

**2022 PROCEEDINGS OF THE EIGHTH  
UF/IFAS ANIMAL SCIENCES  
SYMPOSIUM**



**Embassy Suites by Hilton St. Augustine Beach  
300 A1A Beach Boulevard  
St. Augustine, FL 32080**

**Thursday, October 6<sup>th</sup>**

**&**

**Friday, October 7<sup>th</sup>**



## **WELCOME!**

Welcome to the Eighth UF-IFAS Animal Sciences Graduate Symposium. This symposium was conceived and organized to build an inclusive community, to train our students, to share science, and to foster collaboration in our research efforts. The program contains a mixture of student presentations of proposed, ongoing, and completed research. The presentations will be given by UF graduate students and undergraduate students who interned at UF over the summer. Thursday will include oral and poster presentations, a reception, as well as the Distinguished Lecturer and a group dinner. On Friday, we will have a group photo and an opportunity to listen to more stimulating research presentations and another poster session. Please take this time to learn about other research activities in our department and to relax and enjoy the company of friends and colleagues.

Sincerely, Antonio Faciola – on behalf of the Symposium Committee

## ACKNOWLEDGMENTS

The faculty and students of UF and IFAS thank the following for their support of the Eighth Animal Sciences Symposium:

Dr. Nicole Stedman, Dean of the Graduate School and Associate Provost, University of Florida

Dr. Elaine Turner, Dean of the College of Agricultural and Life Sciences, University of Florida

Dr. Robert Gilbert, Dean for Research and Director of the Florida Agricultural Experiment Station, IFAS, University of Florida

Dr. John Arthington, Department Chair, UF/IFAS Animal Sciences

## Sponsor



Representative - **Kari Estes**, M.S., Research Associate, Animal Nutrition and Health

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Daniella Heredia  
Gabriela Macay  
Adeel Manzoor  
Cecilia Rocha  
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## ANS Symposium 2022 Distinguished Lecturer



**Pamela Ruegg, DVM, MPVM**

David J. Ellis Chair in Antimicrobial Resistance  
Professor, Large Animal Clinical Sciences, College of Veterinary Medicine  
Michigan State University

Dr. Ruegg holds the David J. Ellis Chair in Antimicrobial Resistance and Professor of Large Animal Clinical Sciences in the College of Veterinary Medicine at Michigan State University. From 2018-2020 she served as the chair of the Dept. of Animal Science at Michigan State University. From 1998-2017, she was a professor and extension milk quality specialist in the Dept. of Dairy Science at the University of Wisconsin, Madison and remains an emeritus Professor in that Department. She received her undergraduate degree and D.V.M. from Michigan State University, worked in mixed animal practice in Wisconsin and then returned to academia to complete a residency and Masters of Preventive Veterinary Medicine from the University of California, Davis. She has had varied professional experiences including private veterinary practice, academic positions at both Atlantic Veterinary College in Prince Edward Island, Canada, and the College of Veterinary Medicine at MSU and corporate technical service. Her areas of expertise include preharvest milk quality and safety in dairy cows, udder health and antimicrobial usage on dairy farms and assessment of milking performance and dairy animal health records. She maintains a research program that is focused on ensuring antibiotic stewardship on dairy farms by reducing antibiotic usage on dairy farms and identifying cow, environmental and pathogen factors that can be manipulated to reduce mastitis risk. Dr. Ruegg is active in a number of industry organizations and is a past-president of the National Mastitis Council. Her extension program has focused on developing programs that help farmers maintain healthy cows while reducing antimicrobial usage and improving milk quality and safety on dairy farms. From 2014-2018, she led a team that was responsible for development of >2,000 hours of curriculum for the Nestle Dairy Farming Institute in Heilongjiang Province, PRC. Throughout her career she has received a number of awards for research, extension, and international outreach programs. She has published >130 peer reviewed articles and is frequently cited, with a current H-index of 54.

# 8<sup>th</sup> UF/IFAS ANIMAL SCIENCES SYMPOSIUM

Embassy Suites by Hilton St. Augustine Beach  
300 A1A Beach Boulevard, St. Augustine, FL 32080  
October 6<sup>th</sup> and October 7<sup>th</sup>

## Program

### THURSDAY, OCTOBER 6

- 11:30        **Registration Check-in** - Registration Table next to the Anastasia Room
- 12:15        **Welcome** - Dr. Antonio Faciola, Graduate Coordinator, Anastasia Room
- 12:20        **Program** - Faculty, UF/IFAS, Department of Animal Sciences
- 12:30        **Session 1.** Chairs: Tulio Souza and Stathis Sarmikasoglou
- 12:30 Abstract #22: Leticia Casarotto Trevisan (Dahl)
- 12:45 Abstract #23: Cecilia Constantino Rocha (Binelli)
- 01:00 Abstract #24: Mariana Marinho (Santos)
- 01:15 Abstract #25: Emily Lindner (Miller-Cushon)
- 01:30 Abstract #26: Maria Camila Lopez Duarte (Gonella-Diaza)
- 1:45        **Break**
- 2:00        **Session 2.** Chairs: Lais de Oliveira and Daniel de Oliveira
- 2:00 Abstract #27: Daniel Onan Martinez (Dahl)
- 2:15 Abstract #28: Juan Vargas (DiLorenzo)
- 2:30 Abstract #29: Mikayla Johnson (Faciola)
- 2:35 Abstract #30: Wilmer Cuervo (Dilorenzo)
- 2:40 Abstract #31: Daniella Heredia (Gonella-Diaza)
- 2:45 Abstract #32: Carlos Niño De Guzman (Vyas)
- 2:50 Abstract #33: Ignacio Fernandez Marenchino (Dilorenzo)
- 3:00        **Break - Pick-up room key from front desk**
- 3:30        **Distinguished Lecturer: Dr. Pamela Ruegg, Michigan State University**
- 4:30        **Session 3. Poster Presentations & Embassy Suites Complimentary Reception**
- Abstract #01: Ashley Beard (Mateescu)
- Abstract #02: Fahad Rafiq (Mateescu)
- Abstract #03: Elizabeth Chmielewski (Scheffler, T.)
- Abstract #04: Samia Farooq (Vyas)
- Abstract #05: Fernanda Hentz (Batistel)
- Abstract #06: Joao Losano (Daigneault)
- Abstract #07: Alyssa Ohmstede (Warren)
- Abstract #08: Gabby Allen (Scheffler, J.)
- Abstract #09: Martha Siregar (Faciola)
- Abstract #10: Katie Gingerich (Miller-Cushon)

6:45           **Dinner** - Anastasia Room

**FRIDAY, OCTOBER 7**

7:00           **Breakfast**

**8:20           Program** - Faculty UF/IFAS, Department of Animal Sciences, Anastasia Room

**8:30           Session 4.** Chairs: Mikayla Johnson and Arslan Tariq

8:30   Abstract #34: Aakilah Hernandez (Mateescu)

8:45   Abstract #35: Maddy Smythe (Brooks)

9:00   Abstract #36: Kamryn Joyce (Gonella-Diaza)

9:15   Abstract #37: Ting Liu (Jeong)

9:30   Abstract #38: Julia Ciosek (Brooks)

9:45           **Group Picture**

**10:15          Session 5. Poster Presentations**

Abstract #11: Bruna Souza (Santos)

Abstract #12: Meghan Campbell (Binelli)

Abstract #13: Daniel de Oliveira (Batistel)

Abstract #14: Daniel Clein (Miller-Cushon)

Abstract #15: Benjamin Po (Vyas)

Abstract #16: Szu-Wei “Daniel” Ma (Faciola)

Abstract #17: Giovanni Ladeira (Rezende)

Abstract #18: Hadley Rahael (Brooks)

Abstract #19: Tyeler Gilliam (Brooks)

Abstract #20: Morgan Brown (Gonella Diaza)

Abstract #21: Benjamin Kline (Brooks)

11:00          **Session 6.** Chairs: Gabriela Macay and Araceli Maderal

11:00   Abstract #39: Araceli Maderal (Dilorenzo)

11:15   Abstract #40: Andrea Nunez Andrade(Mateescu)

11:30   Abstract #41: Mauro Venturini (Gonella-Diaza)

11:45   Abstract #42: Gabriel Zayas (Mateescu)

12:00   Abstract #43: Zachary Seekford (Bromfield)

12:05   Abstract #44: Barclay Powell (Brooks)

12:10   Abstract #45: Adeel Manzoor (Scheffler)

12:15   Abstract #46: Federico Podversich (Dilorenzo)

12:20   Abstract #47: Derick Rosler (Batistel)

12:25          **Closing remarks – UF Deans and Animal Sciences Graduate Committee**

12:35          **Awards Presentation** - Oral, Poster, and Flash-talk awards.

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# **ABSTRACTS**

## Using Single Cell RNA Sequencing as an Approach to Understanding Thermotolerance in Beef Cattle

Ashley M. Beard, Raluca G. Mateescu\*

Department of Animal Sciences, University of Florida, Gainesville

Thermotolerance in beef cattle has become a growing concern among producers due to climate change and the negative implications heat stress can have on various production traits. There is a lack of understanding on the overall transcriptome as well as potential molecular pathways related to heat stress in beef cattle. Traditional RNA sequencing aids in measuring the average gene expression across all cells within a sample. Although this technique does give general insight on the transcriptome, heterogeneity is present in different cell types, and analyzing these differences using traditional RNA sequencing is relatively impossible. A newer approach deemed single-cell RNA sequencing (scRNA-seq) helps explore the complex systems within different cell types by enabling cell-specific molecular characterization. Since heat stress in cattle is alleviated primarily through the skin via perspiration to avoid overheating, skin biopsy samples were collected on cattle with both low and high thermotolerance-related phenotypes. Using these tissue samples, nuclei can be isolated and applied to scRNA-seq analyses. Following isolation, nuclei will be fixed to stabilize the cells and deter any RNA degradation. Utilizing combinatorial cDNA barcoding within the nuclei, individual transcriptomes will be uniquely labeled by passing the fixed nuclei through four rounds of barcoding. Samples are first distributed into individual wells and sample-specific barcodes are applied to fixed nuclei with a reverse transcription (RT) reaction. The nuclei are then pooled together and distributed across the plate and ligation is used to attach a second barcode to the cells. This process is repeated once more to attach the third barcode to the nuclei. Nuclei are pooled together again and divided across several sublibraries, where they can then be lysed, and a fourth sublibrary-specific barcode is added by PCR. After the four unique barcoding rounds, library preparation appends adapters in which the samples can now be sequenced for in-depth cell-specific transcriptome analyses.

## The Analysis of the Genetic and Phenotypic Relationships Between Thermotolerance, Feed Efficiency, and Reproductive Performance in Brangus Heifers

Fahad Rafiq, Aakilah S. Hernandez, Andrea N. Nunez, Eduardo E. Rodriguez, Gabriel A. Zayas, Ashley M. Beard, Nicolas DiLorenzo, and Raluca G. Mateescu\*

Department of Animal Sciences, University of Florida, Gainesville, Florida

A major limiting factor of production efficiency in tropical and sub-tropical environments is climatic stress, and the ever-changing climate exacerbates this limiting factor. In beef cattle, it is an emerging challenge to determine the nature and magnitude of the genetic and phenotypic relationships between thermotolerance, feed efficiency, and reproductive performance in Brangus heifers. In this study, we used a set of 326 heifers to measure thermotolerance. We selected extreme (N=140) with high and low thermotolerance measures to study the relationship between thermotolerance, feed efficiency, and reproductive performance. We recorded the vaginal temperature every fifteen (15) minutes over three days and monitored environmental conditions to generate a temperature-humidity index (THI). A Principle Component Analysis was used on several traits related to body temperature to generate a principle component 1 (PC1), which represents the increase in body temperature from low to high THI. The PC1 was used to identify heifers with extreme high and low thermotolerance. These heifers entered a feed efficiency trial for 56 days at NFREC in Marianna, FL. Some of the other recorded traits are shown in Table 1. The Pastecs package in R software was used to produce descriptive statistics. The results in the descriptive analysis show some variation which can be further investigated in further analyses.

Table 1. Descriptive statistics for Thermotolerance, Feed Efficiency and General characteristics in a Brangus Heifer population.

Trait	Number	Mean	Standard Deviation	Coefficient of Variation
Hair Length	325	0.93	0.46	0.49
Coat Score	325	1.85	0.90	0.48
Chute Score	324	1.28	0.47	0.36
Exit Score	323	1.76	0.56	0.32
Body condition Score	324	7.18	0.55	0.08
Residual Feed Intake	133	0.00	3.55	-
Dry Matter Intake Percentage	133	3.64	0.47	0.13

## Metabolic and Color Differences Between Angus and Brahman *Longissimus Lumborum*

Elizabeth Chmielewski, Brianna Hawryluk, Chloe Gingerich, Morgan McKinney, Patricia Ramos, Tracy Scheffler\*

Department of Animal Sciences, University of Florida, Gainesville

Beef color, an important factor for consumer purchasing decisions, can be affected by metabolic activity. Mitochondria (mt) consume oxygen in postmortem muscle through cytochrome c oxidase activity (COX). Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and NADH to lactate and NAD<sup>+</sup>. Previously, we demonstrated Angus *longissimus lumborum* (LL), compared with Brahman, blooms brighter at the onset of display. Thus, our objective was to evaluate COX and LDH activity in Angus and Brahman LL postmortem and the effect on color development. Angus and Brahman steers (n=14 per breed) were harvested, then 1h and 14d LL samples were collected. For COX activity, 1h samples (n=5 per breed) were plated with a reaction media of reduced cytochrome c and buffer at pH 7.0, 6.7, 6.4, 6.1, and 5.8. Activity was reported as nmol/min/mg protein. For LDH activity, 1h and 14d samples were processed and supernatants were plated with reaction media (NAD<sup>+</sup> or NADH), the reaction was initiated with pyruvate or lactate, and activity was reported as nmol/min/mg tissue. Data were analyzed in SAS-JMP Pro 16. Reaction media pH affected COX activity (P<0.0001), with similar activity between pH 7.1 and 6.4, but activity progressively decreasing with lower pH. Breed did not influence COX activity (P=0.24). Brahman exhibited higher LDH activity towards lactate (P=0.007) and pyruvate (P=0.02), compared with Angus. LDH activity decreased from 1h to 14d for LDH activity towards lactate (P=0.002) and pyruvate (P<0.001). The decrease in COX activity below pH 6.4 shows declining pH significantly hinders mt OC. Elevated LDH activity towards lactate in Brahman evidences a greater capacity for glycolytic metabolism, whereas the increased in LDH activity towards pyruvate demonstrates increased capacity to replenish NADH. Thus, increased postmortem OC and a greater capacity for anaerobic metabolism suggests elevated metabolic activity may explain why LL from Brahman, compared with Angus, bloomed less efficiently upon exposure to oxygen.

## **Evaluating the effects of maturity at harvest, microbial inoculation, and ensiling durations on nutritive value, and fermentation characteristics of sorghum hybrids**

Samia Farooq<sup>1</sup>, Marcelo Wallau<sup>1</sup>, C. Cornejo<sup>1</sup>, R. Trump<sup>1</sup>, F. Amaro<sup>1</sup>, J. Portuguez<sup>1</sup>, C.A. Niño de Guzmán<sup>1</sup>, L. Mu<sup>1</sup>, K. Arriola<sup>1</sup>, H. Sultana<sup>1</sup>, Luiz Ferraretto<sup>2</sup>, D. Vyas\*<sup>1</sup>.

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<sup>2</sup>University of Wisconsin, Madison, USA.

Sorghum is considered an important forage crop in many regions including Southeast US because of its morpho-physiological adaptation to water scarcity providing greater biomass yield despite uneven distribution of rainfall. We aimed to analyze the effects of types of hybrids (H1, H2, H4, H5), maturity at harvest (Low and High DM), microbial inoculation (CON, INO), and ensiling duration (30 d, 90 d) on ensiling characteristics, fermentation profile and nutritive value of sorghum silage. Thirty-two treatments (2 maturities × 4 hybrids × 2 inoculants × 2 ensiling durations) with 4 replicates per treatment combination (total 128 mini-silos) were used for the study. Sorghum hybrids harvested at different DM were inoculated without or with mixture of *Lactobacillus buchneri* and *Lactococcus lactis* containing 150,000 cfu/g applied at 30 mL/kg of forage and ensiled for 30 and 90 d. Data was analyzed using MIXED procedure of SAS. Microbial inoculation resulted in lower lactate concentration and subsequently higher silage pH, when compared with CON. Acetate concentrations were greater with INO compared with CON, regardless of different hybrids, maturity, and ensiling duration. No hybrid effect was observed; however, INO silage had lower butyrate concentrations compared with CON suggesting that both CON and INO silages were better preserved. Total yeast and mold counts were lower for INO silage of every hybrid compared with CON. Greater acetate concentrations and lower yeast and mold counts resulted in improved aerobic stability of INO silage regardless of ensiling duration; however, greater stability was observed for CON silage after 30 d compared with 90 d of ensiling. Microbial inoculation resulted in greater lactic acid bacteria counts compared with CON. In conclusion, microbial inoculation resulted in greater acetate concentrations and improved aerobic stability.

## Expression of fatty acid transporters in the gastrointestinal tract of cattle

Fernanda Hentz, Bruno Emanuel Barreta, Fernanda Batistel\*

Department of Animal Sciences, University of Florida, Gainesville

High producing dairy cows consume on average 1 kg of fatty acids daily from dietary feedstuffs and supplemental fat sources. Digestibility of total fatty acids in ruminants is relatively low (67-75%) compared to non-ruminants (80-90%). In addition to efficient lipid digestion, fat digestibility depends on efficient absorption of the lipid hydrolysis products. Although major differences exist in the type of lipids reaching the small intestine of ruminants and non-ruminants, as well as in bile salt composition, current models of ruminant fatty acid absorption are based primarily on non-ruminant studies. The transporters involved in fatty acid absorption have been scarcely evaluated in cattle, and a better understanding of the absorption process may lead to improvements in fatty acid digestibility. Therefore, this research aimed to investigate the presence and distribution of fatty acid transporters in the gastrointestinal tract of cattle. Protein was extracted using a commercial buffer and quantified using a UV-Vis spectrophotometer. Protein expression of transporters involved with fatty acid absorption (solute carrier family 27 member 4 [SLC27A4]) and intracellular trafficking of fatty acids (intestinal and heart fatty acid binding protein [I-FABP and H-FABP], microsomal triglyceride transfer protein [MTTP]) was determined using a chemiluminescence western protein analyzer. Chemiluminescence peak areas were normalized to total protein concentration. Protein expression of the transporters in the different gastrointestinal tract sections of cattle is presented in Table 1.

**Table 1.** SLC27A4, I-FABP, H-FABP, and MTTP protein expression in different gastrointestinal tract sections of cattle.

Chemiluminescence <sup>1</sup>	Rumen	Duodenum	Proximal jejunum	Medial jejunum	Distal jejunum	Ileum
SLC27A4	4244.6	4704.4	4577.8	4564.8	4796.1	2863.5
I-FABP	1919.0	0	35632.0	14994.7	22280.7	78140.3
H-FABP	0	0	0	83780.3	37583.0	44788.2
MTTP	0	8609.8	64954	10011.1	0	8587.3

<sup>1</sup>Chemiluminescence peak areas were normalized to the total protein concentration in each tissue.

## Detection of novel bioenergetic traits in bovine sperm for improved semen quality analyses

Joao Losano<sup>1</sup>, Christopher Souders<sup>2</sup>, Christopher Martyniuk<sup>2</sup> and Bradford Daigneault<sup>1\*</sup>

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<sup>2</sup>Department of Physiological Sciences, University of Florida

Sperm transport through the female reproductive tract requires energy for motility and fertilization. Motility assessment of bovine sperm is conducted as an industry standard to estimate semen quality prior to insemination. However, samples with similar post-thaw motility result in different pregnancy outcomes. Identification of novel sperm traits that are more predictive of fertilizing potential would improve reproductive success. Thus, we aimed to improve and optimize methods of determining sperm bioenergetics over time. Post-thawed sperm from 3 bulls (A,B,C) were assessed individually or pooled (AB, AC; n = 5 samples; 4-16 replicates). Sperm were isolated by Percoll<sup>®</sup> gradient and evaluated for kinematics (CASA: total motility [TM] and progressive motility [PM]) and bioenergetics (Seahorse assay: basal respiration [oxygen consumption rate; OCR] and Energy Map [EM]), at 0 and 24 hr of storage at 23°C. TM and PM was nearly identical ( $\pm 5\%$ , SEM) after thawing. Similarly, no differences in OCR were detected at 0 hr. However, after 24 hr of sperm storage, pool AC presented higher OCR than other samples (0.56;  $P < 0.0001$ ) and pool AB presented higher OCR than Bull C ( $P < 0.0001$ ). Furthermore, sperm kinematic variability was higher at 24 hr (TM and PM;  $P < 0.0001$ ), demonstrating a difference in sperm quality detection over time. Despite a drop in motility, OCR was higher at 24 hr for all samples, except C. When combined, OCR was also higher at 24 vs 0 hr ( $P < 0.0001$ ). At 0 hr, no differences in EM were detected, indicating that sperm are metabolically similar after thawing. At 24 hr, a metabolic divergence between samples was detected ( $P < 0.0001$ ), indicating a shift in bioenergetic profiles over time. In conclusion, the bioenergetic status of bull sperm over 24 hr provides missing information regarding sperm energy requirements. These novel sperm traits have potential to detect variability between samples and may be useful for adoption in sperm analysis prior to insemination.

## Evaluation of indigestible neutral detergent fiber to estimate hay digestibility in horses

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Indigestible neutral detergent fiber (**iNDF**) is commonly used to estimate dry matter digestibility (**DMD**) in ruminants; however, this internal marker has not been routinely applied to horses. This study compared fecal output and DMD measured in horses with total fecal collection (**TC**) to that estimated with different methods of determining iNDF. Mature Quarter Horse geldings ( $552 \pm 14$  kg) were fed 5 hays in a 5 x 5 Latin square design experiment. Hays offered at 1.6% BW included: alfalfa (*Medicago sativa*, **ALF**), orchardgrass (*Dactylis glomerata*, **ORC**), and bermudagrass (*Cynodon dactylon* 'Coastal') harvested at 4 (**BG-E**), 6 (**BG-M**), and 8 weeks of regrowth (**BG-L**). During the last 3 d of each 12-d period, all feces were collected (**TC**) and subsampled to form three replicate 24-h composite samples for each horse. Hay and fecal samples were dried and ground, placed in sealed 25- $\mu$ m filter bags, and incubated for 288-h in four different inocula: *in situ* in rumen-cannulated cows, *in vitro* in rumen fluid (**RF**), *in vitro* in cecal fluid (**CF**), and *in vitro* in equine fecal inoculum (**EFI**). *In vitro* incubations were completed in ANKOM Daisy<sup>II</sup> incubators. Incubated samples were subsequently analyzed for remaining neutral detergent fiber content (ie, iNDF). Data were compared with mixed model ANOVA and linear regression. Mean iNDF recovery for *in situ*, RF, CF, and EFI was 94.46%, 84.80%, 88.30%, and 91.30% (SEM 1.6%), respectively. Fecal output estimated by *in situ* and EFI did not differ from that measured by TC, but was overestimated by RF and CF methods ( $P < 0.05$ ). An interaction of hay\*method was observed for DMD. For ALF and BG-M, all iNDF methods estimated DMD similar to TC. For ORC and BG-E, only DMD estimates from *in situ* and EFI were similar to TC. All iNDF methods underestimated DMD for BG-L. Within a method, statistical ranking of DMD among the 5 hays was most similar between TC, *in situ* and EFI. Across a variety of hay types, use of iNDF to estimate DMD was the most similar to TC when iNDF was determined with *in situ* and EFI methods.

## **Investigating the Control of Salmonella during the Production of Ethiopian Qwanta using Culturally Acceptable Interventions**

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Foodborne disease is a major cause of mortality and morbidity in developing countries. When general nutrition is poor, and access to animal-sourced foods (ASFs) are limited, the safety and preservation of food is particularly important. Qwanta is a homemade Ethiopian whole muscle dried beef product made utilizing an intricate cutting technique to create elongated, thin strips of meat that are seasoned, and air dried at ambient temperatures. The relative risk of this preservation method is not well understood; it does not utilize heat, and only modest amounts of salt (~1%) are used. Quantifying inherent risk and introducing available and culturally acceptable interventions may improve both availability and safety of ASF. The objective of this study is to evaluate interventions during qwanta production to further mitigate the risks of foodborne diseases. Qwanta strips ( $20 \pm 5.0$  g) were cut from top round (<2% fat), inoculated with five strains of *Salmonella enterica* (Anatum, Dublin, Newport, Saintpaul, and Typhimurium), and either dipped in vinegar, wine, or 0.9% saline (control). Strips were seasoned with a mixture of salt and Berbere, an Ethiopian spice. Strips were placed in ambient air-drying chambers for 0, 1, 4, and 7d post-inoculation, weighed, and plated for enumeration. Final weight loss was not affected by treatment; however, all treatments achieved  $a_w < 0.57$  after 7 days of drying. Qwanta dipped in a 1.4 log reduction for *S. enterica*. Vinegar showed an increased inactivation of *S. enterica* by  $2.2 \pm 0.11$  log CFU/g, compared to the control group ( $P < 0.0001$ ). The inclusion of vinegar significantly increases pathogen inactivation and could be a viable intervention during the production of qwanta. Additional sensory testing should be utilized to ensure that the inclusion of vinegar does not affect the desired taste of Ethiopian qwanta.

## Effects of different cobalt sources on methane and total gas production and ruminal fermentation parameters in vitro

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Four different cobalt (Co) sources, supplemented in a high-producing dairy cow diet, were investigated in vitro for their effects on methane and total gas production as well as on ruminal fermentation parameters. Two experiments were conducted. A total mixed ratio (TMR) of, on average, 52:48 forage:concentrate was provided. Cobalt carbonate was used as positive control at 90.6 mg/g of DM of diet (**CON**); and three alternative Co sources: 84.9 mg/g of DM of diet (**LCO**), 88.5 mg/g of DM of diet (**MCO**), and 102.3 mg/g of DM of diet (**HCO**). All diets provided the same amount of total Co. Methane and total gas production were measured in batch culture (experiment 1), in which each treatment was incubated with 52 mL buffer ruminal content and 500 mg of TMR pre-weighed in ANKOM bags for 48 h as fermentation endpoint. The experiment was done as a complete randomized block design with 3 runs. Run was used as a blocking factor. Each treatment had 4 replicates, in which methane and total gas were collected at 2, 4, 8, 12, 18, 24, 36, and 48 h after inoculation. Ruminal fermentation parameters, including pH, NH<sub>3</sub>-N, lactate, individual and total VFA, microbial protein growth, and nutrient degradability were evaluated using a dual-flow continuous culture system (experiment 2). Treatments were essentially the same as previously described and were randomly arranged to 8 fermenters in a replicated 4x4 Latin square design with 4 experimental periods, each consisting of 7 d of diet adaptation and 3 d of sample collection. Each fermenter was fed 106 g of DM per day equally divided between 2 feeding times. On days 8-10, samples were collected for pH, lactate, NH<sub>3</sub>-N, VFA and nutrient degradability measurements. Analyses of results are still ongoing. We speculate that different Co sources may affect methane production as well as ruminal fermentation because of their different Co availability.

## Understanding changes in dairy calf social behavior using social network analysis

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Social housing of dairy calves is becoming more common and provides welfare and performance benefits in early life. However, group housing may pose challenges for identification and management of illness. It is estimated that 20 % of dairy calves will experience bovine respiratory disease (BRD), which has long-term health and productivity consequences, and recent evidence suggests that many cases of subclinical respiratory disease go undiagnosed. Evidence across species suggests a relationship between disease status and the expression of social behaviors, suggesting that changes in social behavior may have value as a novel indicator of cattle health and welfare. The objective of this study was to evaluate the effects of subclinical and clinical respiratory disease on proximity-based social networks in dairy calves. Given evidence of social withdrawal in cattle during parturition and after disbudding, we hypothesized that calves would alter their position within their social network when experiencing disease. Heifer and bull calves ( $n = 120$ ) were enrolled from birth and pair-housed within the first 48 hours of life. At approximately 2 weeks of age, calves entered group housing (12 groups; 10 calves/group) where they remained until after weaning at 8 weeks of age. Calf position within the pen was recorded continuously, using an ultra-wideband positioning system. Clinical health was evaluated twice weekly and subclinical BRD was evaluated weekly using thoracic ultrasounds. Data analysis for this project is ongoing. Using animal position data, proximity-based social networks will be constructed and effects of health status on network metrics will be tested. Results from this study will provide insights into changes in social behavior as a means to identify disease in group-housed animals.

**Effect of supplementing rumen protected arginine (RPA) on performance of transition cows**

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The objectives were to determine the effects of supplementing RPA from 21 d before to 21 d after calving on productive performance and health in dairy cows. One-hundred and two Holstein cows were blocked by parity (nulliparous or parous) and energy-corrected milk (ECM) yield at 250 d of gestation and, within block, randomly assigned to control (CON) or RPA. Control cows were supplemented with 30 g/d of metabolizable protein (MP) from soybean meal, whereas RPA received 30 g/d of MP as arginine, which increased the supply of metabolizable arginine from 5.7 to 7.5% of the MP. Production and health were evaluated until 84 d postpartum. Data were analyzed with mixed-effects models that included the fixed effects of treatment (CON vs. RPA), parity (nulliparous vs. parous cows), week postpartum, and the interactions between treatment and parity, and treatment and week, and the random effects of block and cow nested within treatment. Production data are presented in Table 1. Supplementing RPA improved yields of colostrum and ECM.

Table 1. Performance of dairy cows supplemented with RPA

Item	CON	RPA	SEM	P-value
Prepartum				
DM intake, kg/d	11.9	11.6	0.3	0.35
NE balance, Mcal/d	2.1	2.2	0.7	0.93
First 21 d postpartum				
DM intake, kg/d	16.1	16.3	0.4	0.47
Colostrum, kg	5.3	7.8	1.0	0.02
ECM, kg/d	37.8	40.9	1.2	0.02
BW change, kg/d	-1.9	-2.2	0.2	0.28
NE balance, Mcal/d	-8.8	-9.9	1.0	0.29
22 to 84 d postpartum				
DM intake, kg/d	20.3	20.4	0.4	0.85
ECM, kg/d	40.0	42.6	1.0	0.02
BW change, kg/d	-0.5	-0.6	0.1	0.25
NE balance, Mcal/d	-2.1	-3.2	0.7	0.13

## Search for Non-genomic Markers as Puberty Predictors in *Bos Indicus* Cattle

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Brahman cattle is used for crossbreeding due to their maternal ability, heat, and parasite tolerance. Brahman displays delayed attainment of puberty compared to taurine breeds. Objective was to identify molecular markers in the blood of prepubertal heifers and bulls that predict early puberty attainment. We hypothesize that Brahman that attain puberty have a blood metabolite signature that differs from animals that remain prepubertal. Plasma was collected 90 (-90d) and 30 (-30d) days prior to the beginning of the breeding season from 156 yearling Brahman heifers and 28 bulls. Animals were classified as pubertal (pub) or prepubertal (prepub) based on reproductive variables such as the reproductive tract score and pregnancy to insemination for heifers and scrotal circumference and semen quality for bulls. Plasma from 48 pub and 48 prepub heifers, and 9 pub and 7 prepub bulls were submitted for quantitative mass spectrometry metabolomics analysis. There was a clear clustering of animals according to puberty status (orthogonal projections of latent structures discriminant analysis). Concentrations of the top three metabolites explaining clustering was analyzed by t-tests. As heifers approached maturity, there was a 3.03-, 11.76- and 2.08-fold change ( $p < 0.0001$ ) in the concentrations of PC ae C42:3, ProBetaine and PC ae C42:2 measured at -30 vs. -90d. Similarly for bulls, concentrations of HipAcid, C4 and SM (OH) C24:1 were 1.52-, 1.53- and 1.45-fold greater ( $p < 0.0001$ ), respectively. Comparing pub vs. prepub heifers on -90d, there was a 1.26- ( $p < 0.001$ ), 0.62- ( $p < 0.01$ ) and 1.11-fold change ( $p < 0.05$ ) for hArg, Putrescine and AABA concentrations, respectively. Regarding bulls on -90d, there was a 1.57- ( $p < 0.01$ ), 1.32- ( $p < 0.03$ ) and 1.15-fold change ( $p < 0.03$ ) for FA(18:1), FA(18:2) and DG(16:1\_18:2) concentrations, respectively. In conclusion, we discovered potential markers for prediction of puberty attainment based on plasma metabolomic signatures in Brahman.

## Effect of pH on Fatty Acid Profile of Rumen Bacteria Cell Membrane

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The rumen pH of high-producing dairy cows varies substantially throughout the day because of the high concentration of fermentable carbohydrates. Although it is well established that non-rumen bacteria alter the proportions of saturated and unsaturated fatty acids in the cell membrane to survive environmental changes in pH, limited information is available about rumen bacteria. We hypothesized that rumen bacteria alter the fatty acid profile of the cell membranes under different pH conditions in a similar form. Our objective is to characterize and compare the fatty acid profile of the cell membrane of rumen bacteria under normal and slightly acid pH. Twelve pure cultures of rumen bacteria were anaerobically cultivated under two different pH (6.5 and 5.8) in a completely randomized design. Cultures were terminated after reaching half of the exponential and stationary phases and stored for further analysis. Bacterial growth was measured utilizing a spectrophotometer. The logistic function was used to predict the growth rate and lag phase by nonlinear regression. Then, maximum growth, lag phase, and growth rate were used as input to a least-square analysis of variance. Overall, the pH of 5.8 decreased bacteria growth compared with the pH of 6.5. Our next steps are a) to isolate the cell membrane of bacteria and determine the fatty acid composition using gas chromatography and b) to evaluate the bacteria cell membrane integrity via flow cytometry. At the end of this experiment, we expect to observe a higher proportion of monounsaturated fatty acids in the cell membrane of bacteria under lower pH conditions and no changes in cell membrane integrity between treatments.

## Effects of dairy calf social housing on the development of social networks

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Social housing of dairy heifers has implications for animal welfare and social development early in life, yet there are gaps in our knowledge of longer-term effects on social bonds. The objective of this study was to examine the effects of pre-weaning social housing, with heifers reared individually or in pairs, on social networks following introduction to social groups on pasture. Heifers previously raised in individual pens ( $n = 17$ ) or paired pens ( $n = 20$ ) were mingled between treatments and housed in groups ( $10 \pm 2$  heifers/group; total of 5 groups observed) on pasture following weaning. When heifers were  $60 \pm 5$  days of age, we conducted live observation over a period of 5 observation days (6 h/observation day: 0800 to 1100 h and 1200 to 1500 h) for a total of 30 h observation/group. Using instantaneous scans at 10 min intervals, we recorded activity (feeding, lying, or standing) and social proximity (< 1 body length from another calf, between 1 and 3 body lengths of another heifer, or alone: > 3 body lengths from another heifer) of all heifers, with identity of nearest neighbors also recorded. Data collection for this project is ongoing. Upon completion of data collection, we will analyze social interaction data using social network analysis, generating networks separately for each activity as well as cumulatively, to reflect all interactions. Social network analysis is an analytical technique to characterize interactions within the group, which will generate individual centrality measures reflecting the degree of association between heifers. We will test effects of early life social housing treatments on centrality measures, and will also assess differences in networks generated for each behavior. Findings from this research will provide insight into the long-term effects of early social contact on social preferences and ability.

## **Effect of Psyllium Supplementation on Gastrointestinal Sand Clearance and Fecal Hemicellulose Concentration in Southern White Rhinoceros**

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Strategies for feeding rhino populations in managed settings have largely been influenced by management practices of domestic horses. Ingestion of sand by equids housed in areas with sandy soils can cause serious and potentially life-threatening issues. Feeding psyllium husk is likely one of the most common treatments used within both the domestic horse and zoological communities for mitigating sand accumulation in the large intestine. The objective of this study was to quantify the change in fecal sand output of white rhinoceroses as well as the change in fecal hemicellulose concentration when psyllium was fed as a prophylactic supplement. Three individually housed white rhinoceroses (Moose, Tsavo and Bully; ages 10, 10, and 32; weights 1830, 1910, and 2070 kg; respectively) were fed a psyllium supplement in the diet at approximately 0.266 g/kg BW for 5 days. Voluntarily voided feces were collected over the course of 10 days: prior, during, and after treatment. Feed and fecal samples were analyzed for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and hemicellulose calculated by difference. Acid Insoluble Ash (AIA) from fecal samples was analyzed as a proxy for fecal sand output. Data was analyzed as a repeated measures design with fixed effect of treatment phase and blocked by animal. Fecal AIA concentration did not significantly change ( $P > 0.05$ ) between treatment phases. Hemicellulose concentration within the organic matter fraction of the feces appeared to increase slightly following administration of psyllium and trended toward significance when analyzed by phase ( $P = 0.097$ ). In conclusion, while psyllium may be resisting degradation within the gastrointestinal tract, the current dosing rate does not appear to practically increase gastrointestinal sand clearance.

## **Effects of different slow-release urea compounds on ruminal fermentation and nutrient utilization in a dual-flow continuous culture system**

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Slow-release urea (SRU) is a type of feed additives that could slowly release ammonia-N in rumen, potentially improving N uptake by ruminal microorganisms, reducing N waste, and improve N utilization. The objective of this study was to evaluate the effects of different sources of SRU on ruminal fermentation, nutrient degradation, and N utilization in a dual-flow continuous culture system. Eight fermenters were used in a replicated 4 × 4 Latin square design with four treatments and four experimental periods. Treatments were defined by the source of non-protein N, as follows: 1) Control with regular urea (**CON**); 2) Partial inclusion of SRU compound 1, (**SRU1**); 3) Partial inclusion of SRU compound 2, (**SRU2**); 4) Partial inclusion of SRU compound 3, (**SRU3**). The last three days of each period were used for sample collection. Samples for pH, lactate, volatile fatty acid (VFA) and ammonia-N (NH<sub>3</sub>-N) kinetics were collected from the fermenters at 0, 1, 2, 4, 6, and 8 h after morning feeding. Daily composite samples for lactate, VFA, NH<sub>3</sub>-N, and nutrient degradability were collected at 3, 6, 9, and 24 h after morning feeding. Data were analyzed using the MIXED procedure of SAS. There was no effect of treatment on pH ( $P = 0.28$ ) or NH<sub>3</sub>-N kinetics ( $P = 0.24$ ) for samples collected from the fermenters, nor was there an interaction between treatment and time ( $P > 0.27$ ). There was also no effect ( $P = 0.85$ ) of treatment on NH<sub>3</sub>-N concentration in 24 h composite samples. In conclusion, there was no effect of the inclusion of SRU compounds on NH<sub>3</sub>-N or pH. However, further analyses of lactate, VFA, nutrient degradation, and N utilization are still undergoing.

## Genome-wide CNV and CNVR mapping in U.S. Holstein dairy cattle

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Copy number variations (CNVs) are structural genomic variants that can play a functional role in the genetic architecture of complex traits. Herein, we aimed to map CNVs from thousands of Holsteins genotyped using the BovineHD BeadChip (777,962 SNPs, Illumina). CNVs were identified in 3,529 Holsteins from 16 herds located in 7 U.S. states (CA, FL, MN, NY, OH, TX, WI), which should provide good representativeness of the U.S. Holstein genetic diversity. After SNPs quality control (MAF > 0.05; call rate > 0.90), CNVs were derived from 546,802 autosomal markers using the Log R ratio (LRR) and B Allele Frequency of each SNP in a hidden Markov model implemented in PennCNV software. Since the LRR may be influenced by the surrounding concentration of guanine-cytosine (GC), these values were corrected based on the GC content at 500 kb up and downstream of each SNP. A total of 3,394 non-redundant CNV events ranging in size from 1 kb to 4.22 Mb and averaging 116.76 kb were identified in 2,484 Holsteins with a mean of 8 CNVs per individual and ranging from 1 to 28. Additionally, the number of CNVs per chromosome ranged from 23 (BTA23) to 225 CNVs (BTA12). At the population level, individual CNVs were compiled using CNVRanger package into 820 CNVRs classified as deletions (480), duplications (310), and complexes (30) that covered 3.34% of the bovine autosomal genome. Chromosome coverage by CNVRs ranged from 1.46% (BTA24) to 9.10% (BTA20). Interestingly, 817 CNVRs overlapped 19,410 QTLs, associated with milk (5,477), production (4,657), meat and carcass (4,058), reproduction (2,469), health (1,423), and exterior. (1,326) traits. Furthermore, 611 CNVRs overlapped 1,711 genes, most of them (83.87%) classified as protein-coding genes. These findings will provide opportunities for a better understanding of the genetic architecture of complex traits and for the development of novel selection strategies in Holstein dairy cattle.

**Using Artificial Intelligence Gait Parameter Analysis to objectively predict competition scores while accommodating for subjective performance judging parameters.**

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Traditionally, competition scores are used for decisions impacting training and breeding plans for sport horse breeds. Performance scoring in dressage, a discipline focused on balance and responsiveness of the horse, is impacted by the subjective opinion of the trained judges responsible for evaluating the performance. To examine the variability introduced into these scoring systems by diverse human evaluators, we utilized the software, DeepLabCut (DLC) to track anatomical locations in videos captured of sport horses (n=160) competing at the same venue during two different years (2019 and 2022). We used a custom gait analysis pipeline built in MatLab to process raw X, Y coordinates for each anatomical landmark labeled by DLC. These custom AI-based tools yielded 8 gait parameters such as forelimb extension, hindlimb swing, stride length, and fetlock angle. We examined the relationship between dressage scores and these six chosen parameters relative to the achieved subjective score awarded at these two events by two different judging teams. Between the two events, our analysis revealed that speed and maximum forelimb extension had a significant effect on the model in 2019 (n= 68, p=0.0156) while the same parameters had no significant influence on the predicted dressage scores at the same venue in 2022 (n=92, p=0.4194) judged by a different team of officials. Application of these new AI-based tools for locomotor analysis in horses to identify quantitative measures that more accurately assess performance traits will improve breeding and training decisions resulting in improved horse welfare and economic sustainability of the horse industry.

## Testing Heart Rate Variability for Horses Under a Novel Stimulus

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Due to their history as prey animals, equid species depend on a reactive response when frightened to survive in the wild. However, this same response may present more dangers than benefits for domesticated horses who are frequently within close proximity to human handlers. We obtained a quantitative phenotypic measurement of the startle response through observing changes in equine heart rate and gross motor behaviors. For this study, weanling and two-year-old horses were acclimated to an arena and later introduced to a sudden novel stimulus to elicit a startle response. Comparisons examined the elapsed time between each successive heartbeat from the moment of startle to ten second post-startle. Clustering of variation in the cardiac startle response categorized young horses as accelerators, decelerators, and intermediate response types. Horses clustered into the accelerator group exhibited an increased heart rate, indicating less time between each heartbeat, in response to the novel stimulus. Horses categorized as decelerators expressed a decreased heart rate initially, with longer heartbeat intervals. The intermediate group of horses displayed both a strong rise and fall of their heart rate within the time observed. By quantifying equine startle response through cardiac variables, the extent of individual horses' responses become quantitative phenotypes of this phenotype for future development of genetic tests to predict the susceptibility of each horse to excessive startle responses. Such tests allow breeders to make informed training and marketing decisions based on each horse's temperament predispositions and will enable individualized management strategies to improve animal welfare.

## Effects of Induced Luteolysis on Luteal Size, Luteal Blood Perfusion, and Plasma Progesterone Concentration in Beef Cows

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Luteolysis is the regression of the corpus luteum (CL) caused by episodic pulsatile uterine secretions of prostaglandin (PGF) that occurs between day 16-18 of the estrous cycle in cows. Functional luteolysis is associated with loss of the CL's ability to produce progesterone, while structural luteolysis involves the degradation of luteal cells and a decrease in CL size. We hypothesized that progesterone (P4) concentration, luteal blood perfusion (LBP), and CL area (CL-A) would decrease as we induced luteolysis by administering PGF to cycling cows. The 7-Day CoSynch + CIDR protocol was used to synchronize multiparous cows. Ten days after estrus detection was conducted, two treatments (PGF n=10, Con n=5) were applied. Videos of active CLs were taken transrectally with gray mode and color-Doppler ultrasonography (6.6 MHz, 0.7 of PRF, and 60% gain) twice daily over a period of 4 consecutive days. Also, one blood sample was collected for plasma P4 daily. An image of the CL was captured at maximum size and measured to calculate CL-A using MyLab™Desk/ Desk3. ImageJ software was used to determine LBP. Plasma P4 (PGF=  $5.55 \pm 0.40$  ng/ml; Con=  $5.5 \pm 0.5$  ng/ml), CL-A, (PGF= $2.70 \pm 0.18$  cm<sup>2</sup>; Con=  $2.7 \pm 0.2$  cm<sup>2</sup>), and LBP (PGF=  $17.39 \pm 2.44$  %; Con=  $19.8 \pm 3.4$  %) were similar between groups at 0h. Twenty-four hours after treatments administration a rapid decreased in the Plasma P4 (PGF=  $1.39 \pm 0.40$  ng/ml; Con= $5.6 \pm 0.56$  ng/ml; P <0.01), CL-A (PGF= $1.99 \pm 0.18$  cm<sup>2</sup>; Con=  $2.65 \pm 0.25$  cm<sup>2</sup>; P= <0.01), and LBP (PGF= $17.39 \pm 2.44$  %; Con=  $21.69 \pm 3.45$  %; P= <0.01) was observed in the PGF group compared to control. Plasma P4 (PGF=  $0.58 \pm 0.40$  ng/ml; Con= $7.1 \pm 0.5$  ng/ml), CL-A (PGF=  $1.47 \pm 0.18$  cm<sup>2</sup>; Con=  $2.4 \pm 0.2$  cm<sup>2</sup>), and LBP (PGF=  $3.66 \pm 2.73$  %; Con=  $24.7 \pm 3.4$  %) remained different until the end of the experiment (72h) when comparing PGF and control groups. In conclusion, the results show that a reduction in CL size and blood perfusion is associated with significant reductions in P4 concentration.

## Quantifying Anatomical Lengths in Domestic Cattle with Artificial Intelligence

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Poor health has a substantial impact on cattle production systems, reducing both the value and quality of life of the affected animal. Specifically, lameness impacts both economic and animal welfare indicators, lowering production rates. The need for quick identification of bovine locomotor health, and skeletal growth rates in young calves can enable early detection of problems and targeted management strategies that mitigate losses due to lameness. We used the software package DeepLabCut (DLC) to label key anatomic landmarks on individual adult and juvenile beef cattle filmed at high speed (120fps) while in motion. The landmarks provided a sizable  $x, y$  coordinate output for each landmark, requiring processing in a custom pipeline to yield quantitative morphometric measures including lengths of key skeletal proportions related to growth. Preliminary work investigated the conformational lengths of the back and metatarsus and observed a significant difference in the back-to-metatarsus length ratio between adult and juvenile cattle ( $P = 0.0348$ ). The juveniles depict a smaller ratio value implying their metatarsus length is longer compared to their back length than the adults. Proportionately longer limbs are a common paedomorphic feature, and the rate of loss of this juvenile trait may indicate production-relevant changes in the skeletal growth curve in young cattle. Our ongoing work will utilize DLC to investigate back angles and temporal locomotor parameters often used to identify lameness. Rapid and automated identification of lameness could enable rapid management interventions to reduce the negative impacts on animal health.

## Reducing water use to cool cows using “Smart” technologies

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When trying to achieve optimum production, lactating and dry dairy cows must be provided with relief from heat and humidity. The most practical and common methods to alleviate the negative effects of heat stress on lactating and dry dairy cows are grouped into three main areas: shade, ventilation, and active cooling. Our objective was to determine if an automated “smart” system (Agpro, Paris, TX) for control of soaker output is as effective as the conventional approach to control of soakers that relies on set timing after a threshold temperature is reached. Forty-two cows were dried off ~45 d before expected calving and randomly assigned to one of three treatments: 5 min interval (30s on, 5 min off) cooling during the entire dry period with shade, fan, and soakers (**CL**, n = 14), Agpro smart cooling during the entire dry period with shade, fan and soakers (**SS**, n = 14), and heat stress during the entire dry period, with only shade until calving (**HT**, n=14). Daily dry matter intake (DMI) of individual cows, pen water intake and water usage from soakers were measured daily. Blood samples for hematocrit was taken weekly. Respiration rate (RR) and rectal temperature (RT) were measured thrice weekly. Calf birthweight and gestation length were recorded upon parturition. Data were analyzed using the GLIMMIX procedure in SAS. No differences were observed for gestation length, calf birthweight, dry period length and hematocrit among the treatments. However, SS cows had a greater overall DMI than CL and HT (20.3 vs. 19.1 vs. 18.2 ± 1.2 kg/d; P = 0.05). Water usage per cow was higher in the CL compared to SS and HT groups (469.1 vs. 148.6 vs. 168.1 ± 102.1 L/cow/d). Despite lower water usage in SS, RR (48 vs. 52 vs. 66 ± 2.9 bpm; P < 0.001) and RT (38.3 vs. 38.4 vs. 38.8 ± 0.03 °C; P < 0.001) were affected by treatment with HT having the higher values relative to CL and SS. The SS system decreased the total water usage by cow, effectively cooled the animals, saved water, and maintained animal welfare.

## **Interferon-tau responsiveness and proliferation of luminal endometrial cells are associated with pregnancy outcomes in beef cattle**

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It remains unknown why approximately one third of cows artificially inseminated (AI) or receiving embryo transfer (ET) based on estrus present a negative pregnancy outcome. Our overarching hypothesis is that such outcome is related to a dysfunctional endometrial luminal epithelium. We developed a model to test bovine uterine epithelial cell (BUEC) function from individual cows in vitro, during an ongoing presumptive gestation. Hypothesis was that IFN-t responsiveness and proliferation/migration of BUECs are greater in cows that display estrus and maintain pregnancy by AI (Study-1) and by ET (Study-2). Cows that displayed estrus (Day 0 = day of estrus; D0) after synchronization received AI (n=22) or ET (n=16) and were determined pregnant (preg) or non-pregnant (nonpreg) on D30. On D4, endometrial cytology was conducted and BUECs cultured. At confluence, BUECs were treated with 0 or 10ng/mL of IFN-t for 24h, harvested and analyzed for the gene expression of *ISG15* by qPCR. Proliferation/migration of BUECs was evaluated by wound healing assay and immunohistochemistry for Ki67. On Study-1, preg cows tended to have a greater IFN-t responsiveness (249.5±58.5%) than nonpreg (116.3±47.7%; P=0.09). The proportion of proliferating cells was greater in preg cows (19.4±3.2 vs. 6.6±2.7%; P=0.012). On Study-2, relative expression of *ISG15* on BUECs treated with IFN-t was greater in preg (0.039±0.002) compared to nonpreg cows (0.030±0.003; P=0.05). In conclusion, both IFN-t responsiveness and proliferation/migration of BUECs were greater in cows that remained pregnant after AI or ET. Further research is warranted to confirm these findings and to unravel mechanisms controlling BUEC functions associated with fertility.

## Lactation performance in dairy cows supplemented with microbial additives

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Objectives were to determine the effects of two dietary microbial additives (MA) supplemented to diets of dairy cows on productive performance. Seventy-three multiparous and forty-four primiparous Holstein cows were enrolled at 61 (31 to 87) days postpartum in a randomized block design after a 10-d pre-treatment period. Cows were blocked by parity and energy-corrected milk (ECM) yield and, within block, were assigned randomly to a single diet that was top-dressed daily with either 100 g of corn meal containing no microbial additive (CON), 100 g of corn meal containing  $4 \times 10^7$  CFU of *Clostridium beijerinckii* and  $1 \times 10^9$  CFU *Pichia kudriavzevii* (G1), or 100 g of corn meal containing  $4 \times 10^7$  CFU of *C. beijerinckii*,  $1 \times 10^9$  CFU *P. kudriavzevii*,  $1 \times 10^8$  *Butyrivibrio fibrisolvens*, and  $1 \times 10^8$  of *Ruminococcus bovis* (G2). The treatments lasted 140 d, and dry matter intake (DMI), milk yield, and body weight (BW) were measured daily, whereas milk samples (AM and PM) and body condition were evaluated twice weekly. Data were analyzed with mixed-effects models with orthogonal contrasts evaluating the effects of adding MA (CON vs.  $\frac{1}{2}$  G1 +  $\frac{1}{2}$  G2) or type of microbial additive (TMA; G1 vs. G2). Treatment did not affect DMI, daily changes in BW, or changes in body energy. Yields of lactose and protein tended to increase, whereas yields of milk, ECM, fat, and ECM per kg of DMI all increased with MA. The improvements in performance were observed irrespective of type of additive. Supplementing either MA improved production and feed efficiency in dairy cows.

Item	Treatment <sup>1</sup>			SEM	P-value	
	CON	G1	G2		MA	TMA
DMI, kg/d	22.2	22.4	22.4	0.3	0.50	0.83
Milk, kg/d	39.9	41.3	41.5	0.6	0.04	0.78
ECM, kg/d	37.9	39.3	39.9	0.6	0.009	0.45
ECM/DMI, kg/kg	1.72	1.76	1.80	0.02	0.03	0.19
BW change, kg/d	0.40	0.39	0.39	0.05	0.81	0.98
Fat yield, kg/d	1.312	1.367	1.402	0.029	0.03	0.36
Protein yield, kg/d	1.150	1.187	1.194	0.018	0.06	0.78
Lactose yield, kg/d	1.947	2.008	2.013	0.030	0.07	0.90

<sup>1</sup> G1 and G2 from Native Microbials Inc.

## **Effects of pre-weaning social housing on longer-term heifer performance and reproductive development**

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Social housing for dairy calves can improve early life performance and behavioral outcomes, compared to conventional individual housing. However, there are gaps in our knowledge of longer-term effects of social housing on heifer growth and development into maturity. The objective of this study was to assess effects of social housing on dairy heifer growth, expression of estrus behavior, and age at puberty. Based on evidence of short-term feed intake and growth benefits of social housing, we hypothesized that socially reared heifers will have greater body size and height at puberty. Additionally, based on evidence across species of effects of social experience on reproductive behavior and development, we predicted that socially reared heifers will express more estrus behavior and reach puberty earlier. Heifers were housed either individually (IH;  $n = 50$ ), in pairs (PH;  $n = 50$  pairs), or group (GH; 10 calves/pen;  $n = 50$ ) from birth until 9 weeks of age. In ongoing data collection, we are characterizing a range of outcomes related to growth and reproductive development beginning at 6 months of age. Body weight, hip height, and anogenital distance (AGD) are recorded weekly until breeding. Heifers are placed with an Estroprotect breeding indicator (Estroprotect, Spring Valley, WI), which is examined weekly for evidence of mounting and standing estrus (score 1-4 based on color change indicating rubbing; 1 = no change, 4 = solid color change). Ultrasounds are performed the week following a positive Estroprotect reading (score 3 or 4) to confirm the presence of a corpus luteum. Blood progesterone levels are also assessed to confirm the timing of first estrus. Preliminary results from a subset of data (PH;  $n = 18$ ; IH;  $n = 19$  heifers) suggest that PH heifers tend to be taller (129.3 vs. 128.0 cm; PH vs. IH;  $SE = 0.52$ ;  $P = 0.07$ ), but body weight, AGD, and age of onset to estrus did not differ between previous housing treatment. Results from this study will provide evidence of the longer-term production consequences of early life social housing.

## Plasma cell-free DNA concentration increase during luteolysis in beef cows

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Plasma cell-free DNA (cfDNA) is the result of cell death and can be used as a biomarker and a liquid biopsy for cancer diagnosis. Luteal cells undergo apoptotic process during luteolysis. We hypothesized that cfDNA concentrations increase during luteolysis. Multiparous Angus cows were randomly assigned into two treatments: a single dose of Prostaglandin F2 Alpha analog (PGF, n=10) or saline (Con, n=5). Ten days after ovulation, treatments were administered (0 hour), and the CL was evaluated twice a day using gray-mode and color-doppler ultrasonography. We used ultrasonograms to evaluate CL area (CL-A) and luteal blood perfusion (LBP). Additionally, daily blood samples were collected for plasma Progesterone (P4) and cfDNA concentration until 72 h. Data were analyzed using Proc MIXED of SAS. As expected, CL-A (0h:  $2.7 \pm 0.2 \text{ cm}^2$ ; 72 h:  $2.4 \pm 0.2 \text{ cm}^2$ ), LBP (0h:  $19.8 \pm 3.4 \%$ ; 72 h:  $24.7 \pm 3.4 \%$ ), and P4 (0h:  $5.5 \pm 0.5 \text{ ng/ml}$ ; 72 h:  $7.1 \pm 0.5 \text{ ng/ml}$ ) remained stable in the control group until the end of the experiment. In the PGF group, CL-A (0h:  $2.7 \pm 0.17 \text{ cm}^2$ ; 24h:  $1.9 \pm 0.1 \text{ cm}^2$ ; 72 h:  $1.4 \pm 0.17 \text{ cm}^2$ ), LBP (0h:  $25.9 \pm 2.4 \%$ ; 24h:  $17.3 \pm 2.4\%$ ; 72 h:  $3.6 \pm 2.7\%$ ), and P4 (0h:  $5.5 \pm 0.3 \text{ ng/ml}$ ; 24h:  $1.3 \pm 0.3 \text{ ng/ml}$ ; 72 h:  $0.5 \pm 0.3 \text{ ng/ml}$ ) significantly decreased by 24 h and continue to decreased until the end of the experiment ( $P < 0.01$ ). Plasma cfDNA concentration was significantly higher in the PGF group 24 hours after treatment administration compared to the control group (Con=  $168.3 \pm 83.7 \text{ ng/100}\mu\text{L}$ , PGF=  $297.17 \pm 66.2 \text{ ng/100}\mu\text{L}$ ;  $P < 0.01$ ). By the end of the experiments, cfDNA concentrations increased even more in the PGF group (Con=  $110.0 \pm 83.7 \text{ ng/100}\mu\text{L}$ , PGF=  $305.69 \pm 66.2 \text{ ng/100}\mu\text{L}$ ;  $P < 0.01$ ). In conclusion, cfDNA plasma concentration increased during induced luteolysis, which can lead us to presume that cfDNA could be used as a luteolysis biomarker. Although further research would be necessary to corroborate the origin of this cfDNA.

## **Effect of BlueLite and heat stress on productivity in lactating cows**

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Heat stress generates a negative effect on milk production and feed intake in dairy cattle. Strategies to mitigate the effects of heat stress include osmolyte, electrolyte and energetic supplements in the diet. BlueLite is such a product that has been shown to improve metabolic responses, thus in the present study, we evaluated if BlueLite improves the response in productivity of early lactation cows (Avg: 52 DIM, STD: 28) under heat stress. Fifty-two early lactation cows were enrolled in the four weeks study, yielding four groups of 13 cows each to receive one of the following treatments; Heat stress-No BlueLite, Heat stress-BlueLite, Cooling-No BlueLite and Cooling-BlueLite, following a 2x2 factorial design. Cows were kept in a free-stall barn equipped with individual automatic gates in order to collect individual feed intake. Supplement treatment consisted in 113g of BlueLite a day divided in two feedings (56.5g AM and 56.5g PM) with the ration. The barn had a section with a cooling system including, fans and soakers that was considered the Cooling Treatment whereas the second section only had shade and was considered the Heat Treatment. Productivity parameters as daily milk production, feed intake, body weight, rectal temperature and respiration rate were collected during the entire study. In addition, milk components like SCC, fat, protein and lactose were measured using AfiLab<sup>TM</sup> milk analyzer after each milking. Energy corrected milk yield averaged 42.7 (CL), 41.3 (CL-BL), 36.9 (HT), and 39.5 (HT-BL)  $\pm$  1.6 kg/d, with a significant time X treatment interaction ( $P < 0.02$ ). These results suggest that Blue-Lite partially mitigates the negative impact of heat stress on milk yield.

## Effects of increasing doses of non-protein nitrogen mixtures on in vitro fermentation and methane production

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Supplementation of non-protein nitrogen (NPN) mixtures showed similar in vitro and in vivo responses to the inclusion of 1% urea in a corn silage-based diet. However, there is no information regarding increasing inclusion of NPN mixtures. The current study aimed to evaluate the effects of increasing doses of NPN mixtures on in vitro fermentation and methane production in a corn silage-based diet. Treatments were control (without NPN supplementation) and increasing levels (from one to four-fold) of urea-biuret (UB) and urea-biuret-nitrate (UBN) mixtures. Level one was selected using 1% of urea as a reference. Ruminant fluid was collected from two Angus-crossbred steers fed ad libitum corn silage, gin trash, and mineral mix, plus 100 g/d of a urea-biuret-nitrate mixture for at least 35 d before collection. Incubations were performed in three different days (Replicates). Final pH, concentration of volatile fatty acids (VFA) and ammonia nitrogen (NH<sub>3</sub>-N), in vitro organic matter digestibility (IVOMD), and total gas and methane (CH<sub>4</sub>) production were determined at 24 h. Increasing UB and UBN inclusion linearly increased ( $P < 0.05$ ) pH and concentration of NH<sub>3</sub>-N while linearly reducing ( $P < 0.05$ ) total gas and CH<sub>4</sub> production. The concentration of total VFA showed a quadratic and lineal ( $P < 0.05$ ) response to increasing doses of UB or UBN, respectively. A lineal and quadratic ( $P < 0.05$ ) response was observed in IVOMD with increasing doses of UB and UBN, respectively. Increasing inclusion of NPN mixtures improves fermentation and reduces CH<sub>4</sub> under in vitro conditions using a low crude protein substrate. Future research should be conducted to confirm these results under in vivo conditions.

## Effects of trace mineral and forage sources on mineral solubility, ruminal fermentation, digestibility, and N utilization

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The objective of this study was to evaluate the effects of two different forms of Cu, Zn, and Mn on ruminal fermentation in West and East coast lactating dairy diets. Eight dual-flow continuous culture fermenters were used in a duplicated 4 × 4 Latin square with a 2 × 2 factorial arrangement, combining two factors: (1) diet type [West (W) or East (E) coast] and (2) mineral source [sulfate (S) or hydroxy (H)]. Treatments were (1) WH [West coast diet plus Intellibond<sup>®</sup> Cu, Zn, and Mn]; (2) WS [West coast diet plus sulfate Cu, Zn, and Mn]; (3) EH [East coast diet plus Intellibond<sup>®</sup> Cu, Zn, and Mn]; (4) ES [East coast diet plus sulfate Cu, Zn, and Mn]. The experiment consisted of four 10 d periods and last 3 d of each period were used for sample collection samples for analyses of lactate, volatile fatty acids (VFA), and ammonia nitrogen (NH<sub>3</sub>-N), nutrient digestibility, Cu, Zn, and Mn solubility. Effects of diet type, mineral source, and their interaction were analyzed with the MIXED procedure of SAS. Sulfate mineral sources reduced daily average NH<sub>3</sub>-N concentration when supplemented with the W diet but had the opposite effect with the E diet. When S sources were fed, there was a reduction on pH only with the W diet. Sulfate mineral sources allowed for greater daily average concentrations of soluble Mn and Zn, and greater concentrations of Cu, Mn, and Zn during the first 8 hours after feeding, compared to H sources. Moreover, increase in concentration of soluble Zn resulting from S sources during the first 8 h following feeding, tended to be greater with W diets. Digestibility estimates of DM, CP, NDF, and ADF, were influenced by diet but not by mineral source. Our results indicate that H sources have a lower ruminal solubility than S sources, and that there may be an impact on ruminal fermentation depending upon the type of diet, particularly when it comes to NH<sub>3</sub>-N and pH.

## **Effect of the interaction of tannins and essential oils on ruminal fermentation, gas production and protozoa viability in beef steers.**

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Cow-calf and backgrounding operations produce 83% of methane (CH<sub>4</sub>) emitted by beef production in US. Thus, CH<sub>4</sub> mitigation is a compulsory goal in those systems. It has been suggested that tannins (TAN) and essential oils may reduce CH<sub>4</sub> and protozoa as natural host of Archaea. However, previous studies are inconclusive regarding this effect, and only determine total number of protozoa while their viability is undetermined. Thus, we aimed to evaluate the interaction of tannins and essential oils on ruminal fermentation and protozoa viability. To that aim, ruminal fluid (RF) from 2 cannulated steers (800Lb) receiving corn silage, cotton gin trash, (78, 20, and 2% respectively) and 100g/d of a non-protein-nitrogen (NPN) mix. RF was filtered, diluted, and incubated with the diet (CON) and 0.3% of DM of tannins (TAN), or 0.02% of cashew nutshell extract as essential oil source (CNSE) or the combination (CNSETAN). At 0 and 48-h protozoa were isolated, and their viability was determined using the trypan blue exclusion method. Volatile fatty acids (VFA), CH<sub>4</sub>, ammonia nitrogen (N-NH<sub>3</sub>), ruminal pH and gas production were determined. TAN, CNSE and particularly CNSETAN reduced total protozoa (P<0.01) and their viability (P< 0.01). Only TAN reduced N-NH<sub>3</sub> (P 0.02), tend to increase pH (P 0.07), and tend to reduce gas production (P 0.08). TAN and CNSETAN increased butyric and total VFA (mM) (P<0.05), and tend to increase propionic (P 0.08), and acetic acid (P 0.05). Despite CH<sub>4</sub> is still being determined, previous data from our lab indicate CNSETAN significantly reduces CH<sub>4</sub>. TAN and interaction with CNSE (CNSETAN) influence the ruminal fermentation and reduced gas production, which corresponded with a reduced protozoa viability, suggesting beneficial effects on backgrounding fibrous diets and a potential interaction of TAN, essential oils and NNP in ruminal fluid.

## Impact of supplementing rumen-protected methionine during the periconceptional period in fetal and postnatal growth in beef cattle

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Evidence suggests that changes in maternal nutrition in the periconceptional period have effects during the postnatal growth period in cattle. We hypothesized that feeding rumen-protected methionine (RP-Met) during the periconceptional period will program bovine gestation to enhance fetal and postnatal growth. A total of 114 cows were fed a roughage-based diet and randomized to receive corn gluten supplemented with 15 g of RP-Met (**RP-Met**; Smartamine, Adisseo) or not (**Con**) from d -7 to +7 relative to artificial insemination (AI) with sex-sorted semen. Estrus synchronization was conducted using the 7-Day CoSynch + CIDR protocol. Pregnancy diagnosis, embryo, and fetal morphology were conducted using transrectal ultrasonography 30 and 60 days after AI. After calving, 40 calves (Con = 19; RP-Met = 21) were considered for birth weight (**BW**) analysis. Female calves (n=34) were weighed at 2, 4, and 6 months and weaning. Pregnancy per AI at day 30 (Con = 50%, RP-Met = 55%) and day 60 (Con = 50%; RP-Met = 53.4%) were not different between groups ( $P \geq 0.05$ ). No difference was observed in embryonic and fetal morphology. A treatment by sex interaction was observed ( $P = 0.04$ ) in BW. Male calves (n=6) from cows fed RP-Met showed greater BW than those from the Con group (Con =  $31.9 \pm 2.3$  kg; RP-Met =  $41.4 \pm 2.3$  kg) but no difference was observed in females (n=34). Female calves in the RP-Met had a higher ( $P = 0.04$ ) weight at 2 months of age (Con =  $75.39 \pm 2.07$  kg; RP-Met =  $82.24 \pm 1.98$  kg), and a tendency to be heavier ( $P = 0.08$ ) at 4 months (Con =  $143.9 \pm 2.99$  kg; RP-Met =  $151.63 \pm 2.84$  kg). No difference in weight was observed at 6 months of age and weaning. More data is currently being collected from female calves to observe the effect of RP-Met in puberty attainment and feed efficiency.

## Effects of feeding inoculated corn and ryegrass silage on the performance of lactating Holstein cows

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Including mixed microbial inoculants for the ensiling of corn and other forages is becoming a common practice in the dairy industry. Mixing a homofermentative lactic acid bacteria such as *Lactococcus lactis* and an obligate heterofermentative bacteria such as *Lentilactobacillus buchneri* has shown an improvement in the fermentation parameters as well as in the aerobic stability of the silage during the feed-out phase. The study aimed to evaluate the effects of inoculated corn and ryegrass silage in the total mixed ration (TMR) on the performance of Holstein dairy cows during the transition period (d -21 to d +42). Animals (n=54) were blocked by previous ECM yield (305-d) and randomly assigned to one of the following treatments: 1) CON (TMR containing corn and ryegrass inoculated with water) or 2) INO (TMR containing corn and ryegrass silage inoculated with *Lactococcus lactis* and *Lentilactobacillus buchneri* with 150,000 cfu/g of fresh forage). Cows were fed ad libitum once daily at 0070, and dry matter intake (DMI) was recorded daily. Cows were milked twice daily, and milk and components' yields were recorded. Data were analyzed using the GLIMMIX procedure of SAS. Feeding inoculated silage in the TMR had no effects on milk yield and DMI; however, 3.5% FCM (44.9 vs. 42.0 kg/d,  $P=0.03$ ) and ECM (41.9 vs. 44.5 kg/d,  $P=0.05$ ) yield were greater compared to cows fed TMR with CON silage. Milk fat yield (1.50 vs. 1.62 kg/d,  $P = 0.03$ ) was increased, and milk lactose concentration tended to increase during the first week of lactation (4.53 vs. 4.63%,  $P=0.06$ ). In conclusion, feeding inoculated silage in the TMR increased 3.5% FCM, ECM, and milk fat yields.

## Effects of feeding *Saccharomyces cerevisiae* fermentation product on ruminal fermentation, methane production, and nutrient utilization in beef cattle

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*Saccharomyces cerevisiae* has been shown to modify ruminal fermentation, enhance feed efficiency and performance, and potentially reduce enteric methane emissions. This study aimed to evaluate the effects of feeding a *S. cerevisiae* fermentation product on ruminal fermentation parameters, methane production, and nutrient utilization in beef steers. Six ruminally cannulated Angus-crossbred steers ( $783 \pm 29$  kg of BW) were used in a crossover design, with 42 d per period. Steers were fed a basal diet comprised of 65.9% cracked corn, 14.4% bermudagrass hay, 9.9% distiller's grains plus solubles, 4.9% cottonseed hulls, and 4.9 % of vitamin and mineral supplements on a dry matter (DM) basis. The animals were sorted daily and individually fed 250 g/d of corn gluten feed containing either 0g (CTL) or 28g of *S. cerevisiae* culture (NS). Feeding NS did not affect ( $P > 0.05$ ) in vitro fermentation parameters [i.e., pH, ammonia nitrogen (NH<sub>3</sub>-N), volatile fatty acids (VFA), total gas, and methane production] nor in vitro organic matter digestibility (IVOMD). No effect ( $P > 0.05$ ) of supplementing NS was observed on ruminal pH or concentration of NH<sub>3</sub>-N in vivo. However, acetate to propionate ratio increased ( $P = 0.02$ ) and total VFA, propionate and butyrate concentrations decreased ( $P = 0.04$ ,  $P = 0.03$  and  $0.003$ , respectively) in vivo with the inclusion of NS. Additionally, molar proportions of acetate increased ( $P = 0.02$ ), while butyrate ( $P = 0.03$ ) and propionate ( $P = 0.09$ ) decreased in vivo when NS was included. Apparent total tract digestibility of DM and starch increased ( $P \leq 0.05$ ), and protein tended to increase ( $P = 0.08$ ) as well with NS inclusion. Thus, supplementation of *Saccharomyces cerevisiae* fermentation product at 28 g/d altered rumen fermentation in vivo and improved nutrient utilization.

## **Genome Wide Association Studies for Sweat Gland Properties in a Multibreed Angus-Brahman Population**

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Thermal stress in subtropical regions is a major limiting factor in beef cattle productions with around \$370 million being lost annually due to reduced performance. Cattle utilize sweating to dispense most of their excess heat allowing them to return to their thermoneutral zone. The objective of this study was to conduct a genome-wide association study on different sweat gland properties (area, depth, and length) in the University of Florida's Multibreed Angus-Brahman herd to identify genetic variants related to thermotolerance. Skin samples were collected along the shoulder from 337 heifers of varying Angus and Brahman percentages. Cows were genotyped with the Bovine GGP F250k array. The biopsies were processed into histology slides and then ImageJ software was used to measure sweat gland properties. Quality control was conducted using BLUPF90 software including a call rate of 0.90 and a minor allele frequency of 0.01 which left 125,035 SNPs available for the single-step genome wide association analysis. BLUPF90 software was used to fit a mixed model to test the effects of each marker. GWAS performed on sweat gland properties featured peaks on chromosome 5, 7, and 9 (depth, length, area, respectively). Markers positioned on these chromosomes were significant and located in genes KRT2, NWD1, and ROS1. These variants are associated with proliferation of keratinocytes and endothelial cells, and growth factor receptors. Further analysis of these variants should be completed to investigate its relationship with cattle sweating abilities.

## Quantifying Locomotor Phenotypes in Fragile Foal Syndrome Carriers Using Artificial Intelligence

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The domestic horse has unmatched within-species polymorphism in locomotor pattern and provides an advantageous model to study genetic collagen diseases and their effect on locomotion. Fragile Foal Syndrome (FFS) was chosen for this investigation due to its point missense mutation in the *PLOD1* gene that causes disorganization in collagen fibers. We determined a FFS carrier frequency higher than the reported rate of previous studies. To further understand the effect of the carrier genotype on gait quality, first a critical need for tools that can reliably and accurately quantify various locomotor parameters was addressed. We utilized the software package, DeepLabCut (DLC) to apply anatomical landmarks to video recordings of trotting horses. Geometric parameters calculated within a custom gait analysis pipeline written in MatLab processed the raw output files produced by DLC. Preliminary work investigating sport horses demonstrated the effectiveness of this approach in detecting gait parameters like limb extension and fetlock angle. On-going studies will apply this new phenotyping approach to investigate the joint range of motion in a population of horses carrying the variant for the heritable connective tissue disorder FFS. Improved quantification of locomotor phenotypes, and our understanding of the physiology of this and other known connective tissue disorders in the horse will aid in improving breeding decisions as losing a foal to these diseases causes major economic and emotional hurt to the breeder.

## Transcriptome response of oocytes to seasonal heat stress in beef cows

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Reduced reproductive performance due to heat stress is an increasing issue in cattle. Examining the effects of heat stress on the molecular mechanism of bovine oocytes can help with better understanding their alterations in heat-stressed cattle. We aimed to study the response of oocytes to seasonal thermal stress. Dry beef cows (n=11) were kept together during the study, and Ovum pick-up (OPU) was conducted on all animals in the winter and summer. Cumulus-oocyte-complexes (COCs) were isolated from the OPU fluid. Denuded oocytes were further isolated. Rectal temperature data were also collected on each OPU. Environmental data were collected daily three weeks before each OPU using the Florida Automated Weather Network. Data was analyzed using the Glimmix procedure of SAS and are presented as mean  $\pm$  standard error of the mean. RNA was extracted from five pools of oocytes followed by library preparation and sequencing (NovaSeq; Illumina). As expected, environmental conditions were contrasting: average air temperature (11.5°C vs. 27.5°C), average max air temperature (16.9°C vs. 33.7°C), relative humidity (83.5% vs. 82.3%), and temperature-humidity index (53.39 vs. 79.16) for winter and summer, respectively. Average rectal temperature was higher ( $P = 0.03$ ) in summer ( $39.2 \pm 0.2^\circ\text{C}$ ) than winter ( $38.8 \pm 0.2^\circ\text{C}$ ). Of the total differentially expressed genes, oocytes during the summer showed up and down regulation of 446 and 940 transcripts, respectively compared to the winter season (Fold Change  $\geq 2$ ; FDR p-value  $\leq 0.05$ ). The upregulated transcripts are found to be involved in protein digestion and absorption, and oocyte meiosis pathways. Some of the down-regulated transcripts are involved in cytokine-cytokine receptor interaction and focal adhesion pathways. In conclusion, exposure of cows to thermal stress can influence alteration in the oocyte transcriptome which may have negative impacts on follicular physiology.

## Transcriptome Analysis Reveals the Molecular Mechanisms Underlying the Host Adaptation of Extended-spectrum Beta-lactamase Producing *Escherichia coli*

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Extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Enterobacteriaceae* causes numerous infections in humans and animals worldwide. In the previous study, we found that two genes, *fliD* and *pglA*, were necessary to break the host barrier resulting in the colonization of *E. coli* in multiple hosts. In this study, we investigated the functions of these two genes using an animal model *Caenorhabditis Elegans*. The *C. elegans* infected with *fliD* and *pglA* mutant showed better survival rates compared to wildtype (wt), indicating that these two genes are required for the bacterial colonization. Then, transcriptomic analysis was conducted to identify cellular pathways that were altered with wildtype bacterial infection. A total of 18,913 and 8,297 genes were differentially expressed (DEGs) between wt vs. *fliD* and wt vs. *pglA*, respectively. Of the significantly altered pathways, lysosome activity was the most significantly altered by the infection of both mutants. Through a gene set enrichment analysis, we found 21 up-regulated DEGs with *fliD* mutant infection, belonging to 6 functional orthologs (FOs), including *Aash1*, *BTS*, *CTS2*, *GBA*, *NPC2*, and *SLC11A2* that were involved in lysosome functions. Interestingly, we found 27 down-regulated DEGs with *pglA* mutant infection, belonging to 16 FOs, such as *ATPeV0A*, *ATPeV0B*, *ATPeV0D*, and *ATPeV1H*, that were involved in lysosome functions as well. Our findings suggest that *fliD* and *pglA* genes are necessary for avoiding endocytic pathways, but these genes may be involved in different cellular pathways.

**Juvenile Idiopathic Epilepsy in Arabian Horses is not a Single Gene Disorder**  
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Valued for their temperament, beauty, athletic ability, and exhibition in the show ring, Arabian horses are an important component of the horse industry. Juvenile Idiopathic Epilepsy (JIE), a seizure disorder, specifically impacts Arabian foals from birth to six months of age. Affected foals exhibit tonic-clone seizures lasting as long as five minutes and risking secondary complications like blindness and disorientation. Some foals outgrow this condition, while others die or suffer lifelong complications if not treated. Previous work suggested a strong genetic component to JIE and proposing JIE to be a single-gene trait. In this work we conducted a GWAS in 62 cases of JIE and 118 genetically matched controls, identifying several loci suggesting JIE is not caused by a single locus. Coat color (chestnut, grey) phenotypes were used as positive control traits to assess the efficacy of GWAs in this population. The identification of polymorphisms contributing to JIE could provide a basis for genetic testing for the disease, a tool that may illuminate new treatment options, and help to eliminate production of affected foals.

## Evaluation of a *Bacillus* spp. probiotic on beef cattle performance, nutrient digestibility, and enteric methane emissions

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Direct-fed microbials have been used in livestock production to improve efficiency and reduce the environmental impact. Among others, spore-forming bacteria such as *Bacillus* spp. present handling and processing advantages for use as a probiotic. Several studies utilizing *Bacillus* spp. demonstrated positive effects in dairy cattle production; however, research is scarce regarding its effects in beef cattle. The objective of this study was to evaluate the effects of a probiotic composed of a mixture of *B. subtilis* and *B. licheniformis* on beef cattle performance, nutrient utilization, and enteric methane emissions. For this purpose, a total of 108 Angus crossbreed heifers ( $318 \pm 40.35$  kg of BW) were used in a randomized block design. Animals were fed a sorghum silage-based diet containing the following treatments: 1) control (CTL; no additive) or 2) *Bacillus* spp. probiotic [BOV; 310 mg/kg of dry matter (DM)]. The experiment consisted of 7 days of adaptation followed by 77 days of performance data collection, 5 days of apparent total tract digestibility measurements, and 5 days of enteric methane emissions measurements. No effect of treatment was observed ( $P > 0.05$ ) for DM intake, average daily gain, or gain-to-feed ratio. Similarly, apparent total tract digestibility of DM, organic matter, neutral detergent fiber and crude protein was not affected ( $P > 0.05$ ) by the probiotic inclusion. No differences ( $P > 0.05$ ) were found in enteric methane emissions between the two treatments. In this study, *Bacillus* spp. did not improve cattle performance, nutrient utilization, or mitigate enteric methane emissions in beef heifers when included at 310 mg/kg of dietary dry matter when fed a sorghum silage-based diet.

## **GWAS and Breed Composition Analysis on Epidermis Properties in a Multibreed Angus-Brahman Population**

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Heat stress in cattle has recently received growing attention because of anticipated increases in environmental temperature by global warming. Heat stress limits the production efficiency of cattle, and it is one of the principal causes of economic loss for beef cattle producers in these environments. Thermotolerance can be defined as the ability to maintain optimal growth, feed intake, and reproduction under the presence of heat stress, and it varies among individual animals and breeds. The objectives of the analysis were to investigate the amount of variation on epidermis thickness, test the effect of breed composition and age group on skin histology traits and to conduct a genome-wide association study on skin properties of beef cattle, focusing on the epidermis thickness. Skin biopsy samples were collected from 318 heifers from a UF multibreed population (animals ranging from 100% Brahman to 100% Angus), genotyped with the Bovine GGP F250K chip. Quality control was conducted with BLUPF90 software, including a call rate of 0.90 and a MAF < 0.01. BLUPF90 software was used to fit a single locus mixed model to test the effect of each marker. Breed group and age group were included as fixed effects. There is a significant effect in breed group ( $P < 0.0001$ ) and in age group ( $P < 0.0001$ ). This study shows there is a large amount of variation in epidermis thickness across and within breed groups. Significant SNPs for the thickness of the epidermis were found in the HBEGF gene, which is a protein coding involved in several processes, including epidermal growth factor receptor signaling pathway and there is a variation across breed. Skin histology traits are fundamental for the ability to lose heat more efficiently and allow the maintenance of normal body temperatures under extreme conditions. This study could contribute toward improving cattle's adaptation to thermal stress.

## **Plasma Molecular Biomarkers for Residual Feed Intake Prediction in Beef Bulls**

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Residual Feed Intake (RFI) was proposed as a measure of Feed Efficiency (FE) independent of growth and body weight (BW). RFI is calculated as the difference between actual and expected feed consumption, based on mean metabolic weight and rate of weight gain. Omics technologies are being explored to find Biomarkers for RFI prediction. Our hypothesis was that animals with contrasting RFI have differences in the plasma metabolome. FE was evaluated in 302 bulls from three different producers. After 14 days of adaptation, the bulls were weighed and allocated into pens equipped with GrowSafe® feeders. All animals had ad libitum access to the same diet for 56 days, individual daily feed intake was recorded, BW data were collected every two weeks, and a blood sample was taken on days 0 and 56. Finally, the bulls were ranked according to their RFI, and the top and bottom 30 were used for metabolomics analysis (Biocrates platform–MX Quant Kit®). Data visualization and multivariate statistical analyses were performed with MetaboAnalyst 5.0®. Data were normalized using Log to identify dissimilarities among groups. Principal Component Analysis (PCA) showed moderately separable clustering between treatments, while separate Partial Least Squares – Discriminant Analysis (sPLSDA) showed significant separation. A Variable importance of projection (VIP) plot of the sPLSDA data ranked the top 10 metabolites based on their contribution to the discriminant model. The VIP plot indicates that for low RFI animals the most abundant metabolites on day 0 were (Cer(d18:1/24:1), TG(20:2\_32:1), SM(OH)C22:1, SM(OH)C22:2, and SM C24:0), while on day 56 were (Cer(d18:1/18:0), Cer(d18:1/23:0), TG(14:0\_34:2), c16:2, hEXcER(D18:1/2), c16:1, TG(18:3\_38:5), and LYSOLpCAc16:0). In conclusion, animals with contrasting RFI status have a different plasma metabolome. Further research is needed to understand the biological role of these metabolites and to establish the viability of their use in a prediction model.

## **Identification and Functional Annotation of Signatures of Selection in a Brangus commercial herd**

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Cattle in Florida experience higher levels of stress brought about by humidity and high temperatures. To alleviate this stress, producers in Florida tend to use crossbreeding and create new composite breeds. A popular composite breed used in Florida is Brangus, which is a 5/8 Angus 3/8 Brahman composite. The formation and subsequent selection of these new breeds can lead to Signatures of Selection. Signatures of Selection are genomic regions that have been fixed or increased in frequency in the population because of their functional importance. The objectives of this study were to identify genomic inbreeding and Signatures of Selection in Florida Brangus population and to assign possible functional roles to these regions. We genotyped 2905 replacement heifers and 1042 steers from the Seminole Brangus herd with the GGFP250K. To identify Signatures of Selection, Runs of Homozygosity (ROH) islands were identified using PLINK and genomic inbreeding based on ROH was calculated. ROH islands are regions with reduced genetic diversity and high homozygosity due to selected loci that are under strong positive or negative selective pressures. The genes in these ROH Islands were used in a gene network analysis using CytoScape, to characterize their biological and cellular functions. Literature and Cattle QTL database searches identified traits related to these different significant genomic regions. Genomic inbreeding showed low levels of inbreeding averaging at around 5% inbreeding in the population. However, outliers did exist with inbreeding levels up to 30%. Replacement heifers had statistically significantly higher rates of inbreeding compared to the steers. ROH islands on BTA 1, 3, 6, 13, 14, and 16 were identified as Signatures of Selection, due to the increased frequency of ROH in these regions. The Cattle QTL database search of these regions revealed traits involved in the onset of puberty, growth traits, carcass traits, reproductive traits, and meat quality traits.

## Granulosa cell inflammation reduces progesterone biosynthesis after *in vitro* luteinization.

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Uterine disease in dairy cows is caused by pathogenic bacteria including *Escherichia coli*. Fertility, follicle growth and estradiol secretion are reduced in cows with uterine disease. Treatment of bovine granulosa cells *in vitro* with *E. coli* lipopolysaccharide (LPS) causes a reduction in *CYP19A1* expression and decrease in estradiol synthesis. It is not known if the effects of LPS on granulosa cell steroidogenesis are carried over onto subsequent luteal cells after differentiation. We hypothesized that LPS treatment of granulosa cells will alter the steroidogenic capacity of subsequent luteal cells after *in vitro* luteinization. Bovine granulosa cells spontaneously differentiate into luteal cells *in vitro*; using this model, granulosa cells were isolated from 2-8 mm antral follicles and cultured in basal medium containing 10% fetal calf serum, 1 ng/mL FSH, and 1  $\mu$ M androstenedione for 48 h. Cultured granulosa cells ( $n = 6$ ) were treated with LPS (0.1 to 10  $\mu$ g/mL) or medium alone for 24 h. Subsequently, cells were cultured in treatment-free medium containing 11% fetal calf serum, 10  $\mu$ M forskolin, and 2  $\mu$ g/mL insulin to aid in luteinization. Cells were passaged every 48 hours for 9 days to collect supernatants and cells for analysis. Treatment with LPS increased expression of granulosa cell *IL6* and *CXCL8*, and reduced estradiol synthesis and *CYP19A1* expression after 24 h. Treatment of granulosa cells with LPS reduced subsequent progesterone synthesis of luteal cells. Specifically, treatment with 1 or 10  $\mu$ g/mL of LPS reduced progesterone accumulation on d 3, 5, and 7 post-treatment. Treatment of granulosa cells with LPS did not affect the expression of steroidogenic enzymes *STAR* or *HSD3B1*. Inducing granulosa cell inflammation with LPS altered the steroidogenic capacity of subsequent luteal cells after *in vitro* luteinization. Understanding how uterine infection affects granulosa cells and subsequent luteal cells will help us determine mechanisms by which fertility is reduced in cows after disease resolution.

## **Preliminary Identification of a Quantitative Trait Locus for Body Size Proportion and Head Length in the American Quarter Horse**

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The action of artificial selection during domestication produced diverse morphological traits in the horse. Skeletal development and growth phenotypes impact the desirability and function of horses in diverse disciplines, often key characteristics in the development of breeds. We utilize body measurements taken at two years of age from 91 American Quarter Horses bred and owned by the University of Florida. To investigate variations in body size and head shape within this population, we summarized the 32 body measurements collected from each horse using a principal component analysis followed by a varimax rotation. Body Factor 1 explains 19.5% and Factor 2 explains 16.2% of overall variation across the 32 measures describing body size proportions and head length. The Body Factor 1 trait describes body proportions, heavily loading on the measures of height at the dock and croup. DNA was sampled from blood and hair through previously published methods and genotyped at GeneSeek using the Affymetrix Axiom Equine HD 670k array. We then conducted a genome wide association study with 311,085 high quality SNPs (poly-high resolution, and a MAF > 0.05). This preliminary analysis suggests a quantitative trait locus for the Body Factor 1 trait on chromosome 6 ( $P = 1.356e-09$  and Bonferroni =  $4.51e-07$ ) and Body Factor 2 suggests a quantitative trait locus on chromosome 7 ( $P=4.721e-07$  and Bonferroni= $0.0635$ ) Further investigation will aim to identify functional variants within these QTL's, identify additional relevant loci and develop predictive models for these phenotypes in the horse.

**Thermal inactivation of *Listeria monocytogenes*, *Salmonella enterica*, and Mesophilic Aerobic Bacteria in sous-vide processed beef steaks and regrowth during storage at different temperatures**

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In sous vide cooking, meat is vacuum-packed in plastic bags and cooked under water at lower temperatures for a longer time. However, overloading the cooker or using lower than recommended temperatures may lead to inadequate pathogen lethality. This study aimed to evaluate the effect of sub-optimal cooking temperatures on the inactivation of *Listeria monocytogenes*, *Salmonella enterica*, and Mesophilic Aerobic Bacteria (MAB) and determine the extent of outgrowth during subsequent refrigeration. Beef steaks were inoculated (8 log cfu/g) with *Salmonella* and *Listeria*. Steaks were cooked sous vide style at 60°C, 57.5°C, and 52.5°C in a 1:9 ratio of water and enumerated for pathogens at predetermined times. Following these results, additional steaks were cooked at a time to leave 3 log cfu/g of respective pathogens. After crash chilling, samples were stored at 3°C and 8°C for 2, 4, 6, and 8 d and enumerated. These cooking temperatures take different times for 5 log reduction in *Salmonella* (24 min (60°C), 30 min (57.5°C), 90 min (52.5°C), *Listeria* (40 min (60°C), 60 min (57.5°C), 315 min (52.5°C)) and MAB (40 min (60°C), 75 min (57.5°C), 315 min (52.5°C)). Regardless of cooking temperature, no increase of *Salmonella* was seen in 8 d of storage. In fact, *Salmonella* reduced from d 0 ( $3.8 \pm 0.47$  log cfu/g) to d 4 ( $2.5 \pm 0.47$  log cfu/g) and stayed constant thereafter. Storage temperature did not affect pathogens' growth. However, *Listeria* in samples cooked at 52.5°C increased 0.2 log cfu during 8 d storage compared to higher temperatures, but it is not considered of practical significance with shorter storage time. MAB growth was neither affected by refrigeration temperature nor storage duration. In conclusion, a 5-log reduction of *Salmonella* and *Listeria* was observed within commonly recommended times, albeit with a narrow margin for safety.

**Evaluation of the effects of an *Aspergillus oryzae* prebiotic on mineral metabolism, in backgrounding beef steers fed a Bermudagrass hay-based diet**

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Thirty-six Angus crossbred steers ( $10 \pm 1$  mo,  $238.0 \pm 14.7$  kg of BW) were used to evaluate the effects of the inclusion of an *Aspergillus oryzae* prebiotic (AO) in a Bermudagrass (BG) hay-based diet on mineral metabolism. Basal diet consisted of Bermudagrass hay, and a concentrate supplement offered at 0.7% of BW. Mineral concentration of the basal diet was analyzed prior to the beginning of the experiment. Treatments were 1) No mineral or prebiotic supplementation (Negative Control, NC), 2) Mineral supplementation without prebiotic (Positive Control, PC), and 3) Mineral supplementation plus AO at 2 g/d (AOP). In the PC and AOP groups, mineral supplementation intended to achieve 100% of the requirements. The experiment was conducted as a generalized randomized block design with 3 periods (12 steers/period). Each period consisted of 30 d of diet adaptation, followed by 7 d of adaptation to the crates, and 5 d of collection. During dietary adaptation, steers were collectively housed in a 752 m<sup>2</sup> pen, equipped with 2 C-Lock Smart Feed bunks to measure individual DMI, and were sorted daily and individually fed a concentrate carrying the treatments. During sampling, steers were held in metabolism crates of 1.67 m<sup>2</sup>, equipped with meshed poly-vinyl coated floors, and individual access to feed and water. Total feces and urine were collected 3 times per d, and individual DMI was measured. Blood and liver samples were collected at enrollment and at the end of the experiment to quantify mineral concentration, immunological parameters, and enzymes related with mineral metabolism.

## **Impact of secretory immunoglobulin A on bacterial growth and metabolism**

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Recent research demonstrated that secretory immunoglobulin A (SIgA) modulates the activity of commensal bacteria in the small intestine of non-ruminant animals. Although large amounts of SIgA are present in cattle colostrum and saliva, its effect on rumen bacteria is unknown. Therefore, the purpose of this study was to establish a proof of concept for the modulation of rumen bacteria by SIgA. We first validated a protocol to isolate and purify SIgA from bovine colostrum and then administered SIgA to pure cultures of rumen bacteria. A combination of fiber, starch, protein, and lactate-utilizing bacteria were used in this study. For each experimental run, each strain was revived and transferred twice before starting the experiments. Inoculum volumes were 1% (v/v) of fresh culture. The treatments were: the buffer used to store SIgA (positive control), SIgA solutions (10, 20, 30  $\mu\text{g}/\text{mL}$ ), and an autoclaved SIgA solution (20  $\mu\text{g}/\text{mL}$ ; negative control). Bacterial growth was measured from the increase in optical density of the control tubes using a spectrophotometer. The logistic function was used to predict the growth rate and lag phase by nonlinear regression. Then, maximum growth, lag phase, and growth rate were used as input to a least-square analysis of variance. The addition of SIgA to the pure cultures enhanced bacterial growth for all tested bacteria except for the lactate-using bacteria *Streptococcus bovis* JB1. Our next steps are a) to evaluate the impact of SIgA on the production of short-chain fatty acids and cellulose digestion; and b) to sort SIgA<sup>+</sup> and SIgA<sup>-</sup> bacteria and assess the effect of SIgA on rumen bacteria at the molecular level. At the end of this project, we expect to provide proof-of-concept evidence for the modulation of commensal rumen bacteria by SIgA.