Thank You For Your Attendance

Abstract Booklet

Graduate Research Forum
2014

May 29, 2014
8:30am – 5:00pm

Division of Animal Sciences
College of Agriculture, Food and Natural Resources

Co-Sponsored By:
Animal Science Graduate Student Association
4:00pm - 5:00pm
ASRC Room 147

Keynote Address

“The evolution of genomes and genomics”

Delivered by
Dr. Jim Womack
Member, National Academy of Science
Chancellor’s Distinguished Visitor
Acknowledgements

I would like to thank everyone who helped make this year’s graduate research forum a success, including all those in attendance, and particularly those who participated.

I would like to acknowledge the efforts of the following people for their help in the preparation of the graduate forum:

- Dr. Thomas McFadden
- Dr. Jerry Taylor
- Benjamin Beaton
- Cinda Hudlow
- Amber Neely
- Anita Ryan
- Maria Haag

The Judges:
- Dr. Jared Decker
- Dr. Rodney Geisert
- Dr. Jonathon Green
- Dr. Duane Keisler
- Dr. Bill Lamberson
- Dr. Carol Lorenzen
- Dr. Allison Meyer
- Dr. Rocio Rivera
- Dr. Justin Sexten
- Dr. Peter Sutovsky
- Dr. Eric Walters
- Dr. Kevin Wells

The Sponsors:
- The Division of Animal Sciences
- The Animal Science Graduate Student Association

Additionally, the ASGSA would like to extend special appreciation to Dr. Jim Womack as this year’s invited speaker from Texas A&M University. Thanks for joining us from College Station!

Last, but certainly not least, the Animal Science Graduate Research Forum would not be possible without the help of the staff and faculty members within the division.

Lynsey Whitacre
2014 Graduate Research Forum Chairperson

Welcome to Graduate Research Forum 2014

Welcome and thank you for attending the 2014 University of Missouri Animal Science Graduate Student Research Forum. The Forum is an annual event sponsored by the Division of Animal Sciences and the Animal Science Graduate Student Association (ASGSA). Each graduate student and Post-Doctoral Fellow in the Division is invited and encouraged to share his or her research by presenting a poster or an oral presentation.

The goal of the Animal Science research program is to study fundamental principles, asking the question “why?” and to then apply research findings to increase production efficiency within the livestock industry. Animal Science faculty members represent a continuum of scientists investigating effects of variation in DNA sequence, to animal production, animal nutrition, extension specialists, and genetic engineering. They all work toward a common objective of implementing technology applicable to animal agriculture that would benefit producers and consumers. The Division of Animal Sciences currently ranks in the top ten departments at the University of Missouri in external funds awarded. The faculty has been noted for their excellence in research, and is committed to communicating their research findings to others.

This year’s Graduate Forum will provide 21 posters and 16 oral presentations covering reproductive biology, nutrition, genetics, meat science, and environmental physiology. Dr. Jim Womack, Professor at Texas A&M University and National Academy of Science Member will be this year’s keynote speaker.

Thank you for attending, and enjoy this year’s forum!

Benjamin Beaton,
ASGSA President, 2014 Graduate Research Forum Co-Chair

Lynsey Whitacre,
2014 Graduate Research Forum Chairperson
Use of bovine pregnancy associated glycoproteins (bPAGs) to predict late embryonic mortality in beef cows

K.G. Pohler¹, J.A. Green¹, L. A. Moley¹ H. B. Graff³ R.F.G. Peres² J.L.M. Vasconcelos² and M.F. Smith¹

¹Division of Animal Sciences, University of Missouri, Columbia
²FMVZ – UNESP, Botucatu, SP, Brazil
³Agropecuária Fazenda Brasil, Barra do Garças, MT, Brazil

Embryonic mortality (EM) is generally considered to be the primary factor limiting conception rates in cattle and can be characterized as occurring early (< day 28) or late (≥ day 28) during gestation (day 0 = estrus). In cattle, the incidence of early EM is approximately 25% and the incidence of late EM can be as low as 3.2 % or as high as, 42.7%. Relatively little is known about the causes or mechanisms associated with late EM, most of which occurs around the time of placentome formation. Mechanisms associated with reproductive loss around the time of placentation may be associated with inadequate placental development or function. Giant binucleate trophoblast cells constitute 15-20% of the ruminant placenta trophoblast population. They appear around days 19 to 20 of gestation in cattle, and secrete bPAGs along with other products. The objective of this experiment was to examine the relationship between circulating concentrations of bPAGs and late EM in Nelore beef cows and to develop a cutoff model to accurately predict animals that will experience EM. Cows that maintained a pregnancy to 100 of gestation (n = 347) had significantly (P < 0.0001) higher circulating concentrations of bPAGs on day 28 of gestation compared to cows that did not maintain a pregnancy (EM) to day 100 (n = 39). Based on positive and negative predicative value analysis, a circulating concentration of bPAGs above 7.9 ng/ml was 95 % accurate in predicting embryonic maintenance (to day 100) and a concentration of bPAGs below 0.72 ng/ml (minimal detectable bPAG concentration = 0.28 ng/mL) was 95 % accurate in predicting EM (between days 28 to 100) at day 28 of gestation. In summary, maternal circulating concentrations of bPAGs seem to be a good marker for predicting EM between days 28 to 100 of gestation and may aid in elucidating the molecular mechanisms leading to late EM.
Identification of A-to-I RNA Editing Sites in Honey Bee
(*Apis mellifera*) Using RNA-Seq Data

Shu Tao and C.G. Elsik
Division of Animal Sciences, University of Missouri, Columbia

Honey bee (*Apis mellifera*) is one of the most studied insects, due in part to its importance as a model to study molecular mechanisms related to social behavior. A-to-I RNA editing refers to the post-transcriptional conversion of adenosine (A) to inosine (I) through deamination catalyzed by adenosine deaminase acting on RNA (ADAR).

We have performed computational identification of potential A-to-I RNA editing sites in the honey bee brain by using the latest genome assembly and Illumina-generated RNAseq data from ten honey bee individuals (five nurses and five foragers, 2-3 replicates per individual), and discovered 68 candidate RNA editing sites, of which 41 were located in coding exons, 22 in 3' UTRs, 4 in introns, and 1 in 5' UTR. So far, 22 out of 42 candidate editing sites have been successfully validated through Sanger sequencing of genomic DNA and corresponding cDNA fragments in one forager bee individual. Among these 22 validated sites were two conserved A-to-I editing sites in the gene orthologous to *quiver* (*qvr*) in *Drosophila melanogaster*. The first conserved A-to-I editing site changes the codon recodes serine (AGU) to glycine (GGU), and the second conserved site recodes histidine (CAC) to arginine (CGC). Conservation of these specific amino acid conversions across 300 million years of evolutionary divergence may suggest important biological functions. This is the first RNAseq-based *de novo* investigation of A-to-I editing sites in honey bee, and will provide important insight into future functional study of the honey bee brain.

Program Schedule

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RNA sequencing-based allele-specific analysis reveals aberrant genomic imprinting at multiple loci in Large Offspring Syndrome

Z. Chen1, D. Hagen1, C. Childers1, C. Elsik1, T. Ji2, R.M. Rivera1
1Division of Animal Sciences, University of Missouri, Columbia
2Division of Statistics, University of Missouri, Columbia

In ruminants, embryos generated with the use of assisted reproductive technologies (ART) can develop an overgrowth phenotype known as Large Offspring Syndrome (LOS). LOS exhibits a variety of phenotypic abnormalities beyond large birth weight including macroglossia and umbilical hernias. The phenotypes observed in LOS recapitulate those observed in the human loss-of-imprinting (LOI) overgrowth condition Beckwith-Wiedemann Syndrome (BWS). Children conceived with the use of ART have a higher likelihood to develop BWS. The most common molecular lesion in BWS is LOI of the KCNQ1 locus. We have reported that LOS and BWS have phenotypic and epigenetic (KCNQ1 locus dysregulation) similarities and propose the use of LOS to study BWS. The underlying mechanisms for the variable phenotypes observed in BWS/LOS are not known. We hypothesize that LOI occurs at imprinted loci beyond those currently known to be misregulated in BWS and LOS. Total RNA from brain, kidney, liver, and muscle of control (N=4) and LOS (N=4) Bos taurus indicus X Bos taurus taurus day 105 F1 female fetuses was subjected to Illumina sequencing. Identification of known imprinted genes was achieved by comparing the publically available mouse/human list. We found that 21 imprinted genes showed allele-specific expression in day 105 control fetuses. 12 of 21 imprinted genes showed LOI in at least one tissue of at least one LOS fetus but not in controls. There is a positive association between bodyweight and the number of dysregulated imprinted genes in LOS fetuses. Of the 12 imprinted genes identified by this method, several are known to be misregulated in BWS such as PLAGL1, SNRPN, IGF2R, and MEST. We found biallelic expression of PLAGL1 in the kidney is associated with loss-of-methylation of that locus on the maternal allele. Overall, we found LOI at multiple loci in LOS beyond those currently known to be misregulated in BWS/LOS.
Comparison of white blood cell phagocytic efficiency in two genotypes of Katahdin sheep

S. Azarpajouh, T. Wuliji and A.L. Bax
Department of Agriculture and Environmental Sciences, Lincoln University, Jefferson City, MO

Fourteen Katahdin ewes, 7 in each of high resistant footrot gene markers (HR) and low resistance (LR) were selected. Blood samples were collected in vacutainer tubes (2 x 10 ml) contained EDTA weekly for 8 weeks. Blood smears were made on glass slides to determine the percentage of neutrophils in whole blood. Neutrophils were isolated using a Percoll gradient technique, and stained with 0.8 mM Trypan Blue to determine the percentage of viable cells. Subsequently, 1 ml of freshly isolated neutrophils was inoculated with 1 ml of Lactobacillus casei (2 x 10^7/ml) in PBS and incubated with rotation at 37°C for three time periods at 20, 40 and 60 min. Control samples were incubated in PBS with neutrophils alone to account for bacterial growth during the assay. At specific intervals of 20, 40, and 60 min the number of surviving bacteria in the supernatant (extracellular) was determined by culture plate colony counting to estimate the phagocytic efficiency of the neutrophils. The data were analyzed by using mixed model procedures of SAS and P < 0.05 was considered as significant. The average viability of extracted neutrophils was 95% in each individual specimen at inoculation. The percentage of neutrophils in whole blood was not significantly different (P = 0.57) in HR vs. LR genotypes. There were no significant differences (P = 0.63) among the numbers of bacterial colonies after addition of neutrophil and incubation periods in HR vs. LR genotypes. The number of bacterial colonies significantly decreased after 20, 40, and 60 minutes of incubation (P < 0.001). The interaction of time and gene marker groups was not significant (P = 0.23). No bacterial colony growth was observed in control samples. There is no difference in phagocytic efficiency of the white blood cells in footrot resistant and susceptible genotypes of Katahdin sheep.

Effect of tannin-containing legume forages on crude protein degradation in vitro

Nichole F. Johnson, Margaret E Lees, Monty S. Kerley, Harley D. Naumann

Research in our laboratory has demonstrated greater than 10% improvements in feed efficiency by balancing beef cattle diets for post ruminal amino acids. To achieve this, protein sources with low rumen degradability must be utilized. Expensive animal proteins or treated soybean meal (SBM) are the most commonly available sources. Therefore, it is prudent to examine forages with low rumen degradability as alternatives. Tannin-containing forages have been shown to have high proportions of rumen undegradable protein (RUP) due to protein binding properties of condensed tannins (CT). The objective of this study was to evaluate the ability of selected warm-season perennial legumes containing CT to decrease ruminal crude protein (CP) degradation in vitro. We hypothesized the extent of ruminal CP degradation would be inversely related to forage CT concentration. Six legume species were evaluated. Three field replications of each CT-containing forage species and one replication of alfalfa (minimal CT) were mixed in a 1:1 ratio with SBM. Forage: SBM samples were weighed into tubes, inoculated with a 1:3 mixture of strained rumen fluid and McDougall’s buffer, and closed with stoppers fitted with one-way valves. Samples were fermented for 0, 12, 24 and 48 h in a 39°C shaking water bath. Tubes were centrifuged to remove supernatant, dried at 55°C and analyzed for dry matter (DM) and CP disappearance. Supernatant subsamples were taken for ammonia analysis 0, 4, 8, 12, 16, 24, 36, and 48 hours of fermentation, at which times pH was measured. Increased CT concentration decreased the potential extent of CP degradation and tannin-containing forages had greater %DM and CP remaining at all hours compared to alfalfa and a SBM control. When expressed as a percent of potentially digestible CP, alfalfa and SBM had greater CP digestion at 0 and 12 hours indicating protein solubility may decrease with increased CT concentration.
The use of a monoclonal antibody to rapidly purify bovine pregnancy-associated glycoproteins (PAGs)

R.M. Wallace, T.E. Egen, M.F. Smith, and J.A. Green
Division of Animal Sciences, University of Missouri; Columbia, MO

The PAGs are major products of the bovine placenta, but little is known about their specific roles. The ability to isolate enriched preparations of PAGs is critical for studies on PAG function, structure, and for defining the interactions of PAGs with other proteins. However, protein purification tends to be a laborious process. The goal of the present study was to provide a means for the rapid purification of bovine PAGs through the use of immunoaffinity chromatography with an anti-PAG monoclonal antibody. Cotyledons (gestational range: 104-144 days) were collected from pregnant tracts and homogenized. The homogenates were dialyzed and enriched by differential precipitation with increasing amounts of ammonium sulfate (AS). The column was prepared by binding a biotinylated anti-PAG monoclonal (L4) to 8 milliliters (ml) of a suspended avidin matrix. The matrix was equilibrated in tris-buffered saline (TBS) and 200ml of the AS-precipitated fraction, containing 68.5 mg of PAG, was applied repeatedly to the column. The matrix was washed with TBS and bound PAG was eluted sequentially with 0.1 M Glycine, pH 2.9, followed by 0.5 M Glycine, pH 2.9. Eluted fractions were immediately neutralized and fractions containing PAG were identified and concentrated against polyethylene glycol. Western blotting with anti-PAG sera revealed apparent molecular masses of 75 KDa, 70 KDa, and 64 KDa in the final enriched fraction. The immunoreactive bands in the final preparation were subjected to NanoLC-Nanospray QTOF MS plus MS/MS analysis. This analysis revealed that the purified sample contained bovine PAGs 4, 6, 9, and 21. Quantification of PAGs was performed by using an in-house ELISA. The final concentrated material measured 32.5 mg of PAG, which represented a 47% recovery of the total PAG loaded on the matrix. Our results confirm the suitability of an immunoaffinity system for the rapid enrichment of several bovine PAGs from bovine extracts.

Funding provided by the Food for the 21st Century Reproductive Biology Cluster at the University of Missouri.

Performing Genome-wide Association Studies using Whole Genome Sequence Data within the Canine Genome

H.R. Ramey¹, R.D. Schnabel¹, G.S. Johnson², and J.F. Taylor¹
¹Division of Animal Sciences, University of Missouri, Columbia
²Dept. of Veterinary Pathobiology, University of Missouri, Columbia

The currently accepted method for conducting genome-wide association studies (GWAS) is to use high density genotype data produced via genotyping assays. However, with the decreasing cost of next generation sequencing (NGS) technologies there is an interest in many research communities in performing GWAS with whole genome sequence-derived variant data. We examined Bayesian methods for conducting GWAS with base pair resolution genotype data by employing well-established software (GenSel) in a proof-of-concept analysis in the canine. Positive control phenotype/genotype data were used to assess the ability of this analytical framework to successfully perform GWAS using millions of markers. Two positive controls were selected based on previously identified causal mutations in two independent phenotypes, Multiple System Degeneration and brachycephaly. The data analyzed for the two controls spanned two chromosomes (1 and 32) covering 3,746,715 and 1,360,639 loci, respectively. The analysis was performed for a massive genotype dataset, however neither of the positive control mutations was successfully identified. Interestingly, problems with phenotype specification for the positive controls coupled with the additive model fit by GenSel resulted in failure to identify the causal mutations as being responsible for the phenotypes. The brachycephaly phenotype analysis revealed inconsistencies in the genotype-phenotype relationship for the putative causal mutation in brachycephalic, normal and dolichocephalic breeds. The previously identified mutation associated with brachycephaly in small breeds was found to be variable and not predictive of the phenotype. This result stimulated our interest in exploring the cause of brachycephaly in the Pug breed to enable a characterization of the suite of mutations underlying this phenotype within dogs. Our findings cast doubt on the causality of previously reported brachycephaly variants and further highlight the complexity of cranial morphology in domestic dog breeds.
Dietary fat source and energy concentration alters semimembranosus pH and fatty acid profile of belly fat

K.E. Shircliff, M. S. Kerley, G. L. Allee, and B. R. Wiegand
University of Missouri, Columbia

Finishing barrows (PIC, 337 x C22) were used to test the effect of added fat on carcass characteristics and fat quality. Barrows (n=40) reared individually were blocked by weight and randomly allotted to 1 of 5 dietary treatments (rep=8) containing soybean meal with choice white grease (CWG), high protein dried distiller's grains (DDGS) with CWG, 30% DDGS with no added fat, 30% DDGS plus CWG or 30% DDGS plus 3% butter oil. At 24 hours post mortem, loin eye area (LEA), 10th rib fat, last rib fat, semimembranosus and longissimus pH, semimembranosus and longissimus temperature and objective loin color using a Minolta Chroma Meter CR-410 were measured. Fat tissue samples for fatty acid (FA) analysis were collected posterior to the sternum and anterior to mammary tissue on the belly edge. Fatty acid composition was determined via the Folch method and iodine values (IV) were calculated according to the AOCS (1998) equation. Statistical analysis was performed using the GLM procedure of SAS and significance was defined at P<0.05. Dietary treatment did not significantly effect LEA, 10th rib fat, last rib fat, semimembranosus and longissimus pH, semimembranosus and longissimus temperature or longissimus color. Semimembranosus pH of barrows fed high protein DDGS was significantly different from pigs fed soybean meal with CWG (P=0.0036) and pigs fed 30% DDGS with CWG (P=0.0374). Dietary treatment altered IV with the control diet having the lowest IV (64.72) and 30% DDGS plus CWG having the highest IV (73.79) and all other treatments differing with intermediate values (P<0.0001). Changes in iodine value were proportional to shifts in concentrations of saturated to monounsaturated and polyunsaturated fatty acids. As expected, the addition of dietary fat changed the fatty acid profile of belly fat by increasing unsaturation. Further, the low energy diet increased pH of semimembranosus 24 h postmortem.

Zinc requirements of Pekin ducks and turkeys fed corn-soybean meal based diets.

Y. Albalkhi and D.R. Ledoux
University of Missouri, Columbia

Zinc (Zn) requirements for turkeys (70 mg/kg Zn) listed in the current National Research Council recommendations (NRC, 1994) are based on data generated before 1994, whereas requirement values for Pekin ducks (40 mg/kg Zn) are based on extrapolations from broiler data generated before 1994. Therefore, it is reasonable to question whether the current Zn recommendations for modern strains of turkeys and Pekin ducks are appropriate. This research will be conducted to determine Zn requirements of male Pekin ducks and turkeys fed diets from hatch to day 21. For the duck study, 150 day-old male ducks will be purchased from a commercial hatchery, weighed, wing-banded, and randomly divided into 6 treatment groups, with 5 replicate pens per treatment, and 5 birds per pen. The basal diet (BD) will be a commercial corn-soybean meal type diet formulated to meet the nutritional requirements, except for Zn, of growing ducks as recommended by NRC (1994). It is estimated that the unsupplemented BD will contain between 20-27 mg Zn/kg diet. Zinc sulfate will be added to the Zn-deficient BD to provide levels of Zn as follows: 0, 10, 15, 20, 25, and 30 mg/kg diet. Prior to start of the experiment, the BD and treatment diets will be analyzed for Zn content. Procedures for the turkey study will be similar, except that zinc sulfate will be added to the Zn-deficient BD to provide levels of Zn as follows: 0, 35, 40, 45, 50, and 55 mg/kg diet. Response variables to be evaluated will include growth performance, serum and tissue Zn concentrations, serum growth hormone levels, and histopathologic evaluation of selected tissues. Zinc requirement will be determined using the broken-line assay and regression of Zn intake on tissue Zn concentrations.
Bale diameter and feeder design affects hay waste

D.J. Tomczak, W.J. Sexten, and A.M. Meyer
University of Missouri, Columbia

Forty-eight mid-gestation spring-calving cows were stratified by BW (583 ± 77.2 kg), BCS (5.4 ± 0.6), and age (5.6 ± 2.5 yr) into 6 pens to evaluate influence of bale diameter and feeder design on hay waste. Tall fescue round hay bales (85.5% DM, 8.22% CP, 66% NDF, 152 cm height) were classified as Small (128.3 ± 3.19 cm), Medium (160.7 ± 6.38 cm), or Large (187.7 ± 3.52 cm) diameter, and placed in hay feeders equipped with cradle chain (CONE) or without (RING) in a 3 x 2 factorial design randomly assigned to a 6 x 6 Latin square. We hypothesized hay waste would increase as initial bale diameter increased in RING and not differ in CONE. Bales were placed on the circular end in round bale feeders (230 cm diameter, 170 cm height) with 16 feeding stations and metal sheeting on top (50 cm) and bottom (60 cm). Small, medium, and large bales were replaced every 2, 3, and 5 d, respectively to ensure ad libitum hay access. Waste was collected daily, and residual forage was collected prior to new bale feeding. CONE (15.8%) reduced (P < 0.10) waste as a percent of initial bale weight compared to RING (18.3%). Waste was increased (P < 0.05) for large (19.4%) compared to small (14.2%), while medium (17.6%) did not differ (P > 0.05) from large or small. Bales were not fed for equal number of days, so data were analyzed as an incomplete 6 x 6 Latin square to evaluate feeder effects relative to access time. Waste was not different (P > 0.10) due to increased access time to small in CONE however waste was reduced (P < 0.05) as access time increased for small in RING. As access time increased to medium and large waste was reduced (P < 0.05). In conclusion, CONE tended to decrease waste. Increasing access time due to increased bale diameter increased waste in all cases, except small CONE.

CRISPR/CAS9 Gene Stacking via CRISPR/Cas9

B.P. Beaton, E.M. Walters, A.M. Lichtenauer, M.D. Steinhoff, R.S. Prather, and K.D. Wells
Division of Animal Sciences & National Swine Resource and Research Center, University of Missouri, Columbia

Genetic engineering facilitates heritable modification of target genomes. Modifications can be site-specific or random. Though inefficient, homologous recombination allows for precise genome modification. Random integration can be very efficient, but lacks the opportunity to control the orientation and location of the introduced DNA. Thus, an efficient method for site-specific integration into a predetermined location would have utility. It is known that the rate-limiting step that accounts for the low efficiencies related to homologous recombination is target breakage. With gene editing techniques increasing in availability and ease of use, designed nucleases can be used to create target specific cleavage followed by the insertion of exogenous DNA through homologous recombination. This process can be repeated to allow sequential addition of transgenes to the same location, “gene stacking.” Gene stacking facilitates sequential addition of transgenes within a locus. The specific objective of this project was to utilize the CRISPR/Cas9 system to create a double strand break to direct single-copy integration of an exogenous DNA into the target site at an improved frequency. To evaluate the feasibility of this strategy, we stacked two transgenes (Hygromycin and hCD39) within a previously modified GGTA1 locus (GGTA1 KO harboring a Neomycin cassette and a hCD55 transgene cassette). We observed an increase in gene targeting from 9.8% up to 66.7% in response to the use of Cas9. In addition, we stacked four transgenes (Neomycin, Hygromycin, hCD39, and hCD55) into wildtype cells to disrupt the GGTA1 locus in a single step. Here we increased gene targeting from 0.0% up to 100.0% in response to the use of Cas9. In conclusion, the feasibility of CRISPR/Cas9-mediated gene stacking has been demonstrated in primary porcine fibroblasts. The resulting fibroblasts are compatible with somatic cell nuclear transfer as a day 45 fetus was generated, and pregnancies are on-going to produce transgene-stacked piglets.
Lessertia frutescens (Sutherlandia) suppresses multiple intracellular signaling pathways associated with inflammatory responses in murine macrophages

Wei Lei, Jimmy Browning Jr, Peggy A. Eichen, Yijia Zong, Dennis Chuang, Chi-Hua Lu, William R. Folk, Grace Sun, Kevin L. Fritsche

University of Missouri, Columbia

Sutherlandia is an herb native to southern Africa and traditionally used in various inflammatory conditions. Previously, we reported that an ethanolic extract of Sutherlandia (SE) had anti-inflammatory activity in murine macrophages. This study was designed to investigate the impact of SE on inflammation-related intracellular signaling pathways, and to identify the bioactive component(s) in this herb. RAW 264.7 cells were exposed to various concentrations of SE for 1 hr, then an inflammatory response was triggered by co-treatment with LPS and IFNγ. The phosphorylated and total STAT1, ERK1/2, p38, and JNKs in cell lysates were determined by Western blot. Exposure to SE significantly decreased the phosphorylation of STAT1 and ERK1/2 by up to 80% and 70%, respectively. Meanwhile, SE had only a limited effect on the phosphorylation of p38 and JNK. We used nitric oxide (NO) production as a screening tool to evaluate the anti-inflammatory activities of Sutherlandia-specific constituents: sutherlandiosides A-D, sutherlandins A-D. None of these compounds were able to reduce LPS/IFNγ-induced NO production. In summary, our crude Sutherlandia extract suppressed several key signaling pathways related to inflammatory responses in macrophages, and we continue to search for the constituent(s) responsible.


Structural variation at the KIT locus is responsible for spotted coat color phenotypes in Hereford and Simmental cattle

L.K. Whitacre¹, J.E. Decker¹, J.W. Kim, J.F. Medrano¹, R.D. Schnabel¹, D.C. Schwartz², S. Zhou², the US Consortium for Genetic Improvement of Feed Efficiency in Beef Cattle, and J.F. Taylor¹

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The Spotted locus is responsible for several unique coat color phenotypes in cattle including the Hereford pattern, which is characterized by a white face, underbelly, legs, and tail switch. Since the breed is fixed for this phenotype, we postulated that a historic selective sweep occurred during breed formation to fix the dominant Spotted allele while simultaneously fixing alleles at nearby hitchhiking loci. Spotted was linkage mapped to BTA6 between 64.7 Mb and 85.5 Mb (Grosz & McNeil, 1999). We examined this region in 811 fullblood Herefords using Illumina BovineHD genotypes. Analysis of the SNP minor allele frequencies across the region identified four stretches of homozygosity individually greater than 75 kb. The largest of the four is 430.9 kb and extends into KIT, a tyrosine kinase responsible for the migration of neural crest derived melanocytes along the dorsolateral pathway to their final destination in the skin. To pinpoint Spotted, Hereford sequence was compared to that of Angus cattle, putatively homozygous for the “non-spotted” allele. From the pairwise alignments of these genome sequences, we predicted SNPs, indels, and structural variants that were fixed different between Angus and Hereford. We found two significant Hereford specific duplications within the selective sweep regions, one ~50 kb upstream of KIT and one within the first KIT intron. Both of these regions appear to be duplicated about 6X in Hereford. We analyzed the genomes of 13 other breeds and found these duplications present within all fullblood Simmental sequence and in the sequence of a Beefmaster (~25% Hereford in composition) with a white face. The copy number in Simmental and Beefmaster appears to be 3X. We predict either the upstream duplication is interrupting a long-range regulatory element of KIT or the intron duplication reduces transcription of KIT. However, similar intronic duplications in other breeds may discount the validity of the latter hypothesis.
Effects of postruminal amino acid supply on growth performance and feed cost of beef steers

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Seventy-two steers were used to evaluate supplying excessive AA flow during growth phase on performance, feed cost per gain, and blood metabolites of beef cattle. Steers were randomly assigned to three alfalfa haylage-based diets (30% diet DM) with increasing ruminally undegraded AA; A diet was formulated to meet the requirement (BAL) and two diets formulated to exceed (EX1 or EX2) 100% of the most limiting AA during the 84d growing phase (GP). Steers were fed a corn-based diet during 84d finishing phase (FP) and slaughtered when gain cost exceeded gain value. Initial and final BW did not differ among treatments (P>0.8). Calves fed EX1 tended greater ADG (P<0.1) during the first 21d on feed, however this compensatory growth, was not maintained by the end of 84d GP (P=0.50). ADG during FP and overall, DMI during GP and FP, and FCR during GP and overall did not differ among treatments (P>0.6), however, EX1 had improved FCR than BAL and EX2 during FP (P=0.02). Feed cost per unit of gain ($/Kg of gain) increased (P<0.001) as postruminal AA was included in the diets. Blood glucose did not differ (P=0.74), whereas EX2 had greater blood urea nitrogen compare to BAL and EX1 (P<0.001). Our laboratory has demonstrated diets formulated to meet absorbable AA requirement, but have not determined the effect of exceeding absorbable AA requirement during growth phase on overall performance. AA supply exceeding requirement during growth phase may impact finishing phase efficiency. Calves consuming diets with postruminal AA supplied above requirements may respond with greater compensatory growth as occurred in this study, however improved growth was not sustained over the feeding period and feed cost per gain were increased.

Recruitment of intraepithelial leukocytes to the uterine surface by embryo interleukin-1 beta (IL1BE), a novel IL1 expressed by the elongating pig embryo during establishment of pregnancy

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Pigs have a 30% reduction in their potential litter during the first month of gestation due to early embryonic mortality. Most loss occurs during the second week of gestation, when embryos express a novel cytokine, “embryo” interleukin-1 beta (IL1BE). IL1BE affects the uterine luminal epithelium (LE) and may be critical for embryonic survival. IL1BE protein is 92% similar to pig interleukin-1 beta (IL1B), a pro-inflammatory cytokine expressed by macrophages that can recruit leukocytes in tissues. In this study, IL1B and IL1BE's capacity to recruit uterine intraepithelial leukocytes was evaluated. Endometrium was dissected from three cycling Large White Landrace gilts and in duplicate, incubated for 4 hr in either LPS (10μg/mL), 100ng/mL of recombinant beta-galactosidase (BGal; control protein), IL1B or IL1BE. Endometrium was prepared for microscopic evaluation and stained to visualize the LE. Pictures were taken using a Leica light microscope and the number of intraepithelial leukocytes counted within a 300 X 150μm rectangle using Image J. Based on appearance, two leukocyte phenotypes were observed (small and large). SAS was used to test for an effect of treatment (Trt) on the leukocyte populations. Within the LE, LPS and IL1BE (5.8 ± 0.7 and 5.0 ± 0.7; respectively; LSM ± SEM) increased the total number of leukocytes over BGal (3.7 ± 0.7) (P < 0.05; Trt). IL1BE and LPS (2.6 ± 0.4 and 2.6 ± 0.5; respectively) increased the number of small leukocytes over IL1B (1.4 ± 0.4) (P = 0.05; Trt). Furthermore, within the LE, LPS, IL1B and IL1BE (3.2 ± 0.5, 2.9 ± 0.5, 2.3 ± 0.5; respectively) increased the number of large leukocytes over BGal (1.6 ± 0.5) (P < 0.01; Trt). IL1BE, a novel embryo IL1, may have the capacity to recruit specific leukocyte populations to the uterine surface during establishment of pregnancy in the pig.
Growth adjustment to diet improves profitability within the feedlot

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Feed efficiency improvement can increase beef production profitability. Our objective was to compare feeding system (SYS) affect on efficiency, carcass composition and feedlot profitability. We hypothesized sorting cattle by initial ADG and feeding subsequent diets formulated to match growth potential would increase gain efficiency and profitability compared with traditional feeding programs. Heifers (n = 287; 224.70 ± 1.4 kg) purchased through livestock markets were fed a diet formulated to meet effective energy (EE) and AA requirements for 2.1 kg ADG over 42 d following a 14 d receiving period. From the 42-d ADG, heifers were blocked as high (1.98 to 2.71 ± 0.18 kg/d), mid (1.67 to 1.97 ± 0.08 kg/d) and low (0.54 to 1.70 ± 0.22 kg/d) ADG and were stratified across SYS. Diets were formulated without forage inclusion for matched (M) and nontraditional (NTRAD) SYS. High (HM), mid (MM) and low (LM) blocks within the M SYS were formulated to meet EE and AA requirements. High, mid and low blocks were not separated in either traditional (TRAD) or NTRAD SYS. The TRAD SYS was fed a diet formulated to meet NRC requirements, while the NTRAD SYS was fed the MM diet. Diets were fed from day 43 until slaughter, however, HM and MM SYS were transitioned as needed to the LM diet to adjust for decreasing finishing period nutrient requirements. Heifers were slaughtered when gain cost exceeded value of gain, visually-assessed back fat ≥ 0.76 cm, and estimated HCW ≥ 243.2 kg. Final BW and ADG were not different (P > 0.10) among SYS. Additionally, HCW, LM area, back fat thickness, yield grade and quality grade were not different (P > 0.10) among SYS. Nontraditional and M SYS had less DMI (7.32 and 7.40 vs. 8.36 kg ± 0.30; P ≤ 0.001) and feed conversion ratios (5.97 and 5.86 vs. 6.59 ± 0.30; P ≤ 0.001) than TRAD. Diet cost for TRAD was less ($0.327/kg; P ≤ 0.001) than both NTRAD ($0.347/kg) and M ($0.346/kg) SYS. No SYS resulted in profit; however, loss per heifer was less (P = 0.02) for M (-$6.20) compared with TRAD (-$51.88), where loss per heifer for NTRAD was not different (P ≥ 0.10) from either M or TRAD. In conclusion, forage removal combined with balancing for EE and AA growth performance requirements can increase feedlot efficiency and profitability.
Use of PG-600™ and OvuGel™ in summer weaned first parity sows

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Modern swine production relies on sows returning to estrus within 7d post-weaning, and OvuGel™, a vaginally delivered triptorelin acetate, can lead to synchrony in time of ovulation for these sows and facilitate timed AI. Parity one sows, those weaning large litters and those lactating under high thermal load are less likely to return to estrus in a timely fashion and, therefore, might be expected to have an inferior response to OvuGel™. PG-600™ is used to treat anestrus sows, including parity one females lactating under thermal load. At weaning in August, 2013, 544 parity one sows were divided into two experimental groups, Control and PG-600™. Control sows (n=270) were treated with OvuGel™ per label instructions at 96h post-weaning and mated using post-cervical AI with 3x10^6 cells 22-24h later. PG-600™ sows (n=274) were treated the same as Control sows except they were first given PG-600™ IM at weaning according to label instructions. Detection of estrus was performed daily for 8 weeks following weaning. A row by column Chi Square test was used to evaluate proportion of sows returning to estrus and proportion of irregular returns (<18 or >24d). A higher percentage of Control sows returned to estrus after their first mating than PG-600™ (24.81% vs 12.04%, respectively; P<0.0001), but of those the percentage of irregular returns did not differ by treatment (35.82% vs 27.27%; P<0.3927). Given that sows weaned large litters (12.62±0.09 and 12.55±0.09 for Control and PG-600™, respectively) during August, a large proportion of sows experiencing delayed post-weaning estrus was expected. If these sows responded to PG-600™ the wean-to-estrus interval would be shortened, and a higher proportion should have become pregnant to the timed AI, which they did. Sows not conceiving, however, would have been expected to exhibit different patterns of return to estrus, and they did not.
Gene expression in the kidneys of broilers fed dietary ochratoxin A for different time periods

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This experiment evaluated the effects of dietary ochratoxin A (OA) fed for different lengths of time on performance, organ weight, serum parameters, and gene expression in the kidneys of chicks. One hundred and eighty day-old male broiler chicks (Ross) were randomly assigned to a $3 \times 3$ factorial arrangement of treatments (3 levels of OA; 0, 1 and 2 mg OA/kg diet and 3 time periods; 7, 14 and 21d) and 4 replicate pens of 5 birds each per treatment. At day 21, birds fed 2 mg OA/kg diet had decreased growth performance, and increased relative kidney weight and serum uric acid levels. Birds fed 1 mg OA/kg diet had decreased serum total protein, albumin and aspartate aminotransferase concentrations. For RNA-Sequencing analysis (RNA-Seq), kidney samples were collected weekly from 3 controls and 3 chicks fed 1 mg OA/kg. The libraries were sequenced by Illumina’s TruSeq RNA protocol. NextGENe\textsuperscript{\textregistered} NG Release V 2.17 (beta) was used for read alignment and transcript quantification. A total of 27,638,976, 50-bp RNA-Seq reads were produced over the three time periods. Transcripts (40,782) were built de novo and annotated by homology to either \textit{G. gallus} or \textit{H. sapiens}. At days 7 and 14, genes associated with carbohydrate and amino acid metabolism were downregulated and genes associated with the immune system were upregulated. Genes associated with lipid metabolism and xenobiotic biodegradation were also downregulated at day 14. These metabolic changes disappeared at day 21, suggesting that the damage to the kidney and other related organs was contained. In conclusion, the supplementation of 1 mg OA/kg diet caused altered time-dependent renal gene expression in chicks.

Development of a multiplex assay for simultaneous quantification of endocrine analytes

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Since the advent of the radioimmunoassay in the 1950s, numerous immunologically-based methods have evolved for sample analysis. Although each immunological method possesses unique assets and liabilities, all share limited abilities in the number of analytes resolvable simultaneously; most procedures are limited to one analyte determined per replicate per sample. The objective of this study was to adapt existing technology for hormonal analysis in livestock. We sought to complete this objective through the use of existing technologies (Illumina BeadXpress, Luminex xMAP and immuno-PCR). The Illumina BeadXpress and Luminex xMAP both share similar characteristics; each platform consisted of a ‘reader machine’ and a bead-set. Each bead-set contained microscopic beads with unique identifying signatures. As a test of proof of concept, the surface of a bead set was conjugated to an LH antibody. We were able to successfully establish a hormonal assay on the Luminex platform, but not on the Illumina platform. However, the proprietary nature of both the Luminex and Illumina platforms limited assay flexibility. Therefore, we next attempted to establish a hormonal assay for livestock using quantitative immuno-PCR. Unlike bead based assays, immuno-PCR exploits the PCR chemistry for detection of analytes. Additionally, the unique characteristics of DNA provide the potential to multiplex a number of analytes that far exceeds existing technology. Initial experiments with immuno-PCR provide evidence that the assay performs with linearity over six orders of magnitude. Furthermore, background binding of secondary reagents was found to be an ongoing problem and must be investigated further. The immuno-PCR assay continues to be developed and refined with the objective of hormonal assay multiplexing. The technological leap in capabilities provided by successful multiplexing can be used for understanding the complex interaction of endocrine and metabolic signals in the dynamically changing animal.
Construction of Gene Targeting Vectors for RAG1 and RAG2

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Severe Combined Immunodeficiency (SCID) is a condition characterized by the absence of T cells and lack of B cell function. SCID affects approximately 1 out of every 100,000 infants. Autosomal recessive SCID can occur due to a mutation within the Recombination Activating Genes (RAG1/RAG2) that play a role in recombination of immunoglobulins and T-cell receptors. Due to the anatomical and physiological similarities between humans and pigs, a swine model of both RAG-1 and RAG-2 deficiency would have utility. A first step in the creation of a swine SCID model is to assemble targeting vectors. To accomplish this goal, genomic DNA from porcine fetal fibroblasts was used to amplify a 6,840bp PCR product including the porcine RAG-1 gene. This fragment was cloned into TOPO pCR-XL (Invitrogen, Carlsbad, CA). So that a mammalian G418 resistance cassette could be used for selection of targeting events, this plasmid was modified to remove the endogenous AphII gene (provides G418 resistance). pKW4 contains LoxP (locus of X-ing over) sites that flank a G418 resistance cassette (based on mammalian codon usage) which is driven by a Phosphoglycerate Kinase (PGK) promoter (Lorson et.al. 2011). This cassette was inserted into the RAG-1 gene to create the targeting construct pAB6. For RAG2, a 9,466bp PCR product including the RAG-2 gene was amplified and cloned into TOPO pCR-Blunt II (Invitrogen, Carlsbad, CA). The LoxP flanked G418 resistance cassette from pKW4 was inserted into the second exon of the RAG-2 gene sequence creating the targeting construct pAB13. Further, diagnostic screening strategies were developed and validated to discriminate gene-targeting events from random integration. We report here two targeting vectors and validated screening methods for gene targeting in porcine fetal fibroblasts that have been validated for cloning.

Genome-Wide Association Study Across Six Breeds for Tenderness and Other Carcass Traits

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The aim of this project is to design a commercialized genomic test that strongly predicts meat tenderness and other carcass traits in multiple breeds and crossbred cattle. We are identifying single nucleotide polymorphisms (SNPs) that have significant association with tenderness. Once quantitative trait loci (QTL) are identified, we will fit haplotypes (strings of alleles inherited as a unit) within these regions as effects in a genomic prediction model. We hypothesize that using haplotypes will provide higher accuracy than using single markers. We are using 651 Angus, 695 Charolais, 1,095 Hereford, 283 Limousin, 301 Maine-Anjou, and 516 Simmental samples with phenotypes and genotypes from the Carcass Merit Project. SNPs were filtered so that SNPs with a minor allele frequency with less than 1% and a call rate less than 90% were removed. Individuals were also filtered by excluding those with more than 10% missing genotypes. BEAGLE was then used to phase the genotype data and impute the missing genotypes. We used 3,993 Angus, 101 Charolais, 1,225 Hereford, 2,366 Limousin, 11 Maine-Anjou, and 1,913 Simmental purebred animals along with the CMP animals when phasing genotypes. In GEMMA, we fit a univariate linear mixed model to test SNP associations with each phenotype individually. We also fit multivariate linear models for testing marker associations with multiple phenotypes simultaneously while controlling for population stratification and for estimating genetic correlations among complex phenotypes. Once we have identified these significant SNP associations, we will identify the haplotypes defined by the lead SNP and the 4 flanking markers. We will fit these haplotypes as effects (presence or absence) in a BayesB analysis to estimate genomic prediction equations of tenderness and correlated traits.
Determining the effect of a 5 or 7 day CIDR in a progesterone monitored study on pregnancy rates in lactating dairy cows

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Producers continue to observe low pregnancy rates in dairy cows throughout the United States and across the globe. Despite the use of artificial insemination (AI) protocols with a series of hormone injections, dairy cows continue to achieve low pregnancy rates while milk production increases. Milk production demands may contribute to early embryonic loss and the lack of a standing estrus response. First insemination lactating Holstein and Guernsey cows (n= 105) on the Presynch Ovsynch56 protocol were monitored for progesterone levels, 4 and 2 days prior to insemination during the estrous cycle. The protocol can be extended to insert a 5 or 7 day Controlled Internal Drug Release (CIDR) followed by AI one week later. The CIDR will maintain an increased level of progesterone until the time of removal, 3 days prior to AI, where progesterone levels will decrease. Implementing a CIDR will reduce semen cost and synchronization time. Therefore, development of an extended Presynch Ovsynch56 protocol with the addition of a 5 or 7 day CIDR could greatly benefit the dairy industry. However, further research is needed to determine if the adapted protocol will consistently meet the needs of the dairy producer and increase pregnancy rates.

Contribution of a chelated trace mineral supplement as a methionine source for dairy cows

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This experiment sought to determine whether the methionine contained in a trace mineral supplement made a meaningful contribution toward meeting the methionine requirement of the dairy cow. Four multiparous ruminally-cannulated lactating Holstein dairy cows were used in a 4 x 4 Latin square design with 7-d periods. Treatments were administered at a rate of 0.08 g of 2-hydroxy-4-methylthio-butanoic acid (HMTBa)/kg of BW on d 0 of each experimental period: 1) HMTBa chelated to Zn, 80% HMTBa, dosed ruminally (MIN; MINTREX® Zn, Novus International, St. Charles, MO, USA), 2) Ca-salt of HMTBa, 84% HMTBa, dosed ruminally (MHA; MHA® feed supplement, Novus International), 3) HMTBa free acid, 88% HMTBa, dosed ruminally (ALR; ALIMET®, Novus International), and 4) HMTBa free acid, 88% HMTBa, dosed post-ruminally (APR; ALIMET®, Novus International). Approximately 5 kg of rumen mat contents from each animal were removed through the cannula, mixed with the appropriate treatment dose, and replaced in the rumen. For post-ruminal treatment, a 50cc syringe was placed in the omasal canal, and contents were expelled into the abomasum; all other animals received a post-ruminal infusion of water. Plasma concentrations of methionine did not differ between MIN, MHA, and ALR treatments; however, APR resulted in a greater (P<0.001) concentration of plasma methionine compared with other treatments. A treatment x time interaction (P<0.001) was observed for plasma methionine. Greater plasma concentrations of HMTBa (P<0.001) were found in APR than in MIN, MHA and ALR; however, these did not differ between each other (P>0.10). There was a treatment x time interaction (P<0.001) for HMTBa. In conclusion, the availability of methionine in plasma did not differ between treatments administered ruminally; however, significant increases were observed when treatments were administered post-ruminally. These results suggest that MIN may be used to contribute toward the methionine requirement for dairy cows.
Effect of reduced hair coat on performance of feedlot steers during summer heat stress

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Effective procedures are needed for heat stress reduction under feedlot conditions and over the entire summer period. This necessitates identification and development of predictors of heat strain. A 94-day study, using crossbred Angus steers (n=36; average body weight =284 +/- 29kg) was conducted in 2013. Animals were housed in groups of nine with ~50% shade coverage. Hair coat was removed using standard “torching” procedure from half of the steers. Ambient temperature (Ta) and relative humidity were recorded using Hobo loggers (Onset Computer, Bourne MA) in sun and shade. Ranges of Ta and THI were 12.2 to 36.6°C and 54.4 to 85.3, respectively. Steers were provided a corn-based feedlot diet and water ad libitum, and core temperature (Tcore) was measured hourly using intraruminal telemetric boluses (Smartstock, Pawnee, OK). A feed intake (FI) system (GrowSafe Systems Ltd., Airdrie, AB, Canada) provided FI. Data analysis was conducted using ANOVA, (JMP Statistical Software; SAS Institute; Cary, NC) to determine the effect of a reduced hair coat on FI, feed efficiency (FI/ADG), and Tcore. Analysis revealed no effect of torching on daily FI (P=0.85), but an increase in feed efficiency in non torched versus torched (P<0.01). Analysis of mean daily Tcore over the entire period by animal and hour of day showed a 0.21°C lower Tcore value for torched versus non-torched animals (P<0.01). Likewise, maximum daily Tcore was 0.25°C lower in torched versus non-torched animals, with no difference (P>0.10) in daily minimum Tcore. These results indicate that reducing the hair coat of steers during summer months may offer a cosmetic benefit along a reduction in core temperature due to an increase in cutaneous heat loss. However, overall feed efficiency was slightly reduced as a result of this procedure. Additional studies are needed to determine the reason for this reduction.

The Effect of Initial Assignment of Parent Population Boer Does into High and Low Parasite Resistance Groups on Subsequent Doe Parasite Resistance, Survival Rate, Reproductive Efficiency, and Kid Performance and Survival Rates – 2 Year Summary

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In the United States, goat numbers have increased by about one-third in the past decade because of their economic value as efficient converters of low-quality forages into quality meat, milk, and hides for specialty markets. However, goats are more susceptible to internal parasites than other types of livestock. Therefore, the objective of this study was to evaluate the effect of initial assignment of parent population Boer does into high and low parasite resistance groups on subsequent doe parasite resistance, survival rate, and reproductive efficiency, and kid performance and survival rates. Parasite resistance Expected Progeny Differences were used to rank and sort mixed age Boer does (n = 146) into one of two selection lines: 1) high line (HL; n = 74) to be selected for high resistance to internal parasites or 2) low line (LL; n = 72) to be selected for low resistance to internal parasites. High line and LL Boer does were mated to corresponding HL and LL Kiko bucks to produce crossbred Kiko × Boer progeny for two consecutive y. After initial allocation, fecal egg counts, FAMACHA© scores, and packed cell volumes were measured periodically on all does and were utilized to determine if an animal required deworming. For does, number of times dewormed, survival rate, kidding date, litter size, and kid weaning weights were similar (P ≥ 0.27) across lines. Kidding rates and kid birth weights were greater (P ≤ 0.04) for LL compared with HL; however, HL does weaned more kids (P ≤ 0.05) compared with LL does. A sex effect (P ≤ 0.01) was observed for kid birth and weaning weights, where male kids weighed more compared to female kids. Therefore, after two y, initial assignment of parent population Boer does to high and low parasite resistance lines had no effect on subsequent doe parasite resistance or survival rate, but mixed effects on doe reproductive efficiency, kid performance, and kid survival rates; however, these findings represent short-term effects from an ongoing, long-term selection study.
Optimizing Design for Gut Microbe Identification by Sequencing

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The microbiome plays an important role in the physiology of animals across all species. Gut microbes assist with digestion, and the population is dependent on the animal's diet, genetics, and environmental conditions. In order to avoid PCR-bias, raw environmental samples, ones collected directly from the rumen for example, have been amplified via shotgun sequencing. The sequences can then be aligned to 16S rDNA gene database(s) to allow for the maximum microbial population identification. The objectives of this study were to address how the depth of barcoding effects the detection of difference between groups and the effects of operational taxonomic unit (OTU) group discovery. Rumen fluid samples were collected on 16 crossbred ewes which had been fed forage (n=8) or concentrate (n=8) diets. DNA was extracted and genomic libraries, multiplexed 4 per lane, were diluted and sequenced using Illumina's HiSeq 2000. Resulting reads were quality filtered and aligned to two distinct reference databases of 16S rDNA genes. Reads with a ≥97% sequence identity to the 16S rDNA genes in the databases were used to classify taxa present in each sample. The taxa were then categorized into respective OTUs (n = 349). Variances of taxa present in each OTU were scaled to represent various depths of barcoding and were then used to calculate the group means in a power analysis. Results demonstrate that as variability of reads in a particular OTU increases more sequences are needed to find significance between two groups. Secondly, distributions of taxa present were used to calculate the probability that a particular microbial taxa would be represented when varying the depth of barcoding. Not surprisingly, the higher depth of barcoding results in a lower discovery rate of rare OTU groups.

Development of a Pregnancy Associated Glycoprotein Assay to Accurately Detect Late Embryonic Mortality in Cattle

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The establishment and maintenance of pregnancy is a complex physiological process. In cattle, fertilization rate is high (i.e. 90%); however, there is a 20 to 25% loss of embryos by the time of maternal recognition of pregnancy (day 15-16; day 0 = estrus). Furthermore, there is an additional loss of embryos (i.e. 4 to 8%) that occurs after day 28 (late embryonic mortality). The highest incidence of late embryonic mortality occurs around the time of the fetal-maternal attachment with an incidence of approximately 4 to 10% (Vasconcelos et al., 1999; Perry et al., 2005). Bovine pregnancy associated glycoprotein (PAGs) are members of a large gene family and have been used to detect pregnancy in a variety of ruminant animals (Souusa et al., 2006). In a study by Green et al. (2005), bPAGs first appeared in maternal serum between d 24 and 28 post inseminations and peaked during the last week of gestation at 588.9±249.9 ng/ml. Pohler et al (2013) reported that beef cows that lost an embryo after day 28 had lower circulating concentrations of PAGs in maternal serum compared to cows that maintained pregnancy. The long-term goal is to utilize PAGs to accurately predict which cows will either undergo late embryonic mortality or maintain pregnancy in order to investigate late embryonic mortality in cattle. Some PAG members may be better predictors of late embryonic mortality than others. Our objectives are 1) develop an accurate semi-quantitative ELISA for detecting (PAGs) in bovine serum for the purpose of identifying females that will undergo late embryonic mortality after day 28 of gestation, 2) determine whether the pattern of secretion of circulating concentrations of PAGs in maternal serum from day 22 to parturition differs by using different polyclonal and monoclonal antibodies in an ELISA and RIA, 3) determine if there is a difference in the half-life of PAGs secreted during early gestation, 4) determine if estrus expression, circulating concentrations of estradiol at insemination, and (or) postovulatory circulating concentrations of progesterone affect circulating concentrations of PAGs after day 24 of gestation, 5) determine the relationship between ovulatory follicle size and circulating concentrations of PAGs on days 20 to 60 of gestation when using different polyclonal or monoclonal antibodies in the ELISA or RIA.
Frequency of Heteroplasmy in Canine Mitochondria

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Mitochondrial transmission has been traditionally thought to follow a strict matrilineal inheritance pattern. This inheritance pattern reduces genetic diversity in the mitochondrial genome by preventing recombination, making the mitochondria particularly useful for evolutionary research. However, recent advances in sequencing capabilities are challenging this tenet by showing evidence of higher than expected heteroplasmy frequencies. Mitochondrial heteroplasmy, or the presence of more than one genomic haplotype, is commonly thought to be due to leakage of paternal mitochondria during fertilization. The discovery that mitochondrial inheritance may not be strictly maternal has major implications for evolutionary studies. For this reason, it is important to determine mitochondrial heteroplasmy frequencies across species. In this study our objective was to determine the frequency of heteroplasmy in the canine mitochondrial genome. Using sequence data generated with Illumina HiSeq and Illumina Genome Analyzer IIx we analyzed 87 dogs, 14 wolves, two coyotes, two jackals, and one dingo for the presence of mitochondrial heteroplasmy. We scanned the 16kb canine mitochondrial genome for variant patterns indicative of heteroplasmy. Results show about five percent of animals are heteroplasmic. Animals were also placed into haplogroups to determine if mitochondrial genomes were shared by animals of the same breed. Furthermore, phylogenetic analysis was performed to determine the evolutionary relationships amongst the animals.

Embryonic growth between d 33 and 45 of pregnancy in lactating dairy cows differing in hormone and metabolite concentrations

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The objective was to examine the relationship between postpartum hormones/metabolites and embryonic growth from d 33 to 45 of pregnancy. Holstein cows (n=56; 86±17 d postpartum at AI) were examined by transrectal ultrasonography on d 33, 35, 38, 40, 42, and 45 of pregnancy. Length (l) and width (w) of the embryo and amnionic vesicle were measured and the volumes for the embryo \( e_{vol} \) and amnionic vesicle \( a_{vol} \) were calculated \[ \text{volume} = \frac{4}{3} \pi \cdot (0.5 \cdot l) \cdot (0.5 \cdot w) \cdot (0.5 \cdot w) \]. There was an effect of day of pregnancy \( (P<0.001) \) because \( e_{vol} \) and \( a_{vol} \) increased from d 33 to d 45 \((0.14±0.01 \text{ cm}^3 \text{ vs. } 0.60±0.06 \text{ cm}^3 \text{; and } 1.52±0.05 \text{ cm}^3 \text{ vs. } 10.57±0.59 \text{ cm}^3, \text{ respectively}) \). Across all days, the \( a_{vol} \) of male embryos was larger than female \((4.25±0.20 \text{ vs. } 3.70±0.20 \text{ cm}^3) \) but \( e_{vol} \) was similar for male vs. female. Plasma hormone and metabolite concentrations were not affected by day of pregnancy \( (P>0.1) \) but differed for individual cows \((P<0.001; \text{ range } = 51 \text{ to } 82 \text{ mg/dL for glucose, } 4.7 \text{ to } 13.5 \text{ ng/mL for P4, } 2.3 \text{ to } 13.4 \text{ ng/mL for GH, } 50 \text{ to } 131 \text{ ng/mL for IGF1, and } 0.2 \text{ to } 0.7 \text{ ng/mL for INS).}

Individual cows were categorized as being above or below the median for each blood hormone/metabolite concentration. Cows that were above or below the median for glucose, P4, GH, or INS were similar for \( e_{vol} \) and \( a_{vol} \) \((P>0.1). \) There was a category by day interaction \((P<0.05) \) for IGF1, however, cows with IGF1 above the median \((\text{mean } = 102.4±16.4 \text{ ng/ml}) \) had greater \( e_{vol} \) on d 42 compared with low \( \text{IGF1} \) cows \((\text{mean } = 69.9±13.0 \text{ ng/mL \text{ (1.11±0.04 vs. } 0.98±0.04 \text{ cm}^3 \text{; above vs. below).} \) \( a_{vol} \) was not affected by IGF1 category. Conclusions were that male embryos have greater amnion vesicle volume from d 33 to 45 of pregnancy. Plasma concentrations of IGF1 were positively associated with a larger embryo on d 42.
Autophagy and Ubiquitin-Proteasome System Contribute to Sperm Mitophagy after Mammalian Fertilization

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Maternal inheritance of mitochondria and mitochondrial DNA (mtDNA) is universally observed in humans and most animals. Sperm mitophagy, the elimination of sperm-borne mitochondria after fertilization assures normal preimplantation development and may prevent mitochondrial disease arising from heteroplasmy. We hypothesize that mammalian sperm mitophagy depends on ubiquitin proteasome system (UPS) determinants present in the sperm mitochondria and on the ooplasmic autophagy receptors that recognize such ubiquitin-tagged sperm determinants. We fertilized in vitro matured porcine oocytes with boar spermatozoa pre-labeled with MitoTracker and processed them for immunofluorescence at distinct time points up to 36 hr after in vitro fertilization (IVF). We also assessed sperm mitophagy in rhesus monkey oocytes with boar spermatozoa pre-labeled with MitoTracker and processed them for immunofluorescence at distinct time points up to 36 hr after in vitro fertilization (IVF). We also assessed sperm mitophagy in rhesus monkey oocytes fertilized by intracytoplasmic sperm injection (ICSI). Both pig and rhesus zygotes displayed the association of SQSTM1 with sperm mitochondria. We identified three proteins of mitochondrial origin that co-purified with the synthetic, SQSTM1-derived, ubiquitin-binding UBA domain. We further identified that the accumulation of GABARAP-containing ubiquitinated-protein aggregates was greatly increased and intact sperm mitochondrial sheaths were observed in the embryos treated with MG132. Furthermore, specific pharmacological inhibition of the ubiquitin-binding protein dislocase VCP also delayed the process of sperm mitophagy. Additional autophagy-associated ubiquitin receptors including LC3, HDAC6 and BNIP3L, were detected in the sperm mitochondria and other sperm accessory structures. Sperm mitophagy in higher mammals may rely on a combined action of SQSTM1-dependent autophagy and VCP-mediated presentation of ubiquitinated sperm-mitochondrial proteins to 26S proteasome.

Lethal Haplotype Detection and Characterization in a Beef Cattle Population

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Increasingly inbred population structures in artificially inseminated livestock species adversely impacts reproductive fitness. In beef cattle (Bos taurus), selective breeding has increased the frequency of several known segregating recessive polymorphisms that cause embryonic loss and has also increased identity by descent throughout the genome. By identifying haplotypes for which homozygotes are not observed despite a sufficient sample size considering haplotype frequency, developmentally lethal recessive loci can be localized without the observation of loss-associated phenotype (e.g., failure to implant, first trimester abortion). In this study, haplotypes were constructed for 3881 registered Angus individuals with 54545 SNP genotypes using findhap v2, which exploits the complex pedigree among individuals in this population. Haplotypes that were not observed in homozygous form in the population despite a high frequency and pedigree-based expectation of homozygous inheritance are candidates for harboring recessive lethal alleles. Of these genotyped individuals, 86 have been resequenced to an average 30× coverage to identify loss-of-function alleles genome-wide and have had variants called using a custom in-house developed pipeline. For any candidate haplotype found in these bulls, phased whole-genome genotypes will be used to identify the variants that are responsible for lethality. This will provide insight into the biological foundations of inbreeding depression and the genomic architecture of developmentally important genes in selectively bred populations. Selective breeding programs can utilize these detected lethal haplotypes and causal variants to reduce the negative impact of inbreeding on fertility and to maximize overall genetic gains.

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Effects of melamine in young barrows

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A study was conducted to investigate the toxicity of melamine (MEL) fed to young barrows, and to determine residual levels of MEL in selected tissues. Thirty 14-d post-weaning barrows (initial weight = 10.6 ± 1.2 kg) were allotted to 1 of 6 dietary treatments containing 0, 0.25, 0.50, 0.75, 1.0, or 1.25% MEL. A completely randomized design was used with 5 replicate pens of one pig/pen assigned to each treatment. Compared with controls, BWG and ADG were lower (P = 0.0205) in pigs fed ≥1.00% MEL. A decrease in gain to feed with increasing dietary MEL concentrations contained both linear (P = 0.0076) and quadratic (P = 0.0407) components. Pigs receiving 1.25% MEL had a lower (P = 0.0427) gain to feed value than controls. Linear decreases in serum glucose (P = 0.0124) and serum calcium (P = 0.0053) were observed as dietary MEL levels increased. In contrast, aspartate aminotransferase increased (P = 0.0049) linearly as MEL inclusion in the diet increased. An increase in residual MEL levels in the kidney contained both quadratic (P < 0.0001) and linear components (P < 0.0001). There was a linear (P < 0.0001) increase in MEL concentrations in bile with increasing dietary concentrations of MEL. Pigs fed diets containing ≥0.25% MEL had kidney MEL residue levels greater (P < 0.0001) than controls, whereas pigs fed ≥0.50% had MEL residue levels in bile and muscle that were greater (P < 0.0001) than controls. In summary, results indicate that ≥1.00% MEL was toxic to pigs fed dietary MEL treatments for 21 d.

Preimplantation Embryo Development In Vitro is Improved with Glycine Supplementation

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Recently, it has been shown that glycine metabolism is important for cancer cells to remain highly proliferative. Like cancer cells, preimplantation porcine embryos rapidly divide and have a similar metabolism. The aim of this project was to determine the effect of glycine supplementation on preimplantation embryo development. Cumulus-oocyte complexes were matured and then metaphase II oocytes were selected and fertilized in mTBM for 5 hours and placed into Porcine Zygote Medium MU1 with 0 mM, 0.1 mM (control), 1 mM, 5 mM, 10 mM or 20 mM glycine. Oocyte maturation occurred in 5% CO₂ in air at 38.5°C, fertilization was in 5% CO₂ in air at 38.5°C, and development to day 6 was in 5% CO₂, 5% O₂, and 90% N₂ at 38.5°C. The percent of embryos that developed to blastocyst was not improved by supplementing the embryo culture media with glycine. On day 6, embryos were stained with bisbenzimide to visualize nuclei and then total cell number was determined. The mean number of nuclei for each treatment was 41.5±4.8a, 47.2±4.5ac, 58.2±4.4bc, 65.4±4.1bd, 71.9±4.7d, and 67.8±5.7bc, cells (n=20, 22, 23, 27, 21, 15) in 0 mM, 0.1 mM, 1 mM, 5 mM, 10 mM, and 20 mM glycine, respectively. TUNEL staining was used to determine if there was a significant difference in the number of apoptotic nuclei in embryos cultured in 0.1 mM compared to 10 mM glycine. There was a decrease in the apoptotic positive nuclei in embryos cultured in 10 mM glycine (8%±1.4%) vs. 0.1 mM glycine (14%±1.5%) (p=0.003). Gene expression analysis was completed to determine differences due to glycine culture. Genes involved with glycine/serine metabolism, ROS, and one carbon pool were down regulated. We conclude that glycine concentration in the culture medium is critical to preimplantation embryo development.
Evaluation of Culture and Electroporation of Feline Fetal Fibroblasts Used to Assess the Effect of Zinc Finger Nucleases on Gene Targeting Efficiency

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To generate genetically engineered animals by somatic cell nuclear transfer, methods must be established for transfection, selection and culture of primary cells. Parameters for transfection and selection of feline fetal fibroblasts (FFF) are not well established. The objectives of this project were to evaluate conditions for square-wave electroporation, and G418 selection. In the culture conditions used (DMEM supplemented with 12.5% fetal bovine serum, at 38.6°C in an atmosphere of 5.0% O₂, 5.5% CO₂), the minimum dose of G418 that produced no background colonies was between 120 to 130 μg/mL. Electroporation was evaluated in 2 mm cuvettes using a single 1 ms pulse of varying voltages (V; 100, 150, 200, 250, 300, 350) and multiple 1 ms pulses (1, 2, 3, 4 or 5) at 300V for delivery of a red fluorescent protein reporter gene. Cell survival, percent transfected of surviving cells, and total number of transfected cells were evaluated. Survival decreased with increased voltages and with increased pulse number. The percentage of the surviving cells that were transfected increased with voltage and pulse number (maximum of 58%-63%). The maximum number of transfected cells resulted from a single pulse at 300V (range). FFFs were transfected with a targeting vector alone, or a targeting vector cotransfected with plasmids that encoded gene-specific ZFNs in order to assess targeting efficiency by homologous recombination (HR) with and without ZFNs. Cotransfection of ZFNs increased HR rates by as much as 50 fold. FFFs can be successfully cultured and transfected for genetic modification.

Marker-free production of transgenic livestock

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Transgenic animals can be used to produce biomedical models of human conditions. Similarly, transgenic animals can be used to produce organs, tissues, cells, or other products that may have therapeutic value. One standard method used to produce transgenic livestock is the reconstruction of embryos from transfected cells via somatic cell nuclear transfer. For this project, the overriding goal is to produce transgenic pigs that harbor a full-length, human insulin (hINS) gene for eventual applications in xenotransplantation. This effort presents the opportunity to evaluate methods of transfection that do not require selection for drug resistance. Our lab has developed electroporation protocols that can result in 70-100% transfection rates. We hypothesize that drug selection will not be required to isolate stable integration events when the new electroporation protocols are used. The first experiment will quantify the impact of selection omission on the efficiency of isolation of integration events. This will be accomplished by comparing the number and proportion of colonies that harbor a transgene after application or omission of selection. The minimal proportion of “positive” colonies that will be considered practical will be approximately 1%. Based on these results, a second series of transfections will be performed with the appropriate hINS construct (with or without selectable marker) to produce porcine cells that will be used to produce offspring via somatic cell nuclear transfer. Future experiments will be directed toward marker-free strategies for co-transfection of multiple transgenes.
Comparison of Global Levels of DNA methylation and Mecp2 between fully grown GV oocytes of young and aged female mice

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In mammals, fertility and chronological age show an inverse correlation. Oocyte quality is a contributing factor to this multifactorial phenomenon. DNA methylation (DNAm) is an epigenetic modification that functions primarily to repress genes and promote chromatin compaction. We previously demonstrated that introducing high levels of FSH by exogenous administration of equine chorionic gonadotropin (eCG) leads to decreased levels of global DNAm in mouse oocytes. Follow-up studies demonstrated that even though aged female mice have higher levels of serum FSH, global levels of DNAm were higher in oocytes from aged females than levels seen in oocytes from young females. Results also demonstrated a dissimilar pattern of chromatin configuration between oocytes of young and old mice, suggesting the increased level of DNAm may be involved in this phenomenon. Chromatin remodeling occurs through epigenetic modifiers that act on the DNA and associated histone proteins. Methylated DNA attracts methyl DNA binding domain proteins such as Mecp2 (Methyl-CpG binding protein 2). We hypothesized that levels of Mecp2 would be increased in oocytes of aged mice. To test this, fully grown oocytes were retrieved from the ovaries of 10 aged (85-88 weeks [n=3], 77-79 weeks [n=7]) and 7 young (12-16 weeks [n=5], 6-8 weeks [n=2]) females and Mecp2 detected by confocal microscopy. Image J software was used to obtain values (pixel intensity) for Mecp2 in the germinal vesicle (GV) and averaged for comparison. Results show that Mecp2 is distributed across the genome but does not colocalize with heterochromatin, chromocenters, or the nucleolar ring. No significant differences in levels of Mecp2 were detected between oocytes from any age group (n=17, n=23, n=13, and n=7 respectively). From these results we conclude that Mecp2 is not involved in the apparent difference in chromatin arrangement between oocytes of aged and young mice.

Effects of feeding stockpiled tall fescue versus tall fescue hay to late gestation beef cows on cow and calf performance

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We hypothesize that cows grazing stockpiled tall fescue (STF) during late gestation have increased nutrient intake resulting in improved fetal development and subsequent calf health and performance compared with cows fed poor quality hay. Forty-eight mature, spring-calving crossbred beef cows (683 ± 16 kg) were allocated by BW, BCS, age, and expected calving date to either strip-graze endophyte-infected STF (59.7% NDF, 12.3% CP; 4.05 ha pastures, n = 4) or consume ad libitum endophyte-infected tall fescue hay (HAY; 64.9% NDF, 6.2% CP; uncovered drylots, n = 4) during late gestation (d 188 ± 14). The trial consisted of 2 periods (Period 1: d 0-35; Period 2: d 36-78). Cows grazing STF were moved to drylots 1 week pre-calving and fed ryelage (58.6% NDF, 12.3% CP). Data were analyzed with treatment as a fixed effect for cow measures; calf sex and date of birth were included as fixed effects for calf data. There were no differences (P ≥ 0.44) in initial BW, BCS, or BF. Change in BW during period 2 was affected by treatment (P = 0.02), where HAY cows lost BW and STF cows gained BW. Overall BW change tended (P = 0.10) to follow that of period 2. Treatment affected (P ≤ 0.02) period 1 and overall BCS change, where HAY cows lost BCS and STF cows gained BCS. Although change in BF was not different (P ≥ 0.20), BF at d 78 tended (P < 0.10) to be greater for STF cows. In addition, calves born to STF cows tended (P = 0.07) to weigh more at birth compared with HAY, indicating that fetal growth may have been restricted in HAY cows. Further analyses are planned to determine effects of treatments on circulating cow and calf metabolites, future calf performance, and calf health parameters.