Graduate Student Competition: ADSA-ASAS Northeast Section Graduate Student Oral Competition

288 Assessment of acute pain during and after knife and band castration of beef calves at three different industry-relevant ages. Daniela M. Meléndez*[1,2], Sonia Marti[1], Eugene D. Janzen[2], Diego Moya[1,2], Ed Pajor[2], and Karen S. Schwartzkopf[1], *[1]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [2]University of Calgary, Calgary, Alberta, Canada.*

The aim of this study was to identify which age and method of castration causes less acute pain and distress in beef calves. One hundred five Angus bull calves were blocked by age and body weight, and randomly assigned to 1 of 3 treatments: control (C; n = 12); band castration (B; n = 12); and surgical castration (S; n = 12); at 1 wk, 2 and 4 mo of age. Physiological and behavioral parameters were collected before, during and after castration to assess acute pain. Physiological measures included blood count, salivary cortisol, haptoglobin, substance P and infrared thermography. Behavioral measures consisted of visual analog score, stride length, hobo data loggers and behavioral scoring of walking, standing, lying, tail flick, foot stamping and head turning. Overall, no physiological or behavioral parameters differed significantly for calves castrated at 1 wk of age. Salivary cortisol tended ($P = 0.07$) to differ at 1 wk of age when S calves had greater concentrations than B calves. Conversely, both physiological and behavioral indicators of pain/distress were clearly observed when calves were castrated at 2 and 4 mo of age regardless of the method used. A time × treatment interaction ($P < 0.0001$) was observed for salivary cortisol in 4 mo old calves, with B and S calves having greater concentrations than C calves 60 min after castration, while B had greater concentrations than S and C calves 120 min after castration. Based on behavioral data, S calves at 2 and 4 mo of age, stood ($P < 0.0001$) and walked ($P = 0.04$) more but lie down and ate less ($P = 0.01$; $P = 0.002$) compared with B and C only at 2 mo of age. At 4 mo of age, S calves tail flicked more ($P = 0.0006$) and had shorter stride length ($P = 0.036$) than B and C calves. The indicators of acute pain/discomfort assessed in this study suggest that the most welfare-friendly age and method of castration is band castration at 1 wk of age.

Key Words: castration, pain, beef

289 The influence of dietary strong ions on rumen ion concentrations. Tracy L. Catterton* and Richard A. Erdman, *Department of Animal and Avian Sciences, University of Maryland, College Park, MD.*

Dietary cation-anion difference (DCAD) is being used as a basis for diet formulation in the dairy industry. While extensive research has been conducted on the effects of DCAD concentration on animal performance and rumen characteristics, the effects of the individual strong ions that contribute to DCAD have yet to be determined. In addition to DCAD, there is evidence that cation source (Na vs. K) affects animal performance (Iwaniuk et al., 2015). We hypothesized that dietary strong ion concentrations (Na, K, and Cl) would affect the rumen ion concentrations. Our objective was to determine if rumen Na, K, and Cl concentrations were affected by dietary mineral content. Literature data were collected from 2 dairy cattle studies (Bailey, 1961; Bennink et al., 1978) that included 11 different diets with (mean ± SE) concentrations of 47 ± 35, 358 ± 312, and 108 ± 188 mEq/kg dietary Na, K, and Cl, respectively. Rumen samples, collected by rumen cannula, were taken between 0 to 9 h (Bennink et al., 1978) and 1 to 14 h post-feeding (Bailey, 1961). Principal components analysis (PCA) suggested that rumen Na and K were PCA negative, while rumen Cl and diet K clustered together. Subsequent multiple regression analysis (PROC GLMSELECT) showed that dietary K and Cl were significant factors ($P < 0.01$) associated with changes in rumen Na, K, and Cl, and diet Na was associated ($P \leq 0.06$) with changes in rumen Na and K. As rumen K increased, rumen Na decreased ($P < 0.001$). We concluded that manipulation of diet strong ion concentrations could be used to alter rumen Na, K, and Cl concentrations and, thus, alter the rumen environment.

Table 1 (Abstr. 289).

	Rumen ion		
Item	Na	K	Cl
Regression statistics			
Mean, mEq/L	122.0	46.9	18.7
Root MSE	17.4	11.8	4.04
Adj. R^2	0.61	0.60	0.63
Regression coefficients			
Intercept, mEq/L	151.9	63.4	9.35
Diet Na, mEq/kg	0.254	−0.83	NS
Diet K, mEq/kg	−0.07	0.042	0.251
Diet Cl, mEq/kg	0.064	−0.08	−0.193
Rumen Na, mEq/L	—	−0.21	NS
Rumen K, mEq/L	−0.47	—	NS
Rumen Cl, mEq/L	NS	0.22	—
Time post-feeding, h	NS	NS	−0.38

Key Words: rumen environment, strong ions, dairy cattle

290 Disbudding and dehorning practices in dairy calves among Ontario bovine veterinarians. Charlotte B. Winder*[1], Stephen J. LeBlanc[1], Derek B. Haley[1], Kerry D. Lissemore[1], M. Ann Godkin[2], and Todd F. Duffield[1], *[1]University of Guelph, Guelph, Ontario, Canada, [2]Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Guelph, Ontario, Canada.*

An online survey was conducted in the fall of 2014 to explore current practices, including analgesic use, in the disbudding or dehorning (D/D) of dairy calves by veterinarians in Ontario. Members of the Ontario Association of Bovine Practitioners (n = 238) were invited to participate. Ninety-three veterinarians (39%) from 51 clinics (63%) responded, with 94% of clinics reporting veterinarians or veterinary technicians (VT) performing D/D for an estimated mean of 29% (SD = 20) of their dairy clients. Twenty-five percent of clinics employed VT to perform this service. Of these clinics, VT accounted for an estimated mean of 69% (SD = 29) of the clinic's total D/D. Veterinarians who perform D/D were asked to report calf age at time of D/D. Seventy-five percent of veterinarians disbudded calves < 4 weeks of age. Of these veterinarians, 99% used local anesthetic, 56% used a sedative, and 50% used an NSAID. Ninety-one percent of veterinarians disbudded calves 4–8 weeks of age. Of these veterinarians, 99% used a local anesthetic, 61% used a sedative, and 54% used an NSAID. Finally, 76% of vets performed D/D in calves > 8 weeks of age. Of these veterinarians, 97% used a local anesthetic, 66% used a sedative, and 59% used an NSAID. Injectable meloxicam accounted for 89% of all NSAIDs given in all age groups. Common reasons for NSAID use were: pain control, known withdrawal time, and reasonable cost. Common reasons for the lack of NSAID use

were: objections to cost, and client requests excluding cost. Nearly all (98%) veterinarians practicing for at least 10 years reported changing D/D practices over this time period. Common changes included: use or increased use of an NSAID (60%), use or increased use of sedation (34%), and use or increased use of local anesthetic (29%). Reasons for changes included: concern for the welfare of the calf, information from continuing education, and improved calf handling. Use of NSAID in this survey is higher than previously reported, as was the proportion of calves disbudded < 4 weeks of age. This indicates a trend toward improved analgesia and adoption of best practices for welfare in the D/D of dairy calves.

Key Words: calf, disbud, welfare

291 Identification of early pregnancy and fetal landmarks via transabdominal ultrasound in sheep. Amanda K. Jones*[1], Rachael E. Gately[2], Katelyn K. McFadden[1], Steven A. Zinn[1], Kristen E. Govoni[1], and Sarah A. Reed[1], *[1]Department of Animal Science, University of Connecticut, Storrs, CT, [2]Department of Environmental and Population Health, Tufts Cummings School of Veterinary Medicine, North Grafton, MA.*

Field application of small ruminant ultrasound is primarily used for pregnancy detection and fetal number beginning at mid-gestation. Efforts to estimate fetal age and number early in small ruminant pregnancies are limited. We hypothesized that detection of pregnancy and fetal landmarks before d 45 via transabdominal ultrasound is reliable in the sheep, and may be used to determine fetal age during early in sheep. To test this hypothesis, 106 Western Whiteface ewes were exposed to 1 of 4 rams. The day a ewe was marked by a ram was considered d 0 of pregnancy. Transabdominal ultrasound (Easi-Scan, BCF Technology, Rochester, MN) was performed 3 times/wk with a 5 MHz rectal transducer in the right non-haired abdominal region of the ewe starting at d 26.0 ± 0.3 (range d 22 to d 30). Pregnancy was confirmed in 88 ewes, with the remaining identified as non-pregnant. Fluid-filled uterine cross-sections provided first evidence of pregnancy from d 26.0 ± 2.9 onward. Pregnancy was confirmed by the presence of a fetus with a heartbeat on d 28.5 ± 0.4. Singleton pregnancies were detected later than multiple pregnancies (singletons: d 31.2 ± 0.9, twins: d 27.6 ± 0.9, triplets: d 26.3 ± 1.0, $P < 0.01$). The uterine horn of fetal origin had no effect on identifying singleton pregnancies ($P = 0.52$). Placentome evagination was first observed at d 33.8 ± 0.4, separation of limb buds at d 35.2 ± 0.7, fetal genitalia spots at d 37.9 ± 0.7, mature placentomes at d 40.6 ± 0.4, and ribs at d 42.9 ± 1.4. Additionally, 3 fetal losses were identified by d 40.0 ± 0.7 of pregnancy (3.4% early embryonic loss rate). Accuracy of counting fetuses (77.6%) increased with decreasing number of offspring. That is, identification of singletons, twins and triplets was 95, 80 and 41%, respectively. Pregnancy detection via transabdominal ultrasound was accurate as early as d 28 in sheep and sensitive enough to consistently identify early embryonic developments before d 45. Distinguishing these fetal landmarks may allow for estimating fetal age to improve early detection of pregnancy and breeding management of sheep.

Key Words: ultrasound, pregnancy, sheep

292 Isolation and characterization of chemical components of *Leucaena leucocephala* with anti-methanogenic properties by using in vitro gas production technique. D. Dineshkumar*[1], A. L. Abdalla[1], C. L. Linander[1], A. P. Massarioli[2], A. L. Abdalla Filho[1], P. P. Santos[1], A. S. Natel[1], S. M. Alencar[2], and H. Louvandini[1], *[1]Centre for Nuclear Energy in Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, Brazil, [2]College of Agriculture "Luiz de Queiroz," University of Sao Paulo, Piracicaba, Sao Paulo, Brazil.*

Studies emphasized that group of plant secondary metabolites (saponins, flavonoids and tannins) seems to present the ability to manipulate rumen fermentation lessening the CH_4 formation. The purposes of this research were to find out individual bioactive compound with anti-methanogenic activity. However, we anticipate that advances technology such as GC-MS will provide unprecedented data on the distribution of component existing in plant extracts. *Leucaena leucocephala* plant samples were extracted with methanol solvent using ultra sonication. Crude solvent free extract (8.58 g) was then extracted with different organic solvent with increasing polarity provided extracts of hexane (1.03 g), chloroform (0.34 g), ethyl acetate (0.48 g), butanol (0.77 g) and residual crude fractions (1.99 g) respectively. Assessment of phytoconstituents in such organic extracts was subjected to find out individual bioactive compound with the modified GC–MS (Shimadzu gas chromatography-mass spectrometer (GCMS-QP2010) method. 38 components were identified from the chromatograms of the different organic solvent extracts. Dried 0.5g of ground leucena plant, alfafa plant (positive control) and the different crude methanolic extracts with 3 different levels (125, 250, and 500 µg/mL) were tested for anti-methanogenic properties in terms of in vitro gas production and nutrient degradability. We found significant organic solvent effects for CH_4 production. Hexane extract reduces Net CH_4/OMD ($P < 0.001$) compared with other solvent extracts tested. Nutrient degradability, ruminal parameters and VFA production were non-significantly differed between the treatment groups. But, there was an improvement on nutrient degradability compared with *Leucaena leucocephala* and control plants. However, we found no doses and interaction between solvent and dose effects among the treatment groups. This study explained hexane extract from whole plant methanolic extract is effective against anti-methanogenic activity in modifying ruminal degradation of nutrients. The most active components still have to be identifying by fractionation of hexane extract.

Key Words: *Leucaena leucocephala*, anti-methanogenic properties, GC-MS

293 Immune cells populate mesenteric adipose tissues of Holstein Friesian cows. Bridget A. Aylward*[1], Megan Clark[1], Amanda Barnard[1], Jen Wilson[1], Candice Gittens[1], Tanya Gressley[1], Erin Brannick[1], Marie Fecteau[2], and Robert Dyer[1], *[1]Department of Animal and Food Sciences, College of Agricultural and Natural Resources, University of Delaware, Newark, DE, [2]Department of Clinical Studies, New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, Kennet Square, PA.*

In many animal species, anti-inflammatory immune cells are normal residents of lean adipose depots and produce a barrier of related cytokines that protect against metabolic syndrome. Our objective was to determine if similar immune cells reside in mid-jejunum mesenteric adipose tissue (MAT) of randomly selected lean Holstein Friesian cows from an abattoir. Body condition scores were determined before slaughter (mean BCS = 2.74). Stromal cell fractions (SCF) were prepared from washed, minced MAT digested with type I collagenase. Following digestion and cleaning of the sample, cells were stained with bovine immune cell marker specific monoclonal antibodies expressed on macrophages, dendritic cells, T lymphocytes and T regulatory lymphocytes (T regs). Background controls consisted of cells stained with irrelevant, isotype-matched control antibodies. SCF composition was analyzed across 10,000 cells/sample using a Becton Dickson FACS Calibur Flow Cytometer. Marker specific fluorescence was compared with background fluorescence in controls by ANOVA. Marker expression is summarized

in the table below. Results from this work indicate that populations of innate (macrophage and dendritic cells) and adaptive (lymphocytes and T regs) immune response cells do in fact reside in MAT of lean cows (Table 1). Furthermore, expression of MHC class II in the context of dendritic cells or macrophages and T lymphocytes suggests adaptive immune responses do occur in MAT. Subpopulations of $FoxP3^+$ regulatory T lymphocytes were the dominant effector lymphocyte, implying that anti-inflammatory functions could contribute to MAT homeostasis in lean cows and potentially protect against development of metabolic disease.

Table 1 (Abstr. 293). Presence of immune cells mesenteric adipose tissue (MAT) of Holstein Friesian cows

Presumptive cell type	Marker	Percent of SCF
Macrophages	CD11b (n=9)	8.71 ± 0.75*
	CD172 (n=8)	12.57 ± 1.20*
	CD11b/CD172 (n=8)	6.74 ± 0.84*
	CD3/CD11b (n=4)	2.48 ± 0.62
Dendritic cells	MHC II (n=5)	10.62 ± 0.86*
	CD11c (n=9)	9.43 ± 0.57*
	MHC II/CD11c (n=5)	2.59 ± 0.52
	CD11c/CD3 (n=4)	1.76 ± 0.44
T lymphocytes	CD3 (n=17)	8.99 ± 0.33*
	CD8 (n=4)	3.58 ± 0.89
	CD3/CD8 (n=4)	1.07 ± 0.27
T regulatory lymphocytes	FoxP3 (n=6)	8.00 ± 0.56*
	CD4/FOXP3(n=6)	0.04 ± 0.12

*Expression greater than background ($P \leq 0.05$).

Key Words: immunology, adipose

294 Effects of under- and over-feeding during gestation on organ development of offspring at days 45 and 90 of gestation.

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Poor maternal nutrition during gestation can lead to intrauterine growth restriction resulting in offspring with low birth weight and altered organ development, negatively affecting production. We hypothesized that poor maternal nutrition during gestation would alter fetal body weight and organ mass of lambs during gestation. Western Whiteface ewes (n = 82) were bred to 1 of 4 rams and confirmed pregnant by ultrasound. Ewes were fed 100%, 60%, or 140% of NRC requirements for TDN beginning at d 30.2 ± 0.2 of gestation and offspring from these ewes will be referred to as CON, RES, and OVER, respectively. Fetal weights and organs were collected at d 45, 90, and 135 of gestation and within 24 h of birth. To date, organ weights have been collected from d 45 and 90. Data are expressed as percent of fetal weight and were analyzed using PROC MIXED in SAS. At d 45, fetal weight tended to be different (P = 0.067) between treatments with OVER offspring smaller than CON (P = 0.021) and RES intermediate (CON = 11.0 ± 0.6 g; RES = 10.1 ± 0.5 g; OVER = 9.2 ± 0.5 g). Liver weight at d 45 tended to be different (P = 0.055) with RES offspring larger than OVER (P = 0.017) and CON intermediate (CON = 6.7 ± 0.5%; RES = 7.6 ± 0.5%; OVER 6.1 ± 0.4%). We did not observe a difference in the weight of heart and kidney at d 45 ($P \geq 0.148$). Although fetal weights were not different at d 90 (P = 0.329) liver weights were greater in OVER offspring vs CON and RES ($P \leq 0.05$; CON = 5.5 ± 0.2%; RES = 5.7 ± 0.2%; OVER = 6.3 ± 0.2%). At 90 d an effect of maternal diet was not observed for kidney, heart, pancreas, adrenal, or renal fat weights ($P \geq 0.153$). In conclusion, maternal under- and over-feeding affects liver development during early gestation, a period of rapid liver growth, which may lead to altered health and growth of the offspring later in life.

Key Words: maternal nutrition, sheep, organ development