skills, experience, knowledge, and overall aspirations. The world wants to hear what professionals and experts have to say, and given the real-time connectivity of online social media today, learning how to create a powerful personal brand couldn't be more important.

Key Words: personal brand, success, professional development

0196 Bridging the gaps. J. D. Crosswhite*, *North Dakota State University, Fargo.*

The animal science community is constantly changing to meet the needs of the consumer. The same is true for animal science departments striving to meet the three pronged goals of land grant universities. The landscape of research in animal science is always evolving as our knowledge of basic science expands. The competitiveness of research funding is higher than ever, and this puts increased pressure on the researchers vying for these funds. In addition to this, the generation gap between the average consumer and production agriculture is increasing, making extension education as important as ever. These challenges are making split appointment positions within academia harder to accomplish, as the education of students taking animal science classes is still a major focus. This environment has opened up the opportunity for individuals with a completed master's degree to become instructors. Having a 100% teaching appointment allows these lecturers the unique opportunity to focus all of their attention on bridging the gap between a research mindset and production animal science within the classroom. Thus, opening up time and opportunity for research and extension faculty.

Key Words: lecturer, classroom, teaching

0197 Doctoral programs in animal science: Strategies for targeting academic careers. J. S. Caton*, *Department of Animal Sciences, North Dakota State University, Fargo.*

Objectives of this review are to discuss successful doctoral student professional development strategies for targeting academic positions in the animal sciences and related fields. Entry level positions for academic careers are most often 2-way split appointments containing proportions of research, teaching, and extension responsibilities. Occasionally, institutions will offer 1- or 3-way split appointments. Positions will usually range from 9 to 12 mo appointments on a tenure-track, though variations exist. Successfully targeting these types of career positions requires deliberate planning and action by the doctoral student and mentoring team. Carefully selecting an advisor, institution, doctoral training committee, and other mentoring and training structures are essential early components in the process. Research experiences need to contain both discovery and application based aspects, present opportunities for leadership and collaborative team efforts, be solid in experimental design and methodologies, demonstrate focus in targeted areas, and breadth across species, mechanisms, methods, and systems. Data should be published in multiple venues, including refereed manuscripts. Mentoring and training in teaching at the university level needs to be real and relevant. Experience in formal and informal aspects of teaching are needed and should be supported with both student and peer teaching evaluations when possible. Mentoring in extension needs to include significant clientele contact, evidence of proficiency with a breadth of communication techniques, and clear goals and assessments. Effective training in perusing and securing grant funds to support research, teaching, and extension activities should be evident. Leadership, collaborative skills, and professionalism should be developed and effectively demonstrated. Evidence of effectively managing research teams, mentoring undergraduate students, and overseeing undergraduate research projects helps demonstrate preparedness for the transition from doctoral student to assistant professor. Strategically targeting and successfully accomplishing specific professional development activities within research, teaching, and extension will foster excellence and help secure effective and successful academic careers in animal sciences and related fields.

Key Words: academic careers, doctoral programs, professional development strategies

ASAS UNDERGRADUATE STUDENT POSTER COMPETITION

0198 Antimicrobial activity of tropical spice extracts against *Escherichia coli* O157:H7. E. Olasoji[*1], I. M. Ogunade[2], D. Kim[2], and A. T. Adesogan[2], *[1]Department of Food Science, University of Florida, Gainesville, [2]Department of Animal Sciences, IFAS, University of Florida, Gainesville.*

This study examined the antibacterial effects of spices (Alligator Pepper, Yellow Nutmeg, Tumeric, Green Pepper, Nutmeg, Ginger, African Guinea Pepper, Bayleaf, and Rosemary) on *Escherichia coli* O157:H7 (EC). Stock solutions containing 0.2 g of each spice per mL of ethanolic extract were prepared. The antimicrobial activity of each extract was examined using the agar disc diffusion method. Approximately 100 μL of EC culture was surface-plated on MacConkey agar supplemented with cefixime and tellurite. An aliquot (10 μl) of each spice extract was pipetted onto a 6.2-mm sterile paper disc on the agar surface and incubated for 24 h at 35°C. The inhibition zones around the discs were measured in millimeters. The Minimum Inhibition Concentration (MIC) of each of the spice extracts was determined by macrobroth dilution method. The experiment was repeated twice. Results were analyzed using the GLIMMIX procedure of SAS. The inhibition zones

observed for Green Pepper (19.33mm), Alligator Pepper (18.67mm), Rosemary (18.83mm), Tumeric (15.33mm), Nutmeg (16.50mm), African Guinea Pepper (16.67mm), Bayleaf (15.50) and Ginger (15.67mm) were greater ($P = 0.0001$) than that of the Control (11.67mm). African Guinea Pepper had the lowest MIC (2 mg/mL) while others inhibited EC at MIC less than 16 mg/mL. In conclusion, extracts of these tropical spices showed antimicrobial activity against EC.

Key Words: *Escherichia coli* O157:H7, inhibition zone, spices

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0199 Effect of low- and high-fat dry distillers grains supplementation on forage intake and digestibility in beef heifers. E. L. Stephenson*[1], A. L. Jones[2], J. S. Luther[1], and A. E. Radunz[1], *[1]University of Wisconsin, River Falls, [2]University of Wisconsin, Madison.*

The objective of this study was to evaluate low-medium quality intake and apparent total tract digestibility supplemented with of low-fat vs. high-fat corn dried distillers grains with solubles (DG) in yearling Angus, Hereford, and Angus-cross beef heifers ($n = 30$; 399 ± 16 kg). Heifers were stratified by BW and breed composition and then assigned to 1 of 3 treatments: (1) no supplementation (CON); (2) supplementation of low-fat DG (LDG; 5.04% EE); (3) supplementation of high-fat DG (HDG; 9.09% EE). Heifers were provided ad libitum intake of low to medium quality chopped grass hay (7.82% CP; 1.14 Mcal NE_m/kg). Both LDG and HDG were supplemented at 0.8% body weight (BW) to provide a similar CP intake of 0.22% of BW. Hay was fed twice a d at 0800 and 1600 and supplement was fed once a d before hay feeding at 0700. Individual feed intake was recorded for 37 d. Two-day BW were collected at the beginning and end of the trial. To determine apparent total tract digestibility feed-offered, feed refusals, and fecal grab samples were collected on d 27 from a subset of 3 heifers per treatment and then DM, OM, CP, ADF, NDF, and EE analysis was performed and ADIN was used an indigestible marker. Heifers supplemented with DG (LDG or HDG) had greater BW gain and ADG ($P < 0.0001$) and lower ($P = 0.002$) total DMI in g/kg of BW compared with CON, however no differences were detected ($P \geq 0.14$) in BW gain, ADG, or total DMI in g/kg of BW between HDG and LDG. Furthermore, a change in BCS was not detected ($P = 0.46$) among treatments. Heifers that were supplemented LDG had lower DM, N, and NDF apparent digestibility than heifers supplemented HDG and heifers with no supplementation ($P \leq 0.004$). Apparent digestibility of OM and ADF did not differ among treatments ($P \geq 0.05$) while heifers supplemented LDG had lower apparent digestibility of EE ($P = 0.001$) and HDG supplementation was intermediate as compared with CON. Supplementation of DG regardless of fat content reduced total DMI and improved BW gain for yearling beef heifers, but supplementing the LDG vs. HDG resulted in lower DM, N, and NDF digestibility fed with low to medium quality grass hay.

Key Words: corn distillers grains, protein supplementation, forage digestibility

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0200 Nutritive and digestibility parameters of invasive grasses in northwest Missouri. F. C. Huneke*, M. H. Richardson, A. M. Snyder, and J. D. Allen, *Northwest Missouri State University, Maryville.*

The purpose of this study was to determine nutritive and digestibility parameters of invasive grasses located in improved pastures in northwest Missouri. Samples from 18 grass species were gathered at reproductive maturity in 3 of 6 grazing pastures located at the R. T. Wright farm of Northwest Missouri State University (3 samples/species). Grasses were dried, ground, and subsequently subjected to the following laboratory analyses: NDF, ADF, and ash. Samples were also analyzed for 12-, 24-, and 48-h in situ digestibility using 2 cannulated Holstein dairy cows fed a consistent corn silage based diet. Dry matter was relatively similar (range 23.2 to 33.8 ± 4.78%DM) across species except for Stinkgrass (*Eragrostis cilianensis*; 41.7% DM) and Prairie cordgrass (*Spartina spectinata*; 45.3% DM; $p < 0.01$). Prairie cordgrass also had the greatest ($p < 0.01$) NDF and ADF (64.3 and 30.8%, respectively) when compared with the other species (range 49.1 to 60.7 ± 2.17% NDF and 22.6 to 30.8 ± 1.51% ADF). Ash content also varied (range 7 to 13.1 ± 1.48%; $p < 0.05$). Twelve hour in situ digestion was similar across species ($p > 0.10$), however, species diverged in 24- and 48-h digestion (range 42.2 to 65.4 ± 3.41% and 52.9 to 73.7 ± 3.70%, respectively, $p < 0.01$). In situ digestion developed 3 distinct digestion groups among the species, with Little barley (*Hordueum pusillum*), Foxtail barley (*Hordeum jubatum*), and Prairie cordgrass being the least ($p < 0.01$) digestible over a 48-h period (58.9, 56.4, and 52.9%, respectively). Results indicate that nutritive quality of invasive grasses varies by species, which may disrupt overall forage quality.

Key Words: cattle, digestibilty, invasive species, nutrient composition

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0201 Poor maternal nutrition during gestation alters mesenchymal stem cell (MSC) metabolism in offspring. N. H. Sereda*[1], S. M. Pillai[1], M. L. Hoffman[1], S. A. Zinn[1], Y. K. Park[2], J. Y. Lee[2], and K. E. Govoni[1], *[1]Department of Animal Science, University of Connecticut, Storrs, [2]Department of Nutritional Sciences, University of Connecticut, Storrs.*

Poor maternal nutrition due to excess or reduced nutrient intake during gestation has negative effects on fetal growth and metabolism including reduced bone and muscle, and increased adipose tissue. There is evidence that poor maternal

diet during gestation impairs the function of MSC, stem cells that contribute to bone, muscle, and adipose development, in the offspring. It was hypothesized that poor maternal nutrition during gestation would negatively alter MSC metabolism in offspring. Eighteen pregnant ewes were individually housed and randomly assigned to one of three diets (100%, 60%, or 140% of NRC requirements for TDN) at d 31 ± 1.3 of gestation. One lamb per ewe was euthanized within 24 h of birth (100% = CON, 60% = RES, 140% = OVER; n = 6/treatment) and MSC were isolated from the bone marrow of left tibia and femur. For analysis of glycolytic and mitochondrial functions, cells were plated at 30,000 cells/well and incubated at 37°C for 48 h. Assays were performed using Glycolysis Stress and Cell Mito Stress Test Kits and analyzed using the Seahorse XF^{e}24 Extracellular Flux Analyzer. Data were normalized for total cellular DNA and analyzed using PROC MIXED in SAS. Basal respiration was reduced in RES and OVER, compared with CON (127.4 ± 7.48, 90.17 ± 9.75, 87.51 ± 8.48 ρmol O_2^{-1}·min^{-1}·μg; CON, RES, OVER, respectively; $P \leq 0.008$). Compared with CON, RES and OVER had reduced ATP production (121.09 ± 6.08, 84.86 ± 11.05, 77.7 ± 6.44 ρmol O_2^{-1}·min^{-1}·μg; CON, RES, OVER, respectively; $P \leq 0.006$) and reduced maximal respiration (149.29 ± 17.05, 90.64 ± 23.81, 67.93 ± 10.15 ρmol O_2^{-1}·min^{-1}·μg; CON, RES, OVER, respectively; $P \leq 0.03$). Spare respiratory capacity was reduced in OVER compared with CON (P = 0.02) while RES were intermediate (21.9 ± 10.8, 0.47 ± 12.3, and −19.56 ± 9.24 ρmol O_2^{-1}·min^{-1}·μg; CON, RES, OVER, respectively; $P \leq$ 0.25). There were no significant differences between groups for proton leak, non-mito-derived OCR, coupling efficiency, MSC glycolysis, glycolytic reserve, non-glucose-derived extracellular acidification rate, and glycolytic reserve capacity ($P \geq 0.18$). In conclusion, maternal over- and under-nutrition during gestation reduced the basal metabolic state of offspring MSC, and the ability of these cells to up-regulate ATP production during energetic deficits. The altered MSC metabolism may contribute to impaired muscle, bone, and adipose growth and maintenance in offspring.

Key Words: mesenchymal stem cells, metabolism, nutrition

0202 The abundance of myosin heavy chain Ilb mRNA in porcine Longissimus dorsi muscle was not affected by dietary lysine level.

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Successful swine production is about raising leaner pigs, because the lean meat (i.e., the skeletal muscle) is the most desired component of pork. Myosin, the most abundant contractile protein, constitutes approximately 45% of the total myofibrillar proteins, among which myosin heavy chain IIb (MyHC-IIb) isoform appears to be the determining protein contributing to pig muscle growth. This study was conducted to determine how dietary lysine level affects the expression of MyHC-IIb mRNA in pig skeletal muscle, as lysine is the first limiting amino acid in typical swine diets. Nine crossbred barrows (94.4 ± 6.7 kg) were randomly assigned to one of three groups fed either Diet 1 (lysine-deficient), 2 (lysine-adequate), or 3 (lysine-excess), which contained 0.43, 0.71, or 0.98% total lysine, respectively. After 5 wk on the trial, pigs were slaughtered, and approximately 2 g muscle sample was collected from the middle portion of *Longissimus dorsi* of each pig. Real-time RT-PCR technology was employed to determine the abundance of MyHC-IIb mRNA in each sample using the ΔΔCT quantitative method. Results showed that there was no difference ($0.42 < P < 0.90$) in the MyHC-IIb mRNA abundance between the pigs fed Diet 1 and Diet 2, as well as between the pigs fed Diet 3 and Diet 2. These results suggest that changing dietary lysine level to either below or above the adequate requirement level did not affect the abundance of MyHC-IIb mRNA in Longissimus dorsi of finishing pigs. Since our previous study using these pigs showed that the loin eye areas of the pigs fed Diets 2 and 3 were increased by 18 and 9% when compared with Diet 1, respectively, we further hypothesized that the level of dietary lysine has a significant effect on the abundance of MyHC-IIb protein in the Longissimus dorsi of finishing pigs. Therefore, our next study will be conducted to quantify the MyHC-IIb protein in these Longissimus dorsi samples.

Key Words: myosin heavy chain Ilb, lysine, pig

0203 Identification of loci on chromosome 3 associated with susceptibility to bovine paratuberculosis using genotypes imputed to whole genome sequence in Holstein cows.

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Bovine paratuberculosis or Johne's disease (JD) is an infectious disease of ruminants caused by Mycobacterium avium paratuberculosis (Map) infection. JD continues to increase in prevalence in dairy cattle resulting in loss of profitability and increased animal suffering and death. Several studies have been conducted to identify loci associated with susceptibility to JD with the goal of using selection to reduce the prevalence of the disease. One study with 219 Holstein cows identified a locus on chromosome 3 (BTA3) associated with risk of JD. The objective of this study was to test the association with this locus, identify new quantitative trait loci (QTL), and better characterize the QTL regions in a new population of dairy cows using genotypes imputed to whole genome sequence.

Ileo-cecal lymph nodes were removed from 205 Holstein dairy cows from an Idaho abattoir at harvest, were PCR tested for the presence of Map and DNA was extracted and genotyped with the Illumina BovineSNP50 BeadChip. Cows with genotype call rates < 90% were removed, leaving 190 cows of which 70 were positive for Map (cases) and 120 were negative for Map (controls). Whole genome sequence-level genotypes were imputed from run 4 data from the 1000 bull genomes project. Indels and SNPs with MAF < 0.01 were removed leaving 708,788 biallelic SNPs for analysis on BTA3 with Efficient Mixed Model Association expedited (EMMAX) additive and allelic models. Little evidence for population stratification was evident as l = 1.01. The additive model identified 10 QTL that were moderately associated ($p < 5.5x10^{-5}$) with JD on BTA3, and the allelic model identified 21 QTL (20 that were moderately associated with JD, and one QTL strongly associated ($p = 5.13 \times 10^{-7}$), but did not include the previously identified locus on BTA3. The use of imputed genotypes aided in identifying new QTL, more narrowly defining the QTL regions and testing if QTL were replicated in new cattle populations.

Key Words: bovine paratuberculosis, genetics, chromosome 3

0204 Effect of the total Western diet via direct or ancestral exposure on estrous cycling in third-generation offspring in mice. K. Contreras*[1], J. Cuthbert[1], S. Phatak[1], D. Larson[2], and A. Benninghoff[1], *[1]Utah State University, Logan, [2]USTAR Applied Nutrition Research, Logan, UT.*

Obesity is a contributing factor to many diseases, such as cancer and diabetes. Moreover, mounting evidence points to a role for obesity as a risk factor for infertility and other reproductive dysfunctions. In a previous study, mice fed a diet containing 60% of energy from fat experienced abnormal estrous cycles, with significant extended time in the diestrus phase (Brothers et al., 2010 *Cell Metab*. 12(3): 295–305). Animal model studies investigating the contribution of obesity to other adverse health outcomes usually do not account for typical Western dietary patterns with respect to macro- and micronutrient content. Previously, our group developed the Total Western Diet (TWD) for rodents, which models the typical American diet with respect to macro- and micronutrient content on an energy density basis, as opposed to other simple high fat diets traditionally used in pre-clinical studies. The primary objective of this study was to determine the impact the TWD on estrous cycling in mice. Based on prior observations with a very high fat diet, we expected mice fed TWD would have abnormal estrous cycles. C57BL/6J mice were bred for three generations, during which they were fed an optimized diet (AIN93G), TWD or a simple high fat diet (45% fat DIO) in the F_0 only, F_0 through F_3 or the F_3 generation only ($n = 14$ to 17 mice all but one group, which had $n = 6$). Vaginal cell smears were obtained over 10 d from individual F_3 offspring at 23 wk of age. Samples were stained, fixed and then examined via light microscopy to assess estrous stage. The percent time in proestrus, estrus, metestrus and diestrus were calculated for each individual, and data were analyzed using a mixed model analysis of variance with cage as a random nested factor. No significant differences were observed among any of the diet treatment groups for any of the estrous cycle stages ($p > 0.18$). These data disagree with prior observations that a high fat diet prolonged time in diestrus, although the current study used diets with fewer calories from fat (35% for TWD and 45% for DIO) compared with the study by Brothers et al. (2010), which employed a 60% fat diet. As part of the present study, other ongoing work will assess impacts of these test diets on other reproductive parameters, including birth rate, cannibalism rate, weaning weight and biomarkers of oocyte quality.

Key Words: Western diet, estrous cycle, obesity

0205 Maternal over-feeding during gestation alters islet size and number in the pancreas of 135-d-old fetuses. M. C. Wynn*, M. L. Hoffman, S. M. Pillai, A. K. Jones, K. K. McFadden, S. A. Reed, S. A. Zinn, and K. E. Govoni, *Department of Animal Science, University of Connecticut, Storrs.*

Maternal over-feeding during gestation can lead to increased circulating insulin concentrations, increased carcass adiposity and reduced insulin sensitivity in the offspring. However, the mechanisms behind these changes are not well known. We hypothesized that maternal over-feeding during gestation would affect structures of the endocrine and exocrine pancreas of fetuses at d 135 of gestation. For this study, ewes were fed either 100% (control-fed; $n = 6$) or 140% (over-fed; $n = 7$) of NRC requirements for TDN starting at d 30 ± 0.02 of gestation. Ewes were euthanized at d 135 of gestation and the pancreas from each fetus ($n = 11$ fetuses per treatment group) were collected. Fetal body weight as well as pancreas weights were recorded and pancreas tissue was embedded in optimal cutting temperature medium for histological analysis. A total of 8 fetuses from control-fed ewes (CON) and 9 fetuses from over-fed ewes (OVER) were used for histological analysis. Three 5 µm thick sections per fetus were stained with Harris Hematoxylin and Eosin Y. Sections were imaged using a Zeiss Axiovert 200M microscope with 3 to 4 images taken per section. Islet and duct size and number were quantified using ImageJ across 5 and 10 images per fetus, respectively. Data were analyzed using a student's *t* test. An effect of maternal diet was not observed for fetal BW or pancreas weight expressed as a percent of BW at d 135 of gestation ($P \geq 0.71$). In OVER lambs, islet size was 63% greater compared with CON (5404 ± 477 µm and 8836 ± 300 µm for CON and OVER, respectively; $P < 0.01$). There was an 18% decrease in islet number in OVER lambs compared with CON (8.8 ± 0.5 and 7.2 ± 0.4 islets for CON and OVER lambs, respectively; $P =$

0.03). No effects of maternal over-feeding were observed for offspring duct size or number ($P > 0.13$). The observed effect of maternal over-feeding on islet size and number could be due to changes in cell function and differentiation as a result of being exposed to excessive nutrients during gestation. In conclusion, maternal over-feeding during gestation alters the fundamental structural aspects of the endocrine pancreas of the fetus, which may contribute to altered pancreas function during gestation and later in life. Further studies are needed to determine the link between changes in pancreas structure and function later in life.

Key Words: maternal nutrition, pancreas, sheep

0206 Comparison of high definition Zenmuse X3 and X5 video cameras onboard unmanned aerial vehicles for future use in precision ranching.

C. F. Solecki* and J. S. Church, *Thompson Rivers University, Kamloops, BC, Canada.*

The use of unmanned aerial vehicles (UAVs) in agriculture to increase the efficiency of management is a new and rapidly advancing field. Being able to locate and identify cattle with UAVs would enable producers to better utilize spatiotemporal data from range livestock to better manage both livestock herds and range. UAVs provide a method of capturing aerial video observation data of cattle on extensive range that is more flexible, affordable, and safer for the pilot than traditional aircraft. In this study we used common industry cattle ear tags of two sizes (large tags = 7.5 × 5 cm, small tags = 5.5 × 3 cm) of varying colors as well as back tags (23 × 14 cm, used in behavioral studies) to assess the visual acuity of two commercially available aerial high definition cameras. The aim of this project was to assess the capabilities and limitations of these aerial cameras specifically designed for UAV use, with the goal of determining their practical use in the field as tools for observing and identifying cattle. A set of images from each camera was obtained using the DJI Inspire 1 Pro flight platform (Da-Jiang Innovations Science and Technology Co. Ltd., Shenzhen, China). Images were captured at an initial height of 5 m and progressing upward at 5 m intervals to 80 m above ground level (ABL). An onboard GPS module on the UAV was used to monitor and record the height of the aircraft at each interval. Recorded images were then assessed on a computer monitor to determine whether identification of numbering and lettering on the tags was possible. Through qualitative visual assessment of these aerial photographs, it was determined that the capabilities of the Zenmuse X5 were significantly superior than that of the Zenmuse X3 and will therefore be of greater use in future identification of animals using UAVs. We concluded that identification by cattle ear tags using a UAV is likely not a practical application due to the maximum ABL that is required to identify numbering and lettering on tags (15 m using the Zenmuse X5); however, identification of numbering on back tags to identify individual animals was possible up to an ABL of 70 m using the Zenmuse X5 camera.

Key Words: cattle, drone, ear tags

0207 Leucine supplementation increases mouse mammary cell proliferation in vitro.

M. M. McGuckin*, R. Manjarin, and D. G. Peterson, *California Polytechnic State University, San Luis Obispo.*

The objective of this study was to determine if leucine supplementation increases mammary epithelial cell proliferation in vitro, and whether this effect is associated with the upregulation of leucine transporters, cell cycle protein regulators, and leucyl-tRNA synthetase, which mediates leucine-induced activation of the mammalian target of rapamycin complex 1. Growth of HC11 mouse mammary epithelial cells in either control (CON; 0.38 mmol/L leucine) or leucine-supplemented HMEM media (LEU; 1.52 mmol/L leucine) was assessed by the MTT proliferation assay over 6-d, followed by RNA extraction and quantification of gene expression. Transcript abundance of leucine transporter LAT1 (*SLC7A5*), cyclin D (*CCND1*), leucyl-tRNA synthetase (*LARS2*), and reference genes hypoxanthine guanine phosphoribosyl transferase (*HPRT*), RNA polymerase 3 (*POL3*) and ribosomal protein L3 (*RPL3*) was determined using reverse transcription quantitative PCR. Data were analyzed using a linear mixed model procedure of SAS that included the fixed effect of treatment and the random effect of replicate. Compared with CON, proliferation of leucine-treated cells tended to increase at d 5 ($P < 0.1$), and was significantly higher at d 6 ($P < 0.01$). Similarly, expression of genes encoding LAT1 and leucyl-tRNA synthetase was increased ($P < 0.05$) in cells exposed to LEU. Messenger RNA abundance of cyclin D did not differ between treatments. Taken together, these results suggest that leucine supplementation increases the proliferation of mouse mammary epithelial cells in vitro, which may be mediated by the upregulation of LAT1 and leucyl-tRNA synthetase at the gene expression level. These results represent a novel contribution to our understanding of how amino acid nutrition regulates mammary gland growth, and to the critically needed tools for development of mechanistic models of nutrient utilization for improving efficiency of milk production.

Key Words: mammary gland, proliferation, leucine, growth

0208 Effects of maternal nutrition during gestation on placental steroid metabolizing enzyme activity in sheep. K. J. McCarty*[1], M. P. T. Coleson[1], S. M. Pillai[2], M. L. Hoffman[2], A. K. Jones[2], K. E. Govoni[2], S. A. Reed[2], S. A. Zinn[2], and C. O. Lemley[1], [1]*Mississippi State University, Starkville,* [2]*Department of Animal Science, University of Connecticut, Storrs.*

Normal pregnancy in sheep relies heavily on placental secretion of steroids during the last half of gestation. Maternal nutrient restriction or over-nutrition has been shown to increase or decrease peripheral concentrations of steroids, respectively. The objective was to determine the effects of maternal nutrition on placental steroid metabolizing enzyme activity in sheep. Pregnant ewes ($n = 60$) were allocated to receive either 100% [control-fed (CON; $n = 20$)], 60% [restricted-fed (RES; $n = 20$)], or 140% [over-fed (OVER; $n = 20$)] of NRC requirements for TDN beginning at d 30.2 ± 0.2 of gestation. Ewes from each nutritional treatment were slaughtered at d 45 ($n = 20$), 90 ($n = 20$), or 135 ($n = 20$) of gestation. At slaughter the maternal (caruncle) portion of the placenta was collected for enzymatic activity analysis. Activity of cytochrome P450 1A (CYP1A), cytochrome P450 3A (CYP3A), and UDP-glucuronosyltransferase (UGT) were determined using specific luminogenic substrates. Activities were expressed relative to mg of tissue protein. Data were analyzed using MIXED procedure of SAS and the model statement included nutritional treatment, day of gestation, and their respective interaction. Main effects are discussed in the absence of nutritional treatment by gestational day interactions. Activity of CYP1A in the caruncle was not different across gestational d ($P = 0.15$) or nutritional treatment ($P = 0.94$). Similarly, activity of CYP3A in the caruncle was not different across gestational d ($P = 0.29$) or nutritional treatment ($P = 0.98$). Activity of UGT was not different across gestational d ($P = 0.26$). Activity of UGT in the caruncle tended ($P = 0.07$) to be different across nutritional treatment, whereby activity was increased ($P = 0.02$) by 170% in OVER compared with CON ewes. In addition, activity of UGT in the caruncle tended ($P = 0.10$) to be increased by 86% in OVER compared with RES ewes, while activity was not different ($P = 0.50$) between RES and CON ewes. In conclusion, over-fed ewes had an increase in activity of UGT in the caruncle compared with control-fed and restricted-fed ewes. Therefore, the increase in caruncle UGT could be involved in stimulating additional steroid metabolism during gestation, thereby contributing to the decrease in peripheral concentrations observed in over-fed animals.

Key Words: cytochrome P450, maternal nutrition, steroid

0209 Relationship between antioxidants and residual feed intake in grazing heifers. J. N. Kidrick*, E. Felton, K. S. Shaffer, and K. M. Barnes, *West Virginia University, Morgantown.*

Residual feed intake (RFI) has been established to be a more accurate measure of feed efficiency; however, questions remain about the specific factors that contribute to individual variation. It has been determined that heat production from metabolic processes, body composition, and physical activity explain a large proportion of the variation but not 100%. Additionally, because RFI is an expensive trait to measure, identification of an easily measured biomarker of RFI would be beneficial in animal selection. Our current objective was to determine if antioxidant status contributes to variation in RFI. Serum was collected from a group of genetically similar heifers that were maintained on the same diet. During the feeding period, feed intake and body weight were recorded and used in the calculation of ADG and RFI. Serum nitric oxide ($n = 48$), glutathione peroxidase ($n = 34$), and total antioxidant capacity ($n = 34$) were measured using colorimetric assay kits. Nitric oxide levels were estimated using a nitrate/nitrite kit. Serum nitric oxide tended ($P = 0.08$) to be positively ($r = 0.26$) correlated with RFI. Glutathione peroxidase and total antioxidant capacity were not correlated with RFI, but total antioxidant capacity did tend ($P = 0.06$) to be negatively ($r = -0.33$) correlated with ADFI. Our data indicate that total antioxidant status may not be an adequate indicator of RFI. Increased intake may require a greater utilization of antioxidants, thus the reduced levels we observed in high intake heifers. The positive correlation we observed between RFI and nitric oxide could indicate less tissue turnover in the more efficient animals (low RFI). Therefore nitric oxide does show some potential as a biomarker of RFI.

Key Words: antioxidants, residual feed intake, nitric oxide

0210 Effects of spices on in vitro enteric methane production. S. Taylor*, I. M. Ogunade, D. Kim, K. G. Arriola, and A. T. Adesogan, *Department of Animal Sciences, IFAS, University of Florida, Gainesville.*

This experiment was conducted to study the effects of spices (*Rosmarinus officinalis*, RO and *Allium cepa*, OP) on in vitro rumen fermentation, methane production and digestibility of corn silage-based ration. A corn silage-based total mixed ration (TMR; 0.5 g/sample) for dairy cows was treated with each of the spices at doses of 0 (Control), 5 (Low), 10 (Med) and 15% (High) of the TMR or with monensin (1.2 mg/g of TMR). Each mixture was incubated in triplicate in 50 mL of a rumen fluid-buffer inoculum (ratio 1:2) in a 120-mL gas-tight culture bottle at 39°C for 24 h in each of two runs. In vitro DM digestibility (DMD), fermentation parameters, gas

and methane production were measured. Data were analyzed using the GLIMMIX procedure of SAS. The high dose of RO decreased DMD and acetate-propionate ratio relative to the Control, whereas, all doses of OP increased DMD and decreased acetate-propionate ratio relative to Control and monensin treatments. Gas volume (mL/g DM) and methane (mL/g DMD) were reduced ($P < 0.05$) by the High dose of RO and monensin, but not by Low and Med doses, relative to the control. Compared with monensin, adding OP at Low, Med and High doses reduced ($P < 0.05$; mL/g DMD) the gas volume (158 vs. 140, 136 vs. 110) and methane production (13.48 vs. 9.98, 9.46, and 10.38), respectively. Ruminal pH was increased by monensin and reduced by the High dose of OP. In conclusion, the High rate of *Rosmarinus officinalis* reduced methane production but decreased TMR digestibility whereas, all doses of of *Allium cepa* reduced methane production and increased TMR digestibility compared with the control and monensin treatments.

Key Words: methane, monensin, spices

0211 An exploratory observational study to quantify ante- and post-mortem complete blood count variables in fed beef cattle. C. L. Rogers*[1], T. J. McEvers[1], J. T. Richeson[1], S. L. Roberts[2], and T. E. Lawrence[1], [1]*West Texas A&M University, Canyon,* [2]*Department of Agricultural Sciences, West Texas A&M University, Canyon.*

Our objective was to quantify changes in complete blood count (CBC) variables before and after terminal marketing of fed beef cattle. Steers ($n = 39$; BW = 622 ± 6.6 kg) were used to obtain individual blood samples the morning before harvest (0600 h; BASAL), the morning of harvest (0600 h; ANTE), and on exsanguination at a nearby commercial abattoir (1500 h; POST). Blood was collected via jugular venipuncture (BASAL and ANTE) or proprietary method (POST) and analyzed within 4 h using an automated hemocytometer. Repeated measures analysis was used to determine changes in the concentration (K/μL) of total white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), basophils (BASO), platelets (PLT), and red blood cells (RBC, M/uL), hemoglobin (HGB, g/dL), and mean corpuscular HGB concentration (MCHC, g/dL). The percentage of NEUT (NP), LYMPH (LP), MONO (MP), EOS (EP), and BASO (BP) relative to WBC was also calculated. Moreover, hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular HGB (MCH, pg), and NEUT to LYMPH ratio (N:L) were determined. No differences were detected ($P \geq 0.10$) among CBC variables between BASAL and ANTE except for MONO, MP, MCV, and MCHC ($P \leq 0.01$). However, marked and frequent alterations in CBC variables were observed between BASAL and POST; increased ($P \leq 0.05$) NEUT (1.94 K/uL), BASO (0.003 K/uL), MCV (2.13 fL), NP (24.21%), and N:L (1.04) were coupled with decreased ($P \leq 0.05$) PLT (175.87 K/uL), LYMPH (2.53 K/uL), WBC (1.12 K/uL), MONO (0.37 K/uL), EOS (0.16 K/uL), MCHC (1.67 g/dL), HGB (0.37 g/dL), MCH (0.15 pg), LP (20.07%), MP (2.94%), and EP (1.24%). Likewise, ANTE to POST resulted in increased ($P \leq 0.04$) values for NEUT (1.72 K/uL), BASO (0.004 K/uL), MCV (1.47 fL), NP (22.78%), BP (0.03%), and N:L (0.98) concomitant with decreased PLT (172.66 k/uL), LYMPH (2.38 K/uL), WBC (1.31 K/uL), MONO (0.49 K/uL), EOS (0.16 K/uL), MCHC (1.10 g/dL), HGB (0.32 g/dL), MCH (0.09 pg), LP (17.63%), MP (4.05%), and EP (1.14%). Few variables differed from BASAL to ANTE, yet NEUT and N:L increased and EOS decreased between ante- and post-mortem collection times. This indicates that terminal marketing is stressful, but further research is needed to delineate the specific stressors such as handling, transportation, lairage time or immobilization, that may be most impactful during terminal marketing.

Key Words: cattle, complete blood count, slaughter

0212 Body fat distribution is a determinant of pulmonary arterial and central venous pressures in feedlot cattle. K. M. Freeman*, A. K. Gulick, B. C. Bernhard, R. J. Rathmann, J. O. Sarturi, and J. M. Neary, *Texas Tech University, Lubbock.*

The objective of this study was to determine if adiposity is associated with mean pulmonary arterial and central venous pressures in feedlot cattle during the finishing phase. These pressures are measures of the severity of pulmonary hypertension and venous congestion due to right heart failure, respectively. Pressures were measured in a cohort of crossbred yearling steers ($n = 23$, initial BW = 364 ± 52 kg) at an altitude of 975m, 6 d before slaughter. Steers were fed for 171 d. Measures of body fat evaluated included 12th rib fat thickness, USDA quality grade, KPH fat percentage, and empty body fat percentage (EBFP). These served as proxies for subcutaneous fat, intramuscular fat, visceral fat, and total body fat, respectively. The EBFP was calculated from hot carcass weight, 12th rib fat thickness, USDA quality grade, and longissimus muscle area. KPH fat was evaluated subjectively by a USDA grader. Mean (± SD) pulmonary arterial and central venous pressure were 54 ± 6 mmHg and 28 ± 5 mmHg, respectively, and were positively associated ($P < 0.01$, $r^2 = 0.26$). Cattle with a KPH fat of 2% ($n = 18$) had mean central venous and pulmonary arterial pressure that were 8 ± 2 mmHg ($P < 0.01$, $r^2 = 0.41$) and 8 ± 3 mmHg ($P < 0.01$, $r^2 = 0.26$) greater than cattle with a KPH fat of 1.5% ($n = 5$), respectively. When controlling for mean pulmonary arterial pressure as a covariate ($P = 0.20$), cattle with KPH fat of 2% had a mean central venous pressure that was 7 ± 2 mmHg greater than a KPH fat of 1.5% ($P = 0.01$, $R^2 = 0.43$). This indicates that KPH fat is deleterious to cardiac function independent of its effect on mean pulmonary arterial pressure. None of the other body fat measures evaluated were associated with either central venous or

pulmonary arterial pressures ($P > 0.40$). These findings are in agreement with the many peer-reviewed studies that have reported visceral adiposity to be a strong risk factor for cardiovascular disease in humans. Increasing visceral adiposity may, in part, explain why mean pulmonary arterial and central venous pressures increase in cattle through the feeding period.

Key Words: heart failure, adiposity, visceral, steer

0213 The effects of lavender oil on stalled horses subjected to a stressor. S. R. Adkins*, A. I. Apel, K. D. Vogel, and D. N. Smarsh, *University of Wisconsin, River Falls.*

The use of alternative medicine, such as aromatherapy, has increased in recent years within the equine industry. Lavender oil is commonly thought to have a calming effect, however, there is limited data in horses to confirm these claims. In addition, there are questions regarding the efficacy of such products. Therefore, the two objectives of this research were to conduct a basic chemical analysis of lavender essential oil products, and to assess the potential anti-anxiety effects of lavender essential oil on stalled horses subjected to a stressor. In the first part of this study, gas chromatography analysis was performed on lavender oil products from two companies looking at linalool concentrations. Both oils were of the *Lavendual augstifolia* species, and results were then compared with standards set by the International Standards Organization and published third-party tests. In the second part of this study, 18 horses (12 Quarter Horse geldings and 6 Thoroughbred geldings, aged 10 ± 5 yr) were organized into a Latin Square design with each horse acting as its own control. Horses were individually stalled for a total of 2 h, during which heart rate (HR) and salivary samples were collected at 10, 30, 50, 60, 80, 90, 100, and 110 min. The treatment group was administered lavender oil by aromatic application for 15 min at 40 min and 95 min. The control group did not have an application of lavender oil. The stressor used was a sound recording of sirens played for 10 min. Salivary cortisol and HRs were later analyzed. Gas chromatography results confirmed that both lavender oil products tested did contain linalool. There was an overall effect of time ($p < 0.0001$) on HR, with HR lower at 10, 30, and 50 min as compared with 60 min ($p < 0.0001$), and higher at 80, 90, 100, and 110 min as compared with 60 min ($p < 0.0017$). There was an overall effect of treatment on salivary cortisol ($p = 0.04$), and a trend for an overall effect of treatment on HR ($p = 0.0518$). While the stressor did increase HR, the application of lavender oil did not have a direct effect on HR or cortisol at any specific time point. Further research is needed to identify an effective dose of lavender oil via aromatic application as a means of reducing stress in horses.

Key Words: horse, lavender, anti-anxiety

0214 FSH dependent and IGF-1 independent phosphorylation of β-catenin is similar in bovine and human granulosa cells. C. R. Smith*[1], B. H. Aloqaily[2], C. A. Gifford[3], B. I. Gomez[2], and J. A. Hernandez Gifford[2], *[1]Oklahoma State University, Stillwater, [2]Oklahoma State University, Stillwater, [3]Department of Animal Science, Oklahoma State University, Stillwater.*

Estradiol is a steroid hormone and is required for female fertility. Ovarian granulosa cells (GC) are the major source of estradiol, and synthesis of estradiol relies on the pituitary gonadotropin FSH and local ovarian signaling molecule, insulin-like growth factor 1 (IGF-1). In GC, FSH signals primarily through protein kinase A (PKA), which contributes to β-catenin phosphorylation at Ser^{675} and Ser^{552}. Beta-catenin is a co-transcriptional protein required for the maximal aromatase gene expression and subsequent estradiol production. Ovarian derived IGF-1 signals primarily through protein kinase B (AKT) to regulate steroidogenesis. The objective of the current study was to identify which one of these kinases is responsible for β-catenin activation, and if the phosphorylation has a species specific pattern. Bovine GC and human GC line (KGN) were cultured and treated with IGF-1 (50 ng/mL), FSH (100 ng/mL), or the FSH agonist Forskolin (FSK) (10 μM) for 24 h. At the termination of the treatment period, cells were collected for protein quantification of total and phosphorylated β-catenin abundance via Western blot analysis and protein abundancies were analyzed using densitometry software and the values were analyzed using GLM procedure of SAS. In bovine GC total β-catenin protein increased with FSH, IGF-1, and FSH+IGF-1 ($n = 3$; $P < 0.1$) treatments compared with control. Similarly, in KGN cells FSK increased total β-catenin abundance. However, IGF-1 did not stimulate β-catenin abundance when compared with control. These species differences may be due to a downregulated IGF-1 receptor in the KGN cell line. Phosphorylation of β-catenin Ser^{675} did not differ among treatment groups regardless of cell types. In contrast, phosphorylated Ser^{552} on β-catenin was dramatically increased by FSH or co-treatment of FSH+IGF-1 ($n = 3$; $P < 0.1$), and by FSK and FSK+IGF-1 ($n = 3$; $P < 0.001$) in bovine and KGN cells, respectively. These results suggest that activation β-catenin via phosphorylation at Ser^{552} affected by FSH but not IGF-1 signaling. Follicle stimulating hormone signaling induces β-catenin phosphorylation in cattle and human, indicating that phosphorylated β-catenin is essential in estradiol production regardless of species.

Key Words: β-catenin, FSH, IGF-1

0215 Receptor (chemosensory) transporter protein-4 expression and regulation in bovine granulosa cells. C. N. Horsley[*1], B. H. Aloqaily[1], J. A. Hernandez Gifford[1], and C. A. Gifford[2], *[1]Oklahoma State University, Stillwater, [2]Department of Animal Science, Oklahoma State University, Stillwater.*

In the ovary, the female egg is developed within a follicle, and a follicle is composed of thecal and granulosa cells (GC). These two cell types coordinate development of the oocyte and well as synthesize the steroid hormones progesterone and estrogen. Both FSH and insulin-like growth factor-1 (IGF-1) are known to regulate follicle development and drive steroid hormone production. Receptor (chemosensory) transporter protein-4 (RTP4) belongs to a gene family whose function is to transport G protein coupled receptors. Work in our laboratory suggests that the message for *RTP4* is found in GCs, but the function(s) and regulation of RTP4 is unclear. The objective of current study was to evaluate the effect of FSH and IGF-1 on expression of RTP4 in bovine GC and determine RTP4 localization in bovine ovaries. A rabbit was immunized using 18 AA in an antigenic region of the amino terminus of RTP4. Rabbit serum was collected pre-immunization and at sacrifice (immune serum). Cross-sections from 3 bovine ovaries were incubated with pre-immunized serum and immune serum (1:600) and were developed using biotinylated anti-rabbit IgG for visualization. Primary bovine granulosa cells (n = 3/treatment) were treated with vehicle control, FSH (100 ng/mL; 24 h), IGF-1 (50 ng/mL; 24 h), Forskolin (10 μ M; 24 h) and a combination of the treatments for 24 h and protein lysates collected. Fifty μg of protein was separated by SDS-PAGE. Pre-immunized and immune serum was incubated on separate blots and detected using biotinylated anti-rabbit IgG and horseradish peroxidase development, protein abundancies were analyzed using densitometry software and the values were analyzed using GLM procedure of SAS. Pre-immunized serum displayed no signal in ovarian cross-sections while immunized serum exhibited a robust signal that was ubiquitously expressed in the ovary. Western blot analysis revealed a unique band in immunized serum that showed greater expression ($P < 0.05$) in IGF-1-treated samples compared with controls or FSH-treated samples. However, the unique band was observed at unexpected size; thus, further experiments are necessary to elucidate the size of RTP4 in ovarian tissue. These data suggest that RTP4 is present in the ovary and is regulated by IGF-1 indicating that RTP4 may play a role in steroidogenesis or follicular development.

Key Words: ovary, RTP4, steroidogenesis

0216 Protein expression and localization of receptor (chemosensory) transporter protein 4 in the endometrium during early pregnancy in sheep and cattle. K. S. Wilson[*1], J. A. Hernandez Gifford[1], T. L. Ott[2], and C. A. Gifford[3], *[1]Oklahoma State University, Stillwater, [2]Department of Animal Science, Pennsylvania State University, University Park, [3]Department of Animal Science, Oklahoma State University, Stillwater.*

Reproduction in domestic ruminants continues to challenge efficiency in livestock operations. During early pregnancy, the conceptus produces a unique type I interferon, interferon-tau (IFNT), which acts both locally and systemically to regulate IFN-stimulated genes (ISG) in the maternal uterus and circulating immune cells. Previous work demonstrated that mRNA levels for receptor (chemosensory) transporter protein-4 (*RTP4*) was regulated by pregnancy in peripheral immune cells, the endometrium, and corpus luteum. Receptor transporter protein-4 belongs to a class of G protein coupled receptor transporters, but the function(s) of RTP4 in reproduction remains unclear. Though, mRNA levels for RTP4 are spatially and temporally regulated during early pregnancy in ruminants, there is no information regarding RTP4 protein expression. The objective of the current experiment was to evaluate RTP4 protein expression and localization in the endometrium during early pregnancy in cattle and sheep. A rabbit was immunized using 18 AA in an antigenic region of the amino terminus of RTP4. Rabbit serum was collected pre-immunization and at sacrifice (immune serum). Cross-sections of a D15 pregnant ewe were incubated with pre-immune serum and immune serum (1:600 primary antibody concentration), and protein presence was detected after incubation with biotinylated anti-rabbit IgG and horseradish peroxidase development. No signal was detected for pre-immunized serum, while immune serum exhibited a robust signal in the luminal and glandular epithelium and in the stromal compartments. In the second experiment, cross-sections from D17 pregnant and cyclic Holstein heifers were utilized for immunofluorescence detection of RTP4 protein. Pre-immune serum showed no fluorescence, but immune serum exhibited strong fluorescence in the luminal epithelium, glandular epithelium, and stroma during pregnancy. Interestingly, previous work using in situ hybridization did not detect a presence of *RTP4* mRNA in the luminal epithelium, but the RTP4 protein was clearly expressed in the luminal epithelium in the current experiment. These results demonstrate that RTP4 protein is expressed in the uterus and is regulated by pregnancy. Because RTP4 protein is both expressed in maternal tissues and regulated by pregnancy, it seems likely that RTP4 plays a role in establishment of pregnancy in domestic ruminants.

Key Words: uterus, interferon-tau, RTP4

0217 Follicle-stimulating hormone regulation of proenkephalin in granulosa cells. A. D. Gullic*[1], B. I. Gomez[1], B. Couger[1], C. A. Gifford[2], and J. A. Hernandez Gifford[1], [1]*Oklahoma State University, Stillwater,* [2]*Department of Animal Science, Oklahoma State University, Stillwater.*

Estradiol biosynthesis by ovarian granulosa cells (GC) is crucial for normal female reproductive function and is mediated primarily by FSH. Binding of FSH elicits a multitude of signaling cascades to enhance or inhibit the expression of target genes to regulate estradiol production. The objective of the current experiment was to utilize global expression analysis to identify genes significantly regulated by FSH. Primary rat GC were cultured in the presence or absence of FSH (100 ng/mL) for 24 h and gene expression was analyzed via microarray. Of the 1104 FSH-regulated genes, the opioid precursor proenkephalin *(Penk)* was downregulated ($P < 0.001$) with FSH treatment. Endogenous opioid peptides originate from three protein precursors Penk, proopiomelanocortin (Pomc), and prodynorphin (Pdyn). Stimulation of the μ, δ, and κ opioid receptors in GC downregulate basal estradiol concentrations in cultured GC. Therefore, we hypothesized that Penk blocks FSH-induced estradiol production. Real-time PCR was used to confirm *Penk* regulation in response to FSH treatment. Rat GC were treated with and without FSH for 24 h ($n = 3$) for quantification of *Penk*. Relative fold change values were evaluated using the GLM procedure of SAS and means were separated using the PDIFF function when the model was significant. In agreement with microarray data, FSH downregulated *Penk* 12.81-fold ($P < 0.01$) when compared with control. To determine if the opioid pathway disrupts FSH target genes, KGNGC, a human granulosa tumor cell line, were pre-treated with vehicle or β-endorphin (100 nM), a ligand for the μ opioid receptor, for 5 h followed by treatment with or without 5 μM forskolin (FSK) for 24 h ($n = 3$). Steady state mRNA levels for aromatase (*Cyp19a1)* were quantified via real-time PCR. As expected, FSK increased ($P < 0.01$) *Cyp19a1* 33.5-fold compared with vehicle control whereas treatment with β-endorphin was similar to controls ($P = 0.97$). Co-stimulation of KGN GC with β-endorphin and FSK did not decrease *Cyp19a1* ($P < 0.01$). Results from these experiments indicate that FSH regulates opioid peptides in granulosa cells and work in the literature suggests that opioids regulate steroidogenesis. In the current experiment, stimulation of the μ receptor and subsequent opioid signaling pathway did not inhibit the ability of FSK to increase *Cyp19a1* indicating that opioid regulation of steroidogenesis could be through mechanisms other than disruption of the cAMP signaling pathway or through other opioid receptors.

Key Words: FSH, opioid, aromatase

0218 Optimization of probes and PCR conditions for the correlation between 4 genes and production of high citrate in milk. V. A. Smith*[1], R. Manjarin[1], and R. Jimenez-Flores[2], [1]*California Polytechnic State University, San Luis Obispo,* [2]*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

We have previously demonstrated the existence of 4 gene products associated to key metabolic pathways that are necessary for the production of citrate in milk, namely isocitrate dehydrogenase 1 (*IDH1*), pyruvate dehydrogenase β (*PDHB*), pyruvate kinase (*PKM2*), and solute carrier family 25 member 1 (*SLC25A1*). Following sequencing of genome of a small sample of cows in the Cal Poly herd, it was shown several single nucleotides (SNP) within these genes that were significantly associated with increased milk citrate content, and therefore that could be potentially selected on to influence the outcome of citrate in milk. We now aimed to design primers for identifying the SNPs in the various genes involved in citrate production, and to optimize the conditions for PCR amplification. Primers for *IDH1*, *PDHB*, *PKM2* and *SLC25A1* were designed based on publicly available bovine cDNA and on expressed sequence tag (EST) sequences deposited in the National Center for Biotechnology database using Primer Express software with default settings. Primer pairs were optimized for concentration using a primer optimization matrix and a relative standard curve was used to determine the efficiency. The standard curve was constructed using cDNA synthesized from a RNA pool made of all samples using the following amounts of cDNA (in duplicate): 40 ng, 4 ng, 0.4 ng, 0.04 ng and 0.004 ng. Specific hybridization of the primers was validated by agarose gel electrophoresis of the PCR products. Non-template controls were included to validate that primers were not amplifying contaminating DNA. Our work demonstrates that each set of primers has singular characteristics that deeply influence the efficiency of PCR conditions. We have also developed accurate PCR conditions for the 4 genes of interest. These results are fundamental for our future studies where SNPs identification will be correlated with citrate levels in milk.

Key Words: milk, citrate, SNPs

ASAS/ASN JOINT SYMPOSIUM: GUT MICROBIOTA, DIET AND HEALTH

0219 Modulation of the gut microbiota: An ecological perspective. J. Walter*, *University of Alberta, Edmonton, Canada.*

Diverse strategies have been used for several decades to improve human and animal health through the modulation of