.

The MID calves had heavier ($P = 0.033$) birth weight versus LOW calves, while HIGH calves were similar ($P > 0.10$) to both MID and LOW groups. The HIGH calves had greater ($P = 0.047$) KPH versus LOW calves, and tended ($P = 0.097$) to be greater than the MID calves. No other carcass variables were significant ($P > 0.10$). Similar to the IGF-1 data, a heifer's GH concentration may aid in the prediction of her ability to rebreed in a timely manner. Possibly due to the small dataset and using only a single GH measurement, no other parameters proved to be correlated to serum GH concentrations.

***Leptin Data.*** Means and correlation coefficients for heifer leptin serum concentrations are presented in Table 3. No differences ($P > 0.10$) were detected for either number of female or male parities across leptin GROUP. Pregnancy interval tended to be greater ($P = 0.082$) in MID versus LOW heifers, and HIGH heifers were similar ($P > 0.10$) to both MID and LOW heifer groups. Percentage of calves weaned tended ($P = 0.086$) to be greater in MID versus HIGH heifers, while LOW heifers were similar ($P > 0.10$) to all other groups. The data indicated no differences ($P > 0.10$) between leptin GROUP and birth or weaning weight, or any carcass parameters. Additional observations are required to properly evaluate serum leptin concentrations in regards to predicting heifer longevity and progeny performance.

## IMPLICATIONS

The authors acknowledge that the current dataset is limited in scope and observations, and therefore limited conclusions can be ascertained in regards to predictive capabilities of IGF-1, GH, or leptin in selecting replacement heifers. The current dataset is also based on a single sample collected at time of heifer selection, and though this limits interpretation, it would be similar to sampling methods conducted by producers at time of heifer selection. The data suggests that IGF-1, GH, and leptin may provide useful information regarding breeding heifer productivity.

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**Table 2.** Heifer and progeny performance data based on heifer serum IGF-1 and GH concentrations determined at approximately 1 yr of age.

| | Hormone concentration group[1] | | | | *P*-values | | | Correlation Coefficient[2] | |
|---|---|---|---|---|---|---|---|---|---|
| Item | HIGH | MID | LOW | SE | HIGH vs. MID | HIGH vs. LOW | MID vs. LOW | r = | *P*-value |
| | | | | | *IGF-1* | | | | |
| Parity, n | 2.85 | 3.10 | 2.92 | 0.205 | 0.41 | 0.82 | 0.47 | 0.128 | 0.28 |
| Female | 1.56 | 1.67 | 1.33 | 0.200 | 0.66 | 0.45 | 0.14 | 0.123 | 0.30 |
| Male | 1.29 | 1.41 | 1.54 | 0.231 | 0.72 | 0.46 | 0.63 | 0.005 | 0.97 |
| Pregnancy interval, d | 364.8 | 370.7 | 369.2 | 2.02 | 0.045 | 0.14 | 0.53 | -0.273 | 0.020 |
| Weaning, % calves born | 97.6 | 96.5 | 100.0 | 2.08 | 0.71 | 0.45 | 0.18 | -0.165 | 0.17 |
| Progeny | | | | | | | | | |
| Birth wt, kg | 34.4 | 36.2 | 36.7 | 0.776 | 0.12 | 0.050 | 0.55 | -0.264 | 0.024 |
| Weaning wt, kg | 273.9 | 270.7 | 277.6 | 5.22 | 0.69 | 0.66 | 0.30 | -0.127 | 0.29 |
| Carcass wt, kg | 349.7 | 358.4 | 352.7 | 6.67 | 0.46 | 0.81 | 0.54 | 0.088 | 0.51 |
| Ribeye area, $cm^2$ | 88.5 | 90.6 | 89.1 | 1.51 | 0.42 | 0.83 | 0.48 | 0.055 | 0.68 |
| Ribeye area:HCW ratio | 1.78 | 1.78 | 1.78 | 0.024 | 0.96 | 0.93 | 0.96 | -0.047 | 0.72 |
| Backfat, cm | 1.59 | 1.35 | 1.41 | 0.058 | 0.019 | 0.090 | 0.45 | 0.352 | 0.007 |
| KPH, % | 2.33 | 2.26 | 2.15 | 0.080 | 0.60 | 0.22 | 0.35 | 0.067 | 0.62 |
| Marbling score[c] | 523.8 | 485.1 | 476.9 | 17.08 | 0.20 | 0.14 | 0.73 | 0.078 | 0.56 |
| Yield grade[d] | 3.01 | 2.74 | 2.84 | 0.087 | 0.073 | 0.28 | 0.41 | 0.256 | 0.057 |
| Retail yield, %[e] | 49.62 | 50.27 | 50.12 | 0.189 | 0.050 | 0.14 | 0.57 | -0.275 | 0.036 |
| | | | | | *GH* | | | | |
| Parity, n | 3.31 | 3.12 | 2.67 | 0.202 | 0.61 | 0.11 | 0.064 | 0.180 | 0.12 |
| Female | 1.31 | 1.54 | 1.31 | 0.190 | 0.52 | 0.99 | 0.30 | 0.133 | 0.26 |
| Male | 2.00 | 1.58 | 1.31 | 0.222 | 0.31 | 0.11 | 0.31 | 0.042 | 0.72 |
| Pregnancy interval, d | 376.6 | 370.3 | 366.6 | 0.82 | 0.096 | 0.013 | 0.13 | -0.163 | 0.17 |
| Weaning, % calves born | 97.1 | 98.6 | 99.1 | 2.09 | 0.70 | 0.64 | 0.87 | -0.331 | 0.005 |
| Progeny | | | | | | | | | |
| Birth wt, kg | 36.6 | 36.9 | 34.8 | 0.79 | 0.86 | 0.23 | 0.033 | 0.054 | 0.65 |
| Weaning wt, kg | 278.4 | 269.3 | 270.3 | 5.32 | 0.37 | 0.46 | 0.89 | -0.006 | 0.96 |
| Carcass wt, kg | 362.9 | 353.4 | 345.4 | 6.70 | 0.47 | 0.23 | 0.45 | 0.113 | 0.40 |
| Ribeye area, $cm^2$ | 91.5 | 89.1 | 88.1 | 1.54 | 0.43 | 0.32 | 0.67 | -0.001 | 0.99 |
| Ribeye area:HCW ratio | 1.77 | 1.76 | 1.80 | 0.024 | 0.97 | 0.65 | 0.56 | -0.152 | 0.25 |
| Backfat, cm | 1.37 | 1.39 | 1.46 | 0.06 | 0.89 | 0.49 | 0.42 | 0.095 | 0.48 |
| KPH, % | 2.47 | 2.21 | 2.12 | 0.079 | 0.097 | 0.047 | 0.46 | 0.223 | 0.092 |
| Marbling score[3] | 484.1 | 502.0 | 477.2 | 16.60 | 0.58 | 0.85 | 0.34 | 0.111 | 0.41 |
| Yield grade[4] | 2.84 | 2.78 | 2.85 | 0.089 | 0.75 | 0.96 | 0.64 | 0.187 | 0.17 |
| Retail yield,[5] % | 50.13 | 50.14 | 50.05 | 0.194 | 0.98 | 0.86 | 0.78 | -0.186 | 0.16 |

[1]Groups based on univariate analysis of serum hormone concentrations. Upper 25% = HIGH, middle 50% = MID, and lower 25% = LOW.
[2]Pearson correlation coefficients.
[3]300 = slight (Se), 400 = small ($Ch^-$), 500 = modest ($Ch^0$), 600 = moderate ($Ch^+$)
[4]Calculated as: Yield grade = 2.5 + (2.5 backfat) + (0.0038 carcass wt) + (0.2 KPH) – (0.32 ribeye area)
[5]Calculated as: Retail yield = 51.34 - (5.78 backfat) + (0.0093 carcass wt) – (0.462 KPH) + (0.740 ribeye area)

**Table 3.** Heifer and progeny performance data based on heifer serum Leptin concentrations determined at approximately 1 yr of age.

| | Hormone concentration group[1] | | | | *P*-values | | | Correlation Coefficient[2] | |
|---|---|---|---|---|---|---|---|---|---|
| Item | HIGH | MID | LOW | SE | HIGH vs. MID | HIGH vs. LOW | MID vs. LOW | r = | *P*-value |
| Parity, n | 2.92 | 2.99 | 3.12 | 0.204 | 0.78 | 0.48 | 0.60 | -0.157 | 0.18 |
| Female | 1.48 | 1.49 | 1.56 | 0.201 | 0.76 | 0.96 | 0.79 | -0.064 | 0.59 |
| Male | 1.44 | 1.43 | 1.59 | 0.250 | 0.99 | 0.63 | 0.56 | -0.084 | 0.48 |
| Pregnancy interval, d | 368.3 | 371.4 | 367.2 | 2.04 | 0.24 | 0.69 | 0.082 | 0.096 | 0.42 |
| Weaning, % calves born | 94.7 | 99.4 | 97.4 | 2.07 | 0.086 | 0.35 | 0.44 | -0.104 | 0.39 |
| Progeny | | | | | | | | | |
| Birth wt, kg | 36.9 | 35.7 | 36.6 | 0.79 | 0.25 | 0.84 | 0.31 | -0.053 | 0.65 |
| Weaning wt, kg | 268.3 | 271.5 | 274.9 | 5.34 | 0.65 | 0.39 | 0.60 | -0.110 | 0.36 |
| Carcass wt, kg | 351.3 | 361.1 | 346.3 | 6.54 | 0.36 | 0.66 | 0.12 | -0.232 | 0.079 |
| Ribeye area, $cm^2$ | 89.4 | 90.4 | 88.7 | 1.50 | 0.69 | 0.79 | 0.43 | -0.154 | 0.25 |
| Ribeye area:HCW ratio | 1.80 | 1.76 | 1.80 | 0.023 | 0.35 | 0.97 | 0.27 | 0.173 | 0.19 |
| Backfat, cm | 1.44 | 1.41 | 1.39 | 0.061 | 0.72 | 0.60 | 0.82 | -0.049 | 0.72 |
| KPH, % | 2.25 | 2.17 | 2.19 | 0.082 | 0.52 | 0.68 | 0.82 | -0.121 | 0.37 |
| Marbling score[3] | 502.7 | 484.2 | 468.6 | 17.05 | 0.50 | 0.25 | 0.52 | 0.004 | 0.98 |
| Yield grade[4] | 2.82 | 2.83 | 2.79 | 0.089 | 0.94 | 0.86 | 0.76 | -0.083 | 0.54 |
| Retail yield, [5] % | 50.07 | 50.10 | 50.24 | 0.195 | 0.92 | 0.61 | 0.61 | 0.082 | 0.54 |

[1]Groups based on univariate analysis of serum Leptin concentrations. Upper 25% = HIGH, middle 50% = MID, and lower 25% = LOW.
[2]Pearson correlation coefficients.
[3]300 = slight (Se), 400 = small ($Ch^-$), 500 = modest ($Ch^0$), 600 = moderate ($Ch^+$)
[4]Calculated as: Yield grade = 2.5 + (2.5 backfat) + (0.0038 carcass wt) + (0.2 KPH) – (0.32 ribeye area)
[5]Calculated as: Retail yield = 51.34 - (5.78 backfat) + (0.0093 carcass wt) – (0.462 KPH) + (0.740 ribeye area)

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# EFFECTS OF RUMINALLY DIGESTED AND UNDIGESTED SNAKEWEED EXTRACTS ON FEMALE SPRAGUE-DAWLEY RATS

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**ABSTRACT:** Two studies were conducted to examine effects of snakeweed (SW) extracts (evaporate residues) on serum components and reproduction in female Sprague-Dawley rats. In Exp. 1, 36 rats at day 5 (d 1) of pregnancy were offered SW extracts (ethanol and hexane) at 20% and 30% of diet (25g/rat). In Exp. 2, 36 rats at day 5 (d 1) of pregnancy were offered ruminally digested SW extracted (ethanol and hexane) at 20% and 30% of diet (25g/rat). Each rat was assigned a non-SW control rat fed 5001 Rat Chow. Rats were fed for 10 d and BW was recorded on d 1 and 11. Blood samples were collected via heart puncture and rats were euthanized on d 11. Experimental design was completely random with split-plot when appropriate. In Exp. 1, rats consuming 20% ethanol SW extract (20% ESW) had decreased ($P < 0.05$) feed intake on d 1 to 3 and increased ($P < 0.05$) intake on d 10, while those fed the 20% hexane SW extract (20% HSW) had increased ($P < 0.05$) feed intake from d 6 through d 10 compared with control rats. Rats consuming 30% ESW resulted in decreased ($P < 0.05$) feed intake on d 1 to 3 and increased ($P < 0.05$) intake on d 8 to 10. Rats consuming 30% HSW decreased intake on d 1, 2, 3, and 5 and increased intake ($P < 0.05$) on d 7, 9, and 10 compared with controls. Rats consuming 20 and 30% HSW had increased ($P < 0.05$) aspartate and alanine aminotransferase concentrations. Blood urea nitrogen, albumin, and creatinine increased ($P < 0.05$) in treated rats compared with controls. In Exp. 2, rats consuming 20% ethanol extract of digested SW (20% EDSW), 20% hexane extract of digested SW (20% HDSW), and 30% ethanol extract of digested SW (30% EDSW) increased ($P < 0.05$) feed intake compared with controls and 30% HDSW decreased ($P < 0.05$) feed intake on d 1, 2, and 7. Alkaline phosphatase, BUN, globulin and creatinine increased ($P < 0.05$) in rats consuming SW extracts compared with controls. In exp 1 and 2, Serum P4 and number of pups were similar ($P > 0.05$) among treatments. Results indicate that SW, both ruminally digested and undigested may contain potential chemical compounds that cause a mild toxicity and ruminal digestion altered SW hepatotoxicity.

**Key words**: snakeweed, Sprague-Dawley rats, toxicity

## INTRODUCTION

Snakeweed (**SW**; *Gutierrezia sarothrae*) is a noxious plant in the western United States, northern Mexico and southern Canada (Smith et al., 1991). Approximately 60% of New Mexico and 22% of Western Texas have been invaded by SW with an estimated $40 million in losses (Torell et al., 1988). Snakeweed is unpalatable to animals, but under severe conditions like drought, animals are more likely to graze this toxic plant (Gardner et al., 1999). Clinical signs of snakeweed poisoning include loss of appetite, weight loss, reduction in growth rate, low birth weight, weakened newborns, abortion, and death (Dollahite et al., 1957). Researchers have found that saponins are the primary toxicant in SW (Dollahite et al., 1962; Smith et al., 1994). Rats are sensitive to SW toxicity and ingestion of snakeweed by female rats prior to mating caused reproductive failure and changes in blood serum profile (Flores-Rodriguez et al., 1989, 1990; Edrington et al., 1990, 1993a, 1993b).

The objective of our study was to investigate effects of ruminally digested and undigested SW extracts on pregnancy, serum constituents, body organs, and feed intake of pregnant female Sprague-Dawley rats.

## MATERIALS AND METHODS

All procedures described were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Snakeweed samples were harvested from the Chihuahuan Desert Rangeland and Research Center, located 37 km north of Las Cruces, New Mexico. Snakeweed was harvested in July, 2007 during the pre-bloom stage via hand clipping. Approximately 5 to 10 cm of the distal portion of the plants were harvested. Snakeweed samples were stored frozen at -20°C and were ground to pass through a 2- mm screen.

Snakeweed extraction was performed (Estell et al., 1994) with some modifications. Two solvents were used, hexane and ethanol. Eighty five percent of the solvents were removed by evaporation (the 4 L solvent used for extraction resulted in 600 mL extract). The extract for each solvent was stored in amber colored jars at 2 to 4°C. Solvents used during the extraction process varied in polarity. The hexane fraction (least polar solvent) was expected to contain most of the essential oils (Molyneux et al., 1980) and the ethanol fraction (most polar solvent) was expected to contain various saponins (Chavez-Gonzalez, 1988).

In Exp. 1, mature pregnant female Sprague-Dawley rats at 5 d post introduction of males were purchased (Hilltop Lab Animals Hilltop Lab Animals, Inc.Hilltop Drive, Scottdale, PA), weighted and placed individually in cages (Ancare

model R20 Polycarbonate cage dimensions, 10.5" X 19" X 8"). Rats were randomly assigned to 1 of 4 treatments: 20% hexane SW extract (20% **HSW** = 5mL extract; n = 6), 30% hexane SW extract (30% HSW = 7.5mL extract; n = 6), 20% ethanol SW extract (20% **ESW** = 5mL extract; n = 6), and 30% ethanol SW extract (30% ESW = 7.5mL extract; n = 6) with 5001 Rat Chow as the remainder of the diet. These levels represent feeding 25 g rat chow with extracts representing SW at 20% and 30% of the total diet. Each treated rat was assigned a control rat (control; n = 6/ treatment). Control rats received 5001 Rat Chow diet (23.9 % CP; 5 % fat, 5.1 % CF; 5 % ash) mixed with soybean oil to account for the fat portion in the extract. Snakeweed extract was applied by top dressing the diet and mixing thoroughly to have a homogenous diet. Diets were offered for 10 d. Initial and final BW were recorded. After the 10-d feeding period, rats were anesthetized using isoflurane and a blood sample was collected via heart puncture. Rats were euthanized by decapitation. Heart, liver, kidney, and carcass weights were recorded. Serum was analyzed for a variety of components (Vet. Chem. 20, TRICOR lab, Albuquerque, NM) including LDH, AST, ALT, ALK, CK, total protein, albumin, globulin, creatinine, BUN, glucose, total bilirubin, direct bilirubin, and triglyceride. Serum was analyzed for Na, K, Ca, P, Cl, and Mg. Serum enzymes give an indication of liver damage, while creatinine and BUN are both indicators of kidney damage. Serum progesterone (**P4**) was determined by RIA as described by Schneider and Hallford (1996; CV = 3.8%).

In Exp. 2, SW that were first digested, were then extracted as described previously for Exp. 1. Snakeweed was digested using in vitro ruminal fermentation technique (May and Galyean, 1996). Rumen fluid was obtained from a cannulated Angus cow offered sorghum hay (11.3 % CP; 1.8 % fat; 56.1 % TDN; 6.6 % ash, NRC, 1996). Rats were randomly assigned to 1 of 4 treatments: 20% hexane digested snakeweed extract (20% **HDSW** = 5mL extract; n = 6), 30% hexane digested snakeweed extract (30% HDSW = 7.5mL extract; n = 6), 20% ethanol digested snakeweed extract (20% **EDSW** = 5mL extract; n = 6), and 30% ethanol digested snakeweed extract (30% EDSW= 7.5mL extract; n = 6) with 5001 Rat Chow as the remainder of the diet.

***Statistical Analysis.*** Data were analyzed using the Mixed procedure (SAS Inst. Inc., Cary, NC) as a completely randomized design and rats served as the experimental unit. Feed intake was measured as a split-plot (repeated measures) with treatment in the whole plot and day and treatment by day interaction in the sub-plot. When F value is significant ($P < 0.05$) means were separated by contrasts.

## RESULTS AND DISCUSSION

In Exp. 1, rats treated with 20% ESW, 30% ESW, and 30% HSW had lower ($P < 0.05$) feed intake during the first 3 d compared with controls while 20% HSW showed no effect ($P > 0.05$) during the first 5 d of feeding compared with their control (Table 1). Whereas, feed intake increased ($P < 0.05$) for treated rats compared with controls during d 8 to 10.

**Table 1.** Feed intake (g) of rats offered diets containing 20% ethanol snakeweed extract (20% ESW), 20% hexane snakeweed extract (20% HSW), 30% ethanol snakeweed extract (30% ESW), and 30% hexane snakeweed extract (30% HSW) over a 10-d feeding period[1], Exp.1

| | TRT | | TRT | | TRT | | TRT | | |
|---|---|---|---|---|---|---|---|---|---|
| Day[2] | Con[3] | 20% ESW | Con | 20% HSW | Con | 30% ESW | Con | 30% HSW | SE[4] |
| 1 | 21.0[a] | 10.8[b] | 19.3 | 18.3 | 22.3[a] | 10.7[b] | 22.6[a] | 10.6[b] | 1.45 |
| 2 | 19.2[a] | 14.8[b] | 17.5 | 15.7 | 18.3[a] | 11.8[b] | 17.6[a] | 13.2[b] | 0.94 |
| 3 | 20.1[a] | 15.5[b] | 16.9 | 17.2 | 17.1[a] | 13.8[b] | 18.9[a] | 14.5[b] | 0.93 |
| 4 | 19.8 | 17.6 | 15.6 | 16.9 | 17.7 | 15.5 | 17.9 | 14.9 | 1.54 |
| 5 | 19.6 | 19.0 | 17.2 | 19.7 | 16.5 | 15.7 | 19.4[a] | 14.8[b] | 1.27 |
| 6 | 17.2 | 20.5 | 15.8[a] | 22.8[b] | 15.2 | 18.6 | 17.6 | 18.6 | 1.25 |
| 7 | 17.2 | 19.9 | 16.6[a] | 22.3[b] | 16.1 | 18.8 | 17.7[a] | 21.4[b] | 1.21 |
| 8 | 18.6 | 20.9 | 15.8[a] | 23.0[b] | 16.9[a] | 20.8[b] | 19.7 | 21.7 | 1.35 |
| 9 | 17.7 | 21.0 | 16.1[a] | 23.7[b] | 16.1[a] | 20.3[b] | 17.5[a] | 22.4[b] | 1.33 |
| 10 | 15.4[a] | 21.4[b] | 14.9[a] | 24.3[b] | 14.4[a] | 20.7[b] | 17.1[a] | 23.8[b] | 1.23 |

[1]Data were analyzed as repeated measures, means were reported by day.
[2]Day 1 represents first day of feeding period.
[3]Control rats
[4]Standard error (n = 6).
[a,b]Row values with different superscripts between control and respective SW treatment differ ($P < 0.05$)

Rats treated with 20% ESW showed an increase ($P < 0.05$) in feed intake on d 10, while those treated with 30% ESW showed an increase ($P < 0.05$) in feed intake on d 8, 9, and 10 compared with their control. Rats treated with 20% HSW showed an increase ($P < 0.05$) starting d 6 through d 10, and those treated with 30% HSW showed an increase ($P < 0.05$) in feed intake d 7, 9, and 10 compared with controls. Our results indicated a decrease in feed intake during first days of feeding and then an increase. These data suggest that rats may adapt to extract after offering it for a period of time.

In Exp. 2, rats consuming digested SW extracts had greater ($P < 0.05$) feed intake compared with their controls except for 30% HDSW where feed intake was less ($P < 0.05$) than control rats on d 1, 2, and 7, while all other days were numerically less ($P > 0.05$; Table 2). As previously mentioned, the hemolytic experiment indicated that saponins were located in the hexane fraction and not the ethanol as expected. This can explain the lower feed intake in the hexane extract-treated rats (Table 2). Many studies showed that feed intake diminished with SW feeding reflecting poor palatability or toxicity or both of SW (Flores-Rodriguez et al., 1990; Edrington et al., 1993a,b). By comparing ethanol and hexane extracts with their control we noticed a decrease ($P < 0.05$) in feed intake with the hexane extract compared with ethanol (Table 2). The 30% HDSW had the greatest effect ($P < 0.05$) reducing feed intake than 20% HDSW.

In Exp. 1, AST and ALT both increased ($P < 0.05$) in 20% and 30% HSW treated rats compared with controls whereas, there was no difference ($P > 0.05$) in the 20% and 30% ESW compared with their controls (Table 3). In addition, the 30% HSW had a greater ($P < 0.05$) AST and ALT compared with 30% ESW. All serum enzymes in Exp. 2 were similar ($P > 0.05$) among all treatments compared with control except ALK which increased ($P < 0.05$) in treated rats compared with controls (Table 4). This increase was greater in hexane extract-treated rats compared with ethanol extract-treated both compared with controls. Our results agree with Flores-Rodriguez et al. (1990) and Edrington et al. (1993c) who showed an increase in ALK with SW feeding. Edrington et al. (1993a) reported an increase in ALT, a decrease in AST, and a decrease in AST: ALT ratio when SW was ingested by male rats. Our results showed an increase in both enzymes when feeding the hexane extract (Table 3). The hexane extract has been found to cause red blood cell hemolysis (data not shown). Saponins have been found to cause blood cell hemolysis (Dollahite et al., 1962). Blood urea nitrogen increased ($P < 0.05$) in rats treated with extract containing 30% SW (30% ESW and 30% HSW) compared with control whereas the low levels (20% ESW and 20% HSW) tended to increase also (Table 3). Creatinine increased ($P < 0.05$) in all treated rats except for 20% HSW (Table 3). For digested snakeweed extracts (Exp. 2), Blood urea nitrogen increased ($P < 0.05$) in all treated rats compared with control whereas creatinine increased ($P < 0.05$) in 30%HDSW compared with controls (Table 4). Increased ALT and AST strongly suggests hepatocellular damage and BUN and creatinine suggests

**Table 2.** Feed intake (g) of rats offered diets containing 20% ethanol digested snakeweed extract (20% EDSW), 20% hexane digested snakeweed extract (20% HDSW), 30% ethanol digested snakeweed extract (30% EDSW), and 30% hexane digested snakeweed extract (30% HDSW) over a 10-d feeding period[1], Exp. 2

| | TRT | | TRT | | TRT | | TRT | | |
|---|---|---|---|---|---|---|---|---|---|
| Day[2] | Con[3] | 20% EDSW | Con | 20% HDSW | Con | 30% EDSW | Con | 30% HDSW | SE[4] |
| 1 | 21.5 | 22.9 | 22.2[a] | 12.6[b] | 21.0 | 21.3 | 22.9[a] | 14.2[b] | 1.33 |
| 2 | 19.3[a] | 23.5[b] | 19.9 | 18.1 | 15.1[a] | 23.7[b] | 18.3[a] | 14.9[b] | 0.93 |
| 3 | 17.9[a] | 23.6[b] | 18.6 | 19.8 | 15.9[a] | 24.1[b] | 18.6 | 16.0 | 1.83 |
| 4 | 18.4[a] | 23.1[b] | 15.3[a] | 20.9[b] | 15.5[a] | 24.4[b] | 14.9 | 15.3 | 1.50 |
| 5 | 19.0[a] | 23.0[b] | 18.9 | 20.5 | 16.5[a] | 24.8[b] | 17.0 | 15.6 | 1.26 |
| 6 | 19.8 | 19.5 | 19.3 | 19.5 | 18.2 | 25.0 | 19.5 | 14.7 | 1.95 |
| 7 | 17.4[a] | 24.8[b] | 18.2[a] | 22.6[b] | 18.5[a] | 24.7[b] | 20.4[a] | 15.9[b] | 1.47 |
| 8 | 20.2 | 23.7 | 19.7 | 22.3 | 18.3[a] | 23.2[b] | 19.6 | 16.4 | 1.64 |
| 9 | 20.2 | 24.2 | 21.3 | 21.8 | 18.9[a] | 25.0[b] | 19.4 | 16.9 | 1.74 |
| 10 | 10.6[a] | 24.1[b] | 13.3[a] | 21.4[b] | 8.7[a] | 24.7[b] | 15.1 | 15.0 | 2.17 |

[1]Data were analyzed as repeated measures, means were reported by day.
[2]Day 1 represents first day of feeding period.
[3]Control rats.
[4]Standard error (n = 6).
[a,b]Row values with different superscripts between control and respective SW treatment differ ($P < 0.05$).

**Table 3.** Blood serum components and % organ weights of rats offered diets containing 20% ethanol undigested snakeweed extract (20% ESW), 20% hexane undigested snakeweed extract (20% HSW), 30% ethanol undigested snakeweed extract (30% ESW), and 30% hexane undigested snakeweed extract (30% HSW) over a 10 d feeding period, Exp. 1

| | TRT | | TRT | | TRT | | TRT | | |
|---|---|---|---|---|---|---|---|---|---|
| Item[1] | Con[2] | 20% ESW | Con | 20% HSW | Con | 30% ESW | Con | 30% HSW | SE[3] |
| BUN, mg/dL | 17.0[a] | 21.2[a] | 18.3[a] | 19.5[a] | 14.5[a] | 20.7[b] | 14.7[a] | 23.8[b] | 1.9 |
| Trigy, mg/dL | 369.5 | 140.3 | 176.8 | 151.3 | 250.3 | 154.0 | 221.3 | 90.0 | 71.6 |
| Creat, mg/dL | 0.3[a] | 0.6[b] | 0.3[a] | 0.4[a] | 0.3[a] | 0.9[b] | 0.3[a] | 0.6[b] | 0.1 |
| AST, U/L | 165.7[a] | 280.7[a] | 141.3[a] | 340.2[b] | 218.2[a] | 136.7[a] | 168.2[a] | 331.5[b] | 73.5 |
| ALT, mmol/dL | 49.0[a] | 68.2[a] | 35.2[a] | 68.0[b] | 45.7[a] | 39.0[a] | 40.7[a] | 66.8[b] | 14.2 |
| Ca, mmol/dL | 9.9[a] | 10.4[a] | 10.3[a] | 10.2[a] | 9.8[a] | 10.6[b] | 9.9[a] | 10.4[b] | 0.2 |
| Cl, mmol/dL | 95.8[a] | 93.5[a] | 98.5[a] | 94.5[b] | 99.3[a] | 93.3[b] | 95.7[a] | 91.8[b] | 1.3 |
| Heart | 0.54[a] | 0.53[a] | 0.65[a] | 0.58[a] | 0.49[a] | 0.47[a] | 0.48[a] | 0.39[b] | 0.03 |

[1]BUN; blood urea nitrogen, Trigy; triglycerides, Creat; creatinine, AST; aspartate aminotransferase, ALT; alanine aminotransferase, Ca; calcium; Cl; chlorine.
[2]Control rats.
[3]Standard error (n = 6).
[a,b]Row values with different superscripts between control and respective SW treatment differ ($P < 0.05$).

**Table 4.** Blood serum components and % organ weights of rats offered diets containing 20% ethanol digested snakeweed extract (20% EDSW), 20% hexane digested snakeweed extract (20% HDSW), 30% ethanol digested snakeweed extract (30% EDSW), and 30% hexane digested snakeweed extract (30% HDSW) over a 10 d feeding period, Exp. 2

| | TRT | | TRT | | TRT | | TRT | | |
|---|---|---|---|---|---|---|---|---|---|
| Item[1] | Con[2] | 20% EDSW | Con | 20% HDSW | Con | 30% EDSW | Con | 30% HDSW | SE[3] |
| BUN, mg/dL | 16.2[a] | 21.4[b] | 14.3[a] | 22.3[b] | 13.8[a] | 21.2[b] | 15.7[a] | 24.7[b] | 2.59 |
| Trigy, mg/dL | 212.0 | 244.6 | 229.8 | 154.5 | 188.7 | 131.0 | 197.5 | 53.0 | 56.7 |
| Creat, mg/dL | 0.4[a] | 0.4[a] | 0.4[a] | 0.5[a] | 0.3[a] | 0.4[a] | 0.4[a] | 0.7[b] | 0.09 |
| ALK*, U/L | 59.3[a] | 105.6[b] | 60.3[a] | 145.8[b] | 47.8[a] | 104.8[b] | 48.0[a] | 171.8[b] | 16.47 |
| K*, mmol/dL | 4.6[a] | 5.3[a] | 4.9[a] | 5.8[a] | 5.5[a] | 5.5[a] | 4.8[a] | 6.5[b] | 0.39 |
| Ca, mmol/dL | 9.9[a] | 10.2[b] | 9.5[a] | 10.6[b] | 9.2[a] | 10.1[b] | 9.7[a] | 10.2[a] | 0.29 |
| Liver** | 5.99[a] | 6.64[a] | 6.69[a] | 9.50[b] | 5.49[a] | 5.58[a] | 5.68[a] | 6.16[a] | 0.57 |
| Heart | 0.55[a] | 0.50[a] | 0.59[a] | 0.55[a] | 0.49[a] | 0.44[a] | 0.55[a] | 0.41[b] | 0.03 |

[1]BUN; blood urea nitrogen, Trigy; triglycerides, Creat; creatinine, ALK; alkaline phosphatase, K; potassium, Ca; calcium.
[2]Control rats.
[3]Standard error (n = 6).
*A difference ($P < 0.05$) was found between the two 30% treatments.
**A difference ($P < 0.05$) was found between the two 20% EDSW and 20% HDSW treatments.
[a,b]Row values with different superscripts between control and respective SW treatment differ ($P < 0.05$).

renal damage. These results agree with Edrington et al. (1992, 1993a, 1993b) who reported an increase in BUN, creatinine, Alk, and ALT when feeding SW to rats.

Triglycerides numerically ($P > 0.05$) were decreased in all SW treatments (Exp. 1 and 2) except the 20% EDSW were it showed an increase (Table 3 and 4). Lowering triglycerides levels is a good indication of the ability of saponins to inhibit the pancreatic lipase activity resulting in delayed intestinal absorption of dietary fats (Han et al., 2000).

Our data showed an increase ($P < 0.05$) in Ca concentrations with feeding the high levels of extracts (Table 3). Chlorine concentrations decreased ($P < 0.05$) with all treatments except for 20% ESW compared with controls (Table 3) which could be another indication of renal damage caused by SW extracts. Additional evidence of renal damage was increased ($P < 0.05$) serum K and Ca concentrations (Table 4). Potassium concentrations increased ($P < 0.05$) in 30% HDSW compared with control, and no effects ($P > 0.05$) were found in other treatments compared with their controls. Calcium concentration increased ($P < 0.05$) in 20% EDSW, 20% HDSW, and 30% EDSW compared with control, and no effect ($P > 0.05$) in 30%HDSW compared with control. Our results also agree with Edrington et al. (1993), who found that ingested SW had an effect on increasing ALK, creatinine, K, and Ca in serum clinical profile in rats and with Flores-Rodriquez et al. (1989) who found that feeding SW to rats at levels 12.5% and 25% of commercial rat feed caused an increase in Ca and K concentrations in the blood serum. Increased ALK, BUN, and creatinine, Ca, and K concentrations in serum strongly suggest hepatocellular or renal damage (Dollahite and Anthony, 1957; Dollahite et al., 1962; Edrington et al., 1991).

Treatment effect ($P > 0.05$) on percent body organ weights was noted (Table 3 and 4). In Exp. 1, the 30% HSW had a lower ($P < 0.05$) percent heart weight than control (Table 3). In Exp. 2, offering 20% HDSW to rats increased ($P < 0.05$) liver weight as percent of carcass weight; whereas, percent heart weight decreased with 30% HDSW (Table 4) compared with the respective controls. It is again the hexane extract that caused an increase in liver weight and a decrease in heart weight. This change in weight could be an indication on the presence of saponins and other toxic compounds that caused toxic effect on liver while decreasing heart weight could be influenced by a change in nutrient metabolism as a result of an insult on the liver. Data support that components of SW extracts have some toxic effect.

In conclusion, results showed an alteration in SW toxicity by ruminal digestion. Effects on blood serum constituents and feed intake were changed for digested compared with undigested SW extracts. The rumen environment may have an effect on altering SW toxicity to chemicals that have different effects on animal body.


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# EXPRESSION PATTERNS OF THE MEMBRANE PROGESTERONE RECEPTORS (α, β, AND γ) IN EARLY PREGNANCY

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**ABSTRACT:** Progesterone (P4) is required for maintenance of pregnancy and elicits physiological effects via binding to a nuclear receptor in various target tissues. Additionally, non-canonical membrane progesterone receptors (MPR) have been identified, and numerous isoforms have been identified, including MPR alpha (MPRA), beta (MPRB) and gamma (MPRG). The P4 signaling through the MPR has not been investigated in early pregnancy, and could effect initial maternal/fetal interactions. The objective of this study was to assess gene expression of MPR in maternal and fetal ovine tissues during early pregnancy via real-time quantitative PCR (qPCR). Hypothalamus (HYP), pituitary gland (PIT), and caruncle (CAR) were collected from non-pregnant (NP) ewes on d 10, and from pregnant ewes on d 20, 25, and 30 of gestation. Fetal tissue (FET) was also collected from pregnant ewes. RNA was isolated, cDNA synthesized and subjected to qPCR to determine MPR expression levels. Expression levels were normalized by standard methods, and subjected to ANOVA with Newman-Keuls post hoc test to determine significant differences ($P < 0.05$). In HYP, MPRA was highest expressed, compared with MPRG in PIT, MPRA and MPRG in CAR, and MPRB in FET. In HYP, no gestation-induced alterations in any MPR were observed, though MPRG tended ($P = 0.07$) to decrease with advancing pregnancy. All three MPRs decreased with advancing pregnancy compared with NP in the PIT. The MPRA decreased at early implantation (D20) and once attachment was established (D30) in CAR, where MPRG expression increased at early and mid-implantation (D25), but returned to basal levels by D30. No MPRB changes were observed in CAR. The MPRB decreased during mid-implantation, with D20 and D30 expressing similar levels. The expression of MPRA and MPRG was similar across all times assessed. During early onset of pregnancy, when P4 concentrations increase and fetal-maternal interactions are established, correlates with numerous tissue-specific alterations in MPR expression.

**Key words:** early pregnancy, membrane progesterone receptors, progesterone, sheep

## INTRODUCTION

Progesterone (P4) is critical in maintenance of pregnancy in all mammals. While the nuclear P4 receptor has been studied extensively, insight into the membrane P4 receptors (MPR) is lacking. Multiple isoforms of the MPR exist and are expressed in numerous species (Zhu et al., 2003a,b; Ashley et al., 20060. The identification of structurally distinct membrane receptors for progesterone underscores the diversity of how steroids can signal. These unique receptors, MPR alpha (MPRA), beta (MPRB) and gamma (MPRG), are seven transmembrane domain proteins that, upon binding progesterone or progestins (synthetic forms of P4), rapidly activate G-proteins (Thomas, 2008). Currently, the biological role(s) these receptors play in reproduction and pregnancy remains elusive. Our study aimed to characterize the expression of three MPR isoforms, alpha, beta and gamma in early pregnancy. We examined via real-time quantitative PCR, the gene expression of MPRA, MPRB, and MPRG in the hypothalamus, pituitary, and caruncle of sheep, comparing non-pregnant (d 10 of estrous cycle) and d 20, d 25, and d 30 pregnant ewes. Additionally, fetal membranes were analyzed on d 20, d 25, and d 30 for MPR expression.

## MATERIALS AND METHODS

All experimental procedures using animals were reviewed and approved by New Mexico State University Animal Care and Use Committee.

***Animals and Tissue Collection.*** Estrus was synchronized in Rambouillet-cross ewes during the mid to late luteal phase with two injections of dinoprost tromethamine (5 mg i.m.; Lutalyse; Pfizer, New York, NY) administered 4 h apart. Upon detection of estrus (d 0) with a vasectomized ram, ewes were placed into experimental groups. Ewes (n = 5/d) were anesthetized with sodium pentobarbital (20 mg/kg, i.v.) on either d 20, 25 or 30 of gestation and also from mid-luteal, nonpregnant (d 10 of the estrous cycle) control ewes. The reproductive tract was removed using a mid-ventral laparotomy, tissues were collected and snap frozen in liquid nitrogen, and stored at -80°C for subsequent RNA isolation. The ewes were then euthanized by exsanguination.

***RNA Isolation.*** Total RNA was extracted from maternal and fetal membrane tissue using Tri Reagent BD (Molecular Research Center Inc., Cincinnati, OH) according to manufacturer's directions. The RNA was eluted in RNAse-free water and treated with TURBO DNA-free kit (Ambion, Foster City, CA) to eliminate genomic DNA. The quantity and purity of RNA was determined using a Nanodrop ND-1000 spectrophotometer and subsequently stored at -80°C.

***Real-time Quantitative Polymerase Chain Reaction (qPCR).*** The cDNA was synthesized using the iScript cDNA

**Table 1.** Primer sequences utilized in quantitative real-time PCR analysis of cDNA.

| Target | Forward Primer | Reverse Primer |
|---|---|---|
| MPRA | 5'-GCAGGCCAAGTCTGAG TTCT-3' | 5'-GCAGGAAGATGGTCTG CACT-3' |
| MPRB | 5'-CTACCCGGTCATGAG GAAGA-3' | 5'-AGATGATCTGGAGGG TGTGG-3' |
| MPRG | 5'-ACACCTTCAGCTCCAT GTCC-3' | 5'-AGTCATGGAAGGTGG TGCTC-3' |
| GAPDH | 5'-TGACCCCTTCATTGAC CTTC-3 | 5'-CGTTCTCTGCCTTGAC TGTG-3' |

Synthesis Kit (BioRad, Hercules, CA) per manufacturer's instructions. The cDNA was subjected to real-time PCR to determine relative expression of our genes of interest (Table 1). Real-time PCR (qPCR) was performed using a CFX96 Touch Real-Time PCR Detection System (BioRad). Reactions included iQ SYBR Green supermix (BioRad), forward and reverse specific primers and cDNA. The specific primers employed are included in Table 1, and used at a final concentration of 525 nM each. The qPCR conditions were 95ºC for 3 min. followed by 40 cycles of 95ºC (30 s), 55ºC (30 s), and 72ºC (15 s). After PCR cycling, a melt curve was performed per manufacture's conditions. Expression of GAPDH did not change across days or pregnancy status and was used to normalize each target mRNA by using the ΔCq method (Schmittgen and Livak, 2008). Data are represented by graphing $2^{-\Delta Cq}$ values calculated for each gene of interest. For each mRNA target, the amplicon was sequenced to ensure that each gene of interest was correctly amplified. Amplification efficiencies were determined using a 10-fold dilution series of cDNA for each primer set and each amplified at 95 to 110% efficiency.

***Statistical Analysis.*** Data were subjected to ANOVA analysis appropriate for a completely randomized design. When a significant effect ($P < 0.05$) was detected, day means were separated using Newman-Keuls test on normalized Cq values using Prism (Version 5 from GraphPad Software, Inc., LaJolla, CA).

## RESULTS AND DISCUSSION

All MPR were detected in all tissues at each time point tested. In hypothalamus (HYP), MPRA was the most highly expressed MPR (Figure 1). No alterations in MPRA expression was detected based on progressing pregnancy compared with non-pregnant (NP). To date, the only data on MPRA in ovine HYP is evaluation during the estrous cycle, and similar to the current study, no significant alterations were noted (Ashley et al., 2009). However, MPRB expression decreased in HYP at d 30 compared with NP and MPRG expression also tended ($P = 0.07$) to decrease at d 30. Expression of all MPR was greatest in NP ewes compared with pregnant in the pituitary gland (PIT). The MPRG was the most highly expressed of the three isoforms in PIT, and abundance declined with advancing pregnancy (Figure 2). In parallel, MPRA and

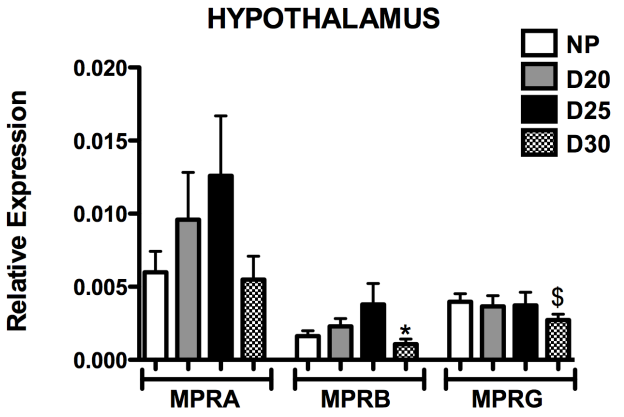


**Figure 1.** Expression of mRNA for membrane progesterone receptors (MPR) in the hypothalamus. Expression of MPR alpha (MPRA), beta (MPRB) and gamma (MPRG) were analyzed via real-time PCR in non-pregnant sheep (NP) on d 10 of the estrous cycle, and pregnant sheep on d 20, 25, or 30 of gestation. *$P < 0.05$ compared with NP. $^{\$}P = 0.07$ compared with NP.

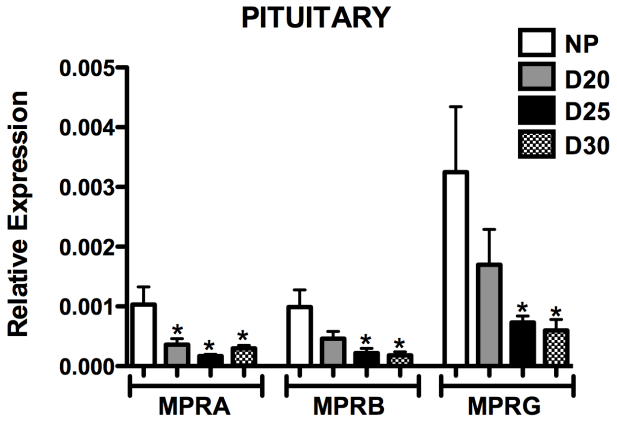


**Figure 2.** Expression of mRNA for membrane progesterone receptors (MPR) in the pituitary gland. The expression of MPR alpha (MPRA), beta (MPRB) and gamma (MPRG) were analyzed via real-time PCR in non-pregnant sheep (NP) on d 10 of the estrous cycle, and pregnant sheep on d 20, 25, or 30 of gestation. *$P < 0.05$ compared with NP.

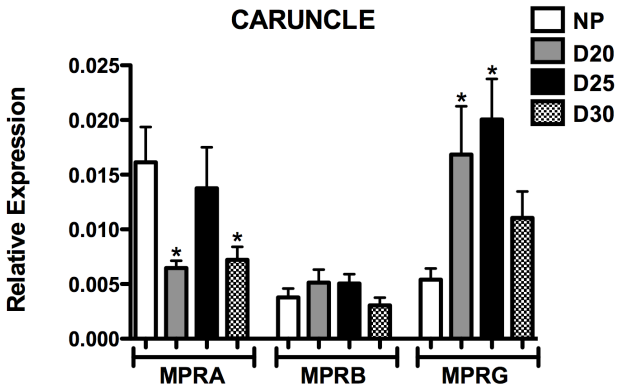


**Figure 3.** Expression of mRNA for membrane progesterone receptors (MPR) in the caruncle. The expression of MPR alpha (MPRA), beta (MPRB) and gamma (MPRG) were analyzed via real-time PCR in non-pregnant sheep (NP) on d 10 of the estrous cycle, and pregnant sheep on d 20, 25, or 30 of gestation. *$P < 0.05$ compared with NP.

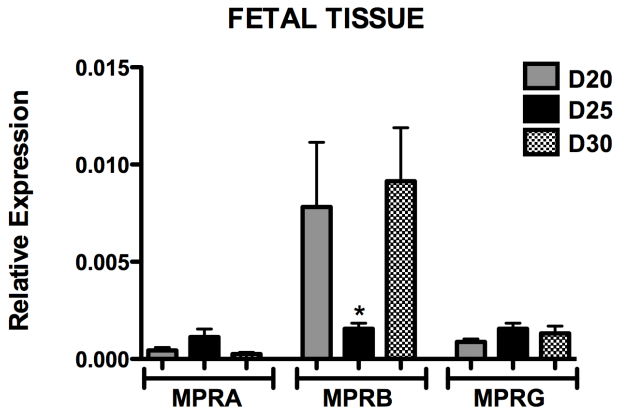


**Figure 4.** Expression of mRNA for membrane progesterone receptors (MPR) in fetal membranes. The expression of MPR alpha (MPRA), beta (MPRB) and gamma (MPRG) were analyzed via real-time PCR in fetal tissues isolated on d 20, 25, or 30 of gestation. * $P < 0.05$ compared with d 20.

MPRB expression also decreased as pregnancy advanced. The MPRA and MPRG were highly expressed in the caruncle (CAR; Figure 3). Abundance of MPRA was less at d 20 and d 30 of pregnancy compared with NP and d 25. The abundance of MPRB did not change with pregnancy in CAR, though MPRG abundance increased at d 20 and d 25 before returning to levels similar to NP by d 30. In fetal membranes (FM), MPRB was expressed at greater levels than the other two isoforms. FM MPRB declined at d 25 compared with d 20 and d 30 of gestation. No alterations in MPRA or MPRG were observed in FM.

The role(s) that MPR may play in early pregnancy are completely unknown, as are the tissues where effects may be elicited. Our study is one of the first to examine MPR in early pregnancy, and demonstrates altering expression based upon pregnancy in numerous tissues. Expression of MPR in HYP was unchanged during early implantation (d 20 and d 25; Figure 1). Once attachment was established (d 30), expression of MPRB significantly decreased, and MPRG tended ($P = 0.07$) to decline as well. MPRA expression was similar at all time points. The decline in PIT expression of all MPR during early implantation and placentation is intriguing, especially as P4 concentrations are increased during this time (Figure 2). It is possible that the effects of MPR in PIT, which remain unknown, are undesirable during early pregnancy hence down-regulation is warranted. Future mechanistic studies are required to determine what role MPR have in the HYPO and PIT to elucidate why down-regulation in these tissues occurs in the context of increased P4.

In CAR, MPRA decreased on d 20 and d 30 compared with NP, where on d 25, expression levels were similar (Figure 3). MPRG expression increased during implantation (d 20 and d 25), then returned to basal levels by d 30. MPRB expression remained unchanged. It is possible that altering expression of MPRA and MPRB, alone or in combination, serves to promote fetal attachment at the CAR. The FM tissue expressed lowered levels of MPRB only on d 25, and by d 30, expression returned to similar levels as on d 20. MPRA and MPRG expression remained unchanged in FM. The comparison of FM and CAR suggests specific, differing downstream signaling cascades are regulated by the MPR isoforms, and may be significant in regulating fetal attachment and subsequent placentation. More research is required to delineate these signaling events to determine physiological significance.

Since the discovery of MPR in 2003, research into their biological functions has grown tremendously, with a majority of studies completed in cell lines. As the first MPR identified, MPRA has been studied more than MPRB and MPRG and is associated with many biological functions such as oocyte maturation, stimulation of sperm hypermotility, down-regulation of GnRH secretion, alterations in T cell functions and myometrial contractility in humans (Dressing et al., 2011). However, the physiological function in ruminants is not known. Characterizing fluctuations in expression of these MPRs may aid in helping to define their biological roles. While further studies are required to characterize the actions of the MPR, the expression of these atypical P4 receptors in numerous reproductive tissues provides an exciting venue for research into mediation of their effects.

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# FACTORS AFFECTING SERUM IGF-I AND TRIIODOTHYRONINE CONCENTRATIONS AS RELATED TO FAT DEPOSITION IN FEEDLOT LAMBS

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**ABSTRACT**: To identify factors affecting serum IGF-I and T3 concentrations after weaning and their relationship to fat deposition, 115 $F_1$ lambs (males and females) weaned at 90 d (BW = 17 ± 3.7 kg), sired by Charollais (CH), Dorper (DP), Hampshire (HM), Suffolk (SF) and Texel (TX) rams bred to estrus synchronized-Pelibuey (PB) and Blackbelly (BB) ewes, were utilized. Lambs were individually fed an ad libitum mixed ration in 80 pens (1.25 x 2.45 m) and 35 stalls (0.5 x 1.45 m) and weighed every 14 d until they reached a minimum BW of 42 kg for males and 40 kg for females. Serum concentrations of IGF-I and triiodothyronine (T3) at d 14, 42, and 70 of the feeding trial were quantified by RIA. Log transformed hormone concentrations were analyzed with PROC MIXED of SAS, fitting a linear model with fixed effects of breed of sire (SB), breed of dam (DB), sex, number weaned, management (pen vs. stall), day of blood sampling, 2-way interactions, and BW at sampling as first and second order covariates. Sire within SB was fitted as a random effect and repeated measures within animal were assumed correlated with an ARH(1) covariance structure. There were management and sex by day and SB by DB interactions ($P < 0.05$) for IGF-I. Concentrations of IGF-I were greater ($P < 0.05$) for lambs in pens than for lambs in stalls later on the trial and increased ($P < 0.05$) with time for males but not for females. Lambs from the CH x PB cross had the least ($P < 0.05$) IGF-I concentrations. Pearson correlations ($P < 0.05$) for IGF-I at d 14, 42, and 70 were -0.42, -0.47 and -0.42 with backfat; -0.54, -0.65 and -0.51 with kidney fat (KF); and -0.36, -0.43 and -0.26 with percent of total carcass fat (PTCF). There were day main effects and sex by management and sex by SB interactions effects ($P < 0.05$) for T3. Mean T3 was greatest ($P < 0.05$) at d 70 and was greater ($P < 0.05$) in females than in males in stalls but not in pens. Also, T3 concentrations were greater ($P < 0.05$) in females than in males for the CH, DP, and SF sired breeds, but not for HM and TX. Pearson correlations for T3 were important ($P < 0.05$) only at d 70 with KF (0.23) and at d 14 and 70 with PTCF (-0.40 and -0.21). Serum IGF-I concentrations in lambs, more than T3, were related to fat deposition as affected by sex, breed, management and time on feed.

**Key words:** insulin-like growth factor-I, sheep breeds, triiodothyronine

## INTRODUCTION

In addition to age, sex, genotype, level of feeding and some other factors, metabolic hormones influence growth and body composition. Insulin-like growth factor-I is involved in postnatal growth through anabolic actions of growth hormone (Liu and LeRoith, 1999) and its effects on longitudinal bone, muscle, and cartilage growth (Duclos et al., 1999; Zapf and Froesh, 1999; Yakar et al., 2002). Polymorphisms in the bovine (Ge et al., 2001) and swine (Estany et al., 2007) *IGF-I* gene are associated with circulating IGF-I concentrations, growth traits, and fatness.

Triiodothyronine (**T3**) and thyroxine (**T4**), along with IGF-I, are also important in normal growth and development of animals. Storer et al. (2005) reported that preweaning concentrations of T3 and T4 appear to be related to growth patterns in lambs. It is known that a positive association exists between the thyroid hormones and weaning weight (García et al., 2005). Strath et al. (1982) showed that serum T3 concentrations are significantly influenced by the degree of phenotypic expression of the double-muscling in cattle, with greater concentrations in the phenotypically extreme muscled animals compared with the phenotypically normal ones. Ayala et al. (2007) reported significant canonical correlations of serum concentrations of IGF-I, T3, and T4 in lambs with their postweaning growth and carcass traits. In a preliminary study, Jiang et al. (2012) found polymorphisms in porcine *TRH* and *TRH receptor* genes to be associated with growth and fatness traits.

Given the role that metabolic hormones play in growth and features of growth and their potential use and manipulation in lamb production systems through marker assisted selection (van der Werf et al., 2007) and programmed nutrition (Desai et al., 2005), it is important to have a good understanding of the factors and conditions that affect their variability in serum concentrations. So, the objective of the present research was to study a multiple set of factors that could influence serum concentrations of IGF-I and T3 during the postweaning feeding period of terminal crossbred lambs and their relation to different indicators of fat deposition.

## MATERIALS AND METHODS

***Animals.*** One hundred and fifteen $F_1$ lambs (males and females) weaned at 90 d (BW = 17 ± 3.7 kg), sired by 5 Charollais (**CH**), 5 Dorper (**DP**), 4 Hampshire (**HM**), 5 Suffolk

(**SF**) and 5 Texel (**TX**) rams bred to estrus synchronized-Pelibuey (**PB**) and Blackbelly (**BB**) ewes, were utilized. From a total crop of 218 lambs weaned, corresponding to the synchronized and following estrous cycles, 12 females and 12 males per sire breed (**SB**) were selected from the central part of the normal distribution, except for DP and HM with 2 females less each and SF with 1 male less. Previous to the performance test, lambs received a vitamin E/Se injection and were vaccinated and treated for internal and external parasites (ivermectin + A, D and E vitamins). Lambs were individually allocated in 80 pens (1.25 x 2.45 m) and 35 stalls (0.5 x 1.45 m), fed an ad libitum mixed ration that, on average for the first 70 d, included corn (46 %), alfalfa hay (35 %), dry distillery grain (4.5 %), soybean meal (4.5 %), bypass fat (1.6 %), cane molasses (1.2 %), cotton seed meal (4.5 % ), wheat bran (1.5 %), salt (0.4 %), and minerals premix (0.8 %). and weighed every 14 d until they reached a minimum BW of 42 kg for males and 40 kg for females. Kidney fat was weighed at slaughter and backfat measured between the 12 and 13$^{th}$ rib of the chilled carcass. Four half carcasses from each sex and SB were dissected to estimate their composition (lean, fat, bone and other tissues).

***Blood Collection.*** Lambs were fasted 12 h before bleeding. Blood samples were collected every 14 d by jugular venipuncture. All samples were collected into 10-mL vacuum serum separator tubes which were allowed to stand at room temperature for 30 min before centrifugation at 4°C for 15 min at 1,200 x *g*. Serum was then transferred to plastic vials and stored frozen until analyzed.

***Hormone Analysis.*** Serum concentrations of IGF-I and T3 at d 14, 42, and 70 of the feeding trial were quantified by RIA as described by Berrie et al. (1995) and Wells et al. (2003), respectively. Within and between assay coefficient of variation for IGF-I and $T_3$ was 9 and 6 % or less, respectively.

***Statistical Analysis.*** Log transformed hormone concentrations were analyzed with PROC MIXED (SAS Inst. Inc., Cary, NC), fitting a linear model with fixed effects of breed of sire (SB), breed of dam (**DB**), sex, number weaned, management (pen vs. stall), day of blood sampling, 2-way interactions, and BW at sampling as first and second order covariates. Sire within SB was fitted as a random effect and repeated measures within animal were assumed correlated with an ARH(1) covariance structure. Pearson correlation coefficients among serum concentrations of hormones and fat measures were estimated.

## RESULTS

There were management and sex by day and SB by DB interactions ($P < 0.05$) for IGF-I. Concentrations of IGF-I increased more ($P < 0.05$) with time for lambs in pens than in stalls (Figure 1a), and they increased ($P < 0.05$) with time in the case of males but not in the case of females (Figure 1b). Lambs from the CH x PB cross had the least ($P < 0.05$) IGF-I concentrations compared with the rest of the crosses evaluated (Figure 2).

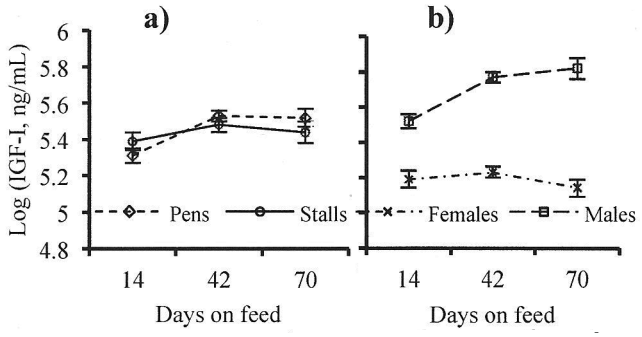


**Figure 1.** Least squares means (± SE) for the log of the IGF-I concentrations in lambs, according to a) management group and days on feed, and b) sex and days on feed.

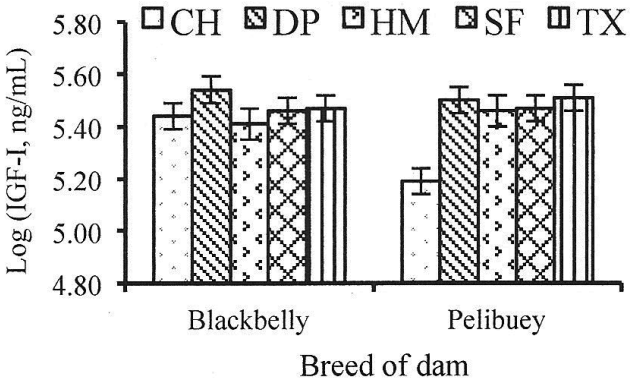


**Figure 2.** Least squares means (± SE) for the log of the IGF-I concentrations in lambs, according to sire breed (CH = Charollais, DP = Dorper, HM = Hampshire, SF = Suffolk, TX = Texel) and dam breed.

Pearson correlations ($P < 0.05$) for IGF-I at d 14, 42, and 70 were -0.42, -0.47, and -0.42 with back fat; -0.54, -0.65, and -0.51 with kidney fat (**KF**); and -0.36, -0.43, and -0.26 with percent of total carcass fat (**PTCF**). There were day main effects and sex by management and sex by SB interactions effects ($P < 0.05$) for T3. Mean was greatest ($P < 0.05$; Figure 3a) at d 70 and was greater ($P < 0.05$; Figure 3b) in females than in males in stalls but not in pens. Serum concentrations of T3 were greater ($P < 0.05$) in females than in males for the CH, DP and SF sired breeds, but not for HM and TX.

Pearson correlations for T3 were important ($P < 0.05$) only at d 70 with KF (0.23) and at d 14 and 70 with PTCF (-0.40 and -0.21).

## DISCUSSION

Although the statistical model used to analyze serum concentrations in the present study did adjust for BW at time of blood sample collection, results without including BW in the model were about the same (data not shown). For IGF-I, it is clear that the effects of the factors studied, and that were statistically significant, were positively related to the degree of leanness and negatively related to the degree of fatness,

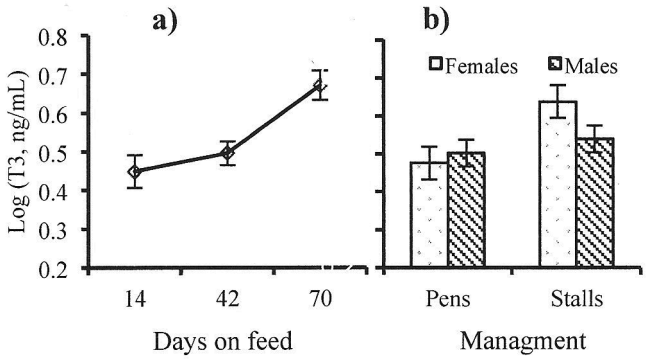


**Figure 3.** Least squares means (± SE) for the log of the T3 concentrations in lambs, according to a) days on feed, and b) sex and management.

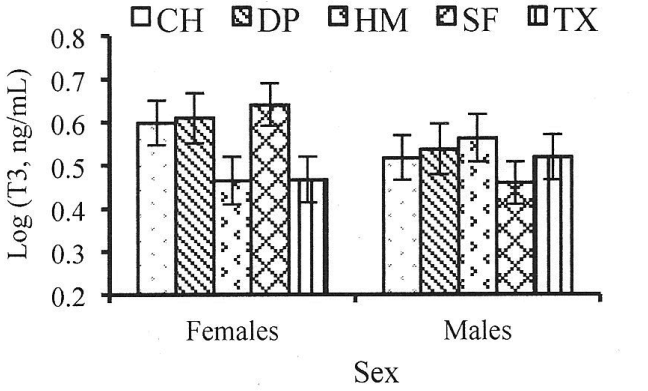


**Figure 4.** Least squares means (± SE) for the log of the T3 concentrations in lambs, according to sire breed (CH = Charollais, DP = Dorper, HM = Hampshire, SF = Suffolk, TX = Texel) and sex.

but the effect of one factor was conditioned on other factors. For instance, serum concentrations of IGF-I increased with days on feed, but only for lambs in pens and for males, which were the ones with less fat deposition (data not shown). The same was true for breed effects. Lambs sired by Charollais rams tended to deposit more fat, although the effects were not statistically significant and were not conditioned on dam breed. All these effects are reflected in the negative correlation coefficients between IGF-I concentration and fat deposition indicators, from -0.26 to -0.65. Studies in pigs (Owens et al., 1999; Therkildsen et al., 2004) have shown that the serum IGF-I concentrations are positively related to and can explain around 35 to 40 % of the variation in BW and cross-sectional area of *longissimus dorsi* at d 90 of age; however, at d 140 of age could only explain 10 to 15 % of the variation in performance.

Even though IGF-I is known to play a role in many cell types, a great proportion of its study has focused on muscle development and growth in meat-producing animals (Oksbjer et al., 2004). During the last decades, various clinical and experimental observations have indicated the importance of the somatotropic axis in the regulation of body mass and adipose tissue growth and development (Etherton and Bauman, 1998; Etherton, 2000). Expression of IGF-I mRNA in cultured preadipocytes is increased by GH, but despite a high expression, its function in adipose tissue, and especially in adipocytes, is not fully understood (Louveau and Gondret, 2004). The effect of IGF-I seems to involve decreased serum insulin levels rather than direct effects (Frick et al., 2000). Louveau and Gondret (2004) suggested a possible antagonist relationship between IGF-I and leptin.

Cameron (1992) analyzed correlated responses in physiological traits in rams of lines of Texel-Oxford sheep selected for high or low carcass lean content and the lean line had greater serum concentrations of IGF-I at the end of the performance test (20 wk of age), although glucose and IGF-I decreased in response to fasting for 56 h. He suggested that the lean line preferentially synthesized protein rather than depositing fat during normal feeding, and when the animals were fasted, there could be relatively greater use of fat as energy source in the lean line, rather than using products from protein catabolism as glucose precursors, given that concentrations of β-hidroxybutirate, NEFA, triglycerides, creatinine, and urea increased.

Regarding the increased T3 concentrations with days on feed, Kahl and Bitman (1983) reported that T3 concentrations increased gradually (38 and 96 %) in both sexes of Holstein calves during 6 to 22 wk, but they saw that oncentrations were greater for males than for females and that were positively correlated with BW during that initial growth period. This was not the case for the effect of sex in the present study. Levels of T3 were greater for females than for males when sired by CH, DP, and SF rams, and when they were in stalls but not in pens. This last situation could be due to the limited space within stalls. Bowers et al. (1993) reported that confinement of wether lambs in metabolism stalls (41 x 95 cm) compared with pasture, increased adrenal function, T4 and motivation for movement, but not T3 serum concentrations. In our study, lambs were involuntarily subjected to nutritional stress during the prenatal and preweaning periods, which could be a factor (Desai et al., 2005) in differences observed with respect to other studies.

## IMPLICATIONS

Serum IGF-I concentrations in lambs, more than T3, were related to fat deposition as affected by sex, breed, management, and time on feed. These results should encourage more studies in sheep to determine genetic and phenotypic variation in the IGF system in order to find alternative selection criteria to ensure a harmonic increase in performance traits and, at the same time, meat quality.

## ACKNOWLEDGMENTS

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# HEIFERS WITH LOW ANTRAL FOLLICLE COUNTS HAVE LOW BIRTH WEIGHTS AND PRODUCE PROGENY WITH LOW BIRTH WEIGHTS

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**ABSTRACT:** Research has demonstrated that heifers with increased numbers of ovarian antral follicle count (AFC) have improved hormonal profiles and improved fertility. Interestingly, heifers with low AFC had lower birth weights, suggesting that genes influencing growth and development also influence the development of the reproductive tract and establishment of the ovarian reserve. To determine the relationship of AFC to heifer BW, reproductive tract characteristics, and first calf performance, composite (MARC III x Red Angus) heifers (n = 362) were used over a 3 yr period. In yr 1 heifers grazed winter range or corn residue and were offered no supplement or 0.45- 0.90 kg/d (31% CP, DM Basis) during development. In yr 2 and 3 heifers grazed winter range and were fed a dried distillers grain-based (DDG) or corn gluten feed-based supplement offered at 0.59% and 0.78% BW, respectively, throughout development. Supplements were formulated to be isocaloric but differed in undegradable protein. All heifers in yr 2 and 3 were fed ad libitum meadow hay while grazing dormant pasture. Prior to breeding, heifers were transrectally ultrasounded to determine AFC and classified as high (≥ 26 follicles; HIGH) moderate (16 to 25; MOD) or low (≤ 15; LOW). There was no diet x AFC classification interaction. HIGH heifers have greater ($P \leq 0.05$) birth, weaning, and adjusted 205-d weaning BW compared with LOW. Pre-breeding BW, total AFC, and proportion of mature BW at breeding were greater ($P < 0.01$) for HIGH compared with LOW heifers. Overall pregnancy rate was similar ($P = 0.36$) among AFC classifications. Progeny birth BW was greater (P = 0.03) for calves born to HIGH compared with LOW heifers. Taken together these data indicate a relationship between AFC and BW through the first breeding season and progeny calf BW. The low birth BW in heifers with low AFC and in their progeny continues to support a possible link between genes that influence growth and development and establishment of the ovarian reserve. USDA is an equal opportunity provider and employer.

**Key words**: antral follicle count, beef, progeny

## INTRODUCTION

New born beef ovaries contain between 10,000 to 350,000 healthy follicles and the number decreases approximately 20% within the first year of life (Erickson, 1966). Longevity of a beef cow is related to reproductive success (Cushman et al., 2009) and thus cows with smaller ovarian reserve may deplete their ovarian reserve sooner resulting in earlier removal of a cow from the herd.

Size of the ovarian reserve has been predicted via ultrasonography and recorded as antral follicle count (**AFC**; Ireland et al., 2008). The size of the ovarian reserve has been correlated with fertility with low AFC heifers having reduced pregnancy rates compared with high AFC heifers (Cushman et al., 2009). Furthermore, maternal diet can impact progeny ovarian reserve. Initial reports indicated a correlation between birth weight and ovarian reserve in sheep (Da Silva et al., 2002, 2003); however, recent reports demonstrate maternal diet can influence ovarian reserve without affecting birth BW in heifers (Mossa et al., 2009; Sullivan et al., 2009). The objective of this study was to determine the relationship between AFC and heifer BW, reproductive characteristics and first calf performance.

## MATERIALS AND METHODS

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in this experiment.

Weaned MARC III (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgauer) x Red Angus (n = 264; yr 1 = 91; yr 2 = 90; yr 3 = 83) were utilized in this experiment. Heifers grazed a common fall pasture and were offered 2.0 kg/d (10.5% CP, DM basis) supplement for 30 prior to the initiation of winter development treatment. In yr 1 heifers were randomly assigned to either graze corn residue or dormant winter range from mid-November through mid-February. Heifers were offered 0.45 to 0.90 kg/d (31% CP, DM basis) supplement while grazing corn residue or dormant winter range. After the 119 d treatment period heifers were placed in a common group on dormant forage pastures and grazed for approximately 100 d until the initiation of the breeding season. Heifers were offered 0.45 kg/d protein supplement during the 100 d grazing period. If weather impeded grazing, heifers were offered free-choice brome hay with CR heifers consuming 4.2 kg/d and WR heifers consuming 3.5 kg/d.

In yr 2 and 3 heifers were randomly assigned to 1 of 2 groups and received either a dried distillers grain based or corn gluten feed based supplement offered at 0.59% (27%

CP, DM) and 0.78% BW (20% CP, DM), respectively, from mid-November through May. Supplements were formulated to be isocaloric but differed in rumen undegradable protein. All heifers were fed ad libitum meadow hay through winter while grazing dormant pasture.

Prior to breeding, heifers underwent transrectal ultrasonography. A single technician scanned each ovary using an Aloka-500 linear array transrectal probe (7.5-MHZ transducer, Aloka Ultrasound, Wallingford, CT) and counted small (3 to 5 mm), medium (6 to 10 mm), and large (> 10 mm) follicles. Follicles counted on each ovary were summed to determine AFC. Heifers were placed in an AFC classification based on AFC and considered low (≤ 15 follicles; **LOW**), moderate (16 to 25 follicles; **MOD**), or high (≥ 26 follicles, **HIGH**). Uterine horn diameter, presence of CL, and ovarian length and height were also determined. Each heifer received a RTS based on the methods reported by Martin et al. (1992).

Estrus was synchronized with two injections of PGF (Lutalyse, Pfizer Animal Health, New York, NY) administered 14 d apart. Estrus detection was performed 5 d following the second injection. Heifers observed in estrus were artificially inseminated approximately 12 h after initial estrus detection. Approximately 10 d after AI heifers were placed with fertile bulls for 45 d. In yr 1 due to poor response of synchronization, all heifers not AI were injected with PGF 10 d after the second injection was administered to resynchronize estrus. Conception rates for both AI and total pregnancy rates were performed via rectal palpation approximately 45 d following AI and bull removal, respectively.

***Statistical Analysis.*** Data were analyzed using the MIXED and GLIMMIX procedures (SAS Inst. Inc., Cary, N.C.). Initial analysis included AFC classification and development treatment as fixed effects and yr and age as random effects. The AFC classification x development treatment interaction was not significant and was removed from the model. Progeny calf data model included maternal AFC classification as the fixed effect and calf sex and year as random effects. A *P*-value of ≤ 0.05 was considered significant.

## RESULTS AND DISCUSSION

Data for the effect of heifer AFC classification on BW, ADG, and reproductive characteristics and performance are reported in Table 1. High AFC heifers had greater ($P$ = 0.04) birth BW compared with LOW heifers (36.2 vs. 34.4 ± 0.6 kg). These data agree with Cushman et al. (2009) reporting an approximate 3 kg increase in birth BW for HIGH compared with LOW heifers. Weaning BW was 13 kg (± 5.1 kg) greater ($P$ < 0.01) for HIGH compared with LOW heifers. Furthermore, when adjusting for age by calculating weaning BW based on a 205-d adjustment, BW remained greater ($P$ = 0.02) for HIGH compared with LOW heifers. Body weight was greater ($P$ < 0.01) at pre-breeding for HIGH compared with MOD and LOW heifers; however, at pregnancy diagnosis after summer grazing, BW was similar ($P$ = 0.77) between AFC classifications. Previous literature regarding the relationship of birth weight and ovarian reserve has been reported in sheep and cattle (Da Silva et al., 2002, 2003; Cushman et al., 2009). However, these studies did not demonstrate a relationship between ovarian reserve and BW at weaning or prebreeding as is reported in the current study. Although not correlating ovarian reserve and BW, Silva et al. (2006) did report a genetic correlation of 0.15 for cow stayability and 550 d BW in Nelore cows.

Average daily gain was greater ($P$ = 0.02) for HIGH heifers compared with LOW heifers prior to weaning (1.03 vs. 0.98 ± 0.03 kg/d). Furthermore, average daily gain during the same period adjusted for 205-d weaning remained greater ($P$ = 0.04) for HIGH compared with LOW heifers (1.08 vs. 1.03 ± 0.04 kg/d). Postweaning ADG to pre-breeding tended ($P$ = 0.08) to be greater for HIGH compared with LOW heifers.

Reproductive tract score, proportion of heifers with a CL present at AFC, and AI pregnancy rates did not differ ($P$ > 0.30) among AFC classifications. In addition, overall pregnancy rates, although not significant, had a tendency ($P$ = 0.15) to be approximately 9% greater for HIGH compared with MOD and LOW heifers. Our data are similar to previous reports of a significant increase in overall pregnancy rates for HIGH compared with LOW heifers (Cushman et al., 2009). In that study 406 heifers were AFC classified and HIGH and LOW heifers were utilized with approximately 180 HIGH and 85 LOW heifers. The current study had fewer heifers available (n = 264) thus, this may be the difference in ability to detect significance in the current study.

At calving, HIGH heifers gave birth to larger ($P$ = 0.02) calves compared with LOW heifers (Table 1). However, the effect of maternal AFC on calf birth weight appears to be sex specific as there was no difference is birth weight to bull calves born to heifers regardless of AFC classification (not reported). However, heifer calves born to HIGH heifers had a 3 kg increase ($P$ < 0.01) in birth BW compared with MOD and LOW heifers (not reported). Birth weight has been reported to impact survivability in several species with reduced birth BW causing increased mortality rates (Moule, 1956; McCormick, 1985; Bellows et al., 1987). Too great of an increase in birth weight (dystocia) has more commonly been the cause of early death in beef calves than reduced weight (Bellows et al., 1987).

Potential longevity and profitability of a heifer is related to the time of first calving. Furthermore, heifers that calve within the first 21 d of the calving season are likely to remain in the herd longer that heifers calving in the second or later 2-d periods (Kill et al., 2012). There was no difference ($P$ = 0.74) in Julian calving date among classification groups and a similar ($P$ = 0.57) proportion of heifers calved in the first 21 d of the calving season for each AFC classification in the current study.

## IMPLICATIONS

Profitability of a beef cow-calf producer is related to longevity of cows, with most cows leaving the herd due

**Table 1.** Effect of Antral follicle count[1] (AFC) classification on heifer BW, ADG, and reproductive performance

| Item | HIGH | MOD | LOW | SEM | *P*-value |
|---|---|---|---|---|---|
| n | 103 | 113 | 48 | | |
| Birth BW, kg | 36.2 [a] | 34.9[a,b] | 34.4[b] | 0.6 | 0.04 |
| Weaning BW, kg | 237[a] | 235[a,b] | 224[b] | 5.1 | < 0.01 |
| Adjusted 205-d BW, kg | 256[a] | 251[a,b] | 246[b] | 7.5 | 0.02 |
| Initial Development BW, kg | 255[a] | 247[a,b] | 238[b] | 3.4 | < 0.01 |
| Pre-breeding BW, kg | 386[a] | 367[b] | 363[b] | 7.1 | < 0.01 |
| Preweaning ADG, kg/d | 1.03[a] | 1.00[a,b] | 0.98[b] | 0.03 | 0.02 |
| Adjusted preweaning ADG, kg/d | 1.08[a] | 1.05[a,b] | 1.03[b] | 0.04 | 0.04 |
| Postweaning ADG, kg/d | 0.63 | 0.58 | 0.60 | 0.02 | 0.08 |
| RTS[2] | 4.27 | 4.29 | 4.12 | 0.17 | 0.24 |
| AFC | 32.5[a] | 20.4[b] | 12.3[c] | 0.90 | < 0.01 |
| Pregnancy diagnosis BW, kg | 451 | 447 | 445 | 17.1 | 0.77 |
| CL present, % | 20.1 | 28.8 | 24.5 | 17.13 | 0.30 |
| Mature BW at Breeding, % | 65.9[a] | 62.9[b] | 62.1[b] | 0.09 | < 0.01 |
| AI conception rate, % | 62.5 | 68.9 | 67.5 | 11.17 | 0.78 |
| Pregnancy rate, % | 96.1 | 89.4 | 89.4 | 3.95 | 0.15 |
| Calving results | | | | | |
| calf birth BW, kg | 36.0[a] | 35.3[a,b] | 33.9[b] | 1.4 | 0.02 |
| calving date, Julian | 84 | 82 | 83 | 3.4 | 0.74 |
| calved first 21-d, % | 77.9 | 75.9 | 67.8 | 8.6 | 0.57 |
| calf weaning BW, kg | 216 | 217 | 208 | 11.3 | 0.30 |
| calf adjusted 205-d BW, kg | 244 | 243 | 236 | 10.8 | 0.29 |

[a-b]Means with different superscripts differ P ≤ 0.05.
[1]Heifer AFC determined via ultrasonography 1 mo prior to breeding season; HIGH ≥ 26 follicles; MOD 16-25 follicles; LOW ≤ 15follicles (Adapted from Ireland et al. 2008).
[2]RTS= reproductive tract score (Martin et al., 1992).

to reproductive failure. Selecting heifers with increased reproductive characteristics such as high AFC has been reported to increase pregnancy rates. We report high AFC heifers have increased BW through pre-breeding, improved ADG prior to development, and give birth to larger heifer calves compared with low AFC heifers. Taken together these data indicate a relationship between AFC and BW through the first breeding season and progeny calf BW. The low birth BW in heifers with low AFC and in their progeny continues to support a possible link between genes that influence growth and development and establishment of the ovarian reserve.

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# OVIDUCTAL PROTEIN AND OVARIAN HORMONE CONCENTRATIONS DURING THE FIRST FIVE DAYS OF THE ESTROUS CYCLE IN FIRST AND THIRD ESTROUS EWE LAMBS AND MATURE EWES [1]

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**ABSTRACT:** The objectives of this study were to determine if ampullary (AMP) and isthmic (IST) protein concentrations patterns, and progesterone (P4) and estradiol (E2) concentrations patterns differ over the first five days of the estrous cycle among first (pubertal; FE) and third estrous (TE) ewe lambs and mature ewes (ME). Crossbred, spring-born ewe lambs (n = 40) and mature (4- to 6-yr-old; n = 20) were assigned randomly at estrus to treatments arranged in a 3 (cycle type; CT) x 5 (day of cycle) factorial (n = 4 ewes per treatment). Observation of estrus occurred twice daily with the aid of mature, epididymectomized rams beginning in October. Each ewe was bi-laterally salpingectomized on either Day 0 (estrus), 1, 2, 3 or 4 after estrus. A jugular blood sample was collected from each ewe immediately before surgery on these days. Right and left AMP and IST segments were flushed with 4 and 2 mL of Delbecco's PBS (pH = 7.2), respectively. PMSF was added to a final concentration of 10 mM to each flushing and flushings were flash frozen in liquid N2. Protein in flushings was assayed using the BCA method (Pierce, Rockford, IL). Serum samples were assayed for P4 and E2 concentrations by RIA. There was an interaction ($P < 0.01$) between CT and day of cycle for AMP and IST protein concentrations. These interactions were caused by greater ($P < 0.05$) protein concentrations in the AMP and IST of ME than in FE ewes on Day 1 and lower ($P < 0.05$) concentrations in ME than in FE ewes on Day 4. There was an interaction ($P < 0.01$) between CT and day of cycle for P4 and E2 concentrations and P4:E2 ratios. Progesterone increased ($P < 0.05$) more rapidly from D 2 to 4 in ME than in FE and TE ewes. Estradiol increased ($P < 0.05$) from D 2 to 3 more rapidly in ME than in FE and TE ewes; whereas, E2 deceased ($P < 0.05$) from D 3 to 4 in ME and TE ewes, while E2 increased ($P < 0.05$) from D 2 to 4 in FE ewes. Progesterone:estradiol ratios increased ($P < 0.05$) from D 2 to 4 in ME and TE ewes than in FE ewes. In conclusion, concentration patterns of AMP and IST protein and ovarian steroid hormones during the first 5 d of the estrous cycle differ between FE and ME ewes. These results indicate the possibility that reduced fertility in ewe lambs at their first estrus may be caused by an inappropriate protein milieu in the AMP and IST that may be detrimental to normal early embryonic development.

**Key words:** ewe lambs, ovarian steroids, oviductal protein

## INTRODUCTION

Failure of young female ruminants to become pregnant early in their lifetime can reduce reproductive efficiency of production systems (Short et al., 1994). Fertility associated with breeding at puberty (first estrus) is significantly lower than that of breeding at a later estrus in cattle (Byerley et al., 1987) and sheep (Hare and Bryant, 1985).

The oviduct plays an important role in the early events that are required for the establishment of pregnancy. It is the site of gamete transport, sperm capacitation, fertilization, and early embryonic development. The oviduct provides the optimum medium for these processes to occur (Harper, 1994). The possibility that the oviduct and its secretions may contribute to less than normal fertility rates associated with breeding at puberty in sheep or other economically important domestic species has not been addressed.

In a preliminary study involving a few animal we found oviductal protein concentrations were lower in first estrous ewe lambs that in third estrous ewes, and mature ewes 24 to 30 h and during the first 72 h after estrus (Berardinelli and Adair, 2000). Whether this difference is maintained over the first 5 d of the cycle, the period during which an embryo is present in the oviduct, is not known. Therefore, in this study that includes more animals we evaluated patterns of ampullary (**AMP**) and isthmic (**IST**) protein concentrations, and progesterone (**P4**) and estradiol (**E2**) concentrations during the first 5 d of the estrous cycle in first (pubertal; **FE**) and third estrous (**TE**) ewe lambs and mature ewes (**ME**). Specifically, we tested the hypotheses that AMP and IST protein concentration patterns, and P4 and E2 concentration patterns do not differ among FE and TE ewe lambs and ME ewes over the first five days of the estrous cycle.

## MATERIALS AND METHODS

Animals were handled and cared for according to a protocol approved by the Montana State University Large Animal Care and Use Committee.

***Animals and Treatments.*** Two trials were conducted in consecutive years. Western white-faces, crossbred ewe lambs

[1] This study was supported by the NRI Competitive Grant no. 94-04277 from the USDA National Institute of Food and Agriculture the Montana Agric. Exp. Sta.

born mid to late April and mature ewes (4 to 6 yr of age) were used in both trials conducted at Fort Ellis Sheep Research Station, Bozeman, MT in late October and early November. Ewes and ewe lambs were maintained in single pasture, fed mixed-grass hay ad libitum, and were given free access to water and a vitamin-mineral supplement.

In both trials, ewe lambs and mature ewes were observed for behavioral estrus twice daily with the aid of mature, epididymectomized rams. Ewe lambs and mature ewes were randomly assigned to treatment as they exhibited estrus in a 3 X 5 factorial arrangement as shown in Table 1.

Mature ewes and ewes lambs that were assigned to TE had to have shown at least one estrous cycle of normal length (16 to 18 d). Once a FE or TE ewe lamb or a ME ewe exhibited estrus she was assigned randomly to a d of surgery. First estrus or TE ewe lambs and ME ewes that were assigned to be salpingectomized on D 0 (estrus) were immediately removed from feed and water. Whereas, FE and TE ewes lambs and ME ewes that were assigned to salpingectomy of D 1, 2, 3 or 4 were removed from feed and water 12 to 20 h before surgery.

***Salpingectomy and Oviduct Processing.*** Bi-lateral salpingectomies were preformed aseptically via mid-ventral laparotomy under halothane anesthesia. The reproductive tract of each ewe was exposed and the ovaries examined for the presence of corpora lutea, corpora hemorrhagica, corpora albicantia, and large antral follicles (> 10 mm in diameter). Ovaries of each FE and TE ewe lambs and ME ewes contained at least one CA, a regressing CL, and either a CH or a LAF in the process of ovulating.

Immediately before removal of an oviduct, ligatures were placed at the utero-tubal, isthmic-ampullary, and ampullary-infundibular junctions of each oviduct to prevent migration of fluids between segments of the oviduct. After removal, each oviduct was trimmed of connective tissue, measured for length, and weighed. Ampullary and isthmic portions of each oviduct were flushed with 4 mL and 2 mL, respectively, of Delbecco's PBS (pH = 7.2). Flushings from the AMP and IST were passed through a 0.45 μm filter into 12 x 75 mm cryogenic tubes. Filtration of flushings was necessary to removal cellular debris associated with the flushing process. PMSF was added to a final concentration of 10 mM to each flushing and flushings were flash frozen in liquid N2. Flushings were and stored at –80° C until assayed for protein content and concentration.

**Table 1.** Experimental design, arrangement of treatment and number of ewe lambs and each per treatment in both trials

| Day of cycle | First estrus (FE) | Third estrus (TE) | Mature estrus (ME) |
|---|---|---|---|
| 0 | 6 | 6 | 6 |
| 1 | 6 | 6 | 6 |
| 2 | 6 | 6 | 6 |
| 3 | 6 | 6 | 6 |
| 4 | 6 | 6 | 6 |

***Blood Sampling.*** A jugular venous blood sample (10 mL) was collected by venipuncture from each ewe immediately before removal of the oviducts during surgery. Samples were placed on ice, allowed to clot at 4° C overnight and centrifuged at 1,850 x *g* for 30 min. Serum was decanted into 12 mm x 75 mm plastic culture tubes, capped and stored at -22° C, until assayed for P4 and estradiol-17β (E2).

***Oviductal Protein Assays and Concentrations.*** Flushings were thawed at 4° C overnight. The quantity of fluid in each cryotube was measured volumetrically. Protein concentrations in 100 μL of each AMP and IST flushing were assayed for protein using BCA assay kits (Pierce, Rockford, IL). Concentrations were adjusted to one mL and the protein content of each AMP and IST sample was obtained by multiplying the flushing volume times the concentration per mL. Protein content of each AMP and IST was divided by their respective weight, length, and weight to length ratio to obtained protein concentrations per gram, per cm, and per $g \cdot cm^{-1}$.

***Steroid Assays.*** Progesterone concentrations were assayed in duplicate using solid-phase RIA kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA) validated for ovine serum in our laboratory (Berardinelli et al., 2001). Intra- and inter-assay CV for a serum pool that contained 2.2 ng/mL of progesterone were 10.2 and 15.4%, respectively. Estradiol-17β was quantified in duplicate using double antibody RIA using kits (Siemens Medical Solutions Diagnostics) and validated for ovine serum in our laboratory (Berardinelli et al., 2001). The sensitivity of this assay was 0.22 pg/mL and the inter- and intra-assay CV were 13 and 9%, respectively.

***Statistical Analyses.*** The initial analyses for AMP and IST protein concentrations included side (right and left), however, there was no difference between right and left sides among animals. Therefore, data for right and left sides of AMP and IST protein concentrations were pooled for further analyses. Data for total AMP and IST protein concentrations and P4 and E2 concentrations, and P4:E2 ratios were analyzed for a CRD by separate ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC). The model included cycle type (FE, TE, and ME), day of the estrous cycle (0, 1, 2, 3, and 4) and the interaction of cycle type by day of the estrous cycle. Means were separated using Bonferroni's multiple comparison tests were used to evaluate treatment means. Differences were considered significant at $P < 0.05$.

## Results

There was an interaction ($P < 0.01$) between cycle type and day of cycle for AMP protein concentrations. Protein concentrations in the AMP of TE and ME ewes increased ($P < 0.05$) between D 0 and D1; whereas, AMP protein concentrations in FE ewe lambs did not increase over these days (Table 2). Thereafter, AMP protein concentrations as in TE and ME ewes decreased ($P < 0.05$) while AMP protein concentrations increased ($P < 0.05$) in FE ewe lambs at D 4 (Table 2).

There was an interaction ($P < 0.01$) between cycle type and day of cycle for IST protein concentrations. Protein concentrations in the IST of ME ewes increased ($P < 0.05$) between D 0 and D1 and decreased ($P < 0.05$) by D2 and remained low through D 4 (Table 3); whereas, IST protein concentrations in TE ewe lambs decreased ($P < 0.05$) after D 1. Protein concentration in the IST of FE ewe lambs did not differ during the first 5 d of the estrous cycle (Table 3).

There was an interaction ($P < 0.01$) between cycle type and day of cycle for P4 concentrations. Progesterone concentrations increased ($P < 0.05$) from D 1 through D 4 in FE and TE ewe lambs and in ME ewes (Table 4). However, the increase in P4 concentrations was much more ($P < 0.05$) rapid from D 2 to 4 in ME ewe than in FE and TE ewes (Table 4).

There was an interaction ($P < 0.01$) between cycle type and day of cycle for E2 concentrations. Estradiol concentrations increased ($P < 0.05$) from D 2 to 3 more rapidly in ME than in FE and TE ewes; whereas, E2 deceased ($P < 0.05$) from D 3 to 4 in ME and TE ewes, while E2 tended to increased ($P < 0.08$) from D 2 to 4 in FE ewes (Table 5).

There was an interaction ($P < 0.01$) between cycle type and day of cycle for P4:E2 ratios expressed in pg/mL of P4 to pg/mL of E2. Progesterone:estradiol ratios increased ($P < 0.05$) from D 0 to 4 in FE and TE ewe lambs and in ME ewes (Table 6). However, the increase in P4:E2 ratios was more rapid ($P < 0.05$) from D 2 through 4 in TE and ME ewes than in FE ewes (Table 6).

## DISCUSSION

We evaluated patterns of ampullary (AMP) and isthmic (IST) protein concentrations, and progesterone (P4) and estradiol (E2) concentrations during the first five days of the estrous cycle in first (pubertal; FE) and third estrous (TE) ewe lambs and mature ewes (ME). Such differences in oviductal protein and ovarian steroid patterns may explain or lead to further experimentation for a mechanism involving the oviduct that is related to the cause of lower fertility rates associated with breeding during the pubertal transition in sheep.

Indeed, AMP and IST protein concentrations differ mostly between FE ewe lambs and ME ewes, with TE ewe lambs showing intermediate changes during the first five days of the estrous cycle. Oviductal secretions contain proteins, some of which represent a serum transudate, while others are synthesized and secreted by the epithelium. Oviduct-specific glycoproteins of high molecular weight have been identified and characterized in all mammalian species studied to date including; swine, bovine, and ovine (Oliphant et al., 1982; Buhi et al., 2000). These proteins are secreted at a time when sperm capacitation, fertilization, and early embryonic growth occurs; they bind to zona pellucidae and sperm; are found in the perivitelline space; are associated with plasma membranes of blastomeres; enhance in vitro embryonic development; and may provide protection from proteolytic enzymes during oviductal transport (Buhi et al., 2000). Therefore, the differences in the patterns of oviductal proteins between FE ewe lambs and ME ewes during the first five days of the

**Table 2.** Least squares means for ampullary protein concentrations (ug/mg) in first (FE) and third (TE) estrous ewe lambs and mature (ME) ewes during the first 5 days of the estrous cycle[1]

| | Cycle type | | |
|---|---|---|---|
| Day of cycle | FE | TE | ME |
| 0 | 0.354[a] | 0.300[a] | 0.238[a] |
| 1 | 0.400[a,b] | 0.520[b,c] | 0.710[c] |
| 2 | 0.435[a,b] | 0.422[a,b] | 0.309[a] |
| 3 | 0.297[a] | 0.206[a] | 0.276[a] |
| 4 | 0.715[c] | 0.380[a,b] | 0.296[a] |

[1]SEM = 0.106.
[a-c]Least squares means without a common superscript differ, $P < 0.05$.

**Table 3.** Least squares means for isthmic protein concentrations in first (FE) and third (TE) estrous ewe lambs and mature (ME) ewes during the first 5 days of the estrous cycle[1]

| | Cycle type | | |
|---|---|---|---|
| Day of cycle | FE | TE | ME |
| 0 | 0.270[a] | 0.255[a] | 0.221[a] |
| 1 | 0.278[a] | 0.332[a,b] | 0.400[b] |
| 2 | 0.148[a,c] | 0.100[c] | 0.121[c] |
| 3 | 0.250[a] | 0.140[a,c] | 0.175[a,c] |
| 4 | 0.240[a] | 0.180[a,c] | 0.145[c] |

[1]SEM = 0.063.
[a-c]Least squares means without a common superscript differ, $P < 0.05$.

**Table 4.** Least squares means for progesterone concentrations (ng/mL) in first (FE) and third (TE) estrous ewe lambs and mature (ME) ewes during the first 5 days of the estrous cycle[1]

| | Cycle type | | |
|---|---|---|---|
| Day of cycle | FE | TE | ME |
| 0 | 0.04[a] | 0.04[a] | 0.06[a] |
| 1 | 0.04[a] | 0.12[a] | 0.08[a] |
| 2 | 0.35[a,b] | 0.42[a,b] | 0.35[a,b] |
| 3 | 0.78[b] | 0.67[b] | 1.58[c] |
| 4 | 1.43[c] | 1.64[c] | 1.81[c] |

[1]SEM = 0.35.
[a-c]Least squares means without a common superscript differ, $P < 0.05$.

**Table 5.** Least squares means for estradiol-17β concentrations (pg/mL) in first (FE) and third (TE) estrous ewe lambs and mature (ME) ewes during the first 5 days of the estrous cycle

| | Cycle type | | |
|---|---|---|---|
| Day of cycle | FE | TE | ME |
| 0 | 1.60 [a] | 2.14 [a] | 2.73 [a] |
| 1 | 1.60 [a] | 1.70 [a] | 1.60 [a] |
| 2 | 1.61 [a] | 3.53 [a,b] | 2.95 [a] |
| 3 | 2.83 [a] | 5.82 [b] | 9.50 [c] |
| 4 | 4.85 [a,b] | 2.53 [a] | 3.75 [a] |

[1]SEM = 2.35.
[a-c]Least squares means without a common superscript differ, $P < 0.05$.

**Table 6.** Least squares means for progesterone to estradiol-17β ratios (pg/mL:pg/mL) in first (FE) and third (TE) estrous ewe lambs and mature (ME) ewes during the first 5 days of the estrous cycle[1]

| | Cycle type | | |
|---|---|---|---|
| Day of cycle | FE | TE | ME |
| 0 | 25 [a] | 6 [a] | 37 [a] |
| 1 | 25 [a] | 74 [a] | 52 [a] |
| 2 | 217 [a] | 122 [a] | 48 [a] |
| 3 | 138 [a] | 293 [a,b] | 165 [a] |
| 4 | 437 [b] | 1064 [c] | 979 [c] |

[1]SEM = 240.
[a-c]Least squares means without a common superscript differ, $P < 0.05$.

estrous cycle may play essential roles in processes regulating early embryonic development and survival.

Additionally, we found that the ovarian steroid milieu differs between FE ewe lambs and ME ewes over the first five days of the estrous cycle. In particular is the timing of the dramatic increase in both P4 and E2 on D 3 after estrus in ME and a lack of change in these steroids in FE ewe lambs. It is well known that oviductal protein secretion is primarily regulated by ovarian steroids (Buhi et al, 2000). Although we did not test the relationship between ovarian steroids and oviductal protein concentration it does seem that temporal changes in these steroids, especially E2 may be responsible for changes in the oviductal protein patterns between FE ewe lambs and ME ewes.

In conclusion, oviductal protein and ovarian steroid concentration patterns over the first five days of the estrous cycle in sheep differ between ewe lambs that exhibit their first estrus and mature cyclic ewes. These results indicate the possibility that reduced fertility in ewe lambs at their first estrus may be caused by an inappropriate protein milieu in the AMP and IST that may be detrimental to normal early embryonic development.

## IMPLICATIONS

Oviductal fluid is a complex mixture of proteins derived from both serum and oviductal epithelial cell secretion. Further analyses of the proteins found in FE ewe lambs and ME ewes may show dramatic shifts in type and quantities of specific oviduct proteins. Changes such as these may give us insight into developmental changes that occur in the oviduct during sexual development, and a possible mechanism for reduced fertility associated with breeding domestic females at puberty.

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# PLASMA PROGESTERONE CONCENTRATION IN BEEF HEIFERS RECEIVING EXOGENOUS GLUCOSE, INSULIN, OR BOVINE SOMATOTROPIN

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**ABSTRACT:** Three experiments evaluated plasma concentrations of glucose, insulin, IGF-I, and progesterone (P4) in pubertal beef heifers receiving exogenous glucose, insulin, or sometribove zinc. All heifers utilized had no luteal P4 synthesis but received a controlled internal drug releasing device containing 1.38 g of P4 to estimate treatment effects on hepatic P4 degradation. In Exp. 1, 8 nulliparous Angus × Hereford heifers (initial BW = 442 ± 14 kg; initial age = 656 ± 7 d) were randomly assigned to receive, in a crossover design containing 2 periods of 10 h: 1) intravenous (i.v.) insulin infusion (1 μg/kg of BW; INS) or 2) i.v. saline infusion (0.9%; SAL). Treatments were administered via jugular venipuncture in 7 applications (0.15 μg of insulin/kg of BW per application) 45 min apart (from 0 to 270 min). Blood samples were collected immediately before each infusion, as well as at -120, -60, 330, 390, and 450 min relative to the first infusion. Heifers receiving INS had greater ($P < 0.01$) plasma insulin, reduced ($P \leq 0.04$) plasma glucose and IGF-I, but similar ($P = 0.62$) plasma P4 concentrations compared with SAL heifers. In Exp. 2, the same heifers were assigned to receive, in a similar experimental design as Exp. 1: 1) i.v. infusion containing insulin (1 μg/kg of BW) and glucose (0.5 g/kg of BW; INS+G) or 2) SAL. Heifers receiving INS+G had greater ($P \leq 0.02$) plasma insulin, glucose, and P4, but reduced ($P = 0.01$) plasma IGF-I concentrations compared with SAL heifers. In Exp. 3, the same heifers were assigned to receive, in a crossover design containing 2 periods of 14 d: 1) subcutaneous injection containing 250 mg of sometribove zinc (BST), or 2) SAL. Blood samples were collected 3 h apart (from 0900 to 1800 h) on d 6, 8, and 10 relative to treatment administration (d 1). Heifers receiving BST had greater ($P < 0.01$) plasma glucose and IGF-I, and similar ($P \leq 0.67$) plasma insulin and P4 concentrations compared with SAL heifers. Results from this series of experiments suggest that concurrent increases in glucose and insulin are required to reduce hepatic catabolism and increase plasma concentrations of P4 in bovine females.

**Key words:** beef heifers, glucose, insulin-like growth factor-I, insulin, progesterone

## INTRODUCTION

Nutrition, more specifically energy intake, is the environmental factor that most influences reproductive function in beef females (Mass, 1987). Several studies demonstrate that energy intake can be positively associated with hastened attainment of puberty, decreased postpartum interval, and greater pregnancy rates (Wiltbank et al., 1962; Schillo et al., 1992; Pescara et al., 2010). Moreover, beneficial effects of energy intake on cattle reproduction are regulated, at least partially, by circulating hormones and metabolites such as glucose, insulin, and IGF-I (Wettemann et al., 2003).

As an example, insulin modulates circulating concentrations of progesterone (**$P_4$**; Lopes et al., 2009), a steroid required for resumption of estrous cycles and establishment and maintenance of pregnancy (Looper et al., 2003). More specifically, insulin stimulates luteal $P_4$ synthesis (Spicer and Echternkamp, 1995) and alleviates hepatic steroid catabolism (Lemley et al., 2008). Our research group recently reported that cows in adequate nutritional status receiving intravenous (**i.v.**) glucose infusion to increase circulating insulin concentrations had greater plasma $P_4$ concentrations compared with cohorts receiving saline, which was attributed to reduced hepatic $P_4$ degradation given that cows were ovariectomized and supplemented with exogenous $P_4$ (Vieira et al., 2010). However, glucose supplementation also increases circulating concentrations of other hormones associated with reproductive and hepatic functions, including glucose itself and IGF-I (Jones and Clemmons, 1995).

Therefore, we hypothesized that the insulin- stimulated decrease in hepatic $P_4$ catabolism may also be dependent on circulating glucose and IGF-I. Based on this rationale, 3 experiments were conducted to evaluate plasma concentrations of glucose, insulin, IGF-I, and $P_4$ in beef females receiving exogenous insulin, insulin + glucose, or ST.

## MATERIALS AND METHODS

Animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee. All experiments were conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns, OR) from January to March 2011.

***Experiment 1.*** Eight pubertal, nulliparous Angus x Hereford heifers (initial BW = 452 ± 12 kg; initial age = 656 ± 7 d) were assigned to an estrus synchronization protocol (d -16 to 0 of the study). On d -16 heifers received a 100-μg treatment of GnRH (Cystorelin, Merial Ltd., Duluth, GA) and a controlled internal drug releasing device containing

1.38 g of $P_4$ (CIDR, Pfizer Animal Health, New York, NY), $PGF_{2\alpha}$ treatment (25 mg Lutalyse, Pfizer Animal Health) and CIDR removal on d -9, and a second GnRH treatment (100 µg) on d -7. On d 0, heifers received another $PGF_{2\alpha}$ treatment (25 mg) and a CIDR that remained in heifers throughout Exp. 1 (d 0 to 14). Transrectal ultrasonography examinations were performed immediately and 48 h after the second GnRH (d -7) and $PGF_{2\alpha}$ (d 0) treatments to verify ovulation and corpus luteum (CL) regression, respectively. All heifers utilized in this experiment responded to the hormonal treatment.

Heifer BW was recorded at the beginning and end of the experiment (d 0 and 14). On d 5, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 10 h each (d 6 and 8): 1) i.v. insulin infusion (1 µg/kg of BW; INS), or 2) i.v. saline infusion (0.9%; SAL). Bovine insulin solution was dissolved into 10 mL of physiological saline immediately prior to infusions and administered via jugular venipuncture in 7 applications (0.15 µg/kg of BW per application) 45 min apart (0, 45, 90, 135, 180, 225, and 270 min), whereas SAL heifers concurrently received 10 mL of physiological saline. Blood samples were collected immediately before each infusion, as well as at -120, -60, 330, 390, and 450 min relative to the first infusion. All heifers were fasted for 12 h prior to the beginning of each period, and remained fasted during sampling, to prevent any confounding effects between feed intake and infusion treatments on circulating concentrations of $P_4$ (Vasconcelos et al., 2003).

***Experiment 2.*** Immediately after the end of Exp.1 (d 14), the same heifers (mean BW = 456 ± 14 kg) received a new CIDR and evaluated via transrectal ultrasonography to confirm the absence of a CL.

Heifer BW was recorded at the beginning and end of the experiment (d 14 and 28). On d 20, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 10 h each (d 20 and 22): 1) i.v. infusion containing insulin (1 µg/kg of BW) and glucose (0.5 g/kg of BW; INS+G), or 2) i.v. saline infusion (0.9%; SAL). Glucose and bovine insulin solution were dissolved into 10 mL of physiological saline immediately prior to infusions. Similarly to Exp. 1, infusion was administered via jugular venipuncture in 7 applications (0.07 g/kg and 0.15 µg/kg of BW per application for glucose and insulin, respectively) 45 min apart. Blood samples were collected immediately before each infusion, as well as at -120, -60, 330, 390, and 450 min relative to the first infusion. As in Exp. 1, heifers were fasted for 12 h prior to the beginning and during the sampling.

***Experiment 3.*** Immediately after the end of Exp. 2 (d 28), heifers (mean BW = 462 ± 14 kg) received a new CIDR and were evaluated via transrectal ultrasonography to confirm the absence of CL.

Heifer BW was recorded at the beginning and end of the experiment (d 28 and 55). On d 28, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 14 d each (d 28 to 42 and 42 to 56): 1) s.c. injection containing 250 mg sometribove zinc (BST; Posilac, Elanco, Greenfield, IN), or 2) subcutaneous (**s.c.**) saline injection (0.9%; SAL). Treatments were applied once, at 0800 h, during the first day of each period (d 28 and 42). Heifer also received a new CIDR at the beginning of the second period concurrently with treatment administration (d 42).

Four blood samples were collected, 3 h apart (from 0900 to 1800 h) from heifers on d 33, 35, and 37 (period 1) and 47, 49, and 51 (period 2) of the experiment. Similarly to Exp. 1 and 2, all heifers were fasted for 12 h prior to the beginning and during each collection day.

***Diets.*** During all experiments, all heifers were individually offered (as-fed basis) 12 kg of mixed alfalfa- grass hay, 1.0 kg of ground corn, and 0.5 kg of camelina meal in the morning (0700 h). Heifers also received a complete commercial mineral and vitamin mix and water for ad libitum consumption.

***Blood Analysis.*** All blood samples were harvested for plasma and stored at −80°C until assayed for concentrations of glucose (#G7521; Pointe Scientific, Inc., Canton, MI), insulin (B1009; Endocrine Technologies Inc., Newark, CA), IGF-I (SG100; R&D Systems, Inc., Minneapolis, MN), and $P_4$ (11-PROHU-E01; Alpco Diagnostics, Salem, NH).

***Statistical Analysis.*** All data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Heifer was considered the experimental unit for all analysis. The model statement used for Exp. 1 and 2 contained the effects of treatment, time, the resultant interaction, in addition to period as independent variable. Data obtained prior to treatment application (-120, -60, and 0 min prior to infusion) were averaged and used as covariate. Heifer was used as random variable. The specified term for the repeated statement was time, and heifer (treatment × period) was included as subject. The covariance structure utilized was autoregressive, which provided the lowest Akaike information criterion and hence the best fit for all variables analyzed. Results are reported as covariately adjusted least square means if the covariate was significant ($P \leq 0.05$), and were separated by LSD. The model statement used for Exp. 3 contained effects of treatment, day, time, and all interactions, in addition to period as independent variable. Heifer was used as random variable. The specified term for the repeated statement was time, and heifer (treatment × day × period) was included as subject. The covariance structure utilized was autoregressive, which provided the lowest Akaike information criterion and hence the best fit for all variables analyzed. Results are reported as least square means and separated using LSD. For all analysis, significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $\leq 0.10$. Results are reported according to treatment effects if no interactions were significant or according to highest-order interaction detected.

## RESULTS AND DISCUSSION

***Experiment 1.*** Heifer BW did not change ($P = 0.51$; data not shown) during the experimental period, indicating that heifers were in adequate nutritional status. As expected, mean plasma insulin concentration during the experimental period was greater ($P < 0.01$) for INS compared with SAL (Table 1).

A treatment × time interaction was detected ($P = 0.01$) for plasma glucose (Figure 1). After the initial infusion, plasma glucose decreased for INS heifers (time effect; $P < 0.01$) and did not change for SAL heifers (time effect; $P = 0.53$). Moreover, mean plasma glucose concentration during the experimental period was reduced ($P < 0.01$; Table 1) for INS compared with SAL heifers. In agreement, Kegley et al. (2000) also reported that i.v. insulin infusion reduced circulating glucose concentrations in beef cattle, given that insulin directly estimates the uptake of glucose by body tissues (Nelson and Cox, 2005).

Mean plasma IGF-I concentration was reduced ($P = 0.04$) for INS heifers compared with SAL heifers during the experimental period (Table 1). The goal of Exp. 1 was to evaluate if insulin administration would increase plasma $P_4$ concentrations in beef heifers in adequate nutrient balance, by reducing hepatic $P_4$ catabolism, independently of circulating concentrations of glucose and IGF-I. However, no treatment effects were detected ($P = 0.62$) for plasma $P_4$ concentrations (Table 1). Therefore, insulin itself may not be capable of alleviating hepatic $P_4$ catabolism and consequently increasing circulating concentrations of this hormone. Accordingly, research studies documenting the role of insulin on hepatic expression of $P_4$ catabolic enzymes (Lemley et al., 2008) and resultant plasma $P_4$ concentrations (Vieira et al., 2010) included glucose infusion into the experimental design.

***Experiment 2.*** Similarly to Exp. 1, BW did not change ($P = 0.55$; data not shown) during the experimental period. As expected by the experimental design, mean plasma glucose and insulin concentrations during the experimental period were greater ($P \leq 0.01$) for INS+G compared with SAL heifers (Table 2).

Similarly to Exp. 1, INS+G heifers had reduced ($P = 0.01$) mean plasma IGF-I concentrations compared with SAL heifers during the experimental period (Table 2). Other researchers reported that cattle receiving i.v infusion of insulin and glucose had similar (Molento et al., 2002) or greater circulating IGF-I concentrations compared with cohorts receiving saline (Butler et al., 2003).

During the experimental period, INS+G heifers had greater ($P = 0.02$) mean $P_4$ concentration compared with SAL heifers (Table 2). The goal of Exp. 2 was to evaluate if supplemental glucose modulates the effects of insulin infusion on plasma $P_4$ concentrations by reducing hepatic $P_4$ catabolism. In fact, we also expected that INS+G heifers would have greater plasma IGF-I, whereas IGF-I also influences hepatic function and could potentially modulate hepatic steroid catabolism (Jones and Clemmons, 1995). Nevertheless, results from Exp. 2 suggest that i.v. insulin infusion increased plasma $P_4$ concentrations by reducing hepatic $P_4$ catabolism only when supplemental glucose is provided. Therefore, results from Exp. 2 combined with those reported by Lemley et al. (2008) and Vieira et al. (2010) suggest that circulating glucose modulates the effects of insulin on hepatic steroid catabolism and subsequent circulating $P_4$ concentrations in bovine females in adequate nutritional status.

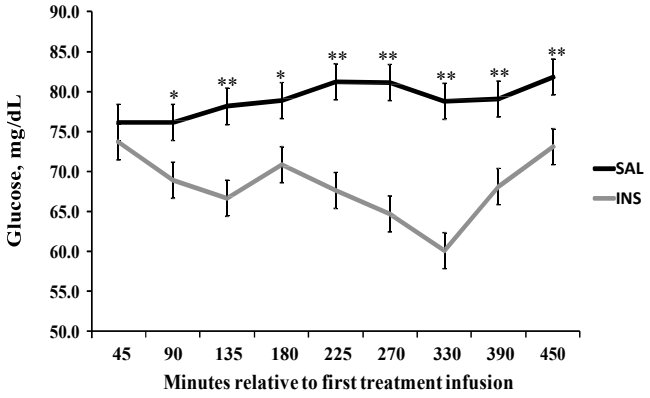


**Figure 1.** Plasma glucose concentrations of heifers receiving i.v. infusions containing 10 mL of physiological saline (0.9%; SAL) or 0.15 µg/kg of BW of insulin (INS). A treatment × time interaction was detected ($P < 0.01$). Treatment comparison within time: ** $P < 0.01$, * $P = 0.01$.

***Experiment 3.*** Similarly to Exp. 1 and 2, BW did not change ($P = 0.72$; data not shown) during the experimental period. As expected, BST heifers had greater ($P < 0.01$) mean plasma IGF-I concentrations compared with SAL heifers (Table 3), given that sometribove zinc has been shown to increase IGF-I synthesis and circulating concentrations in cattle (Bilby et al., 1999). Heifers receiving BST had greater ($P < 0.01$) plasma glucose but similar ($P = 0.76$) plasma insulin concentrations compared with SAL heifers (Table 3). In the present study, the increase in plasma glucose concentrations in BST heifers despite similar insulin concentrations can be attributed to decreased insulin sensitivity caused by sometribove zinc administration (Dunshea et al., 1995).

**Table 1.** Plasma concentrations of glucose, insulin, IGF-I, and progesterone ($P_4$) in beef heifers receiving i.v. infusion of insulin (1µg/kg of BW; INS; n = 8) or saline (0.9%; SAL; n = 8) in Exp. 1

| Item | INS | SAL | SEM | $P =$ |
|---|---|---|---|---|
| Glucose, mg/dL | 68.20 | 79.00 | 1.30 | < 0.01 |
| Insulin, ng/mL | 1.40 | 0.99 | 0.10 | < 0.01 |
| IGF-I, ng/mL | 145.00 | 154.00 | 3.00 | 0.04 |
| $P_4$, ng/mL | 3.74 | 3.84 | 0.27 | 0.65 |

**Table 2.** Plasma concentrations of glucose, insulin, IGF-I, and progesterone ($P_4$) in beef heifers receiving i.v. infusion containing insulin (1 µg/kg of BW) and glucose (0.5 g/kg of BW; INS+G; n = 8) or saline (0.9%; SAL; n = 8) in Exp. 2

| Item | INS+G | SAL | SEM | $P =$ |
|---|---|---|---|---|
| Glucose, mg/dL | 133.90 | 76.80 | 16.40 | 0.01 |
| Insulin, ng/mL | 3.65 | 2.12 | 0.32 | < 0.01 |
| IGF-I, ng/mL | 134.00 | 142.00 | 2.00 | 0.01 |
| $P_4$, ng/mL | 2.88 | 2.52 | 0.11 | 0.02 |

**Table 3.** Plasma concentrations of glucose, insulin, IGF-I, and $P_4$ in beef heifers receiving s.c. injection containing 250 mg sometribove zinc (BST; n = 8) or saline (0.9%; SAL; n = 8) in Exp. 3.

| Item | BST | SAL | SEM | *P* = |
|---|---|---|---|---|
| Glucose, mg/dL | 73.00 | 69.60 | 1.60 | < 0.01 |
| Insulin, ng/mL | 1.44 | 1.65 | 0.51 | 0.76 |
| IGF-I, ng/mL | 248.00 | 143.00 | 6.00 | < 0.01 |
| $P_4$, ng/mL | 3.07 | 3.13 | 0.15 | 0.67 |

The main goal of Exp. 3 was to determine if circulating IGF-I also modulates hepatic $P_4$ catabolism and consequent $P_4$ concentrations given that this hormone directly regulates hepatocytes activity (Jones and Clemmons, 1995). However, mean plasma $P_4$ concentrations were similar ($P = 0.67$) between BST and SAL heifers (Table 3), suggesting that hepatic $P_4$ catabolism in bovine females in adequate nutritional status is not directly regulated by circulating IGF-I.

## IMPLICATIONS

Results collectively suggest that the effects of insulin on hepatic $P_4$ degradation and circulating $P_4$ concentrations in bovine females in adequate nutritional status are dependent on circulating glucose, but not IGF-I. In addition, results reported herein indicate that nutritional alternatives to increase circulating concentrations of glucose and insulin may benefit reproductive function of females in adequate nutritional status by increasing circulating concentrations of $P_4$.

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# THE RELATIONSHIP OF FEED EFFICIENCY AND VISCERAL ORGAN SIZE IN GROWING LAMBS FED A CONCENTRATE OR FORAGE-BASED DIET

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**ABSTRACT:** We hypothesized that a portion of individual differences observed for feed efficiency can be attributed to gastrointestinal tract (GIT) size, which would vary based on diet. Growing wethers (n = 82; 51.3 ± 1.2 kg BW) were fed a concentrate (CONC; 12.1% CP, 17.6% NDF, 2.98 Mcal/kg ME) or forage-based pelleted diet (FOR; 16.2% CP, 36.3% NDF, 2.31 Mcal/kg ME) for 49 d. Individual intake was measured with the GrowSafe System to determine residual feed intake (RFI). The 20% most (low RFI, n = 8) and 20% least (high RFI, n = 8) efficient lambs from each diet were slaughtered (66.6 ± 2.3 kg BW; n = 32 total), and the viscera was dissected and weighed. Data were analyzed as a 2 x 2 factorial with RFI class (low vs. high RFI), diet type (FOR vs. CONC), and the interaction in the model. Organ mass was not affected ($P > 0.10$) by the RFI class x diet type interaction. Low RFI lambs tended to have greater ($P = 0.09$) pancreas and spleen mass than high RFI, although RFI class did not affect ($P > 0.15$) other organ actual (g) or proportional (g/kg BW) mass. Lambs fed FOR vs. CONC had greater ($P \leq 0.01$) actual and proportional reticulum, omasum, large intestinal, and kidney mass and tended to have greater ($P \leq 0.09$) actual and proportional small intestinal mass. However, CONC fed lambs had greater ($P \leq 0.05$) rumen, heart, liver, and proportional rumen mass than FOR fed lambs. All other visceral organs were unaffected ($P > 0.11$) by diet type. Proportional abomasum mass tended to be positively correlated ($P = 0.08$) with RFI, whereas pancreas and spleen mass tended to be negatively correlated ($P \leq 0.09$) with RFI. Intake was positively correlated ($P \leq 0.04$) with reticulum, omasum, abomasum, large intestinal, kidney, and proportional omasum mass, and tended to be positively correlated ($P \leq 0.10$) with total GIT, small intestinal, and proportional large intestinal mass. Proportional spleen and heart mass tended to be negatively correlated ($P \leq 0.09$) with intake. Results of this study suggest that visceral organ size in growing lambs is more affected by diet type than individual feed efficiency.

**Key words:** feed efficiency, feed intake, gastrointestinal tract

## INTRODUCTION

Rising costs of limited feed resources within the sheep and cattle industries have increased feed costs, decreasing producer profitability. In fact, feed costs account for 50-70% of total sheep production costs (Nash, 1991), and 60-65% of total beef production costs (Sainz and Paulino, 2004). One way to reduce feed costs is to select for animals within the flock or herd with reduced intake and improved feed efficiency. Residual feed intake (**RFI**) is a measure of feed efficiency that is moderately heritable and genetically independent of mature size (Arthur et al., 2001; Crews, 2005). Despite the increased interest in RFI and feed efficiency in both research and industry settings, physiological mechanisms underlying differences in individual feed efficiency remain largely unknown. The gastrointestinal tract (**GIT**) is not only essential for nutrient digestion and absorption, but also is a major energy and nutrient sink, accounting for 40 to 50% of energy expenditure by the whole body (Ferrell, 1988; Caton et al., 2000). Despite some recent research in the area (Basarab et al., 2003; Mader et al., 2009; Meyer et al., 2012a), the role of the GIT in feed efficiency is unclear. Herd and Arthur (2009) suggested that individual differences in RFI can be attributed in part to tissue metabolism, turnover, and stress (37%), digestibility (10%), heat increment of feeding (9%), body composition (5%), and feeding patterns (2%), all of which have contributions from the GIT.

We hypothesized that a portion of individual differences observed for feed efficiency can be attributed to GIT size and function, which would vary based on diet. The objective of this study was to determine GIT and visceral organ size in high and low efficiency growing lambs fed either a concentrate or forage-based diet.

## MATERIALS AND METHODS

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

***Animals and Diet.*** Growing wethers (n = 82; 51.3 ± 1.2 kg BW) of Rambouillet, Hampshire, and Suffolk breed types were randomly allocated by BW to receive either a pelleted concentrate-based diet (**CONC**; Table 1) or pelleted forage-based diet (**FOR**; Table 1). Lambs were acclimated to diets using a 20% increase in proportion of new feed to old feed for 21 d until the diet consisted of 100% treatment diet ad libitum. Individual feed intake was then measured by the GrowSafe System for a 49-d trial period.

Two-day average initial and final BW were obtained to calculate ADG. From these data, expected feed intake was determined by regressing ADG and metabolic midweight on

**Table 1.** Composition of pelleted diets fed to growing lambs

| Item | FOR[1] | CONC[2] |
|---|---|---|
| Ingredient, % DM | | |
| Alfalfa pellets | 67.7 | -- |
| Corn | -- | 50.2 |
| Wheat middlings | 27.5 | 31.0 |
| Corn gluten | -- | 10.0 |
| Cane molasses | 2.50 | 2.50 |
| Salt | 1.34 | 1.76 |
| Calcium carbonate | 0.60 | 2.30 |
| Dried distillers grains with solubles | -- | 1.0 |
| Calcium sulfate | -- | 0.75 |
| Potassium chloride | -- | 0.19 |
| Trace minerals and vitamins[3] | 0.34 | 0.36 |
| Analyzed nutrient composition | | |
| DM, % as fed | 92.3 | 91.6 |
| CP, % DM | 16.2 | 12.1 |
| NDF, % DM | 36.3 | 17.6 |
| ADF, % DM | 25.1 | 6.6 |
| ME, Mcal/kg[4] | 2.31 | 2.98 |

[1]FOR = pelleted forage diet.
[2]CONC = pelleted concentrate diet.
[3]Includes Selenium 1600, Sheep TM ORG-Zn, Flavor APF-168, Vitamin E 20000 IU/#, and CHS/PN VT-FDLT.
[4]Calculated from NRC (2007) values.

actual feed intake. Residual feed intake was then calculated as the expected feed intake subtracted from the actual feed intake (Cammack et al., 2005).

***Measurement of Visceral Organ Size.*** Based on their RFI, the 20% most efficient (low RFI; n = 8) and 20% least efficient (high RFI; n = 8) wethers from each diet type were slaughtered (66.6 ± 2.3 kg BW; n = 32 total) at the University of Wyoming Meat Laboratory, when GIT and visceral organ data were collected. Using procedures of Meyer et al. (2012b), viscera were removed and dissected. The liver and pancreas were removed and weighed, then the stomach complex and intestine were dissected, gently stripped of fat and digesta, weighed, and measured (small intestine only). The stomach complex was divided into the reticulum, rumen, omasum, and abomasum based on anatomical structures. Additionally, the small intestine was dissected using the following demarcations. The duodenum began at the pylorus and ended at the point adjacent to the junction of the gastrosplenic vein and mesenteric vein. The jejunum began here and ended 300 cm (non-stripped intestine) after a point adjacent to the mesenteric vein, 10 cm caudal from its junction with the ileocecal vein. The ileum comprised the remaining small intestine and concluded at the cecum.

***Statistical Analyses.*** Data were analyzed as a 2 x 2 factorial in PROC MIXED (SAS Inst. Inc., Cary, NC) with RFI class (low vs. high RFI), diet type (FOR vs. CONC), and their interaction in the model. Means were separated using LSD and were considered significant when $P \leq 0.05$ or were considered tendencies when $P < 0.10$. In the absence of interactions ($P > 0.10$), main effects are reported. Data were also analyzed using the CORR procedure of SAS to determine the relationship of RFI and feed intake with lamb GIT and visceral organ size.

## RESULTS

Effects of RFI class and diet type on viscera mass and intestinal length are reported in Tables 2 (actual masses and lengths) and 3 (proportional masses and lengths). The interaction of RFI class x diet type did not affect ($P \geq 0.11$) visceral organ mass or small intestinal length. Low RFI lambs tended to have greater ($P = 0.09$) pancreas and spleen actual mass (g) than high RFI, although RFI class did not affect ($P \geq 0.21$) actual empty GIT, stomach complex, small intestinal, large intestinal, omental and mesenteric fat, liver, lung, heart, or kidney mass. Proportional mass (g/kg BW) of all visceral organs was unaffected ($P \geq 0.16$) by RFI class. Additionally, actual (cm) and proportional (cm/kg BW) small intestinal length was not influenced ($P \geq 0.44$) by RFI class in this study.

Lambs fed the FOR diet had greater ($P \leq 0.01$) actual reticulum, omasum, large intestinal, and kidney mass and tended to have greater ($P \leq 0.09$) actual small intestinal mass compared with CONC. However, CONC fed lambs had greater ($P \leq 0.05$) actual rumen, liver, and heart mass than FOR fed lambs. Actual empty GIT, total stomach complex, abomasum, small intestinal section, omental and mesenteric fat, pancreas, spleen, and lung mass were unaffected ($P \geq 0.12$) by diet type. Lambs fed the FOR diet also had greater ($P \geq 0.01$) proportional reticulum, omasum, large intestinal, and kidney mass and tended ($P = 0.09$) to have greater proportional small intestinal mass than CONC. Proportional rumen mass was greater ($P = 0.01$) for CONC compared with FOR fed lambs. Despite this, there was no effect ($P \geq 0.14$) of diet type on empty GIT, total stomach complex, abomasum, small intestinal section, omental and mesenteric fat, liver, pancreas, spleen, lung, o heart mass. Diet type also did not impact ($P \geq 0.32$) actual or proportional small intestinal length.

Residual feed intake was positively correlated with intake (r = 0.64; $P < 0.001$). Actual pancreas (r = -0.31; $P = 0.09$) and spleen (r = -0.31; $P = 0.08$) mass tended to be negatively correlated with RFI, whereas proportional abomasum mass tended to be positively correlated (r = 0.31; $P = 0.08$) with RFI. No other actual or proportional visceral organ masses or small intestinal lengths were correlated with RFI ($P \geq 0.12$).

Feed intake was positively correlated with actual reticulum (r = 0.47; P = 0.006), omasum (r = 0.60; $P < 0.001$), abomasum (r = 0.36; $P = 0.04$), large intestine (r = 0.48; $P = 0.005$), and kidney (r = 0.57; $P < 0.001$) mass and tended to be positively correlated with actual empty GIT (r = 0.30; $P = 0.09$) and small intestinal (r = 0.32; $P = 0.08$) mass. Additionally, intake was positively correlated with proportional omasum mass (r = 0.43; $P = 0.01$) and tended

**Table 2.** Effects of residual feed intake (RFI) class and diet type on lamb visceral organ mass and small intestinal length

| | RFI Class[1] | | | Diet Type[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | Low | High | SEM | CONC | FOR | SEM | RFI | Diet | RFI x Diet |
| Live BW, kg | 66.2 | 66.5 | 3.4 | 67.3 | 65.8 | 3.4 | >0.99 | 0.76 | 0.73 |
| Organ mass, g | | | | | | | | | |
| Gastrointestinal tract | 3,223 | 3,241 | 129 | 3,193 | 3,272 | 129 | 0.92 | 0.67 | 0.96 |
| Stomach complex | 1,641 | 1,711 | 73 | 1,749 | 1,603 | 73 | 0.50 | 0.16 | 0.99 |
| Reticulum | 163 | 164 | 7 | 151 | 176 | 7 | 0.89 | 0.01 | 0.91 |
| Rumen | 1,039 | 1,070 | 51 | 1,172 | 936 | 51 | 0.67 | 0.002 | 0.88 |
| Omasum | 138 | 149 | 9 | 120 | 167 | 9 | 0.38 | 0.001 | 0.83 |
| Abomasum | 300 | 327 | 16 | 305 | 322 | 16 | 0.25 | 0.47 | 0.48 |
| Total small intestine | 1,064 | 1,014 | 42 | 986 | 1,092 | 42 | 0.42 | 0.09 | 0.76 |
| Duodenum | 113 | 104 | 9 | 108 | 109 | 9 | 0.51 | 0.90 | 0.96 |
| Jejunum | 422 | 402 | 24 | 385 | 439 | 24 | 0.56 | 0.12 | 0.52 |
| Ileum | 528 | 507 | 35 | 493 | 543 | 35 | 0.67 | 0.31 | 0.42 |
| Large intestine | 518 | 515 | 28 | 457 | 576 | 28 | 0.94 | 0.005 | 0.53 |
| Omental and mesenteric fat | 2,350 | 2,331 | 197 | 2,519 | 2,162 | 197 | 0.95 | 0.21 | 0.83 |
| Liver | 1,308 | 1,224 | 50 | 1,355 | 1,176 | 50 | 0.24 | 0.02 | 0.83 |
| Pancreas | 95.0 | 82.7 | 5.0 | 87.5 | 90.2 | 5.0 | 0.09 | 0.71 | 0.87 |
| Spleen | 111 | 97 | 6 | 107 | 102 | 6 | 0.09 | 0.54 | 0.90 |
| Lungs | 707 | 730 | 49 | 726 | 711 | 49 | 0.74 | 0.83 | 0.86 |
| Heart | 330 | 306 | 13 | 337 | 299 | 13 | 0.21 | 0.05 | 0.54 |
| Kidneys | 192 | 196 | 8 | 174 | 214 | 8 | 0.74 | 0.001 | 0.91 |
| Intestine length, cm | | | | | | | | | |
| Total small intestine | 2,801 | 2,760 | 74 | 2,748 | 2,813 | 74 | 0.69 | 0.54 | 0.54 |
| Duodenum | 299 | 276 | 21 | 290 | 285 | 21 | 0.44 | 0.86 | 0.43 |
| Jejunum | 1,026 | 1,008 | 53 | 983 | 1,052 | 53 | 0.81 | 0.37 | 0.18 |
| Ileum | 1,475 | 1,475 | 81 | 1,474 | 1,475 | 81 | >0.99 | 0.99 | 0.11 |

[1]Low = 20% lowest RFI lambs of each diet (most efficient); High = 20% highest RFI lambs of each diet (least efficient).
[2]CONC = pelleted concentrate diet; FOR = pelleted forage diet.

to positively correlated with proportional large intestinal mass (r = 0.29; *P* = 0.10). Proportional spleen (r = -0.31; *P* = 0.08) and heart (r = -0.31; *P* = 0.09) mass also tended to be negatively correlated with intake.

## DISCUSSION

To the knowledge of the authors, this is the first study investigating a relationship between visceral organ size and RFI in growing lambs. Results indicate that pancreas and spleen mass may be greater in more efficient lambs, though this may be due to BW, as the relationship was not maintained when organ mass was expressed relative to BW. Although pancreas mass has not been previously associated with feed efficiency in ruminants (Mader et al., 2009), given the many digestive and metabolic functions of the pancreas, increased pancreas function could improve metabolic efficiency. A companion abstract found no differences in pancreatic α-amylase or trypsin activity between low and high RFI lambs, however (Doscher et al., 2012). Spleen mass was also positively correlated with G:F in a previous study, indicating a similar relationship to that observed currently (Mader et al., 2009). Increased spleen mass may suggest greater blood volume or altered red blood cell dynamics or immune response in more efficient lambs.

Feed intake was positively correlated with organ mass, as previously reported in sheep (Burrin et al., 1990; Noziere et al., 1999; Meyer et al., 2012b). Despite this, no clear relationship emerged between feed efficiency and GIT size or liver, lung, heart, or kidney mass. Previous work in feedlot cattle by Mader et al. (2009) also observed no relationship of RFI and visceral organ masses, despite negative correlations between G:F and total visceral organ and visceral fat masses. Conversely, another study observed greater liver and empty GIT masses in high RFI compared with low RFI steers (Basarab et al., 2003). In addition, recent data from our lab in feedlot cattle demonstrated a positive relationship between RFI and small intestinal mass and negative relationships between RFI and small intestinal mucosal density and DNA concentration (Meyer et al., 2012a). This suggests that more efficient cattle may have smaller intestinal size with a more dense mucosa. Differences between the current study in lambs and previous work in cattle may be due to species, or multiple breeds in the current study may have diminished differences due to RFI class. Because visceral organ masses were also affected by diet type, in agreement with previous work (McLeod and Baldwin, 2000), changes caused by diet type may have also minimized differences due to RFI class.

**Table 3.** Effects of residual feed intake (RFI) class and diet type on lamb proportional visceral organ mass and small intestinal length

| | RFI Class[1] | | | Diet Type[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | Low | High | SEM | CONC | FOR | SEM | RFI | Diet | RFI x Diet |
| Organ mass, g/kg BW | | | | | | | | | |
| Gastrointestinal tract | 49.2 | 50.5 | 2.50 | 48.1 | 51.6 | 2.50 | 0.72 | 0.33 | 0.94 |
| Stomach complex | 24.9 | 26.4 | 1.2 | 26.3 | 25.1 | 1.2 | 0.35 | 0.48 | 0.88 |
| Reticulum | 2.49 | 2.55 | 0.11 | 2.28 | 2.76 | 0.11 | 0.71 | 0.003 | 0.95 |
| Rumen | 15.7 | 16.5 | 0.8 | 17.6 | 14.6 | 0.8 | 0.48 | 0.01 | 0.95 |
| Omasum | 2.12 | 2.30 | 0.14 | 1.81 | 2.61 | 0.14 | 0.39 | <0.001 | 0.98 |
| Abomasum | 4.55 | 5.08 | 0.29 | 4.56 | 5.08 | 0.29 | 0.21 | 0.22 | 0.69 |
| Total small intestine | 16.3 | 15.9 | 1.0 | 14.9 | 17.3 | 1.0 | 0.79 | 0.09 | 0.76 |
| Duodenum | 1.72 | 1.62 | 0.14 | 1.61 | 1.73 | 0.14 | 0.60 | 0.56 | 0.83 |
| Jejunum | 6.56 | 6.51 | 0.66 | 5.88 | 7.19 | 0.66 | 0.96 | 0.17 | 0.94 |
| Ileum | 8.01 | 7.79 | 0.46 | 7.40 | 8.40 | 0.46 | 0.75 | 0.14 | 0.41 |
| Large intestine | 7.93 | 8.02 | 0.53 | 6.87 | 9.09 | 0.53 | 0.91 | 0.006 | 0.56 |
| Omental and mesenteric fat | 35.0 | 35.7 | 2.5 | 38.0 | 32.7 | 2.5 | 0.84 | 0.15 | 0.88 |
| Liver | 19.9 | 19.2 | 1.1 | 20.4 | 18.7 | 1.1 | 0.67 | 0.27 | 0.82 |
| Pancreas | 1.45 | 1.30 | 0.09 | 1.32 | 1.43 | 0.09 | 0.29 | 0.44 | 0.91 |
| Spleen | 1.73 | 1.50 | 0.11 | 1.63 | 1.60 | 0.11 | 0.16 | 0.86 | 0.99 |
| Lung | 10.6 | 11.2 | 0.7 | 10.8 | 11.1 | 0.7 | 0.53 | 0.77 | 0.81 |
| Heart | 5.04 | 4.73 | 0.22 | 5.05 | 4.72 | 0.22 | 0.34 | 0.31 | 0.71 |
| Kidney | 2.99 | 3.06 | 0.20 | 2.63 | 3.42 | 0.20 | 0.79 | 0.008 | 0.99 |
| Intestine length, cm/kg BW | | | | | | | | | |
| Total small intestine | 43.2 | 43.4 | 2.5 | 41.6 | 45.0 | 2.5 | 0.94 | 0.34 | 0.67 |
| Duodenum | 4.58 | 4.32 | 0.36 | 4.35 | 4.56 | 0.36 | 0.62 | 0.69 | 0.43 |
| Jejunum | 16.1 | 16.3 | 1.6 | 15.1 | 17.3 | 1.6 | 0.95 | 0.32 | 0.68 |
| Ileum | 22.4 | 22.8 | 1.3 | 22.1 | 23.1 | 1.3 | 0.83 | 0.58 | 0.13 |

[1]Low = 20% lowest RFI lambs of each diet (most efficient); High = 20% highest RFI lambs of each diet (least efficient).
[2]CONC = pelleted concentrate diet; FOR = pelleted forage diet.

In summary, visceral organ size in growing lambs was more affected by diet type than individual feed efficiency status, as measured by RFI, in the current study. Despite this, pancreas and spleen size and function differences may exist between high and low efficiency lambs. Further research is necessary to determine the role of the visceral organs, and especially the GIT, in ruminant animal metabolic efficiency.

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# PRODUCTION, MANAGEMENT, AND ENVIRONMENT

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# COMPARISON OF RECEIVING STRATEGIES ON FEEDLOT PERFORMANCE IN BEEF CALVES AT WEANING

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**ABSTRACT:** One hundred and twenty four newly weaned Angus, Hereford, and Angus × Hereford bull and heifer calves (initial BW = 233 ± 14.9 kg) were utilized to evaluate two feedlot receiving management strategies at Colorado State University's Agriculture, Research, Development and Education Center in Fort Collins, CO on feedlot performance over the first 30 d upon arrival to the feedlot. Cattle were blocked by gender and stratified by BW, breed, and age, and assigned to one of 14 pens (8 to 10 head/pen). Pens were then assigned to one of two dietary treatments. Dietary treatments included: 1) a dried distillers grain-based total mixed ration (DDG) initiated upon arrival, or 2) long-stem grass hay followed by a total mixed ration containing no DDG (HAY). Calves receiving the HAY treatment received only grass hay for the first d after arrival, long stem grass hay and total mixed ration combination the following 2 d, followed by a grain based total mixed ration on d 4. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets. Calves were weighed on d 0 and 30, and DMI was determined daily. Initial BW was similar ($P = 0.99$) across treatments; however, d 30 BW was greater ($P < 0.001$) for DDG vs. HAY calves. As a result, ADG was greater ($P < 0.001$) for DDG vs. HAY calves (0.59 vs. 0.41 ± 0.04 kg/d, respectively). Gain-to-feed ratio was greater ($P < 0.05$) for DDG vs. HAY calves (0.22 vs. 0.17± 0.013, respectively), and feed-to-gain ratio tended ($P = 0.05$) to be greater in HAY vs. DDG calves. Daily DMI tended ($P = 0.06$) to be greater in DDG vs. HAY calves (2.70 kg·hd$^{-1}$·d$^{-1}$ vs. 2.35 kg·hd$^{-1}$·d$^{-1}$ ± 0.256, respectively). In summary, providing a DDG-based receiving ration to newly weaned calves upon arrival to the feedlot resulted in greater feed intake, gain, and feed efficiency over a 30 d period than traditional long-stemmed grass hay followed by a non-DDG total mixed ration.

**Key words:** beef calves, dried distillers grains, feedlot performance, receiving diet, weaning

## INTRODUCTION

Weaning is one of the most stressful times in a calf's life. Nutritional strategies can affect calf performance, morbidity, and mortality (Hutcheson and Cole, 1986). Therefore, enticing calves to eat as soon as possible after arrival to a feedlot can improve performance and reduce losses associated with illness. At Colorado State University's (**CSU**) agriculture research centers, 2 different weaning strategies have been used to entice calves to eat after weaning upon arrival in feedlot pens: 1) feeding only long-stem grass hay the first day then a mix of grass hay and a total mixed ration (**TMR**) on following days; each day reducing the grass hay and increasing the TMR, and 2) feeding only a TMR with dried distillers grains. Therefore, the objective of this experiment was to evaluate the effect of feeding strategy on cattle performance, morbidity, and mortality 30 d immediately post-weaning.

## Materials and Methods

Prior to the initiation of this experiment, care, handling, and sampling of animals as described herein were approved by the CSU Animal Care and Use Committee.

One hundred and twenty four newly weaned Angus, Hereford, and Angus × Hereford bull and heifer calves (initial BW = 233 ± 14.9 kg) were selected from an initial group of 134 calves. Twenty five days prior to weaning, calves were vaccinated with 7-way (UTRABAC 7, Pfizer Animal Health, New York, NY), a modified live virus (Bovi-Shield Gold FP5 L5 HB, Pfizer Animal Health), and BW was collected. No implant was given at any time because the majority of these calves were retained as breeding animals.

Following processing, mean weights were computed for each of the 3 breed and gender classifications. Animals that were beyond ± 2 SD from the mean BW were excluded from the experiment. A random number was assigned to each animal and animals with the lowest random number were excluded from the trial until only 124 animals needed for the experiment remained. Animals were blocked by gender and stratified by breed and BW across treatments. Treatments were randomly assigned to feedlot pens.

The morning of weaning, cows and calves were gathered from a group pasture in Southeast Wyoming at the CSU Y Cross Ranch. Calves were sorted from cows and transported via 2 semi-truck trailers approximately 2 hrs to CSU's Agriculture Research, Development, and Education Center (Fort Collins, CO). Upon arrival, calves were processed. Processing included a booster of modified live virus (Bovi-Shield Gold FP5 L5 HB, Pfizer), BW collection, a tattoo in each ear, DNA sample collection and placement of calves into treatment pens. The experiment was conducted during the months of September and October, and calves were housed in soil surfaced pens (8 bulls or 10 heifers per pen)

measuring 40 x 6.1 m with a single automatic water fountain shared between every 2 pens. Feed was delivered to calves in fence-line (6.1 m in length) concrete feed bunks allowing for 61 or 76.25 cm (heifers and bulls, respectively) of linear bunk space per calf and which had a 3.5-m-wide concrete apron adjacent to the feed bunk and water fountain to provide a solid area for steers to stand while eating or drinking. Thirty days after initiation of the experiment at 1300 h a final BW was collected. A 3% shrink was applied to final BW because calves had ad libitum access to water and one- half of a day's feed delivery.

During the trial, all diets were fed once daily at 0700 h. Feed bunks were evaluated at 1700 h on the previous day and bunks devoid of feed were noted. At 0600 h the next day bunks were swept and orts were collected and weighed. The target was to have 0.5 to 1.0 kg/pen of feed remaining in the bunk. If bunks were devoid of feed at 1700 h or for 2 consecutive mornings; the daily feed amount was increased by 0.45 kg of feed (**AF**) per calf. When 5 kg or more were remaining in the bunk at 0600 h, the feed call was decreased by the amount remaining in the bunk. On the final morning of the experiment, pens received half of the daily feed amount that was fed the prior morning. Dietary treatments included: 1) a dried distiller's grain-based TMR (**DDG**) initiated upon arrival, or 2) long-stem grass hay followed by a TMR containing no DDG (**HAY**). Calves receiving the HAY treatment received only grass hay for the first d after arrival, long-stem grass hay and TMR combination the following 2 d, followed by a grain-based TMR on d 4. The proximate analysis of the grass hay on a DM basis was CP 11.96%, ADF 36.39%, NDF 60.26%, $NE_m$ 1.08 Mcal/kg, and $NE_g$ 0.529 Mcal/kg. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets (Table 1).

The experiment was conducted as a randomized block design with 2 treatments and 2 gender blocks with a total of 7 replicates per treatment. Live BW, ADG, G:F, feed to gain ratio, and DMI data were analyzed on a pen mean basis using the MIXED model procedures (SAS Inst. Inc., Cary, NC). Treatment and gender were included in the model as fixed effects. Pen was included in the model as a random effect. Initial BW was included as a covariate when analyzing final BW.

## RESULTS AND DISCUSSION

Feedlot performance of calves by treatment is included in Table 2. Initial BW was similar ($P = 0.99$) across treatments; however, d 30 BW was greater ($P < 0.001$) for calves receiving DDG vs. HAY treatment. As a result, ADG was greater ($P < 0.001$; 0.59 vs. 0.41 ± 0.04 kg/d, respectively) for DDG vs. HAY calves. The greater performance of the DDG treatment could be due to increased energy density of the ration in the first 4 d, and the for increased DMI ($P = 0.06$) over the 30-d period. This is consistent with Fluharty and Loerch (1996) who found calves consuming higher energy diets performed

**Table 1.** Ingredient composition of diets for both treatments on a DM basis

| | Diet | |
|---|---|---|
| Item[1] | HAY[2] | DDG[3] |
| Corn Silage | 28.69 | 29.06 |
| Cracked Corn | 28.67 | 31.34 |
| Wheat Straw | 3.78 | 13.29 |
| Alfalfa hay | 36.53 | 0.00 |
| Dry Distillers Grains (DDG) | 0.00 | 22.73 |
| Calcium Carbonate | 0.00 | 1.25 |
| Supplement | 2.33 | 2.33 |
| DM, % AF | 60.01 | 60.04 |
| CP | 14.50 | 14.50 |
| NPN[4] | 1.09 | 1.09 |
| NDF | 31.24 | 35.60 |
| NEm, Mcal/kg | 1.62 | 1.64 |
| NEg, Mcal/kg | 1.06 | 1.06 |
| Calcium | 0.68 | 0.75 |
| Phosphorus | 0.29 | 0.38 |
| Ca:P ratio | 2.36 | 1.96 |

[1] Percentage of DM unless stated otherwise.
[2]HAY treatment received only grass hay for the first d after arrival, long stem grass hay and total mixed ration combination the following 2 d. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets.
[3]DDG treatment received only the total mixed ration for the entire 30-d period.
[4]CP equivalent.

**Table 2.** Feedlot performance of beef calves comparing receiving strategies at weaning

| | Treatment | | | |
|---|---|---|---|---|
| Item | HAY[1] | DDG[2] | SEM | Prob. > F |
| Initial BW, kg | 233 | 233 | 14.9 | 0.99 |
| Final BW, kg | 258 | 269 | 1.2 | 0.001 |
| ADG, $kg \cdot hd^{-1} \cdot d^{-1}$ | 0.41 | 0.59 | 0.04 | 0.001 |
| DMI, $kg \cdot hd^{-1} \cdot d^{-1}$ | 2.35 | 2.70 | 0.256 | 0.06 |
| G:F | 0.17 | 0.22 | 0.013 | 0.05 |
| Feed to gain | 2.66 | 2.14 | 0.171 | 0.05 |

[1] HAY treatment received only grass hay for the first d after arrival, long stem grass hay and total mixed ration combination the following 2 d. Beginning on d 4, calves across all treatments had access to iso-caloric and iso-nitrogenous diets.
[2] Dry Distillers Grains (DDG) treatment received only the total mixed ration for the entire 30-d period.

better during the first week after being received into the feedlot.

Gain-to-feed ratio was greater ($P < 0.05$; 0.22 vs. 0.17± 0.013, respectively), and feed-to-gain ratio tended ($P = 0.05$) to be greater in HAY vs. DDG calves. Daily DMI tended ($P = 0.06$) to be greater in DDG vs. HAY calves (2.70 $kg \cdot hd^{-1} \cdot d^{-1}$ vs. 2.35 $kg \cdot hd^{-1} \cdot d^{-1} \pm 0.256$, respectively). Wagner et al. (2012) found no improvement in ADG or DMI when comparing a dry-rolled corn receiving diet to a wet distillers grian receiving diet.

There was no calf mortality during the current experiment, which is consistent with Fluharty and Loerch (1996). And, 1 calf was treated for illness during the 30-d period, which is historically consistent with calves weaned from this cowherd.

The results are hard to interpret due to the lack of a TMR without distiller's grains and without grass- hay for the first 4 d. This was due to a limited number of animals available for this experiment. Subsequent experiments should investigate this effect.

## IMPLICATIONS

Providing a DDG-based receiving ration to newly weaned calves upon arrival to the feedlot likely results in greater feed intake, gain, and feed efficiency over a 30-d period than traditional long-stemmed grass hay followed by a non-DDG TMR. This may result in fewer days on feed and better overall feedlot performance.

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# DROUGHT MANAGEMENT: REPLACING HAY WITH A FIELD PEA-CO-PRODUCT SUPPLEMENT FED DAILY OR ON ALTERNATE DAYS

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**ABSTRACT**: One hundred-seven, 3-10 year old, third trimester-early lactation cows were randomized to treatment and weight blocks, in a 113 day study to evaluate a 25% hay reduction and a blended RDP-RUP supplement replacement for hay fed either daily (D) or on alternated days (Alt-D) as a drought management strategy. Control cows (C) were fed an all hay diet (Alfalfa-Bromegrass; 10.2% CP) or a hay and wheat straw (4.7% CP) diet in which the amount of hay fed per cow was reduced 25% and replaced with a field pea-BMS-DDGS supplement (22.8% CP) that was fed either daily (D) at 0.25% of initial BW or 0.50% of initial BW on alternate days (Alt-D). The 0.635 cm pelleted supplement contained a blend of RDP and RUP from field peas (70.0% RDP), distillers dried grain with solubles (65.0% RUP), and barley malt sprouts (64.0% RDP). Data were analyzed using MIXED procedures of SAS. Unsupplemented C cows were fed an average 18.8 kg of mixed hay daily compared with supplemented cows that were fed 9.40 kg of hay, 2.41 kg wheat straw, and 1.53 kg of the pea-co-product supplement totaling 13.34 kg daily (As-Fed). Using the blended RDP-RUP supplement as a replacement for hay, fed either D or on Alt-D, did not affect ending cow weight (P = 0.301), body condition score ($P = 0.624$), 12th rib fat depth (P = 0.415), or pre-breeding estrous cyclicity ($P = 0.678$). Subsequently, hay conserving strategies did not affect fall calf weaning weight (P = 0.634), gain ($P = 0.621$), or ADG ($P = 0.644$). Daily cost per cow was $1.13, $1.19 and $1.19 for the C, D and Alt-D methods, respectively. The data suggest that blending the selected RDP and RUP ingredient sources supplied sufficient ammonia nitrogen to the rumen on the non-supplementation day and appeared to provide adequate nutrient flow when fed on alternate days; and proved to be an effective drought management strategy. Conversely, when hay is plentiful the conservation strategy would be a cost effective method for stockpiling hay as a hedge against drought or to make hay available as a cash crop.

**Key words**: beef cattle, drought management, field pea, rumen degradable protein-rumen undegradable protein

## INTRODUCTION

Distillers dried grains with solubles (DDGS) are difficult to pellet (personal communication with CHS Nutrition production manager). Koch and Landblom (2010) documented that, when field pea and barley malt sprouts were blended with DDGS, electrical use declined and pellet quality improved.

Nutrient-dense co-products can replace a large amount of forage, but when used in alternate day feeding systems, the supplement must not induce a rapid decline in rumen pH postfeeding. The starch degradation rate of field pea (*Pisum sativum*) is similar to that of corn (Robinson and McQueen, 1989) and rumen protein degradation (RDP) is estimated to range from 78 to 94% (Aufrere et al., 1994; NRC 1989).

Distillers dried grains with solubles are a source of rumen undegradable protein (**RUP**), energy, and minerals (Stock et al., 2000. As a percent of CP, DDGS contain approximately 65% RUP, which can be beneficial when balancing cattle diets for Metabolizable protein (Patterson et al., 2003).

Barley malting co-products consist primarily of dried malt sprouts and some thin light test weight barley. Barley malt sprouts (**BMS**) possess medium CP (16%), moderate energy (74%), and a NEg of 1.15 Mcal/kg indicating that the fiber component is of moderate to high digestibility (Lardy and Anderson, 2009).

Supplementation, as infrequently as every 6th day, with ingredients high in rumen undegradable protein (**RUP**) have been utilized effectively by ruminants fed low-quality forage without adversely affecting DMI, N efficiency, bacterial CP synthesis, or animal performance (Bohnert et al., 2002a, b). Atkinson et al. (2009) evaluated ruminal protein degradation and supplementation frequency on intake, N retention and nutrient flux across visceral tissues of lambs fed a low-quality forage diet. This study evaluated diets containing either predominantly RDP or RUP, which were compared with a blended diet containing a 50:50 blend of RDP and RUP that was fed daily or on alternate days. Forage OM, NDF, ADF, and N were unaffected by treatment, and neither protein degradability or supplement frequency had any effect on N retention.

Moderate body condition score of 5 (1 to 9 scale) has been suggested as the most functional target condition for mature beef cows at calving (Houghton et al., 1990; Richards et al., 1986). Feeding pre- and postpartum beef cows a blended field pea-BMS-DDGS supplement, as a replacement for hay, may be a desirable drought management strategy, but has not been evaluated. The objective of this research project was to evaluate a drought strategy in which a significant

amount of daily forage is replaced with a blended RDP/RUP supplement fed either daily or on alternate days to determine the effect on ending cow body weight, ending BCS, ending fat depth, estrous activity at the start of the breeding season, and the subsequent effect on calf gain and weaning weight.

## MATERIALS AND METHODS

All experimental protocols were approved by the North Dakota State University Animal Care and use Committee.

***Animals and Treatments.*** Multiparous (3 to 10 yr) range beef cows (n = 107) were randomly assigned in a 113 day study to three treatments: 1) all hay control diet (**C**), 2) 25% of forage DM replaced with blended RDP/RUP field pea/co-product supplement fed daily at the rate of 0.25% of BW (**D**), and 3) 25% of forage DM replaced with a blended RDP/RUP field pea/co-product supplement fed on alternate days at the rate of 0.50% of BW (**ALT-D**). There were four pen blocks per treatment (light, medium, medium-heavy, and heavy) and nine cows per pen.

***Diets and Adjustment for Temperature.*** In this field study, the diets fed were formulated to contain a calculated balanced energy concentration (DM Basis) across treatments using medium-quality alfalfa-bromegrass (*Medicago sativa* and *Bromus inermis*) hay (10.2% CP), wheat straw (4.7% CP), and the experimental pelleted 22.8% CP field pea-co-product supplement (field pea – 49.80%, barley malt sprouts – 22.0%, DDGS – 20.0%, molasses – 5.0%, dical – 2.45%, salt – 0.50%, trace mineral premix – 0.15%, and vitamin premix – 0.025%). The forages were delivered to the cows daily using a Haybuster forage processor equipped with a Digi-Star EZ 2000 electronic scale. Within a 7-day feeding period, alfalfa-bromegrass hay was fed 6 days and wheat straw was fed 1 day. Forages were fed on the ground and orts were not weighed back. The field pea-BMS-DDGS supplements were fed in portable bunks at the rate of 0.25% of trial starting BW for the D supplemented treatment and 0.50% of trial starting BW for the ALT-D treatment.

Winter temperature and wind speed in North Dakota can fluctuate widely from pleasant temperatures and light wind to strong wind, blizzards, and subzero temperatures. During the 16 week study, the amount of daily dry matter fed within each weight block was determined based on the estimated energy content of the supplement, alfalfa-bromegrass hay, and wheat straw. To arrive at the desired calculated NEm energy balance across treatments, the initial DMI for each weight block was determined using the NRC (1996) formula for DMI. The initial late gestation daily NEm/cow for each starting weight block was calculated to be 10.10, 10.80, 11.67, and 12.52 Mcal/day for the light, medium, medium-heavy, and heavy weight blocks, respectively. Since temperature fluctuations in western North Dakota can be extreme, the amount of hay DMI was adjusted at the beginning of each week for temperature based on the local weather forecast for the upcoming week. Dry matter increases used, due to declining temperature, were as follows: 12.2 ºC and above – no increase, 12.2 ºC to -15.0 ºC + 7% increase, -15.0 ºC to -17.8 ºC + 10% increase, -17.8 ºC to -23.3 ºC + 16% increase, and -23.3 ºC to -28.9 ºC + 20.0% increase. These dietary adjustments only affected the amount of hay delivered to each BW block. During the entire study, the RDP/RUP levels, which were established based on cow starting BW, did not change. Gestation diets were fed from the first week of January to the 3rd week of March, when the diets were reformulated for lactation by removing wheat straw and increasing hay DM. The daily lactation NEm balance for weight blocks was calculated to be 15.80, 18.19, 19.06, and 19.97 Mcal/day for the light, medium, medium-heavy, and heavy weight blocks, respectively. The lactation diets were fed until the last week of April, when the cow-calf pairs were moved to crested wheatgrass pastures.

***Data Collection and Assay Procedures.*** Measurements of cow performance included changes in cow BW, BCS, 12th rib fat depth, number of cows in estrus at the start of the breeding season, and subsequent weaning weight. Visual BCS and ultrasound fat depth measurements were collected each time the cows are weighed.

The number of cows cycling at the start of a 45 d breeding season was based on the circulating progesterone concentration derived from two blood serum samples collected 10 days apart just prior to the start of the breeding season. Circulating concentrations of progesterone were analyzed in all serum samples using methodology described by Engel et al. (2008). Intra- and interassay CV for progesterone assays were 2.47 and 5.9%.

***Statistical Analysis.*** The data was analyzed using the generalized least squares MIXED analysis procedure (SAS Inst. Inc., Cary, NC). Main effects included dietary treatments and pen served as the experimental unit. Pretrial cow gestation interval days were used as a covariate to adjust cow starting and ending weight, and gain.

## RESULTS AND DISCUSSION

According to the project objective, a drought management strategy was evaluated in which the amount of forage fed daily was reduced and replace with a nutrient-dense field pea-BMS-DDGS supplement fed either daily at 0.25% of BW or on alternate days at 0.50% of BW (Table 1). For the 113 day late gestation-early lactation period, C cows consumed an average 18.8 kg of alfalfa-bromegrass hay and the D and ALT-D day supplemented cows consumed an average 9.4, 2.41, and 1.53 or 3.07 kg/d of alfalfa-bromegrass hay, wheat straw, and field pea-BMS-DDGS supplement, respectively. The diet cost/cow/day was 1.13, 1.19, and $1.19 for the C, D, and ALT-D treatments, respectively.

Cow performance was not negatively affected by forage reduction or supplementation frequency (Table 2). Using pretrial gestation interval as a covariate, cow starting weight, ending weight, and gain did not differ between treatments ($P > 0.10$). Cow BCS changed from a prepartum starting condition of approximately 6 across treatments ($P = 0.81$) to an ending postpartum BCS of 5.39, 5.47, and 5.14 for the C, D, and ALT-D treatments, respectively, that did not differ ($P = 0.624$). Ultrasound fat depth mimicked BCS and did not differ between treatments ($P = 0.415$).

**Table 1.** Forage, supplement consumption, and feed cost following hay replacement with a field pea based co-product supplement fed daily or on alternate days

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Item | Control | Daily | Alternate Day | SEM | *P*-Value |
| Hay Intake: | | | | | |
| Hay, kg/Cow/Day [a-b] | 18.8[a] | 9.4[b] | 9.44[b] | 0.34 | 0.001 |
| Straw/Cow/Day, kg | - | 2.41 | 2.41 | - | - |
| Total Forage/Cow/Day, kg | 18.8 | 11.81 | 11.85 | - | - |
| Supplement Intake: | | | | | |
| Supplement/Cow/Day, kg | - | 1.53 | 3.07 | - | - |
| % of Cow Body Wt., % | - | 0.249 | 0.497 | - | - |
| Cost/Cow/Day, $ | 1.13 | 1.19 | 1.19 | | |

[a,b] Means within a row with different superscripts differ ($P \leq 0.001$).

[1]Treatments: Control = all hay; Daily = Blended RDP/RUP Field Pea Co-product supplement fed daily at 0.25% of starting cow BW; Alternate Day = Blended RDP/RUP Field Pea Co-product supplement fed on alternate days at twice the daily rate or 0.50% of starting cow BW.

**Table 2.** Cow and calf performance following hay replacement with a field pea-based co-product supplement fed daily or on alternate days

| | Treatments[1] | | | | *P*-Value | |
|---|---|---|---|---|---|---|
| Item | Control | Daily | Alternate Day | SE | Covariate | Trt |
| PreTrial Gestation Interval, Days | 197.8[a] | 198.7[a] | 195.3[b] | 1.23 | | 0.061 |
| Cow Performance: | | | | | | |
| Cow Start Wt., kg | 651.3 | 654.9 | 665.7 | | 0.634 | 0.473 |
| SE | (33.09) | (33.13) | (33.17) | | | |
| Cow End Wt., kg | 610.7 | 595.5 | 643.7 | | 0.085 | 0.301 |
| SE | (21.84) | (23.87) | (25.66) | | | |
| Cow Gain, kg | -33.70 | -40.22 | -37.95 | | 0.963 | 0.900 |
| SE | (10.22) | (10.88) | (11.47) | | | |
| Cow ADG, kg | -0.2982 | -0.3398 | -0.3359 | | 0.963 | 0.900 |
| SE | (0.0904) | (0.0963) | (0.1015) | | | |
| Body Condition Score: | | | | | | |
| Start BCS | 6.10 | 6.00 | 5.95 | 0.23 | | 0.810 |
| End BCS | 5.39 | 5.47 | 5.14 | 0.34 | | 0.624 |
| BCS Decrease | -0.71 | -0.53 | -0.81 | 0.12 | | 0.191 |
| 12th Rib Fat Depth: | | | | | | |
| Start Rib Fat Depth, mm | 6.42 | 6.31 | 6.53 | 0.67 | | 0.973 |
| End Rib Fat Depth, mm | 3.97 | 4.92 | 4.67 | 0.74 | | 0.415 |
| Rib Fat Depth Decline, mm | -2.46 | -1.39 | -1.86 | 0.47 | | 0.190 |
| Pre-Breeding Progesterone - Percent Cycling, % | 70.35 | 79.06 | 75.06 | 6.85 | | 0.678 |
| Calf Performance: | | | | | | |
| Calf Birth Wt., kg [a,b] | 41.7[a] | 39.5[b] | 43.3[a] | 0.72 | | 0.014 |
| Calf Weaning Age, days | 213.9 | 214.9 | 211.6 | 1.23 | | 0.061 |
| Calf Weaning Wt., kg | 299.9 | 304.6 | 305.1 | 6.34 | | 0.634 |
| Calf Wt. Gain, kg | 258.2 | 265.1 | 261.9 | 6.31 | | 0.621 |
| Calf ADG, kg | 1.21 | 1.23 | 1.24 | 0.027 | | 0.644 |

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: Control = all hay; Daily = Blended RDP/RUP Field Pea Co-product supplement fed daily at 0.25% of starting cow BW; Alternate Day = Blended RDP/RUP Field Pea Co-product supplement fed on alternate days at twice the daily rate or 0.50% of starting cow BW.

Postpartum circulating progesterone concentrations did not differ ($P$ = 0.678); however, there was a numerically greater number of cows in estrus at the start of the breeding season among supplemented cows compared with the control cows.

Calf birth weight for calves whose dams received daily supplement were lighter ($P$ = 0.014) than calves from either C or ALT-D supplemented cows. Calf weaning weight ($P$ = 0.634) and preweaning gain ($P$ = 0.621) did not differ.

Although hay is the most common feed fed to gestating and lactating beef cows, the cost per unit of energy is often considerably more expensive than high energy feedstuffs such as corn (Loerch 1996; Schoonmaker et al., 2003). Radunz et al. (2010) documented that energy source, i.e. grass hay, corn, or DDGS, fed during gestation did not affect pre- and postpartum cow performance, but energy partitioning associated with corn and DDGS shunted nutrients to the fetus and increased birth weight. In the present study, ending ultrasound fat depth did not differ; however, fat depth decline was numerically greater among the ALT-D and C hay groups, and calf birth weight among these two groups was greater ($P$ = 0.014) compared with the daily supplemented group. Energy, CP, and amino acids are essential for late gestation fetal growth (Ferrell et al., 1976). Although the current data is not conclusive, it appears that there may have been greater lipid mobilization from fat stores among cows in the C and ALT-D groups combined with greater energy and N flow when the ALT-D supplemented cows received a double amount of supplement resulting in greater calf birth weight. This may also be associated with feeding excess protein on the days of supplementation, and the possible loss of nitrogen compared with daily supplemented cows through excretion of excess nitrogen in the urine.

## IMPLICATIONS

These data are in agreement with others and suggest that nutrient-dense field pea-BMS-DDGS supplements formulated to contain blended RDP and RUP, and fed on alternate days, can be used to supply adequate protein and energy to pre- and postpartum cows fed restricted hay diets. The supplementation strategies tested maintained an ending postpartum BCS 5 and also suggested that restricted hay intake diets can be competitively priced.

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# EFFECT OF BEEF HEIFER DEVELOPMENT SYSTEM ON ADG, REPRODUCTION, AND FEED EFFICIENCY DURING FIRST PREGNANCY

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**ABSTRACT:** A 3-yr study was conducted to determine the effect of heifer development system on ADG, reproductive performance, and subsequent feed efficiency as a pregnant heifer. Crossbred Angus heifers (n = 299) were assigned by initial BW to graze corn residue (CR) or developed in a drylot (DL). Corn residue heifers grazed native pasture 33 d prior to grazing CR 74 d. Corn residue heifers were then placed on dormant forage pastures 66 d and then with DL heifers for approximately 40 d for synchronization and AI. Heifers assigned to DL grazed dormant forage for 98 d and then placed in a DL for 112 d. The following winter, a subset of pregnant CR and DL heifers (n = 114) were stratified by BW and development system, and placed in a Calan Broadbent individual feeding system for 84 d during late gestation. Prebreeding BW was greater ($P$ = 0.01) for DL heifers compared with CR heifers (349 vs. 314 ± 9 kg). At pregnancy diagnosis BW remained greater ($P$ = 0.05) for DL compared with CR heifers (422 vs. 403 ± 10 kg). Drylot heifers had greater ($P$ = 0.01) overall ADG during development compared with CR heifers. There was no difference ($P \geq 0.33$) in percent cycling (42 vs. 52 ± 15%), AI pregnancy (69 vs. 63 ± 7%), or final pregnancy rates (93 vs. 91 ± 3%) for CR and DL heifers, respectively. At the beginning of the second winter, initial BW was similar ($P$ = 0.76) between development systems. However, pre-calving BW tended ($P$ = 0.08) to be greater for DL heifers compared with CR. Gestation length, calving date, and calf birth BW were similar ($P \geq 0.37$) between development systems. Dry matter intake and RFI were similar ($P \geq 0.33$) between treatments. Drylot heifers tended to have greater ($P \leq 0.09$) ADG (0.80 vs. 0.70 ± 0.14 kg/d) and G:F compared with CR. Heifers developed on CR had reduced BW through early pregnancy, however reproductive performance was similar to DL developed heifers.

**Key words:** beef cattle, feed efficiency, heifer development

## INTRODUCTION

Previous literature suggests development of heifers to approximately 65% mature BW is required to maximize pregnancy rates (Patterson et al., 1992). These early data would also indicate an inverse correlation between postweaning growth rate and age at puberty (Patterson et al., 1992; Funston et al., 2012). The single greatest cost of heifer development is feed. Reducing harvested forage use could decrease heifer development costs. Decreasing development BW 5-10% below contemporaries resulted in a $19 to 45/heifer decrease in development cost (Feuz, 2001; Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011).

In addition to reduced feed inputs, reports indicate decreased BW through pregnancy diagnosis in heifers developed to reduced proportions of mature BW (Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011) and reduced BW through 5-years of age in heifers restricted feed 140 d post weaning (Roberts et al., 2009) compared with their nonrestricted contemporaries. This reduction in BW would suggest reduced maintenance requirements and thus decreased production costs. Furthermore, reduced input production systems report similar reproductive performance when compared with contemporaries fed to a higher plane of nutrition or to a greater proportion of mature BW (Freetly et al., 2001; Funston and Deutscher, 2004; Martin et al., 2008; Roberts et al., 2009; Funston and Larson, 2011). The objective of this study was to determine the effect of heifer development system on ADG, reproductive performance, and subsequent feed efficiency as a pregnant heifer.

## MATERIALS AND METHODS

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment.

***Developing Heifer Management.*** Weaned crossbred Angus heifers (n = 299) were received at the West Central Research and Extension Center (**WCREC**), North Platte, NE. After a 14 d acclimation period heifers were blocked by BW and randomly assigned to either graze corn residue (**CR**) or developed in the drylot (**DL**). Corn residue heifers grazed native pasture 33 d prior to grazing CR 74 d. Subsequently, CR heifers were placed on dormant pastures 66 d prior to being placed in the DL for approximately 40 d for synchronization of estrus and AI. Drylot heifers grazed native forage pastures 98 d then were placed in the DL for 112 d. Drylot diet was formulated for heifers to reach 65% mature BW at the beginning of the breeding season. During winter grazing (native pasture and corn residue) all heifers were offered 0.45kg/d (28% CP, DM basis) distillers based supplement. Forty d prior to AI, CR and DL heifers were managed together and fed a common diet.

Prior to the breeding season blood samples were collected 10 d apart via coccygeal venipuncture to determine plasma progesterone concentration. Plasma progesterone concentration was determined through direct solid phase RIA

(Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA). Heifers with plasma progesterone concentrations >1.0 ng/mL were considered pubertal.

Estrus was synchronized utilizing the melengestrol acetate-prostaglandin (**MGA-PGF**) synchronization protocol with heifers fed MGA (Pfizer Animal Health, New York, NY) for 14 d and administered a single injection of PGF (Lutalyse, Pfizer Animal Health) 19 d after the end of MGA feeding. Estrus detection was performed for 5 days following PGF administration. Each year heifers were randomly AI to 1 of 4 bulls approximately 12 h after standing estrus. Approximately 10 d following the last d of AI heifers were exposed to bulls (1bull:50 heifers) for 60 d. Artificial insemination and overall pregnancy rates were determined 45 d after AI and 45 d after bull removal, respectively, via transrectal ultrasonography.

***Primiparous Heifer Management.*** All heifers remained in a common group through the summer grazing native pasture. After final pregnancy diagnosis, a subset of heifers (yr 1 = 38; yr 2 = 40; yr 3 = 36) confirmed AI pregnant were placed in a Calan Broadbent individual feeding system during late gestation. Heifers were allowed approximately 25 d to adapt to the individual feeding system followed by an 84 d feeding trial. Heifers were offered ad libitum grass hay and either no supplement; 0.83 kg/d distillers based supplement; or 0.83 kg/d dried corn gluten based supplement. Supplements were formulated to be isocaloric and isonitrogenous, but differed in rumen undegradable protein. Feed offered was recorded daily and refusals removed and weighed weekly. Residual feed intake (**RFI**) was calculated as the predicted DMI – actual DMI, with DMI calculated based on NE values of the feed to account for different energy levels of the supplement compared with the control diet.

After calving, heifers remained at WCREC through breeding. Artificial insemination utilized a fixed-timed AI protocol and pairs were transported 43km to a commercial ranch in the Nebraska Sandhills for summer grazing. A single bull was placed with heifers approximately 10 d after AI for 60 d. Pairs were returned to WCREC prior to weaning for final pregnancy diagnosis.

***Statistical Analysis.*** Data were analyzed using the MIXED and GLIMMIX procedures (SAS Inst. Inc., Cary, NC). Year was considered the experimental unit in heifer development data with development system as the fixed effect. A subset of animals from each development system were placed in 1of 4 pens where individual feeding occurred. This development by pen classification was included as a random variable and considered the experimental unit for individual feeding data with developmental treatment and barn diet as fixed effects and year considered a random effect. Heifer development x second winter treatment interaction was not significant and removed from the model. For the individual feeding period, heifer development x barn treatment interaction was not significant, thus all data are presented as the effect of heifer development system. A $P$-value ≤ 0.05 was considered significant.

## RESULTS AND DISCUSSION

***Heifer Development BW Gain and Reproduction.*** Data for heifer development BW gain are reported in Table 1. Body weight was similar for CR and DL heifers at the beginning of the experiment (220 vs. 220 ± 3 kg; $P = 0.93$). However, prior to breeding, DL heifers were 35 kg (±9 kg) heavier ($P = 0.01$) than CR developed heifers. Body weight remained greater for DL heifers at AI pregnancy diagnosis (370 vs. 348 ± 11 kg; $P = 0.02$) and final pregnancy diagnosis (422 vs. 403 ± 9 kg; $P = 0.05$) compared with CR heifers. These data agree with previous literature (Funston and Larson, 2011) reporting DL developed heifers having greater ($P < 0.05$) BW from the end of the development period through pregnancy diagnosis compared with heifers developed on CR and winter range. However, in that study DL heifers did not graze dormant forage pastures prior to entering the DL and were thus in the DL between 150- 204 d, whereas DL heifers in the current study grazed dormant forage pasture for 98 d and then placed in the DL for 112 d.

Overall ADG was greater ($P = 0.01$) for DL heifers compared with CR heifers. Dry lot developed heifers also had greater ($P = 0.04$) ADG from February to April compared with CR heifers which would coincide with the time the DL heifers were removed from dormant pasture and placed in the DL, while the CR heifers were moved from CR to native dormant pasture. Average daily gain was similar (0.81 vs. 0.58 ± 0.13 kg/d; $P = 0.25$) between CR and DL heifers while in the DL prior to breeding and remained similar through AI pregnancy diagnosis ($P = 0.24$).

Although ADG was greater through development for DL compared with CR heifers, there was no difference ($P = 0.46$) in the proportion of heifers pubertal prior to the breeding season (Table 1). Martin et al. (2008) also reported no significant difference in attainment of puberty for heifers fed to 51 vs. 57% mature BW. However, Funston and Larson (2011) reported decreased puberty in heifers developed on winter range and CR compared with DL developed heifers (56 vs. 65% mature BW, respectively). In the current study, DL developed heifers were developed to 63% mature BW (554 kg mature BW) compared with 57% ($P = 0.01$) for CR developed heifers. There were no differences ($P \geq 0.33$) in AI pregnancy (69 vs. 63 ± 7%), or final pregnancy rates (93 vs. 91 ± 2%; Table 1) for CR and DL, respectively. Although previous research indicates maximal reproductive rate when heifers were developed to approximately 65% mature BW prior to breeding (Patterson et al., 1992), more recent data suggests developing heifers to 50 to 57% mature BW at breeding will result in similar pregnancy rates as heifers developed to a greater percent of mature BW (Martin et al., 2008; Funston and Larson, 2011; Funston et al., 2012). This is in agreement with data reported in the current study.

***Primiparous Heifer Feed Efficiency.*** Data for primiparous heifers placed in the Calan Broadbent individual feeding system during late gestation are reported in Table 2. Initial BW was similar ($P = 0.76$) between DL and CR heifers. However, pre-calving BW tended to be greater ($P = 0.08$) for

**Table 1.** Effect of winter heifer development system on BW, ADG, and reproductive performance

| Item | CR[1] | DL[2] | SEM | *P*-value |
|---|---|---|---|---|
| n | 3 | 3 | | |
| Initial BW, kg | 220 | 220 | 3 | 0.93 |
| Prebreeding BW, kg | 314 | 349 | 9 | 0.01 |
| AI pregnancy check BW, kg | 348 | 370 | 11 | 0.02 |
| Pregnancy check BW, kg | 403 | 422 | 9 | 0.05 |
| | | | | |
| ADG | | | | |
| Overall[3], kg/d | 0.43 | 0.59 | 0.03 | 0.01 |
| Dec- Feb[4], kg/d | 0.04 | 0.06 | 0.12 | 0.64 |
| Feb-April[5], kg/d | 0.55 | 1.09 | 0.04 | 0.04 |
| April-May[6], kg/d | 0.81 | 0.58 | 0.13 | 0.25 |
| May- July[7], kg/d | 0.52 | 0.39 | 0.06 | 0.24 |
| July-September[8], kg/d | 0.77 | 0.75 | 0.03 | 0.61 |
| | | | | |
| Pubertal[9], % | 42 | 52 | 15 | 0.46 |
| AI pregnant, % | 69 | 63 | 7 | 0.33 |
| Pregnant, % | 93 | 91 | 2 | 0.41 |
| Mature BW, % | 57 | 63 | 2 | 0.01 |

[1]CR= heifers grazed dormant pastures 33 d, corn residue 74 d, and were placed on dormant winter pastures 66 d prior to entering the drylot 40d before AI.
[2]DL= heifers grazed dormant pastures 98 d prior to entering the drylot 112 d before AI.
[3]ADG from initiation to prebreeding.
[4]ADG while grazing dormant pasture and corn residue (CR) or dormant pasture (DL).
[5]ADG while grazing dormant pasture (CR) or while in drylot (DL).
[6]ADG while in the drylot.
[7]ADG from breeding to AI pregnancy detection.
[8]ADG from AI pregnancy to final pregnancy detection.
[9] Considered pubertal if blood serum progesterone concentrations were > 1 ng/mL.

**Table 2.** Effect of winter heifer development system on late gestation ADG, feed efficiency and reproductive performance through the subsequent breeding season

| Item | CR[1] | DL[2] | SEM | *P*-value |
|---|---|---|---|---|
| n | 3 | 3 | | |
| Initial BW, kg | 449 | 450 | 9 | 0.76 |
| Pre-calving BW, kg | 506 | 516 | 9 | 0.08 |
| DMI, kg/d | 10.14 | 10.14 | 0.10 | 0.96 |
| NE DMI, kg/d | 4.97 | 4.97 | 0.07 | 0.92 |
| ADG, kg/d | 0.70 | 0.80 | 0.14 | 0.09 |
| RFI, NE | 0.036 | -0.048 | 0.135 | 0.33 |
| G:F | 0.068 | 0.078 | 0.013 | 0.07 |
| | | | | |
| Gestation length, d | 276 | 276 | 0.66 | 0.77 |
| Birth date, Julian | 60 | 61 | 1.42 | 0.37 |
| Calf birth BW, kg | 72 | 74 | 1.58 | 0.39 |
| Calving ease | 1.38 | 1.48 | 0.11 | 0.53 |
| Prebreeding BW, kg | 441 | 442 | 6 | 0.89 |
| Pregnancy check BW, kg | 482 | 474 | 26 | 0.64 |
| Pregnant, % | 83 | 81 | 17 | 0.83 |

[1]CR= heifers grazed dormant pastures 33 d, corn residue 74 d, and were placed on dormant winter pastures 66 d prior to entering the drylot 40d before AI.
[2]DL= heifers grazed dormant pastures 98 d prior to entering the drylot 112 d before AI.

DL compared with CR heifers at the end of the 84-d individual feeding period. There was no difference ($P \geq 0.92$) in DMI or DMI based on feed NE. Average daily gain and G:F tended to be greater ($P = 0.09$; = 0.07, respectively) for DL compared with CR developed heifers. There were no differences ($P = 0.33$) between treatments for RFI based on NE values of the diet. Furthermore, gestation length, calf birth BW, and calving ease did not differ ($P \geq 0.37$) among treatments. Pre-breeding BW did not differ ($P = 0.89$) between treatments as first calf heifers and proportion pregnant at weaning was also similar ($P = 0.83$) for DL compared with CR developed heifers.

## IMPLICATIONS

Traditional DL development systems would place heifers in DL shortly after weaning. In this experiment, developing heifers on dormant pasture followed by DL increased heifer BW from the end of the development period through pregnancy diagnosis compared with CR developed heifers. However, reproductive and calving performance was similar between treatments. Reproductive performance was maintained by developing heifers with reduced harvested forage.

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# EFFECTS OF WEANING AGE AND WINTER DEVELOPMENT ENVIRONMENT ON HEIFER PERFORMANCE[1]

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**ABSTRACT:** Our objective was to determine if early weaning (about 125 d) vs. normal weaning (about 250 d) and wintering replacement heifers in drylot vs. rangeland affected heifer growth and reproductive performance. Heifer calves from the 2009 and 2010 calf crops (n = 104 and 73, respectively) were allocated to the 2 weaning treatments and then stratified by age into the 2 winter development treatments forming a 2 by 2 factorial of treatments. Heifers wintered in drylot received mixed grass and alfalfa hay (year 1: 11.6% CP, 52.5% TDN; year 2: 12.3% CP, 53.4% TDN) plus 1.8 kg of a dried distiller's grain (DDGS)-based supplement/hd/d (year1: 22.7% CP, 75.8% TDN; year2: 25.4% CP, 76.7% TDN). Heifers wintered on rangeland also received 1.8 kg/hd/d of the same supplement. Over the winter, each treatment was allocated to a separate pen or pasture. After estrus synchronization and timed AI, all heifers were placed on rangeland to graze through the summer. During the summer of year 1, heifers were allocated by winter treatment to 2 pastures, and in year 2 all 4 treatment combinations were allocated to separate pastures. Responses measured were BW, ADG, puberty at initiation of estrus synchronization, and pregnancy detection. Pubertal status was indicated by serum progesterone $\geq$ 1 ng/mL. A winter by weaning treatment interaction affected ($P$<0.001) BW and ADG both years. During the winter months, range heifers were lighter and grew slower than drylot, but BW did not differ due to winter treatments at the end of the summer. However, early weaned heifers remained lighter than normal weaned heifers at the end of the summer. Weaning treatment affected ($P$ = 0.03) fall pregnancy rate (93.2% ± 4.0 and 74.7% ± 7.98 for early- and normal-weaning, respectively) in year 2. In year 1, there was a difference ($P$ = 0.006) between drylot and range heifers (92.7% ± 3.52 and 72.8% ± 6.47, respectively) in the proportion that obtained puberty before estrus synchronization. In conclusion, a producer needs to consider important interactions between weaning and winter management practices when establishing a replacement heifer development program that best fits the goals of their operation.

**Key words:** beef heifer development, heifer performance, weaning

## INTRODUCTION

There have been multiple research projects on different heifer development programs to evaluate effectiveness of alternative options (Olson et al., 1992; Arthington and Kalmbacher, 2003; Salverson et al., 2005). Past research has suggested that rangeland may be an effective resource to develop heifers that are destined to become range cows (Olson et al., 1992; Salverson et al., 2005).

The objective of this study was to evaluate how two ages at weaning, early and normal, and two winter development environments, rangeland and drylot, affected heifer growth and development. We hypothesized that heifers wintered on rangeland would have lower ADG and would be lighter at initation of breeding compared with the heifers wintered in drylot, but would have greater compensatory growth throughout the summer months. We also hypothesized that both treatments would have similar reproductive performance as they both received supplements to achieve optimal development by breeding.

We also hypothesized that normal-weaned range heifers would have better grazing distribution than early- weaned range heifers allowing them to have a better ADG throughout the summer. This would be a result of the normal-weaned heifers being with their mothers longer. We hypothesized that as a result of the previous hypothesis, the normal weaned heifers would have better reproductive performance than the early-weaned heifers.

We further hypothesized that wintering heifers in drylot would have the same results for both early-weaned and normal-weaned heifers. We also hypothesized that winter-range development would work best for normal- weaned heifers compared with early-weaned heifers when developing replacement heifers.

## MATERIALS AND METHODS

All animal procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

***Design and Treatments.*** Heifer calves from the 2009 and 2010 calf crop (n = 104 and 73, respectively) were split into two groups to either be early weaned (**EW**, about 125 d of age) or normal weaned (**NW**, about 250 d of age).

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These groups were based on cow assignments to weaning treatments for another study that was ongoing. In that study, cows had been stratified into two groups and then each group was randomly assigned to either be early- or normal-weaned when they entered the study, then sex of the calf determined which post-treatment experiment the calves went into. Within the two weaning-age groups, heifers were stratified by age into two winter development treatments. These groups were either wintered in drylot (**D**) or wintered on rangeland (**R**) from weaning to breeding. This created the following four treatment combinations in a 2 × 2 factorial treatment structure: 1) early weaned and wintered in drylot (**ED**), 2) early weaned and wintered on rangeland (**ER**), 3) normal weaned and wintered in drylot (**ND**), and 4) normal weaned and wintered on rangeland (**NR**). Heifers wintered in drylot received mixed grass and alfalfa hay (Table 1) ad libitum plus 1.8 kg of a dried distiller's grain (**DDGS**)-based supplement/hd/d (Table 1). Heifers wintered on rangeland also received 1.8 kg/hd/d of the same supplement. During the winter when the ground was snow covered to a depth that precluded grazing, range heifers received the same hay as the drylot heifers. Heifers in the ER treatment combination consumed 226 kg/hd of hay in year 1 and 305 kg/hd of hay in year 2. Heifers in the NR treatment combination consumed 219 kg/hd of hay in year 1 and 294 kg/hd of hay in year 2. Over the winter, each treatment combination was allocated to a separate pen or pasture. After estrus synchronization and timed AI, all heifers were placed on rangeland to graze through the summer. During the summer of year 1, heifers were allocated by winter treatment to 2 pastures, and all 4 treatment combinations were allocated to separate pastures in the summer of year 2.

***Collections.*** Heifer BW were recorded at EW (August 18, 2010; August 17, 2011), NW [November 3, 2010; November 2, 2011 (NW heifers only)], middle of the winter treatment period (March 9, 2010; February 4, 2011), first blood sampling (May 14, 2010; May 18, 2011), breeding (June 19, 2010; June 9, 2011), July pregnancy detection (July 29, 2010; July 26, 2011), end of summer grazing period (September 1, 2010; August 24, 2011), and fall pregnancy detection (November 3, 2010; October 20, 2011).

**Table 1.** Nutrient analyses of grass/alfalfa hay and DDGS[1]

| | Feedstuff | | | |
|---|---|---|---|---|
| | Year 1 | | Year 2 | |
| Item | Hay | DDGS | Hay | DDGS |
| DM, % | 87.1 | 93.4 | 89.3 | 91.8 |
| | ----- % of DM ----- | | | |
| CP, % | 11.6 | 22.7 | 12.3 | 25.4 |
| NDF, % | 62.6 | 33.4 | 56.5 | 32.8 |
| TDN, % | 52.5 | 75.8 | 53.4 | 76.7 |
| Ca | 0.93 | 2.01 | 1.17 | 1.72 |
| P | 0.21 | 0.67 | 0.18 | 0.75 |
| S | 0.11 | 0.50 | 0.14 | 0.48 |

[1] DDGS = dried distillers grains with soluble- based cube

***Puberty.*** Pubertal status of the heifers at the beginning of the breeding season was determined by analysis of serum progesterone. Blood samples were collected via jugular or coccygeal venipuncture into a 10-mL Vacutainer tube at d -10 (May 14, 2010; May 18, 2011), d 0 (May 25, 2010; May 30, 2011) and d 15 [June 9, 2010 (yr 1 only)], with d 0 being the initial start of estrus synchronization. Blood set at room temperature for 1 h to clot and was then centrifuged for 20 minutes. Serum was harvested and frozen at -20º C until analysis. Serum progesterone concentrations were analyzed by a previously validated radioimmunossay (Engel et al., 2008). Heifers were defined as having reached pubertal status if serum progesterone was ≥ 1 ng/mL in either serum sample.

***Breeding.*** On d -7 (May 25, 2010 and May 30, 2011) heifers received an estrus synchronization protocol and were bred by timed AI (June 19, 2010 and June 9, 2011) [100 µg GnRH (Cystorelin, Merial Marysville, Kansas) and Controlled Internal Drug Releasing device (**CIDR**) inserted on d -7; 25 mg PG (Lutalyse, Pfizer Kalamazoo, Michigan) and CIDR removal on d 0); timed AI with 100 µg GnRH at 72 hr post CIDR removal]. A mistake was made in yr 1 and the CIDR were reinserted on d 8 to d 15 and heifers were bred on June 19, 2010. Timed AI was followed by a 45-day exposure to natural service to complete the breeding season. Semen-tested bulls were used with a bull:heifer exposure ratio not exceeding 1:28 both years. Conception to AI was determined by trans-rectal ultrasonography on d 40 after AI in year 1 and d 47 after AI in year 2 (July 29, 2010 and July 26, 2011). Overall pregnancy rate was determined by rectal palpation in yr 1 and trans- rectal ultrasonography in yr 2 on d 90 (November 3, 2010 and October 20, 2011) after the breeding season.

***Statistics.*** Heifer BW and ADG were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included weaning treatment, winter treatment, and their interaction as independent variables. Each time heifers were weighed (or the intervals between weighing for ADG) and their interaction with weaning and winter treatments were included as repeated measures. Animal was included as a random effect and was considered the experimental unit.

Pregnancy rates and puberty status were analyzed using the GENMOD procedure of SAS with the use of the logit structure for binomial data. The model included independent variables of weaning treatment and wintering treatment as well as their interaction.

## RESULTS AND DISCUSSION

Weaning treatment, winter treatment, and weigh period interacted for BW and ADG during both years ($P < 0.001$) (Tables 2, 3, 4, and 5). In both years, R heifers were lighter and grew slower than D heifers during the winter months. Within each winter treatment the EW heifers were also lighter than the NW heifers. Once spring green-up occurred, R heifers had an increase in ADG and continued to gain more than the D heifers throughout the summer. At the end of the study in

**Table 2.** Effect of weaning and winter treatments on BW in 2010 yearlings

| | Early Weaned | | Normal Weaned | |
|---|---|---|---|---|
| Date | Drylot | Range | Drylot | Range |
| Birth, kg | 38.2 ± 1.17 | 38.2 ± 1.26 | 38.5 ± 1.28 | 39.3 ± 1.37 |
| 8/18/2009, kg | 168.3 ± 5.03 | 168.3 ± 5.42 | 168.8 ± 5.52 | 169.6 ± 5.90 |
| Weaning[1], kg | 168.3 ± 4.28[a] | 168.3 ± 4.61[a] | 234.0 ± 4.68[b] | 238.7 ± 5.02[b] |
| 3/9/2010, kg | 281.3 ± 4.42[c] | 227.1 ± 4.76[a] | 316.0 ± 4.84[d] | 262.2 ± 5.16[b] |
| 5/14/2010, kg | 305.3 ± 4.03[b] | 274.9 ± 4.33[a] | 360.8 ± 4.44[c] | 299.5 ± 4.71[b] |
| Breeding (6/19/10), kg | 318.8 ± 4.03[a] | 319.9 ± 4.34[a] | 364.4 ± 4.42[c] | 350.5 ± 4.71[b] |
| Preg. Check (7/29/10), kg | 347.1 ± 4.18[a] | 359.0 ± 4.49[a] | 391.9 ± 4.58[b] | 388.9 ± 4.88[b] |
| 9/1/2010, kg | 377.7 ± 4.43[a] | 399.7 ± 4.76[b] | 425.8 ± 4.85[c] | 427.6 ± 5.17[c] |

[a-c] Means within a row with different superscripts differ ($P < 0.05$).
[1] August 18, 2009 for early-weaned and November 3, 2009 for normal-weaned.

**Table 3.** Effect weaning and winter treatments on ADG in 2010 yearlings

| | Early Weaned | | Normal Weaned | |
|---|---|---|---|---|
| Date | Drylot | Range | Drylot | Range |
| 8/18/09 to 3/9/10 | 0.56 ± 0.012[c] | 0.29 ± 0.014[a] | 0.73 ± 0.014[d] | 0.46 ± 0.015[b] |
| Weaning to 3/9/10 | 0.56 ± 0.013[c] | 0.29 ± 0.015[b] | 0.65 ± 0.014[d] | 0.19 ± 0.016[a] |
| 3/9/10 to Breeding (6/19/10) | 0.37 ± 0.022[a] | 0.91 ± 0.024[c] | 0.48 ± 0.024[b] | 0.87 ± 0.025[c] |
| Breeding (6/19/10) to 9/1/10 | 0.80 ± 0.030[a] | 1.07 ± 0.033[b] | 0.83 ± 0.033[a] | 1.04 ± 0.035[b] |

[a-c] Means within a row with different superscripts differ ($P < 0.05$).

**Table 4.** Effect of weaning and winter treatments on BW in 2011 yearlings

| | Early Weaned | | Normal Weaned | |
|---|---|---|---|---|
| Date | Drylot | Range | Drylot | Range |
| Birth, kg | 38.4 ± 1.17 | 38.4 ± 1.11 | 37.4 ± 1.35 | 37.8 ± 1.31 |
| 8/17/2010, kg | 175.9 ± 7.00 | 175.5 ± 6.67 | 168.6 ± 8.08 | 171.5 ± 7.82 |
| Weaning[1], kg | 175.9 ± 5.20[a] | 175.5 ± 4.96[a] | 246.7 ± 6.01[b] | 244.9 ± 5.81[b] |
| 2/4/2011, kg | 276.6 ± 4.90[b] | 236.1 ± 4.67[a] | 303.3 ± 5.66[c] | 262.8 ± 5.48[b] |
| 5/18/2011, kg | 331.7 ± 5.22[b] | 295.4 ± 4.98[a] | 325.7 ± 6.03[b] | 327.5 ± 5.84[b] |
| Breeding (6/9/11), kg | 317.7 ± 4.85[a] | 304.8 ± 4.63[a] | 339.1 ± 5.60[b] | 341.8 ± 5.43[b] |
| Preg Check (7/26/11), kg | 329.4 ± 4.76[a] | 334.4 ± 4.57[ab] | 345.3 ± 5.50[b] | 369.5 ± 5.32[c] |
| 8/24/11, kg | 372.9 ± 5.57[a] | 381.9 ± 5.37[a] | 403.1 ± 6.43[b] | 419.6 ± 6.23[b] |

[a-c] Means within a row with different superscripts differ ($P < 0.05$).
[1] August 17, 2010 for early-weaning and November 2, 2010 for normal-weaned.

**Table 5.** Effect of weaning and winter treatments on ADG in 2011 yearlings

| | Early Weaned | | Normal Weaned | |
|---|---|---|---|---|
| Date | Drylot | Range | Drylot | Range |
| 8/17/10 to 2/4/11 | 0.59 ± 0.016[c] | 0.35 ±0.015[a] | 0.79 ± 0.019[d] | 0.53 ± 0.018[b] |
| Weaning to 2/4/11 | 0.59 ± 0.019[c] | 0.35 ± 0.018[b] | 0.60 ± 0.022[c] | 0.19 ± 0.022[a] |
| 2/4/11 to Breeding (6/9/11) | 0.33 ± 0.023[a] | 0.55 ± 0.022[b] | 0.29 ± 0.026[a] | 0.63 ± 0.025[c] |
| Breeding (6/9/11) to 8/24/11 | 0.72 ± 0.044[a] | 1.01 ± 0.043[b] | 0.84 ± 0.018[a] | 1.03 ± 0.049[b] |

[a-c] Means within a row with different superscripts differ ($P < 0.05$).

year 1, there was no difference in BW between the two NW groups, and they were both significantly heavier than the EW groups. At the end of year 2, there was no difference between wintering treatments; however EW heifers were still lighter than NW heifers. This agrees with other studies by Lusby et al. (1981); Olson et al. (1992); and Arthington and Kalmacher (2003).

In year 1, more D heifers had obtained puberty before the breeding season than R heifers ($P = 0.006$; 92.7% ± 3.52 vs. 72.8% ± 6.47, respectively). However, after heifers were initially exposed to progestin (immediately before the CIDR were re-inserted), there was no difference in pubertal status ($P > 0.05$; 96.8 ± 1.76%). In year 2 there was no difference ($P > 0.05$) in the percentage of heifers that obtained puberty between treatments (99 ± 282%). This could have been due to less-harsh winter conditions and earlier green-up in year 2, allowing the R heifers to obtain an adequate percentage of mature BW to reach puberty. Other studies have also shown that as long as heifers obtain an appropriate percentage of mature BW by initiation of breeding, winter gain should not affect puberty at breeding (Lemenager et al., 1980; Clanton et al., 1983; Lynch et al., 1997).

The AI conception rate did not differ among treatments in either year 1 or 2 ($P > 0.05$; 53.7 ± 7.05% and 48.2 ± 8.45%, respectively). This was likely because there were no differences in percentage of heifers that were pubertal at initiation of breeding. In yr 1, there was also no difference in overall pregnancy rate between treatments ($P > 0.05$; 86.7 ± 5.03%). This supports previous finding by Lynch et al. (1997), Martin et al. (2008), and Funston and Larson (2011). However, in year 2 more ($P = 0.03$) EW heifers were pregnant at fall pregnancy diagnosis than NW heifers (93.22% ± 0.040 and 74.65% ± 0.080, respectively).

## IMPLICATIONS

Wintering heifers on rangeland or early weaning could be a beneficial option for certain heifer development programs. A producer needs to look at important interactions between weaning and winter treatment when selecting a development program that best fits the goals of their operation.

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# FIXED-TIME AI IN LACTATING BEEF COWS AFTER GNRH ON DAY 9 OF A 14-DAY CIDR

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**ABSTRACT:** Most progestin-based estrus synchronization protocols focus on inducing a new follicular wave before progestin removal by administering GnRH at the initiation of the protocol. However, lack of response to GnRH due to stage of the estrous cycle when given and incomplete corpus luteum regression upon progestin removal contribute to failure to conceive to timed-AI (TAI). Our objectives were 1) to determine the effectiveness of an extended controlled internal drug release (CIDR) protocol with 2 induced follicular waves, and 2) determine the efficacy of initiating the 14-d CIDR treatment with GnRH analogue (Factrel) or prostaglandin $F_{2\alpha}$ (PG) injections. Lactating beef cows at 4 locations (n = 264, location 1; n = 99, location 2; n = 139, location 3; n = 128, location 4) were randomly assigned one of 3 treatments. Cows in the 14-d GnRH treatment received a CIDR (1.38 g progesterone) and 100 µg GnRH analogue i.m. on d 0, 100 µg GnRH analogue i.m. on d 9, CIDR removal with 50 mg PG i.m. on d 14, and 100 µg GnRH analogue with TAI 72 ± 2 h after CIDR removal. Cows in the 14-d PG treatment were assigned to the same protocol as 14-d GnRH cows except that 25 mg PG i.m. was given at CIDR insertion instead of GnRH. Cows in the control treatment, 5-d CO-Synch + CIDR (5-d CO-Synch), received a CIDR and 100 µg GnRH analogue i.m. on d 9, CIDR removal and 25 mg PG i.m. on d 14, 25 mg PG i.m. 6 ± 1 h later, and 100 µg GnRH analogue i.m. with TAI 72 ± 2 h after CIDR removal. Pregnancy status to TAI was determined 40 ± 2 d after TAI by ultrasonography. There was no treatment × location interaction ($P > 0.1$). Pregnancy rate to TAI across locations were greater ($P < 0.05$) in the 14-d PG cows (70.4%, n = 208) than 14-d GnRH (54.4%, n = 214) and 5-d CO-Synch cows (53.5%, n = 208). There was no increase in pregnancy rate to TAI in the 14-d GnRH treatment compared with the 5-d CO-Synch. However, replacement of GnRH with PG at the initiation of the 14-d PG treatment improved TAI pregnancy rates compared with the 5-d CO-Synch treatment resulting in a more efficacious estrus synchronization protocol.

**Key words:** dry distillers grains, estrus synchronization, prostaglandin $F_{2\alpha}$

## INTRODUCTION

Research incorporating multiple follicular waves within an estrus synchronization protocol, such as the 14-day CIDR-PG protocol for heifers, allows multiple follicular waves to grow without induced ovulation using exogenous GnRH (Leitman et al., 2009). While this protocol results in acceptable timed-AI (**TAI**) pregnancy rates, it is quite time consuming (33 d).

The failure in response to GnRH is a common problem with many short-term progestin based estrus synchronization protocols that initiate only 1 follicular wave since only 66% of cycling cows are in a stage of the estrous cycle where there is a follicle responsive to GnRH (Geary et al., 2000). The inclusion of 2 GnRH injection within a 14 d controlled internal drug release (**CIDR**) insert increased TAI pregnancy rates compared with a standard 5-d CO-Synch + CIDR protocol in beef cows (Giles et al., 2011).

During the luteal phase, a spontaneously formed corpus luteum (**CL**) will elicit enough progesterone (**P4**) to suppress luteinizing hormone (**LH**) pulse frequencies (Roberson et al., 1989), but low-level exogenous P4 (e.g. CIDR), in the absence of a CL, does not suppress similar LH release. This environment could be created in a 14 d CIDR protocol by inclusion of $PGF_{2\alpha}$ at the initiation of exogenous P4 treatment. Increasing LH pulse frequency would theoretically increase the number of LH receptors on the growing follicle. This could ensure ovulation from GnRH, CL formation, and responsiveness to $PGF_{2\alpha}$ leading to a more synchronized estrus.

The first objective for this experiment was to compare TAI pregnancy rates between two 14 d CIDR estrus synchronization protocols with $PGF_{2\alpha}$ or GnRH at the time of CIDR insertion to a short-term estrus synchronization protocol. The second objective was to validate synchronization of multiple follicular waves and determine $PGF_{2\alpha}$ responsiveness of the accessory CL formed as a result of ovulation on d 9 between the two 14 d CIDR treatments.

## MATERIALS AND METHODS

All experimental procedures with animals were approved by the Colorado State University Animal Care and Use Committee.

***Experimental Design.*** Angus, Angus cross, and Hereford cows (n = 630) at 4 locations (n = 264, location 1; n = 99, location 2; n = 139, location 3; n = 128, location 4) were randomly assigned to one of 3 treatments (Figure 1) on d 0 of treatments. All animals were evaluated for body condition score (**BCS**) on d 9 of treatment using a 1 to 9 BCS system by one evaluator. Cows in the 14-d GnRH treatment received

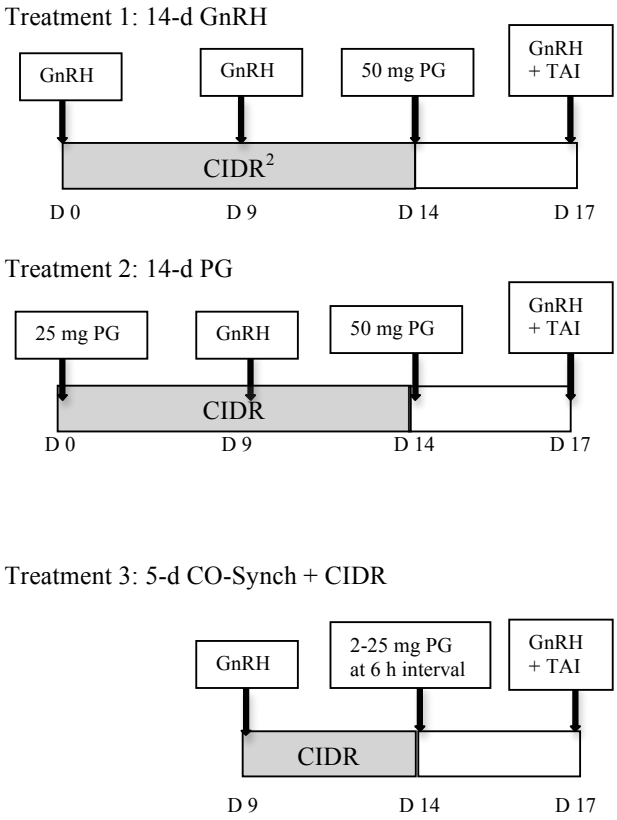


**Figure 1.** Estrus synchronization treatments administered to lactating beef cows. GnRH; 100 µg GnRH analogue administered i.m. (Factrel, Fort Dodge Animal Health). CIDR; Controlled internal drug release device (EAZI-BREED CIDR, Pfizer Animal Health). PG; prostaglandin F2α administered i.m. (Lutalyse, Pfizer Animal Health). TAI; Timed-AI

a CIDR (EAZI-BREED CIDR, Pfizer Animal Health, New York, NY; 1.38 g progesterone) concurrent with 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) i.m. on d 0, 100 µg GnRH analogue i.m. on d 9, and CIDR removal concurrent with a single 50 mg dose of prostaglandin $F_{2\alpha}$ (**PG**, Lutalyse, Pfizer Animal Health) i.m. on d 14. Cows in the 14-d PG treatment received a CIDR concurrent with 25 mg PG i.m. on d 0, 100 µg GnRH analogue i.m. on d 9, and CIDR removal concurrent with a single 50 mg dose of PG i.m. on d 14. Cows in the control treatment, 5-d CO-Synch + CIDR, received a CIDR concurrent with 100 µg GnRH analogue i.m. on d 9, CIDR removal concurrent with 25 mg PG i.m. on d 14, and 25 mg PG i.m. 6 ± 1 h later. Cows in all treatments received 100 µg GnRH analogue i.m. concurrent with TAI 72 ± 2 h after CIDR removal.

***Ovarian Response.*** Ovarian structures and response to hormone treatments were determined via transrectal ultrasonography (3.5 MHz linear transducer GP-DV, E.I. Medical, Loveland, CO) in a random subset of cows from at location 1 (n = 127) and all cows at location 2 (n = 99) on d 0, 3, 9, 12, 14, and 17. Visible ovarian structures (CL and follicles) ≥ 5 mm were recorded. A successful response to GnRH analogue on d 0 (for 14-d GnRH treatment) was defined as presence of a follicle ≥ 9 mm on d 0, and absence of that follicle by d 3 and/or replacement with a CL on d 9. A lack of response to GnRH analogue on d 0 was defined as a cow having no follicle present on d 0 or presence of a follicle ≥ 9 mm on d 0 that had grown or remained statically present by d 3. A successful response to PG on d 0 (for 14-d PG treatment) was defined as presence of a CL on d 0 and absence by d 3, and absence by d 9 as well. Lack of response to PG on d 0 was defined as a cow having presence of a CL on d 0 and continued presence of a CL on d 3 and 9. A lack of response was also defined as a cow with absence of a CL on d 0, but presence of a new CL by d 3 and/or 9. The same procedure used for successful response to GnRH analogue on d 0 was used for determining a successful response to d 9 GnRH analogue (for all 3 treatments). Ultrasonography performed on d 14 was used to determine presence of a CL at time of PG administration. Ultrasonography performed on d 17 with TAI was used to determine response to PG administered on d 14.

***Pregnancy Diagnosis.*** Pregnancy status to TAI was diagnosed between 37 and 40 d after TAI using transrectal ultrasonography (as described above). Cows were exposed to intact bulls 9 to 10 d after TAI.

***Statistical Analyses.*** Data were analyzed via logistic regression using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC) for determining differences in TAI pregnancy rates, BCS, and postpartum interval (**PPI**, days from calving to TAI) among treatments. The initial model included location, treatment, parity (primiparous and multiparous), BCS, PPI, service sire, AI technician, and their first order interactions. However, the final model only used the significant factors ($P < 0.05$) treatment and PPI. The factors location and location × treatment were also included in the model although not significant ($P > 0.1$).

## RESULTS

Number of cows, BCS, and PPI for treatments are presented combined across locations in Table 1. Mean (± SE) BCS did not differ ($P > 0.1$) among treatments, or among treatments within locations. However, mean BCS were different ($P < 0.05$) by location. Mean (± SE) PPI did not differ ($P > 0.1$) among treatments, or among treatments within locations. However, mean PPI differed ($P < 0.05$) by location. Pregnancy rates to TAI did not differ ($P > 0.05$) among treatments at location 1 or 2 (Table 2). However, at locations 3 and 4, pregnancy rates to TAI were greater ($P < 0.05$) in the 14-d PG treatment (76.6%, location 3; 75.8%, location 4) than both the 14-d GnRH (55.3%, location 3; 55.8%, location 4) and 5-d CO-Synch + CIDR (46.7%, location 3; 52.4%, location 4) treatments. There were no differences ($P > 0.1$) between the 14-d GnRH and 5-d CO-Synch + CIDR treatments at location 3 and 4. There was no treatment × location interaction ($P = 0.53$). For combined data from all four locations, pregnancy rates to TAI were greater ($P < 0.05$) in the 14-d PG treatment (70.4, n = 217) than both

**Table 1.** Number, post partum interval (PPI, d from calving to TAI on d 17) and BCS of lactating beef cows for 3 treatments at 4 locations (LS mean ± SE[1-3])

| Treatment | n = | PPI (d) | BCS |
|---|---|---|---|
| 14-d GnRH | 205 | 75 ± 1.21 | 4.8 ± 0.05 |
| 14-d PG | 217 | 76 ± 1.16 | 4.9 ± 0.05 |
| 5-d CO-Synch + CIDR | 208 | 78 ± 1.16 | 4.9 ± 0.05 |

No differences in PPI or BCS between treatments ($P > 0.1$).
[1]14-d GnRH; 14 d CIDR (EAZI-BREED CIDR, Pfizer Animal Health) with 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health) i.m. d 0 and 9 and 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) i.m. on d 14 with CIDR removal.
[2]14-d PG; 14 d CIDR with 25 mg $PGF_{2\alpha}$ i.m. d 0, 100 µg GnRH analogue i.m. d 9 and 50 mg $PGF_{2\alpha}$ i.m. on d 14 with CIDR removal.
[3]5-d CO-Synch + CIDR; 5 d CIDR with 100 µg GnRH analogue i.m. d 9, 25 mg $PGF_{2\alpha}$ i.m. on d 14 with CIDR removal and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later.
Cows in all treatments received 100 µg GnRH analogue i.m. with timed-AI at 72 ± 2 h after CIDR removal.

**Table 2.** LS means for timed-AI (TAI) pregnancy rates of lactating beef cows by treatment (mean ± SE)

| Location and treatment | n = | TAI PR[4] (%) |
|---|---|---|
| Location 1 | | |
| 14-d GnRH[1] | 85 | 56.5 ± 5.5 |
| 14-d PG[2] | 91 | 65.6 ± 5.1 |
| 5-d CO-Synch + CIDR[3] | 88 | 53.4 ± 5.3 |
| Location 2 | | |
| 14-d GnRH | 30 | 51.7 ± 9.3 |
| 14-d PG | 36 | 61.8 ± 8.3 |
| 5-d CO-Synch + CIDR | 33 | 61.3 ± 8.4 |
| Location 3 | | |
| 14-d GnRH | 47 | 55.3 ± 7.3[a] |
| 14-d PG | 47 | 76.6 ± 6.4[b] |
| 5-d CO-Synch + CIDR | 45 | 46.7 ± 7.5[a] |
| Location 4 | | |
| 14-d GnRH | 43 | 55.8 ± 7.6[a] |
| 14-d PG | 43 | 75.8 ± 6.8[b] |
| 5-d CO-Synch + CIDR | 42 | 52.4 ± 7.9[a] |
| Combined across all locations[5] | | |
| 14-d GnRH | 205 | 54.4 ± 4.0[a] |
| 14-d PG | 217 | 70.4 ± 3.6[b] |
| 5-d CO-Synch + CIDR | 208 | 53.5 ± 4.0[a] |

[a,b]Within a column, means without common superscripts differ ($P < 0.05$).
[1]14-d GnRH; 14 d CIDR (EAZI-BREED CIDR, Pfizer Animal Health) with 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health) i.m. d 0 and 9, and 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) i.m. on d 14 with CIDR removal.
[2]14-d PG; 14 d CIDR with 25 mg $PGF_{2\alpha}$ i.m. d 0, 100 µg GnRH analogue i.m. d 9 and 50 mg $PGF_{2\alpha}$ i.m. on d 14 with CIDR removal.
[3]5-d CO-Synch + CIDR; 5 d CIDR with 100 µg GnRH analogue i.m. d 9, 25 mg $PGF_{2\alpha}$ i.m. on d 14 with CIDR removal, and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later.
[4]PR; Pregnancy rate to TAI determined 37 to 40 d after timed-AI.
[5]There was no treatment × location interaction ($P = 0.53$)
Cows in all treatments received 100 µg GnRH analogue i.m. with TAI at 72 ± 2 h after CIDR removal.

the 14-d GnRH (54.4%, n = 205) and 5-d CO-Synch + CIDR (53.5%, n = 208) treatments.

At locations 1 and 2, luteolysis in response to $PGF_{2\alpha}$ administered to the 14-d PG treatment (n = 79) on d 0 occurred in 76.9% (40/52) of all cows with presence of a CL on d 0 (Table 3). Of the cows in the 14-d GnRH treatment (n = 72) that received GnRH analogue on d 0, 54.2% (39/72) of the cows responded to the treatment and ovulated a follicle. By d 9, 64.6% (51/79) of cows in the 14-d PG treatment had absence of a CL. The TAI pregnancy rate of these animals was 68.6%. Response to GnRH analogue administered d 9 was greater ($P < 0.05$) in the 14-d GnRH (76.4%) and 14-d PG (83.5%) treatments than the 5-d CO-Synch + CIDR (57.9%) treatment. There were no differences ($P > 0.05$) in response to GnRH analogue on d 9 between 14-d GnRH and 14-d PG treatments. Mean (± SE) follicle sizes (mm) for d 9 for the 14-d GnRH, 14-d PG, and 5-d CO-Synch + CIDR treatments were 13.5 ± 0.43, 14.7 ± 0.47, and 11.9 ± 0.34 mm, respectively. Mean d 9 follicle size was greater ($P < 0.05$) in the 14-d PG treatment than the 5-d CO-Synch + CIDR and tended ($P = 0.06$) to be greater than the 14-d GnRH treatment.

Synchronization of two follicular waves from response to GnRH analogue on d 0 and 9 occurred in 40.3% (29/72) of cows in the 14-d GnRH treatment. However, of the 14-d GnRH treatment cows that responded to d 0 GnRH analogue (n = 39), 74.4% (29/39) responded to d 9 GnRH analogue as well, forming two synchronized follicular waves with TAI pregnancy rates of 55.2% (16/29). There were no differences ($P > 0.05$) between percentage of cows in the 14-d GnRH (88.9%), 14-d PG (86.1%), and 5-d CO-Synch + CIDR (86.8%) treatments with CL presence on d 14. However, there were greater percentages ($P < 0.05$) of cows in the 14-d GnRH (12.5%) and 5-d CO-Synch + CIDR (19.7%) treatments with CL presence on d 17 with TAI than the 14-d PG treatment (3.8%).

## DISCUSSION

Inducing three follicular waves within a GnRH-$PGF_{2\alpha}$ based synchronization protocol has increased TAI conception rates in dairy cows (Friedman et al., 2011). Previous research on synchronizing two follicular waves under the influence of P4 increased TAI pregnancy rates in lactating beef cows

**Table 3.** Combined ultrasonography results from ovarian structures and response to treatments at locations 1 and 2 for lactating beef cows

| | Treatment | | |
|---|---|---|---|
| | 14-d GnRH[1] | 14-d PG[2] | 5-d CO-Synch + CIDR[3] |
| Variable n = | 72 | 79 | 76 |
| CL lysed from d 0 $PGF_{2\alpha}$[4] | - | 76.9 (40/52) | - |
| Response to GnRH d 0 (%)[5] | 54.2 (39/72) | - | - |
| Response to GnRH d 9[5] | 76.4[a] (55/72) | 83.5[a] (66/79) | 57.9[b] (44/76) |
| Response to GnRH d 0 and 9 | 40.3 (29/72) | - | - |
| Mean follicle size (mm) d 9 | 13.5 ± 0.43[a] | 14.7 ± 0.47[a] | 11.9 ± 0.34[b] |
| CL presence d 14 (%) | 88.9 (64/72) | 86.1 (68/79) | 86.8 (66/76) |
| CL presence d 17 (%) | 12.5[a] (9/72) | 3.8[b] (3/79) | 19.7[a] (15/76) |

[a,b]Within a column, means without common superscripts differ ($P < 0.05$).
[1]14-d GnRH; 14 d CIDR (EAZI-BREED CIDR, Pfizer Animal Health) with 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health) i.m. d 0 and 9 and 50 mg $PGF_{2\alpha}$ (Lutalyse) i.m. on d 14 with CIDR removal.
[2]14-d PG; 14 d CIDR with 25 mg $PGF_{2\alpha}$ i.m. d 0, 100 µg GnRH analogue i.m. d 9 and 50 mg $PGF_{2\alpha}$ i.m. on d 14 with CIDR removal.
[3]5-d CO-Synch + CIDR; 5 d CIDR with 100 µg GnRH analogue i.m. d 9, 25 mg $PGF_{2\alpha}$ i.m. on d 14 with CIDR removal and another 25 mg $PGF_{2\alpha}$ i.m. 6 ± 1 h later.
[4]Percentage of cows in 14-d PG treatment with presence of a CL d 0 that lysed CL by d 3.
[5]A successful response to GnRH given on d 0 or 9 was defined as presence of a follicle ≥ 9 mm on either d 0 or 9 and absence of that follicle 3 d later.
Cows in all treatments received 100 µg GnRH analogue i.m. with timed-AI at 72 ± 2 h after CIDR removal.

compared with the 5-d CO-Synch + CIDR protocol (Giles et al., 2011), but they did not evaluate ovarian responses to multiple GnRH analogue injections within this protocol, which is necessary for further understanding of follicular dynamics.

Tracking of follicular waves at locations 1 and 2 confirmed that most cows that responded to d 0 GnRH analogue injection (n = 39) also responded (74.4%) to the d 9 GnRH analogue synchronizing two follicular waves. The overall response in synchronization of 2 follicular waves in the 14-d GnRH treatment (40.3%; 29/72) was lower than expected, but lack of success could be explained by the overall response of animals to the GnRH analogue on d 0 (54.2%; 39/72). This lack of response could have led to poor synchronization of follicular waves for the succeeding GnRH analogue administration on d 9. However, most cows not responding to the d 0 GnRH analogue (45.8%; 33/72), ovulated in response to the GnRH analogue administered on d 9 (78.8%; 26/33), and had acceptable TAI pregnancy rates (57.7%; 15/26).

Data from ultrasonography at locations 1 and 2 reinforced the idea that successful response to GnRH within an estrus synchronization protocol would increase TAI pregnancy rates. However, within these two locations, the increased response to d 9 GnRH analogue in the 14-d GnRH treatment (76.4%) compared with the 5-d CO-Synch + CIDR treatment (57.9%) was not associated with greater TAI pregnancy rates within these treatments. While the increased response to d 9 GnRH analogue was validated in the 14-d GnRH treatment, the similarity in TAI pregnancy rates between these two treatments was verified by the similarities between the two treatments at locations 3 and 4. These data suggest differential efficacy of the 14-d GnRH protocol compared with previous experiments to determine its success for increasing TAI pregnancy rates compared with the 5-d CO-Synch + CIDR protocol (Giles et al., 2011).

The reduced fertility of oocytes in persistent bovine follicles has been documented repeatedly (e. g., Mihm et al., 1994). This decreased fertility has led to estrus synchronization protocols that avoid ovulation of these poor quality oocytes. The persistent follicle formed from administering prolonged low biological doses of P4 (e.g., CIDR or melengestrol acetate) has been avoided in currently recommended protocols. However, the 14-d PG treatment takes advantage of these follicles by forcing ovulation via GnRH and setting up a fresh follicular wave.

Presence of low levels of exogenous P4, in the absence of a spontaneously formed CL, is more similar to the endocrinological environment of the follicular phase of the estrous cycle than the luteal phase (Kinder et al., 1996). However, the threshold level of P4 maintained from low dose progestins still prevents ovulation or atresia of a dominant follicle and forces extended growth of a persistent follicle (Kinder et al., 1996). Creating this environment, while preventing ovulation of the dominant follicle, could increase exposure of the existing follicle to LH pulse frequencies, which would agree with research finding greater numbers of LH receptors on granulosa cells of persistent follicles than normal dominant follicles (Cupp et al., 1993). Therefore, in the 14-d PG treatment, along with ensuring the presence of a responsive follicle at time of GnRH analogue on d 9 of a 14 d CIDR, the follicle that ovulated might have a greater number of LH receptors and luteinize more rapidly than a normal ovulated follicle.

Validating GnRH analogue responsiveness at locations 1 and 2 was determined from the increased response to d 9 GnRH analogue in the 14-d PG treatment (83.5%; 66/79) compared with the 5-d CO-Synch + CIDR treatment (57.9%; 44/76). The increased response to d 9 GnRH analogue in the 14-d PG treatment was associated with a non-significantly ($P > 0.1$) greater TAI pregnancy rate (69.7%; 46/66) than for cows responding to d 9 GnRH analogue in the 5-d CO-Synch + CIDR treatment (56.8%, 25/44). The overall greater TAI pregnancy rates ($P < 0.05$) in the 14-d PG treatment than the 5-d CO-Synch + CIDR treatment also support this interpretation.

Coincident with the benefits of increased response to d 9 GnRH analogue within the 14-d CIDR treatments, the measure of CL responsiveness within the 3 treatments at CIDR removal and $PGF_{2\alpha}$ also affected their success rate. While the presence of high affinity $PGF_{2\alpha}$ receptors have been located on the early developing bovine CL (Wiltbank et al., 1995), responsiveness of the early CL (d 0 to 5) appears to be inhibited by blocks within the downstream cascade of events of $PGF_{2\alpha}$ binding to its receptor on the ovary. Increased protein kinase C epsilon (**PRKCE**) gene expression, the gene responsible for protein kinase C (**PKC**) translation, has been found in the d 10 bovine CL compared with the early stage CL, and has been associated with greater sensitivity to $PGF_{2\alpha}$ (Mahusudan et al., 2009). Increased presence of PKC inhibitors has also been identified in the early bovine CL, and may contribute to preventing responsiveness to $PGF_{2\alpha}$ (Wiltbank et al., 1995).

Inclusion of two 25 mg $PGF_{2\alpha}$ injections at 8 ± 2 h intervals with CIDR removal in the 5-d CO-Synch + CIDR protocol (Bridges et al., 2008) ensures luteal regression of a d 5 CL; a single 50 mg dose of $PGF_{2\alpha}$ has also been investigated with varying efficacy (Bridges et al., 2008, 2011; Kasimanickam et al., 2009). Within the 14-d PG treatment, a single 50 mg dose of $PGF_{2\alpha}$ apparently was sufficient to cause luteolysis. Follicles forced to ovulate on d 9 may have had additional LH receptors to enhance the luteinization rate and achieve $PGF_{2\alpha}$ responsiveness at CIDR removal. The increased CL presence on d 17 (d of TAI) in the 14-d GnRH (12.5%; 9/72) and 5-d CO-Synch + CIDR treatments (19.7%; 15/76) compared with the 14-d PG treatment (3.8%; 3/79) is consistent with increased CL responsiveness to $PGF_{2\alpha}$ of cows in the 14-d PG treatment. While mean follicle size and percentage of cows with presence of follicle ≥ 12 mm on d 17 did not differ between the 14-d GnRH (15.8 mm, 86.1%), 14-d PG (16.2 mm, 83.5%), and 5-d CO-Synch + CIDR (15.3 mm, 88.2%) treatments, the sustained CL presence could offset timing of ovulation and decrease successful conception to TAI. This was reflected in combined data by the increased TAI pregnancy rates in the 14-d PG treatment compared with the 14-d GnRH and 5-d CO-Synch + CIDR treatments.

## IMPLICATIONS

Determining the value of incorporating estrus synchronization protocols for TAI into a beef operation requires analysis of labor requirements and other costs. When considering adoption, the benefits of the 14-d PG treatment include increasing TAI pregnancy rates by 17 percentage points (32%) and are substantial when compared with short-term Beef Reproductive Task Force recommended 5-d CO-Synch + CIDR protocol. Coinciding with the improved d 9 response to GnRH, the 14-d PG treatment also had the best response to $PGF_{2\alpha}$ upon CIDR removal, resulting in an overall superior estrus synchronization protocol for maximizing TAI pregnancy rates in lactating beef cows.


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# POSTWEANING FEED RESTRICTION EFFECTS ON STEER FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS[1]

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**ABSTRACT:** The objective was to evaluate impacts of 2 levels of supplemental feed provided to cows during late gestation and 2 levels of feed provided to their sons during postweaning development on subsequent feedlot performance and carcass characteristics. Bull calves (n = 56 in 2010; n = 51 in 2011) were born from dams receiving adequate (1.8 kg/d) or marginal (1.2 kg/d) winter supplementation. After weaning, bulls were developed on ad-libitum (Control) or 27% less feed (Restricted) for ~140 d. Bulls were then band-castrated and placed on an 80% corn finishing diet ad libitum. Individual intakes were measured with a GrowSafe system for the final 100-150 d of the finishing period. Cattle were harvested at a commercial packing plant and carcass data were collected. Dam winter supplementation effects were not detected ($P \geq 0.22$). Postweaning phase ADG exhibited a postweaning treatment × year interaction ($P < 0.01$). Restricted calves had similar ADG in both years (0.64 vs 0.68 ± 0.03 kg/d) and gained less than Control calves. Control calves had greater ADG in 2010 than in 2011 (1.16 vs 1.03 ± 0.03 kg/d). Postweaning treatment did not impact feed intake during the finishing phase ($P = 0.29$; 13.0 vs 12.8 ± 0.22 kg/d for Restricted vs Control; as-fed basis). During the finishing phase, ADG exhibited a postweaning treatment × year interaction ($P < 0.01$). Restricted steers had similar ADG in both years (1.25 vs 1.27 ± 0.05 kg/d) and gained more than Control steers. Control steer ADG was less in 2010 than in 2011 (0.92 vs 1.13 ± 0.05 kg/d). Compared with Control steers, Restricted steers had lower ($P \leq 0.08$) final BW (603 vs 623 ± 9 kg), HCW (357 vs 373 ± 6 kg), and yield grade (2.71 vs 2.89 ± 0.10). However, back fat thickness (1.09 vs 1.14 ± 0.05 cm), ribeye area (85.4 vs 85.8 ± 0.98 $cm^2$), and marbling score (5.59 vs 5.50 ± 0.12) were not different ($P \geq 0.34$). Calves restricted during postweaning development gained more efficiently, and when harvested on a common date, had lower carcass weights and yield grade, but similar fat thickness, ribeye area and quality grade compared with their ad libitum-fed counterparts.

**Key words:** finishing, postweaning development, uterine programming,

## INTRODUCTION

For range-based cow-calf producers, harvested feedstuffs are a major input cost. A long term study at Fort Keogh has evaluated the influence of 2 levels of nutritional input during heifer development and cow winter supplementation on lifetime productivity in beef females (Roberts et al., 2009). Dietary treatments imposed on cows in this experiment resulted in a uterine programming effect in their heifer progeny (Roberts et al., 2010). Bull calves in this experiment also receive 2 levels of nutritional input during the postweaning period, but little work has been done to assess feedlot performance and carcass characteristics. Therefore, the objective was to evaluate impacts of 2 levels of supplemental feed provided to cows during late gestation and 2 levels of feed provided to their sons during postweaning development on subsequent feedlot performance and carcass characteristics.

## MATERIALS AND METHODS

Research protocols were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee.

Cattle used in this study were a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise). Beginning in 2001, cows in this herd were randomly assigned to be fed levels of harvested feed from December to March of each year that were expected to result in either marginal (**MARG**) or adequate (**ADEQ**) nutrition while grazing dormant winter forage through this period, based on average quality and availability of winter forage (Roberts et al., 2009). Each group of cows was managed on separate pastures during the winter to allow differential feeding. Beginning in December of each year (based on pasture and weather conditions), cows were supplemented with alfalfa hay every other day. Quantity fed was equivalent to 1.8 or 1.1 kg hay/day for each ADEQ or MARG cow, respectively. In early March of each year, cows were transferred to small calving pastures and fed 10.2 (ADEQ) or 8.1 (MARG) kg alfalfa hay/cow each day.

Bull calves born from ADEQ or MARG dams during spring 2009 (n = 203; avg. birth date Apr 9) and spring 2010

[1]USDA-ARS is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the authors and does not imply its approval to the exclusion of other products that may be also suitable.

(n = 201; avg. birth date Apr 1) were allotted by weight at weaning to 6 pens (n = 33 or 34 per pen) in 2009 and 8 pens (n = 24 or 26 per pen) in 2010. During the first 56 d postweaning, bulls were fed ad-libitum with a diet of 65% silage, 20% grain (50:50 barley:corn in 2009; 100% corn in 2010), 10% ground alfalfa hay, and 5% supplement, as fed basis). Average age at the endof this adaptation period was 244 and 254 d for bulls born in 2009 and 2010, respectively. Half of the pens of bulls in each year were then assigned to continue being fed ad libitum (**Control**) and the remaining pens were fed 80% (as-fed basis) of that consumed while fed ad-libitum (**Restricted**). Bulls were weighed every 28 d and amount of feed provided to Restricted bulls were adjusted to be 80% of that consumed by Control bulls on a common BW basis. Feeding treatments were imposed for 140 d. Diet during this period consisted of 65% silage, 20% corn, 10% ground alfalfa hay, and 5% supplement, as fed. An exception to this diet occurred during the first 28-d period for bulls born in 2010, where no corn was available due to a feedmill equipment breakdown. During this time, corn was replaced with additional silage (diet contained 85% silage). At the end of the 140-d postweaning treatment, ultrasound measurements of LM area, back fat, and percent intramuscular fat were taken as described by Roberts et al. (2007), using an Aloka SSD-500 ultrasound equipped with a 17.2 cm, 3.5 MHz, linear array transducer (Aloka Co. Ltd., Wallingford, CT) and the Beef Image Analysis software (Designer Genes Technologies LLC, Gustine, TX).

For the present study, a subset of bulls (n = 56 in 2010; n = 51 in 2011) were band-castrated after the 140-d trial and placed on a corn finishing diet ad libitum (78.9% corn, 12.7% alfalfa hay, 4.2% silage, and 4.3% supplement, as fed). These bulls were selected from sires with offspring represented in each dam treatment and individual treatment classifications. Individual intakes were measured with a GrowSafe system for the final 100-150 days of the finishing period, and steers were weighed every ~28 d. Cattle were harvested at a commercial packing plant (Nov 18, 2010; Dec 7, 2011; Cargill, Fort Morgan, CO) and carcass data were collected by an independent company (Diamond T Livestock Services, Inc.).

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included dam winter supplementation treatment, postweaning treatment, their interaction, year, year by postweaning treatment interaction, and dam age (2, 3, and 4+ years of age). Calf age was used as a covariate, and sire was a random effect. Means were separated using PDIFF.

## RESULTS AND DISCUSSION

Dam winter supplementation effects were not detected ($P \geq 0.22$). Postweaning treatment × year interactions were observed for postweaning phase ADG, postweaning final BW, and end of postweaning LM area ($P \leq 0.07$; Table 1). Restricted calves had similar postweaning ADG in both years and gained less than Control calves. Control calves had greater ADG in 2010 than in 2011. These differences in ADG were reflected in postweaning final BW. Restricted calves had similar BW in both years and were lighter than Control calves. Control calves weighed less at the end of the postweaning period in 2011 compared with 2010. Longissimus muscle area at the end of the postweaning period was similar in both years for Restricted calves, and was smaller than for Control calves. Control calves had larger LM area in 2010 than in 2011. Differences observed for growth and LM area between Control calves from the 2 yr appear to result from the feedmill equipment failure that resulted in the unavailability of corn during the first 28-d period for bulls born in 2010. Interestingly, similar effects were not observed in the Restricted calves.

Postweaning treatment and year both impacted other ultrasound measures at the end of the postweaning period. Restricted calves had smaller back fat thickness and less intramuscular fat ($P < 0.01$) than Control calves (Table 2). Calves had less back fat and intramuscular fat ($P = 0.02$) in 2011 than in 2010 (Table 3).

Postweaning treatment did not impact feed intake during the finishing phase ($P = 0.29$; 13.0 vs 12.8 ± 0.22 kg/d for

**Table 1.** Postweaning treatment × year interactions for postweaning ADG ($P < 0.01$), postweaning final BW ($P = 0.04$), end postweaning LM area ($P = 0.07$) and finishing phase ADG ($P < 0.01$)

| | Year | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2010 | | | | 2011 | | | |
| | Postweaning Treatment[1] | | | | | | | |
| Item | Restricted | SE | Control | SE | Restricted | SE | Control | SE |
| Postweaning ADG, kg/d | 0.64[a] | 0.02 | 1.17[b] | 0.03 | 0.68[a] | 0.03 | 1.03[c] | 0.03 |
| Postweaning final BW, kg | 342[a] | 5.9 | 420[b] | 6.7 | 340[a] | 6.5 | 395[c] | 6.6 |
| End postweaning LM area, $cm^2$ | 68.3[a] | 1.21 | 80.3[b] | 1.37 | 66.9[a] | 1.33 | 74.6[c] | 1.37 |
| Finishing phase ADG, kg/d | 1.25[a] | 0.04 | 0.92[b] | 0.05 | 1.27[a] | 0.05 | 1.13[c] | 0.05 |

[1]140-day postweaning treatment. Control animals fed ad libitum, Restricted animals fed 80% of ad libitum intake at similar BW.

[2]Measured via ultrasound at the end of the 140-d postweaning period.

[a-c] Means in the same row with different superscripts differ, $P \leq 0.08$.

Restricted vs Control; as-fed basis). During the finishing phase, ADG exhibited a postweaning treatment × year interaction ($P < 0.01$; Table 1). Restricted steers had similar ADG in both years and gained more than Control steers. Control steer ADG was less in 2010 than in 2011. While the concept of compensatory growth observed in this experiment is not novel (Fox et al., 1972), the present study indicates that the response may to be due to improved conversion, and not increased intake.

Postweaning treatment had varying effects on final BW and carcass characteristics. Compared with Control steers, Restricted steers had lower ($P \leq 0.08$) final BW, HCW, and yield grade (Table 2). However, back fat thickness, ribeye area, and marbling score were not different ($P \geq 0.34$). In general, carcass composition data in this experiment were consistent with to those found by Fox et al. (1972) at finish weight. Final BW were similar ($P = 0.60$) in both years of the study, as were all carcass characteristics ($P \geq 0.15$) except for LM area, which was larger in 2010 than 2011($P = 0.01$; Table 3).

Calves from 2- and 3-year-old dams were lighter at the end of the postweaning period and also had less back fat and smaller LM area than calves from mature dams ($P < 0.01$; Table 4). Dam age did not impact postweaning ADG, end-of-postweaning intramuscular fat percentage, finishing phase ADG, carcass LM area, or carcass marbling score ($P \geq 0.19$). However, calves from 2-year-old dams had lighter final BW and HCW than calves from mature dams, while calves from 3-year-old dams were intermediate ($P \leq 0.03$). Back fat thickness was smaller ($P < 0.01$) for calves from 2- and 3-year old calves than for calves from mature dams, which was also reflected in a similar pattern for yield grade ($P < 0.01$), with calves from mature dams having a greater yield grade than calves from young cows.

Calves restricted during postweaning development gained more efficiently, and when harvested on a common date, had lower carcass weights and yield grade, but similar fat thickness, ribeye area and quality grade compared with their ad libitum-fed counterparts.

**Table 2.** Postweaning treatment impacts on postweaning ultrasound measures, feedlot performance, and carcass characteristics

| | Postweaning Treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | Restricted | SE | Control | SE | *P*-value |
| Postweaning phase | | | | | |
| Fat thickness, cm[2] | 0.23 | 0.01 | 0.35 | 0.01 | < 0.01 |
| Intramuscular fat, %[2] | 2.87 | 0.06 | 3.10 | 0.06 | < 0.01 |
| Finishing phase and carcass characteristics | | | | | |
| Final BW, kg | 603 | 8.8 | 623 | 9.3 | 0.05 |
| Hot carcass weight, kg | 357 | 5.5 | 373 | 5.9 | 0.01 |
| Backfat thickness, cm | 1.09 | 0.05 | 1.14 | 0.05 | 0.34 |
| LM area, cm$^2$ | 85.4 | 0.90 | 85.8 | 0.98 | 0.78 |
| Marbling score | 5.59 | 0.11 | 5.50 | 0.12 | 0.53 |
| Yield grade | 2.71 | 0.09 | 2.89 | 0.10 | 0.08 |

[1]140-d postweaning treatment. Control animals fed ad libitum, Restricted animals fed 80% of ad libitum intake at similar BW.
[2]Measured via ultrasound at the end of the 140-d postweaning period.

**Table 3.** Year impacts on postweaning ultrasound measures, feedlot performance, and carcass characteristics

| | Year | | | | |
|---|---|---|---|---|---|
| Item | 2010 | SE | 2011 | SE | *P*-value |
| Postweaning phase | | | | | |
| Fat thickness, cm[1] | 0.31 | 0.01 | 0.27 | 0.01 | 0.02 |
| Intramuscular fat, %[1] | 3.09 | 0.06 | 2.88 | 0.07 | 0.02 |
| Finishing phase and carcass characteristics | | | | | |
| Final BW, kg | 609 | 10.5 | 617 | 11.0 | 0.60 |
| Hot carcass weight, kg | 364 | 6.6 | 365 | 6.9 | 0.92 |
| Backfat thickness, cm | 1.18 | 0.06 | 1.06 | 0.06 | 0.15 |
| LM area, cm$^2$ | 87.8 | 1.00 | 83.4 | 1.19 | 0.01 |
| Marbling score | 5.74 | 0.12 | 5.35 | 0.15 | 0.15 |
| Yield grade | 2.73 | 0.11 | 2.87 | 0.11 | 0.40 |

[1]Measured via ultrasound at the end of the 140-d postweaning period.

**Table 4.** Dam age impacts on postweaning growth, feedlot performance and carcass characteristics

| | Dam Age | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | 2 | SE | 3 | SE | 4+ | SE | *P*-value |
| Postweaning phase | | | | | | | |
| ADG, kg/d | 0.91 | 0.03 | 0.84 | 0.03 | 0.87 | 0.02 | 0.19 |
| Final BW, kg | 360[a] | 6.6 | 365[a] | 6.9 | 397[b] | 4.3 | < 0.01 |
| Fat thickness, cm | 0.28[a] | 0.02 | 0.26[a] | 0.02 | 0.33[b] | 0.01 | < 0.01 |
| Intramuscular fat, % | 2.97 | 0.09 | 2.90 | 0.09 | 3.08 | 0.06 | 0.23 |
| LM area, $cm^2$ | 71.1[a] | 1.36 | 70.2[a] | 1.43 | 76.2[b] | 0.88 | < 0.01 |
| Finishing phase and carcass characteristics | | | | | | | |
| ADG, kg/d | 1.11 | 0.04 | 1.19 | 0.04 | 1.13 | 0.03 | 0.40 |
| Final BW, kg | 593[a] | 12.2 | 613[ab] | 12.7 | 633[b] | 8.4 | 0.01 |
| Hot carcass weight, kg | 354[a] | 7.7 | 365[ab] | 8.0 | 376[b] | 5.3 | 0.03 |
| Backfat thickness, cm | 1.04[a] | 0.07 | 1.04[a] | 0.07 | 1.27[b] | 0.05 | < 0.01 |
| LM area, $cm^2$ | 86.5 | 1.36 | 84.9 | 1.44 | 85.4 | 0.84 | 0.70 |
| Marbling score | 5.54 | 0.17 | 5.53 | 0.18 | 5.57 | 0.10 | 0.97 |
| Yield grade | 2.58[a] | 0.13 | 2.77[a] | 0.13 | 3.05[b] | 0.09 | < 0.01 |

[1]Measured via ultrasound at the end of the 140-d postweaning period.
[a,b] Means in the same row with different superscripts differ, $P \leq 0.05$.

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# THE EFFECTS OF LIMIT-FEEDING A FINISHING RATION TO FEEDLOT STEERS ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS

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**ABSTRACT:** The objectives of this study were to evaluate limit-feeding feedlot steers on performance and carcass characteristics. Angus cross steers (n = 168) were randomly blocked into 1 of 24 pens (6 pens/treatment; 7 steers/pen) and assigned to one of four dietary treatments: 1) 85% ad libitum intake (85 ADLIB) 2) 90% ad libitum intake (90 ADLIB) 3) 95% ad libitum intake (95 ADLIB); or 4) 100% ad libitum intake (ADLIB). Diets consisted of a corn-based finishing ration and the percentage of intake was calculated from the ADLIB treatment on a weekly basis. Animals were slaughtered when their respective 12th rib fat depth reached 1.02 cm. Data were analyzed using the MIXED procedure of SAS. As expected, steers on the ADLIB diet had greater DMI ($P \leq 0.0001$) when compared with all other treatments. Steers on the 95% ad libitum diet had greater DMI ($P \leq 0.0001$) compared with the 85 and 90% ad lib diets, however, there was no difference ($P = 0.26$) between the 85 ADLIB and 90 ADLIB treatments. Total ADG over the course of the study was greater ($P = 0.002$) for steers on the ADLIB treatment, however, there were no differences ($P \leq 0.36$) between the other treatments. Steers on the 90ADLIB diets had a greater G:F ($P = 0.01$) compared with steers on 95 ADLIB, while the 85ADLIB and ADLIB treatments were intermediate. Days on feed were greater ($P = 0.0003$) for steers on the 85 ADLIB treatment when compared with the 95 ADLIB and ADLIB intake treatment; however, there was no difference ($P = 0.29$) between the 95 ADLIB and ADLIB treatments. Total feed costs did not differ ($P = 0.47$) between treatments. Steers in the 85 ADLIB group had less ($P = 0.01$) KPH fat compared with all other treatments; however, no differences ($P \geq 0.17$) were detected in HCW, 12th rib fat, REA, yield grade, or quality grade. Limit-feeding steers decreases DMI without sacrificing carcass quality; however, because days on feed are increased, there appears to be no economic advantage to limit-feeding feedlot steers.

**Key words:** beef, limit-feeding, steers

## INTRODUCTION

Increased corn, fuel, and fertilizer prices coupled with increased environmental standards have had a tightening effect on the livestock industry. The economic and environmental constraints have spurred additional research into limit feeding cattle to try to reduce input costs and decrease manure output from livestock. Limit feeding has been reported to increase feed efficiency in feedlot steers.

Previous research has indicated that limit feeding improved feed efficiency of feedlot steers at a reduced rate of gain (Hicks et al., 1990, Faulkner and Berger, 2001). Likewise, improvements in feed efficiency were reported at 4.4 and 14.5% for the steers were limited to 90 and 80% of ad libitum feed intake, respectively (Murphy and Loerch, 1994). In addition to increased feed efficiency, limit-fed cattle have been reported to maintain carcass quality while reducing subcutaneous fat by 15 to 25% (Radunz, 2010).

We hypothesized that in the current climate of volatile grain markets, limiting feed intake of finishing steers would be a viable alternative to reduce feed costs. Specifically, our objectives were to evaluate feedlot performance, carcass quality, and economic potential of steers fed 85 to 100% of ad libitum intake.

## MATERIALS AND METHODS

All procedures involving animals during this study were approved by the Purdue Animal Care and Use Committee.

***Animals and Diets.*** One hundred sixty-eight Angus-cross steers were used in a randomized complete block design to evaluate the effects of limit feeding finishing rations. A typical finishing ration (Table 1) was formulated to meet NRC (2000) requirements for finishing steers. Steers were randomly assigned to 1 of 4 management treatments: 1) fed to ad libitum intake (**ADLIB**), 2) fed to 95% of ad libitum intake (**95ADLIB**), 3) fed to 90% of ad ad libitum intake (**90ADLIB**), or 4) fed to 85% of ad libitum intake (**85ADLIB**). Steers were blocked and randomly assigned to pens (7 steers/pen; 6 pens/treatment). Steers in the ad libitum treatment were fed to a daily bunk score of 0.5 to 1 on the SDSU feedbunk scoring system (Loy, 1997). Amount of feed offered to the restricted pens was determined on a weekly basis from the amount consumed by the ad libitum pens. All steers were implanted with Revalor I-S (Intervet, Inc., Millsboro, DE) on 1 d before initiation of the study. Steers were housed in 6.1- x 3.3-m pens, inside a curtain-sided, slatted-floor finishing barn.

***Sampling.*** Feed samples were collected every 1 d and stored at -20° C until further analyses were conducted. Samples were dried in a forced air oven at 60ºC for 48 h, ground to pass a 1-mm screen (Udy Cyclone mill, UDY Corp., Fort Collins,

**Table 1.** Ingredients and chemical composition the diet fed to finishing steers (DM basis)

| Item | |
|---|---|
| Ingredient, % of diet DM | |
| Dry Rolled Corn | 60.7 |
| Dried distillers grains[2] | 24.8 |
| Corn silage | 12.2 |
| Limestone | 1.9 |
| Sodium chloride | 0.22 |
| Mineral/vitamin premix[3,4] | 0.09 |
| Thiamine-10 premix | 0.07 |
| Nutrient Composition, % of diet DM | |
| CP | 15.4 |
| NDF | 21.4 |
| ADF | 10.0 |
| Ash | 4.3 |
| $NE_g$, Mcal/-kg | 1.45 |
| DM | 79.1 |

[1]Dried distillers grains contained (DM basis): 29.5% CP, 13.9% fat, 14.3% ADF, 0.85% P, 0.04% Ca, 1.03% K, 0.27% Mg, 0.71% S, and 0.26%Na.
[1]Provided NRC (2000) recommended levels of trace minerals and vitamins A, D, and E.
[2]Akey beef premix No. 4 (Akey Inc., Lewisburg, OH) contained 9% Mg, 4% S, 0.02% Co, 1% Cu, 0.09% I, 2% Fe, 4% Mn, 0.03% Se, 4% Zn, as well as 4,400,000 IU of vitamin A, 550,000 IU of vitamin D, and 5,500 IU of vitamin E/kg of premix.
[3]Diet formulated to contain 15 mg/kg of thiamine (22 g of thiamine/kg of premix).
[4]Based on values obtained from complete mixed feed samples in our laboratory.

Co), and analyzed for DM, OM, and ash (AOAC, 1990). Neutral and acid detergent fiber fractions were determined by ANKOM 2000 Fiber Analyzer (ANKOM Corp., Fairport, NY). Nitrogen was determined via block digestion (AOAC, 1990; method 976.06) and steam distillation with MgO using a 2300 Kjeltec Analyzer Unit (FOSS TECATOR AB, Foss North America, Eden Prairie, MN). Crude protein was calculated by multiplying N % by 6.25. Net energy for growth was determined by a commercial laboratory (AgSource Soil & Forage Laboratory, Bonduel, WI).

***Performance and Carcass Data.*** Initial weights were the average of 2 weights taken on consecutive days to start the study. Dry Matter Intake, ADG, and G:F were calculated from bi-monthly data. However, DMI, ADG, and G:F were all calculated into performance during the first half of the study, second half of the study, and overall study performance. Steers were harvested when they reached approximately 1.1 cm 12th rib fat depth (**UBF**) which was determined via ultrasound. Measurements were taken with an Aloka 500-V B-mode ultrasound instrument equipped with a 3.5-MHz, 12.5-cm linear transducer (Aloka American, Ltd., Wellington, CT). No steers were allowed to remain on study in a pen as an individual; therefore, if all but 1 steer within a pen had reached 1.1 cm of UBF, all steers were slaughtered regardless of the UBF of the remaining animal. Days on feed and feed costs were determined once the steers reached the slaughter criteria. Individual weight and UBF were recorded every 14 d during the first 90 d of the experiment, and every 1 d from d 90 to d 181 to monitor performance and to aid in harvest selection. However, no individual steers were allowed to remain on study in a pen; therefore, if all but 1 steer within a pen had reached 1.1 cm of UBF, all steers were slaughtered regardless of the final UBF of the animal. Final BW was an average of BW taken on two consecutive days before transporting cattle for slaughter at a commercial beef packing facility (Tyson Fresh Meats, Inc., Joslin, IL). Carcass data was collected by trained plant personnel after a 24-h chill (except for HCW which were taken immediately after exsanguinations) and included: 1) subcutaneous fat depth at the 12th to 13th rib (**BF**); 2) LM area; 3) KPH fat as a percentage of carcass weight (**KPH**); 4) dressing percentage; 5) USDA quality grade; and, 6) marbling score. Yield grade was calculated using the formula reported by Aberle et al. (2001). Total costs were calculated by determining the cost of the as- fed ration per head and compounding the cost by the days on feed multiplied by a moderate yardage fee of $0.50/d.

***Statistical Analyses.*** Performance and carcass data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) for a randomized complete block design. The fixed effect of treatment was included in the model, with pen serving as the experimental unit and block included as the random effect.

## RESULTS

As expected by experimental design, daily DMI ($P \leq 0.001$) was greatest for ADLIB steers compared with all other treatments. Additionally, 95ADLIB steers had greater DMI than both 90ADLIB and 85ADLIB steers; however, there was no difference ($P = 0.26$) between 90ADLIB and 85ADLIB steers.

Average daily gain during the first half of the study (d 0 to d 89) was greatest ($P = 0.002$) for the ADLIB fed steers compared with the 90ADLIB and 85ADLIB fed steers, while 95ADLIB steers were intermediate. There were no differences ($P = 0.17$) detected for ADG during the second half of the study (d 90 to d 177) due to treatment. Steers fed the ADLIB diet had greater overall ($P = 0.002$) ADG compared with all other treatments; however, there were no differences in ADG between steers of the other treatments ($P \leq 0.36$).

Steers fed the 90ADLIB treatment had greater ($P = 0.004$) G:F compared with 95ADLIB and 85ADLIB, with ADLIB being intermediate. During the second half of each treatment period, steers managed to be fed 90ADLIB tended ($P = 0.06$) to have a greater G:F than 95ADLIB and ADLIB fed steers. Feed efficiency over the entire study was greatest ($P = 0.01$) in 90ADLIB when compared with 95ADLIB with 85ADLIB and ADLIB being intermediate.

No differences were detected for final BW ($P = 0.18$), or total feed costs ($P = 0.06$) due to dietary delivery treatments.

No differences were detected in HCW ($P = 0.42$), 12th

**Table 2.** Effect of limit-feeding on feedlot performance

| | Treatment[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | 85 ADLIB | 90 ADLIB | 95 ADLIB | ADLIB | SEM | *P*-value |
| Initial Weight, kg | 585.59 | 593.25 | 584.23 | 590.88 | 12.6 | 0.78 |
| Final Weight, kg | 598.25 | 605.92 | 596.62 | 604.02 | 12.7 | 0.56 |
| DMI/h/d, kg | 9.29$^{c}$ | 9.44$^{c}$ | 10.12$^{b}$ | 10.62$^{a}$ | 0.22 | <.001 |
| DMI total, kg | 1587.84 | 1615.64 | 1617.59 | 1641.05 | 48.89 | 0.89 |
| ADG Total,kg | 3.23$^{b}$ | 3.60$^{b}$ | 3.43$^{b}$ | 3.79$^{a}$ | 0.11 | 0.01 |
| ADG 1[2], kg | 1.73$^{b}$ | 1.90$^{b}$ | 1.88$^{b}$ | 2.15$^{a}$ | 0.19 | 0.002 |
| ADG 2[3], kg | 1.50 | 1.59 | 1.55 | 1.64 | 0.14 | 0.48 |
| G:F Total, kg | 0.31$^{a,b}$ | 0.34$^{a}$ | 0.28$^{b}$ | 0.29$^{a,b}$ | 0.004 | 0.04 |
| G:F 1[2], kg | 0.16$^{a,b}$ | 0.18$^{c}$ | 0.15$^{a}$ | 0.16$^{b,c}$ | 0.001 | 0.04 |
| G:F 2[3], kg | 0.14 | 0.15$^{a}$ | 0.13$^{b}$ | 0.13$^{b}$ | 0.006 | 0.05 |
| Days on Feed | 177.82$^{a}$ | 172.36$^{a,b}$ | 155.14$^{c}$ | 155.14$^{c}$ | 4.76 | 0.004 |
| Total Costs, $ | 355.01 | 358.29 | 363.20 | 265.76 | 10.68 | 0.10 |

[a,b,c] Means within a row lacking a common superscript letter differ ($P < .05$).

[1]85, 90, 95 = percentage limit-fed until reaching slaughter criteria.

[2,3] ADG1, ADG2, G:F1, G:F2 denote the respective first half and second half of the trial.

**Table 3.** Effect of limit-feeding on carcass characteristics

| | Treatment[1] | | | | | |
|---|---|---|---|---|---|---|
| Item[5] | 85 ADLIB | 90 ADLIB | 95 ADLIB | ADLIB | SEM | *P*-value |
| HCW, kg | 353.32 | 353.60 | 353.38 | 357.89 | 7.75 | 0.96 |
| KPH, % | 1.87$^{b}$ | 2.175$^{a}$ | 2.29$^{a}$ | 2.13$^{a}$ | 0.07 | 0.01 |
| 12$^{th}$ rib fat, cm | 1.20 | 1.30 | 1.31 | 1.21 | 0.24 | 0.93 |
| REA, cm$^2$ | 87.74 | 89.8 | 91.5 | 91.04 | 1.16 | 0.17 |
| USDA YG | 2.77 | 2.75 | 2.68 | 2.65 | 0.13 | 0.92 |
| USDA QG[2] | 16.83 | 17.1 | 17.18 | 17.10 | 0.13 | 0.12 |
| Upper 2/3 % Choice | 13 | 25 | 30 | 29 | 0.06 | 0.19 |
| Percent Choice | 57.83 | 66 | 50 | 56.1 | 2.8 | 0.002 |

[a-c] Means within a row lacking a common superscript letter differ ($P < .05$).

[1]85 ADLIB, 90 ADLIB, 95 ADLIB = percentage limit-fed until reaching slaughter criteria.

[2] Low Choice = 15, Average Choice =16, High choice = 17, Low Prime = 18

rib fat ($P = 0.60$), REA ($P = 0.17$), or yield grade ($P = 0.49$). Steers managed in the 85ADLIB treatment had less (P = 0.01) KPH than all other treatments. There was a tendency for ($P = 0.04$) 95ADLIB steers to have a greater quality grade; however, 90ADLIB steers had the greatest ($P = 0.002$) percentage of cattle grading choice.

## DISCUSSION

Feedlot performance is an early indicator as to the economic return for the livestock producer. In our study, there were several notable effects on feedlot performance across the treatments.

There are many factors that will affect dry matter intake (**DMI**) and associated DM digestibility in finishing feedlot cattle. This includes type of diet, diet delivery, and specific type of delivery. Profitability in feedlot cattle is derived by cattle that grow (ADG), convert (G:F), and have quality carcasses. Average daily gain is directly affected by the dry matter intake (Hicks et al., 1990). Results from the current study suggest that cattle with greater DMI (ADLIB) also had greater gains during the first half of study. There was no difference in ADG during the second period of the study; however, the greater gains during the first period resulted in an overall improvement in ADG over the entire study for cattle that were given ad libitum access to feed. In agreement with this study, Murphy and Loerch (1995) reported a decrease in ADG and increase in DOF for calves restricted to 80 and 90% of ad libitum. Similarly, Loerch and Fluharty (1996)

concluded that as growth rate increased, the days required to reach the end of a designated period was decreased.

Early limit-feeding research indicated that cattle restricted to 96% ad libitum intake were 2.6% more efficient than those fed to ad libitum intake (Plegge, 1987). Calves that were fed an ad lib diet during a growing period had the lowest feed efficiency in the finishing period compared with calves that were previously limit-fed (Loerch and Fluharty, 1998). Calves in the current study stayed on their respective treatments for the entire study; however, results were similar to that of Loerch and Fluharty (1998). Calves fed the 90ADLIB diet had similar ADG during period 2 as all other calves, but had greater efficiency during the second phase of the study.

Although restricted calves in the current study had reduced DMI and reduced ration costs on a daily basis, their reduced growth response, especially during the first period, resulted in greater days on feed to reach their finish endpoint. Therefore, total costs associated with finishing steers were not different across treatment.

There were no differences in HCW, 12$^{th}$ rib fat depth, REA, or USDA YG or QG due to treatment. Although there was no difference in the number of cattle grading choice or overall quality grade, there was a strong trend for a greater number of cattle reaching upper 2/3s choice as DMI increased. The 85ADLIB treatment had less than half the percentage of cattle grading upper 2/3s choice compared with the 95ADLIB and ADLIB treatments. This data would suggest that limiting DMI intake to 85% of ad libitum DMI, and therefore limiting energy intake, in finishing rations of feedlot steers may negatively affect carcass quality.

## IMPLICATIONS

Because of the volatility in commodity markets, the beef industry is constantly evaluating ways to increase overall system efficiency. Increasing feed efficiency while reducing feed intake appears to be a viable option. However, the cost savings gained on a daily basis by decreasing DMI appears to be offset by the increased days on feed required to achieve the same level carcass weight and finish. Overall profitability may be affected by decreasing carcass quality in the 85% of ad libitum treatment if cattle are marketed on a grid pricing system.

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# RUMINANT NUTRITION

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# COMPARISON OF DIFFERENT SUPPLEMENTAL COBALT FORMS ON FIBER DIGESTION AND COBALAMIN LEVELS[1]

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**ABSTRACT:** Cobalt (Co) is essential for rumen microbial metabolism to synthesize methane, acetate and methionine. It also serves as a structural component of vitamin $B_{12}$, which functions as a coenzyme in energy metabolism. A study was conducted to determine if Co form (cobalt carbonate vs. cobalt glucoheptonate) supplemented above NRC requirements would improve fermentability of a low quality forage diet and change serum cobalamin concentrations. Twenty ruminally-cannulated cows (577 ± 13 kg) were individually fed in a completely randomized experimental design. Cows were fed a grass hay diet (7.9% CP, 56% TDN, 63% NDF, 87% DM) at 2.25% of BW for a 62 d study, which consisted of 3 periods; acclimation (AC), treatment (TR), and residual (RE). Cows were stratified by age (5 ± 0.37 yr) and lactational history, and assigned to receive 12.5 mg supplemental Co in 1 of 2 forms: 1) 27.2 mg of cobalt carbonate (CC, n =11 cows) or 2) 50 mg of cobalt glucoheptonate (CGH, n = 9 cows). Supplementation was administered daily via a gelatin capsule placed directly into the rumen 2 h after feeding. During the last 96 h of each period, forage fermentability was measured using an in situ nylon bag technique. Serum samples were collected 4 and 6 h following feeding, 24 h before the end of each period. Measurements taken in the AC period were used as covariates for analysis in the TR and RE periods. A treatment × period interaction ($P = 0.03$) was exhibited for in situ OM fermentability at 96 h; (TR period, 68.44 and 70.83 ± 0.81 %, and RE period, 67.61 and 66.82 ± 0.75 %, for CC and CGH, respectively). Once inclusion of Co in the CGH group was removed, fermentability was reduced by 4.01 % compared with 0.82 % in the CC cows. The NDF disappearance (OM basis) was lower for the TR period compared with the RE period at 48 h ($P < 0.001$; 62.95 and 65.18 ± 0.39 %, respectively). However, by 96 h the NDF disappearance was greater for TR period than the RE period ($P = 0.02$; 70.44 and 68.89 ± 0.44 %; respectively). No differences were detected for cobalamin serum cocentrations or rate of fiber fermentation. The outcomes of this research signify that while there are no residual effects of Co supplementation on fermentation, there is an indication that CGH supplementation does enhance the overall extent of fermentation. The extent of fiber disappearance is also improved with Co supplementation regardless of form.

**Key words:** beef cattle, cobalt, forage digestibility

## INTRODUCTION

Cobalt (**Co**), an essential trace element, has long been recognized to have several important functions in the ruminant. One such function includes the vital role Co plays in rumen microbial synthesis of vitamin $B_{12}$ (McDowell, 2000). However, ruminate microorganisms are only capable of synthesizing vitamin $B_{12}$ when adequate Co is present in the diet. Therefore, dietary Co is the limiting factor for ruminal microorganism syntheses of vitamin $B_{12}$ (Sutton and Elliot, 1972). Vitamin $B_{12}$ serves as a growth factor for many ruminal microorganisms (Tanner and Wolfe, 1988) and is an essential co-factor for gluconeogenesis. Gluconeogenesis is the process in which the liver and to a lesser extent the kidney convert fermentation by-products or glucogenic amino acids to glucose through a series of enzymatic reactions which require vitamin $B_{12}$ as a co-factor (Seal et al., 1992; Brockman, 1993). Tiffany et al. (2002) reported increased concentrations of ruminal vitamin $B_{12}$ levels and propionate when Co was supplemented to steers fed a high energy, Co deficient diet. Along with its structural role in vitamin $B_{12}$ synthesis, several studies have also suggested that Co may improve fiber digestion in the rumen.

The effectiveness of supplemental Co is dependent on the biological availability of Co not only to ruminal microorganisms but ultimately the host ruminant. Thus, the source in which Co is fed may be of importance to both vitamin $B_{12}$ production and the various roles Co is responsible for in the ruminant animal. Ammerman et al. (1982) found cobalt oxide to be of lower nutrient value when compared with more rumen soluble sources such as cobalt carbonate or cobalt sulfate. However, limited information is known about organic Co sources and what, if any, benefits apply to supplementing an organic source of Co to ruminant diets. The objective of this study was to determine the effect of supplementing an organic Co source on in situ fiber fermentation and serum vitamin $B_{12}$ concentrations.

## MATERIALS AND METHODS

The Fort Keogh Livestock and Range Research Laboratory Institutional Animal Care and Use Committee

[1]USDA-ARS is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the authors and does not imply its approval to the exclusion of other products that may be also suitable.

approved all animal handling and experimental procedures used in the current study.

Twenty crossbred ruminally-cannulated cows (577 ± 13 kg) were stratified by age (5 ± 0.37 years old) and prior lactation history (3 cows did not raise a calf in 2011). Cows were assigned to one of two different forms of Co supplementation: 1) 12.5 mg Co supplied as 27.2 mg of cobalt carbonate (**CC**, n = 11 cows) 2) 12.5 mg Co supplied as 50 mg of cobalt glucoheptonate (**CGH,** n = 9 cows; Zinpro Corp., Eden Prairie, MN). To alleviate weaning stress, calves were weaned on d -14, at which time cows were moved to a common feedlot pen and fed a chopped grass hay diet, free choice. On d -7, cows were randomly assigned to an individual pen (6 x 10 m) with ad libitum access to water. Water analysis found undetectable concentrations of Co and no other known possible antagonistic factors were detected. Throughout the trial all cows were fed a chopped grass hay diet (Table 2) at 2.25% BW and 2 g of Fort Keogh Range Mineral (Table 2) top dressed into each bunk once daily (0700 h). Cows were managed in individual pens for 62 d, which included three periods; acclimation (**AC**), treatment (**TR**), and residual (**RE**) period. During the AC period, all cows were fed only the basal diet with no supplemental Co, d 0 thru d 17. On d 18 thru d 44 (TR period) cows received one of the two Co treatments daily, 2 h after feeding (0900 h). Cobalt supplement was administered directly into the rumen via the rumen cannula in a gelatin capsule (Torpac Inc., Fairfield, NJ). From d 45 thru d 62 (RE period) cows received no Co supplementation to determine any carry-over effects from the previous treatment period.

To estimate diet digestibility, in situ NDF disappearance (**ISNDFD**) and in situ OM disappearance (**ISOMD**) was measured. Grass hay samples were collected from stock piled hay and ground to pass a 2 mm screen (Model 4 Wiley mill, Arthur H. Thomas Co, Philadelphia, PA). Dacron bags (10 cm x 20 cm: pore size 53 ± 10µm: Ankom Technology Corp., Fairport, NY) were individually filled with 5 g of ground grass hay and sealed. During the last 4 d of each period (AC d 14, TR d 41, and RE d 58) a 96 h in situ was initiated. At each incubation time (96, 48, and 24 h), 5 filled Dacron bags along with a sealed blank bag were placed in a 35 cm x 45 cm polyester mesh bag (Household Essentials, Hazelwood, MO) which was anchored with ~ 1 m of string and a rubber stopper. The bags were then placed into the rumen in the vicinity of the mat and liquid interface. The empty bag (i.e., blank) was used as a correction factor for ruminal contamination entering the bags. The empty bag residue weight was subtracted from each sample bag at the same incubation time. Upon removal from the rumen, all the bags were subjected to an initial rinse by submerging them into a bucket filled with cold tap water to arrest microbial fermentation. Five filled bags and 1 blank bag were not inserted into the rumen but were subject to the cold water rinse (Wiley et al., 1991), which were 0 h bags. All bags were then transported to the lab and individually rinsed in cold tap water until the effluent was clear, after which bags were frozen (-20° C), lyophilized, and weighed. Residue remaining in the bag was analyzed for DM, OM and NDF using the methods of Van Soest et al. (1991).

Disappearance of NDF was determined with a batch processor (ANKOM 200 fiber analyzer, ANKOM Technology).

On d 17, 44 and 61 of the corresponding AC, TR, and RE periods, blood samples were collected via coccygeal venipuncture (Corvac, Sherwood Medical, St Louis, MO) 4 and 6 h after feeding. Blood samples were centrifuged at 1,500 × *g* for 30 min; serum was decanted and stored at -20° C until analysis. Cobalamin was assayed by an independent lab using a Dualcount Solid Phase Vitamin $B_{12}$ / Folic Acid procedure (Siemens Healthcare Diagnostics, Los Angeles, CA Cat # KDSP1, interassay CV 4.2% and intraassay CV 3.3%) and expressed as pg/mL.

Particulate-associated carboxymethylcellulase (**CMCase**) activity was measured on residue from in situ bags incubated at 96, 48, 24 and 0 h for all three periods. A single dacron bag/cow•incubation period$^{-1}$ was randomly pulled after lyophilization for CMCase analysis. Approximately 0.4 g of residue was weighed out into a 50 mL centrifuge tube, after which 8 mL of 10 mM sodium phosphate buffer (pH 6.8) containing 20 µg/mL of lysozyme (Sigma Aldrich, St. Louis, MO) was added. Following an addition of 1 mL of carbon tetrachloride, the mixture was vortexed and incubated in a 37° C water bath for 3 h. The tubes were then centrifuged at 29,000 x *g* at 4° C for 15 min and supernatant was decanted and frozen for later analysis. The CMCase assay has been previously adapted to utilize a 96-well micro plate system (Xiao et al., 2004, 2005). Thirty microliters of the sample supernatant in duplicates was pipetted into a 96 well PCR plate. An addition of 30 µL of 2% (wt/vol) sodium carboxymethylcellulose (Sigma Aldrich) containing 0.1 mg/mL of thirmersol was then added to each well. The plate was then incubated utilizing a thermocycler, programmed to heat to 50°C for 30 min followed by cooling and holding at 20° C. The plate was then removed and 60 µL of 3,5-dinitrosalicylic acid reagent (Miller et al., 1960) was added to each well. To develop color, the plate was incubated again in the thermocycler programmed to heat to 95° C for 5 min followed by cooling to 4° C for 1 min and holding at 20° C (King et al., 2009). Following color development, a 100 µL aliquot from each duplicated sample was transferred to the wells of a flat- bottom 96-well (Costar, Corning INC, Corning, NY). Absorbance was measured on a plate reader (Synergy HT, BioTek Instruments Inc., Winooski, VT) at 540 nm. D-Glucose was used as the standard, and CMCase activity was expressed as micromole of glucose released per gram of OM per min. CMCase activity was corrected by subtracting the background glucose activity from every sample (Bhatti and Firkins, 1995).

Data were analyzed using Proc Mixed procedures (SAS Inst. Inc., Cary, NC). The experiment was a completely randomized design with three periods (AC, TR and RE). The restricted maximum likelihood method was used for estimating the variance components and degree of freedom was adjusted using the Kenward Roger option. The model included the fixed effects of treatment and period (TR and RE) and their interaction. Data was analyzed by hour. The covariance structure used was variance components.

The Repeated statement included Period with the subject being cow. Corresponding measurements obtained in the acclimation period were used as covariates in the treatment and residual period. Significance was set at $P \leq 0.05$. On the first day of treatment supplementation, one cow in the CGH treatment group was given a CC bolus, thus was moved to the CC treatment group, increasing this group to 11 cows. Unfortunately, one cow in the CGH treatment group was found to be a true outlier, thus was removed from the study decreasing this group to 8 cows.

## RESULTS

An interaction for ISOMD ($P = 0.03$) at 96 h was measured between source of Co and period (TR and RE, respectively). Cows receiving CGH exhibited a greater percentage of ISOMD when incubated at 96 h in the rumen, during the TR period compard to the RE period (TR period 68.4 and 70.8 ± 0.8% and RE period 67.6 and 66.82 ± 0 .8 % for CC and CGH, respectively; Figure 1). Shorter incubation durations (24 and 48 h), or Co source did not affect ISOMD (Table 1). Disappearance of in situ NDF (OM basis) was less during the TR period compared with the RE period at 48 h ($P < 0.001$) however by 96 h, the TR period had significantly greater disappearance ($P = 0.02$). No differences were found between treatments for ISNDFD. No differences were detected for fiber fermentation rate (Table 1).

Carboxymethylcellulase activity was measured to assess the effect supplemental Co or treatment period had on microbial activity. A trend was found between the two periods at 96 hours of in situ incubation, with the TR period having greater CMCase activity ($P = 0.09$; 0.34 and 0.23 ± 0.04 µmol of glucose released/g OM• $min^{-1}$;Table 1). Similar results for CMCase were measured when analyzed on a dry matter basis ($P = 0.09$; 0.32 and 0.22 ± 0.04 µmol of glucose released/g DM•$min^{-1}$ for TR and RE periods, respectively).

Serum Cobalamin concentration was assayed as an indication of the effectiveness of Co source on serum vitamin $B_{12}$ concentrations. Neither sampling time (4 and 6 h after feeding) nor Co source resulted in differences in serum cobalamin.

## DISCUSSION

Although several studies have documented the importance of Co supplementation in high concentrate diets fed to young growing cattle (Schwarz et al., 2000; Stangl et al., 2000; Tiffany and Spears, 2005), much is yet to be learned about Co requirements for ruminants consuming high fiber diets or if Co source differentially affects ruminal microbial function. Hussein et al. (1994) reported no effects on in vitro fermentation when Co was supplemented at rates 0, 5, and 10 mg of Co/kg of substrate DM after 24 and 48 h incubation periods. Lopez-Guisa and Satter (1992) supplemented both Co and Cu at concentrations greater then NRC recommendations and reported that the greater dietary content aided digestion of low quality forage diets. However, both minerals were supplemented together not allowing for determination of the impact of Co or Cu alone. They, like several others, have attributed the enhanced digestion to the increased affinity between the fiber particles to the microbes. Often the microbial cell wall is negatively charged therefore attachment to a similarly charged fiber particle is difficult. As a divalent cation, Co functions as a link between negatively charged microbes and similarly charged fiber particles (Somers, 1973; Lopez-Guisa and Satter, 1992; Hussein et al., 1994).

Most trace minerals have been linked to increased efficiency of absorbance, availability and metabolism when organic forms are supplemented (Nockels et al., 1993; Kegley and Spears, 1994; Du et al., 1996). With the current study, organic Co improved ISOMD and ISDMD at 96 h during the TR period compared with the RE period. When Co was not fed in the form of CGH, ISOMD and ISDMD at 96 h was 4.0 % lower compared with the supplementation period. This is compared with the CC supplemented cattle that varied by only 0.8 % between the periods when they were and not supplemented Co.

The current study found greater ISNDFD when CGH and CC forms of Co were supplemented after 96 h of in situ incubation, thus the extent of NDF digestion was enhanced regardless of Co form. Few studies have investigated the effectiveness of Co to influence digestibility after 48 h of disappearance. Our results suggest that limited Co availability in the rumen may limit further break down of the highly lignified fiber. This could be especially important for cattle consuming high roughage or grazing late season dormant vegetation as diets are often resistant to digestion and are characterized by a slower rate of passage. This finding is further supported by changes in CMCase activity that were measured, where a trend for greater CMCase activity with Co supplementation was found when compared with no supplementation after 96 h of fermentation. Thus, enzymatic activity derived from the microbial population was greater in later hours of fermentation when cows were supplemented with Co. This story is not completely clear since the RE period 48 h incubation interval had increased ISNDFD compared with supplemented cows. The reason for this is unclear.

Similar Cobalamin serum concentrations were found; however, it may have been more informative to had collected liver biopsy samples where excess Co is thought to be stored. While it has been well documented that sheep and cows fed Co deficient diets have lower serum and plasma vitamin $B_{12}$ concentrations (Somers and Gawthorne, 1969; Kennedy et al., 1991; Stangl et al., 1999), little is known about specific serum or plasma concentrations needed for optimal production. It is also unclear how the dietary Co requirements for the microbial population as well as the host animal differ, if at all. This is due to the complexity of Co/vitamin $B_{12}$ metabolism and the later absorption. Tiffany et al. (2003) showed a linear increase of plasma vitamin $B_{12}$ as Co supplementation increased from 0 and to 1.0 mg/kg of DM and even greater increases were measured with greater concentrations of Co supplementation, 0.1 to 1.0 mg/kg of DM; however no differences between Co supplemental sources (Co carbonate and Co propionate) in plasma vitamin $B_{12}$ were detected. With supplementation targeting the high

**Table 1.** In situ organic matter disappearance (ISOMD), In situ NDF disappearance (ISNDFD), rate (k) of fiber fermentation and particle-associated carboxymethylcellulase (CMCase) specific activity of low quality grass hay extrusa, and serum cobalamin concentrations in cows during a treatment period (TR) with cobalt supplementation and a residual period (RE) with no supplement. Cows were supplemented daily with one of two treatments; 27.2 mg of cobalt carbonate (CC) or 50 mg of cobalt glucoheptonate (CGH)

| | Period (P) | | | Supplement (S) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | TR | RE | SEM | CC | CGH | SEM | P | S | P x S |
| No. of cows | 19 | 19 | - | 11 | 8 | - | - | - | - |
| ISOMD, % | | | | | | | | | |
| 24 h | 48.21 | 47.38 | 0.88 | 47.90 | 47.70 | 1.0 | 0.49 | 0.88 | 0.22 |
| 48 h | 62.42 | 63.60 | 0.40 | 63.16 | 62.86 | 0.44 | 0.05 | 0.61 | 0.83 |
| NDFD, % Omb | | | | | | | | | |
| 24 h | 48.32 | 48.48 | 0.90 | 48.46 | 48.35 | 1.01 | 0.90 | 0.94 | 0.29 |
| 48 h | 62.95 | 65.18 | 0.39 | 64.22 | 63.91 | 0.42 | <0.001 | 0.58 | 0.94 |
| 96 h | 70.44 | 68.89 | 0.44 | 69.40 | 69.93 | 0.48 | 0.02 | 0.41 | 0.12 |
| k, $h^{-1}$ | 4.79 | 4.77 | 0.02 | 4.77 | 4.79 | 0.02 | 0.40 | 0.52 | 0.40 |
| CMCase, µmol glucose·of OM·$min^{1}$ | | | | | | | | | |
| 24 h | 0.36 | 0.44 | 0.06 | 0.38 | 0.42 | 0.06 | 0.30 | 0.61 | 0.64 |
| 48 h | 0.49 | 0.46 | 0.06 | 0.41 | 0.55 | 0.07 | 0.71 | 0.15 | 0.66 |
| 96 h | 0.34 | 0.23 | 0.04 | 0.31 | 0.26 | 0.05 | 0.09 | 0.47 | 0.83 |
| Cobalamin, pmol/$ml^{1}$ | | | | | | | | | |
| 4h post feeding | 180.8 | 180.7 | 13.7 | 180.8 | 180.6 | 14.5 | 1.00 | 0.99 | 0.70 |
| 6h post feeding | 194.2 | 174.0 | 14.1 | 170.6 | 197.6 | 14.9 | 0.32 | 0.19 | 0.73 |

[1]One animal from the CC supplement group was removed from the Cobalamin data due to exceptionally high Cobalamin concentrations compared with contemporaries (n = 10).

**Table 2.** Chemical composition of hay and top dressed Fort Keogh Range Mineral fed throughout the 62 d experiment

| | Grass Hay | Mineral |
|---|---|---|
| DM, % | 87.42 | 94.42 |
| CP, % | 7.92 | 10.27 |
| NDF, % | 63.32 | 13.05 |
| ADF, % | 40.40 | |
| Ca, % | 0.28 | 11.13 |
| P, % | 0.18 | 5.55 |
| K, % | 1.58 | 4.38 |
| Na, % | 0.08 | 0.74 |
| Co, % | n/d | 0.001 |
| Zn, % | 0.002 | 0.42 |
| Cu, % | 0.0005 | 0.22 |
| Fe, % | 0.01 | 0.31 |
| Mn, % | 0.005 | 0.29 |

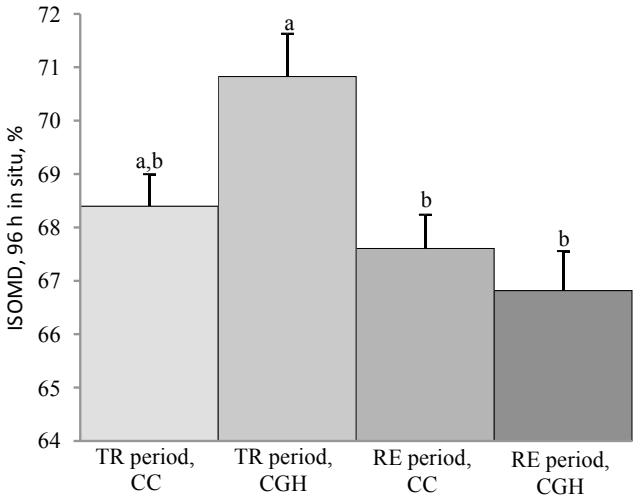


**Figure 1.** In situ organic matter disappearance (ISOMD) at 96 h of low quality grass hay extrusa from cows during a treatment (TR) period with cobalt supplementation and a residual period (RE) when no supplement was offered. During the TR period cows were supplemented daily with one of two treatments to provide 12.5 mg Co; 27.2 mg of cobalt carbonate (CC) or 50 mg of cobalt glucoheptonate (CGH, Zinpro Corp., Eden Prairie, MN). Letters above bars indicate statistical significance; means without a common superscript letter differ ($P = 0.03$).

levels of the current study it was not surprising we did not find differences in the serum cobalamin concentrations.

Our research indicates an increased extent of fiber digestion in later incubation hours. Very few studies have increased disappearance with any supplementation this late in the fermentation process thus Co may be a valuable resource for maximize forage fermentation. Cows in this study were not as challenged as a cow grazing late season vegetation might be in the Northern Great Plains, thus this may be an area of further investigation.

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# COMPARISON OF METHIONINE CHELATED VERSUS SULFATE TRACE MINERALS ON RATE AND EFFICIENCY OF GAIN AND PREGNANCY RATES IN BEEF HEIFERS

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**ABSTRACT**: Objectives of this experiment were to compare rate and efficiency of gain, and conception rates of yearling heifers supplemented with Cu, Zn and Mn as either metal methionine hydroxy analogue chelated trace mineral (CTM; provided as MINTREX) or the same trace minerals in $SO_4$ form. The experimental design utilized 3 ranches, each having 2 replications per treatment with pen as the experimental unit for ADG, DMI and G:F. Individual heifer was the experimental unit for pregnancy diagnosis. Ranch A contained 498 Angus heifers, Ranch B, 240 Red Angus composite heifers, and Ranch C, 1,742 Angus composite heifers. All heifers were fed silage based diets that contained approximately 13.5% CP, 64% TDN (DM basis) and had no significant levels of $SO_4$, Mo or Fe in feed or water. Diets contained an average of 24 ppm Cu, 70 ppm Zn and 64 ppm Mn. Diets were fed for 181 d (Ranch A), 149 d (Ranch B) and 151 d (Ranch C) prior to breeding. Heifers were weighed once at trial initiation (initial BW 270 kg ± 2.8), end of drylot feeding, at breeding and at pregnancy diagnosis. Ranch A heifers were bred by AI followed by natural service (45 d breeding), Ranch B heifers were bred by natural service (50 d breeding) while Ranch C heifers were bred by AI once. Pregnancy was determined via ultrasound using trained technicians. No ranch x treatment interactions were detected for any measurements ($P \geq 0.47$) and no differences ($P \geq 0.46$) were detected between treatments for total gain, ADG, G:F or the number of heifers that conceived during the first 21 d on Ranches A or B. Ranch effects were significant ($P < 0.001$) for gain, ADG, G:F and overall pregnancy rate, but not for conception in the first 21 d of breeding. Conception rate increased ($P = 0.03$) for CTM fed heifers from Ranch C with one AI breeding. Conception rates during the first 21 d of breeding did not differ ($P = 0.12$) between treatments but overall pregnancy rate was greater ($P = 0.05$) for heifers supplemented with CTM vs. $SO_4$ form. Under the conditions of this experiment, results suggest that supplementation with CTM contributed to increased pregnancy rates in heifers.

**Key words:** chelated minerals, fertility, mineral nutrition

## INTRODUCTION

Heifers must calve by 24 mo to achieve maximum lifetime productivity (Patterson et al. 1992). One suggested cause of pregnancy failure in heifers is mineral deficiency of Cu, Zn, and Mn (NRC, 1996 and Paterson and Engle 2005). Copper, Zn, and Mn act together. Supplementation of both Cu and Zn together increased liver storage of each mineral more than when only one mineral was supplemented (Wellington et al., 1998) and supplementation of both Zn and Mn increased immunity compared with supplementation with Zn alone (Chirase et al., 1994). Sulfate, Mo and Fe are antagonists to Cu absorption (Suttle 1974, 1991). Bio-availability of organic minerals in beef cattle has been questioned (NRC, 1996) and research has produced inconsistent results (Suttle, 2010). Nockels et al. (1993) reported that CuLys and ZnMet were more bio-available than $CuSO_4$ and $ZnSO_4$. Kegley and Spears (1994) reported that CuLys and $CuSO_4$ had similar bio-availability. Research concluded that feeding CTM as MINTREX resulted in improved feedlot performance when $SO_4$ was present (Vazquez-Anon et al., 2007) Kincaid et al. (1986) and Rabiansky et al. (1999) reported that CuLys was more available than $CuSO_4$ when S and Mo were present. Organic mineral supplementation has resulted in greater pregnancy rates in cows, but with inconsistency across age groups, breeding methods, and time (Stanton et al. 2000, Ahola et al. 2004 and Arthington and Swenson 2004). Research has explored CuLys, ZnMet and MnMet, but limited research has compared supplementation with methionine chelated form of Cu, Zn, and Mn to $SO_4$ form to satisfy requirements for rate and efficiency of gain or pregnancy in heifers. The objectives of this study were to compare rate of gain, efficiency of gain and pregnancy rates in heifers supplemented with either CTM form or $SO_4$ form of Cu, Zn and Mn. The null hypothesis tested was that no differences would exist in drylot performance or in pregnancy rates between forms of supplemental trace minerals.

## MATERIALS AND METHODS

***Animals.*** Three ranches were utilized for this experiment. Ranches were located in Dillon, MT (498Angus heifers), Terry, MT (240 Red Angus x Composite heifers) and Dayton, WY (1,742 Angus x Composite heifers).

***Design and Treatment.*** The experimental design was a randomized block (3 ranches) with each ranch having two replications per treatment. Treatments were diets supplemented with trace minerals provided in either sulfate form of Cu, Zn and Mn or methionine chelated form of Cu, Zn and Mn. Pen was the experimental unit for drylot performance

measures with approximately 125 heifers per pen at Ranch A, approximately 60 heifers per pen at Ranch B, and 333 to 537 heifers per pen at Ranch C. Diets were formulated using NRC (1996) recommendations for protein and $NE_g$ so that heifers would achieve 65% of mature BW by the time of breeding. Diets, water and supplements were analyzed at a commercial laboratory (MidWest Labs, Omaha, NE) for CP, energy and mineral content. Heifers were fed silage based diets that contained approximately 13.5% CP, 64% TDN (DM basis) and had minimal concentrations of $SO_4$, Mo or Fe in either feed or water. Diets contained an average of 24 ppm Cu, 70 ppm Zn and 64 ppm Mn (Table 1), consistent with common industry practice in the area. Diets were fed as a total mixed ration once daily during the feedlot phase of the trial and then fed in free choice mineral feeders when cattle were on pasture in the days between the end of the feeding period and pregnancy diagnosis. Prior to breeding, Ranch A heifers received the treatment supplements for 181 d, Ranch B received treatment supplements for 149 d, and Ranch C received treatment supplements for 151 d.

***Measurements and Collections.*** Initial BW of heifers was collected on December 11, 2010 at Ranch A (BW 257 kg ± 2.0), on December 15, 2010 at Ranch B (BW 269 kg ± 2.8), and February 8 to 11 and 14, 2011 at Ranch C (BW 295 kg ± 1.5). Body weight was recorded upon completion of the drylot phase of the experiment after 181 d at Ranch A (BW 341 kg ± 2.6), after 149 d at Ranch B (BW 390 kg ± 3.9), and 77 to 81 d at Ranch C (BW 348 kg ± 1.6). Ranch A heifers were bred via AI by a trained technician June 3 to 5, 2011 with bulls introduced on June 15, 2011 for 45 d. Ranch B heifers were bred via natural service for 50 d starting on May 20, 2011 with a bull to heifer ratio of 1:20. Ranch C heifers were placed on pasture immediately after drylot phase conclusion, but remained segregated by treatment and continued to receive the assigned treatment until breeding commenced, at which time they were returned to the feedlot and commingled to facilitate breeding. Ranch C heifers (BW 400 kg ± 0.0) were bred by AI from July 17 thru August 1, 2011 and were bred only once. At each ranch, heifers were commingled at breeding and remained so until the date of pregnancy diagnosis. Body weight and date of conception was recorded at the time of pregnancy diagnosis which was August 22, 2011 at Ranch A, August 10, 2011 at Ranch B, and September 12-16, 2011 at Ranch C. Due to management practices some heifers that were included in the feedlot trial were removed from the reproductive phase of the experiment. Heifers were not removed from treatments in equal numbers. Heifers remaining in the experiment for the reproductive phase numbered 474 at Ranch A, 236 at Ranch B, and 1,621 at Ranch C.

***Statistical Analysis.*** Using Statistix 9 (Analytical Software, Tallahassee, FL) ANOVA was performed for the effects of ranch, treatment and potential treatment x ranch interactions for gain, ADG and G:F using pen as the experimental unit. Pregnancy rate differences between treatments were analyzed with Chi-square using SAS software (SAS Inst. Inc., Cary, NC) and Statistix 9 (Analytical Software, Tallahassee, FL).

## RESULTS AND DISCUSSION

***Drylot Performance Measures.*** Drylot performance data includes data from heifers present for the feedlot phase of the experiment, but removed from the reproductive phase of the experiment. There were no ranch x treatment interactions ($P \geq 0.76$) for total gain, ADG, or G:F (Table 2). There were no differences ($P \geq 0.57$) due to form of mineral for total gain, ADG, or G:F at any individual ranch or across ranches. Ranch was significant ($P < 0.001$) for gain, ADG and G:F. No treatment differences in rate or efficiency of gain were expected based on prior research. Spears et al. (1989), Ward et al. (1993) and Wellington et al. (1998) all noted no differences in gains or ADG in growing steers and heifers when organic and inorganic forms of Cu and Zn were tested against each other in the presence or absence of antagonists.

***Pregnancy Rate.*** When all data were combined there was no ranch x treatment interaction ($P \geq 0.47$) for pregnancy rates attributed to the first 21 d of breeding (Table 3). No difference was detected ($P = 0.12$) between form of mineral fed on percent pregnant resulting from the first 21d of breeding (Table 3). Overall pregnancy rate was greater ($P = 0.05$) in the CTM fed heifers (Table 3). Ranch was not a significant factor ($P = 0.10$) for pregnancies achieved during the first 21d of breeding, but was significant ($P \leq 0.001$) in overall pregnancy and reflects the decreased breeding season at Ranch C where heifers had only one opportunity to conceive. Heifer pregnancy rates attributed to the first 21d of breeding and overall pregnancy rate did not differ ($P \geq 0.46$) between treatments at Ranches A and B. Ranch C heifers had 7% greater conception rate following one AI breeding when supplemented with CTM ($P = 0.03$). Among Ranch C heifers, no differences ($P \geq 0.77$) in BW between mineral treatments existed at time of breeding or pregnancy diagnosis. Similar results to the current experiment have been noted by Stanton et al. (2000) who utilized 300 Angus cows supplemented with three mineral treatments in the presence of antagonists; a low level inorganic mineral supplement containing Cu, Zn, Mn, and Co, a high level of inorganic supplement containing Cu, Zn, Mn, and Co, and a high level of complexed organic Cu, Zn, Mn, and Co. Cows supplemented with the high level of organic minerals had significantly greater conception rates to AI breeding than cows supplemented with the other two treatment minerals, however overall reproductive performance did not differ. Similarly, Ahola et al. (2004) conducted a study in which 178 crossbred multiparous cows were fed two treatment supplements; 100% inorganic mineral containing Cu, Zn, and Mn compared with a 50% complexed:50% inorganic mineral supplement containing Cu, Zn, and Mn. In year 1 overall reproductive performance did not differ between treatments, but the trend ($P = 0.08$) was for those cows supplemented with the high level of complexed minerals to have a greater rate of pregnancy to AI breeding. In year 2 of the trial, cows fed the complexed mineral treatment had 10% greater conception to AI breeding than those fed the inorganic mineral treatment. Arthington and Swenson (2004) conducted an experiment using 160 Braford cows over a

**Table 1.** DM analysis of diets fed daily to heifers by block

| Item | Ranch A | Ranch B | Ranch C |
|---|---|---|---|
| CP,% | 12.67 | 13.28 | 15.20 |
| ADF,% | 37.83 | 25.22 | 34.78 |
| TDN,% | 59.42 | 67.55 | 63.95 |
| $NE_m$, (Mcal/lb) | 0.58 | 0.68 | 0.64 |
| $NE_g$, (Mcal/lb) | 0.35 | 0.41 | 0.36 |
| S, % | 0.23 | 0.23 | 0.21 |
| P, % | 0.30 | 0.39 | 0.32 |
| K, % | 1.89 | 1.43 | 1.50 |
| Mg, % | 0.31 | 0.26 | 0.31 |
| Ca, % | 1.55 | 0.92 | 1.53 |
| Na, % | 0.18 | 0.20 | 0.20 |
| Fe, ppm | 309 | 416 | 184 |
| Mn, ppm | 70 | 59 | 64 |
| Cu, ppm | 20 | 23 | 28 |
| Zn, ppm | 62 | 69 | 80 |

**Table 2.** Summary of differences in feedlot performance of heifers fed CTM[1] or $SO_4$ form of Cu, Zn and Mn

| | | | | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ranch A | | Ranch B | | Ranch C | | SE | Trt[2] | Ranch | R[3] x Trt |
| Treatment | $SO_4$ | CTM | $SO_4$ | CTM | $SO_4$ | CTM | | | | |
| No. hfs[4] | 251 | 246 | 120 | 119 | 870 | 872 | | | | |
| DoT[5] | 181 | 181 | 149 | 149 | 77 | 77 | | | | |
| IBW[6],kg | 249 | 251 | 268 | 270 | 288 | 294 | 2.82 | | | |
| EBW[7],kg | 341 | 340 | 389 | 391 | 347 | 349 | 4.11 | | | |
| Gain,kg | 92 | 89 | 121 | 121 | 59 | 55 | 2.77 | 0.60 | <0.001 | 0.88 |
| ADG,kg | 0.50 | 0.49 | 0.81 | 0.81 | 0.76 | 0.70 | 0.03 | 0.57 | <0.001 | 0.76 |
| G:F,kg | 0.16 | 0.16 | 0.26 | 0.26 | 0.23 | 0.25 | 0.04 | 0.91 | <0.001 | 0.85 |

[1] CTM = Cu, Zn and Mn as metal methionine hydroxy analogue chelated trace mineral provided as MINTREX
[2]Trt = treatment
[3]R = Ranch
[4]hfs = heifers
[5]DoT = days on test
[6]IBW = Initial BW
[7]EBW = BW at end of feeding in the drylot

**Table 3.** Summary of differences in pregnancy rates of heifers fed CTM[1] or $SO_4$ form of Cu, Zn and Mn

| | | | | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ranch A | | Ranch B | | Ranch C | | SE | Trt[2] | Ranch | R[3] x Trt |
| Treatment | $SO_4$ | CTM | $SO_4$ | CTM | $SO_4$ | CTM | | | | |
| % Pregnant | 85 | 86 | 92 | 91 | 59 | 66 | 0.02 | 0.05 | <0.001 | 0.47 |
| % Preg[4] 1st 21d | 58 | 57 | 54 | 51 | 59 | 66 | 0.02 | 0.12 | 0.10 | 0.54 |

[1] CTM = Cu, Zn and Mn as metal methionine hydroxy analogue chelated trace mineral provided as MINTREX
[2]Trt = treatment
[3]R = Ranch
[4]Preg = pregnant

three year period where sulfur antagonism was present. Cows were assigned to one of four treatments, which included a treatment using complexed organic forms of Cu, Zn, and Mn supplement and an inorganic form of Cu, Zn, and Mn. Over the course of the trial it was determined that mineral source had no effect on cow BW, cow BCS, or calf weaning weight. However, pregnancy rates were greater in year two ($P < 0.05$) and calving intervals were shorter in years 1 and 3 for young cows that were fed the organic forms of Cu, Zn, and Mn, but older cows did not differ regardless of mineral type or source. When comparing similarities between the present experiment and prior research, it appears that chelated, organic forms of Cu, Zn and Mn can contribute to greater pregnancy rates to AI. The current experiment supplemented heifers with CTM for a period of 149 to 181 d. Ahola et al. (2004) and Arthington and Swenson (2004) both supplemented cattle with complexed, organic forms of Cu, Zn and Mn over a period of 2 to 3 yr. In both experiments, overall pregnancy rates were not different due to differences in mineral form in all years, indicating that organic forms of minerals did not always achieve greater pregnancy rates when fed over extended periods of time. While CTM supplementation was a factor in achieving greater pregnancy rates to AI breeding at Ranch C as well as to the overall pregnancy rate differences when ranches were combined, the high level of significance that ranch exhibited in the analysis indicates that location or management also plays a key factor in the ability of the CTM to have an effect on animal reproductive performance.

## IMPLICATIONS

This experiment suggests that rate and efficiency of gain, when diets contained adequate levels of dietary protein and energy, were not affected by form of mineral supplementation. However, under the conditions of management at Ranch C, yearling heifers exhibited greater conception to AI breeding when supplemented with CTM (provided as MINTREX) compared with those supplemented with $SO_4$ form of Cu, Zn, and Mn.

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# DOSE RESPONSE EFFECTS OF LAIDLOMYCIN PROPIONATE PLUS CHLORTETRACYCLINE OR MONENSIN PLUS TYLOSIN ON GROWTH PERFORMANCE, CARCASS MERIT AND HEALTH OF GROWING-FINISHING BEEF STEERS

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**ABSTRACT:** Doses of laidlomycin propionate (LP) plus chlortetracycline (LP/CTC) or monensin (M) plus tylosin (T) were evaluated in an experiment with growing-finishing beef steers. No ionophore (CON), low, moderate, and high levels of LP (50, 100 and 150 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) and M (150, 300 and 450 $mg^{-1}$•$animal^{-1}$•$d^{-1}$), respectively were compared. Chlortetracycline (350 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) or T (90 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) were fed in combination with LP or M, respectively. Four hundred twenty steers (average initial BW= 357 kg) were randomized by initial BW to 6 blocks (7pens/block). Steers were on feed for 153 d (3 heavy weight blocks) or 175 d (3 light weight blocks). Linear (L) and quadratic (Q) effects of ionophore dose were evaluated for growth performance and carcass measurements. Carcass-adjusted final BW, ADG, and HCW indicated a Q increase ($P \leq 0.05$) with dietary LP level. Dry matter intake and carcass-adjusted DM feed:gain (F/G) ratio were not affected by LP dose. Linear or Q responses were not observed with M level for DMI, carcass-adjusted BW, growth performance, DM F/G and HCW. Increasing M level increased number of Prime/Choice carcasses (L and Q); increased marbling score L and Q); $12^{th}$ rib fat thickness (Q); and calculated yield grade (Q). Across dose levels, mean ionophore effects indicated LP increased ($P \leq 0.05$) carcass-adjusted final BW, ADG, DMI and HCW compared with M, with no difference between ionophores for carcass-adjusted DM F/G ratio. Growth performance responses of feedlot cattle can be modified by MFA program and dietary level of ionophore.

**Keywords:** beef cattle, ionophore level, laidlomycin, monensin, propionate

## INTRODUCTION

Defining the optimal dose of an ionophore for a particular application, diet, or cattle type is a critical component for realizing the most cost-effective response. Nonetheless, few if any, previous research studies have examined the response of either laidlomycin propionate (**LP;** Cattlyst 50, Pfizer Animal Health, Madison NJ) or monensin (**M;** Rumensin 91; Elanco Animal Health, Greenfield, IN) dose when fed concurrently and in combination with chlortetracycline (**CTC;** Aureomycin 100; Pfizer Animal Health) or tylosin (**T;** Tylan 100; Elanco Animal Health), respectively. Availability of alternative medicinal feed additive (**MFA**) programs might allow cattle feeders greater flexibility to improve health, growth performance, and meet current economic challenges. The objective of this study was to determine the dose-response effects of feeding either LP plus CTC (**LP/CTC**) at LP levels of 50, 100 and 150 $mg^{-1}$•$animal^{-1}$•$d^{-1}$or M plus T (M/T) at M levels of 150, 300 and 450 $mg^{-1}$•$animal^{-1}$•$d^{-1}$ on DMI, BW gain, feed efficiency, carcass quality and yield, and overall health in growing – finishing beef steers.

## MATERIALS AND METHODS

Approximately 490 cross bred steers were purchased from sale barn facilities and shipped to the research site in Wellington, CO. Cattle were received and acclimated at the research site until processing. Cattle were processed by receiving group. All steers were administered Component TE-S (VetLife Animal Health, Shawnee Mission, KS) as the initial implant and vaccinated according to standard procedures. Individual animal BW was collected on two consecutive days and each animal was individually identified. Steers were randomly assigned to one of 42 feeding pens based on BW and receiving group. Pens were blocked on the basis of location along the feeding alley into groups of 7 adjacent pens (6 blocks total) with 10 animals allotted to each pen.

Treatments were randomly assigned to pens within each block. Mean initial BW across all study pens ranged from 323 to 391 kg such that it was necessary to set different reimplant and slaughter dates. Across all blocks, a terminal implant of Revalor S (Merck Animal Health, DeSoto, KS) was administered approximately 90 to 100 d prior to the projected slaughter date. Accordingly the heavier three blocks were reimplanted after 60 d on feed and the lighter three blocks after 86 d on feed.

Dose response effects of feeding no ionophore (**CON**), low, moderate, and high levels of LP (50, 100 and 150 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) and M (150, 300 and 450 $mg^{-1}$•$animal^{-1}$•$d^{-1}$), respectively were compared. Chlortetracycline (350 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) or T (90 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) were fed in combination with LP or M, respectively. Monensin levels were not stepped up during the initial weeks of the study. Pens were fed once daily to allow for maximal DMI without an accumulation of unconsumed feed in the bunk. Test articles were added to treatment diets using a commercial micro-ingredient machine (Lextron Microtech Inc., Greeley, CO).

Diets were formulated by the consulting nutritionist to meet or exceed nutrient requirements for growing-finishing large frame beef steers (NRC, 1996). Throughout the study, steers were observed daily by experienced pen riders for sickness or other health-related conditions. Animals that died during the course of the study were necropsied by a veterinarian and cause of death documented. Slaughter dates were determined based on visual appraisal of cattle BW and degree of finish. Heavier BW blocks were slaughtered after 153 d on feed and lighter blocks after 175 d on feed. Final individual animal BW were obtained by pen within each block after which cattle were loaded on trucks and shipped to a commercial packing plant facility. Cattle were individually identified and were tracked throughout the packing plant where carcass data was collected by trained technicians. Carcasses were graded by USDA graders after approximately 24 hours in the cooler. At this time LM area, 12$^{th}$-rib fat thickness, percentage of kidney, heart and pelvic fat, and marbling score were also determined by an independent carcass data collection service.

Design structure used in the study was a randomized block with a 2 x 4 factorial arrangement of treatments with main effects of MFA program and dietary inclusion level of ionophore (LP or M). Pen was the experimental unit for all responses evaluated. Pen-based performance parameters, that included BW and HCW, daily BW gains, DMI, and DM feed:gain ratios (**F/G**) were evaluated by mixed model (Proc Mixed) analysis (SAS Inst. Inc., Cary, NC). The statistical model included effects for block and treatment group with treatment being a fixed effect and blocks a random effect. Linear (**L**) and quadratic (**Q**) effects of ionophore level were determined using orthogonal contrast coefficients (Steel and Torrie, 1960). For all statistical analyses, probabilities equal to or less than 10% *($P < 0.10$)* were considered significant. All performance data were calculated on the basis of excluding those animals that died or were removed from study during the course of the trial. Categorical (or count per pen) data that consisted of USDA quality grade and yield grade, liver abscess data, and numbers of animals that died or were removed or treated for disease were analyzed using the GLIMMIX procedure of SAS with the same model as for performance and carcass data.

## RESULTS AND DISCUSSION

Ingredient and calculated nutrient composition of the transition and finishing diets are shown in Table 1. Inclusion level of wet distillers grains (**WDGS**) which was acquired from a single source during the trial was increased as cattle were transitioned to the finishing diet. Approximately 1 wk after the study started, a major snow storm hit the study site. This storm lasted for over 2 d during which time in excess of 40 cm of snow was recorded. The primary consequence of this storm was to set all cattle back on feed intake, so that cattle remained on the transition diets for a longer time than originally scheduled. In addition, as the snow melted, pen conditions became wet and muddy for a period of time. Although this event caused substantial stress at the time, it was concluded there were no lasting adverse effects that significantly affected the outcome of the study.

**Table 1.** Ingredient and nutrient composition of basal diets (100% DM basis)

| | Transition diets | | | | Finishing |
|---|---|---|---|---|---|
| Item | 1 | 2 | 3 | 4 | Diet |
| Days fed | 6 | 5 | 7 | 7 | 128 to 150 |
| Ingredient | | | | | |
| Steam-flaked corn, % | 44.8 | 48.5 | 52.9 | 52.5 | 59.2 |
| Alfalfa hay, % | 40.0 | 30.0 | 20.0 | 12.6 | 8.1 |
| Wet distillers grains, % | 10.0 | 15.0 | 20.0 | 25.1 | 25.0 |
| Tallow, % | 0.0 | 1.5 | 1.9 | 1.9 | 2.5 |
| Supplement, % | 5.1 | 5.1 | 5.2 | 5.2 | 5.2 |
| Microingredients, % | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Nutrient composition[1] | | | | | |
| Dry matter, % | 71.5 | 67.5 | 63.8 | 58.7 | 57.1 |
| NEm, Mcal/lb | 0.79 | 0.86 | 0.91 | 0.94 | 0.98 |
| NEg, Mcal/lb | 0.52 | 0.57 | 0.61 | 0.63 | 0.66 |
| Crude protein, % | 16.2 | 16.3 | 16.5 | 17.0 | 15.8 |
| Non protein nitrogen, | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 |
| Ether extract, % | 3.8 | 5.6 | 6.5 | 6.9 | 7.00 |
| Neutral detergent fiber, % | 21.3 | 18.2 | 15.0 | 14.5 | 12.4 |
| Calcium, % | 1.14 | 1.01 | 0.90 | 0.81 | 0.68 |
| Phosphorus, % | 0.32 | 0.34 | 0.37 | 0.39 | 0.39 |
| Magnesium, % | 0.24 | 0.23 | 0.22 | 0.22 | 0.22 |
| Potassium, % | 1.44 | 1.25 | 1.07 | 0.96 | 0.74 |
| Sulfur, % | 0.23 | 0.24 | 0.26 | 0.26 | 0.27 |

[1]Expressed on 100% DM basis

***Effects of LP Dose on Growth Performance, DM Intake and Carcass Characteristics.*** Dose-response effects for LP on growth performance, DMI and selected carcass measurements are shown in Table 2. Growth performance parameters indicated a Q relationship of LP dose for BW and ADG wherein the 150 $mg^{-1}$•$animal^{-1}$•$d^{-1}$ level resulted in the greatest ADG and heaviest final BW. On a percentage basis, carcass-adjusted ADG was improved by 4.5, 1.8 and 6.1%, for the 50, 100 and 150 $mg^{-1}$•$animal^{-1}$•$d^{-1}$ levels, respectively compared with CON. Dry matter intake was not affected by LP dose whereas F/G from study initiation to reimplant responded quadratically *($P$ = 0.08)*. Feed efficiency results for the entire trial indicated a tendency for improvements of 3.0 and 4.3% for the 50 and 150 mg LP levels, respectively, compared with the CON and the 100 mg LP/CTC level showing only a marginal improvement compared with CON and a much diminished response compared with 50 and 150 mg LP/CTC. Hot carcass weight was Q increased *($P \leq 0.05$)* with LP dose. Likewise, USDA quality grades exhibited a Q response with the 50 and 150 LP/CTC groups having approximately 78 and 70% of the carcasses, respectively, grading USDA Choice (data not shown). Carcasses in the CON and 100 mg LP/CTC had a lower level of Choice carcasses with approximately 57% of the total carcasses in this category. No dose effects of LP were observed for USDA yield grades. The total incidence of liver abscesses approached statistical significance *($P$ = 0.11)* for a Q response to LP dose with the 100 mg LP/CTC having the lowest overall incidence of liver abscesses as well a lower level of A+ abscesses. The total incidence of liver abscesses, as well as the incidence within each severity category was greater in the CON group than in the groups receiving CTC (data not shown).

Overall results indicated that the 100 mg LP/CTC had only marginal response relative to CON and 50 mg LP/CTC. Based on previous dose response data for LP (Spires et.al., 1990), the 100 mg LP/CTC would have been expected to exhibit a greater response relative to the CON and 50 mg LP/CTC than noted in the present study. An examination of the data suggested a possible reason for a diminished response being related to animal health issues specific to pens in this treatment group.

***Effects of M Dose On Growth Performance, DM Intake and Carcass Characteristics.*** Dose response effects for M on growth performance, DMI and selected carcass measurements are shown in Table 3. Trends were present with the two greater M levels indicating greater numerical ADG and improved F/G compared with the 150 mg M/T group. In general, growth performance measurements were similar between the 300 mg and 450 mg M levels. At the onset of the study, it had been expected, based on previous research data and field experiences, that DMI would have shown a significant response to M dose. Although DMI numerically decreased with increasing M level, this was not statistically significant.

Hot carcass weight and dressing percentage were not affected by M level (Table 3); however, USDA quality grades exhibited both a significant L and Q response (data not shown). In general, this response appeared to be driven by the very high percentage of carcasses (81.4%) grading USDA

**Table 2.** Effects of dietary laidlomycin propionate (LP) dose fed with chlortetracycline (CTC) on body weight, growth performance, DM intake and carcass characteristics of growing-finishing steers

| | LP dose, $mg^{-1}$•$animal^{-1}$•$d^{-1}$ | | | | SE[1] | *P*-Value[2] | |
|---|---|---|---|---|---|---|---|
| Item | 0 | 50 | 100 | 150 | | L | Q |
| Body weight,kg. | | | | | | | |
| Initial | 357 | 357 | 357 | 357 | 9.9 | 0.84 | 0.81 |
| Reimplant | 484 | 496 | 489 | 502 | 6.2 | 0.16 | 0.04 |
| Actual final[2] | 638 | 649 | 640 | 648 | 8.6 | 0.89 | 0.29 |
| Carcass-adjusted final | 639 | 652 | 641 | 660 | 8.3 | 0.38 | 0.05 |
| Daily gain, $kg^{-1}$•$animal^{-1}$•$d^{-1}$ | | | | | | | |
| Initiation to reimplant | 1.74 | 1.90 | 1.82 | 2.00 | 0.05 | 0.15 | 0.02 |
| Actual final | 1.71 | 1.78 | 1.75 | 1.85 | 0.03 | 0.12 | 0.05 |
| Carcass-adjusted final | 1.72 | 1.80 | 1.75 | 1.85 | 0.04 | 0.34 | 0.06 |
| DM intake, $kg^{-1}$•$animal^{-1}$•$d^{-1}$ | | | | | | | |
| Initiation to reimplant | 10.01 | 10.26 | 10.18 | 10.28 | 0.19 | 0.93 | 0.60 |
| Initiation to finish | 10.97 | 11.10 | 10.90 | 11.11 | 0.33 | 0.97 | 0.49 |
| DM Feed: gain, kg. | | | | | | | |
| Initiation to reimplant | 5.82 | 5.43 | 5.62 | 5.18 | 0.21 | 0.23 | 0.08 |
| Actual | 6.39 | 6.23 | 6.27 | 6.02 | 0.14 | 0.20 | 0.31 |
| Carcass-adjusted | 6.35 | 6.16 | 6.27 | 6.02 | 0.16 | 0.47 | 0.30 |
| Hot carcass wt., kg. | 407 | 415 | 409 | 420 | 5.3 | 0.38 | 0.05 |
| Dressing percentage, % | 63.81 | 64.01 | 63.71 | 63.75 | 0.27 | 0.50 | 0.61 |
| Marbling score | 434 | 450 | 435 | 447 | 8.8 | 0.77 | 0.22 |

[1]Standard error of least squares means, n = 6.

[2]Probability based on *F* statistic; L = linear effect of ionophore dose; Q = quadratic effect of

**Table 3.** Effects of dietary monensin dose fed with tylosin on body weight, growth performance, DM intake and carcass characteristics of growing-finishing steers

| | Monensin dose, mg$^{-1}$•animal$^{-1}$•d$^{-1}$ | | | | | *P*-Value[2] | |
|---|---|---|---|---|---|---|---|
| Item | 0 | 150 | 300 | 450 | SE[1] | L | Q |
| Body weight, kg. | | | | | | | |
| Initial | 357 | 357 | 358 | 357 | 9.9 | 0.68 | 0.41 |
| Reimplant | 484 | 491 | 491 | 494 | 6.2 | 0.55 | 0.73 |
| Actual final | 638 | 639 | 643 | 645 | 7.7 | 0.46 | 0.82 |
| Carcass-adjusted final | 639 | 639 | 644 | 641 | 8.2 | 0.80 | 0.55 |
| Daily gain, kg$^{-1}$•animal$^{-1}$•d$^{-1}$ | | | | | | | |
| Initiation to reimplant | 1.74 | 1.84 | 1.82 | 1.87 | 0.05 | 0.62 | 0.61 |
| Actual final | 1.71 | 1.72 | 1.75 | 7.75 | 0.03 | 0.44 | 0.85 |
| Carcass-adjusted final | 1.72 | 1.72 | 1.75 | 1.73 | 0.04 | 0.87 | 0.55 |
| DM intake, kg$^{-1}$•animal$^{-1}$•d$^{-1}$ | | | | | | | |
| Initiation to reimplant | 10.09 | 10.05 | 9.83 | 9.84 | 0.19 | 0.32 | 0.51 |
| Initiation to finish | 10.97 | 10.68 | 10.59 | 10.54 | 0.33 | 0.67 | 0.96 |
| DM Feed: gain, kg. | | | | | | | |
| Initiation to reimplant | 5.82 | 5.50 | 5.42 | 5.30 | 0.21 | 0.33 | 0.89 |
| Actual | 6.39 | 6.20 | 6.07 | 6.00 | 0.14 | 0.22 | 0.83 |
| Carcass-adjusted | 6.35 | 6.22 | 6.05 | 6.09 | 0.17 | 0.53 | 0.57 |
| Hot carcass wt., kg. | 407 | 407 | 410 | 408 | 5.2 | 0.80 | 0.56 |
| Dressing percentage, % | 63.81 | 63.65 | 63.79 | 63.35 | 0.27 | 0.43 | 0.39 |
| Marbling score | 434 | 435 | 429 | 476 | 9.0 | <0.01 | 0.02 |

[1]Standard error of least squares means, n = 6.
[2]Probability based on *F* statistic; L = linear effect of ionophore dose; Q = quadratic effect of ionophore dose.

**Table 4.** Main effects of LP/CTC and M/T on body weight, growth performance, DM intake and carcass characteristics of growing-finishing steers

| | MFA treatment [1] | | | | Probability <*F* [3] | |
|---|---|---|---|---|---|---|
| Item | CON | LP/CTC | M/T | SE[2] | Con vs. MFA | LP/CTC vs. M/T |
| Body weight, kg. | | | | | | |
| Initial | 357 | 357 | 357 | 9.9 | 0.87 | 0.06 |
| Reimplant[2] | 484 | 495 | 492 | 6.3 | 0.01 | 0.17 |
| Actual final[2] | 638 | 650 | 642 | 8.0 | 0.13 | 0.07 |
| Carcass-adjusted final[2] | 639 | 651 | 641 | 8.4 | 0.25 | 0.04 |
| Daily gain, kg$^{-1}$•animal$^{-1}$•d$^{-1}$ | | | | | | |
| Initiation to reimplant[2] | 1.74 | 1.91 | 1.84 | 0.05 | <0.01 | 0.07 |
| Actual final[2] | 1.71 | 1.79 | 1.74 | 0.03 | 0.11 | 0.03 |
| Carcass-adjusted final[2] | 1.72 | 1.80 | 1.73 | 0.04 | 0.26 | 0.03 |
| DM intake, kg$^{-1}$•animal$^{-1}$•d$^{-1}$ | | | | | | |
| Initiation to reimplant[2] | 10.09 | 10.24 | 9.90 | 0.18 | 0.92 | <0.01 |
| Initiation to finish[2] | 10.97 | 11.04 | 10.60 | 0.32 | 0.56 | 0.03 |
| DM Feed: gain, kg. | | | | | | |
| Initiation to reimplant[2] | 5.82 | 5.41 | 5.41 | 0.21 | 0.01 | 0.98 |
| Actual[2] | 6.39 | 6.17 | 6.09 | 0.14 | 0.04 | 0.38 |
| Carcass-adjusted[2] | 6.35 | 6.15 | 6.12 | 0.16 | 0.17 | 0.80 |
| Hot carcass wt., kg.[2] | 407 | 415 | 409 | 5.3 | 0.25 | 0.03 |
| Dressing percentage, %[2] | 63.81 | 63.82 | 63.60 | 0.27 | 0.74 | 0.32 |
| Marbling score[2] | 434 | 444 | 447 | 10.4 | 0.25 | 0.68 |

[1]MFA treatments: CON = non-medicated control; LP/CTC = all dose levels of laidlomycin propionate plus chlortetracycline; M/T = all dose levels of monensin plus tylosin.
[2]Standard error of least squares means where due to heterogeneous variance the largest SE value is shown, with n = 6 for CON and n = 18 for M/T and LP/CTC.
[3]Probability based on *F* statistic, with orthogonal contrast for CON vs. LP/CTC + M/T and all levels of LP plus CTC vs. all levels of M plus T.

Prime and Choice in the 450 mg M/T compared with CON and 150 mg M/T and 300 mg M/T groups. This response was also observed for marbling score for which the 450 mg M/T group had the highest degree of marbling (Table 3). Quadratic effects of M dose were observed in calculated yield grades; however, no dose effects were noted for the distribution of carcasses for USDA yield grade. Overall incidence of liver abscesses was not affected by M dose; however, as expected incidence of liver abscesses was decreased by the inclusion of T into the diet (data not shown).

***Main Effects of LP or M, Across Dose Levels, On Growth Performance, DM Intake and Carcass Characteristics.*** The overall effects of LP/CTC and M/T, across all dose levels, on growth performance and DMI are shown in Table 4. Cattle fed LP/CTC had greater ADG and DMI than steers fed M/T. The contrast between CON and MFA diets in general did not show statistical significance *(P* > 0.10*)* for many of the variables measured in the trial. This was a result to the dichotomy of the response observed between LP and M. The response to LP for BW, ADG and DMI was generally greater than the CON whereas the response to M for these variables were generally equal or less than CON. Feed efficiency from study initiation to reimplant and for the entire trial was improved for both LP and M compared with CON; however, F/G did not differ between LP and M *(P* > 0.10*)*.

Main effects of CON, LP and M dose for dressing percent and carcass measurements did not indicate many significant differences for either CON or medicated diets or between LP and M diets. One exception was a decrease in measured LM area for M/T steers compared with LP/CTC steers. In addition, HCW was increased *(P* = 0.03*)* by 6.4 kg for LP/CTC compared with M/T. Overall health of study animals was very good throughout the study. Across all treatment groups, receiving medication for treatment bovine respiratory disease (BRD) was the primary health issue with 6.4% of cattle. Of the 420 steers that began the study, 12 (2.9%) were either removed for health issues or died during the course of the study. Morbidity, mortality and removal rate was not affected by dose level of either LP or M.

## IMPLICATIONS

Dose response curves for lasalocid and monensin (Galyean and Hubbert, 1987) and LP (Spires et.al., 1990) have distinct characteristics for their effects on DMI, ADG and feed efficiency in feedlot cattle. The results of this study indicated wide latitude in potential LP and M doses that can be fed to achieve optimum response from both a live-animal performance as well as carcass yield perspective. Overall, similar or greater responses were observed for LP at a lower dose than M. Further analyses are required to better define the optimal dose for LP and M from an economic perspective relative to feed costs and market prices for cattle either on a live or carcass basis.

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# EFFECT OF CORN OIL OR CORN PROTEIN SUPPLEMENTATION ON PERFORMANCE AND RUMEN FERMENTATION CHARACTERISTICS OF FEEDLOT LAMBS CONSUMING A 90% CONCENTRATE DIET CONTAINING 30% DDGS

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**ABSTRACT:** Feeding values of corn dried distillers grains with solubles (DDGS) are best when included in concentrate diets at 20 to 30% of the diet. When DDGS are included at levels greater than 30%, high ether extract or CP content may cause a decrease of feeding value. This study evaluated effects of corn oil and corn protein supplementation on feedlot diets containing dry rolled corn (DRC) and DDGS. Twenty-one Suffolk cross wethers (average BW = 41.8 ± 1.95 kg) were randomly assigned to 1 of 4 treatments and were individually fed once daily. Experimental diets were DRC based containing 10% alfalfa (DM basis). Treatments included: 1) DDGS at 30% of the diet (30%), 2) DDGS at 60% of the diet (60%), 3) DDGS at 30% of the diet containing similar EE to the 60% diet supplied by corn oil (OIL), and 4) DDGS at 30% of the diet containing similar CP to the 60% diet with protein supplied by corn gluten meal (PROTEIN). Animals were weighed on d 1 and d 56 of the 56 d experiment. Ruminal fluid samples collected on d 48 via oral lavage 4 hours post feeding were analyzed for pH, VFA, ammonia, and bacterial populations. Final BW and DMI were not affected ($P \geq 0.13$) by treatments. Average daily gain and G:F were 69% greater ($P = 0.01$) for 30% than OIL. Ruminal pH tended to be lower for 30% than for 60% or OIL ($P \geq 0.06$). Ammonia concentrations increased 47% when 60% was fed compared with 30% ($P = 0.01$). Total VFA production was not affected ($P > 0.20$) by treatment. Bacterial populations were analyzed using PCR-denaturing gradient gel electrophoresis (DGGE) using the 16S rDNA gene. Bacterial presence or absence was analyzed using Richness index and was not affected ($P = 0.89$) by treatment. Overall, all samples were 60.21% similar in DGGE banding pattern. Animals fed OIL had the least similar DGGE banding pattern (57.73%) when compared with all other treatments. In conclusion, adding enough corn oil to match the EE of 60% DDGS decreased animal performance and resulted in shifts in bacterial populations.

**Key words:** distillers byproducts, rumen bacteria, sheep

## INTRODUCTION

Grain has been fermented to produce ethanol for many years (Klopfenstein, 2008). One of the resulting coproducts of the fermentation process is distillers grains, which are heavily used in feedlot finishing diets (Vasconcelos and Galyean, 2007). The ethanol industry has grown significantly and is fueled by government mandates, subsidies, and tax credits (U.S. Department of Energy, 2012); therefore increased amounts of distillers grains are available. Feeding DDGS at high levels of the diet is driven by increased availability and rising corn prices (USDA, 2012). Vasconcelos and Galyean (2007) also showed that corn is the major energy source fed in feedlots providing incentives to lower costs by exchanging the lower cost distillers grains for corn.

It has been shown that optimal levels of dried distillers grains (**DDGS**) range from 20% of the diet for lambs (Felix, 2011b) and 15 to 30% of the diet for cattle (DM basis; Depenbusch, 2009). However, as DDGS inclusion increases above optimal levels, animal performance decreases (Klopfenstein, 2008). Klopfenstein et al. (2008) also reviewed that during the process of ethanol production, DDGS become 3 times more concentrated in protein, fat, fiber, and P than the original corn grain. Excess fat or CP might cause the negative impact on performance of high DDGS containing diets. Therefore, the purpose of this study is to investigate the effects of high fat and high protein from the DDGS on animal performance when included in the diet at greater than optimal levels.

## MATERIALS AND METHODS

All animal handling and procedures were approved by the Institutional Animal Care and Use Committee of New Mexico State University.

***Animals and Diet.*** Twenty-one Suffolk cross wethers (average BW = 41.8 ± 1.95 kg) were used in a complete randomized design at the NMSU campus animal research facilities in Las Cruces, New Mexico. Lambs were randomly assigned to individual pens and diets. Pens offered a 9.2 $m^2$ area, overhead shade, and automatic water fountains. Experimental diets were DRC based containing 10% alfalfa (DM basis; Table 1). Treatments included: 1) DDGS at 30% of the diet (**30%**), 2) DDGS at 60% of the diet (**60%**), 3) DDGS at 30% of the diet containing similar EE to the 60% diet supplied by corn oil (**OIL**), and 4) DDGS at 30% of the diet containing similar CP to the 60% diet with protein supplied by corn gluten meal (**PROTEIN**). Over the 56 d experiment lambs were weighed twice (d 1 and d 56). Diets were a total mixed ration and fed in rubber buckets once daily. Feed refusals were collected, weighed, and recorded weekly.

**Table 1.** Diet composition (% of diet DM) fed to individually penned lambs.

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | 30% | 60% | OIL | PROTEIN |
| Ingredient | | | | |
| Dry-rolled corn | 54.2 | 27.2 | 53.8 | 49.4 |
| DDGS | 32.9 | 60.0 | 31.4 | 30.0 |
| Alfalfa hay | 9.0 | 9.0 | 9.0 | 9.0 |
| Corn gluten meal | - | - | - | 7.5 |
| Limestone | 1.0 | 0.8 | 1.0 | 1.1 |
| Ammonium chloride | 1.0 | 1.0 | 1.0 | 1.0 |
| Salt | 1.0 | 1.0 | 1.0 | 1.0 |
| Corn Oil | - | - | 1.8 | - |
| Vitamin A-D-E premix[2] | 0.5 | 0.5 | 0.5 | 0.5 |
| Bovatec[3] | 0.3 | 0.3 | 0.3 | 0.3 |
| Trace-mineral salt[4] | 0.2 | 0.2 | 0.2 | 0.2 |
| Chemical composition[5] | | | | |
| CP | 16.5 | 20.3 | 16.1 | 20.3 |
| DIP, % of CP | 9.9 | 13.3 | 9.6 | 11.2 |
| Ether extract | 5.6 | 7.3 | 7.3 | 5.2 |

[1]Treatment 30% = Diet containing 30% dried distillers grains, 60% = diet containing 60% dried distillers grains, OIL = 30% dried distillers grains with added corn oil to be isolipidic with 60%, and PROTEIN = 30% dried distillers grains with added corn gluten meal to be isonitrogenous with the 60% (DDGS corn based).

[2]Vitamin A-D-E premix: Premix containing vitamin A at 30,000 IU/g of premix, vitamin D at 176,000 IU/g of premix, and vitamin E at 275 IU/g of premix.

[3]Bovatec: Premix containing 5% Bovatec 91.

[4]Trace-mineral salt: Premix containing 42.83% white salt and cotton seed meal premix, 22.71% selenium premix (600 mg/kg), 15.82% ethylenedamine dihydroiodide (3.7%), 6.58% $ZnSO_4$, 5.54% $MnSO_4$, 5.00% mineral oil, 1.50% $CuSO_4$, and 0.03% $CoSO_4$.

[5]Calculated values.

***Sampling and Analysis.*** Approximately 40 mL of ruminal fluid was collected via oral lavage (Lodge-Ivey et al., 2009) on d 48 from each lamb and analyzed for pH using a portable pH probe (Accumet AP72, Fisher Scientific, Waltham, MA) within 5 min of collection. Samples were then divided equally into 3 separate tubes, placed on ice, and subsequently frozen at -20° C until further analysis. Two mL of 5% HCl was added to 1 of the 3 tubes of rumen fluid for each animal prior to sample collection for ammonia quantification. Ammonia concentrations were assessed using the protocol by Broderick and Kang (1980) adapted to a microtiter plate (BioTek Instruments Inc., Winooski, VT) at a wavelength of 630 nm. Ruminal VFA profiles were measured using a gas chromatograph (Agilent Technologies 7890 A, Santa Clara, CA) according to the method of Goestch and Gaylean (1983).

Genomic DNA was extracted from ruminal fluid samples using the repeated beat beating plus column extraction method of Yu and Morrison (2004b) and analyzed for overall DNA quality on a 1% agarose gel. Each PCR reaction was performed in 25 µL and amplified on DNA Engine PTC-200 (MJ Research, Watertown MA) for each PCR-DGGE analysis. The PCR mixture contained 1.25 U Platinum taq (Invitrogen, Carlsbad CA) 60 mM Tris-SO4 (pH 8.9), 18 mM ammonium sulfate,1.75 mM $MgCl_2$, 250 µM of each deoxynucleoside triphosphate, 0.0674% BSA, 0.5 µM each primer and approximately 100 ng of template DNA. The V3 reigion of the rrs gene was amplified using primers 357f (5'-CCTACGGGAGGCAGCAG-3') and 519r (5'-ATTAC CGCGGCKGCTGG-3'). The 357f primer has a 40-bp GC clamp attached to the 5' end (CGCCCGCCGCGCGC GGCGGGCGGGGCGGGGGCACGGGGGG) to prevent dissociation of the DNA strands (Yu and Morrison, 2004a). To reduce the production of spurious PCR products, touchdown PCR was performed. The PCR cycle consisted of an initial denaturing at 94ºC for 4 min, 10 cycles of touchdown PCR wherein the starting annealing temperature of 61ºC was decreased 0.5ºC per cycle for 10 cycles to 56ºC. This was followed by 25 cycles with denaturing step at 94ºC for 30 s, annealing at 56ºC for 30 s, and a final primer extension at 72ºC for 30 s. Quality of the PCR products was confirmed visually using a 1.5% agarose gel stained with ethidium bromide. Using the Bio-Rad D-Code system (BioRad, Hercules, CA), DGGE was performed as described by Simpson et al. (1999). To separate PCR fragments, 30 µL of PCR product was resolved on 7.5 % polyacrylamide gel (37.5:1) containing 30 to 60% gradient denaturants [100% denaturants consisting of 40% (vol/vol) formamide and 7 M urea]. Electrophoresis was performed at 60°C and 74 V for 16 hrs. Additionally, 2 standard samples were included in each gel to allow for normalization of band migration and gel curvature among different gels (McCracken et al., 2001). After electrophoresis, gels were

**Table 2.** Performance characteristics for lambs fed dry rolled corn based feedlot diets with dried distillers grains (DDGS).

| | Treatments[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | 30% | 60% | OIL | PROTEIN | All | SEM | *P*-value |
| Animal Performance | | | | | | | |
| Initial BW, kg | 41.79 | 41.09 | 41.53 | 43.00 | - | 1.954 | 0.91 |
| Final BW, kg | 54.12 | 52.13 | 50.04 | 54.82 | - | 2.096 | 0.40 |
| DMI, g | 1351.56 | 1354.78 | 1234.64 | 1317.48 | - | 52.374 | 0.36 |
| ADG, g | 220.30[a] | 197.03[ab] | 152.04[b] | 210.99[a] | - | 15.716 | 0.03 |
| G:F[2] | 0.162[a] | 0.146[a] | 0.121[b] | 0.159[a] | - | 0.0083 | 0.01 |
| Rumen Fermentation | | | | | | | |
| pH | 5.31 | 5.53 | 5.51 | 5.40 | - | 0.082 | 0.18 |
| Ammonia N, mM | 9.42[a] | 19.98[b] | 9.55[a] | 13.92[a] | - | 1.946 | 0.01 |
| Total VFA, mmol | 74.16 | 69.71 | 75.42 | 72.37 | - | 6.209 | 0.92 |
| Acetate, mol/100mol | 50.31 | 52.16 | 51.20 | 51.72 | - | 1.571 | 0.84 |
| Propionate, mol/100mol | 42.79 | 36.00 | 37.77 | 38.74 | - | 2.504 | 0.26 |
| Butyrate, mol/100mol | 4.06 | 6.98 | 7.51 | 5.75 | - | 1.355 | 0.27 |
| A:P[2] | 1.19 | 1.48 | 1.39 | 1.40 | - | 0.134 | 0.44 |
| Rumen Microbiology | | | | | | | |
| Richness[3] | 16.50 | 16.20 | 15.00 | 16.80 | - | 1.740 | 0.89 |
| Similarity, %[4] | 73.85 | 81.63 | 57.73 | 71.66 | 60.21 | - | - |

[1]Treatments = 30% = Diet containing 30% dried distillers grains, 60% = diet containing 60% dried distillers grains, OIL = 30% dried distillers grains with added corn oil to be isolipidic with 60%, and PROTEIN = 30% dried distillers grains with added corn gluten meal to be isonitrogenous with the 60% (DDGS corn based).

[2]G:F = Gain:Feed.

[3]Richness = Indicates the average number of bands detected per treatment in each lanes using the Dice analysis of the denaturing gradient gel electrophoresis (DGGE) profile.

[4]Similarity = Calculated percentage of similarity of each lane on DGGE gel by using clustering algorithms and unweighted pair group method with average linkages (UPGMA). Similarity percent under column All represents similarity percentage of all treatments.

[a,b]Means within a row with unlike superscripts differ ($P < 0.05$).

stained with GelStar (Cambrex, Rockland, ME) according to manufacturer's specifications. All electrophoresis gels were imaged on a Molecular Imager (Chemi Doc XRS+, BioRad, Hercules, CA).

***Statistical Analysis.*** The GLM procedure (SAS Inst. Inc., Cary, NC) was used to analyze performance and fermentation characteristics. Animal was used as the experimental unit, treatments represented fixed effects and random error was accounted for in the error term. Means were calculated using LSMEANS. Treatment effect was considered significant when the probability of a greater $F$ was $\leq 0.05$ and a tendency when $F$ was $\leq 0.10$. When $F$-tests were significant, mean separations were performed using PDIFF.

## RESULTS AND DISCUSSION

Due to government mandates and ethanol subsidies, availability of distillers grains are increasing. Rising corn prices, and the costs associated with corn processing provide incentives for feedlots to use distillers grains at greater levels of the diet. Increasing DDGS above 30% of the diet (DM basis) has been shown to negatively impact performance. The cause for the decrease in performance is still largely not understood.

The effects of treatment on performance, rumen fermentation, and rumen microbial ecology are shown in Table 2. Initial and final BW were not different ($P > 0.40$) among diets. No difference ($P = 0.36$) was detected for DMI between diets. These data are consistent with Felix et al. (2011b), who reported that lambs showed no difference ($P > 0.13$) in DMI with 0, 20, 40, and 60% inclusion of DDGS (DM basis). However, Schauer et al. (2008) showed that DMI increased linearly ($P < 0.001$) with rising inclusion levels in lamb diets. Conflicting studies are found in other species as well. Several cattle studies (Larson et al. 1993 and Depenbusch et al. 2009) indicate a decrease ($P$-values $\leq 0.04$) in DMI with levels of distillers grains greater than 40% while others report conserved DMI at 40% or greater of the diet (Firkins et al., 1985; Ham et al., 1994; and Lodge et al., 1997). Spiehs et al. (2002) suggest that the inconsistency among studies may be explained by the high variability of the coproduct itself. In the same study by Spiehs et al. (2002), they showed that DDGS produced in plants in Minnesota and South Dakota ranged from 28.7 to 31.4% CP and 10.2 to 11.7% fat (DM basis). The NRC (2000) reports DDGS values between 29.5 to 30.4% CP and 9.2 to 10.7% fat (DM basis) based on processing and drying methods. The amount

of sorghum in DDGS may also play a role in decreasing DMI. A difference in DMI was seen when 30% inclusion levels of 100% wet corn distillers grains and 100% wet sorghum distillers grains (**WCDGS and WSDGS** respectively**;** Al-Suwaiegh et al. 2002) were compared in feedlot diets.

There was a decrease ($P$ = 0.01) in ADG for the OIL compared with the other treatments. A decrease ($P$ = 0.01) in G:F for the OIL was also observed. This suggests that at high levels of DDGS, the crude fat may be responsible for the decrease in performance. Differences in 60% and OIL may lie in the form of crude fat in each diet. Although both diets were isolipidic the OIL was supplemented with corn oil. Vanderpol et al. (2008) showed that supplemental corn oil may impede total tract starch digestion relative to fat supplied by WDGS, and may have accounted for the decrease in ADG seen. Corn oil addition to the diet may have also coated feed matter and rendered it unavailable for microbial fermentation (MacLeod and Buchanan-Smith, 1972).

Acidosis is common in feedlots and is caused by high amounts of rapidly fermenting starch in grain. By reducing the amount of corn in the diet with the addition of distillers grains, the amount of starch is also reduced while increasing the fiber content (Klopfenstein, 2008). In our study, ruminal pH was not affected ($P$ = 0.18) by treatments, supporting the pH stabilizing effect of distillers grains. In contrast, pH was greater in cattle when 60% DDGS were included in the diet than 0% inclusion (Felix, 2011a). Ruminal ammonia N concentration was greater ($P$ = 0.01) for 60% than the other treatments. Ruminal ammonia N concentration ranged from 9.42 to 19.98 m$M$. Ruminal ammonia N concentration was not limiting for rumen microbial growth because all treatments presented values above minimum requirements for optimal rumen fermentation (Satter and Slyter, 1974). Although 60% and PROTEIN were isonitrogenous, ammonia concentrations differed ($P < 0.05$) and may be accounted for in the solubility of the different protein sources. Treatments had no effect ($P \geq 0.26$) on ruminal VFA production or molar proportions.

Previous studies involving microbial ecology and distillers grains show a shift in population dynamics. It was shown that Richness and Shannon-Wiener diversity indices increased linearly with inclusion of WDGS (Tracey et al., 2010). Fron et al. (1996) showed that more fibrolytic and lactolytic species were present in diets with inclusion of distillers byproducts. Rumen microbial ecologies were compared for each treatment in our study. Dice analysis calculated from the number of bands detected per treatment in each lane of the DGGE showed no difference ($P$ = 0.89) in richness between treatments. Using clustering algorithms and unweighted pair group method with average linkages (UPGMA) to compare similarity, it was seen that the OIL had the least similar rumen environment, indicating less microbial stability and possibly a negative impact on microbial fermentation.

In summary, the decrease in performance observed when DDGS are fed above the optimal level seems to be caused by the excess fat of DDGS. However, more research is required to explain why added corn oil to the diets depresses performance more drastically than that contained in DDGS.

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# EFFECTS OF DRIED DISTILLERS GRAINS FED FOR PROGRAMMED RATE OF BODY WEIGHT GAIN IN BEEF HEIFERS GRAZING NATIVE RANGELANDS PRIOR TO BREEDING ON GROWTH AND REPRODUCTIVE PERFORMANCE

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**ABSTRACT:** The objective of this study was to evaluate effects of dried distillers grains plus solubles (DDGS) on growth and reproductive performance of beef heifers fed at a programmed rate of BW gain. Eighty-two beef heifers (initial BW = 249 ± 3.2 kg) grazing native range (2 pastures per treatment; 4% CP, 65% NDF, DM basis) were used in a completely randomized experiment and assigned to 1 of 3 treatments 49 d prior to breeding. Treatments were: 1) high level of DDGS (1.81 kg/heifer daily) to provide gain at (0.36 kg/d) for 28 d and then fed (0.68 kg/heifer daily) to achieve a low rate of gain (0.18 kg/d) up to breeding (HL); 2) a low rate of gain for the first 28 d and the high rate of gain up to breeding (LH); 3) moderate rate of gain (0.27 kg/d) throughout the feeding period by feeding (1.13 kg/heifer daily; MOD). Heifers were weighed every 14 d, at which time blood was collected via coccygeal venipuncture and serum progesterone concentrations were determined. Heifers were synchronized at the end of the feeding period using the CO-Synch + CIDR (progesterone insert) with fixed-time AI. Heifers received an injection of GnRH (100 µg, i.m.) and a CIDR was inserted at d -7 relative to timed AI. At d 0, CIDR was removed and heifers received 25 mg of $PGF_{2\alpha}$. Heifers were observed for estrus and bred 12 h after observation of estrus. All heifers not bred by 54 h after CIDR removal was given GnRH (100 µg, i.m.) and AI. Heifers were joined with bulls for 60 d. No difference ($P = 0.11$) in ADG was noted in the first 28 d of supplementation, however, ADG was greater ($P = 0.01$) for HL and MOD groups during the second period of the experiment. Days to reach puberty was less ($P = 0.08$) for the LH treatment compared with the MOD and HL treatment. The LH treatment also had a greater ($P = 0.06$) percentage of pubertal heifers at breeding compared with MOD. Treatment did not affect ($P = 0.35$) overall pregnancy rates. Despite the fact that HL and MOD treatments had greater ADG, the LH growth program increased the percentage of heifers that were pubertal at breeding.

**Key words:** heifers, puberty, reproduction

## INTRODUCTION

Heifer development is a critical component of a sustainable and profitable cow-calf operation (Perry et al., 2009) and heifers need to calve by 24 mo of age to maximize productivity over their lifetime (Patterson et al., 1992). Research has shown that replacement heifers need to achieve 60 to 65% of mature BW by breeding to increase pregnancy rates (Patterson et al., 1992) and conventional heifer development protocols have primarily focused on supplementing heifers to reach this weight. More recent research has shown that pre-breeding BW can be as light as 51 to 57% of mature BW with limited effects on reproductive performance and decreased cost of development (Funston et al., 2012).

However, plane of nutrition in heifers and cows can greatly influence reproductive performance. Lalman et al. (1993) showed that growth patterns can be modified and rate of BW gain can be altered to result in compensatory growth periods and reduce the amount of supplementation necessary to develop heifers. Freetly et al. (2001) found that delaying BW gain until later in the post-weaning period resulted in reduced total energy intake with no effects on calving rate, age at calving, and postpartum interval. In contrast, Byerley et al. (1987) reported that conception rates of pubertal estrus were less than that of the third estrus. Therefore, feeding schemes that allow heifers to reach puberty prior to the breeding season will improve AI conception rates. The focus of this research was to determine if increased BW earlier in the pre-breeding period would result in increased reproductive performance.

Thus, we hypothesized that raising the plane of nutrition earlier in the pre-breeding supplementation program would result in an increased percentage of heifers reaching puberty by breeding. The objective of this study was to determine whether the timing of supplementation in relation to breeding influenced growth and pregnancy percentage in heifers grazing native rangelands.

## MATERIAL AND METHODS

All experimental procedures were reviewed and approved by the New Mexico State University Institutional Animal Care and Use Committee.

***Animals, Diets and Treatments.*** Eighty Angus, Angus-crossed heifers (initial BW = 249 ± 3.2 kg) were used in a completely randomized experiment and assigned to 1 of 3 treatments 49 d prior to breeding. Heifers were randomly assigned to 1 of 6 dormant native range pastures (2 pastures per treatment; 4% CP, 65% NDF, DM basis) predominated by blue grama (Bouteloua gracilis) and wolftail (Lycurus phleoides; Forbes and Allred, 2001) and were supplemented

in bunks, 3 times per week. Treatments were: 1) dried distillers grains plus solubles (**DDGS**) fed at 1.81 kg•heifer$^{-1}$•d$^{-1}$ to provide gain at ADG of (0.36 kg/d) for 28 d and then fed DDGS fed at 0.68 kg•heifer$^{-1}$•d$^{-1}$ to achieve a decreased rate of gain (0.18 kg/d) up to breeding (**HL**); 2) a low rate of gain (0.18 kg/d) for the first 28 d and the high rate of gain (0.36 kg/d) up to breeding (**LH**); 3) moderate rate of gain (0.27 kg/d) for all 49 d achieved by feeding 1.13 kg•heifer$^{-1}$•d$^{-1}$ (**MOD**). Heifers were weighed every 14 d until breeding and given free choice access to a mineral supplement (Corona Range & Livestock Research Center mineral, Hi-Pro Feeds, Friona, TX; NaCl >38.7%, P >8%, Mg >2%, K >2%, Zn >2,000 ppm, Mn >2,500 ppm, Cu >2000 ppm, Se >13ppm, Vitamin A >54,420 units/kg). Chemical composition of grass and DDGS is presented in Table 1.

***Breeding.*** Heifers were synchronized at the end of the feeding period using the CO-Synch + CIDR (progesterone insert) with fixed-time AI. Heifers received an injection of GnRH (100 µg, i.m.) and a CIDR was inserted at d -7 relative to timed AI. At d 0, CIDR was removed and heifers received 25 mg of $PGF_{2\alpha}$. Heifers were observed for estrus and bred 12 h after observation of estrus. All heifers not bred by 54 h after CIDR removal were given GnRH (100 µg, i.m.) and AI. Heifers were joined with bulls for 60 d. Pregnancy and first service conception rates were confirmed with calving dates. First service conception rates were calculated as a percentage of heifers that were confirmed bred.

***Blood Collection and Assay.*** Blood samples were collected via coccygeal venipuncture and analyzed for serum progesterone concentrations. Blood was collected in 7-mL sterile serum separator tubes (CORVAC, Tyco Healthcare Group LP, Mansfield, MA) and kept on ice until centrifugation at 1,500 x *g* for 30 min at 4°C. Serum was stored at -20°C until assays could be conducted. Serum progesterone concentrations were quantified by RIA in duplicate using components of a solid phase kit (Coat-A-Count); Siemens Medical Solutions Diagnostics, Los Angeles, CA as reported by Schneider and Hallford (1996). Intra and inter assay CV was 13.5% and 8.6%, respectively. Age at puberty was determined by 2 consecutive serum progesterone concentrations above 1 ng/mL (Byerley et al., 1987).

***Laboratory Analysis.*** Forage samples were dried for 72 h at 55°C in a forced-air oven (Blue M Electric Company, Blue Island, IL) and ground to pass through a 2-mm screen (Wiley Model 4, Thomas Scientific, Swedesboro, NJ). Ground samples were then analyzed for DM and ash (AOAC, 1990), N (Leco FP-528, Leco Corp., St. Joseph, MI), and NDF using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY). Nutrient analysis of DDGS was determined by a commercial laboratory (SDK Laboratories., Hutchinson, KS).

**Table 1.** Chemical composition of range forage and DDGS fed to beef heifers

| Item | Range Forage | DDGS |
|---|---|---|
| DM | 96.8 | 90.2 |
| OM, % of DM | 88.6 | 82.9 |
| CP, % of OM | 4.99 | 31.0 |
| NDF, % of OM | 71.2 | 37.4 |

***Calculations and Statistical Analysis.*** All data were analyzed using PROC GLM (SAS Inst. Inc., Cary, NC). Pasture was the experimental unit and the model included effects of treatment. When a significant ($P < 0.05$) treatment effect was detected, LS means were separated by the PDiff option of SAS. Reproductive traits were analyzed using the CHI-SQUARE model of SAS and significant differences ($P < 0.05$) were separated using the GENMOD option of SAS.

## RESULTS AND DISCUSSION

Forage and water quality was similar among all pastures. There was no difference ($P = 0.19$) in initial BW or BW after the first 28 d of the experiment (Table 2). Heifers that received the HL and MOD treatment had a greater ($P = 0.03$) final BW when compared with heifers that received the LH treatment. No difference ($P = 0.11$) in ADG was noted in the first 28 d of supplementation, however, ADG was greater ($P = 0.01$) for HL and MOD than LH during the last 21 d of the experiment. It is not fully understood why LH performed below that of the other treatments despite receiving more DDGS. It is possible that the HL group benefitted from a 14-d adaptation period prior to the start of the trial. Specifically, a 1-wk adaptation was provided to allow heifers to acclimate to the DDGS. However, it took an additional week of adaptation for the HL heifers to completely consume their supplement. At the mid-point of the study, intake for the LH group switched to 1.81 kg•heifer$^{-1}$•d$^{-1}$ with no transition period because these heifers readily consumed this greater level of DDGS. It is possible that BW gain suffered during this period due to subclinical S toxicity in the rumen causing reduced performance (Niles et al., 2002). No heifer exhibited outward signs of S toxicity. Nevertheless, cattle can adapt to greater S levels over time (Drewnoski and Hansen, 2011). Overall dietary S (water, forage, and DDGS) was calculated to be 0.36% which is below the maximum tolerable concentration of 0.4% (NRC, 2000).

Days to reach puberty, calculated in Julian days, in the present experiment was less ($P = 0.07$) for the LH treatment compared with the MOD and HL treatment. This differs from research performed by Lynch et al. (1997) in which delaying gain until 56 d prior to breeding resulted in heifers reaching puberty 20 d later than heifers fed for the entire development period. Roberts et al. (2009) also found that heifers developed on a restricted diet (80% of ad libitum) reached puberty at an older age than the control treatment (100% ad libitum). However, the restricted treatment resulted in heifers reaching puberty at a reduced BW, which also occurred in the current experiment. Despite reduced ADG, there was a greater percentage of heifers pubertal ($P = 0.06$) at the start of the breeding season for LH compared with MOD, which seems

**Table 2.** Effect of programmed rate of body weight gain of beef heifers grazing native rangelands and supplemented dried distillers grains plus solubles

| Item[1] | HL | LH | MOD | SE | *P*-value |
|---|---|---|---|---|---|
| BW, kg | | | | | |
| d 0 | 226 | 222 | 229 | 3.1 | 0.43 |
| d 28 | 239 | 231 | 239 | 2.6 | 0.19 |
| d 49 | 246[a] | 234[b] | 248[a] | 1.9 | 0.03 |
| ADG, kg/d | | | | | |
| d 0 – 28 | 0.44[a] | 0.30[b] | 0.37[a] | 0.05 | 0.11 |
| d 28 – 49 | 0.42[a] | 0.19[b] | 0.40[a] | 0.06 | <0.01 |
| d 0 – 49 | 0.43[a] | 0.24[b] | 0.38[a] | 0.03 | <0.002 |
| Days to reach puberty[2] | 382[a] | 369[b] | 387[a] | 7.08 | 0.07 |
| Pubertal at breeding, % | 80.0[a] | 95.2[a] | 66.7[b] | 5.71 | 0.06 |
| Overall pregnancy rate, % | 63.2 | 62.5 | 82.6 | 2.61 | 0.27 |
| First service conception rate[3], % | 36.4 | 64.3 | 66.7 | 2.89 | 0.23 |

[1]Treatments: Heifers were fed the following – HL: 1.81 kg/d, 0.68 kg/d; LH: 0.68 kg/d, 1.81 kg/d; MOD: 1.13 kg/d.
[2]Days to reach puberty: calculated in Julian Days.
[3]First service conception rate: calculated as a percentage of all heifers confirmed pregnant.
[a,b]Means with different superscripts within a row differ ($P < 0.10$).

to contradict what one would expect due to the lower ADG observed for this treatment. This response is likely due to the fact that heifer ADG was 0.01 kg/d during the first 14 d of the second period compared with 0.39 kg/d for the remaining days up to breeding giving an overall lower rate of gain when averaged across this period. This improvement in the number of pubertal heifers for LH during the latter half of the period may have been the result of rumen adaptation to greater dietary S and subsequently an improvement in nutrients reaching the greater brain centers that control reproduction. Heifers in the HL, LH, and MOD treatments reached 54, 52, and 55% of mature BW, respectively. This agrees with Martin et al. (2008) who reported that heifers can be successfully developed to as little as 51% of mature BW. Treatment did not affect ($P \geq 0.23$) overall pregnancy or first service conception rates. This is similar to findings of Lynch et al. (1997) who fed heifers at varying rates of gain during the development period and found that overall pregnancy rate did not differ.

## IMPLICATIONS

Despite the fact that HL and MOD treatments had greater ADG, the LH feeding program increased the percentage of heifers that were pubertal at breeding. Nevertheless, under the constraints of the current experiment, timing and rate of gain did not improve overall pregnancy rate. Due to the limited number of animals in this experiment more work is needed to evaluate effects of delayed supplementation in beef heifers. Further, work is also needed to evaluate the adaptation of heifers to high levels of dried distillers grains plus solubles when grazing native rangeland.

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# EFFECTS OF MEDICINAL FEED ADDITIVE (MFA) PROGRAM FED WITH VARYING LEVELS OF WET DISTILLERS GRAINS (WDGS) ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND HEALTH OF GROWING/FINISHING BEEF STEERS

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**ABSTRACT:** Levels of wet corn distillers grains (WDGS) and medicinal feed additive (MFA) program were evaluated in an experiment with 320 (average initial BW 320 kg) growing-finishing beef steers. Steers were randomized by initial BW in a 2 x 4 factorial arrangement (4 blocks / treatment) with main effects of MFA program consisting of laidlomycin propionate (LP; 11.1 $g^{-1}$• ton DM) plus chlortetracycline (350 $mg^{-1}$•$animal^{-1}$•$d^{-1}$; CTC) or monensin (M; 28 $g^{-1}$• ton DM) plus tylosin (T; 90 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) and 0, 15, 30 and 45% dietary (DM basis) inclusion level of WDGS. A single source of WDGS was used throughout the study with cattle fed once daily. Cattle were on feed for 179 d prior to being shipped to a commercial plant for slaughter where carcass and liver abscess data were collected. Interactions between MFA and WDGS level ($P \geq 0.10$) were not observed for any growth, health or carcass parameter. Growth rate (ADG) between MFA programs did not differ ($P \geq 0.05$); however, DMI tended to be greater ($P \leq 0.10$) for LP/CTC and DM feed:gain (F/G) ratio improved ($P \leq 0.05$) by M/T. Carcass measurements and liver abscess incidence were not affected ($P \geq 0.05$) by MFA program. Linear (L; $P \leq 0.01$) and cubic effects (C; $P \leq 0.06$) of dietary WDGS were present for BW, ADG and HCW with 15% WDGS having greater response than the 0% diet and 30 and 45% WDGS. Dry matter F/G and DMI were not affected by WDGS level. Marbling score was linearly decreased with increased WDGS level. Overall health was not affected by MFA program; however, apparent morbidity from respiratory disease, while low, was increased ($P \leq 0.05$) with greater WDGS levels. Overall morbidity at the 30 and 45% WDGS levels was increased ($P \leq 0.01$) compared with 0 and 15% inclusion levels, with no differences among WDGS levels for mortality. Alternative MFA programs may have application in feedlot diets containing varying levels of WDGS.

**Key words:** distillers by-products, feedlot cattle, ionophore, laidlomycin propionate, monensin

## INTRODUCTION

Federal mandates and subsidies for the use of renewable fuel sources have increased ethanol production from corn and other grains. The resulting increase in the demand and price for corn has adversely impacted feeding costs for beef producers, while increasing the availability of co-products for cattle feeding. Few research studies have examined the relative response of medicinal feed additive (**MFA**) program and dietary level of co-product that may be fed in commercial practice. The objectives of this study were to evaluate the relative response when monensin (**M**) with tylosin (**T**) or laidlomycin propionate (**LP**) with chlortetracycline (**CTC**) were fed in diets providing 0, 15, 30 or 45% of the DM as wet distillers grains (**WDGS**) on health, growth performance and carcass traits in yearling steers fed in confinement.

## MATERIALS AND METHODS

Yearling steers were purchased from sale barn facilities in KS and OK and shipped to the research site in Wellington, CO. Cattle were received and acclimated at the research site until processing. Cattle were processed by receiving group which included vaccination and treatment for parasites. Steers were administered Synovex Choice (Pfizer Animal Health, Madison, NJ) as the initial implant and Synovex Plus (Pfizer Animal Health) as the terminal implant. Individual animal BW, at study initiation, was collected on two consecutive d with each animal being individually identified. Three hundred twenty steers (average initial BW 320 kg) were randomized in a 2 x 4 factorial arrangement. Main effects of MFA program were laidlomycin propionate (LP; 11.1 $g^{-1}$• ton DM) plus CTC (350 $mg^{-1}$•$animal^{-1}$•$d^{-1}$) or M (28 $g^{-1}$• ton DM) plus T (90 $mg^{-1}$•$animal^{-1}$•$d^{-1}$). Main effects of WDGS were dietary inclusion levels of 0, 15, 30 and 45% (DM basis). Steers were randomly assigned to one of 32 feeding pens based on BW and source. Pens were blocked on the basis of location along the feeding alley into groups of 8 adjacent pens (4 blocks / treatment) with 10 animals per pen. During the receiving period, a separate supplement providing CTC at 22 $mg^{-1}$• kg BW was fed for 5 d (Monday – Friday) to steers being fed LP to treat respiratory disease. Monensin concentration was progressively increased during the initial weeks on study from 7 g•$ton^{-1}$ DM to 28 g•$ton^{-1}$ DM in the finishing diet. Tylosin was fed throughout the study to provide 9 g•$ton^{-1}$ DM or approximately 90 $mg^{-1}$•$animal^{-1}$•$d^{-1}$. The WDGS was supplied from a single source throughout the study. Pens were fed according to MFA treatment in the

order of increasing WDGS level. Pens were fed once daily to allow a maximal level of DMI without an accumulation of unconsumed feed in the bunk.

Steers were observed daily for sickness or other health-related conditions by experienced pen riders. Animals dying during the course of the study were necropsied by a veterinarian and cause of death documented. Final individual BW was obtained by pen within each block after which cattle were loaded on trucks and shipped to a commercial packing plant. Carcass parameters measured at the packing plant were hot carcass weights **(HCW)** and the incidence and severity of liver abscesses. Carcasses were graded by USDA graders. At this time LM area, 12th–rib fat thickness, percentage of kidney, heart and pelvic fat, and marbling score were also determined by an independent carcass data collection service. Cattle were on feed for a total of 179 d.

Design structure was a randomized block with a 2 x 4 factorial arrangement of treatments with main effects of MFA program and WDGS level. Pen was the experimental unit for all responses evaluated. The statistical model included effects for block, MFA program, WDGS level and the MFA x WDGS level interaction. Pen-based performance parameters that included BW and HCW, daily BW gains, DMI and DM feed:gain ratios **(F/G)** were evaluated using a mixed model (Proc Mixed) analysis (SAS Inst. Inc., Cary, NC). Linear (**L**), quadratic (**Q**) and cubic (**C**) effects of WDGS level were determined using orthogonal polynomial contrast coefficients (Steel and Torrie, 1960). Categorical (or count per pen data) were analyzed using the GLIMMIX procedure of SAS with the same model as for performance and carcass data.

## RESULTS

Ingredient and calculated nutrient composition of the finishing diets are shown in Table 1.

***Main Effect; MFA Program.*** There were no interactions ($P \geq 0.10$) between MFA program and WDGS level, therefore main effect means for ADG, DMI, F/G, HCW and other carcass parameters are shown in Tables 2 and 3, respectively. No differences between M/T and LP/CTC were observed for BW or ADG; however, DMI was greater ($P \leq 0.10$) in steers receiving LP/CTC. Because DMI was increased with no concomitant increase in growth, F/G was significantly reduced in the LP/CTC group ($P < 0.10$). Carcass parameters, including HCW were not affected by MFA program. Mean 12th -rib fat thickness was approximately 1.3 cm, suggested that cattle were not fed to an excess level of finish. Marbling scores, LM area and calculated yield grades did not differ between MFA programs and were consistent with size and type of cattle used in the study. The number of carcasses greater than 432 kg and subject to a discount was similar between the M/T and LP/CTC treatments (data not shown). The overall incidence and severity of liver abscesses observed during this study were minimal, with approximately 95% of the total livers evaluated having no signs of abscesses or adhesions. Morbidity and mortality of cattle was not affected by MFA program.

**Table 1.** Ingredient and nutrient composition of finishing diets[1]

| | Finishing Diets, as % WDGS in diet (DM basis) | | | |
|---|---|---|---|---|
| Item | 0 | 15 | 30 | 45 |
| Days fed | 156 | 156 | 156 | 156 |
| Ingredient | | | | |
| Steam flaked corn | 77.3 | 69.2 | 55.2 | 41.4 |
| Alfalfa hay | 5.9 | 5.9 | 5.9 | 5.9 |
| WDGS[2] | 0.0 | 15.0 | 30.0 | 45.0 |
| Supplement | 5.2 | 5.2 | 5.2 | 5.2 |
| Tallow | 3.9 | 2.6 | 1.6 | 0.4 |
| Medicated supplement | 2.0 | 2.0 | 2.0 | 2.0 |
| Corn silage | 2.1 | 2.1 | 2.0 | 2.1 |
| Soybean meal | 5.7 | 0.0 | 0.0 | 0.0 |
| Calculated nutrient composition | | | | |
| Dry matter, % | 76.0 | 64.0 | 55.7 | 49.2 |
| NE maintenance, Mcal/lb | 1.01 | 0.99 | 0.97 | 0.95 |
| NE gain, Mcal/lb | 0.67 | 0.66 | 0.65 | 0.63 |
| Crude protein, % | 13.99 | 14.70 | 17.91 | 21.11 |
| Non-protein nitrogen, % | 2.82 | 2.81 | 2.83 | 2.82 |
| Ether extract, % | 6.97 | 6.95 | 7.02 | 6.92 |
| Neutral detergent fiber, % | 4.17 | 8.12 | 12.48 | 16.90 |
| Calcium, % | 0.74 | 0.73 | 0.73 | 0.73 |
| Phosphorus, % | 0.29 | 0.34 | 0.42 | 0.49 |
| Magnesium, % | 0.19 | 0.20 | 0.22 | 0.24 |
| Potassium, % | 0.77 | 0.75 | 0.84 | 0.93 |
| Sulfur, % | 0.16 | 0.22 | 0.30 | 0.39 |

[1]Expressed on a DM basis

[2]WDGS = Wet distillers grains

**Table 2.** Effects of feeding laidlomycin propionate (LP) plus chlortetracycline (CTC) or monensin (M) plus tylosin (T) at four inclusion levels of wet distillers grains (WDGS) on growth performance, DM intake and carcass characteristics of growing – finishing beef steers

| | MFA program[1] | | | Probability[3] |
|---|---|---|---|---|
| Item | M/T | LP/CTC | SE[2] | Prob. > *F* |
| No. pens, n | 16 | 16 | | |
| Body weight, kg | | | | |
| Initial | 321 | 321 | 9.6 | 0.36 |
| Actual final | 654 | 652 | 12.8 | 0.75 |
| Carcass-adjusted final | 653 | 653 | 15.8 | 0.99 |
| Daily gain, kg•d$^{-1}$ | | | | |
| Actual final | 1.86 | 1.85 | 0.03 | 0.70 |
| Carcass-adjusted final | 1.86 | 1.86 | 0.04 | 0.94 |
| DM intake, kg•d$^{-1}$ | | | | |
| Initiation to finish | 9.65 | 10.17 | 0.27 | 0.10 |
| DM Feed: gain, kg. | | | | |
| Actual | 5.18 | 5.51 | 0.19 | 0.04 |
| Carcass-adjusted | 5.20 | 5.50 | 0.23 | 0.09 |
| Hot carcass wt., kg | 411 | 411 | 9.9 | 1.00 |
| Dressing percentage, % | 62.79 | 62.95 | 0.34 | 0.54 |
| Marbling score | 416 | 411 | 6.7 | 0.60 |
| Calculated yield grade | 3.32 | 3.31 | 0.05 | 0.80 |
| Incidence liver abscesses, % | 5.2 | 3.2 | 0.01 | 0.26 |

[1]MFA program = medicinal feed additive program.

[2]Standard error of least squares means, n = 16.

[3]Probability based on *F* statistic for main effect of MFA program.

**Table 3.** Effects of wet distiller's grain (WDGS) level on growth performance and DM intake and carcass characteristics of growing – finishing beef steers

| | Level of WDGS, % DM | | | | | Probability[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 15 | 30 | 45 | SE[1] | Overall | L | Q | C |
| No. pens, n | 8 | 8 | 8 | 8 | | | | | |
| Body weight, kg. | | | | | | | | | |
| Initial | 320 | 320 | 320 | 320 | 9.6 | 0.94 | 0.80 | 0.76 | 0.64 |
| Actual final | 659 | 667 | 644 | 641 | 13.4 | 0.01 | <0.01 | 0.36 | 0.06 |
| Carcass-adjusted final | 661 | 670 | 644 | 637 | 16.3 | <0.01 | <0.01 | 0.17 | 0.05 |
| Daily gain, kg•d$^{-1}$ | | | | | | | | | |
| Actual final | 1.89 | 1.94 | 1.81 | 1.79 | 0.04 | <0.01 | <0.01 | 0.33 | 0.06 |
| Carcass-adjusted final | 1.90 | 1.95 | 1.81 | 1.77 | 0.05 | <0.01 | <0.01 | 0.16 | 0.05 |
| DM intake, kg•d$^{-1}$ | | | | | | | | | |
| Initiation to finish | 10.02 | 10.02 | 9.96 | 9.65 | 0.35 | 0.80 | 0.40 | 0.62 | 0.90 |
| DM Feed; gain, kg. | | | | | | | | | |
| Actual | 5.30 | 5.19 | 5.50 | 5.39 | 0.22 | 0.52 | 0.40 | 0.98 | 0.22 |
| Carcass-adjusted | 5.30 | 5.15 | 5.50 | 5.47 | 0.26 | 0.44 | 0.26 | 0.73 | 0.26 |
| Hot carcass wt., kg | 415 | 421 | 405 | 401 | 10.3 | <0.01 | <0.01 | 0.17 | 0.05 |
| Dressing percentage, % | 62.97 | 63.17 | 62.85 | 62.48 | 0.39 | 0.31 | 0.13 | 0.28 | 0.70 |
| Marbling score | 423 | 422 | 405 | 403 | 9.50 | 0.33 | 0.09 | 0.95 | 0.46 |
| Calculated yield grade | 3.35 | 3.38 | 3.26 | 3.27 | 0.07 | 0.45 | 0.20 | 0.85 | 0.35 |
| Incidence liver abscesses, % | 1.3 | 10.3 | 3.8 | 1.3 | 0.02 | <0.01 | 0.36 | <0.01 | 0.01 |

[1]Standard error of least squares means, n = 8.

[2]Probability based on *F* statistic. L = linear effect of WDGS level; Q = quadratic effect of WDGS level; C = cubic effect of WDGS level

***Main effects: Level of WDGS.*** Final BW and ADG were linearly decreased ($P < 0.01$) by dietary WDGS level. Dry matter intake and F/G were numerically decreased as WDGS level increased. Hot carcass weights linearly decreased ($P \leq 0.01$) as WDGS level increased above 15% with no effect observed for dressing percentage. Marbling score indicated a linear decline ($P \leq 0.09$) with increased WDGS level which was consistent with the decreased number of carcasses grading USDA choice (data not shown). Longissimus dorsi muscle area, 12th-rib fat thickness and calculated yield grade did not indicate a significant response to WDGS level. Level of dietary WDGS had no effect on the number or distribution of carcasses across USDA yield grade categories (data not shown). The proportion of carcasses that were over 432 kg was strongly influenced by WDGS level in the diet. Both L and C effects were observed with a greater number of heavy carcasses being present in the 0 and 15% WDGS level treatments (data not shown). The number of liver abscesses observed in this study was relatively low across all levels of WDGS; however, there were Q and C responses to WDGS level observed with cattle fed the 15% level having the highest overall incidence. There was a significant ($P \leq 0.05$) response to WDGS level in the number of steers pulled and treated for bovine respiratory disease **(BRD)**. More steers were treated for BRD fed the 30 and 45% levels of WDGS than for either the 0% or 15% WDGS treatments. The number of animals treated for non-BRD conditions, again while low in number, was greater in the 30 and 45% WDGS diets ($P = 0.21$), so that the total morbidity rate experienced in the study was greater ($P \leq 0.01$) with the greater dietary levels of WDGS. The numbers of cattle that either died or were removed from study for various causes were not ($P \geq 0.10$) affected by WDGS level.

## DISCUSSION

***MFA Program.*** Previous research examining the response of MFA program with feedlot diets providing WDGS have been variable. Meyer et al. (2009) in a combined study analysis indicated that M and M/T improved F/G by approximately 1.7% and 4.1%, respectively in finishing diets containing 25% WDGS compared with similar non-medicated diets. Depenbusch et.al (2007), however did not measure a response for either M or M/T in steam-flaked corn diets that contained 25% WDGS compared non-medicated diets. In two research studies (Branine, 2008a,b) evaluated feeding either lasalocid (**L**) plus CTC (**L/CTC**) or M/T in dry-rolled or steam-flaked corn diets that provided either 0 or 20% WDGS. In the dry-rolled corn study, feeding L/CTC increased marbling score, DMI and percentage of carcasses grading USDA with no difference in ADG. In this study, a MFA x WDGS level interaction was present for F/G. In the steam-flaked corn study, L/CTC increased ADG, DMI, final BW and HCW and percentage of carcasses grading choice in both 0 and 20% WDGS diets compared with M/T. In a large pen commercial feedlot study (Branine, 2008c) conducted concurrently with the present study at the same location, significant improvements in ADG, DMI, F/G and carcass yield were observed for LP/CTC compared with M/T in diets with 25% WDGS on a DM basis. Collectively, these results suggest that alternative MFA products and programs may be used in feedlot diets containing varying levels of WDGS in place of the conventional M or M/T programs.

***WDGS Level.*** In this study, a linear decline for several performance and carcass parameters was observed as WDGS level increased above 15%, which appeared to be the optimum for animal performance and carcass merit. In other research, where WDGS was fed in flaked corn diets, a similar optimal response level has been shown (Corrigan et al., 2007). Dietary sulfur levels, as determined from laboratory analyses ranged 0.16% DM for the 0%WDGS diet and progressively increased to 0.24, 0.31 and 0.38% DM for the 15, 30 and 45% WDGS diets, respectively. During the study, no clinical cases of polioencephalomalacia (**PEM**) were observed; however, as previously noted, there was an apparent trend for increased incidence of cattle treated for respiratory disease in the 30 and 45% WDGS diets. A meta-analysis examining the effect of dietary sulfur level on feedlot health of cattle as defined by incidence of PEM suggested that dietary sulfur level should remain below 0.46% to minimize risk of PEM (Nichols et.al. 2012). However, a possible relationship between ruminal hydrogen sulfide production resulting from excess levels of dietary sulfur, whether derived from feed and /or water sources as a predisposing factor for incidence of respiratory disease in feedlot cattle may deserve additional consideration.

## IMPLICATIONS

The differential responses of feedlot cattle to various ionophores and other MFA products provide nutritionists and feedlot managers the opportunity to design feeding programs that can best utilize these characteristics to meet a particular need to optimize animal health and productivity. Economic challenges resulting from high diet costs and feeder cattle prices will require a more thorough understanding of how MFA technologies may be best used with a particular diet or cattle type.

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# EFFECTS OF MONENSIN, MICOTIL, AND COMPONENT TE-G WITH TYLAN ON FALL/WINTER GRAZING PERFORMANCE AND FINAL CARCASS CHARACTERISTICS OF STOCKER CATTLE GRAZING WINTER WHEAT PASTURE

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**ABSTRACT:** Fall-weaned crossbred Brangus steers (N = 206; 200 ± 22 kg) were used in a split plot design to determine the additive effects of metaphylactic treatment with Micotil upon arrival, inclusion of Rumensin in an energy supplement, and the use of a combination grazing implant on wheat pasture growth performance and carcass characteristics. Upon arrival, one-half of the steers were randomly treated with Micotil (10 mg/kg of BW) and held in receiving pens for 42 d. Following receiving, steers were stratified by BW and randomly assigned to 1 of 16 wheat pastures (7.3 to 9.7 ha) at a stocking rate of 1.35 steers/ha for a 112-d winter grazing phase. Pastures were blocked and randomly assigned to 1 of 2 supplement treatments that consisted of a corn and wheat middling-based energy supplement that contained 0 or 220 g/metric ton monensin fed at a rate of 0.91 kg·steer$^{-1}$·d$^{-1}$. Prior to turnout, half of the steers within each pasture and within each Micotil treatment were implanted with Component TE-G with Tylan. Following the grazing phase, steers were transported to a commercial feedlot, implanted with Component TE-S with Tylan and fed in a single pen for 137-d prior to harvest. Growth performance and carcass characteristics were analyzed using PROC MIXED procedure of SAS. The model included Rumensin as the whole-plot and Micotil and Component TE-G as the sub-plot. The two- and three-way interactions were not significant ($P > 0.10$). There were no differences ($P > 0.87$) in receiving health parameters or economic impact among Micotil treatments, but none of the steers were treated for respiratory issues. Inclusion of Rumensin in the supplement increased ($P < 0.03$) final BW and grazing ADG compared with the non-medicated supplement (349 vs. 340 kg; 1.07 vs. 1.01 kg/d, respectively). The grazing ADG of implanted steers was 0.13 kg/d greater ($P < 0.01$) than non-implanted steers (1.10 vs. 0.97 kg/d). Steers implanted during grazing had greater HCW and LM area compared with non-implanted steers with no differences in 12th-rib fat or marbling score. Since the interactions between Micotil, Rumensin, and Component TE-G were not significant, we conclude that these technologies are independent and, therefore, additive.

**Key words:** carcass characteristics, grazing implants, Micotil, Rumensin, wheat pasture performance

## INTRODUCTION

Each year in the Southern Great Plains, one of the major beef cattle production systems is growing cattle on winter wheat pasture. Because of the large amounts of rumen degradable N in wheat forage, Horn et al. (2005) and Horn (2006) developed a monensin-containing energy supplement, "Oklahoma Green Gold", to provide: 1) additional digestible energy to help balance the energy:CP ratio of wheat forage; 2) monensin to improve the economics of the supplementation program and to decrease bloat (Branine and Galyean, 1990); 3) additional Ca for growth; and 4) a means of getting other feed additives into cattle when needed. The addition of monensin within the energy supplement has increased ADG by 0.08 kg/d compared with a non-monensin energy supplement (Horn et al., 1981). The inclusion of monensin has been shown to decrease the incidence and severity of bloat in cattle grazing winter wheat pasture (Paisley and Horn, 1998).

Metaphylactic treatment with Micotil upon arrival has been shown to decrease the incidence of Bovine Respiratory Disease (**BRD**) in high-risk risk cattle by 63% (Galyean et al., 1995). Additionally, these authors reported that during the 28-d receiving phase in which cattle grazed wheat pasture, there were no differences in ADG between the metaphylactic treatment and control calves.

Anabolic implants increase rate of live weight gain of stocker cattle by 8 to 18% (Kuhl, 1998), and provide the highest return on investment of any cattle management practice. Implanting steers grazing wheat-ryegrass pastures with zeranol increased ADG and did not influence final carcass quality (McCann et al., 1991). Kuhl et al. (1997) and Paisley et al. (1999) included a combination implant (Revalor-G) as one of several grazing implant treatments and reported that type of grazing implant did not negatively impact final carcass quality grade. Therefore, the objective of this experiment was to examine the additive effects of metaphylactic treatment with Micotil upon arrival, inclusion of Rumensin in an energy supplement, and use of a combination grazing implant (Component TE-G with Tylan) on the performance of steers grazing wheat pasture and their subsequent carcass characteristics.

## MATERIALS AND METHODS

***Cattle Source and Receiving Phase.*** Fall-weaned Brangus steer calves (N = 206; initial BW = 200 ± 22 kg) were received October 6, 2010 at the Oklahoma State University Wheat Pasture Research Unit near Marshall, OK. Upon arrival, steers were tagged, individually weighed, and vaccinated with Bovi-Shield Gold 5 (Pfizer Animal Health; New York, NY) and Ultrabac 8 (Pfizer Animal Health). During processing, every other steer through the chute received a subcutaneous injection (10 mg/kg of BW) of Tilmicosin Phosphate (Micotil 300; Elanco Animal Health, Greenfield, IN). During the 42-d receiving period, steers were kept in one of 2 pens and were fed long-stem prairie hay and limited amounts (≈ 1.25 kg/d) of a rolled corn, ground alfalfa hay and cottonseed meal-based diet (15.96% CP) that contained 22 g/metric ton of monensin for the first 12 d. Beginning on d 7, the amount of the rolled corn diet was decreased and a high-protein, soybean meal and cottonseed meal based (42.68% CP) receiving supplement containing 89 g/metric ton monensin was added to the daily feeding program. Mean ± SD monensin consumption during the final 31 d of the receiving period was 77 ± 9.0 mg/steer. Steers were monitored on a daily basis for morbidity and were pulled and treated based on treatment protocol and antibiotic treatment regimen protocol approved by a consulting veterinarian.

***Study Site, Vegetation, and Treatments.*** Clean-tilled farm ground (144 ha) was planted to hard red winter wheat (Triticum aestivum; variety = Duster) and was divided into 18 contiguous pastures (7.29 to 9.72 ha) for fall/winter grazing. However, 2 of the pastures had unusually high initial forage mass compared with surrounding pastures and were not included in this study. Pastures were paired (blocked) and randomly assigned to one of two supplement treatments that consisted of a corn and wheat middling-based energy supplement (12.7% CP) that contained 0 or 220 g/metric ton monensin. The energy supplements were pelleted and fed at the rate of 0.91 kg·steer$^{-1}$·d$^{-1}$. There were no supplement refusals and steers in all pastures had access to improved water sources.

***Fall/Winter Grazing Phase.*** Of the original 206 steers received, 169 steers (198 ± 18 kg) were allotted for fall/winter grazing at a stocking rate of 1.35 steers/ha. Steers that were ± 2 SD from the initial BW were removed from the data set and were not considered for fall/winter grazing. The fall/winter grazing phase was initiated on November 19 when the steers were individually weighed and allotted to the 16 pastures based on Micotil treatment and previous receiving BW. Half of the steers within each pasture and within each Micotil treatment were implanted with Component TE-G with Tylan (40 mg TBA, 8 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health). Steers were allowed to graze wheat pasture until March 3 (112 d) when the wheat reached first-hollow stem stage of maturity.

***Graze-Out and Finishing Phase.*** Following fall/winter grazing, steers were comingled and allowed to graze two separate areas of wheat pasture (25% of the total ha planted) for 46 d prior to being transported 588 km to a commercial feedlot for finishing. Supplements were not fed during graze-out. Upon arrival at the commercial feedlot, steers were implanted with Component TE-S with Tylan (120 mg TBA, 24 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health). At the end of finishing, steers were fed Zilmax (zilpaterol hydrochloride, Intervet Inc., Merck Animal Health, Summit, NJ). Steers were fed in one pen for 137 d prior to final harvest. Carcass data were collected by Cattlemen's Carcass Data Service (West Texas A & M University, Canyon, TX). Initial BW used for feedlot performance data was the final BW from the end of graze-out. Final BW at the end of finishing was calculated using individual HCW and the mean dressing percentage of 64.29%.

***Statistical Analysis.*** Fall/winter grazing and finishing performance and carcass characteristics were analyzed as a split-plot randomized complete block using the Proc MIXED procedure (SAS Inst. Inc., Cary, NC). Rumensin treatment was the whole-plot (experimental unit = pasture) and Micotil and Component TE-G were included as sub-plots (experimental unit = steer). The model included the main effects of Rumensin, Micotil, and Component TE-G, all two-way interactions and the three-way interaction terms. Random variables included block within landowner, pasture, and pasture within (block landowner supplement). The two- and three-way interactions were not significant ($P > 0.10$); therefore the LSMeans for each main effect are presented.

## RESULTS AND DISCUSSION

***Receiving Phase.*** None of the steers were pulled/treated for BRD. This was surprising given that the steers would be classified as high-risk for BRD due to the fact that they were not weaned prior to shipment and were subjected to the stress of a long haul from Okeechobee, FL. One possible explanation could be that the steers had undergone a very thorough beef quality assurance program at the ranch of origin. Daily weight gain of steers during the 42-d receiving period is shown in Table 1 and was not ($P = 0.87$) influenced by metaphylactic treatment with Micotil upon arrival. Galyean et al. (1995) reported no differences in ADG during the receiving phase between the control steers and steers given a metaphylactic treatment with Micotil while grazing wheat pasture. In contrast, J. E. Sawyer (Texas A&M University, College Station, TX, personal communication) conducted a study that was designed similar to the current experiment and observed that during the 28-d receiving phase, the metaphylactic treated steers had greater ADG compared with the non-treated controls steers by 0.14 kg/d. Guthrie et al. (2004) reported that high-risk steers which received a metaphylactic treatment with Micotil upon arrival had greater ADG (0.25 kg/d) during the 28-d receiving phase in a confined setting compared with non-treated control steers.

***Fall/Winter Grazing Performance.*** There were no differences ($P > 0.27$) among the two- and three-way interactions for either initial or final BW or ADG during the fall/winter grazing period. Therefore, the following discussion will focus on the main effects of Rumensin, Component TE-G, and Micotil.

**Table 1.** Effect of metaphylactic treatment with Micotil upon arrival on steer performance during the 42-d receiving phase

| | Treatment | | | |
|---|---|---|---|---|
| Item | No Micotil | Micotil | SEM | *P*-value |
| Steers, No. | 103 | 103 | - | - |
| Initial BW, kg | 197 | 203 | 2.11 | 0.07 |
| Final BW, kg | 227 | 232 | 2.46 | 0.14 |
| ADG, kg/d | 0.70 | 0.70 | 0.02 | 0.87 |

***Rumensin.*** Inclusion of Rumensin (220 g/metric ton) in the energy supplement did increase ($P < 0.03$) final BW (349 vs. 340 kg) and increased ($P < 0.01$) ADG during the entire 112-d grazing period by 0.06 kg/d (Table 2). The Oklahoma Green Gold Supplement Program has been shown to improve ADG by 0.11 and 0.19 kg/d during fall/winter grazing of wheat pasture (Fieser et al., 2003; Horn, 2006, respectively) compared with steers only consuming a non-medicated mineral mix. Horn et al. (1981) reported that steers consuming an energy supplement with the inclusion of Rumensin increased ADG by 0.08 kg/d compared with steers consuming a non-Rumensin energy supplement while grazing winter wheat pasture.

***Component TE-G.*** Component TE-G with Tylan increased ($P < 0.01$) final BW and ADG during the entire grazing phase (Table 2). Average daily gain during the entire 112-d grazing period was increased 0.13 kg/d (13.4%) by the use of the Component TE-G implant. Paisley et al. (1999) reported that steers implanted with a combination implant while grazing dormant native range improved ADG by 25% (0.35 vs. 0.28 kg/d). Kuhl et al. (1997) observed that implanting steers with a combination implant during the grazing phase improved ADG by 13% (0.87 vs. 0.77 kg/d). Additionally, McMurphy et al. (2011) reported that steers implanted with a combination implant during a 126-d summer grazing period on Old World Bluestem pastures increased ADG compared with steers that were non-implanted or implanted with a Zeranol implant (0.98 vs. 0.81 and 0.93 kg/d, respectively). In continued support of previous reports, implanting stocker cattle significantly increases BW gain during the grazing phase.

***Micotil.*** During the grazing phase (112 d), ADG was 0.04 kg/d lower ($P < 0.05$) for steers that received Micotil on arrival compared with steers that did not receive Micotil (Table 2). This seems contrary to what was expected and a plausible explanation is not known at this time for these results, but there are limited data regarding metaphylactic treatment followed by a long grazing period. In contrast, J. E. Sawyer (Texas A&M University, College Station, TX, personal communication) showed that the metaphylactic treated steers tended to have greater ADG compared with the non-treated controls steers by 0.06 kg/d during the 84-d winter grazing phase on oat pastures. Additionally, Galyean et al. (1995) reported that in trial 3, the metaphylactic-treated steers that were held in a drylot for a 56-d receiving phase had greater DMI and Feed:Gain compared with steers that were not given Micotil upon arrival. The metaphylactic-treated steers did have a numerical increase in ADG by 0.15 kg/d. Guthrie et al. (2004) showed a tendency for the metaphylactic treated steers to have greater ADG at the end of the 191-d finishing phase compared with the non-treated control steers (1.55 vs. 1.52 kg/d).

***Graze-out Performance.*** During the graze-out period, steers gained 1.47 and 1.42 kg/d and 356 and 339 kg/ha for the North and South graze-out areas, respectively. These values are similar to data that has previously been reported at the same research center (Sharman et al., 2012).

***Finishing Performance and Carcass Characteristics.*** There was a Rumensin*Component TE-G interaction ($P < 0.04$) for LM area such that LM area was greater for steers that were implanted compared with steers that were not implanted within the no Rumensin supplemented treatment group, but implanted steers had similar LM area when Rumensin was fed. However, there were no differences ($P > 0.10$) among the two- and three-way interactions for finishing performance or other carcass characteristics. Therefore, the following discussion will focus on the main effects of Rumensin, Component TE-G, and Micotil.

***Rumensin.*** Inclusion of Rumensin in the energy supplement during winter grazing did not affect ($P > 0.33$) initial or final BW or ADG during finishing (Table 3). Inclusion of Rumensin in the energy supplement during winter grazing increased ($P < 0.04$) 12th-rib fat and yield grade compared with the steers that did not receive Rumensin during grazing (Table 3). There was a tendency for marbling score to be greater for the Rumensin steers compared with steers not fed Rumensin during the grazing phase. Hot carcass weight, LM area, or KPH was not affected ($P > 0.25$) by Rumensin treatment during fall/winter grazing. Sharman et al. (2012) reported no differences in feedlot performance or carcass characteristics when steers were supplemented with the Oklahoma Green Gold Supplement prior to finishing compared with the non-supplemented controls.

***Component TE-G.*** Implanting steers with Component TE-G with Tylan during fall/winter grazing increased ($P < 0.01$) initial finishing BW by 22 kg (5.5%; Table 3). This increase in BW was carried through finishing such that the steers that were implanted had heavier ($P < 0.04$) final BW by 17 kg (2.8%). Additionally, there was no difference ($P > 0.39$) in finishing ADG between the grazing implant treatments. Similar results were reported by Sharman et al. (2012) that the use of a combination implant on steers grazing wheat pasture increased initial finishing BW and the weight advantage was carried through the finishing phase with no

**Table 2.** Effect of including Rumensin in an energy supplement, use of Component TE-G with Tylan, and metaphylactic treatment of Micotil during the receiving phase on performance of steers grazing fall/winter wheat pasture for 112-d

| | Treatment | | | Treatment | | | Treatment | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | No Rumensin | Rumensin | SEM | No Implant | Component | SEM | No Micotil | Micotil | SEM |
| Steers, No. | 84 | 85 | - | 85 | 84 | - | 85 | 84 | - |
| Initial BW, kg | 227 | 229 | 2.38 | 227 | 228 | 2.38 | 226 | 230 | 2.38 |
| Final BW, kg | 340[a] | 349[b] | 2.84 | 336[a] | 352[b] | 2.84 | 345 | 344 | 2.84 |
| ADG, kg/d | 1.01[a] | 1.07[b] | 0.02 | 0.97[a] | 1.10[b] | 0.02 | 1.06[b] | 1.02[a] | 0.02 |

[a,b] Within a row and within each main effect, means without a common superscript differ ($P < 0.05$).

**Table 3.** Effect of including Rumensin in an energy supplement, use of Component TE-G with Tylan, and metaphylactic treatment of Micotil during the receiving phase prior to/or during fall/winter grazing on feedlot performance and carcass characteristics

| | Treatment | | | Treatment | | | Treatment | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | No Rumensin | Rumensin | SEM | No Implant | Component | SEM | No Micotil | Micotil | SEM |
| Steers, No. | 81 | 83 | - | 84 | 80 | - | 82 | 82 | - |
| Feedlot Performance | | | | | | | | | |
| Initial BW[1], kg | 409 | 413 | 3.26 | 400[a] | 422[b] | 3.28 | 412 | 410 | 3.24 |
| Final BW[2], kg | 611 | 616 | 5.67 | 605[a] | 622[b] | 5.70 | 617 | 610 | 5.63 |
| ADG, kg/d | 1.48 | 1.48 | 0.03 | 1.50 | 1.46 | 0.03 | 1.50 | 1.46 | 0.03 |
| Carcass Characteristics | | | | | | | | | |
| HCW, kg | 393 | 396 | 3.64 | 389[a] | 400[b] | 3.66 | 396 | 392 | 3.62 |
| 12th-rib fat, cm | 1.62[a] | 1.76[b] | 0.05 | 1.72 | 1.67 | 0.05 | 1.78[b] | 1.60[a] | 0.05 |
| LM area, $cm^2$ | 93.24 | 91.67 | 0.96 | 90.71[a] | 94.20[b] | 0.97 | 92.34 | 92.57 | 0.95 |
| KPH, % | 1.99 | 2.02 | 0.03 | 2.01 | 2.01 | 0.03 | 2.04 | 1.98 | 0.03 |
| Yield grade | 3.16[a] | 3.41[b] | 0.08 | 3.35 | 3.22 | 0.08 | 3.40[b] | 3.17[a] | 0.08 |
| Marbling score[3] | 408 | 428 | 8.39 | 412 | 425 | 7.91 | 423 | 413 | 7.82 |

[1] Initial BW was the final shrunk BW following graze-out.
[2] Final BW was calculated using individual HCW and the mean dressing percentage of 64.29%.
[3] Marbling grid: 300 = Slight[00]; 400 = Small[00]; 500 = Modest[00].
[a,b] Within a row and within each main effect, means without a common superscript differ ($P < 0.05$).

effects on finishing ADG compared with steers that were previously non-implanted.

Implanting steers with Component TE-G with Tylan during fall/winter grazing increased ($P < 0.04$) HCW by 11 kg and increased ($P < 0.01$) LM area compared with the non-implanted steers (Table 3). There were no differences ($P > 0.20$) for 12th-rib fat, KPH, yield grade, or marbling score among the grazing implant treatments. Similar results from the same research center showed that implanting steers on wheat pasture with a Component TE-G implant increased HCW and LM area without negatively impacting 12th-rib fat or marbling score (Sharman et al., 2012).

***Micotil.*** Metaphylactic-treatment of Micotil upon arrival in the receiving phase prior to grazing had no effect ($P > 0.41$) on feedlot performance (Table 3).

Steers receiving a metaphylactic-treatment of Micotil upon arrival in the receiving phase had lower ($P < 0.01$) 12th-rib fat, which decreased ($P < 0.05$) yield grade compared with steers not receiving metaphylactic treatment upon arrival (Table 3). Steers were harvested at a similar time point instead of being harvested at a similar carcass composition; therefore these differences may be reduced if the Micotil steers were on feed for a longer period of time. However, there were no differences ($P > 0.11$) for any other carcass characteristic between Micotil treatments. Similarly, Guthrie et al. (2004) and Corbin et al. (2009) reported that at the end of the finishing phase there were no differences in carcass quality among the metaphylactic treated or control steers. However, no previous studies have examined the effects of metaphylactic-treatment of cattle prior to a fall/winter grazing period before entering the finishing phase.

## IMPLICATIONS

Because none of the two-way interactions or the three-way interaction between Micotil, Rumensin, and Component TE-G were significant, we conclude that these three technologies are independent and, therefore, additive. The use of Rumensin within an energy supplement and a combination implant will improve ADG while grazing wheat pasture without negatively impacting finishing performance or carcass quality. More research is needed to determine the effects of Metaphylactic-treatment with Micotil when followed by an extended grazing phase.

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# EFFECTS OF SUPPLEMENTATION WITH A PRESSED DRIED DISTILLERS GRAIN BLOCK ON BEEF COW PERFORMANCE AND HAY INTAKE DURING LATE GESTATION

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**ABSTRACT**: Multiparous crossbred beef cows (n = 72; BW = 600.2 ± 6.2 kg, BCS = 4.3 ± 0.4, age = 6.9 ± 0.1 yr) in late gestation were blocked by expected calving date and randomly allocated by BW to 1 of 3 treatments: ad libitum chopped grass hay (CON; 8.2% CP and 57.7% NDF, DM basis), CON hay with ad libitum access to a pressed dried distillers grain block (BLOCK; SweetPro 16; 22.4% CP and 6.9% fat, DM basis), and CON hay with 0.57 kg•cow$^{-1}$•d$^{-1}$ of a positive control supplement (POS; 57% corn and 43% DDGS; 19.2% CP and 6.4% fat, DM basis). Each treatment had 6 replicates (n = 4/pen), and the trial consisted of 2 periods (period 1: d 1 to 41; period 2: d 42 to 70). Hay was weighed and fed twice daily and refusals weighed once weekly to determine pen hay intake. All CON and POS cows had ad libitum access to a trace mineralized salt, whereas macro and trace minerals were provided by the BLOCK supplement. Data were analyzed with treatment, block, and treatment x block as fixed effects in the model for performance measures. Week and week x treatment were also fixed effects in the model for hay intake. Means were separated using LSD and considered significant when $P \leq 0.05$ or tendencies when $P < 0.10$. Hay intake was affected ($P = 0.05$) by treatment, where cows receiving the BLOCK treatment consumed less ($P = 0.01$) hay than CON (12.4, 12.7, and 12.6 ± 0.1 kg DMI for BLOCK, CON, and POS, respectively). Although BW change was not affected ($P = 0.29$) by treatment during period 1, BLOCK and POS tended to have greater BW change during period 2 ($P \leq 0.07$) and for the overall trial ($P = 0.005$). During period 1, cow BCS change tended to be affected ($P = 0.11$) by treatment, where BLOCK and POS tended to have greater ($P \leq 0.09$) BCS change than CON. Body condition score change during period 2 was unaffected ($P = 0.46$) by treatment, however, overall BCS change was affected (P = 0.006) by treatment, where BCS change was greatest in POS, intermediate in BLOCK, and least in CON. Additionally, treatment did not affect ($P \geq 0.35$) ultrasonic backfat thickness, LM depth, or marbling score. These data suggest that the ad libitum pressed dried distillers grain block used was not only able to improve cow performance in a similar manner to supplement fed daily, but also reduced hay intake compared with control.

**Key words**: beef cows, gestation, supplementation

## INTRODUCTION

Feed is the largest operating cost in beef production, greatly affecting profitability of cow-calf producers (Miller et al., 2001; Ramsey et al., 2005). Approximately two-thirds of total energy consumed by beef cattle goes toward cow maintenance, making it a considerable portion of total costs in beef production systems (Ferrell and Jenkins, 1985; Montano-Bermudez and Nielson, 1990). Despite this, cattle grazing in the northwestern United States, and especially on high elevation rangelands, typically consume low-quality forage, making protein or energy supplementation or both often necessary to maintain optimal production (Rusche et al., 1993; DelCurto et al., 2000; Lardy and Endecott, 2010). Supplementation can be both expensive and labor intensive; therefore, implementing a supplementation program that minimizes labor and still offers adequate nutrients is essential.

The ethanol industry and resulting high corn prices have increased the prevalence and use of co-product feeds such as dried distillers grains with solubles (**DDGS**) that have greater protein (and increased RUP fraction), fat, and fiber content than many traditional supplements (Klopfenstein et al., 2008). The nutrient content of DDGS thus gives the potential to supplement both RUP and fat to beef cows consuming low-quality forages. Use of these co-products may be limited in the range setting because of the increased labor of daily or alternate day feeding of range cube or loose DDGS-based supplements, however. Our objective was to evaluate effects of supplementation with a self-limiting pressed DDGS block, SweetPro 16, on cow BW, body composition, and hay intake in late gestation. Our next objective was to monitor changes in BW, BCS, ultrasonic fat depth, LM depth, and marbling score during this period.

## MATERIAL AND METHODS

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

***Animals and Treatments.*** Seventy-two late gestation multiparous crossbred beef cows ranging in age from 5 to 9 yr of age (average age = 6.9 ± 0.1 yr) were utilized at the University of Wyoming Beef Unit. Cows were blocked by expected calving date and randomly allocated by BW to 1 of 3 treatments. All treatments had access to ad libitum chopped grass hay (Table 1) for the duration of the 70-d trial, which

consisted of 2 periods (period 1: d 1 to 41; period 2: d 42 to 70). The control (**CON**) treatment received only ad libitum grass hay without any supplementation. In addition to the ad libitum chopped grass hay, cows in the second treatment received ad libitum access to SweetPro 16 (SweetPro, LLC, Walhalla, ND), a pressed DDGS block (**BLOCK**; Table 1). Cows in the positive control treatment also received 0.57 kg•cow$^{-1}$•d$^{-1}$ (as fed) of a supplement formulated to be similar to the nutrient composition of BLOCK (**POS**; 57% corn and 43% DDGS; Table 1). Eighteen pens were used, allowing for 6 replicates of each treatment (4 cows/pen). The BLOCK supplement provided macro and trace minerals, but CON and POS pens were offered ad libitum access to trace mineralized salt throughout the study.

Hay fed to each pen was weighed before twice daily feedings, and hay refusals were weighed once weekly to determine pen hay intake. Disappearance of BLOCK supplement was determined as block weight divided by number of days for complete consumption. New block supplement was given after completion of each block. Two-day BW and BCS by 3 independent evaluators were taken at the initiation and conclusion of each period (d 0 and 1, d 41 and 42, and d 69 and d 70). Ultrasonic backfat thickness, LM depth, and marbling score were also collected at study initiation and conclusion using an Aloka 500V ultrasound machine (Aloka Co., Ltd., Japan) and the Cattle Performance Enhancement Company software system (CPEC, Oakley, KS).

***Nutrient Analysis.*** Hay and supplement samples were taken weekly for nutrient analysis. All samples were analyzed for DM and ash, N (Leco FP-528 N Analyzer, Leco Corporation, Henderson, NV), and NDF and ADF (Ankom 200 Fiber Analyzer, Ankom Technology) as previously described (Moriel et al. 2011). A protein factor of 6.25 was used for CP calculations.

***Statistical Analysis.*** All data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Cow performance data were analyzed with treatment, block (expected calving date), and treatment x block as fixed effects in the model. Week and week x treatment were also included as fixed effects in the model for hay intake. Means were separated using LSD and considered significant when $P \leq 0.05$ or tendencies when $P < 0.10$.

**Table 1.** Nutrient composition of the hay and supplements fed to beef cows during late gestation

| | | Supplement | |
|---|---|---|---|
| Item | Hay[1] | SweetPro 16 block[2] | Positive control[3] |
| DM, % | 82.1 | 72.0 | 89.1 |
| Nutrient, % of DM | | | |
| CP | 8.2 | 22.4 | 19.2 |
| NDF | 57.7 | 11.8 | 22.8 |
| ADF | 41.4 | 7.9 | 11.4 |
| Fat[4] | | 6.9 | 6.4 |
| Ash | 9.8 | 5.1 | 4.5 |

[1]Chopped grass hay fed ad libitum to all treatments.
[2]The SweetPro 16 (SweetPro, LLC, Walhalla, ND), a pressed dried distillers grain block, was fed ad libitum to the BLOCK treatment.
[3]The positive control supplement 57% corn and 43% DDGS and fed to the POS treatment at 0.57 kg•cow$^{-1}$•d$^{-1}$.
[4]Calculated from NRC (2000).

## RESULTS

Over the course of the study, hay DMI (kg and % BW; Table 2) was affected by treatment ($P = 0.05$) and week ($P < 0.001$), but not their interaction ($P > 0.89$). Cows in late gestation that had access to SweetPro 16 block supplementation consumed less ($P = 0.02$) hay DMI (kg) than cows receiving hay only. Although actual hay DMI was not decreased in POS cows, both BLOCK and POS had decreased ($P \leq 0.02$) DMI compared with CON when expressed as a percent of BW. Intake of the SweetPro 16 block ranged from 0.58 to 1.98 kg•cow$^{-1}$•d$^{-1}$ (as fed; average intake = 1.54 kg•cow$^{-1}$•d$^{-1}$) for pens receiving the BLOCK treatment.

Despite there being no effect ($P = 0.29$) of treatment on cow BW change (Table 2) during period 1, treatment tended to affect ($P = 0.07$) BW change during period 2 and affected BW change for the overall study ($P = 0.007$). During period 2, cows that received the BLOCK and POS treatments tended ($P \leq 0.07$) to have greater BW increase than CON. Additionally, BW increase over the entire study was greater ($P \leq 0.005$) for BLOCK and POS compared with CON.

During period 1, cow BCS (Table 2) tended to be affected ($P = 0.11$) by treatment, where cows in the BLOCK and POS treatments tended ($P \leq 0.09$) to have greater BCS increase than CON. There was no effect ($P = 0.46$) of treatment on BCS change during period 2; however, overall BCS was affected ($P = 0.006$) by treatment. Over the entire study, cows that received the POS treatment tended ($P \leq 0.09$) to have the greatest BCS increase, BLOCK tended ($P \leq 0.09$) to be intermediate, and CON had the least ($P \leq 0.05$) BCS increase. Despite these BCS differences, there was no effect ($P \geq 0.35$) of treatment on ultrasonic backfat thickness, LM depth, or marbling score (Table 2).

## DISCUSSION

Although cows in all treatments gained BW and BCS during this study, both supplemented groups (BLOCK and POS) had improved performance compared with CON. This supplementation effect was expected, as supplemented cows consumed more nutrients and were more readily able to surpass nutrient requirements necessary for fetal growth and gain maternal tissue during later stages of pregnancy. Hay intake was decreased for both BLOCK (DMI and % BW) and POS (% BW) supplemented cows, but this decrease in nutrients from hay was more than compensated by consumption of supplement. Body weight gains in cows from all treatments are likely both a result of the rapid fetal growth during late gestation and the increase in nutritional plane that occurred when cows were fed the mid-quality hay fed in this study compared with previous winter grazing, as indicated by the increase in BCS for all treatments.

**Table 2.** Effects late gestation supplementation on hay intake and cow performance and body composition

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | CON | BLOCK | POS | SEM | *P*-value |
| Hay intake | | | | | |
| DMI, kg | 12.7[a] | 12.4[b] | 12.6[ab] | 0.1 | 0.05 |
| DMI, % of BW | 2.05[a] | 2.00[b] | 2.01[b] | 0.02 | 0.05 |
| BW, kg | | | | | |
| Initial | 598 | 593 | 600 | 12 | 0.90 |
| Period 1 change[2] | 45.1 | 53.9 | 51.9 | 4.0 | 0.29 |
| Period 2 change[3] | 13.5[a] | 22.5[b] | 24.4[b] | 3.1 | 0.07 |
| Overall change[4] | 58.5[a] | 76.5[b] | 76.3[b] | 3.7 | 0.007 |
| BCS | | | | | |
| Initial | 4.35 | 4.26 | 4.32 | 0.08 | 0.67 |
| Period 1 change | 0.18 | 0.36 | 0.39 | 0.07 | 0.11 |
| Period 2 change | 0.17 | 0.16 | 0.29 | 0.08 | 0.46 |
| Overall change | 0.34[a] | 0.53[b] | 0.68[c] | 0.06 | 0.006 |
| Backfat thickness, mm | | | | | |
| Initial | 4.24 | 4.59 | 4.75 | 0.31 | 0.50 |
| Overall change | 0.053 | 0.387 | 0.543 | 0.33 | 0.57 |
| Marbling score[5] | | | | | |
| Initial | 482 | 466 | 465 | 21 | 0.82 |
| Overall change | 29.1 | 12.8 | 14.9 | 25.5 | 0.79 |
| LM depth, mm | | | | | |
| Initial | 49.2 | 47.6 | 49.7 | 1.6 | 0.62 |
| Overall change | -1.46 | 0.39 | -2.49 | 1.35 | 0.35 |

[a-c] Within an item, means differ ($P < 0.10$).

[1] Treatments during late gestation included ad libitum chopped grass hay (CON), CON hay with ad libitum access to a pressed dried distillers grain block (BLOCK; SweetPro® 16), and CON hay with 0.57 kg•cow$^{-1}$•d$^{-1}$ of a positive control supplement (POS).

[2] Period 1 = d 1 to 41 of the study.

[3] Period 2 = d 42 to 70 of the study.

[4] Overall = both period 1 and period 2 .

[5] US marbling scores where 400 = Small$^{00}$ and 500 = Modest$^{00}$.

Anecdotal evidence from producers using the SweetPro 16 block suggested that this supplement may decrease hay intake, as confirmed in this study. Previous studies have demonstrated that DDGS supplementation decreases forage intake in beef cattle (Loy et al., 2007; Griffin et al., 2009; Leupp et al., 2009; Wahrmund et al., 2011), although many utilized greater DDGS supplementation levels than observed in this study. While it unknown how consumption of the SweetPro 16 block pressed DGGS block reduced hay intake, several possibilities exist: 1) Increased fat intake because of DDGS inclusion reduced fiber digestibility, reducing passage rate and hay intake (MacDonald et al., 2007); 2) Increased intake of NDF due to DDGS inclusion resulted in greater effective ruminal fill from the supplement (Leupp et al., 2009); 3) Supplementation altered ruminal fermentation end-products, such as propionate (Loy et al., 2007; Leupp et al., 2009), signaling satiety (hepatic oxidation theory; Allen et al., 2009); 4) Supplementation altered ruminal environment or fermentation characteristics by other means (e.g., pH; Loy et al., 2007), resulting in decreased fiber digestibility and reduced hay intake; 5) Supplementation increased ruminally undegradable protein intake, which altered metabolism and circulating hormones, resulting in satiety signals (MacDonald et al., 2007); 6) Inclusion of the ProBiotein additive (prebiotic oligosaccharides, digestive enzymes, and yeast cultured from grains) affected fermentation, digestibility, or signaled satiety; and 7) Extraneous factors from the production process affected ruminal fermentation or induced satiety.

Intake of SweetPro 16 was greater in this study (average = 1.54 kg•cow$^{-1}$•d$^{-1}$) than the expected product intake of 0.32 to 0.57 kg•cow$^{-1}$•d$^{-1}$, which was likely due to the small number of cows and relative small pen size in comparison to a production setting and may have resulted in the observed decrease in hay intake. Although cows receiving the positive control supplement were fed at expected BLOCK intake levels rather than observed levels, DMI as a % BW decreased in these cows, suggesting that a similar supplement may decrease forage intake at lower supplementation levels.

From these data, it can be concluded that ad libitum supplementation with the SweetPro 16 pressed DDGS block may result in improved cow performance while also reducing intake of moderate quality forage and minimizing labor necessary for supplementation. However, cost and intake of the supplemental inputs must be considered to determine financial feasibility in beef production systems.

## IMPLICATIONS

Performance improved and hay intake was decreased in cows allowed ad libitum access to the SweetPro 16 pressed DDGS block, suggesting that this is a viable supplementation option for beef cows during late gestation.

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# METABOLIZABLE PROTEIN SUPPLY ALTERS PREGNANCY AND SUBSEQUENT RETENTION RATE DURING HEIFER DEVELOPMENT WHILE GRAZING DORMANT WINTER FORAGE

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**ABSTRACT:** Type of heifer development system can have major impact on the future productivity and retention rate of the cowherd. Therefore, the objective of this experiment was to determine growth, reproductive performance, retention rate, and economic efficiency of heifers developed in range raised (with 2 CP treatments) or high input (feedlot developed) heifer development system. Spring-born, crossbred heifers (n = 191) were stratified to 1 of 3 treatments at weaning: (1) 0.9 kg/d of a 36% CP supplement containing 64% RDP (CSM), (2) 0.9 kg/d of a 36% CP supplement containing 50% RUP (RUP), or (3) a concentrate diet fed in dry lot (CONC) to gain 0.68 kg/d. Supplementation was initiated in February and terminated at the onset of a 45-d breeding season in May. Heifer BW and hip height (HH) were taken monthly from initiation of supplementation until breeding and again at weaning. Females were removed from the herd for failure to reproduce or wean a calf. Percent of heifers becoming pregnant and remaining in the herd at start of each breeding season was recorded to determine retention rate throughout their 4th breeding season. Breeding BW was greater ($P < 0.01$) for the CONC than CSM or RUP developed heifers. Hip height at breeding was greater ($P < 0.01$) in RUP and CSM relative to CONC heifers. However, palpation BW and HH was similar ($P \geq 0.24$) for all heifer development treatments. At breeding, RUP and CSM heifers reached 51% of mature BW ($P < 0.01$) compared with CONC heifers at 58% of mature BW. Pregnancy rates were 94, 88, and 84% for RUP, CSM and CONC heifers ($P = 0.10$). Net return was $99.71 and $87.18 per developed heifer greater for RUP and CSM heifers, respectively compared with CONC heifers due to differences in pregnancy and development costs. Retention rate at age 4 was greatest ($P \leq 0.01$) for RUP heifers. This study indicates that range developed heifers can be as reproductively successful as heifers developed in a feedlot, while improving future productivity. Furthermore, metabolizable protein supply improves reproduction in heifers developed on dormant native range.

**Key words**: beef heifers, heifer development, pregnancy, retention rate

## INTRODUCTION

Selection and development method of replacement heifers can impact future productivity and longevity of the entire cowherd. Replacement heifers represent a significant cost to beef cattle enterprise. The primary cost associated with developing heifers managed under extensive conditions is purchased feed to augment diets for sufficient gains to achieve puberty before breeding (Ferrell, 1982). Considerable supplemental nutrient input may be necessary to achieve a target BW for heifers developed on poor quality forages. However, developing heifers to lighter target BW may be effective in reducing costs over time while achieving reproductive goals (Hawkins et al., 2000). Roberts et al. (2009) suggested a potential economic advantage to developing heifers on a restricted level of feeding. Clanton et al. (1983) demonstrated that costs and patterns of growth may be altered during the postweaning period without a decrease in the ability of the heifer to conceive. However, developing heifers on a limited diet may have a negative effect on the longevity and future productivity due to lesser body reserves. We hypothesized developing heifers in an ongoing low input management scheme based on maximizing native range would not have a negative impact on heifer pregnancy rates or longevity. The objective of the experiment was to determine growth, reproductive performance, longevity, and analyze the economic efficiency of heifer development in a low input (grazing native range with strategic CP supplemental inputs) or greater input (feedlot developed) heifer development.

## MATERIALS AND METHODS

All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University Institutional Animal Care and Use Committee.

This study used 3 consecutive replacement heifer crops at New Mexico State University's Corona Range and Livestock Research Center (**CRLRC**), Corona, NM. The ranch's average elevation is 1,900 m. Annual precipitation averages 400 mm, with approximately 50% of annual precipitation occurring from July to September.

This study was designed to determine if an advantage exists by developing heifers in an environment similar to that in which they would spend the majority of their productive life. Over 3 yr (2005 – 2007), 191 spring-born, British crossbred heifers (234 ± 1.1 kg at weaning) were used to compare 2 low-input range development methods and a high-input commercial growing diet on reproductive performance, development, and retention rate based on 4 years

of reproductive achievement at CRLRC. Supplementation strategy was dynamic based on season, nutrient deficits and projected nutrient use efficiency. From November to January, all heifers were managed in a common pasture and were fed 1.6 kg·heifer$^{-1}$·wk$^{-1}$ of a cottonseed meal-based 36% CP supplement. At weaning, heifers were assigned to 1 of 3 treatments by weaning weight. Heifer development treatments were: 1) pasture developed and fed a 36% CP cottonseed-meal based supplement containing 64% RDP (**CSM**); 2) pasture developed and fed a 36% CP cottonseed-meal based supplement containing 50% RUP (**RUP**); and 3) steam-flaked corn and corn silage-based growing diet in a dry lot (**CONC**). Supplementation treatments were initiated in February and terminated at breeding (mid-May). Pasture developed heifers were randomly assigned to 1 of 4 pastures and fed 3 d/wk at a rate of 0.9 kg·heifer$^{-1}$·d$^{-1}$. Pastures were 270 ha and contained approximately 400 kg/ha of standing forage. All pastures were stocked at a rate that was 50% less than the NRCS recommended rate so that forage availability was assumed not to limit heifer productivity (USDA-NRCS, 2002). The CONC heifers were shipped to a feedlot and were fed to gain approximately 0.68 kg/d prior to breeding when consuming a corn silage-based growing diet. Upon arrival in the feedlot, heifers were divided into replicate pens with 6 to 7 heifers/pen. Upon termination of the supplement feeding period (late April to early May), feedlot heifers were shipped back to CRLRC and heifers from all treatments were combined in a common pasture and fed CSM at 0.9 kg·heifer$^{-1}$·d$^{-1}$.

**Table 1.** Ingredients and nutrient composition of protein supplements (all units as fed) fed to range developed heifers

| | Supplement[1] | |
|---|---|---|
| Item | CSM | RUP |
| Ingredients | % | |
| Cottonseed meal | 56.94 | 24.80 |
| Urea | 1.20 | 0.70 |
| Wheat middlings | 21.45 | 42.50 |
| Hydrolyzed feather meal | -- | 20.00 |
| Soybean Meal | 10.00 | -- |
| Molasses | 9.00 | 9.00 |
| Potassium chloride | 0.95 | 1.70 |
| Monocalcium phosphate | 0.30 | -- |
| Vitamin A premix | 0.08 | 0.08 |
| Manganese sulfate | 0.06 | 0.05 |
| Trace mineral premix | 0.02 | 0.02 |
| Copper sulfate | 0.01 | < 0.01 |
| Nutrient Composition | | |
| TDN | 65.64 | 64.98 |
| CP | 36.01 | 36.01 |
| RDP | 23.05 | 18.39 |
| RUP | 12.96 | 17.62 |

[1]CSM = 36% CP cottonseed meal base supplement fed 3 d/wk supplying 36% RUP; RUP = 36% CP supplement fed 3 d/wk supplying 50% RUP.

In May of each year, estrus was synchronized using a controlled internal drug-releasing device (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY), Co-Synch protocol. Heifers were administered a single 2-mL i.m. injection of GnRH (Cystorelin, Merial, Iselin, NJ), and a CIDR was inserted. After 7 d, the CIDR was removed and all cows received a single 5-mL i.m. injection of PGF (Lutalyse, Pfizer Animal Health). Approximately 66 h after CIDR removal, all heifers were artificially inseminated and were administered a single 2-mL i.m. injection of GnRH (Cystorelin, Merial, Iselin, NJ). All heifers were then managed together with a bull for a 45-d breeding season. Heifers were evaluated for pregnancy by rectal palpation in October. In each year, BW and hip heights (**HH**) were recorded once monthly from January to May and then again at weaning (pregnancy diagnosis). Weight-to-height ratios were calculated at initiation of supplementation, prior to breeding, and at pregnancy diagnosis. Percent of heifers becoming pregnant and remaining in the herd at start of each breeding season was recorded to determine retention rate. Females were removed from the herd for failure to reproduce or wean a calf.

***Economic Analysis.*** Hypothetical enterprise budgets were used to evaluate economic returns generated by developing 100 heifers in each development system. A grazing fee was assigned based on the average leased price ($12.30/animal unit month, AUM) of private rangeland in New Mexico for the year 2008 (National Agricultural Statistics Service, 2008). Animal unit equivalents used for the heifers was suggested by Vallentine (1990). Cost of supplementation and mineral supplements were calculated to current costs delivered to the ranch. Costs associated with developing heifers in the feedlot included freight to and from the feedlot, yardage, feed, and vaccinations. Returns generated from the sale of non-pregnant heifers were included in the budget. Pregnant heifers were retained, however, for the purpose of the analysis their sale value was assigned using a 10-yr average market value for heifers (Cattle Fax, 1998-2008).

***Statistical Analysis.*** Normality of data distribution was evaluated using PROC UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Continuous data were evaluated by the MIXED procedure of SAS, using pen or pasture as the experimental unit. The model included treatment, year, and treatment × year. The Kenward-Roger degrees of freedom method was used to adjust standard errors and calculate denominator degrees of freedom. Means were separated using least significant difference and considered significant when $P \leq 0.05$. The binomial data were analyzed with PROC GLIMMIX.

## RESULTS AND DISCUSSION

By design, BW among groups of heifers assigned to the 3 development methods were similar at weaning ($P = 0.97$; Table 2) and at initiation of supplementation ($P = 0.98$). However, CONC heifers were heavier ($P < 0.01$) at breeding relative to CSM and RUP heifers. This was expected due to an increased ($P < 0.01$) ADG from the initiation of the study to breeding and greater ($P < 0.01$) overall ADG than

either range developed heifer nutritional strategy (CSM or RUP heifers). Range developed heifers (RUP and CSM) did have a greater ($P < 0.01$) ADG from breeding until pregnancy diagnosis. Heifers developed on dormant winter range may compensate for the minimal pre-breeding (winter) ADG and gain more weight during the breeding season than heifers fed in a dry-lot, due to reduced maintenance requirements and the ability to respond to a seasonal improvement in forage quality associated with summer rains (Marston et al., 1995; Ciccioli et al., 2005).

Similar to BW, heifer HH at the initiation of supplementation was similar ($P = 0.29$; Table 2) between the 3 heifer development methods. However, HH change from initiation of supplementation to breeding was greater ($P = 0.01$) in CSM and RUP heifers compared with CONC heifers, resulting in a greater ($P < 0.01$) HH at breeding in the RUP and CSM heifers. However, HH change from breeding to pregnancy diagnosis and overall HH change were greater ($P < 0.01$) in CONC heifers. This increase in height from breeding to pregnancy diagnosis did not result ($P = 0.18$) in greater overall HH in the CONC heifers. Hip heights at pregnancy diagnosis were similar between the 3 methods; therefore, mature size is not expected to be impacted in heifers managed in a restricted environment. Previous research has reported that level of energy intake does not affect heifer hip height (Buskirk, et al., 1995; Roberts et al., 2007). Dietary protein supplementation increased peak bone mass acquisition in energy-restricted growing rats (Mardon et al., 2009). Protein supplemented heifers grazing dormant forage may prioritize nutrients to skeletal growth instead of BW gain. Initial BW to hip height ratio was similar ($P = 0.94$); however, when compared at breeding and at pregnancy diagnosis CONC heifers had increased ($P \leq 0.01$) BW to HH ratio. The difference found in BW to HH ratio at breeding and pregnancy diagnosis was due to an increased BW in CONC heifers, since RUP and CSM had greater hip height at breeding and no difference at palpation.

Recommended guidelines have been to achieve 60 to 65% of mature BW in beef heifers at breeding to optimize reproduction (Patterson et al., 1992). In contrast, Martin et al. (2008) has shown that heifers in a 60-d breeding season can be as low as 50% of mature BW and realize pregnancy rates of 88.4%. Heifers were 51, 51, and 58% of mature BW at the time of breeding between the CSM, RUP, and CONC heifers, respectively ($P < 0.01$; Table 2). Previous research developing heifers to pre-breeding target BW similar to those

**Table 2.** Effect of grazing native dormant pastures or dry lot developed on heifer body weight and pregnancy rates

| | Supplement[1] | | | | |
|---|---|---|---|---|---|
| Measurement | CSM | RUP | CONC | SEM | *P*-value |
| Heifer BW, kg | | | | | |
| Weaning BW | 223 | 224 | 224 | 3 | 0.97 |
| Initial[2] BW | 255 | 256 | 255 | 3 | 0.98 |
| Breeding BW | 276 | 276 | 315 | 4 | < 0.01 |
| Pregnancy diagnosis BW | 402 | 393 | 403 | 5 | 0.24 |
| Average Daily Gain, kg/d | | | | | |
| Initial to breeding | 0.27 | 0.26 | 0.69 | 0.02 | < 0.01 |
| Breeding to pregnancy diagnosis | 0.85 | 0.80 | 0.61 | 0.01 | < 0.01 |
| Initial to pregnancy diagnosis | 0.62 | 0.58 | 0.64 | 0.01 | < 0.01 |
| Heifer Hip Height, cm | | | | | |
| Initial[2] | 116.64 | 116.38 | 116.13 | 0.53 | 0.80 |
| Breeding | 120.88 | 120.80 | 118.49 | 0.46 | < 0.01 |
| Pregnancy diagnosis | 124.76 | 123.80 | 124.82 | 0.56 | 0.32 |
| Heifer BW/HH ratio, kg/cm | | | | | |
| Initial[2] | 2.19 | 2.20 | 2.19 | 0.02 | 0.94 |
| Breeding | 2.28 | 2.28 | 2.65 | 0.01 | < 0.01 |
| Pregnancy diagnosis | 3.20 | 3.16 | 3.21 | 0.02 | 0.14 |
| Hip Height Change, cm | | | | | |
| Initial to breeding | 4.27 | 4.42 | 2.34 | 0.30 | < 0.01 |
| Breeding to pregnancy diagnosis | 3.89 | 2.97 | 6.32 | 0.36 | < 0.01 |
| Initial to pregnancy diagnosis | 8.13 | 7.39 | 8.66 | 0.38 | < 0.01 |
| Mature Body Weight, % | 51 | 51 | 58 | 0.62 | < 0.01 |
| Reproductive Performance | | | | | |
| Pregnancy Rate, % | 88 | 94 | 84 | 3 | 0.10 |

[1]CSM = 36% CP cottonseed meal base supplement fed 3 d/wk supplying 36% RUP; RUP = 36% CP supplement fed 3 d/wk supplying 50% RUP; CONC = concentrate growing diet fed in dry lot to gain 0.68 kg/d.

[2]Initiation of heifer development treatments.

in the present study reported pregnancy rates from 88 to 90% after a 60- (Funston and Deutscher, 2004) or 45-d breeding season (Martin et al., 2008), respectively. These studies indicate that heifers can be developed at a lower target BW prior to breeding while maintaining adequate pregnancy rates and effectively lowering developmental feed costs. In the current study, pregnancy rates tended to be greater ($P = 0.10$) in RUP heifers compared with CONC heifers. Therefore, developing heifers on a less nutrient dense diet with a lower gain did not negatively impact reproductive performance when supplemented with RUP.

The greatest concern with developing heifers on low energy density diet with slower rate of gain is decreasing heifer pregnancy rates, increased calving difficulties, resulting in a decreased longevity and productivity in the cow herd. Lesmeister et al. (1973) reported that nutritionally restricted heifers have decreased pregnancy rates and those that get bred generally calve later, which leads to a decrease in lifetime productivity. Moriel et al. (2012) suggested that replacement beef heifers consuming low-quality forages should receive low-starch energy supplements daily to enhance their reproductive development. However, heifers developed on lower levels of nutrient intake have been suggested to adapt by improving efficiency and later longevity in the cow herd (Roberts et al., 2008). Heifers fed RUP tended ($P \geq 0.08$; Figure 1) to have greater retention rate in breeding Year 1 and 2, whereas, in Year 3 and 4 retention rates were greater ($P < 0.01$) when compared with other treatments.

***Economic Analysis.*** An enterprise budget comparing the three heifer development systems on their economic impacts is illustrated in Table 3. Gross returns were highest for the RUP heifers and lowest in CONC heifers, resulting from an increase in pregnant heifers. Feed costs for CONC heifers were greatest compared with heifers developed on dormant forage. Therefore, with lower gross returns and greater feed costs, CONC developed heifers had decreased net returns compared with RUP and CSM heifers. Net return was $99.71 and $87.18 per heifer greater for RUP and CSM, respectively compared with CONC. The increase in net revenue was due to an increase pregnancy rates and decreased development costs. Likewise, restricting development by limiting DMI (Roberts et al., 2009) or developing to a lower target breeding BW (Martin et al., 2008) also reported economic advantages in low cost heifer development system. However, the additional benefit of increased retention rate was not considered in the enterprise budget which would have further increased the revenue of developing heifers on dormant forage with a RUP supplement.

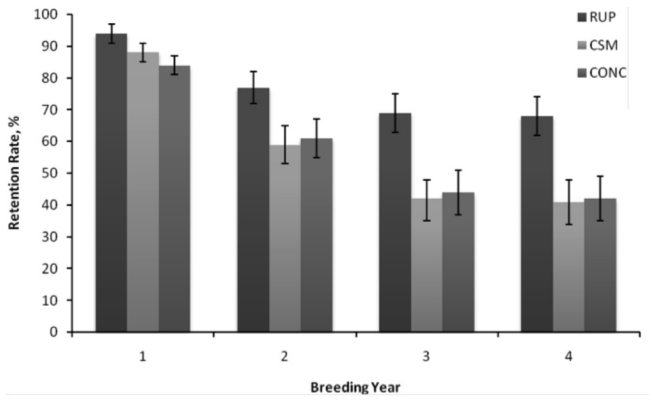


**Figure 1.** Retention rate of heifers developed on native dormant pastures supplemented with 2 different types of protein supplements or in a dry-lot with a concentrate diet (Exp. 2). Breeding year 1 are heifer pregnancy rates. Breeding years 2 through 4 are proportion remaining at end of the 2nd through 4th breeding seasons. Retention tended (*$P \geq 0.08$) to differ among groups in Yr 1 and 2, but was greater for RUP than CSM and CONC cows in Yr 3 and 4 (**$P < 0.01$). CSM = 36% CP cottonseed meal base supplement fed 3 d/wk supplying 36% RUP; RUP = 36% CP supplement fed 3×/wk supplying 50% RUP; CONC = concentrate growing diet fed in dry-lot to gain 0.68 kg/d.

## IMPLICATIONS

Heifers developed on native range fed 50% RUP supplements prior to breeding may increase retention and productivity with an overall lower cost. This study demonstrates that heifers can be grown at a slow rate of gain on semi-arid rangelands with strategic supplementation, resulting in pregnancy rates similar to heifers in a moderate to high rate of gain. Developing heifers on native range and supplementing with RUP had greater retention rates based on greater reproductive success, indicating that they may have become better adapted to their production environment.

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**Table 3.** Enterprise budget for costs and returns from each heifer development

| | Supplement[1] | | |
|---|---|---|---|
| Item | CSM | RUP | CONC |
| Gross Returns ($) | | | |
| Non pregnant heifers | 6,576.96 | 3,214.08 | 8,799.04 |
| Pregnant heifers | 77,890.56 | 83,201.28 | 74,350.08 |
| Total | 84,467.52 | 86,415.36 | 83,149.12 |
| | | | |
| Costs | | | |
| Heifer Purchase Cost | 50,264.00 | 50,264.00 | 50,264.00 |
| Ranch Cost | | | |
| Developing ranch heifers | | | |
| Grazing | 4,419.00 | 4,419.00 | |
| Protein Supplement | 3,561.60 | 4,256.00 | |
| Mineral and Salt | 620.00 | 620.00 | |
| Feedlot Cost | | | |
| Developing feedlot heifers | | | |
| Freight | | | 600.00 |
| Feed | | | 12,600.00 |
| Yardage | | | 2,800.00 |
| Total | 58,864.60 | 59,559.00 | 66,264.00 |
| Net Returns ($) | 25,602.92 | 26,856.36 | 16,885.12 |
| Net Returns ($ per heifer) | 256.03 | 268.56 | 168.85 |

[1]CSM = 36% CP cottonseed meal base supplement fed 3 d/wk supplying 36% RUP; RUP = 36% CP supplement fed 3 d/wk supplying 50% RUP; CONC = concentrate growing diet fed in dry lot to gain 0.68 kg/d.


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# PROTEIN SUPPLEMENTATION OF LOW-QUALITY FORAGE: EFFECTS OF AMOUNT AND FREQUENCY ON INTAKE AND NUTRIENT DIGESTIBILITY BY LAMBS

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**ABSTRACT:** The objectives of this research were to determine the effects of protein supplementation frequency on intake and nutrient digestibility by lambs. Seven lambs were utilized in a 4 × 7 incomplete Latin square design. Dietary treatments were arranged as a 2 × 3 factorial (2 levels of CP and 3 supplementation frequencies); CON = unsupplemented control; D = supplemented daily 0.28% of BW of soybean meal (SBM); 5D = supplemented 1.4% of BW of SBM once every 5 d; 10D = supplemented 2.8% of BW of SBM once every 10 d; ½ D = supplemented at 50% of D; ½ 5D = supplemented at 50% of 5D; ½ 10D = supplemented at 50% of 10D. Full CP refers to D, 5D, and 10D and half CP refers to ½ D, ½ 5D, and ½ 10D dietary treatments. Lambs were supplemented with SBM immediately prior to feeding of low-quality forage (Chewings fescue grass seed straw). Each experimental period was 30 d, with intake measured d 19 to 28. Feces, urine, and blood were collected d 21 to 30. Straw and SBM DMI, total DMI, straw OM intake, OM intake, ADF intake, and NDF intake were not different ($P \geq 0.26$) due to supplementation. Supplementation increased ($P \leq 0.02$) DM, OM, and NDF digestibility compared with the CON. The CON lambs had reduced ($P \leq 0.002$) N intake, urine N excretion, N balance, N digestibility, and digested N retained compared with supplemented lambs. Plasma urea N was increased in the supplemented lambs ($P = 0.004$) compared with the CON lambs as well as for full CP compared with half CP lambs ($P = 0.03$). Lambs supplemented with full CP had increased ($P \leq 0.03$) urine N excretion and N digestibility compared with the half CP lambs; however, digested N retained was not different ($P = 0.94$) due to supplementation amount. As supplementation frequency decreased, N digestibility was also reduced ($P = 0.01$). Both DM and OM digestibility increased ($P \leq 0.04$) as supplementation interval increased. These results suggest that increasing the supplementation interval may be utilized to maintain intake, digestibility, and reduce the labor costs associated with more frequent supplementation.

**Key words:** lambs, nitrogen balance, supplementation frequency

## INTRODUCTION

Grazing livestock in the western United States consume low-quality forage (< 6% CP) from late summer through winter (Bohnert et al., 2002b). Therefore, supplemental CP is required to maintain livestock BW and BCS (Bohnert et al., 2002a; Schauer et al., 2005). Protein supplementation every day can be costly; therefore, decreasing supplementation frequency may reduce labor costs while maintaining livestock performance. Current research suggests that supplementation frequency can be reduced to once every 10 d while maintaining livestock performance (Schauer et al., 2010).

Previous research has indicated that supplementation frequency can be reduced to once every 6 or 10 d due to urea recycling and maintenance of nitrogen use efficiency (Bohnert et al., 2002a; Schauer et al., 2010). However, there is no research currently available to determine if the amount of CP can also be reduced while decreasing supplementation frequency. Therefore, we hypothesized that as supplementation frequency is reduced, ruminants will become more efficient in their N utilization and, with the increased efficiency, the amount of CP supplemented can be reduced. The objectives of the trial were to evaluate infrequent supplementation of differing amounts of CP on sheep nitrogen use efficiency.

## MATERIALS AND METHODS

All procedures were approved by the North Dakota State and Oregon State University Animal Care and Use Committees.

***Animals and Diets.*** Seven wethers (western Whiteface; 31.6 ± 1.7 kg) were used in a 4 × 7 incomplete Latin square design to evaluate the efficiency of lambs fed low-quality forage supplemented CP at different amounts and frequencies. Treatments were arranged in a 2 × 3 factorial design (2 levels of CP and 3 supplementation frequencies); **CON** = unsupplemented control; **D** = supplemented daily 0.28% of BW of soybean meal (SBM); **5D** = supplemented 1.4% of BW of SBM once every 5 d; **10D** = supplemented 2.8% of BW of SBM once every 10 d; **½ D** = supplemented at 50% of D; **½ 5D** = supplemented at 50% of 5D; **½ 10D** = supplemented at 50% of 10D. Full CP refers to D, 5D, and 10D and half CP refers to ½ D, ½ 5D, and ½ 10D dietary treatments. The full CP was estimated to meet the CP requirement of a 30 kg lamb gaining 0.20 kg per day; the half CP was 50% of the corresponding full CP. Trace mineralized salt was provided daily (16.0% Ca, 8.0% P, 21.0% Salt, 2.75% Mg, 3 ppm Co, 5 ppm Cu, 100 ppm I, 1400 ppm Mn, 20 ppm Se, 3000 ppm Zn, 113,500 IU/kg vitamin A, 11,350 IU/kg vitamin D, and 227 IU/kg vitamin E). In addition, an

intramuscular injection of vitamins A, D, and E (100,000, 10,000, and 300 IU of vitamins A, D, and E, respectively; Vitamin E-AD; VetOne, Neogen Corp., Lexington, KY). Lambs had continuous access to fresh water and low-quality cool season hay (Chewings fescue grass seed straw; 4.9% CP).

***Sampling and Laboratory Analysis.*** Wethers were weighed on d 0 and 1 of each 30 d experimental period, with a total of 4 periods. Wethers were housed in an enclosed room with a 13 h light and 11 h dark cycle. Lambs were adapted to diets from d 1 to 18. Soybean meal (SBM; 49.9% CP) was used as the CP supplement and offered to lambs immediately prior to hay feeding along with the trace mineralized salt. Hay was provided daily at 0830 h at 120% of the average daily intake for the previous 5 d. Feed refusals from the previous day were determined prior to feeding.

Dry matter intake was determined on d 19 to 28. Additionally, samples of hay and SBM were collected on d 19 to 28 and dried at 55°C for 48 h to determine DM. Orts were collected on d 20 to 29 and dried at 55°C for 48 h. Total fecal and urine output were collected on d 21 to 30. A subsample of each daily fecal sample (7.5% of total, wet basis) was dried at 55°C for 96 h for calculation of fecal DM. Urine was composited daily by wether (10% of total; wet basis) and stored at 4°C. Sufficient 6 *N* HCL (100 mL) was added daily to urinals to maintain urine pH < 3. On d 21 to 30, 10 mL of blood were collected via jugular venipuncture 4 h after feeding using Vacutainers (VWR, catalogue no. VT6480). Blood was cooled at 4°C for 2 h, centrifuged (3,640 × *g*; 20 min), and plasma harvested and stored (-20°C).

Dried fecal samples were ground through a Wiley mill (2-mm screen) and composited by lamb. Daily samples of hay and SBM were composited for the collection period, and orts were composited by lamb on an equal weight basis (20%; as fed basis). Feed, orts, and fecal samples were analyzed for DM, OM, NDF, and ADF. Feed, orts, fecal, and urine samples were analyzed for N. Plasma samples were analyzed for urea-N.

***Statistical Analysis.*** Data were analyzed as an incomplete 4 × 7 Latin square (Cochran and Cox, 1957) using the Mixed procedure (SAS Inst. Inc., Cary, NC) with Satterwaite approximation. Period, wether, and dietary treatment were included in the model and lamb used as the random variable. Orthogonal contrasts were used to determine dietary treatment effects because of the treatment structure. Orthogonal contrasts were 1) CON vs. CP supplementation; 2) full vs. half CP; 3) linear effect of supplementation frequency (SF); 4) quadratic effect of SF; 5) contrast 2 × contrast 3; and 6) contrast 2 × contrast 4. Plasma urea-N concentrations were analyzed using the MIXED procedure of SAS. Lamb, period, treatment, day, and treatment × day were used in the model. The slice option of the MIXED procedure was used to determine treatment and time differences. The random statement included lamb × period × day. The contrasts above were used to determine treatment sums of squares. Quadratic contrasts were not significant ($P > 0.05$) and will not be presented.

## RESULTS AND DISCUSSION

Supplementation of CP did not affect ($P \geq 0.26$; Table 1) straw DMI, total DMI, straw OM intake, total OM intake, NDF intake, ADF intake, or indigestible ADF (IADF) intake. However, DM digestibility, OM digestibility, and NDF digestibility were increased ($P \leq 0.03$) and ADF digestibility tended ($P = 0.08$) to increase due to CP supplementation. Similar results were observed by Schauer et al. (2010), who observed that as supplementation of CP was reduced to once every 10 d but, total DMI and OM intake were not influenced. However, Bohnert et al. (2002a) observed that total DMI was increased with CP supplementation compared with unsupplemented control wethers. In contrast to the current results, Beaty et al. (1994) observed that steers consuming wheat straw and supplemented three times weekly had reduced straw and total DMI compared with steers supplemented daily. Similar to the current trial, daily supplementation of CP has been demonstrated to increase DM digestibility in steers (DelCurto et al., 1990). Supplementation of CP increased ($P \leq 0.002$) N intake, urinary N excretion, N balance, N digestibility, and daily digested N retained compared with the unsupplemented control. However, fecal N excretion was not affected ($P = 36$) by CP supplementation. These results were expected due to the increase in N available for digestion in the supplemented wethers. In a similar trial to ours, daily NDF intake was 13.1 g/kg BW for unsupplemented controls compared with 13.1, 11.3, and 10.8 for daily, every 5$^{th}$ day, and every 10$^{th}$ day CP supplementation, respectively (Schauer et al., 2010). Supplemental CP has caused an increase in forage intake in other studies. Bandyk et al (2001), DelCurto et al. (1990), and Köster et al. (1996) all observed that forage intake increased when NDF intake of the unsupplemented controls was 8.2, 6.4, and 5.1 g/kg BW per d (respectively). These studies suggest that lambs must consume less than 12.5 g/kg BW per d of NDF for protein supplementation to elicit an increase in forage intake. All lambs in the current trial were consuming more than 12.5 g/kg BW per d of NDF, which may explain the lack of a treatment effect on forage intake. Also, Bohnert et al. (2011) demonstrated that the forage intake response to CP supplementation of low-quality forage may be dependent on forage type, with intake of warm season forages increasing substantially while little to no increase in intake of cool-season forages, like that used in the current study, is commonly noted.

The amount of CP fed (full vs. half) did not affect ($P \geq 0.14$) DM, OM, or fiber intake or digestibility parameters. Similarly, the amount of supplemental CP did not affect ($P = 0.27$) fecal N excretion or daily digested N retained ($P = 0.94$). As was expected, N intake was increased ($P < 0.001$) in full CP wethers compared with half CP wethers. Half CP wethers had reduced ($P \leq 0.002$) urinary N excretion and N digestibility and tended ($P = 0.09$) to have reduced N balance compared with full CP wethers. Full CP wethers had a greater reduction ($P = 0.05$) in fecal N excretion as SF decreased than the half CP wethers. Full CP wethers tended ($P = 0.07$) to have a greater increase in N digestibility as SF reduced compared with half CP wethers. Nitrogen

**Table 1.** Effect of CP amount and supplementation frequency on intake and diet digestibility of wethers

| | Dietary Treatment[1] | | | | | | | | *P*–value[3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON | D | 5D | 10D | ½ D | ½ 5D | ½ 10D | SEM[2] | CON vs. Supp | Full vs. Half | L SF | L SF vs. Amt |
| Intake | | | | | | | | | | | | |
| Straw DMI, g/kg BW | 18.8 | 21.6 | 19.5 | 14.0 | 20.2 | 19.3 | 18.7 | 1.73 | 0.97 | 0.45 | 0.02 | 0.08 |
| Supplement DMI, g/kg BW | 0.0 | 2.8 | 2.8 | 2.8 | 1.4 | 1.4 | 1.4 | | | | | |
| Total DMI, g/kg BW | 18.8 | 24.4 | 22.3 | 16.8 | 21.6 | 20.7 | 20.1 | 1.73 | 0.26 | 0.80 | 0.02 | 0.09 |
| Straw OM intake, g/kg BW | 17.4 | 20.0 | 18.0 | 12.9 | 18.6 | 17.8 | 17.3 | 1.60 | 0.97 | 0.46 | 0.02 | 0.08 |
| Supplement OMI, g/kg BW | 0.0 | 2.5 | 2.5 | 2.5 | 1.3 | 1.3 | 1.3 | | | | | |
| Total OM intake, g/kg BW | 17.4 | 22.5 | 20.5 | 15.4 | 19.9 | 19.1 | 18.6 | 1.60 | 0.26 | 0.79 | 0.02 | 0.09 |
| NDF intake, g/kg BW | 14.9 | 17.8 | 15.9 | 11.6 | 16.0 | 15.4 | 15.0 | 1.40 | 0.78 | 0.73 | 0.02 | 0.07 |
| ADF intake, g/kg BW | 8.6 | 10.2 | 9.0 | 6.5 | 9.1 | 8.8 | 8.5 | 0.84 | 0.88 | 0.73 | 0.02 | 0.07 |
| Indigestible ADF intake, g/kg BW | 3.9 | 4.5 | 4.0 | 2.9 | 4.1 | 4.0 | 3.8 | 0.38 | 0.96 | 0.51 | 0.02 | 0.10 |
| Digestibility, % | | | | | | | | | | | | |
| DM | 37.4 | 40.6 | 45.7 | 49.6 | 43.5 | 43.5 | 43.5 | 1.98 | 0.004 | 0.28 | 0.04 | 0.04 |
| OM | 39.6 | 43.6 | 48.0 | 52.0 | 45.7 | 45.4 | 45.5 | 1.85 | 0.002 | 0.14 | 0.04 | 0.03 |
| NDF | 42.2 | 43.6 | 46.9 | 48.6 | 46.0 | 45.7 | 44.7 | 1.39 | 0.03 | 0.43 | 0.19 | 0.04 |
| ADF | 43.0 | 43.4 | 46.0 | 48.4 | 46.5 | 45.2 | 44.8 | 1.38 | 0.08 | 0.69 | 0.25 | 0.03 |
| N balance | | | | | | | | | | | | |
| N intake, g/kg BW | 0.15 | 0.38 | 0.37 | 0.33 | 0.26 | 0.26 | 0.26 | 0.015 | <0.001 | <0.001 | 0.04 | 0.12 |
| Fecal N excretion, g/kg BW | 0.13 | 0.19 | 0.16 | 0.11 | 0.15 | 0.14 | 0.13 | 0.017 | 0.36 | 0.27 | 0.01 | 0.05 |
| Urine N excretion, g/kg BW | 0.05 | 0.15 | 0.18 | 0.20 | 0.10 | 0.12 | 0.11 | 0.015 | <0.001 | <0.001 | 0.05 | 0.15 |
| N balance, g/kg BW | -0.03 | 0.04 | 0.04 | 0.01 | 0.02 | 0.01 | 0.01 | 0.013 | 0.002 | 0.09 | 0.22 | 0.36 |
| N digestibility, % | 12.2 | 49.5 | 58.4 | 65.8 | 45.5 | 48.9 | 48.4 | 3.40 | <0.001 | 0.002 | 0.01 | 0.07 |
| Daily digested N retained, g/kg BW[4] | -460.6 | 21.8 | 17.9 | 3.9 | 12.4 | 2.4 | 7.3 | 119.95 | 0.002 | 0.94 | 0.93 | 0.96 |

[1]CON = unsupplemented control; D = 0.28 % of BW/d of SBM; 5D = 1.4 % of BW of SBM once every 5 d; 10D = 2.8 % of BW of SBM once every 10 d; ½ D = 50 % of D; ½ 5D = 50 % of 5D; ½ 10D = 50 % of 10D.

[2]n = 4.

[3]CON vs. Supp = control vs. supplemented treatments; Full vs. Half = full vs. half amount of CP; L SF = linear effect of supplementation frequency; L SF vs. Amt = interaction of the linear effect of supplementation frequency and amount of CP.

[4]Calculated as (Daily N rentention, g/kg BW/Daily N digested, g/kg BW) x 100.

digestibility of the half CP lambs was similar to that of the full CP daily supplemented lambs. These results suggest that the amount of CP fed may be reduced and still elicit similar results to the full CP fed wethers.

As SF was reduced, a linear reduction ($P$ = 0.02) in straw DMI, total DMI, straw OM intake, total OM intake, NDF intake, ADF intake, and IADF intake were observed. However, we noted a linear increase ($P$ = 0.04) in DM and OM digestibility as supplementation became less frequent, with NDF and ADF digestibility not affected ($P \geq 0.19$). Frequency of supplementation did not affect N balance ($P$ = 0.22) or daily digested N retained ($P$ = 0.93). Similar results were observed by Bohnert et al. (2002b), as supplementation frequency did not affect the apparent total tract N disappearance of steers. Nitrogen intake and fecal N excretion decreased ($P \leq 0.04$) as SF was reduced. Urinary N excretion and N digestibility increased ($P \leq 0.05$) as SF reduced. In similar trials, as the frequency of supplementation was reduced to once every 10 or 6 d, urinary N excretion and N digestibility was increased (Schauer et al., 2010 and Bohnert et al., 2002a, respectively). As with Bohnert et al. (2002a) and Schauer et al. (2010), the reduction in straw and total DMI can partially be explained by the reduction in forage intake on the days following supplementation (data not shown).

A tendency existed ($P \leq 0.10$) for a CP amount × SF interaction on all intake variables. Lambs offered full CP had a greater reduction in straw intake, total DMI, straw OM intake, total OM intake, NDF intake, ADF intake, and IADF intake as SF decreased compared with the lambs offered half CP. Dry matter, OM, NDF, and ADF digestibility increased as SF decreased for the full CP wethers compared with the half CP wethers, which remained relatively constant ($P \leq 0.04$). These results suggest that with a scenario similar to the current study, the amount of CP supplemented can be reduced to 50 % of that required while maintaining N efficiency similar to full CP supplemented wethers.

There was a dietary treatment × day effect ($P <$ 0.001; Figure 1) for plasma urea N concentration. Plasma urea N concentration was increased on the day post-supplementation for the 5D, 10D, ½ 5D, and ½ 10D wethers ($P \leq 0.05$). Similar results were observed by Bohnert et al. (2002a), with increased plasma urea N concentration on the first day post-supplementation. Also, Bohnert et al. (2002c) observed a similar increase in steer ruminal ammonia N concentration, with concentration greater 24 h post-supplementation compared with the time of supplementation. Both gastrointestinal tract permeability to urea and regulation of renal urea excretion can be altered by low-protein diets and/or restricted feeding (Harmeyer and Martens, 1980 and

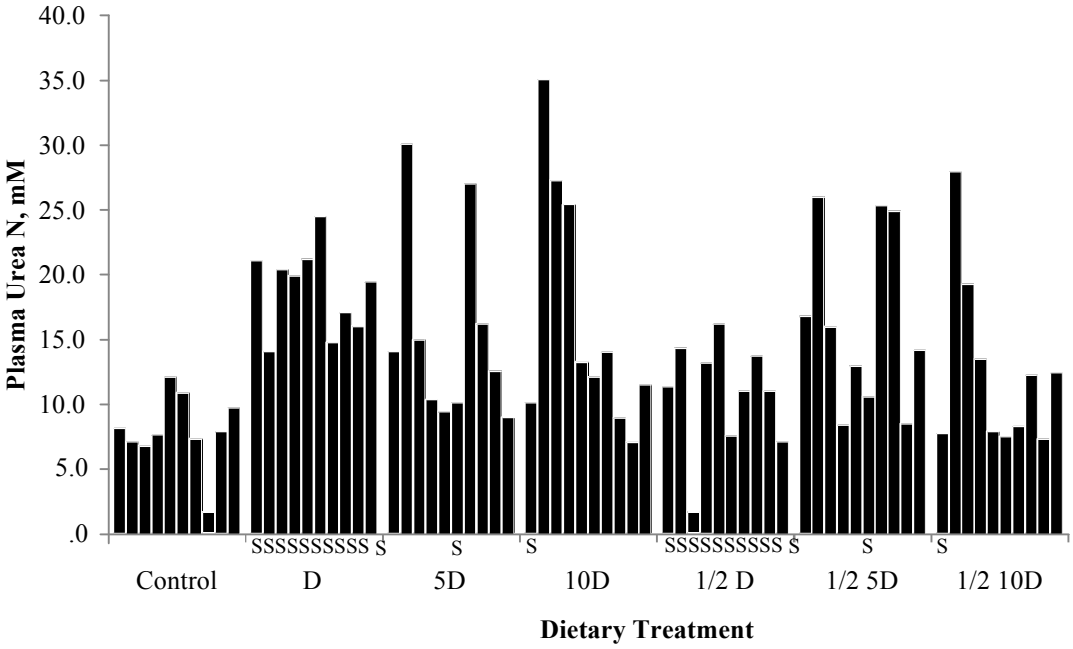


**Figure 1.** Influence of supplementation frequency and amount of CP supplemented on plasma urea N concentrations of wethers. Columns from left to right for each dietary treatment represent d 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of a 10 d supplementation window, respectively. Treatments were CON = unsupplemented control; D = supplemented daily 0.28% of BW of soybean meal (SBM); 5D = supplemented 1.4% of BW of SBM once every 5 d; 10D = supplemented 2.8% of BW of SBM once every 10 d; ½ D = supplemented at 50% of D; ½ 5D = supplemented at 50% of 5D; ½ 10D = supplemented at 50% of 10D. Full CP refers to D, 5D, and 10D and half CP refers to ½ D, ½ 5D, and ½ 10D dietary treatments. Treatment × day interaction (P < 0.001). SEM = 3.80.

Kennedy and Milligan, 1980). Three factors influence the excretion of urea from the kidneys: 1) changes in filtered urea loads correspond with changes in plasma urea concentrations, 2) changes in glomerular filtration rate, and 3) changes in tubular resorption of urea (Harmeyer and Martens, 1980). This would suggest that the lambs in the current trial were more efficient in conserving and recycling urea N as SF and amount of CP decreased.

## IMPLICATIONS

Reducing the amount of CP supplemented and decreasing SF did not negatively impact N retention in wethers. Therefore, our data suggests that reducing the amount of CP while reducing SF is a potential strategic supplementation practice that can maintain N use efficiency. The reduction in SF and amount of CP fed will minimize labor and feed costs during times when only low- quality forage is available.

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# RUMINAL FERMENTATION, KINETICS AND DIGESTIBILITY OF HAIR LAMBS SUPPLEMENTED WITH CULL PINTO BEAN

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**ABSTRACT.** Cull pinto bean (Phaseolus vulgaris) is an ingredient used in ovine feeding in northern Mexico. The objective was to evaluate the effect of three levels of cull pinto bean (CPB) on feed intake, ruminal fermentation, kinetics, and nutrient digestibility of hair lambs. Six crossbred (Dorper × Pelibuey and Charolais × Pelibuey) rumen fistulated lambs averaging 56.6 ± 3.8 kg were used. Lambs were randomly assigned to 1 of 3 treatments. Treatments were (DM basis): 1) 0% of CPB of the supplement (CON); 2) 25% of CPB of the supplement (F25); and 40% of CPB of the supplement (F40). Dry matter intake, ruminal pH, ruminal $NH_3$ and VFA, methane production, Kp, MRT, DMD, CPD, and NDFD were evaluated. Data were analyzed with the MIXED procedure of SAS in a Latin square design 3 × 3 repeated in line. Estimates used for Kp, and MRT were obtained by a non-linear regression model (PROC NLIN, SAS). There was a difference in DMI (kg; $P \leq 0.05$) among treatments, F25 (1.62 ± 0.083) and F40 (1.62 ± 0.083) were reduced probably due to anti-nutritional factors in CPB. Ruminal pH and $NH_3$ (mg/100 ml of ruminal fluid) were not different ($P \geq 0.05$) among treatments. Differences were found ($P \leq 0.03$) for ruminal VFA (mM) concentration (CON: 60.37 ± 2.1; F25: 68.42 ± 2.1; F40: 64.23 ± 2.1). Greater DMI of CON did not affect total VFA. The greater value in F40 could be due to its greater oligosaccharide content. Acetate: propionate ratio was greater ($P \leq 0.0001$) for F40 (CON: 2.34 ± 0.073; F25: 2.2± 0.073; F40: 2.9 ± 0.073). CH4 production (mM/ml) was different ($P \leq 0.032$) among treatments (CON: 29.06 ± 0.75; F25: 27.59 ± 0.75; F40: 32.87 ± 0.75). Kp (%/h) (CON: 3.6 ± 1.9; F25: 2.9 ± 1.9; F40: 5.9 ± 1.9%) and MRT (h) (CON: 36.26 ± 13.7; F25: 57.68 ± 13.7; F40: 36.24 ± 13.7h) were similar ($P \geq 0.42$) among treatments. Digestibility of DM, CP, and NDF was similar ($P \geq 0.26$) among treatments. Cull pinto bean reduce DMI; although, it does not affect ruminal pH and $NH_3$ production. The inclusion of 25% of CPB in the diet of hair lambs allows having an appropriate nutrient digestibility without affecting Kp and MRT, and this increases the molar proportion of VFA without increasing methane production maintaining acetate: propionate ratio.

**Key words:** cull pinto bean, hair lamb, ruminal fermentation and kinetics.

## INTRODUCTION

Cull pinto bean (*Phaseolus vulgaris*) is an ingredient used in ovine feeding in northern Mexico. Its effect on animal performance has been studied. Villalobos et al. (2010) reported that as cull pinto bean level increased in the diet, ADG decreased. In other study Castillo et al. (2011) found similar performance in pregnant and lactating ewes fed with different level of cull pinto bean. Nonetheless the effect of its inclusion on ruminal fermentation and kinetics has not been studied. Because its nutritive characteristics, high content of oligosaccharides (0.4% of raffinose, 3.23 % of estaquiose, and 0.12% of verbacose of DM; Serrano and Goñi, 2004), and the presence of anti-nutritional factors (e.g., lectins, protease inhibitor factor, tannins, saponins; Mejia et al., 2003), it was hypothesized that the inclusion of cull pinto bean in a finishing diet of lambs affects ruminal fermentation and kinetics of hair lambs. The objective was to evaluate the effect of three levels of cull pinto bean (**CPB**) on feed intake, ruminal fermentation, kinetics, and nutrient digestibility of hair lambs.

## MATERIALS AND METHODS

All procedures involving animals were approved by local official techniques for animal care (NOM-051-ZOO-1995: Humanitarian care of animals during mobilization of animals; NOM-024-ZOO-1995: Animal health stipulations and characteristics during transportation of animals). This study was conducted at the Facultad de Zootecnia y Ecología of the Universidad Autónoma de Chihuahua, Chihuahua City, México.

Six crossbred (Dorper × Pelibuey and Charolais × Pelibuey) rumen fistulated lambs averaging 56.6 ± 3.8 kg were used. During the experiment lambs were allocated in individual cages, and fed ad libitum (0800 and 1800 h) with iso-energetic and iso-nitrogenous diets. Composition of the experimental diets is shown in Table 1. Lambs were randomly assigned to 1 of 3 treatments. Treatments were (DM basis): 1) 0% of CPB of the supplement (**CON**); 2) 25% of CPB of the supplement (**F25**); and 40% of CPB of the supplement (**F40**). During each period, animals received 8 d of adaptation to diets. Dry matter intake was evaluated daily during the sampling period (8 d). A ruminal fluid sample was obtained on d 1 (0, 1, 2, 4, 8, 12, 18, 24 h after feeding) of

**Table 1.** Ingredients and chemical composition (DM basis) of diets for hair lambs fed cull pinto bean

| | Treatments[1] | | |
|---|---|---|---|
| Ingredients (%) | CON | F25 | F40 |
| Cull pinto bean | 0 | 26.6 | 43.6 |
| Ground sorghum | 34.7 | 17.6 | 28.3 |
| Dry corn distiller grain | 47.2 | 44.3 | 6.2 |
| Cottonseed meal | 15.7 | 9.1 | 19.5 |
| Salt | 1.2 | 1.2 | 1.2 |
| Mineral Premix[2] | 1.2 | 1.2 | 1.2 |
| Calculated chemical composition (% DM basis) | | | |
| CP | 21.8 | 21.8 | 21.8 |
| ME, (Mcal/kg) | 2.8 | 2.8 | 2.8 |
| Ca | 0.25 | 0.23 | 0.22 |
| P | 0.65 | 0.51 | 0.51 |

[1]CON: 0% of CPB of the supplement; F25: 25% of CPB of the supplement; F40: 40% of CPB of the supplement

[2]Microfos 12:10: P: 12%; Ca: 11.5%; Mg: 0.6%; Mn: 2160ppm; Zn: 2850 ppm; Fe: 580 ppm; Cu: 1100 ppm; I: 102 ppm; Co: 13 ppm; Se: 9 ppm; Vitamins: A: 22000IU/kg; E: 24500 IU/kg.

sampling period to evaluate ruminal pH (UltraBASIC pH/mV Meter; Denver Instrument), ruminal $NH_3$ (Broderick and Kang, 1980) and VFA production. Methane production was estimated according to the method proposed by Wolin (1960). One gram of chromic oxide (99% of purity) was administrated to each lamb in the second day (0800 h) of the sampling period, in order to evaluate Kp and MRT. Feces samples (30 g) were obtained during 6 d (0, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108, 120, 132, 144 h) and frozen until analyzed. Feces samples were processed according to the method of Williams et al. (1962) and then Cr concentration was obtained by atomic absorption spectrophotometry. For digestibility of DM, CP, and NDF, fecal samples were taken directly from the rectum 4 times daily as follows: d 1 at 0800, 1000, 1200 and 1400 h; d 2 at 1600, 1800, 2000 and 2200 h; and d 3 at 2400, 0200, 0400 and 0600 h. Individual fecal samples consisted of approximately 50 g (wet basis). Samples for each animal were composited for analysis and stored at -20ºC. Composited fecal samples were dried in a forced air oven (60ºC) for 5 d. Feed, orts, and fecal samples were ground in a Wiley mill (1-mm screen) and analyzed for DM, OM, CP, ADF and NDF as described previously. Feed and fecal samples were incubated DAISYII system (Ankom Technology Corp., Fairport, NY) during 5 d (Mabjeesh et al., 2000). After incubation, bags were washed four times with cold water during 5 min, and then dried (60ºC) during 24 h. Concentration of ADF was determined in the bag residue, in order to calculate the percentage of indigestible ADF (Penning and Johnson, 1981). Apparent DM digestibility was predicted using insoluble ADF according to the following formula (Schneider and Flatt, 1975): DMD = {100-[100*(%IADF in feed/% IADF in feces)]}. Apparent digestibility of CP and NDF were calculated using the following formula: ND = (100-{100*[(% IADF in feed/% IADF in feces)*(% of nutrient in feces/% of nutrient in feed)]}). Data for DMI, ruminal pH, ammonia and VFA concentration, methane production, DMD, CPD, and NDFD were analyzed with the MIXED procedure (SAS Inst. Inc., Cary, NC) in a Latin square design 3 × 3 repeated in line. Model statement included the effect of treatment, period, repetition and hour (except for DMI, DMD, CPD, and NDFD). Estimates used for Kp, and MRT were obtained by a non linear regression model (Pond et al., 1987), and analyzed by NLIN procedure of SAS. Information obtained was analyzed by ANOVA in order to understand the effect of cull pinto bean inclusion level on Kp and MRT in a Latin square design 3 × 3 repeated in line. Fixed effects were: treatment, period, column and repetition. Information was analyzed by Mixed procedure of SAS. When significant ($P < 0.05$) F-statistics were noted, means were separated using linear and quadratic contrast.

## RESULTS AND DISCUSSION

There was a difference in DMI (kg; $P \leq 0.05$) among treatments, (CON: 1.77 ± 0.083; F25: 1.62 ± 0.083; and F40: 1.62 ± 0.083) F25 and f40 were decreased probably due to anti-nutritional factors in CPB. Paduano et al. (1995) found that ovines can tolerate a low intake of antinutritional factors without having negative effects, although, as antinutritional factors intake increase, DMI decrease, which explain the greater DMI by CON. Similar results have been found by different authors (Encinias et al., 2000; Soto-Navarro et al., 2004), in beef cattle supplemented with field peas; and Villalobos et al. (2010) reported a lineal decrease in DMI in hair lambs as cull pinto bean level increased. Ruminal pH was not different ($P \geq 0.05$) among treatments (CON: 6.19 ± 0.07; F25: 6.23 ± 0.07; and F40: 6.19 ± 0.07). Similar

**Table 2.** Ruminal volatile fatty acid concentration of hair lambs fed with different cull pinto bean level

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Volatile fatty acid | CON | F25 | F40 | S. E. | *P*-value |
| Total VFA | 60.4[b] | 68.4[a] | 64.2[a b] | 2.1 | ≤ 0.05 |
| Acetate | 36.8[a] | 39.8[b] | 42.3[b] | 2.8 | ≤ 0.05 |
| Propionate | 17.5[a] | 21.9[b] | 14.5[a] | 3.9 | ≤ 0.01 |
| Butyrate | 6.1[a] | 6.8[b] | 7.39[c] | 0.6 | ≤ 0.0001 |
| Acetate:propionate ratio | 2.3[a] | 2.2[a] | 2.9[b] | 0.07 | ≤ 0.0001 |

[1]CON: 0% of CPB of the supplement; F25: 25% of CPB of the supplement; F40: 40% of CPB of the supplement.

[a-c]Different superscript in row means difference among treatments ($P < 0.05$).

results were found by Gilbery et al. (2007) with different legume grains levels in receiving diets for beef cattle. Data for $NH_3$ (mg/100 ml of ruminal fluid) showed that there was no difference ($P \geq 0.05$) among treatments (CON: 27.2 ± 1.7; F25: 26.6 ± 1.7; and F40: 27.43 ± 1.7). Ruminal ammonia concentration is due to nitrogen ruminal degradation rate, and the concentration of RDP. Similar results were found by Gilbery et al. (2007) in beef cattle. Differences were found ($P \leq 0.03$) for ruminal VFA (mM) concentration (CON: 60.37 ± 2.1; F25: 68.42 ± 2.1; F40: 64.23 ± 2.1). Greater DMI of CON did not affect total VFA. The greater value in F40 could be due to its greater oligosaccharide content. Acetate: propionate ratio was greater ($P \leq 0.0001$) for F40 (Table 2). Similar results were reported by Reed et al. (2004) where the inclusion of field peas increased VFA ruminal concentration. Nonetheless, Gilbery et al (2007) found that the inclusion of different legume grains in receiving feedlot diets decreased VFA ruminal concentration. Volatile fatty acid production is influenced by different factors as diet type, DMI, management, physiological status, among others. Ruminal concentration of VFA is regulated by a balance among production and absorption. Methane production (mM/ml) was different ($P \leq 0.032$) among treatments (CON: 29.06 ± 0.75; F25: 27.59 ± 0.75; F40: 32.87 ± 0.75). Nowadays information available about methane production related to this study is limited. Pelchen and Peters (1998) reported that there is no difference in methane production when lambs are feeding with diets with a dry matter digestibility between 60 and 80%. In the present experiment, dry matter digestibility averaged 69% and was not different among treatments, although methane production was greater for F40 group. These differences could be due the high presence of oligosaccharides in high level of cull pinto bean diet. Rate of passage (%/h; CON: 3.6 ± 1.9; F25: 2.9 ± 1.9; F40: 5.9 ± 1.9%) and MRT (h) (CON: 36.26 ± 13.7; F25: 57.68 ± 13.7; F40: 36.24 ± 13.7h) were similar ($P \geq 0.42$) among treatments. Dry matter intake is probably the most important variable related with MRT (Colucci et al., 1990). In the present study DMI did not affect Kp and MRT. These results can be explained due to the chemical and physical composition of the diets which were similar among treatments. Bernard et al. (2000) said that a decrease in MRT is related to a decreased organic matter and neutral detergent fiber digestibility, but in the present study digestibility of DM and NDF were similar among treatments without affecting Kp and MRT. Digestibility (%) of DM (CON: 68.9 ± 1.45; F25: 69.5 ± 1.45; F40: 69.4 ± 1.45), CP (CON: 73.6 ± 1.5; F25: 73.8 ± 1.5; F40: 71.8 ± 1.5), and NDF (CON: 48.2 ± 2.1; F25: 48.6 ± 2.1; F40: 44.6 ± 2.1) were similar ($P \geq 0.26$) among treatments. These results showed that in the present study anti nutritional factors did not affect digestibility. Different results were presented by Williams et al. (1984), Singh et al. (2006) and Gilbery et al. (2007), these experiments reported a reduction in digestibility of DM or CP as level of grain legume in the diet increased.

## IMPLICATIONS

Cull pinto bean reduce DMI; although, it does not affect ruminal pH and $NH_3$ production. The inclusion of 25% of CPB in the diet of hair lambs allows having an appropriate nutrient digestibility without affecting Kp and MRT, and this increases the molar proportion of VFA without increasing methane production maintaining acetate: propionate ratio.

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# SWAINSONINE EXCRETION, NUTRIENT DIGESTIBILITY AND NITROGEN RETENTION OF LAMBS FED ALFALFA HAY, LOCOWEED, AND NOVEL FEED ADDITIVES[1]

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**ABSTRACT:** Novel products are needed that could reduce locoweed toxicity, alleviate impaired performance, and prevent possible death when consumed by livestock. This study evaluated the effect of 3 feed additives on swainsonine intake and excretion, nutrient digestibility, and N retention of 40 wether lambs (39 ± 0.4 kg initial BW). Lambs were blocked by initial BW and assigned to 5 dietary treatments in a randomized complete block design (4 blocks). Treatments were a control diet (86% alfalfa hay and 14% corn-based supplement) fed to lambs at 1.8% of BW (as fed) for 20 d (CON), CON with 20 g/d locoweed replacing alfalfa hay (LOCO), LOCO with 50 g/d of feed additive 1 replacing alfalfa hay (AK1), LOCO with 50 g/d of feed additive 2 replacing alfalfa hay (AK2), and LOCO with 50 g/d of feed additive 3 replacing alfalfa hay (AK3). Lambs were housed individually for 14 d in pens and then for 6 d in metabolism crates for total fecal and urine collections. Statistical analysis used the mixed procedure of SAS with lamb as the experimental unit. Intake, fecal, and urinary swainsonine were greater ($P < 0.05$) for LOCO, AK1, AK2, and AK3 than CON. Intake of swainsonine was lower ($P < 0.05$) for AK3 than LOCO, fecal swainsonine was lower ($P < 0.05$) for AK1 than LOCO, and urinary swainsonine was less ($P < 0.05$) for AK1 and AK2 than LOCO. Treatments did not affect (P 2 0.20) DM intake, fecal DM, or DM digestibility. Nitrogen intake was less ($P < 0.05$) for AK1, AK2, and AK3 than for CON and LOCO, but fecal N and urine N was not affected (P 2 0.11) by treatments. Nitrogen digestibility was not different ($P = 0.26$) among treatments, but N retention was less ($P < 0.05$) for AK1 and AK3 than CON. In summary, lamb consumption of locoweed with the feed additives evaluated in the current study does not significantly affect DM and N digestibility. Decreased fecal and urinary swainsonine in lambs receiving AK1 indicated that it may affect metabolism of swainsonine in sheep.

**Key words:** nitrogen retention, sheep, swainsonine

## INTRODUCTION

Locoweed species (Astragalus and Oxytropis spp.) are poisonous plants responsible for large economic losses estimated at $300 million or more in the livestock industry (Nielsen and James, 1992). Swainsonine, the primary toxin in locoweed (Molyneux and James, 1982), is an inhibitor of a-mannosidase, causes accumulation of oligosaccharides in the lysosomes (Dorling et al., 1980), and alters the processing of glycoproteins in the Golgi (Kang et al., 1993). Many nutrient transporters, such as sodium-dependent glucose transporter-1 and intestinal amino acid regulatory proteins (rBAT and 4F2), are glycoproteins in nature (Wright et al., 1994; Mailliard et al., 1995). Therefore, the effects of swainsonine on synthesis, processing, and transport of glycoproteins may alter gastrointestinal function and nutrient absorption (Pan et al., 1993).

Taylor et al. (2000) reported that lower serum cholesterol, triglycerides, and Fe concentration in sheep consuming swainsonine may be caused by altered nutrient digestion and metabolism. Additionally, Reed et al. (2003) demonstrated that ruminal $NH_3$ and in situ dry matter digestion were altered when wether lambs were fed locoweed, Reed (2004) reported negative N balance for wether lambs fed 1.6 mg swainsonine per kg of body weight, while control lambs had a positive N balance. Previous research has also demonstrated that swainsonine induces vacuolization of hepatocytes of rats (Novikoff et al., 1985). Preliminary research (unpublished) indicated that supplementation of novel feed products containing a combination bacterial cell walls, yeast, and enzymes minimized the negative effects of swainsonine on rumen epithelial cells and liver weight of sheep fed locoweed. Therefore, the objectives of this experiment were 1) to evaluate the effects of locoweed on nutrient digestion and N balance of lambs, and 2) to evaluate the potential for three novel feed additives (Agri-King Inc., Fulton, IL) to alter swainsonine excretion and minimize the negative effects of locoweed on nutrient digestibility and N balance.

## MATERIALS AND METHODS

Experimental procedures were approved by the New Mexico State Institutional Animal Care and Use Committee.

***Animals, Design, and Treatments.*** The experiment was conducted in the Physiology and Nutrition Building at New Mexico State University in Las Cruces, New Mexico. Forty wether lambs (39 ± 0.4 kg initial BW) were equally divided into 4 BW blocks, and within each block were randomly assigned to 1 of 5 dietary treatments in a randomized

[1] Authors acknowledge A. Temple and Agri-King, Inc. for supply of feed products and support with sample analysis.

complete block design. The experimental period for each block was 20 d; animals were housed in individual feeding pens from d 1 to 14 for adaptation to dietary treatments, and in metabolism crates from d 15 to 20 for urine and fecal collections. Treatments were a control diet (86% alfalfa hay and 14% corn-based supplement) fed to lambs at 1.8% of BW (as fed) for 20 d (**CON**), CON with 20 g/d locoweed replacing alfalfa hay (**LOCO**), LOCO with 50 g/d of feed additive 1 replacing alfalfa hay (**AK1**), LOCO with 50 g/d of feed additive 2 replacing alfalfa hay (**AK2**), and LOCO with 50 g/d of feed additive 3 replacing alfalfa hay (**AK3**). Locoweed (Astragalus allochrous) was collected in April in southeast New Mexico, allowed to air dry, and passed through a forage chopper (The Western Bear Cat No 5A, by Western Land Roller Co., Hastings, NE) to reduce particle size. The amount of locoweed fed was calculated to supply approximately 2 mg swainsonine per kg of BW daily.

***Sample Collections.*** Feed samples were collected every week after mixing of dietary treatments and stored at -20°C for analysis. Feed refusals were collected daily before the morning feeding, weighed, and then stored at -20°C. Total fecal and urinary outputs were collected daily for 6 days (from d 15 to d 20). Feces was collected into pans attached to metabolism crates and urine was collected via a funnel into 20-L plastic buckets containing 50 mL of HCl (6 M) to trap N and reduce $NH_3$ loss. Total daily fecal output, and 10% of the daily urine output was frozen at -20°C. At the end of the collection period, feed refusals, and fecal and urine samples were thawed, mixed thoroughly for each lamb, and a representative sample was stored at -20°C to be analyzed later.

***Sample Analysis.*** Diets, refusals and fecal composited samples were weighed, dried for 72 h at 55°C in a forced air oven (Blue M Electric Company, Blue Island, IL), allowed to equilibrate overnight at room temperature, and then weighed to measure moisture loss. Dried samples were ground in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. All samples of dietary treatments, feed refusals, feces and urine were analyzed for swainsonine concentration using a modified a- mannosidase inhibition assay as described by Taylor and Strickland (2002). Ground samples were analyzed for DM after being placed in an oven (Model 845, Precision Scientific Group, Chicago, IL) at 105°C for 24 h, and for ash after being placed in a muffle furnace at 550°C for 8 h. Samples were analyzed for NDF and ADF using Ankom protocol (Ankom 200 fiber analyzer, Ankom Technology Cooperation, Fairport, NY) by boiling ground samples in neutral detergent solution with a-amylase enzyme for 75 min followed by three times of 5-min washing in hot water for NDF analysis, and in acid detergent solution for 60 min followed by three times of 5-min washing in hot water for ADF analysis. Diets, refusals, fecal, and urinary samples were analyzed for N by measuring $N_2$ produced upon complete combustion of samples in a thermo-conductivity cell using an N analyzer (Leco, Model FP-528, LECO Corporation, St. Joseph, MI).

***Statistical Analysis.*** The experiment was a randomized complete block design, and all data were analyzed using mixed models (SAS Inst. Inc., Cary, NC) with lamb as the experimental unit. Due to a limited number (10) of the metabolism crates, lambs were equally divided into 4 complete blocks based on BW and date in metabolism crates. The statistical model included treatment, day, and treatment × day interaction as fixed effects, and block was random. When treatment × day interactions were not significant, single degree of freedom contrasts were used to compare CON with the average for all other treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3. Differences among treatments were considered significant when $P < 0.05$.

## RESULTS

Intake, fecal, and urinary swainsonine were greater ($P < 0.05$) for treatments containing locoweed than CON (Table 2). Intake of swainsonine was less ($P < 0.05$) for AK3 than LOCO, fecal swainsonine was lower ($P < 0.05$) for AK1 than LOCO, and urinary swainsonine was less ($P < 0.05$) for AK1 and AK2 than LOCO. Treatments did not affect (P 2 0.20) DM intake, fecal DM, DM digested, or DM digestibility. Intake of OM was greater ($P < 0.05$) for AK3 than LOCO, but fecal OM and OM digestibility were not different ($P$ 2 0.62) among dietary treatments. Intake of NDF was less ($P < 0.05$) for AK1 and AK3 than LOCO, and was greater ($P < 0.05$) for AK2 compared with LOCO. No differences ($P$ 2 0.09) were observed among treatments for fecal NDF and NDF digestibility, but grams of NDF digested was less ($P < 0.05$) for AK1 than LOCO. Intake of ADF was greater ($P < 0.05$) for treatments containing locoweed than CON, less ($P < 0.05$) for AK1 than LOCO, and greater ($P < 0.05$) for AK2 than LOCO. Fecal ADF and ADF digestibility were not different ($P = 0.06$) among dietary treatments, but ADF digested was less ($P < 0.05$) for AK1 than LOCO. Nitrogen intake was less ($P < 0.05$) for treatments containing locoweed than CON, and less ($P < 0.05$) for AK1, AK2, and AK3 than LOCO. However, fecal N and urine N was not affected ($P > 0.11$) by treatments. Nitrogen digestibility was not different ($P = 0.26$) among treatments, but grams of N digested was less ($P < 0.05$) for treatments containing locoweed than CON, and less ($P < 0.05$) for AK1, AK2, and AK3 than LOCO. Retained N and N retention was less ($P < 0.05$) for treatments containing locoweed than CON, and lower ($P < 0.05$) for AK1 than LOCO.

## DISCUSSION

***Effects of Feeding Locoweed.*** Many nutrient transporters are glycoproteins in nature (Wright et al., 1994; Mailliard et al., 1995), and because swainsonine alters the processing of glycoproteins in the Golgi (Kang et al., 1993), it is possible that locoweed in livestock diets could affect nutrient absorption and metabolism. In the current study, no differences in DM, OM, and NDF intake between lambs fed treatments containing locoweed and CON are likely

**Table 1.** Dietary treatments fed to lambs

| Item | CON | LOCO | AK1 | AK2 | AK3 |
|---|---|---|---|---|---|
| Ingredient, g/d | | | | | |
| Alfalfa hay | 620 | 600 | 550 | 550 | 550 |
| Corn grain | 95 | 95 | 95 | 95 | 95 |
| Feed product[1] | 0 | 0 | 50 | 50 | 50 |
| Locoweed[2] | 0 | 20 | 20 | 20 | 20 |
| Molasses | 5 | 5 | 5 | 5 | 5 |
| Nutrient, % DM | | | | | |
| OM | 88.8 | 88.5 | 88.5 | 88.5 | 88.8 |
| NDF | 48.4 | 48.7 | 47.7 | 49.9 | 48.1 |
| ADF | 35.8 | 36.2 | 35.8 | 37.6 | 36.2 |
| CP | 18.3 | 18.4 | 17.3 | 17.5 | 17.7 |
| Swainsonine[3], mg/kg DM | 0 | 149 | 145 | 150 | 123 |

[1]Novel feed products containing a combination of bacterial cell walls, yeast, and enzymes, with rice hulls as the carrier.
[2]Astragalus allochrous (half moon locoweed).
[3]Analyzed using the modified a-mannosidase inhibition assay as described by Taylor and Strickland (2002).

**Table 2.** Intake, excretion of swainsonine, and digestibility of DM, OM, NDF and ADF, and N retention of lambs exposed to locoweed toxicity and supplemented with novel feed additives

| | Treatments[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | CON | LOCO | AK1 | AK2 | AK3 | SEM | *P*-value[2] | Contrast[3] |
| Swainsonine | | | | | | | | |
| Intake, mg/d | 0 | 98.2 | 95.9 | 99.4 | 81.4 | 3.93 | <0.01 | 4,7 |
| Feces, mg/d | 0 | 6.35 | 4.29 | 6.24 | 5.18 | 0.66 | <0.01 | 4,5 |
| Urine, mg/d | 0 | 25.3 | 19.6 | 16.4 | 25.6 | 1.73 | <0.01 | 4,5,6 |
| DM | | | | | | | | |
| Intake, g/d | 660 | 660 | 661 | 660 | 662 | 0.74 | 0.20 | NS |
| Feces, g/d | 239 | 245 | 254 | 246 | 249 | 6.02 | 0.52 | NS |
| Digested, g/d | 421 | 415 | 407 | 415 | 413 | 6.05 | 0.62 | NS |
| Digestibility, % of intake | 63.8 | 62.9 | 61.6 | 62.8 | 62.4 | 0.91 | 0.56 | NS |
| OM | | | | | | | | |
| Intake, g/d | 586 | 584 | 585 | 584 | 588 | 0.81 | <0.01 | 7 |
| Feces, g/d | 204 | 208 | 216 | 209 | 213 | 5.73 | 0.62 | NS |
| Digested, g/d | 382 | 375 | 369 | 376 | 375 | 5.81 | 0.64 | NS |
| Digestibility, % of intake | 65.2 | 64.3 | 63.0 | 64.3 | 63.8 | 0.98 | 0.64 | NS |
| NDF | | | | | | | | |
| Intake, g/d | 319 | 322 | 315 | 330 | 318 | 1.12 | <0.01 | 5,6,7 |
| Feces, g/d | 138 | 148 | 159 | 148 | 155 | 5.86 | 0.16 | NS |
| Digested, g/d | 181 | 173 | 156 | 181 | 164 | 6.14 | 0.03 | 5 |
| Digestibility, % of intake | 56.5 | 53.9 | 49.6 | 55.0 | 51.5 | 1.83 | 0.09 | NS |
| ADF | | | | | | | | |
| Intake, g/d | 236 | 239 | 236 | 248 | 240 | 0.79 | <0.01 | 4,5,6 |
| Feces, g/d | 107 | 115 | 126 | 114 | 122 | 4.56 | 0.06 | NS |
| Digested, g/d | 129 | 124 | 110 | 134 | 118 | 4.73 | 0.01 | 5 |
| Digestibility, % of intake | 54.2 | 51.9 | 46.7 | 53.9 | 48.9 | 1.91 | 0.04 | NS |
| N | | | | | | | | |
| Intake, g/d | 19.3 | 19.4 | 18.3 | 18.5 | 18.7 | 0.05 | <0.01 | 4,5,6,7 |
| Feces, g/d | 6.12 | 6.32 | 6.11 | 6.28 | 6.07 | 0.15 | 0.68 | NS |
| Urine, g/d | 12.5 | 13.3 | 14.6 | 13.0 | 14.1 | 0.56 | 0.11 | NS |
| Digested, g/d | 13.2 | 13.1 | 12.2 | 12.3 | 12.7 | 0.14 | <0.01 | 4,5,6,7 |
| Retained, g/d | 0.82 | -0.22 | -2.39 | -0.75 | -1.47 | 0.69 | 0.02 | 4,5 |
| Digestibility, % of intake | 68.3 | 67.3 | 66.4 | 65.9 | 67.5 | 0.80 | 0.26 | NS |
| Retention, % of intake | 4.41 | -1.76 | -13.9 | -4.87 | -8.34 | 3.83 | 0.01 | 4,5 |

[1]CON = basal diet of 620 g/d of alfalfa hay and 100 g/d of corn-based feed fed to lambs in equal portions twice daily (0730 and 1930) for 20 d; LOCO = 20 g/d of locoweed replaced alfalfa hay in basal diet; AK1 = 20 g/d of locoweed plus 50 g/d of feed product 1 replaced alfalfa hay in basal diet; AK2 = 20 g/d of locoweed plus 50 g/d of feed product 2 replaced alfalfa hay in basal diet; AK3 = 20 g/d of locoweed plus 50 g/d of feed product 3 replaced alfalfa hay in basal diet. Novel feed products were supplied by Agri-King Inc.
[2]Observed significance level for the type 3 test of treatment fixed effects.
[3]Contrast: 4 = CON vs. average of LOCO, AK1, AK2, and AK3 ($P < 0.05$); 5 = LOCO vs. AK1 ($P < 0.05$); 6 = LOCO vs. AK2 ($P < 0.05$); 7 = LOCO vs. AK3 ($P < 0.05$); NS = all contrasts were not significant ($P > 0.05$).

because the total feed offered (as fed) was limited to 1.8% of BW (DM intake was 1.7% of BW) to encourage complete consumption of the diets and to minimize selective refusal of the supplement containing locoweed and feed product. Our findings are in contrast to Pfister et al., (1996), who observed decreases in intake when sheep were fed alfalfa pellets containing 10% locoweed at 1.6% of BW. Also, Obeidat (2004) reported decreases in DM intake when the diet of limit-fed (1.8% of BW) sheep contained 20% locoweed. Although the contrast that compared the average of the locoweed-containing treatments with the CON showed significant differences for ADF intake, N intake, and N digested, these responses appeared to not be due to the locoweed, but due to presence of the novel feed additives. A tendency for greater urinary N excretion, and negative N retention for lambs fed locoweed-containing treatment versus positive N retention for CON lambs is consistent with Reed (2004), and suggests that swainsonine alters N metabolism.

***Effects of Feeding Novel Product.*** Preliminary research (unpublished) demonstrated that novel feed additives containing a combination of bacterial cell walls, yeast, and enzymes minimized subclinical symptoms associated with swainsonine toxicity and appeared to increase the tolerance of sheep to swainsonine. Although the mode of action has not been determined, our hypothesis was that these novel feed products will increase swainsonine excretion and minimize the negative effects of locoweed on nutrient digestibility and N balance. However, the addition of AK1, AK2, or AK3 to the diets of lambs consuming locoweed did not increase the excretion of swainsonine via feces or urine. In contrast, feeding AK1 to lambs decreased swainsonine excretion in the feces and urine. Lambs fed AK2 also had lower urinary swainsonine excretion than CON lambs.

Each novel feed product contained different levels a combination of bacterial cell walls, yeast, and enzymes, with rice hulls as the carrier. Therefore, the effects of AK1, AK2, and AK3 on fiber (NDF and ADF) intake are likely due to differences in fiber concentrations between the novel feed products and the forages (locoweed and alfalfa hay). Similarly, the novel feed products contained less crude protein than locoweed and alfalfa hay, and therefore lambs fed AK1, AK2, and AK3 had lower N intake and lower grams of N digested compared with lambs fed LOCO.

## CONCLUSIONS

The results demonstrate that lamb consumption of locoweed with the feed additives evaluated in the current study does not significantly affect DM and N digestibility. Lower fecal and urinary swainsonine in lambs receiving AK1 indicated that this novel feed product may affect metabolism of swainsonine in sheep.

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# UTILIZATION OF *YUCCA SCHIDIGERA* TO ALTER HYDROGEN SULFIDE GAS PRODUCTION FROM RUMEN FLUID IN VITRO[1]

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**ABSTRACT:** This study evaluated the effects of an additive from *Yucca schidigera* (Ruma-Just, Nova Microbial Technologies) on in vitro fermentation of ground corn grain, total gas and $H_2S$ production, and rumen fluid viscosity. Rumen fluid was collected from a ruminally cannulated cow fed a corn-based diet. Anaerobic fermentations were conducted in 250-mL serum bottles containing 50 mL rumen fluid, 50 mL McDougal's buffer, and 1 g of ground corn grain that was thoroughly mixed with one of 6 treatments. Treatments, in a 3 × 2 factorial arrangement, were ground corn grain substrate mixed with 0%, 0.01%, or 0.02% Ruma-Just and 0% added sulfur, or 0%, 0.01%, or 0.02% Ruma-Just with 0.6% added sulfur as sodium sulfate. Total gas production was recorded, and a sample of gas for $H_2S$ analysis was collected after incubating 9 replicate bottles per treatment for 24 h at 39°C. Contents from in vitro fermentations were frozen at -20°C, and later filtered for IVDMD and rumen fluid viscosity measurements. Gas production decreased in response to 0.01% Ruma-Just when no sulfur was added, and gas production decreased in response to 0.02% Ruma-Just when 0.6% sulfur was added (Ruma-Just × S interaction, $P < 0.01$). Ruma-Just at both 0.01% and 0.02% increased IVDMD when no sulfur was added, but Ruma-Just did not affect IVDMD when 0.6% sulfur was added (Ruma-Just × S interaction, $P = 0.07$). Production of $H_2S$ per gram of substrate was not affected by Ruma-Just when 0% sulfur was added, but $H_2S$ production decreased in response to 0.02% Ruma-Just with 0.6% added sulfur (Ruma-Just × S interaction, $P = 0.01$). By design, addition of 0.6% sulfur increased ($P < 0.01$) $H_2S$ production. Rumen fluid viscosity tended to be lower ($P = 0.10$) for 0.02% Ruma-Just than 0% and 0.01% Ruma-Just. In conclusion, *Yucca schidigera* may decrease total gas and $H_2S$ production in ruminants consuming diets high in sulfur.

**Key words:** hydrogen sulfide, rumen, *Yucca schidigera*

## INTRODUCTION

According to the Renewable Fuels Association (2011), the U.S. produced 13.9 billion gallons of ethanol in 2011. This translates to 5.15 billion bushel of corn and 35.7 million metric tons of distillers' grain products. Dry distiller grains with solubles (DDGS) and wet distiller grains with solubles (WDGS) are high in protein, low in starch, andare useful fed co-products for ruminant livestock. During the ethanol production process, corn is treated with sulfuric acid to facilitate hydrolysis and enhance fermentation. This result in high concentration of sulfur in distillers grains, which when fed to ruminant animals can cause polioencephalomalacia due to ruminal $H_2S$ gas production.

*Yucca schidigera*, which is native to southwestern U.S. and Mexico, contains physiologically active phyto- chemicals, polyphenolics such as resveratrol and stilbenes, and is a rich source of steroidal saponins (Cheeke et al., 2006) that have anti-bacterial, anti-protozoan, antifungal and ionophore-like activities. Goel et al. (2008) showed that saponin can improve overall ruminal activity, and Wallace et al. (1994) showed the ability to indirectly reduce ruminal $NH_3$. Wang et al. (2000) demonstrated that *Y. schidigera* reduced growth of *S. bovis*, *P. bryantii*, and *R. amylophilus,* but increased growth of *S. ruminantium*. Coleman et al. (2010) characterized plant-derived saponin as having antifungal activity against clinical isolates of Candida albicans including hyphae and isolates known to be resistant to clinical drugs. McAllister et al. (1998) showed saponin to kill Giardia lamblia in both humans and animals. Different sources of saponin have different biological effects as demonstrated by the concentration dependent killing on *E. coli* K12 (Sen et al., 1998).

Based on the properties of *Y. schidigera*, it was hypothesized that treatments containing yucca powder will alter rumen fermentation by decreasing total gas and $H_2S$ production and viscosity of rumen fluid. Therefore, this study evaluated the effects of an additive from *Y. schidigera* dried plant powder (Ruma-Just, Nova Microbial Technologies) on in vitro fermentation of ground corn grain, total gas and $H_2S$ production, and viscosity.

## MATERIALS AND METHODS

***Treatments.*** Six treatments combinations in a 3 × 2 factorial arrangement were utilized for in vitro total gas and $H_2S$ production, IVDMD and viscosity measurements. Treatments included ground corn grain substrate thoroughly mixed with 0%, 0.01%, or 0.02% Ruma-Just and 0% added

[1]Authors acknowledge R. Goodall and Nova Microbial Technologies for funding.

sulfur, or 0%, 0.01%, or 0.02% Ruma-Just with 0.6% added sulfur as sodium sulfate. These concentrations of Ruma-Just were equivalent to supplementing 0, 1, or 2 g/d of Ruma-Just to a steer consuming 10 kg/d of feed. A total of 96 in vitro fermentations in serum bottles were used in 4 runs; each run utilized 24 in vitro serum bottles, which allows 3 blank (no rumen fluid), 3 control (rumen fluid, McDougal's buffer and no substrate) incubations, and 3 replicated flasks in each run to evaluate the effects of 6 treatment combinations in a 2 × 3 factorial arrangement.

***In Vitro $H_2S$ Gas Production.*** Rumen content was collected from a ruminally cannulated steer that was adapted to a corn-based diet (Table 1), placed into a pre-heated (39ºC) insulated container, and immediately transported to the laboratory. The in vitro fermentations was conducted in 250-mL serum bottles containing 50 mL strained rumen fluid, 50 mL of McDougal's buffer (Tilley and Terry, 1963), 1 g of a ground dietary substrate (corn grain), and treatments. Each bottle was flushed with $CO_2$, fitted with butyl rubber stoppers, and incubated in a LAB-LINE Orbit ENVIRON- Shaker at 39ºC. Gas production was measured via water displacement after 24 h by inserting a 21-guage needle through the butyl rubber stopper of the sample bottle, connected with tubing to 250-mL inverted buret cylinders. Gas production was corrected to blank incubations containing no rumen fluid.

Following gas production measurements, a sample of gas was collected (using a 5-mL syringe and 21-guage needle) from the head space of each 250-mL air-tight serum bottle and analyzed for $H_2S$ concentration using a Synergy HT plate reader (BioTek, Winooski, VT) according to the procedure of Quinn et al. (2009). In short, 5 mL head space gas was injected into vacuum tube containing 5 mL of alkaline water with a pH of 8.2. Next, 0.5 mL of ferric chloride and 0.5 mL of N, N-dimethyl-p-phenylenediamine were added to each tube, and incubated at 25°C for 30 min. Each sample was read in duplicate at 665 nm after transferring 200 µL to a 96 well microtiter plate. Standards were prepared from RAD 171 (Supelco) and the standard curve was prepared by dividing the known sulfide concentration by 0.9409 to determine the concentration of $H_2S$. Production of $H_2S$ was calculated by multiplying $H_2S$ concentration by total gas production and by correcting for DM fermented (see IVDMD below).

**Table 1.** Composition of the concentrate-based diet fed to the donor animal

| Item | DM basis |
|---|---|
| Ingredient, % | |
| Corn, dry rolled | 82.8 |
| Alfalfa hay, chopped | 10.0 |
| Molasses | 3.0 |
| Cottonseed meal | 2.0 |
| Limestone | 1.0 |
| Urea | 0.9 |
| Salt | 0.3 |
| Vitamin A premix[1] | + |
| Rumensin-80[2] | + |
| Nutrient, % | |
| CP | 13.5 |
| Ca | 0.52 |
| P | 0.30 |

[1]Supplied 2,200 IU vitamin A per kilogram diet DM.
[2]Supplied 33 mg monensin per kilogram diet DM.

***In Vitro Dry Matter Digestibility.*** After measuring and sampling gas, fermentations were stopped at the end of each 24 h incubation period by freezing at -20ºC. Tubes were thawed, and residuals from each tube filtered using polyester bags (5 × 10 cm; pore size = 50 µm, Bar Diamond Inc., Parma, ID). The polyester bags were sealed using a heat sealer, rinsed with cold water, dried at 105ºC, and weighed for analysis of DM. Blank tubes containing no substrate were used in calculation to allow for correcting of DM disappearance.

***Statistical Analysis.*** All data were analyzed statistically as a randomized complete block design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Because of limited space in the incubator for in vitro fermentations (experimental unit), the experiment was blocked by run; each of the 4 runs (blocks) contained 24 in vitro serum bottles. The statistical model included Ruma-Just, sulfur, and the Ruma-Just × sulfur interaction as fixed effects, and block as random effect. Differences were considered significant at $P < 0.10$.

## RESULTS

The effects of Ruma-Just and sulfur on total gas production, $H_2S$ gas production, IVDMD, and rumen fluid viscosity are presented in Table 2. Gas production decreased in response to 0.01% Ruma-Just when no sulfur was added, and gas production decreased in response to 0.02% Ruma-Just when 0.6% sulfur was added (Ruma-Just × S interaction, $P < 0.01$). Ruma-Just at both 0.01% and 0.02% increased IVDMD when no sulfur was added, but Ruma-Just did not affect IVDMD when 0.6% sulfur was added (Ruma-Just × S interaction, $P = 0.07$). Production of $H_2S$ per gram of substrate was not affected by Ruma-Just when 0% sulfur was added, but $H_2S$ production decreased in response to 0.02% Ruma-Just with 0.6% added sulfur (Ruma-Just × S interaction, $P = 0.01$). A similar tendency for a Ruma-Just × S interaction ($P = 0.11$) was observed for $H_2S$ production per gram of fermented substrate. By design, addition of 0.6% sulfur increased ($P < 0.01$) $H_2S$ production. Rumen fluid viscosity tended to be lower ($P = 0.10$) for 0.02% Ruma-Just than 0% and 0.01% Ruma-Just.

## DISCUSSION

In the wake of ethanol production as a source of alternative fuel, corn prices have increased and corn availability has decrease. Therefore, producers have turned to distiller's grains co-product, which is a low cost feedstock and is readily available as an alternative for finishing cattle. During the ethanol production process, corn is treated with sulfuric acid to facilitate hydrolysis and enhance fermentation, and therefore the amount of sulfur in distillers grains may range from 0.31 to 1.93% (Schingoethe et al., 2008). Distillers

**Table 2.** Effects of Ruma-Just (Nova Microbial Technologies) on in vitro fermentation of ground corn grain, total gas and $H_2S$ production and rumen fluid viscosity

| Item | Treatment[1] | | | | | | | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0% Sulfur | | | 0.6% Sulfur | | | | | | |
| | 0% RJ | 0.01% RJ | 0.02% RJ | 0% RJ | 0.01% RJ | 0.02% RJ | SEM | S | RJ | S×RJ |
| Gas,[2] mL | 229.0 | 221.5 | 229.2 | 234.3 | 236.3 | 218.5 | 4.27 | 0.19 | 0.03 | <0.01 |
| Gas,[2] mL/g fermented | 298.3 | 276.0 | 285.2 | 300.9 | 292.8 | 282.4 | 20.9 | 0.18 | <0.01 | 0.13 |
| IVDMD,[3] % | 77.0 | 80.6 | 81.3 | 78.2 | 80.9 | 78.2 | 4.42 | 0.46 | <0.01 | 0.07 |
| $H_2S$, µmol/L gas | 74.0 | 74.3 | 78.4 | 95.2 | 93.4 | 86.9 | 9.94 | <0.01 | 0.82 | 0.11 |
| $H_2S$, µmol/g substrate | 16.9 | 16.5 | 17.9 | 22.4 | 22.1 | 19.2 | 2.57 | <0.01 | 0.39 | 0.01 |
| $H_2S$, µmol/g fermented | 22.1 | 20.6 | 22.5 | 28.9 | 27.5 | 25.1 | 4.61 | <0.01 | 0.25 | 0.11 |
| Viscosity, cP | 2.81 | 2.76 | 2.63 | 2.77 | 2.75 | 2.69 | 0.74 | 0.95 | 0.10 | 0.73 |

[1]Treatments included ground corn grain substrate thoroughly mixed with 0%, 0.01%, or 0.02% Ruma-Just (RJ) and 0% added sulfur, or 0%, 0.01%, or 0.02% RJ with 0.6% added sulfur as sodium sulfate.
[2]In vitro gas production recorded after 24 h of anaerobic incubation at 39ºC.
[3]In vitro dry matter digestibility after 24 h of anaerobic incubation at 39ºC.

grains have been used as a replacement for corn up to 40% in the diet. However, greater percentages can lead to decreased performance, reduced availability and absorption of other minerals and causes polioencephalomalacia in cattle. To decrease animal inefficiency to feed containing sulfur, and sulfur induced polioencephalomalacia, there are numerous practices available such as injection with thiamine. Our result showed that addition of dried plant powder from *Yucca schidigera* (Ruma-Just) decreased $H_2S$ gas production in vitro, and may be an alternate feed additive for high-sulfur diets. However, prior to being implemented in animal feeding practices, these results need to be validated in an in vivo model. Nichols et al. (2011) reported that feeding Ruma-Just to beef steers had little effect on animal performance and carcass characteristics. It is important to consider the source of the saponin as there are findings that saponin reduced feed NDF digestibility and lowered methane production (Holtshausen et al., 2009).

Ruma-Just is dried powder from the *Yucca schidigera* plant known to decrease ruminal $NH_3$ concentrations and have antimicrobial and anti-protozoan properties (Wallace et al., 1994). Yucca extracts are also known to contain saponin-like activity that could decrease ruminal gas production. Our in vitro fermentation results showed that Ruma-Just decreased gas production while increasing IVDMD of ground corn substrate. Hristov et al. (2004) demonstrated that flaked corn grain DM and starch degradability were increased in the presence of saponin surfactant. This is consistent with our finding, which showed that both 0.01% and 0.02% Ruma-Just increased IVDMD. Observed increase in IVDMD and decreased gas production was surprising because digestion and gas production are generally expected to be positively correlated. A tendency for Ruma-Just to decrease rumen fluid viscosity may be attributable to the detergent-like and surfactant property of saponin (Francis et al., 2002), which may acts as a wetting agent, reduce surface tension and prevents trapping of fine particle. Therefore, the use of Ruma-Just as a potential bloat preventative warrants investigation.

In conclusion, dried plant powder from *Yucca schidigera* may decrease total gas and $H_2S$ production in ruminants consuming diets high in sulfur.

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# REMOTE MONITORING OF INDIVIDUAL ANIMAL MINERAL SUPPLEMENT INTAKE BY RANGE CATTLE[1]

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**ABSTRACT:** Individual animal intake and behavior associated with mineral supplementation of cattle on rangelands is largely undocumented. Our objective was to develop instrumentation to remotely obtain such information. We designed and constructed was a solar powered high precision load cell and continuous (every 0.25 s) data acquisition system, configured to a standard range mineral feeder. The system was field tested in November 2010 and October 2011 at the V Bar V Ranch in central Arizona. Load cell precision varied with temperature and wind. Wind speed (kph) ranged from 0 to 23 and averaged 14 ± 0.3 in 2010. Corresponding values were 0 to 32 and 11 ± 0.4 respectively, in 2011. Temperature (C) ranged from -7 to 4 and averaged -2 ± 0.9 in 2010. Corresponding values were -1 to 11 and 2 ± 1.1 respectively, in 2011. In pre-dawn hours when ambient conditions were relatively consistent, baseline variation of the load cells was ± 2 g. This value increased to ± 17 g during mid-day. Individual cow identification was accomplished via time-stamped, motion activated digital photography. Feeding bouts were classified by predetermined acute weight change thresholds and matched to a cow by visual inspection of images. Duration of feeding bouts (min) ranged from 1 to 16 and averaged 3 ± 0.4 in 2010, and 1 to 21 and 4 ± 0.4 respectively, in 2011 ($P > 0.1$). Mineral intake (g/feeding bout) ranged from 0 to 679 and averaged 109 ± 16.0 in 2010, and 0 to 1598 and 226 ± 25.4 respectively, in 2011 ($P < 0.05$). Measured total daily intake (g/cow) ranged from 0 to 1009 and averaged 190 ± 26.0 in 2010, and 0 to 2177 and 663 ± 108.9 respectively, in 2011 ($P < 0.05$). Intake rate (g/min) ranged from 1 to 182 and averaged 45 ± 5.2 in 2010, and 1 to 446 and 82 ± 8.0 respectively, in 2011 ($P < 0.05$). Correlation of the classification protocol was tested between two independent observers. For discrete (n = 5) bouts, $r^2 = 0.95$, SE = 5.3 ($P < 0.05$), and for multiple animal (n = 7) bouts, $r^2 = 0.75$, SE = 18.0 ($P < 0.05$). This system, as tested, is capable of providing individual animal intake of mineral supplement under rangeland management conditions.

**Key words:** Individual animal intake, mineral supplement, remote measurement.

## INTRODUCTION

Mineral supplementation is an essential part of all beef production settings. Being able to provide those necessary minerals in the most efficient manner becomes crucial to ensure optimum production. In intensively managed production settings, this would be through direct incorporation into the formulated diet. In range production settings, this is not possible and therefore managers often choose to provide supplements in a much less precise manner. Due to the physical limitations presented, minerals are often supplied free choice where large quantities of supplement can be transported and left with the herd at one time. This assumes that the mineral will be consumed at proper quantities, implying that the animals will limit their intake, although the degree to which they possess this wisdom is debatable (Tait and Fisher, 1996). Animal behavior is also related to intake. The ability to make mineral supplements available to the herd in the proper location in the pasture is important. At what time of the day mineral consumption is likely to occur, for instance, can influence location in the pasture of the supplement based upon known animal behavior patterns (Tait and Fisher, 1996). Therefore, it becomes obvious that our understanding of individual animal mineral supplement consumption must improve in order to provide range livestock managers with the knowledge and tools that they need to efficiently provide minerals to their herd. With the above being said, our objective was to measure individual animal intakes and the associated behavior with that intake (e.g., number of feeding bouts, rate of consumption, time of day) using remote measurements in an extensive rangeland setting.

## MATERIAL AND METHODS

Animal care was approved by the University of Arizona, Institutional Animal Care and Use Committee (Protocol # 08-131).

In order to observe the cattle in rangeland conditions, the V Bar V Ranch in north central Arizona was used to field test the equipment in two different trials, one occurring

[1]We acknowledge the support of the Arizona Experiment Station and Cargill Animal Nutrition. Mention of a proprietary product does not constitute a guarantee or warranty of the product by the Arizona Experiment Station, University of Arizona, or the authors and does not imply its approval to the exclusion of other products that may also be suitable. We extend our thanks to Bopper and Keith Cannon of the V-V Ranch, Bob Walker of Imperial Instruments, Mark Anderson of Measurement Solutions, and Ted Haller and LaVar Clegg of Interface.

in November 2010 for 5.5 days and a subsequent trial occurring in October of 2011 for 9.5 days. In 2010 the trial occurred in a 1,157 ha pasture with 385 cows. In 2011 the trial was in a 1,295 ha pasture with 315 cows. The cattle present in both trials were *Bos Taurus* or *Sanga* or crosses of the two. Cattle age ranged from two years old to fourteen years old at various reproductive states but none were lactating. In both cases, the herd at the V Bar V was familiar to the mineral supplement being used, having consumed it in multiple pastures for the time preceding the trial. The supplement was in the form of a loose, granular, commercially produced mineral (Table 1).

In both cases the equipment was placed in areas of known animal traffic to ensure that at least part of those present in the pasture would encounter the mineral. At these areas, a feeding enclosure was created. This was accomplished using two 1.52 x 3.058 m cattle panels in a wing formation secured by two 1.83 m steel T posts. This would allow for the entrance of only one to two animals at a given time. When two cows were present at the same time, intake was averaged over both cows. To prevent animals from accessing the feeder through the cattle panel bars, a 30.5 x 121.9 cm x 1 cm plywood was used in 2010 as an additional barrier on one side and a 1.27 x 4.88 m 4 gauge welded wire livestock panel was used on the other side to protect measurement and solar equipment.

To power the measurement equipment in remote environments, solar technology was utilized. This included a Sunforce 50044 60 W Solar panel kit (adjustable to 49° angle for winter; 19° for summer; Sunforce, Montreal, Quebec, Canada). The voltage was regulated by a 10 A SS-10 Morningstar PWM Solar Controller (Morningstar Corp., Newton, PA). This system supplied a 100 AH deep cycle Lifeline GPL-27T battery allowing 3 day autonomy (Lifeline Batteries Inc., Azusa, CA). The solar controller and battery were housed in a Bison Prefab (Bison Profab, Magnolia, TX) 008014 40.6 x 40.6 x 25.4 cm NEMA 3R Aluminum Pole Mount Solar Battery Exclosure (mounted on 6.4 x 130.8 cm pipe over 1.83 m T post with wooden wedges). The solar system proved sufficient in output for both trials conducted.

Great care was taken to ensure that measurement equipment would be precise and yet functional in a rangeland, remote measurement setting. We additionally required a system that would not be rejected by the animals supplemented with the mineral. To meet the above criteria, a custom made steel feeder of 1 cm thick by 52.4 cm diameter was mounted on a 7.6 x 66 cm channel iron square frame with 4.4 cm diameter x 26.7 cm pipe legs (0.3 cm thick walls) welded at a 69° angle from 10.2 x 12.7 cm bases secured with 1.6 x 40 cm rebar pins. Three load cells were then mounted on a 35.9 cm diameter inner circle with 120° between each mounting bolt. The feed pan itself was also custom made of 1 cm thick steel, 52.4 cm in diameter with load cells mounted on a 35.9 cm diameter inner circle with 120° between each mounting bolt. An old rubber tire, cut to 68.6 cm diameter x 24.1 cm deep was fitted to the exterior of the inner circle of the feed pan and secured with 0.6 x 1.3 cm cap screws. Load cells were mounted top and bottom to the feeder base and feed pan with 1 cm-24 x 1.9 cm UNF case hardened bolts with a 1 cm flat washer in between the feed pan and each load cell.

To provide for the precision needed in measuring individual animal intakes, which has been documented to be minimal, the load cells had to be highly sensitive (Tait and Fisher, 1996; Cockwill et al., 2000). For this purpose, we employed Interface (Interface, Scottsdale, Arizona) 2420BLX-250 load cells, possessing a 113 kg capacity. Each 3 mV/V hermetically sealed stainless steel canister load cell, with Bayonet PT-WIH-10-6P connectors, were mounted in the measurement system as above mentioned using Interface stainless steel bases (to provide precision of measurement

**Table 1.** Composition of Nutrena (Minneapolis, Minnesota) NutreBeef Mineral Supplement

| | AZ (UA) Range Mineral | |
|---|---|---|
| Item | Minimum Guaranteed | Maximum Guaranteed |
| Calcium | 13.0% | 15.0% |
| Phosphorus | 6.0% | |
| Salt | 25.0% | 28.0% |
| Sodium | 10.0% | 12.0% |
| Magnesium | 2.0% | |
| Potassium | 1.0% | |
| Copper | 1500 PPM | |
| Selenium | 42 PPM | |
| Zinc | 6000 PPM | |
| Vitamin A | 300000 IU/LB | |
| Vitamin E | 300 IU/LB | |

while mounting to a non-specification surface). The load cells were powered through the amplifier described below and also had a ground wire connected from the solar regulator to the mineral feeder support frame. Interface CT-179-10 2400/WMC (3.058 m) interconnect cables for each load cell were used to connect the load cells to an Interface model JB104SS stainless steel summing junction box and the junction box was connected to the amplifier described below. Interface matching from the junction box to the load cells provided for standardization to single signal output for zero and span tolerance. This output was processed further by an Imperial (Imperial Instruments, Rushville, Illinois) 12 VDC powered (via the solar regulator positive and negative load connections) TM1-H-WP amplifier/conditioner module with Shunt Cal Resistor. Matching for the cells shipped from Interface occurred at the Imperial facility. The amplifier/conditioner module was modified to contain: a 13,333 OHM cal resistor, a 50 K OHM balance limiter to accommodate ± 22.68 kg of tare was adjustment and a gain adjustment covering the range of input times 179 through input times 278. Everything was tested as a complete set and shipped with cables to hook up to an input/output module. A 24 bit Labjack (Labjack, Lakewood, CO) UE9-Pro input/output module (with industrial temperature range of -40 to 85°C) was powered by a USB cable from a box PC, converting the analog signal from the amplifier to a digital signal. The Labjack module was hooked to the amplifier with an analog input 0 and ground. A Habey (Habey USA, Walnut, CA) BIS 6620-I fan- less Box PC with Z510 Intel Atom Processor (-10 to 50°C temperature range) with 1 GB DDR2 SO-DIMM DDR2 added memory & 16GB CF Flash Card was used to store data. The box PC was powered through the positive and negative load connections on the solar regulator. Labview 9.0.1 version software (National Instruments, Austin, TX) recorded the amplified signal weight data and internal solar battery exclosure temperature every 0.25 s for 20 data points for a measurement interval of 5 s followed by a 10 s idle time. Since it was not possible to obtain tare weights reliably, data were recorded every 16 to 17 s to detect changes in mineral weights and to overcome the effects of scale creep and temperature creep. All the electronic components were mounted in the Bison Profab solar battery exclosure.

The system was calibrated in the field with two 22.68 kg scale test weights and a 100 g Precision Weight Set (Ginsberg Scientific, Fort Collins, CO).

Weather data were collected using publicly available data from the Yavapai County Flood Control District. This remote weather station was located 2 km from the mineral feeder in both 2010 and 2011.

At the V Bar V Ranch, all of the cattle are tagged in the left ear with a unique six digit identification number. They are also branded with this same identification number on the left hip. To capture which animal was consuming mineral at the measurement equipment, a Moultrie I65 Digital Trail Camera, 6 Megapixels, 32 MB Memory with added 16GB SD HC Flash Card (Moultrie Feeders, Alabaster, AL) was used. This camera was placed just above and to the rear of the feeder to capture images of which animal was at the feeder at any given time. It was set to capture three pictures per minute when activated by movement at the feeder.

The measurement system generated a significant number of data points. To determine consumption of mineral, data were compared from two different data files, compressed and uncompressed. The compressed file was obtained using GNU Octave 3.2.4 software (GNU Software; http://www.gnu.org/software/octave/) and the software averaged data over the 20 data points obtained in the 5 s weighing interval. Also calculated was a delta value to help identify spikes for feeding events.

The compressed file was used solely to help identify the initiation of feeding events and the uncompressed file was actually used to obtain actual mineral intakes for each feeding event. Feeding events were matched to the camera photos and identified by delta values approaching or greater than delta = 1 in the compressed file and by absolute value differences in the uncompressed file scale weights ≥ 20 g.

End points for feeding event were identified by absolute scale differences being below 20 g for at least 3 data points. To minimize noise in scale weights, beginning weights prior to the feeding events were obtained over an average of 10 data points immediately preceding the initiation of the feeding event. Following the cessation of the feeding event (as defined by absolute scale weight differences < 20 grams), the scale was allowed to settle for 10 data points, then the average scale weight was obtained over 10 data points to obtain the ending weight. With few exceptions, this worked for obtaining individual feeding bout mineral intakes. Cattle which fed with large idle times with no other cows present had beginning and ending weights obtained over the total feeding period. In a few instances when cattle were feeding closely together, we were unable to accommodate the 10 data point post feeding settling period before obtaining ending scale weights.

Once obtained, these individual feeding events, as determined by weights, could then be matched to the pictures taken (both pictures and weights had time and date stamps). This allowed for pairing of individual animal identification numbers and individual feeding bouts.

Descriptive statistics and simple regression (Steel and Torrie, 1980) were performed to provide analysis of data collected.

## RESULTS AND DISCUSSION

The intent of this research was not to obtain exact mineral intake for all cows in the pasture, rather to test our ability to obtain individual intake for cows. Due to the sensitivity of the load cells, precision varied with temperature and wind. Wind speed (kph) ranged from 0 to 23 and averaged 14 ± 0.3 in 2010. Corresponding values were 0 to 32 and 11 ± 0.4 respectively, in 2011. Temperature (C) ranged from -7 to 4 and averaged -2 ± 0.9 in 2010. Corresponding values were

**Table 2.** Feeding behavior, daily intake, and feeding rate for cows consuming free choice mineral in a rangeland setting.

| Item | Avg. | Minimum Value | Maximum Value | CV % | SE |
|---|---|---|---|---|---|
| Individual feeding bouts | | | | | |
| 2010, min | 3 | 1 | 16 | 96.7 | 0.4 |
| 2011, min | 4 | 1 | 21 | 97.2 | 0.4 |
| Daily Mineral Intake | | | | | |
| 2010, g | 190[a] | 0 | 1009 | 99.8 | 26.0 |
| 2011, g | 663[b] | 0 | 2177 | 92.9 | 108.9 |
| Intake Rate | | | | | |
| 2010, g/min | 45[a] | 1 | 182 | 82.3 | 5.2 |
| 2011, g/min | 82[b] | 1 | 446 | 94.5 | 8.0 |

[a,b] Means in columns without a common superscript differ ($P < 0.05$).

-1 to 11 and 2 ± 1.1 respectively, in 2011. In pre-dawn hours when ambient conditions were relatively consistent, baseline variation of the load cells was ± 2 g. This value increased to ± 17 g during times when metal expanded at mid-morning, mid-day and mid- afternoon.

Duration of feeding bouts ranged from 1 to 16 min and averaged 3 ± 0.4 min in 2010, and 1 to 21 min and 4 ± 0.4 min respectively, in 2011 (Table 2; $P > 0.10$). Some cows approached the feeder and were photographed but did not consume any mineral. Repeat visits were made on different days or at different times of the day by four cows in 2010 and three cows in 2011. Feeding periods when mineral was consumed in 2010 included morning (19%) from 729 to 1020 h, late afternoon (46%) from 1506 to 1749 h, and evening (35%) from 1804 to 2208 h. In 2011, the trial was conducted in October instead of November and 86% of the cattle consumed mineral in the early morning from 631 to 857 h, 9% in mid- to late morning from 953 to 1117 h, and 5% in the late afternoon from 1549 to 1718 h. No cattle accessed the feeder in the evening in 2011.

The variability seen in mineral intake and feeding behavior (Table 2) has also been observed in other studies (Tait and Fisher, 1996; Cockwill et al., 2000; Norvell et al., 2011). Mineral intake (g/feeding bout) ranged from 0 to 679 and averaged 109 ± 16.0 in 2010. In 2011, the range increased ($P < 0.05$) to 0 to 1598 g/feeding bout and averaged 226 ± 25.4. Intake rate (g/min) for each feeding bout ranged from 1 to 182 and averaged 45 ± 5.2 in 2010, increasing ($P < 0.05$) to 1 to 446 and 82 ± 8.0 in 2011. Measured total daily intake per cow over all feeding bouts ranged from 0 to 1009 g and averaged 190 ± 26.0 g in 2010, increasing ($P < 0.05$) to 0 to 2177 g and 663 ± 108.9 g in 2011. When expressed as total intake over the course of the two trials, cattle who accessed the feeder consumed an average of 37 ± 5.5 g/d in 2010 and 80 ± 15.3 g/d in 2011. Norvell et al. (2011) reported that cattle consumed mineral at a greater rate during the first 15 d of their trial as opposed to the second 15 d. In 2010, of the 385 cows that were present in the pasture for our trial, 48 accessed the feeder. In 2011, of the 315 cows present, 28 accessed the feeder.

Correlation of the classification protocol for mineral intake was tested between two independent observers, to test the repeatability of the classification protocol. To further identify repeatability, two different test times were chosen; one during discrete feeding bouts where ample time for scale settling was allowed between bouts and another where multiple bouts occurred very close together which showed greater variability among the two observers. For discrete ($n = 5$) bouts, $r^2$ was 0.95 and the SE was 5.3 ($P < 0.05$), and for multiple animal ($n = 7$) bouts, $r^2$ was 0.75 and the SE was 18.0 ($P < 0.05$).

## IMPLICATIONS

The intent of this research was to see if a solar powered data acquisition system could be built to determine individual animal mineral intake in an extensive rangeland setting with low power draw (12 VDC) box PC computers and an USB powered input/output module and continuous weight data collection. We accomplished our goal (Figure 1). Individual weight data point precision varied with temperature and wind, but reasonable estimates of mineral intake were obtained. Future research will determine intake with a small herd to obtain additional individual mineral intake and behavior data. With this knowledge, better management decisions can be made, allowing for greater targeted supplementation of the individual animal's mineral needs.


**Figure 1.** Solar powered high precision data acquisition system for obtaining individual mineral intake on rangeland.

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# SYMPOSIUM

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# IMPACT OF ENVIRONMENTAL STRESS ON FEEDLOT CATTLE

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**ABSTRACT:** In the Midwest and Plains states, the heat waves of 1995, 1999, 2006, 2009, and 2010 were particularly severe with documented cattle losses approaching 5,000 head each year. However, during the summer of 2011, nearly 15,000 head of cattle perished across five states as a result of heat stress. The winters of 1992 to 93, 1996 to 97, 1997 to 98, 2006 to 07, and 2008 to 09 also caused hardship for cattle producers with some feedlots reporting losses in excess of 1,000 head. Up to 50% of the newborn calves were lost in many areas with over 75,000 head of cattle lost in the Northern Plains states during the 1996 to 97 and 2008 to 2009 winters. Late fall and early winter snowstorms in 1992, 1997, and 2006 resulted in the loss of over 25,000 head of feedlot cattle each year in the Central and Southern Plains of the United States. Economic losses from reduced performance of cattle experiencing severe environmental stress likely exceed losses associated from cattle death by 5- to 10-fold. The magnitude of death, and extensive animal suffering, would suggest that we need to be more proactive in managing for and mitigating environmental stress. Use of alternative supplementation programs may need to be considered for cattle challenged by adverse environmental conditions. Use of additional water for consumption and cooling, shade, and/or alternative management strategies need to be considered to help cattle cope with heat stress. During the winter, catastrophic losses typically occur during severe snowstorms, however early winter moisture combined with poor drying conditions may result in greater losses in performance and income due to muddy lot conditions and muddy cattle. Strategies need to be employed to minimize effects of mud, which is the single largest contributor to poor cattle performance in winter and spring. The above-mentioned weather events suggest that there are ample opportunities to improve animal welfare and minimize impact of environmental stress.

**Key words**: environmental stress, livestock management, models

## INTRODUCTION

Ruminants have the ability to generate a substantial amount of heat through fermentation of feedstuffs. In particular, feedlot cattle fed high-energy grain-based diets generate large amounts of metabolic heat, which is usually transferred from the body to the environment using normal physiological processes. This can be detrimental in the summer but a large asset in the winter. Failure to transfer this heat in the summer results in an accumulation of heat within the body and predisposes the animal to heat stress (Gaughan et al., 2010; Mader et al., 2010b), while failure to preserve body heat results in an opposite effect in the winter. Modification of heat flux may be achieved through behavioral changes initiated by the animal, or facilities and/or feed management changes initiated by the caretaker (Mader et al., 2006, 2007, 2008). If winter conditions are severe enough, productivity is compromised as a result of increased maintenance energy requirements associated with exposure to cold, wet, and/or windy conditions. For most cattle, efficiency of feed conversions and maintenance energy requirements are approximately 15 and 25% greater, respectively, in the winter than in the summer (Mader, 2003). If hair coats are wet and muddy, energy requirements for maintenance can easily double, particularly if the animal is not protected from the wind. However, under summer conditions, cattle deaths associated with heat waves are often a greater concern than performance losses.

The primary objective of any environmental mitigation strategy is to aid the animal in the winter to keep body temperature elevated throughout the day, and in the summer to reduce peak body temperature during the day, and/or help the animal drive body temperature down at night (Figure 1).

## MATERIALS AND METHODS

### Cold Mitigation Strategies

***Bedding and Pen Space.*** There are a number of things that can be done in the winter to enhance animal comfort. Bedding, such as crop residues or saw dust, can be used to help insulate cattle from the cold ground during severe cold outbreaks. These are better for bedding than hay-like materials because they are less palatable. Cattle will be less likely to eat the bedding and more likely to stay on the ration provided. Around 1 to 2 kg of bedding per head per day can make a big improvement in productivity. A summary of Colorado and South Dakota data found that gains and feed efficiencies can be improved nearly 7% through the use of bedding. Interestingly, the more significant responses came during the later versus early portion of the feeding period. This is likely due to problems heavier cattle often experience with wet, muddy conditions, which accompany late winter and early spring precipitation events. Lighter cattle, once they are on feed, are generally not impacted as much.

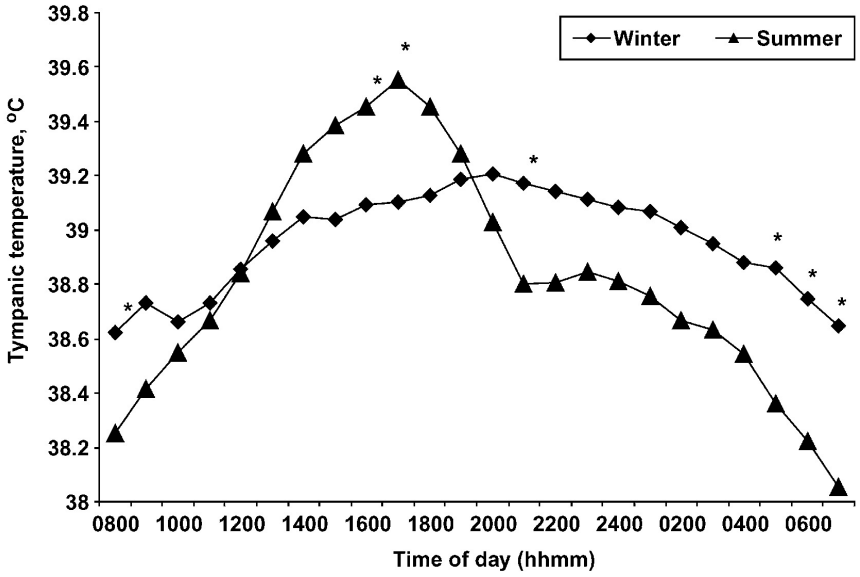


**Figure 1.** Effects of season on tympanic temperature over a 24-h period in feedlot heifers.* Means within an hour differ by season ($P < 0.05$; SE = 0.10). Each point represents the mean of 12 pens of cattle (Mader and Kreikemeier, 2006).

Under today's feed costs, the daily feed cost to maintain an animal that is partially wet, under winter conditions, is 2 to 3 times the cost of the bedding needed to keep the animal dry. Bedding is a cheap alternative, especially if hay, corn, or other feed prices are relatively high when compared with bedding cost. Furthermore, once the animal is dry, bedding usage would decrease, whereas if bedding was not utilized the moisture laden facilities will usually remain wet and the animal stays wet.

If ample pen space is available the benefits from bedding are not as great. In studies conducted in Nebraska, it was found that doubling normal pen space in the winter was as effective as using bedding (Mader and Colgan, 2007). Some cattle operations do not have the luxury of doubling space, nor is there a desire to bed cattle. Nevertheless, at the very least, young animals or animals that are susceptible to getting sick are candidates for bedding. If bedding is used, the bedded areas must be cleaned periodically. In addition, provide the cattle with as much dry area as possible to allow them to spread out and lay down. The more concentrated the animals are under wet conditions, the less chance there will be for surfaces to dry, which will increase maintenance energy requirements. One of the greatest hindrances to cattle performing in non-summer months is mud.

***Windbreaks and Shelters.*** On average, cattle fed in the winter with wind protection have better performance than cattle without wind protection (Mader et al., 1997a). In general, cold stress will stimulate intake, however, with less daylight in the winter combined with the cold conditions, cattle may not aggressively go to feeding areas. Thus feed intake is not always increased. Under these conditions, windbreaks have been found to be useful, especially for heavyweight cattle. It is important to design windbreaks to keep snow away from feeding areas.

New cattle coming into the feedlot, and cattle 30 to 45 d from slaughter, are most susceptible to cold stress. They need shelter and/or bedding to maintain health and stay on feed. It is satisfactory to change to a higher roughage diet when a snowstorm is imminent to minimize overeating or acidosis, but do not be too aggressive in making those changes. A more stable DMI can maintain a more stable rumen environment.

Recent interest has been shown in solid–floor confinement feedlot units, in which bedding is applied year-round in the pens on a weekly basis. These units can cost two to three times traditional outside feedlot units and have shown promise for controlling the total amount of waste that has to be managed and for greater control of environmental factors. These units appear to have the greatest benefit in areas where surface drainage is poor, soil and winter drying conditions enhance mud build-up, and added waste water generated from normal precipitation constitutes a disposal problem. In today's cattle feeding environment, it is becoming increasingly important that we maintain optimum cattle comfort not only for optimizing efficiency but also for enhancing consumer confidence and acceptance. Keeping cattle dry, clean, and comfortable is critical for accomplishing this goal, whether in open lots or in more confined structures.

In summary, to enhance animal comfort in feedlot pens and other areas in the winter, the following guidelines can be utilized: 1) facilities should be designed to properly drain water away from areas where cattle normally accumulate; 2) pushing snow out of pens (preferable after every storm)

or at least to the low end of the facilities will minimize the effects of gradual melting and aid in drying-out resting areas; 3) smooth out or knock down rough, frozen surfaces which may impede access of feed and water by cattle; 4) double per animal space allocation - the added space minimizes mud accumulation and allows for greater access to dry areas for animals to lie down; and 5) if cattle are prone to getting wet then use bedding and/or structures that provide wind protection, while minimizing moisture effects.

**Summer Mitigation Strategies**

***Restricted or Managed Feeding Programs.*** Benefits of using restricted feeding programs under hot conditions have been reported by Mader et al. (2002) and Davis et al. (2003). In addition, Reinhardt and Brandt (1994) found the use of restricted feeding programs to be particularly effective when cattle were fed late afternoon or evening vs morning. Implementing a bunk management regimen, whereby bunks are kept empty 4 to 6 hours during the daytime hours is another management strategy that could be used to minimize peak metabolic heat load occurring simultaneously to peak climatic heat load (Mader and Davis, 2004). Even though this forces the cattle to eat in the evening it does not appear to increase night-time body temperature (**BT**). In restricted feeding studies in which BT was measured, Mader et al. (1999b) housed feedlot steers under thermoneutral or hot environmental conditions. Steers were offered a 6% roughage finishing diet ad libitum (**HE**), offered the same diet restricted to 85 to 90% of ad libitum DMI levels (**RE**), or offered a 28% roughage diet ad libitum (**HR**). Steers fed the HR diet (39.7 ºC) had significantly lower BT under hot conditions than HE (40.6 ºC) and RE (48.3 ºC) fed steers, while RE fed steers had significantly lower BT than HE fed steers. The lower BT of the HR and RE fed steers would indicate that ME intake prior to exposure to excessive heat load influences the ability of cattle to cope with the challenge of hot environments and that lowering ME intake can lower BT (Davis et al., 2003). Arias et al. (2011) reported similar results, in that high concentrate feedlot diets (3.04Mcal ME/kg) promoted greater BT in the summer, while the lower energy, higher roughage diets (2.63 Mcal ME/kg) tended to produce lower BT in the winter.

***Waterer Space Requirements.*** Evaporation of moisture from the skin surface (sweating) or respiratory tract (panting) is the primary mechanism used by the animal to lose excess body heat in a hot environment. Under these conditions, waterer space availability and water intake per head becomes very important. During heat episodes, Mader et al. (1997b) found that as much as three times the normal waterer space (7.5 vs. 2.5 cm of linear space per animal) may be needed to allow for sufficient room for all animals to access and benefit from available water. In general, water consumption per unit of DMI in the summer is 2 times greater than in the winter.

***Sprinkling Systems.*** In addition to pen design and altering feeding regimen, sprinkling can also be effective in minimizing heat stress. Benefits of sprinkling tend to be enhanced if sprinkling is started in the morning, prior to cattle getting hot (Davis et al., 2003). These data also show significant benefits to sprinkling or wetting pen surfaces. Sprinkling of pen surfaces may be more beneficial than sprinkling the cattle. Kelly et al. (1950), reported feedlot ground surface temperatures in excess of 65° C by 1400 h in Southern California. Similar surface temperatures can be found in most High Plains feedlots under dry conditions with high solar radiation levels. Cooling the surface would appear to provide a heat sink for cattle to dissipate body heat, thus allowing cattle to better adapt to environmental conditions vs adapting to being wetted. Wetting or sprinkling can have adverse effects, particularly when the cattle get acclimated to being wet and failed or incomplete sprinkling occurs during subsequent hot days. Increased relative humidity may also be problematic if large areas of the feedlot are sprinkled versus isolated areas in pens.

Sprinkling may increase feedlot water requirements 2- to 3-fold. In addition, mud build-up is associated with sprinkling systems. Intermittent sprinkling is recommended and constitutes 2 to 5 minute application every 30 to 45 minutes or up to 20 minute application every hour to 1.5 hours. Whether cattle that need to be sprinkled (cooled) always go to or get under the sprinklers is unknown.

***Use of Shade.*** Shade has also been found to be beneficial for feedlot cattle exposed to hot climatic conditions, (Mader et al., 1999a). In general, the response to shade is greatest at the onset of heat stress even though shade use increases with time cattle are on feed. Although no heat-related cattle deaths occurred in this study, these results suggest that shade improves performance in the summer particularly when cattle are fed in facilities that restrict airflow and for cattle that have not become, or had the opportunity to become, acclimated to hot conditions.

Greater benefits of using shade are found in areas having greater temperature and/or solar radiation (Hahn et al., 2001). Mitlöhner et al. (2001) found excellent results to providing shade for cattle fed near Lubbock, TX. The overall economic benefit of using shade depends not only on location, but also on cost of structures and maintenance.

***Mitigation Strategy Economic Analysis.*** The economic effects of imposing various environmental stress mitigation strategies have been determined by Mader (2010a,b, 2011) based on the comprehensive climate index (Mader et al., 2010a) and how the respective mitigation strategy changes apparent or "feels-like" temperature. In the summer analysis, moderate sprinkling was utilized versus heavy sprinkling in an effort to minimize the quantity of excess runoff water. The pen area sprinkled was kept to around 25 $ft^2$ per head. In addition to shade and sprinklers, evaluation of the use of fans (with water injection under shade) was conducted to determine the benefits of added evaporative cooling potential through the enhanced airflow under shade. From this analysis, the performance effects of sprinkling and shade on apparent temperatures were similar even though different physiological

cooling properties are involved between the two strategies. Greater amounts of water tend to have a greater benefit than shade while lesser amounts (i.e., misting) tend to have less benefit than shade. Due to the limited heat tolerance of British crossbred cattle, they tended to have greater cost of gain (**COG**) than Holsteins. Thus, heat stress mitigation strategies will be more economical when imposed on the British breeds of cattle. An opposite scenario occurs under cold stress, with Holsteins having greater COG. The response to mitigation strategies were similar among breed types but with a slightly greater break-even (annualized amount that could be spent for the respective mitigation strategy) cost for the British crossbred. An analysis of Brahman cross cattle displayed a lower benefit and one-time setup costs (break-even construction cost) when compared with comparable costs for Holstein steers.

In theory, sprinkling should always produce greater heat stress relief than shade or misting due to the high heat loss associated with the evaporation process. However, limited research data in feedlot cattle suggests that shade provides a greater and more consistent performance response than sprinkling. When cattle are in very close confinement and the probability is high that water gets applied to the animal, then a more positive response to sprinkling/direct water application is found (e. g., dairy units). Well designed and constructed shade and shelters tend to produce greater long-term benefits than sprinklers and/or less stable shade structures.

Heat stress is dependent not only on temperature and solar radiation, but also on humidity and wind speed. Adjustments for solar radiation and wind speed have also been developed and need to be considered when predicting heat stress (Mader et al, 2006). The effects of environmental stress are dependent on not only the magnitude and duration, but also on the rate at which environmental conditions change.

## SUMMARY

Beef cattle are traditionally managed outdoors with exposure to natural and variable environmental conditions. Cattle are particularly vulnerable not only to extreme environmental conditions, but also to rapid changes in these conditions. Depending on season, facility and management alternatives need to be considered to help cattle cope with adverse conditions. In addition to these changes, manipulation of diet energy density and intake may also be beneficial for cattle challenged by environmental conditions.

From a consumer perspective, animal comfort must have a greater priority than in the past. The large number of cattle deaths during adverse weather events is unacceptable. From an animal welfare standpoint, the use of shade or other mitigation strategies in the summer would be recommended for most cattle that are confined and being fed high-energy finishing diets. In the winter, wind protection and/or bedding would be encouraged for cattle fed in wet conditions or in feedlots with wet surfaces. In dry environments in the winter, and in dry-cool environments in the summer, limited environmental stress mitigation is needed. However, with the current feed and cattle price structure, the economic benefits of utilizing shade, windbreaks, etc. for cattle exposed to adverse climatic conditions are greater today than in the past. In addition to the performance response, environmental stress mitigation has significant animal comfort and consumer perception implications.

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