## PHYSIOLOGY AND ENDOCRINOLOGY: INTERRELATIONSHIPS BETWEEN ENVIRONMENTAL, METABOLIC, AND PHYSIOLOGICAL PROCESSES II

**0509** Effects of calcium salts of soybean oil on factors that influence pregnancy establishment in *Bos indicus* beef cows. B. I. Cappellozza\*<sup>1</sup>, R. F. Cooke<sup>1</sup>, T. Guarnieri Filho<sup>1,2</sup>, I. Bueno<sup>2</sup>, D. W. Bohnert<sup>1</sup>, R. L. A. Cerri<sup>3</sup>, and J. L. M. Vasconcelos<sup>4</sup>, <sup>1</sup>Oregon State University–EOARC Burns, Burns, <sup>2</sup>Faculdade de Medicina Veterinária e Zootecnia, UNESP–Univ. Estadual Paulista, Botucatu, Brazil, <sup>3</sup>Faculty of Land and Food Systems–University of British Columbia, Vancouver, Canada, <sup>4</sup>UNESP–FMVZ, Botucatu, Brazil.

This experiment compared fatty acid (FA) concentrations in plasma, reproductive tissues, as well as hormones, and expression of genes associated with pregnancy establishment in beef cows supplemented or not with Ca salts of soybean oil (CSSO) beginning after timed AI. Ninety nonlactating multiparous Nellore (Bos indicus) cows were inseminated on d 0 of the experiment, and divided into 18 groups of 5 cows/group. Groups were randomly assigned to receive (as-fed basis) 100 g of protein-mineral mix + 100 g of ground corn per cow/d, in addition to (1) 100 g/cow daily of CSSO (n = 9), or (2) 100 g/cow daily of kaolin (CON; rumen-inert indigestible substance; n = 9). Groups were maintained in a single *Brachiaria* brizantha pasture with ad libitum access to forage. However, groups were segregated daily and offered treatments individually during the experiment (d 0 to 18). Blood samples were collected and transrectal ultrasonography was performed to verify ovulation and corpus luteum (CL) volume immediately before AI (d 0), on d 7, and 1 d 8. On d 19, 36 cows (18 cows/ treatment, 2 cows/group) diagnosed without the presence of a CL on d 0, but with a CL > 0.3 cm<sup>3</sup> in volume on d 7 and 18, were slaughtered for collection of conceptus, uterine luminal flushing, and tissue samples from the CL and endometrium. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with group as experimental unit. Cows receiving CSSO had greater ( $P \le 0.08$ ) concentrations of linoleic and other  $\omega$ -6 FA in plasma, endometrium, CL, and conceptus compared with CON. On d 7, CSSO-supplemented cows had greater plasma progesterone concentrations (P = 0.02) and CL volume (P = 0.01) compared with CON, whereas no treatment effects were detected ( $P \ge 0.20$ ) for these parameters on d 18 (treatment  $\times$  day interaction; P <0.01). Cows receiving CSSO tended (P = 0.09) to have greater concentrations of IFNt in the uterine flushing media compared with CON. No treatment effects were detected (P > 0.12)for mRNA expression genes associated with pregnancy establishment in endometrial (cyclooxygenase-2 and oxytocin receptor), CL (steroidogenic enzymes), and conceptus (IFNt) samples. In summary, supplementing beef cows with 100 g of CSSO beginning after AI favored incorporation of  $\omega$ -6 FA into their circulation, reproductive tissues, and conceptus, without impacting expression of genes associated with pregnancy establishment on d 19 of gestation.

**Key Words:** beef cows, calcium salts of soybean oil, pregnancy

0510 Metabolomics profiling of four biofluids from dairy cows fed different forages using gas chromatography-time of flight/mass spectrometry.
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The quality of forage can significantly impact milk production and quality. This study aims to investigate the effect of type of forage on metabolites in biofluids of dairy cow and their potential role in lactation. Sixteen multiparous Holstein dairy cows were blocked based on days in milk ( $164 \pm 27.5$  d, mean  $\pm$  SD) and milk yield (29.4  $\pm$  2.16 kg, mean  $\pm$  SD), and were randomly assigned into 1 of 2 treatments. Isonitrogenous diets with a ratio of forage-to-concentrate at 45:55 contained similar concentrate mixtures and 15% corn silage, with 2 different forage sources (DM basis): 23% alfalfa hay and 7% Chinese wild rye hay (AH); and 30% corn stover (CS). After a 65-d feeding, 4 biofluids (rumen fluid, milk, serum, and urine) were collected from all cows to characterize the metabolites using a novel metabolomics method based on gas chromatographytime of flight/mass spectrometry). A total of 165, 195, 218, and 156 metabolites were identified in rumen fluid, milk, serum, and urine, respectively. Among them, 29 metabolites were mutual in all 4 biofluids. The chromatograms among 4 biofluids were obviously different, with a clear discrimination between diets CS and AH. Principal component analysis yielded the separated clusters of metabolite profiles between diets AH and CS. Partial least squares discriminant analysis also showed segregation in the metabolites from 4 biofluids between 2 diets. There were 56, 8, 31, 31 metabolites in 4 biofluids, respectively, that differed significantly between diets AH and CS (VIP > 1 and P < 0.05). These metabolites were mainly involved in AA metabolism and carbohydrate metabolism. Among 56 dissimilar metabolites in the rumen, 90% was lower for diet CS than for AH, while 74% of 31 dissimilar urinary metabolic wastes was higher in CS. The cumulative explanation rate ( $R^2Y$ ) of orthogonal to partial least squares discriminant analysis was 0.899, 0.967, 0.899, and 0.982, respectively, suggesting that biofluid metabolomics combining metabolic profiles with multivariate analysis can be used to investigate the cow complex metabolic alteration in response to different type of forages with successful metabolites models.

Key Words: biofluids, metabolomics, dairy cow

#### **0511 Separation of proteins from the milk fat globule membrane with minimal losses.** W. Holzmüller\*, *Technische Universität München, Freising, Germany.*

Components of the milk fat globule membrane (MFGM) are reported to have functional properties. However, determination of the specific properties of individual MFGM components is hardly possible, since the MFGM is a complex 3-layered structure consisting of phospholipids and proteins. Therefore, to assess the MFGM proteins' functionalities, first an isolation of the MFGM from the bulk milk proteins is required. Second, single MFGM proteins must be separated from the phospholipid phase. The aim of this project was to compare established and novel isolation methods in terms of the capacity to obtain MFGM proteins at high yield. First, the method of washing the cream for several times was used. After each washing step the cream sample was churned and the buttermilk obtained was analyzed regarding MFGM proteins using SDS-PAGE. The intensities of the different proteins (XO/XDH, BTN PAS6/7) were compared with a nonwashed sample. Beside the removal of caseins and whey proteins it was found that a significant loss of MFGM proteins up to 90% occurs, which has been neglected in previous studies. Particle size measurements showed that the fat globules increased during the washing process. This is due to coalescence of the fat globules and consequently results in a loss of MFGM material including membrane proteins. Second, filtration experiments with buttermilk were done. Because of an overlap in size between the casein micelles and the MFGM fragments a pre-treatment of the buttermilk was necessary to realize an isolation of the MFGM. In this study, a new approach based on the coagulation of casein micelles by adding rennet was developed. The supernatant (buttermilk-sweet whey) obtained was used for a subsequent diafiltration to remove the residual whey proteins. All permeates and retentates of each diafiltration step were again analyzed for the remaining MFGM proteins, caseins, and whey proteins by SDS-PAGE to evaluate the isolation procedure. However, significantly higher amounts (~70%) of the total MFGM proteins were recovered when the newly developed MFGM isolation method was used in comparison with the washing method. Concluding, both separation methods are applicable to realize MFGM isolates, but a simultaneous loss of MFGM proteins is hardly avoidable. As far as we oversee the literature, this fact was not sufficiently considered in previous studies. Finally, the results of this work show that different MFGM proteins are enriched in the particular isolates depending on their location in the MFGM and the applied extraction method.

Key Words: milk fat globule membrane proteins

## **0512** Serotonin (5-HT) receptor expression in bovine apocrine sweat gland epithelial cells isolated from cow skin. S. Hamzaoui<sup>1</sup>, J. L. Collier<sup>2</sup>, and R. J. Collier<sup>\*2</sup>, <sup>1</sup>Universitat Autònoma de Barcelona, Bellaterra, Spain, <sup>2</sup>University of Arizona, Tucson.

Apocrine sweat glands in bovine skin are involved in thermoregulation. Human, horse, and sheep sweat gland epithelial cells have been isolated and grown in vitro. However, isolation of bovine sweat gland epithelial cells (BSGEC) has never been reported. Recent studies have demonstrated that serotonin (5-HT) is an important local regulator of lactational homeostasis and involution in bovine, mouse, and human mammary epithelial cells. We hypothesized that, since the mammary gland is a modified sweat gland, that 5-HT receptors may also be present in BSGEC. The present study was conducted to identify a method to isolate bovine sweat glands and culture apocrine BSGEC in vitro and evaluate the expression of 5-HT receptors (1B, 2A, 2B, 4, and 7) in bovine skin, intact apocrine sweat glands and BSGEC. Collagenase digestion, neutral red staining and mechanical shearing were used to identify and isolate the apocrine glands from skin. The isolated material was transferred to complete media (keratinocyte serum-free media, K-SFM), bovine pituitary extract (BPE), and human recombinant epidermal growth factor (EGF) + 2.5% FBS in a T25 flask with media film then incubated at 37°C for 24 h. After sweat glands adhered to the bottom, an additional 2 mL of complete media was added, and the media was changed every 3 d. Isolated apocrine sweat glands and BSGEC were immunostained for cytokeratin and fibroblast specific protein, indicating fibroblast-free cultures. We also determined the mRNA expression of bovine 5-HT receptor subtypes in bovine whole skin, sweat glands, and BSGEC. The mRNAs for 5 receptor isoforms (5-HT 1B, 2A, 2B, 4, and 7) were identified by conventional PCR. We identified isoforms 5HT 1B, 2A, 2B, 4, and 7 in whole skin; 1B, 2B, and 7 in isolated sweat glands and BSGEC. We report a method for the isolation of bovine apocrine sweat glands and suggest that keratinocyte medium supplemented with 2.5% FBS is effective and suitable for the culture of BSGEC. The presence of 5-HT receptors in BSGEC indicates that the serotonergic system is involved in regulation of BSGEC function.

Key Words: apocrine gland, serotonin 5-HT receptors, dairy cow

0513 Responses to an insulin challenge in dairy cows classed as efficient or inefficient based on residual feed intake during mid-lactation and the dry period. K. DiGiacomo\*<sup>1</sup>, E. Norris<sup>1</sup>, L. C. Marett<sup>2</sup>, W. J. Wales<sup>2</sup>, B. J. Hayes<sup>3</sup>, F. R. Dunshea<sup>1</sup>, and B. J. Leury<sup>1</sup>, <sup>1</sup>The University of Melbourne, Parkville, Australia, <sup>2</sup>The Department of Environment and Primary Industries, Victoria, Ellinbank, Australia, <sup>3</sup>The Department of Environment and Primary Industries, Bundoora, Australia.

Cows selected for milk yield are insulin resistant and readily mobilize lipid energy stores during lactation. Upon exposure to a stressor, insulin secretion from the pancreas is inhibited by epinephrine and consequently adds to the hyperglycemic responses to stress. Insulin-induced hypoglycemia is an indirect means of stimulating the hypothalamic-pituitary-adrenal (HPA) axis to release adrenocorticotropic hormone (ACTH) and subsequently cortisol. This experiment was designed to explore the nutrient partitioning and stress hormone responses to an insulin challenge in mid-lactating and dry cows classed as efficient or inefficient based on residual feed intake (RFI). Sixteen multiparous Holstein-Friesian cows  $(589 \pm 37 \text{ kg})$  were selected based on RFI extremes (8 inefficient and 8 efficient). On 2 occasions, during mid-lactation  $(122 \pm 23 \text{ d in milk})$  and the dry period (~38 d dry), an i.v. insulin challenge was conducted. Animals were housed in metabolism stalls and fed lucerne hay cubes ad libitum and recieved 6 kg DM crushed wheat grain (and minerals) per day at milking (18% CP and 10.5 MJ ME/ kg DM) and had food removed 12 h before the challenge. The day before the challenge cows were fitted with interdwelling jugular catheters. Insulin (0.12 U/kg) was infused via the catheter and blood samples collected at regular intervals pre- and postinfusion. Isolated plasma was analysed for cortisol, GH, IGF-1, NEFA, and glucose concentrations. Overall, responses to the insulin challenge were more pronounced during mid-lactation compared with the dry period. Basal plasma IGF-1 concentrations were greater in inefficient compared with efficient cows during mid-lactation (11.2 vs. 15.9 ng/mL respectively, P = 0.006) but not the dry period (P = 0.78). Peak plasma cortisol concentrations were not influenced by efficiency group during either measurement periods. Plasma cortisol and NEFA concentrations were greater, and glucose concentrations lower, in mid-lactation compared with the dry period. This experiment demonstrated a stress (cortisol) response to the insulin-induced hypoglycemia, although this response did not vary with efficiency. The diminished responses to insulin during the dry period are likely due to the varied metabolic states of the animals at these 2 varied stages of production. The greater basal IGF-1 concentrations in inefficient animals may in part explain their inefficiency as IGF-1 mimics the actions of insulin and reduces circulating glucose concentrations, reducing the concentration of glucose available for use in mammary tissues.

Key Words: stress responses, efficiency, insulin

## **0514** Interactions between metabolic load and dairy cow welfare-related parameters in herbage-based feeding systems. R. S. Zbinden<sup>1</sup>, J. J. Gross<sup>\*1</sup>, M. Falk<sup>2</sup>, H. A. van Dorland<sup>1</sup>, A. Münger<sup>2</sup>, F. Dohme-Meier<sup>2</sup>, and R. M. Bruckmaier<sup>1</sup>, <sup>1</sup>Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, <sup>2</sup>Agroscope, Institute for Livestock Sciences ILS, Posieux, Switzerland.

In Switzerland, herbage feeding with only little input of concentrates plays an important role in milk production. The objective here was to investigate the effects of a solely herbage based diet on production, metabolic, endocrine and welfare-related parameters of dairy cows. Twenty-five multiparous Holstein dairy cows were divided into 2 groups according to their previous lactation yield (4679 to 10,808 kg): a control (C, n = 13) and a treatment group (nC, n = 12) from wk 3 antepartum until wk 8 postpartum. Within C and nC, the median of the preceding lactation yields (7752 kg) was used to split cows into a high (CH, nCH) and low yielding (CL, nCL) subgroup. While CH/CL received fresh cut herbage plus additional concentrate according to their estiamted energy and nutrient requirements, no concentrate was fed to nCH/ nCL throughout the experiment. Milk yield and DMI were recorded daily. Blood samples were weekly and analyzed for IGF-1, glucose, NEFA, BHBA, and welfare-related parameters haptoglobin (Hp), serum amyloid A (SAA), β-endorphin (BE), and alkaline phosphatase (AP). Saliva samples were taken biweekly and analyzed for cortisol. Data were analyzed using mixed models. Throughout the study, CH had a higher milk yield (35.9 kg/d) compared with the other subgroups (27.2 to 31.7 kg/d, P < 0.05). Plasma glucose (3.51 vs. 3.72)mmol/L) and IGF-1 (66.0 vs. 78.9 ng/mL) concentrations were lower in nCH/nCL compared with CH/CL cows (P <0.05). Plasma NEFA and BHBA concentrations were higher in nCH (1.1 and 1.6 mmol/L) compared with the other subgroups (0.5 and 0.6 mmol/L, P < 0.05). Saliva cortisol (0.60 vs. 0.68 ng/mL), SAA (0.60 vs. 0.87 µg/mL), Hp (728 vs. 909 U/L), BE (30.0 vs. 32.1 pg/mL), and AP (48.5 vs. 45.9 mg/ mL) were not different among C and nC. In conclusion, in herbage dominated feeding systems without supplementary concentrate especially high yielding dairy cows experience a higher metabolic load during early lactation leading in turn to a reduced lactational performance compared with cows of a similar potential fed according to their needs. Low yielding dairy cows can perform well without concentrate supplementation. Interestingly, the commonly accepted welfare-related parameters cortisol, Hp, SAA, BE, and AP did not indicate a reduced animal welfare induced by metabolic stress.

**Key Words:** welfare, metabolism, herbage feeding, dairy cow

## 0515 Effects of repeated short-term feed restrictions and lipopolysaccharide-induced systemic inflammation on metabolism and performance in dairy cows. J. J. Gross\*<sup>1</sup>, E. Kalaitzakis<sup>2</sup>, O. Wellnitz<sup>1,2</sup>, H. Bollwein<sup>2</sup>, and R. M. Bruckmaier<sup>1</sup>, <sup>1</sup>Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, <sup>2</sup>Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.

A negative energy balance (NEB) may occur later in lactation when feed supply and/or quality are insufficient. We investigated if responses of metabolism, performance and immune system to energy deficiency differ between lactational stages. Fourteen multiparous Holstein dairy cows were grouped according to their previous lactation yield in 2 groups: a control (CON) group and a restricted (RES) group. The trial lasted from wk 3 antepartum until wk 12 postpartum (pp). Cows (CON and RES) were fed with grass ad libitum plus additional concentrate throughout the study, except the RES group, which received only grass during 1-wk feed-restrictions in wk 2, 5, 8, and 11 pp. At the end of the first restriction period, lipopolysaccharide (LPS) from Escherichia coli was infused intravenously (0.5 µg/kg BW) to mimic an inflammatory status interacting with a different metabolic status. Dry matter intake and milk yield were recorded daily. Blood was obtained weekly throughout the study, daily during the restriction periods in wk 2, 5, 8, and 11 pp, and every 0.5 h during the day of systemic LPS challenge. Blood samples were analyzed for glucose, NEFA, BHBA, and IGF-1 concentrations. Data were analyzed using a mixed model including group and wk as fixed effects. During restriction periods, RES had an elevated grass DMI (0.3 to 4.2 kg/d) compared with CON. In-between restriction periods, DMI did not differ between RES and CON. Milk yield was lower for RES in wk 2, 5, 8, and 11 pp (ca. 5 kg/d) compared with CON and recovered between restriction periods. On the day of LPS challenge, milk yield in RES dropped more distinct than in CON (9.5 vs. 11.3 kg/d). CON cows recovered faster in milk yield after the LPS challenge. During wk 2 pp, plasma concentrations of glucose, NEFA, BHBA were not different between RES and CON, while IGF-1 was lower in RES (41.1 vs. 82.6 ng/mL). During the restriction periods in wk 5, 8, and 11, NEFA and BHBA concentrations were elevated in RES (up to 0.67 mmol/L NEFA and 0.74 mmol/L BHBA), while glucose and IGF-1 concentration were lower in RES compared with CON (3.77 vs. 4.03 mmol/L glucose, 68.8 vs. 98.4 ng/mL IGF-1 in wk 8). In conclusion, the experiment showed the changing priority of the lactating mammary gland during different stages of lactation. Performance, metabolic and endocrine changes became less with progress of lactation. Further analyses are in progress.

**Key Words:** feed-restriction, metabolism, grass feeding, dairy cow

## 0516 Effects of heat stress on pancreatic insulin content and β-cell distribution in growing pigs. M. Sanz Fernandez<sup>\*1</sup>, J. S. Johnson<sup>1</sup>, M. Abuajamieh<sup>1</sup>, S. K. Stoakes<sup>1</sup>, S. M. Lei<sup>1</sup>, R. P. Rhoads<sup>2</sup>, and

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Heat stress (HS) induces postabsorptive metabolism changes, independent of inadequate nutrient intake. Despite marked hypophagia, HS inexplicably increases plasma insulin kinetics in a variety of animal models, and we have previously demonstrated that the HS pigs included in this study had increased plasma C-peptide (10%; P < 0.05), an indicator of insulin secretion. Whether higher plasma insulin is due to increased pancreatic insulin production or secretion is unknown. Seventeen crossbred gilts (57  $\pm$  5 kg BW) were subjected to 1 of 2 environmental treatments: (1) constant HS conditions  $(32^{\circ}C, 23\%$  relative humidity) and ad libitum feeding (n = 7), or (2) pair-feeding in thermoneutral conditions (20°C, 36%) relative humidity; PFTN; n = 10) to eliminate the confounding effects of dissimilar feed intake. After 8 d of environmental exposure, pigs were sacrificed and the pancreas was immediately collected. Insulin content was determined after acid-ethanol protein extraction from frozen samples using a commercially available ELISA kit. Fixed samples were sectioned and stained for insulin using an indirect immunoperoxidase immunohistochemical technique. Heat stress tended to decrease (32%; P = 0.08) insulin stained area compared with PFTN conditions. In agreement, pancreatic insulin protein content was numerically reduced (39%; P = 0.18) in HS pigs. There were no differences (P > 0.10) in the quantity of  $\beta$ -cell groups, determined as the number of insulin positive cells clusters. However, when classifying  $\beta$ -cell groups by size, HS decreased (66%; P < 0.01) the percentage of larger  $\beta$ cell clusters (diameter > 100  $\mu$ m), while increasing (4%;  $P \leq$ 0.01) the percentage of smaller clusters (diameter  $< 50 \ \mu m$ ). In conclusion, the similar amount of  $\beta$ -cell groups between treatments, coupled with the decrease in pancreatic insulin content in the HS pigs, indicates that HS-induced changes on plasma insulin concentration might be due to increased pancreatic insulin secretion, rather than production.

Key Words: heat stress, pig, insulin, pancreas

# 0517 Effects of protein supplementation frequency on metabolic responses associated with reproduction of beef cows. M. M. Reis\*<sup>1</sup>, R. F. Cooke<sup>1</sup>, B. I. Cappellozza<sup>1</sup>, R. Marques<sup>1</sup>, T. Guarnieri Filho<sup>1,2</sup>, G. A. Perry<sup>3</sup>, and D. W. Bohnert<sup>1</sup>, <sup>1</sup>Oregon State University–EOARC Burns, Burns, <sup>2</sup>Faculdade de Medicina Veterinária e Zootecnia, UNESP–Univ. Estadual Paulista, Botucatu, Brazil, <sup>3</sup>South Dakota State University, Brookings.

This experiment evaluated if frequency of protein supplementation impacts physiological responses associated with reproduction in beef cows. Fourteen nonpregnant, nonlactating Angus cows were ranked by parity and BW, and allocated to 3 groups. Groups were assigned to a  $3 \times 3$  Latin square design, containing periods of 21 d and the following treatments: (1) daily supplementation of soybean meal (7D), (2) soybean meal supplementation 3 times/wk (3D), and (3) soybean meal supplementation once/wk (1D). Within each period (d 0 to 21), cows were assigned to an estrus synchronization protocol; 100 µg of GnRH + controlled internal device release (CIDR) containing progesterone (P4) on d 1, 25 mg of  $PGF_{2\alpha}$  on d 8, CIDR removal plus 100 µg of GnRH on d 11. Straw was offered for ad libitum consumption. Soybean meal was individually supplemented at 1 kg/cow daily. Moreover, 3D were supplemented on d 0, 2, 4, 7, 9, 11, 14, 16, and 18, whereas 1D were supplemented on d 4, 11, and 18 of each period. Blood samples were collected on d 11 and 18 from 0 to 72 h relative to supplement feeding, and analyzed for plasma urea N (PUN). Samples collected from 0 to 12 h were analyzed for plasma glucose, insulin, and P4 (d 18 only) concentrations. Uterine flush fluid was collected 28 h after supplementation for pH measurement. Data were analyzed with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and cow as experimental unit. A treatment  $\times$  hour interaction (P <0.01) was detected for PUN, which peaked (P < 0.01) for 1D and 3D at 28 h after supplement feeding, whereas the same response was not detected in 7D. Moreover, PUN concentration at 28 h was greater (P < 0.01) for 1D compared with 3D and 7D (42.7, 34.4, and 25.7 mg/dL, respectively), and also greater (P < 0.01) for 3D compared with 7D. No treatment effects were detected ( $P \ge 0.65$ ) for plasma glucose and P4 concentrations, whereas mean plasma insulin concentrations were greater (P < 0.02) in 7D and 3D compared with 1D (4.61, 4.76, and 3.74 µIU/mL, respectively). Uterine flushing pH tended ( $P \le 0.10$ ) to be greater for 1D compared with 3D and 7D (6.204, 6.130, and 6.140, respectively). In conclusion, reducing frequency of protein supplementation to once/ week impacted plasma insulin, PUN, and uterine flushing pH, which are known to modulate reproduction of beef cows.

**Key Words:** beef cows, protein, physiology, reproduction

## 0518 A vaccine-induced acute-phase reaction increases plasma leptin concentrations in beef cattle.

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The objective of this experiment was to evaluate if a vaccine-induced acute-phase reaction also results in increased plasma leptin concentration, which would explain a potential DMI decrease in vaccinated beef cattle. Twelve yearling Angus  $\times$  Hereford calves (9 steers and 4 heifers) were ranked by sex and BW, and allocated to 2 groups (6 calves per group, 4 steers and 2 heifers). Groups were assigned to a crossover design containing 2 periods of 14 d, and the following treatments on d 0 of each period: (1) vaccination against Mannheimia haemolytica (VAC; One Shot; Pfizer Inc., New York, NY), or (2) saline-injected control (CON). Calves were maintained in individual pens, offered grass hay for ad libitum consumption, in addition to 1.3 kg/d (DM basis) of a corn-based supplement. During Period 1, hay and concentrate intake were evaluated daily. During Period 2, blood samples were collected before (0 h) and at 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144, 168, 240, and 336 h after treatment administration. All samples were analyzed for plasma haptoglobin concentration. Samples collected from 0 to 36 h were also analyzed for plasma cortisol, whereas samples collected from 0 to 96 h were analyzed for serum NEFA and plasma leptin concentrations. Values obtained at 0 h served as covariates within each respective analysis. Treatment  $\times$  day interactions were detected (P = 0.05) for hay and total DMI, given that these parameters were reduced ( $P \le 0.02$ ) in VAC compared with CON on d 0 and 1. Mean plasma cortisol was greater  $(P \le 0.01)$  in VAC compared with CON (39.8 vs. 26.3 ng/ mL, respectively; SEM = 2.4). Treatment  $\times$  hour interactions were detected (P < 0.01) for all other blood variables. Serum NEFA concentration was greater ( $P \le 0.03$ ) in VAC compared with CON at 16, 24, 48, and 72 h. Plasma haptoglobin concentration was greater in VAC compared with CON at 8 h, and from 16 to 120 h. Plasma leptin concentration was greater  $(P \le 0.05)$  in VAC compared with CON beginning at 6 h relative to treatment administration. In conclusion, plasma leptin concentration was increased during a vaccine-induced acute-phase reaction, and may explain the decrease in DMI observed herein in vaccinated cattle.

**Key Words:** acute-phase reaction, feed intake, leptin, vaccination

## 0519 A prepartum diet supplemented with rolled sunflower seed increased calf weight, the incidence of dystocia, and colostrum immunoglobulin content in Holstein cows. R. Salehi\*1, M. G. Colazo<sup>2</sup>, M. Oba<sup>1</sup>, and D. J. Ambrose<sup>2</sup>, <sup>1</sup>University of Alberta, Edmonton, Canada, <sup>2</sup>Alberta Agriculture and Rural Development, Edmonton, Canada.

Supplementing dietary fats during late gestation period has certain advantages, but its effects on the incidence of dystocia, calf weight and colostrum quality are sparsely reported. Our objective was to investigate whether prepartum diets supplemented with sunflower or canola seed will affect calf birth-weight, calving-ease and colostrum immunoglobulin content. Pregnant Holstein cows, blocked by BCS, were assigned to 1-of-3 prepartum diets supplemented with canola (n = 43, CAN; high oleic acid), sunflower (n = 46, SUN; high)linoleic acid), or control (no oilseed, n = 43; CON) from 35 d (d 35) before expected calving date until parturition (d 0). The concentrate portion of CAN- and SUN-diets contained 0.99 kg rolled oilseeds (DM basis), providing 0.27 kg/d oleic or 0.31 kg/d linoleic acid. Feed intake was recorded daily from d -35 to d 0, and BCS was evaluated on d -35 and d 0. After parturition, colostrum samples (n = 13 per treatment) were collected at first milking and stored at -20°C until evaluating total fat, protein, fatty acid profile and IgG. Calves (n = 132)were weighed at birth. Colostrum immunoglobulin content was estimated using a Brix refractometer. Cows fed CON had greater (P < 0.05) mean DMI (15.3 ± 0.6 kg/d) than those fed SUN (13.3  $\pm$  0.5) and CAN (13.5  $\pm$  0.5) during prepartum. The BCS on d -35 did not differ among treatments, but cows fed SUN  $(3.5 \pm 0.0)$  and CAN  $(3.5 \pm 0.0)$  had higher BCS on d 0 than CON  $(3.4 \pm 0.0)$ . The difference in BCS between d -35 and 0 was greater in SUN ( $0.22 \pm 0.02$ ) than in CON  $(0.12 \pm 0.02)$  but not CAN  $(0.18 \pm 0.02)$ . Total fat content of colostrum (%) was higher in CON ( $5.8 \pm 0.5$ ) compared with CAN (4.5  $\pm$  0.4) and SUN (3.8  $\pm$  0.4), whereas, total protein (%) was significantly higher in SUN ( $15.0 \pm 0.6$ ) than in CON (12.1  $\pm$  0.8) and CAN (12.5  $\pm$  0.8) fed cows. Mean colostrum immunoglobulin (brix%) was significantly higher in SUN (24.1  $\pm$  0.9) than in CON (20.4  $\pm$  1.0) and CAN (20.0  $\pm$  1.0). Cows given SUN during prepartum delivered heavier (kg) calves  $(44.3 \pm 0.9)$  than those fed CON  $(41.2 \pm 0.8)$  or CAN (42.9  $\pm$  0.8). Moreover, cows fed SUN (35%) during prepartum had a tendency to have higher incidence of dystocia at parturition than those fed CON (17%, P = 0.07) or CAN (18%, P = 0.08). In summary, cows fed supplemental oilseeds during late gestation consumed less DM than those fed a no oilseed control diet, but had greater BCS at parturition. Calf birth weight and the incidence of dystocia were higher in cows fed SUN; colostrum IgG and total protein content were also higher in cows fed SUN.

Key Words: colostrum, immunoglobulin, oilseed

## 0520 Effect of altering the dietary ratio of n-6 to n-3 fatty acids on luteolytic mechanism in dairy cows. L. F. Greco\*<sup>1</sup>, J. T. Neves Neto<sup>2</sup>, A. Pedrico<sup>2</sup>, F. S. Lima<sup>2</sup>, R. S. Bisinotto<sup>1</sup>, N. Martinez<sup>1</sup>, E. S. Ribeiro<sup>1</sup>, W. W. Thatcher<sup>1</sup>, C. R. Staples<sup>1</sup>, and J. E. P. Santos<sup>1</sup>, <sup>1</sup>Department of Animal Sciences, University of Florida, Gainesville, <sup>2</sup>University of Florida, Gainesville.

Objectives were to evaluate the impacts of altering the ratio of dietary n-6 to n-3 fatty acids (FA) on timing of luteolysis, uterine production of prostaglandin  $F_{2\alpha^2}$  and endometrial fatty acid profile and gene expression in dairy cows. Diets were supplemented (1.43% DM) with a mixture of Ca salts of fish oil, safflower oil and palm oil to create different ratios of n-6 to n-3 FA; 4, 5, and 6 parts of n-6 to 1 of n-3 FA (R4; R5; R6). Cows were blocked by milk production from 6 to 10 d in milk (DIM) and then assigned randomly to 1 of the 3 dietary treatments. Cows had the estrous cycles synchronized starting at 40 DIM. An indwelling catheter was inserted in the tail vessel on d 15 of the estrous cycle and blood was sampled every 2 h from estrous cycle d 16 to 23. Progesterone and 13,14,dihydro-15-keto-PGF<sub>2a</sub> metabolite (PGFM) were measured in plasma. Cows had the estrous cycle resynchronized and endometrial tissue was collected for biopsy on d 8 of the cycle. Gene expression and FA profile were measured. Data were analyzed using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment did not influence the length of the estrous cycle or concentrations of progesterone in plasma. Basal PGFM concentrations did not differ (P = 0.66) among treatments. The number of PGFM pulses decreased (P = 0.05) as the ratio n-6 to n-3 FA increase, and they averaged 5.6, 4.3, and 3.8 pulses, for cows fed R4, R5, and R6, respectively. The area under the curve of the largest PGFM pulse increased as the ratio n-6 to n-3 increased (P = 0.02) and were 764, 958, and 1953 pg/h per mL, for cows fed R4, R5, and R6, respectively. The concentrations of arachidonic acid increased (R4 = 8.09, R5 = 10.35, and R6 = 11.04% of the identified FA; P = 0.01) and of eicosapentaenoic acid decreased linearly (R4 = 2.29, R5 = 1.90, and R6 = 1.83% of the identified FA; P = 0.03) in the endometrium by altering the ratio of n-6 to n-3 from R4 to R6. Of the genes evaluated, expression of the oxytocin receptor, estrogen receptor and steroidogenic acute regulatory protein linearly increased as the diet change from R4 to R6. Altering dietary ratio of n-6 to n-3 FA of lactating dairy cows influenced the pattern of prostaglandin synthesis, the FA profile, and gene expression of the endometrium, but did not influence the length of the estrous cycle.

Key Words: dairy cow; fatty acid; luteolysis