# BEEF & FORAGE CENTER INSTITUTE OF AGRICULTURE THE UNIVERSITY OF TENNESSEE

# **University of Tennessee Institute of Agriculture**

**Beef and Forage Center Annual Research Report** 

> Volume 1, 2020 doi: 10.6084/m9.figshare.13341395

Photo Credit: Dr. Phillip Myer

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### Welcome

#### 2020 UT Beef and Forage Center Annual Research Report

Welcome to the first volume of the UT Beef and Forage Center Annual Research Report. The mission of the Center is to facilitate research and communication of science-based information to advance the Tennessee beef and forage industry. The Center functions as an "information hub" serving all in the Tennessee beef and forage industries. The Center also serves as a focal point and catalyst for research, Extension, and teaching efforts related to issues facing beef and forage systems in Tennessee. The report aims to build on this vision by providing an opportunity to highlight the current work related to the Center to producers and stakeholders across the state. Although abbreviated this year, future volumes of the report will provide comprehensive material to convey new knowledge and technology to improve the management, efficiency and production of high-quality beef cattle.

The Beef and Forage Center would like to thank Dr. Kyle McLean for his great effort in leading, editing, and formatting the first iteration of this report so that we can produce a great statewide report. Also, thanks to the contributors to the report and to the staff and students who help with the research, teaching and Extension activities on beef cattle and forages. Finally, thanks to the funders of the grants that help fund the research projects and students/staff working on the projects. We truly appreciate your contributions to our research programs because without this support, the research would not be possible.

Should have any questions about the work reported in this report, please do not hesitate to contact the UT Beef and Forage Center or any of the authors of the individual reports. Thank you for your encouragement and support of beef and forage research in Tennessee.

Sincerely,

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### Effect of Castration on Hematological and Immunological Parameters in Newly Received Stocker Calves in a Commercial Stocker Operation

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**Take Home Message:** We studied what the effects of castration status at arrival to stocker facility were on risk for BRD. Several Complete Blood Count parameters differed between groups, but no association was observed for developing BRD. Findings from this study might help in making decisions on BRD treatment in commercial stocker operation.

Summary: This study determined the effects of castration status on Complete Blood Count (CBC) and cytokines levels, and the association of castration with Bovine Respiratory Disease (**BRD**) in stocker calves at arrival to a commercial stocker operation. Forty newly weaned stocker calves were received at a commercial stocker farm in February 2020. Calves were monitored for 4 weeks and treated based on the clinical signs of BRD. Blood samples were collected on days 0, 7, 14, and 21 for CBC and cytokines analyses using a hematology analyzer and commercial ELISA kits, respectively. Calves were either freshly (FC; n=27) or previously castrated (PC; n=13). Overall, 40% (n = 16) calves developed BRD symptoms in the 4 weeks of the study period including FC (41%; n = 11) and PC (38%; n = 5) calves. Results showed significantly (P < 0.05) lower levels of blood cells and hematocrit in FC calves, as compared to PC calves, indicating potential blood loss and dehydration in FC calves. We observed no significant association (P < 0.05) between castration status and the rate of BRD development.

#### Introduction

The Southeastern US is suitable for raising cattle on forages because of abundant native grasses, temperate climate, and adequate annual rainfall, making cow-calf production common in these states. Beef cattle operations in the southeastern US maintain small herd sizes (<50; 84%), and only 45% of the beef operations follow a defined breeding season (82 to 92% in northcentral and northern Plains in the US).<sup>2</sup> The combination of small herd sizes and undefined breeding season result in many small groups of recently weaned calves entering sale barns where they are commingled and sold. Stocker operators, feedlot operators, or order buyers purchase calves at these auction markets to create a larger group to be backgrounded prior to feedlot entry. These calves are subjected to many stressors including weaning. commingling, castration, transportation, and social restructuring; all of which leads to

disease susceptibility. Bovine Respiratory Disease is a multifactorial disease characterized by depression, fever, nasal discharge, breathing difficulty, and social isolation. Previous studies demonstrated that castration of bulls at arrival significantly increased the risk of BRD.<sup>5,7</sup> Although stocker and feedlot operations could dramatically reduce the risk for BRD by purchasing singlesourced, previously castrated and fully weaned calves, there is still an influx of "highrisk" calves into those facilities.<sup>8</sup> Therefore, the study objective was to determine the effect of castration status on Complete Blood Count and cytokines levels, and to test if there were increased odds for BRD due to castration status at arrival.

#### **Materials and Methods**

Forty newly received mixed-breed steers were observed at a commercial stocker farm in Crossville, TN. Calves were received from an order buyer in February 2020, with average body weight of approximately 500 lbs. At the order buyer, they received vaccinations, broad-spectrum anthelmintic (dewormer), prophylactic antibiotics, castration (if intact), and dehorning (if horned). When calves were transferred to the farm, they were managed according to the farm's standard procedures.

On days 0, 7, 14, and 21 researchers collected blood and rectal temperature. Clinical severity score (CSS; 0: normal to 4: moribund) for each calf was collected according to Pillen et al.<sup>4</sup> Calves were defined as BRD infected if they had a CSS score of 1 to 2 and fever (rectal temperature  $\geq 104^{\circ}$ F), or if they had a CSS score  $\geq 3$ , regardless of rectal temperature. White blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), Hematocrit (HCT), mean corpuscle volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT) was measured by using a hematology analyzer. Serum haptoglobin (HP), IL-8, IL-1 $\beta$ , and TNF $\alpha$  concentrations were determined using commercial ELISA kits.

All calves were either freshly castrated (FC; n=27) at the order buyer or previously castrated (PC; n=13). Calves were categorized as FC (if they had a fresh castration site at arrival) or calves missing testicles (i.e. castrated after birth), or calves with completely healed castration sites were categorized as PC. To test if the FC group differed from PC with CBC value and cytokines value changes, mixed model analysis of variance was performed (Proc GLIMMIX; SAS 9.4). Least square means  $\pm$ standard errors are reported. To test the association of castration status with BRD development, a Chi-square test (Proc FREQ) was used.

#### **Results and Discussion**

Calves were asymptomatic on arrival. During the 4-week study period, 40% (n =16) of calves developed BRD symptoms and were treated. Among the treated calves, 9 were treated once, 4 were treated twice, and 3 were treated three times.

No significant association was found between castration status and likelihood to ever develop BRD (P = 0.89). There was a similar cumulative incidence of BRD in both groups; the FC calves (n = 27) had only a slightly greater incidence of BRD cases (41%; n = 11), as compared to PC calves (38%; n =

5). We observed no significant differences (P = 0.98) in the rate of developing new BRD cases between FC (1.76 new cases per 100 animal-days at risk; 95% CI: 0.96 - 3.25 new cases per 100 animal-days at risk) and PC (1.74 new cases per 100 animal-days at risk; 95% CI: 0.70 - 4.31 new cases per 100 animal-days at risk) calves. Ratcliff et al.<sup>6</sup> also found no significant effects (P < 0.22) of castration method or timing on the number of calves being treated for BRD. On the contrary, Richeson et al.<sup>7</sup> observed a significant (P <0.001) increased risk for developing BRD

once or twice in bull calves compared to steer calves. Intact bulls at arrival had 8.63 times the odds to be treated for BRD one time compared to steers (OR: 8.63; 95% CI: 6.31 to 11.7), and bulls had 4.02 times the odds for developing BRD twice compared to steers (OR: 4.02; 95% CI: 2.98 to 5.41). According to Taylor et al.<sup>9</sup>, failure to castrate calves at a younger age could be confounded with other indicators of poor management (i.e. lack of vaccination protocols, late weaning, etc.) which may create additional risk to health.

**Table 1.** Differences in CBC parameters and cytokines in  $FC^1$  and  $PC^2$  calves at arrival via mixed model analysis of variance. CBC parameters and cytokines levels are presented as LSmean  $\pm$  SE.

CBC Parameters and cytokines	FC (LSmean ± SE) <sup>1</sup>	PC (LSmean ± SE) <sup>1</sup>	P value
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	$10.82\pm0.74^{\rm b}$	$14.35\pm1.07^{\mathrm{a}}$	$< 0.01^{*}$
RBC (106/mm <sup>3</sup> )	$7.06\pm0.34^{b}$	$8.68\pm0.49^{a}$	$0.01^{*}$
HGB (g/dL)	$9.65\pm0.33^{\mathrm{a}}$	$10.65 \pm 0.46^{a}$	0.09
HCT (%)	$26.67 \pm 1.22^{b}$	$31.36\pm1.76^a$	$0.03^{*}$
MCV (μm <sup>3</sup> )	$37.82\pm0.52^{a}$	$36.31\pm0.75^a$	0.11
MCH (pg)	$13.09\pm0.17^{a}$	$12.41 \pm 0.24^{b}$	$0.02^{*}$
MCHC (g/dL)	$34.64\pm0.33^a$	$34.03\pm0.47^a$	0.29
PLT (10 <sup>3</sup> /mm <sup>3</sup> )	$405.44 \pm 33.97^{a}$	$395.08\pm48.96^a$	0.86
Haptoglobin	$13.17\pm0.38^{a}$	$13.04\pm0.55^a$	0.85
IL1β	$2.74\pm0.1106^a$	$2.68\pm0.1594^a$	0.76
IL8	$4.56\pm0.2185^{\mathrm{a}}$	$4.87\pm0.2968^{a}$	0.38
$TNF\alpha^3$	$5.28 \pm 1.62^{\rm a}$	$1.06\pm2.33^{a}$	0.36

<sup>1</sup>FC: Freshly castrated

<sup>2</sup>PC: Previously castrated

<sup>3</sup> For TNFα, untransformed LSmean and SE were reported. \*Significant at  $\alpha < 0.05$ ; <sup>ab</sup>LSmean with different alphabet differed at *P* < 0.05

We observed significant differences in WBC, RBC, HCT, and MCH levels at arrival to the stocker facility between FC and PC calves (Table 1). At day 0 of our study, FC calves had a lower WBC, RBC, and HCT compared to PC calves. These results were also corroborated by Ratcliff et al.<sup>6</sup> In their study on the effect of castration method and timing on newly arrived stocker cattle, RBC, HGB, and HCT were reduced on day 0 and 7 post-castration for bull calves, as compared to steer calves. They suggested that unlike steers

that are previously castrated, bulls are subjected to blood loss and stress after they arrive at the research facility. This suggests that castrating calves at an early age, well before they are transferred to the commercial facility is a better way to manage calves for optimal health.

Circulating cytokines may be used as a way of predicting infection. However, we observed no significant differences in cytokine levels between FC and PC calves. Acute-phase proteins have been found to increase  $\geq 100$ -fold at the time of infection.<sup>1</sup> However, some studies indicate that HP has limited prospect as a biomarker, although acute or chronic infection may be distinguished.<sup>3</sup> Results of this study provide useful insight on hematological and immunological profile in relation to castration status in recently purchased stocker calves. Utilizing hematological parameters in addition to other risk factors might be helpful for prediction of BRD in commercial stocker management.

**Acknowledgements:** This work was supported by the multi-state project NC1192 "Integrated approach to control of bovine respiratory diseases" from the USDA National Institute of Food and Agriculture. Authors would like to acknowledge funding for this work by the UT Center of Excellence in Livestock Diseases and Human Health.

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### Tall Fescue Seed and Isoflavone Consumption Impacts Metabolism in the Rumen and Serum of Beef Steers

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**Take Home Message:** Fescue toxicosis causes a variety of symptoms that reduce overall cattle performance, but may be mitigated with isoflavone consumption. The objective of the current study was to evaluate the influence of isoflavone consumption with tall fescue seed on ruminal and circulating metabolites and determine potential impact on animal growth performance.

Summary: We hypothesized that consumption of tall fescue seed and isoflavone supplementation would affect metabolism of steers. Angus steers were fed endophyteinfected (E+) or endophyte-free (E-) tall fescue seed, and supplemented with (P+) or without (P-) isoflavones. After receiving the treatments daily for 21 days, rumen fluid and blood samples were collected from all steers for determination of metabolites. The overall metabolite composition in the rumen greatly contrasted the metabolite composition of the serum. Abundances of metabolites differed in the rumen between E+ and E- steers, while metabolites in the serum differed between P+ and P- steers. Metabolic pathways involved in amino acid and nucleic acid break down and synthesis differed between treatments. Although rumen metabolic activity was affected by consumption of endophyteinfected tall fescue seed, consumption of isoflavones increased circulating metabolites and metabolic pathway activity related to growth and development to potentially mitigate fescue toxicosis effects.

#### Introduction

Tall fescue is the main component of grazing lands in the Southeast and commonly used by beef cattle producers due to its hardiness in the environment and annual growth pattern. The perseverance of tall fescue is largely attributed to the presence of a fungus living in a mutual relationship with the grass. However, consumption of infected fescue results in reduced feed intake and weight gain, constriction of blood vessels, and elevated body temperature in cattle: collectively, this condition is known as fescue toxicosis.<sup>1,2</sup> Current research is evaluating methods for reducing the impact of fescue

toxicosis on animal production efficiency. Isoflavones, compounds extracted from red clover, have recently shown to reduce the consequences of fescue toxicosis when included in the diet. Isoflavones can increase blood flow by dilating blood vessels and altering the microbes in the rumen.<sup>3,4</sup> By these effects, the metabolism of the rumen microbes and animal host may be impacted and contribute to improvements in animal performance.

#### **Materials and Methods**

The following experimental design is similar to those described in Melchior et al.<sup>5</sup> Purebred Angus steers (n = 36, ~ 8 months of age,  $551 \pm 44$  lbs) were acclimated to a basal diet providing 11.57% crude protein and 76.93% total digestible nutrients. Genotyping of the DRD2 gene was conducted on all steers to determine susceptibility to fescue toxicity, and used as a block for statistical analysis. Within each genotype block, steers were randomly assigned in a 2×2 factorial arrangement of treatments resulting in four treatment groups: endophyte-free tall fescue seed without isoflavones (E-P-), endophytefree tall fescue seed with isoflavones (E-P+), endophyte-infected tall fescue seed without isoflavones (E+P-), and endophyte-infected tall fescue with isoflavones (E+P+). Over the 21 day feed trial, steers in the endophyteinfected seed treatment groups were targeted to receive 0.011 mg ergovaline plus ergovalinine  $\times$  kg of body weight<sup>-1</sup> (BW) per day to maintain consistent dosage of ergot alkaloids. Steers in the isoflavone supplemented groups were provided Promensil daily, via bolus, based on Promensil amounts used and reported by Aiken et al.<sup>3</sup> On day 21, blood samples were collected from the tail vein and rumen fluid was collected via esophageal tubing. Blood was centrifuged to separate and collect the serum. Rumen samples were centrifuged and the supernatant was filtered and collected. Serum and filtered rumen fluid were stored at -112°F until metabolite extraction. Metabolites in the serum and rumen fluid extracted and analyzed similar to previously described methods of Clemmons et al.6 Extraction and analysis by UPLC-MS was performed at the UTK Biological and Small Molecule Mass Spectrometry Core. The Metabolomic Analysis and Visualization Engine program was used to identify metabolite peaks for annotation. Resulting known metabolites were then analyzed in MetaboAnalyst 4.0 and SAS 9.4 to compare overall metabolomes, individual metabolites, and metabolic pathway differences between the rumen and serum, then by treatment groups within each sample type.

#### **Results and Discussion**

The rumen metabolome, the collection of metabolites in the rumen, greatly contrasted the serum metabolomes with little similarity (Figure 1), as highly abundant metabolites in one sample type were lowly abundant or absent in the other sample type. In the rumen, the majority of metabolites detected are reflective of microbial activity and originate their fermentation processes from as feedstuffs are digested.<sup>7</sup> In the serum, metabolites largely originate from the metabolism of other tissues across organ systems for a variety of physiological functions. The circulatory system provides a route for metabolites to travel to other tissues for further use and processing. Therefore, the rumen metabolites



Figure 1. Separation in the overall metabolomes of the rumen fluid and serum was detected by orthogonal partial least squares discriminant analysis, indicating highly different metabolomes in each environment.

may indicate the impact on microbial metabolism, while serum metabolites indicate host metabolic response to fescue toxicosis and isoflavone treatment, and potential downstream affects from the impact on rumen microbial metabolism.

The rumen metabolome was most affected by the consumption of endophyteinfected tall fescue seed. Multiple metabolites such as hypoxanthine, xylose, and uracil were determined to be important by the variable importance in projection (VIP) analysis for the differences observed between seed type treatment groups. Each of these metabolites were significantly lower in steers consuming endophyte-infected seed (Table 1). Previously, these metabolites were higher in abundance in more feed efficient animals.<sup>8</sup> Lower concentrations of these metabolites in steers likely indicates low feed efficiency during fescue toxicosis. Multiple metabolic pathways related to amino acid, carbohydrate, and nucleic acid metabolism, which are crucial for growth and development, were also affected by the seed type consumed. Therefore, the observed reductions in weight gain during fescue toxicosis is potentially attributed to alterations in the rumen metabolome, changing the metabolite and nutrient output, resulting in compromised feed efficiency.

The serum metabolome was highly affected by isoflavone supplementation. Seven metabolites differed in abundance in the serum due to isoflavone treatments, while no metabolites differed in the serum due to seed type consumed (Table 1). However, citrulline had the largest impact on the serum metabolome due to isoflavone treatments according to VIP analysis. Isoflavones reduce protein-degrading bacteria in the rumen which allows for more protein to be used by the host, and less to be degraded into non-protein nitrogen entering the urea cycle.<sup>9</sup> As citrulline is a key component of the urea cycle, the impact of isoflavones in the rumen is detected systemically by effects on downstream metabolites of the urea cycle.<sup>10</sup> These effects were also observed by the numerous amino acid metabolism and biosynthesis pathways impacted in the serum. Therefore, the increased protein available to the ruminant and altered amino acid metabolic processes caused by isoflavone supplementation may provide improved muscle growth and development to mitigate the effects of fescue toxicosis.

In conclusion, consumption of tall fescue seed type affected multiple metabolites and pathways important to ruminant growth

and development that are potentially improved by consumption of isoflavones. Further research is needed for determining the isoflavone dose needed for application to provide benefits in cattle production systems. **Acknowledgements:** Thank you to the

University of Tennessee CVM, Center for Excellence in Livestock and Human Diseases for funding of the project. We acknowledge the USDA-NIFA Hatch/Multistate Project W4177-TEN00524-Enhancing the Competitiveness and Value of U.S. Beef. We thank Ames Plantation, UT Plateau Research and Education Center, Dr. Rebecca Payton, Lezek Wojakiewicz, and Gloria Gellin (USDA-ARS) for their support and assistance during the project.

Metabolites in the Rumen Impacted by Seed Type						
Metabolite	See	ed Type	<b>N 1</b> 1			
	E+	<b>E-</b>	— P Value			
Dihydroxybenzoate	$5.90 \times 10^7 \pm 5.35 \times 10^6$	$8.43{\times}10^7 \pm 6.26{\times}10^6$	0.05			
Adenine	$2.30{\times}10^7 \pm 1.13{\times}10^7$	$6.84{\times}10^7 \pm 1.32{\times}10^7$	0.02			
CMP	$9.17{\times}10^5\pm7.64{\times}10^5$	$3.17{\times}10^6 \pm 8.95{\times}10^5$	0.04			
Deoxyuridine	$8.04{\times}10^5 \pm 2.71{\times}10^5$	$1.74{\times}10^{6}\pm3.18{\times}10^{5}$	0.02			
Glutamate	$7.18{\times}10^7 \pm 2.17{\times}10^7$	$1.57{\times}10^8 \pm 2.54{\times}10^7$	0.05			
Guanosine	$3.00{\times}10^5 \pm 1.44{\times}10^5$	$8.63{\times}10^5 \pm 1.69{\times}10^5$	0.05			
Homoserine/threonine	$1.02{\times}10^7 \pm 8.90{\times}10^5$	$6.65{\times}10^6 \pm 7.60{\times}10^5$	0.05			
Hypoxanthine	$4.40{\times}10^7 \pm 1.66{\times}10^7$	$1.17{\times}10^8 \pm 1.94{\times}10^7$	0.01			
Uracil	$5.76{\times}10^7 \pm 1.19{\times}10^7$	$1.08{\times}10^8 \pm 1.39{\times}10^7$	0.02			
Xanthine	$1.79{\times}10^8 \pm 4.34{\times}10^7$	$3.48{\times}10^8 \pm 5.09{\times}10^7$	0.01			
Xylose	$3.63{\times}10^6 \pm 1.05{\times}10^6$	$8.69{\times}10^6 \pm 1.23{\times}10^6$	0.01			
Meta	bolites in the Serum Imp	acted by Isoflavones				
Metabolite	Isoflavor	ne Treatment	<b>N 1</b> 1			
	P+	Р-	— P Value			
Histidine	$8.50{\times}10^6 \pm 8.94{\times}10^5$	$1.05 \times 10^7 \pm 9.37 \times 10^5$	0.05			
Cytidine	$1.51{\times}10^6 \pm 4.89{\times}10^5$	$2.67{\times}10^7{}\pm5.12{\times}10^5{}$	0.01			
Pantothenate	$6.64{\times}10^6 \pm 1.84{\times}10^6$	$1.51{\times}10^7 \pm 1.93{\times}10^6$	0.01			
Homocysteine	$1.47{\times}10^6 \pm 1.28{\times}10^5$	$2.02{\times}10^{6}\pm1.35{\times}10^{5}$	0.02			
Allantoin	$1.94{\times}10^8 \pm 1.14{\times}10^7$	$2.37{\times}10^8 \pm 1.19{\times}10^7$	0.03			
GABA	$9.68{\times}10^5 \pm 1.40{\times}10^5$	$1.41{\times}10^{6}\pm1.44{\times}10^{5}$	0.05			
Methylhistidine	$8.35{\times}10^5\pm6.25{\times}10^4$	$1.04{\times}10^6\pm6.51{\times}10^4$	0.05			

**Table 1.** Metabolite abundances differed in the rumen due to seed type andin the serum due to isoflavone treatment. Abundances are present as Mean $\pm$  SEM.

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### Protozoal Communities in the Rumen of Angus Steers Differing in Feed Efficiency

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**Take Home Message:** Protozoa are important members of the rumen microbial community as they contribute to nitrogen and microbial protein cycling. The objective of the study was to evaluate the protozoal communities in the rumen of Angus steers and their association with feed efficiency. The number and phylogenetic diversity of protozoa were associated with differences in feed efficiency indicating protozoa as a potential microbial factor in the efficiency of nutrient utilization in cattle.

Summary: The rumen microbiome contributes significantly to the feed utilization of the host animal. Most studies focus on the bacterial communities due to their abundance and genetic and functional diversity they provide to the host. However, protozoal communities account for approximately 50% of the rumen microbial biomass and participate in determining the microbial microbial composition, crude protein turnover, and nitrogen cycling. It is unclear whether these protozoal effects on nutrient use impact feed efficiency. Therefore, in this study, 50 weaned steers seven months of age weighing approximately 573 lbs underwent a 70-day feed efficiency trial using the GrowSafe system. Steers were classified

as high- or low- RFI based on  $\pm$  0.5 standard deviations about the mean RFI. On day 70 of the trial, approximately 1.7 fluid ounces of rumen content were collected from high- and low- RFI steers. Microbial DNA from the rumen content was extracted, sequenced, and used to identify protozoal populations. Differences were observed between low- and high- RFI steers in protozoal community phylogenetic diversity and species abundance. High RFI steers had significantly greater abundances of unassigned protozoa.

#### Introduction

The rumen microbiome (i.e. the combined genetic material of all microorganisms in the rumen) is paramount to effective feed digestion and utilization by the host animal, ultimately affecting its

nutritional status. In cattle, the rumen microbiota estimated are to produce approximately 70% of the total energy substrates absorbed and used by the animal. Much of the research conducted to understand the relationship between the ruminant host and the rumen microbiome has focused on bacterial communities. The bacteria are arguably the most diverse microbial kingdom in the rumen and are the most abundant microbes. Bacteria contribute greatly to the fermentative capacity and metabolic potential of the rumen. Their activities support many critical functions in ruminant nutrition and health, such as protein and fiber breakdown and vitamin production.1 Nevertheless, it cannot be overlooked that rumen protozoa represent equal volume of microbial biomass.<sup>2</sup> Researchers using photomicrograph and electron microscopic techniques have demonstrated protozoa that actively participate in the rumen ecosystem and exhibit behaviors different than that of bacteria, such as starch hoarding.<sup>2</sup> Protozoa participate in commensal and mutualistic relationships with other microbes and can also act as predatory species.<sup>3</sup> Despite these interactions, much is still to be elucidated as to their role in ruminal fermentation, host metabolism, and nutrition.

Ciliate protozoa in the rumen environment have cellulolytic and amylolytic capabilities<sup>4,5</sup> and intensively interact with the entirety of the microbiome. Given the functional importance of ciliate protozoa in the rumen environment, it is critical to not only categorize the diversity and phylogeny of rumen ciliate protozoa, but also determine their association with nutrition and feed efficiency in beef cattle. Our objective was to investigate the ciliate protozoal community in the rumen from steers differing in feed efficiency using deep 18S ribosomal RNA gene (18S rDNA)-based community profiling. We hypothesized that protozoal populations within the rumen would differ between animals of different feed efficiency.

#### **Materials and Methods**

The study was conducted at the University of Tennessee Institute of Agriculture Plateau Research and Education Center in Crossville, TN. Fifty purebred Angus steers 7 months of age, initially weighing  $582 \pm 5.9$  lbs, were used for the study. Two weeks post-weaning, the steers were acclimated to the GrowSafe feeding system (GrowSafe Systems Ltd., Airdrie, Canada) for 10 d prior to data collection onset. During the acclimation, steers were fed a stepup diet before receiving the trial ration (11.57% crude protein and 76.93% total digestible nutrients on a dry matter basis) with 28 mg monensin/kg DM. Following the acclimation period, feed intake was continuously measured using the GrowSafe© system and body weight was measured at 7 d intervals for 70 d. At the end of the feeding trial, the average value of RFI and standard deviation (SD) were calculated and used to rank steers based on feed efficiency. High RFI was defined as  $RFI \ge 0.5$  SD above the group mean and Low RFI was defined as RFI  $\leq -0.5$ SD below the group mean, resulting in 10 Low RFI and 14 High RFI animals. At d 70, approximately 1.7 fluid ounces of rumen content were collected via esophageal tubing.

Microbial DNA in the rumen content was extracted and the DNA was sequenced targeting the variable regions V3 and V4 and rumen ciliate signature regions 1 and 2 of the 18S rRNA gene. DNA was sequenced using the v3  $2\times300$  kit and the Illumina MiSeq sequencing platform (Illumina, Inc., San Diego, CA). The protozoal sequences were processed using the Quantitative Insights Into Microbial Ecology (QIIME) bioinformatics pipeline, version 1.9.1. Protozoal taxonomy was assigned using the Silva reference database, 128 release.

High-RFI <sup>a</sup>	Low-RFI <sup>a</sup>	<i>P</i> -value <sup>b</sup>	<b>FDR</b> <sup>b,c</sup>
0.99 (0.00)	0.99 (0.00)	0.03	0.07
297.93 (1.79)	306.60 (2.71)	0.01	0.03
142.11 (0.44)	144.42 (0.74)	< 0.001	0.03
310.11 (2.64)	315.01 (1.08)	0.15	0.22
1.86 (0.12)	1.77 (0.21)	0.69	0.69
0.0074 (0.00)	0.0079 (0.00)	0.67	0.69
	High-RFI <sup>a</sup> 0.99 (0.00) 297.93 (1.79) 142.11 (0.44) 310.11 (2.64) 1.86 (0.12) 0.0074 (0.00)	High-RFIaLow-RFIa0.99 (0.00)0.99 (0.00)297.93 (1.79)306.60 (2.71)142.11 (0.44)144.42 (0.74)310.11 (2.64)315.01 (1.08)1.86 (0.12)1.77 (0.21)0.0074 (0.00)0.0079 (0.00)	High-RFIaLow-RFIaP-valueb0.99 (0.00)0.99 (0.00)0.03297.93 (1.79)306.60 (2.71)0.01142.11 (0.44)144.42 (0.74)<0.001

<sup>a</sup>Mean (SEM)

<sup>b</sup>Significance determined at  $P \le 0.05$ <sup>c</sup>False discovery rate corrected P value

#### **Results and Discussion**

A total of 7,237 species or operational taxonomic units (OTU) were identified across all samples. Measures of alpha-diversity differed by RFI in this study (Table 1). The diversity metrics that differed were those related to phylogenetic diversity (Faith's Phylogenetic diversity; P = 0.03) and number of observed species (observed OTU; P =0.03), with greater phylogenetic diversity observed in low-RFI steers. These results are antithetical to those regularly observed in bacterial communities, in which bacterial diversity does not typically differ by RFI.<sup>6,7</sup> However, greater ecosystem diversity on a macro scale tends to result in greater adaptability and resilience of an ecosystem.<sup>8</sup> In particular, functional diversity is related to a more productive ecosystem.<sup>9</sup> In the rumen, the greater phylogenetic diversity of protozoa may be reflective of greater community functional capability, given the variation in

utilization and metabolism.<sup>10</sup> substrate However, elimination of rumen protozoa (i.e., defaunation) has been shown to have little impact on feed digestion or fermentation but increases dietary nitrogen utilization efficiency.<sup>3</sup> Interestingly, defaunation studies have demonstrated that when protozoa are removed from the ruminal environment, the structure of the bacterial community is impacted, resulting in reduced bacterial diversity.<sup>3,11</sup> This lack of diversity as a result of defaunation has also been corroborated by meta-analyses indicating reductions in important fibrolytic microorganisms, including anaerobic fungi and the abundant Ruminococcus bacteria albus and flavefaciens.<sup>10</sup> Ruminococcus Therefore, increases in protozoal diversity may be indicative of increases in the abundance and diversity of fibrolytic microbes that would improve fiber digestion and result in improved feed efficiency.

Genus	High-RFI <sup>a</sup>	Low-RFI <sup>a</sup>	<i>P</i> -value <sup>b</sup>	<b>FDR</b> <sup>b,c</sup>
Diplodinium	0.12 (0.06)	0.04 (0.03)	0.05 <sup>d</sup>	0.15 <sup>d</sup>
Entodinium	0.67 (0.09)	0.81 (0.07)	0.13 <sup>d</sup>	0.19 <sup>d</sup>
Isotricha	0.06 (0.06)	0.05 (0.05)	0.28 <sup>d</sup>	0.33 <sup>d</sup>
Ophryoscolex	0.08 (0.06)	0.02 (0.02)	0.13 <sup>d</sup>	0.19 <sup>d</sup>
Trichostomatia	0.02 (0.01)	0.05 (0.03)	0.74 <sup>e</sup>	0.74 <sup>e</sup>
Unassigned	0.05 (0.00)	0.03 (0.00)	< 0.001 <sup>d</sup>	0.03 <sup>d</sup>

 Table 2. Genus-level differences between high- and low-RFI steers.

<sup>a</sup>Mean (SEM)

<sup>b</sup>Significance determined at  $P \le 0.05$ 

<sup>c</sup>False discovery rate corrected P value

<sup>d</sup>Based on ranked data

<sup>e</sup>Based on log-transformed data

The only specific protozoa genera or species that differed by RFI in this study were the unassigned protozoa (Table 2); meaning protozoa that were not able to be identified based on the current databases. This unassigned trend has also been observed in bacterial communities.<sup>7</sup> There was a greater abundance of unassigned protozoa in high-RFI steers compared to low-RFI steers. These unassigned protozoa may provide great insight as to differences in feed efficiency phenotypes in ruminants, particularly given that there is increasing evidence that rare or low abundance species may drive important phenomena in other ecosystems. For instance, Aanderud and others examined rare bacterial species in various ecosystems before and after a soil re-wetting event.<sup>12</sup> The researchers established that following the re-wetting events, formerly rare species often became dominant, sometimes accounting for up to 60% of relative abundance of bacteria.<sup>12</sup> Additionally, those bacterial community changes were associated with drastic alterations in gas production, with up to 20fold increases in CO<sub>2</sub> production and a 150%

reduction in methane production.<sup>12</sup> The same impacts of rare microbial species have been observed in other organisms and ecosystems.<sup>13,14</sup> Similarly, these rare taxa may significantly alter host phenotypes, and further research could divulge deeper understanding of mechanisms driving divergences in feed efficiency phenotypes in cattle.

This study was one of few studies to examine the relationship between feed and ruminal efficiency protozoal communities. The present research provides preliminary insight as to the relationship between the ruminal protozoa and feed efficiency in beef cattle. Future studies, will aim to further define the interactions of protozoa with other microbes in the rumen to help reveal the microbial mechanisms impacting efficiency. feed Acknowledgements: This study was supported by Ascus Biosciences, Inc. (Grant No. A17-0146-003). The authors thank Emily Melchior and the staff at the Plateau Research and Education Center in Crossville, TN for their technical assistance.

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### Moderate or Aggressive Implant Strategies May Alter the Rumen Microbiome and Metabolome to Improve Gain

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**Take Home Message:** The use of varying levels of hormone-based growthpromoting implants in beef cattle could have differential physiological impacts aside from directly increasing muscle growth that could alter the rumen microbiome and metabolome to influence growth and feed efficiency in beef cattle.

Summary: The effects of growthpromoting implants have been well-defined for their ability to impact growth performance in beef cattle. Productionrelevant microbes and microbiomes in the rumen have also been associated with growth traits. However, the role of implants on the rumen microbiome has not been determined. The objective of this study was to determine if different doses of implant hormones cause gain-associated ruminal microbial community changes. To assess this, a completely randomized design was used and 336 fall-born steers, 450 to 470 days of age, from the germplasm evaluation population at the US Meat Animal Research Center (Clay Center, NE) were divided into two treatment groups: 1) a moderate implant strategy of Revalor-IS (80 mg trenbolone acetate and 16 mg estradiol) followed by Revalor-S (120 mg trenbolone acetate and 24 mg estradiol) or 2) an aggressive implant strategy of Revalor-IS followed by Revalor-200 (200 mg trenbolone

acetate and 20 mg estradiol). Steers were fed the same diet (57.0% dry-rolled corn, 30% wet distiller's grains with solubles, 8.0% alfalfa hay, 4.25% supplement, and 0.75% urea, on a DM basis). On d 84 after implant administration, rumen content was collected orogastric tubing. Samples via were sequenced to target and identify bacteria, and protozoa. Untargeted archaea, metabolomics were performed on rumen fluid using the liquid chromatography mass spectrometry. Production data between implant strategies was analyzed using a mixed model analysis of variance (SASv9.4, Cary, NC) followed by separation of least square means. Microbial diversity between strategies did not differ for bacteria, archaea, or protozoa (P > 0.05). Average daily gain was different (P = 0.01; 3.79 vs 3.66  $\pm 0.04$ lbs, aggressive vs moderate, respectively); however, large microbial community shifts were not associated with implant strategy. Two metabolites, acetyllysine and N-

acetylornithine, were significantly different between implant strategies ( $P \le 0.04$ ). Understanding associations between the rumen microbiome and implant strategies may allow improvement of growth in beef cattle.

#### Introduction

Hormone-based implants have been well-defined for their improvement of growth performance in beef cattle. Historically, research involving growth-promoting implant strategies in beef cattle focused primarily on direct and indirect effects of estrogenic and androgenic hormones on the skeletal muscle in addition to blood metabolites. Implants have well-defined ability to improve growth and feed efficiency in growing beef cattle. Hormone-based implants can increase average daily gain (ADG) by 30% and feed efficiency by 15%.<sup>1</sup> These implant strategies can vary from low to aggressive in potency, modulating the amount of hormone available to release over a variety of times in the animal. Differences in implant strategies have been proven to alter growth and feed efficiency metrics in cattle. Multiple factors influence feed efficiency and gain, such as genetics, nutrition, management practices such as implanting, and other physiological factors. These factors can involve variation in the microbiome metabolome, rumen and modulating gain and feed efficiency in cattle. Previous research has demonstrated the capabilities of the rumen microbiome to impact these production-relevant outputs and thus prove crucial to improving gain.<sup>2</sup> In addition, studies are beginning to assess the impact of the rumen metabolome (i.e. the total collection of metabolites in the rumen)

on gain and feed efficiency in beef cattle.<sup>3</sup> The relationship between implant strategies and the rumen microbiome and metabolome has not been classified. Thus, there was a critical need to begin understanding the potential impacts of the moderate or aggressive implant strategy on the rumen microbial communities and metabolome to augment weight gain in steers.

#### **Materials and Methods**

In this study, 336 germplasm evaluation steers from US MARC were placed into feedlot pens. Animals were kept on a high-concentrate diet consisting of 57.0% dry-rolled corn, 30.0% wet distillers' grains with solubles, 8.0% alfalfa hay, 4.25% vitamin and mineral supplement with Rumensin and Tylan, and 0.75% urea, on a dry-matter basis. Steers were then randomly assigned to either a moderate or aggressive implant strategy. The moderate implant strategy (n = 169) consisted of Revalor-IS (80) mg trenbolone acetate and 16 mg estradiol) for 90 days followed by re-implantation of Revalor-S (120 mg trenbolone acetate and 24 mg estradiol). The aggressive implant strategy (n = 167) consisted of Revalor-IS for 90 days followed by re-implantation of Revalor-200 (200 mg trenbolone acetate and 20 mg estradiol). Eighty-five days re-implant of either Revalor-S or Revalor-200, 6.8 fluid ounces of rumen fluid was collected from each animal via orogastric tubing.

Rumen samples containing fiber and fluid were extracted, isolated, and purified using the rumen digesta protocol by Yu and Morrison.<sup>4</sup> The DNA amplicon libraries of the 16S rRNA gene for bacteria (V1-V3 regions) and archaea (V3-V4), and 18S rRNA gene for protozoa (V3-V4) were sequenced using the 2x250, v2 500-cycle kit through the Illumina MiSeq System (Illumina, San Diego, CA). The Illumina fastq sequencing files for bacterial, archaeal, and protozoal communities were then processed through an R pipeline as described by Callahan and others<sup>5</sup> and statistical analyses performed in SAS v9.4 (SAS Institute, Cary, NC) using a mixed model analysis of variance with separation of least square means.

Metabolites were extracted from rumen fluid and underwent analysis through an ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS). Following, metabolites were statistically analyzed in SAS v9.4 using the same model of analysis.

#### **Results and Discussion**

There was an effect of implant strategy on ADG, where steers implanted with the aggressive implant strategy  $(3.79 \pm$ 0.04 lbs) had increased ADG compared to steers implanted with the moderate strategy  $(3.66 \pm 0.04 \text{ lbs}; P < 0.02).$ While statistically significant, this is likely not a production-relevant difference in gain that would impact production decisions related to which strategy was best. However, the duration of the study may have limited the ability to see production-relevant differences in ADG over longer periods of time. Aggressive implant strategies often release hormones longer (approximately 200 days) and, thus, have prolonged impacts which were not seen in this study due to rumen collections occurring 90 days after treatment implants were administered.

There were no significant differences between the moderate and aggressive implant

strategies for the bacterial or archaeal communities. The protozoal genus *Isotricha* was more abundant in samples from the aggressive implant strategy (Figure 1).





The impacts of the *Isotricha* on the rumen microbiome is often related to the breakdown of carbohydrates. In addition, there is potential that the Isotricha use bacterial communities to increase biohydrogenation the increase of saturated fatty acids in the Biohydrogenation benefits rumen. the microbial communities, as the action converts polyunsaturated fatty acids, which are toxic to multiple bacterial communities, to saturated fatty acids. Further, the rumen metabolome was analyzed, identifying 115 total metabolites. Two metabolites, Nacetylornithine and acetyllysine, were found significant and greater in the moderate implant strategy compared to the aggressive implant strategy. Both metabolites are involved in arginine synthesis, which can be indicative of urea cycles and overall nitrogen metabolism in ruminants. These metabolites may have been involved in systemic

metabolism instead of rumen metabolism, as there are little to no differences in the rumen microbiome.

These few differences between the microbial communities and metabolites could potentially be attributed to the similarities in gain between the moderate and aggressive implant treatment groups. Additionally, all animals received varying levels of trenbolone acetate and estradiol, thus, the similarities between treatment implants may induce similar physiological effects that make analyzing the differences

between the moderate and aggressive implant strategies more difficult, as the lack of differences between the treatment implants may be insufficient to alter the rumen environment. Due to the small differences between the varied implant strategies, there is the potential for future research to examine the differences in the rumen microbiome and metabolome between implanted and nonimplanted animals.

**Acknowledgements**: We thank Bob Lee and the USMARC for technical assistance.

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### IN PROGRESS: Differing Planes of Nutrition Affecting the Morphology and Motility of Bovine Spermatozoa in Beef Cattle

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**Take Home Message:** Proper nutrition is critical in bulls to maximize reproductive efficiency and genetic improvement of the calf crop. The interaction of nutrition and reproduction prior to and during the breeding season may affect the overall reproductive performance of bulls.

**Summary:** Bull fertility is highly influential to the profits of cow-calf production. During the year bulls often experience various planes of nutrition which may increase stress and be influential on fertility. These changes in nutrition may also influence the nutrient and overall composition of the ejaculate. Thus, we hypothesized that differing nutritional planes would affect the morphology and motility of bovine spermatozoa due to the compositional change of seminal plasma in bulls. Mature Angus bulls (n=12) were individually housed and randomly assigned to one of two treatments: 1) over-fed (n=6) or 2) restricted (n=6). Bulls were fed the same ration (35%) ground hay, 35% cracked corn, 20% distillers' grain and 10% soybean meal) at differing amounts to achieve the desired body weight (BW) and body condition score (BCS) changes. Scrotal circumference and an ejaculate were collected once a month for evaluation. Spermatozoal motility and morphology of each ejaculate were evaluated on every sampling day. The morning ejaculate had lower progressive forward motility than

the afternoon ejaculate. Morphology defects were influenced by sampling dates, time collected, and treatment. Scrotal circumference increased after February 5th which may relate to seasonality and also included a treatment by sampling date effect. In conclusion, nutritional levels impact the ejaculate including motility and morphology of spermatozoa which may impact fertility and the ability to establish pregnancy.

#### Introduction

Bull nutrition is important for optimizing fertility to maximize pregnancies but malnutrition can alter the ejaculate of bulls. Due to annual variation in forage quality and bull activity, bulls often fluctuate in BCS and BW throughout the breeding season which may alter the composition of their ejaculate and influence their ability to get females pregnant. Seminal plasma makes up about 95 to 98% of the ejaculate and is very important in fertility.<sup>5</sup> Seminal plasma is composed of energy substrates, amino acids, and cytokines which function to transport and protect the spermatozoa through the female

reproductive tract. Seminal plasma also provides the spermatozoa energy for motility in the form of fructose.<sup>5</sup> Singh et al.<sup>4</sup> found that a low energy and protein diet compared to a control diet can impact male fertility by delaying the onset of puberty, causing poor testicular development, decreasing libido, and reducing progressive forward motility of the spermatozoa. Low energy and protein diets can also alter fertility by decreasing the seminal volume ejaculated.<sup>4</sup> Additionally, diets high in energy and protein can decrease sperm motility and increase morphological abnormalities due to adipose deposition which alters scrotal thermoregulation.<sup>2</sup> A high energy and protein diet can also create a stress-induced hormonal imbalance<sup>3</sup>, impair testicular development, and decrease libido.<sup>4</sup> Therefore, our hypothesis was that differing planes of sire nutrition would impact bovine morphology and motility of spermatozoa through component changes of seminal plasma.

#### **Materials and Methods**

Mature Angus bulls (n=11) were utilized to test the effect of diet on seminal plasma and spermatozoa. All bulls were individually housed in an 8 foot  $\times$  40 foot paddock with ad-libitum access to water and were given a trace mineral supplement every day. The diet for all bulls consisted of 35% ground hay, 35% cracked corn, 20% dried distillers' grain and 10% soybean meal. Each bull was randomly assigned to one of two dietary pathways (Fig. 1) with a 21-day adjustment period. Pathway one, (restricted), was developed for six bulls to lose ~3 lbs./day over 84 days. Restricted bulls would then be maintained at a BCS of 4 for 84 days and finally returned to BCS of 6 by gaining ~3

lbs./day. Treatment pathway two (over-fed) bulls were fed to perform inversely to restricted treatment. Over-fed bulls were managed to gain ~3 lbs./day for 84 days, then maintained for 84 days at a BCS of 8, and they were returned to a BCS of 6 by losing ~3 lbs./day. Individual BW and BCS were recorded every 14 days to monitor and manage feed amounts whereas semen was collected via electro-ejaculation twice (once in the am and once in the pm) every 28 days. Immediately after collection, the ejaculate sample was evaluated under a microscope to assess the motility and morphology of the spermatozoa. To test the effects of differing nutritional planes on the bovine ejaculate, a model ANOVA with repeated mixed PROC measures was completed via GLIMMIX (SAS 9.4, Cary, NC). Means were determined to be significantly different when P < 0.05 and a tendency when P < 0.10.



Figure 1. Dietary plane schematic of desired body condition score throughout treatments.



Figure 2. The effects of nutritional levels on body weight within mature Angus bulls.

#### **Results and Discussion**

Body weight was impacted by treatment and time (P < 0.01). Body weight increased over time on the over-fed pathway from 1,574 ± 45.10 lbs. to 1,940 ± 45.10 lbs. (Fig. 2) whereas the restricted diet pathway caused a decrease in body weight over time from 1,671 ± 45.10 lbs. to 1,523 ± 45.10 lbs. (Fig. 2). Body condition score increased on the overfed diet to 7 ( $6.92 \pm 0.18$ ) while decreasing on restricted treatment to a BCS of 4 (4.08 ± 0.18). Scrotal circumference had a tendency for treatment by sampling date interaction (P = 0.056). Scrotal circumferences ranged from 38.7 cm to 42.0 cm (± 0.6) over time (P < 0.0001; Fig. 3) which could indicate a seasonal effect. The second ejaculate (pm) had greater motility (40.43 ± 2.08%) compared with the first ejaculate (am) motility (37.25 ± 2.08%); potentially due to the accumulation of spermatozoa abnormalities during



Figure 3. Effects of sampling date on scrotal circumference.

epididymal storage over the course of 28 days. The removal of abnormal spermatozoa in the morning ejaculate could lead to a higher motility in the afternoon with spermatozoa that have reduced abnormalities. However, there was a significant decrease in motility (P < 0.001) after 84 days to 32.29  $\pm 1.52\%$ possibly due to the effect of nutritional treatments. Similar to motility, sperm morphology was impacted by sampling date for spermatozoa head (P < 0.01) and midpiece (P < 0.01) abnormalities as well as total normal spermatozoa (P < 0.0001; Table 1). There was also a tendency for tail defects by sampling date (P < 0.09; Table 1) with the highest abnormalities occurring after 84 days.

**Table 1.** Differences in head and taildefects in spermatozoa between morning<br/>and afternoon ejaculate samples.

	AM	РМ	<i>P</i> – Value	SE
Head	29.11 <sup>b</sup>	35.78 <sup>a</sup>	< 0.01	1.23
Tail	2.22 <sup>a</sup>	1.47 <sup>b</sup>	0.02	0.26
Total Normal	48.93	45.82	0.09	2.29

Means without a common letter differ with a P-value < 0.05

The percentage of mid-piece abnormalities (P < 0.0012) tended to be greater in the restricted diet bulls ( $39 \pm 3.1\%$ ) after 84 days compared to over-fed bulls ( $18 \pm 3.1\%$ ). When evaluating morphological defects for morning and afternoon samples, there were significant spermatozoa head (P < 0.001) and tail (P = 0.023) abnormalities as well as a tendency for total normal spermatozoa (P = 0.086; Table 1), with a greater number of head (35.78

 $\pm$  1.23%) and total defects (45.82  $\pm$  2.29%) corresponding in the afternoon sample.

**Table 2.** The effect of restricted vs overfed nutrition on midpiece defects in spermatozoa

spermatozoa.					
	High Low		<i>P</i> – Value	SE	
Midpiece	12.18	17.35	0.10	2.05	

A tendency (P = 0.105) was observed when evaluating the mid-piece abnormalities between over-fed and restricted diets, with the restricted diet having greater abnormalities than the over-fed (17.35 ± 2.05%; Table 2). This could potentially be due to nutritional deficiencies and environmental stresses similar to our dietary treatments causing abnormal spermatogenesis<sup>1</sup>.

Our upcoming results will finalize our dataset and include motility and morphology of spermatozoa, scrotal circumference and pro-/anti-inflammatory cytokines within seminal plasma. In conclusion, determining the impacts of reproductive outcomes from differing levels of nutrients influenced by the paternal diet can lead to improved feeding programs and strategies. The implications will provide additional evidence to maximize reproductive efficiency and future genetic potential through the impacts of the sire diet

**Acknowledgements:** We would like to express our appreciation to Matt Backus, Hugh Moorehead, and Kevin Thompson, as well as, the rest of the crew at MTREC. We could not have conducted this research without their assistance.

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### IN PROGRESS – DuraCor <sup>™</sup> Controls Perilla Mint and Other Broadleaf Weeds in Pastures

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**Take Home Message:** DuraCor TM, a broadleaf herbicide that does not contain 2,4-D, provides control equal to or better than industry standards with flexibility in rates and surfactant options. DuraCor TM also controlled other troublesome broadleaf weeds in tall fescue pastures.

**Summary:** Herbicidal options are needed to avoid cattle losses from ingestion of Perilla mint. DuraCor <sup>TM</sup> (a mixture of aminopyralid and florpyrauxifen-benzyl) was applied at three rates (12, 16, and 20 fluid ounces) using 2 different adjuvants (Activator 90 and MSO with Leci-tech). These treatments were compared to several industry standards for control comparison of perilla mint under field conditions. All treatments provided similar levels of control, which was >95% for all treatments. Injury to tall fescue was not observed (data not shown).

#### Introduction

Perilla mint, also known as purple mint, is a noxious weed that can poison cattle if not controlled. Perilla Mint (Perilla frutescens) is a herbaceous annual with small whitish or purplish flowers and usually no more than 1 to 2 feet tall (Figure 1). It is common throughout zones A, B, C and D in pastures, waste places and roadsides.<sup>2</sup>

Perilla mint causes more cattle deaths in Tennessee than any other toxic plant.<sup>4</sup> Perilla is very poisonous to cattle and other ruminants, as well as horses. All plant parts are toxic, especially the flowering structures. Dried plants in hay can be toxic, but the greatest risk is associated with consumption of fresh plant material, especially if flowers and fruit are present. Perilla mint contains ketones that cause acute respiratory distress syndrome in cattle (ARDS), also called panting disease. Treatment is often ineffective. Although these plants can occur anywhere in a pasture or feed lot. typically favor semi-shaded they environments, and are most frequently located around farm structures, edges of woods and along fence rows.<sup>4</sup> Russell et al.<sup>3</sup> reported good perilla mint control from aminopryalid+24D applied post-emergence, but incomplete control from preemergent applications.



Figure 1. Image of perilla mint from study in Tennessee.

The focus of this research was the newly released product DuraCor <sup>TM</sup> .<sup>1</sup> DuraCor <sup>TM</sup> <sup>TM</sup> herbicide provides extended control of 140+ weeds while maintaining grass safety. That allows nutritious grasses to thrive and opens the way for more productivity from every acre. DuraCor <sup>TM</sup> stops the weeds that are up and growing while providing residual control of those that germinate later. DuraCor <sup>TM</sup> has a low use rate and high ease of use. Unlike many other pasture management products, DuraCor <sup>TM</sup> is nonrestricted and does not contain 2,4-D or dicamba.

#### **Materials and Methods**

The field study was located in the Bear Hollow community of Claiborne County in East Tennessee. The site was a hilltop mixed tall fescue and common bermudagrass pasture currently being grazed by beef cattle.

Plots were placed side by side in a single line down a partially shaded fence row. Each plot was 6.7 feet by 25 feet with a 2-foot untreated running check on each side. Treatments were replicated four times. The entire study was enclosed by an electric fence.

Treatments were applied on 27 May 2020 by hand using a  $CO_2$  powered backpack plot sprayer equipped with 8002 extended range flat fan nozzles. Application rate was 20 gallons per acre and a 0.45 gallon spray mix was used. Treatments were applied at 3 miles per hour with a boom height of 30 inches.

Temperature at application was 77 degrees Fahrenheit with a soil temperature of 64 degrees Fahrenheit and 68% relative humidity. Wind speed was between 1 and 3 miles per hour. The first light rain occurred between 12 and 48 hours after application.

Plant heights at application were as follows: Perilla mint 3-12 inches, Wingstem 10-20 inches, Ladysthumb 4-12 inches, White clover 1-6 inches. Plant density was at least 3 plants per square foot for all species evaluated except wingstem, which was 1 plant per square foot. The growing conditions over the course of the study were excellent.

Treatments included a  $3 \times 2$  factorial of DuraCor <sup>TM</sup> (9% acid equivalent aminopyralid and 0.76% acid equivalent florpyrauxifen-benzyl) at 12, 16 and 20 fluid ounces; each with 0.25% Activator 90 (NIS) at 0.25% v:v and MSO with Leci-Tech at 1.0% v:v. Additional treatments included TerraVue (71% acid equivalent aminopyralid and 6% acid equivalent florpyrauxifenbenzyl) with MSO leci-tech at 1% v:v, GrazonNext HL (8% aminopyralid and 41% 24D amine) with Activator 90 at 0.25% v:v, and 2,4-D amine at 24 fl oz with Activator 90 at 0.25% v:v.

ACTIVATOR 90 is a non-ionic surfactant (Loveland Products). MSO® CONCENTRATE with LECI-TECH® is a methylated seed oil containing emulsifiers, along with Leci-Tech technology, to provide uniform mixing for excellent performance as well as drift reduction, enhanced penetration and droplet adhesion (Loveland Industries). Using the MSO at a higher concentration would suggest more plant uptake under challenging conditions such as larger plants or dry weather conditions.

After herbicide application, plots were visually evaluated from 0 to 100, with 0 being no visual symptoms and 100 being

	Rate		% Control of Perilla Mint			
Treatment			6/8/2020 12 DAT	6/23/2020 27 DAT	7/3/2020 37 DAT	7/29/2020 63 DAT
DuraCor TM	12	fl oz/a	57 5a	71 3b	07b	QQa
ACTIVATOR 90	0.25	% v/v	57.5	/1.5	71	77
DuraCor TM	12	fl oz/a	57 5a	80 <sup>ab</sup>	99ª	99 <sup>a</sup>
MSO WITH LECI-TECH	1	% v/v	57.5			
DuraCor TM	16	fl oz/a	<b>61</b> 2a	71.3 <sup>b</sup>	99ª	99 <sup>a</sup>
ACTIVATOR 90	0.25	% v/v	01.5"			
DuraCor TM	16	fl oz/a	<b>50.0</b> %	76.3 <sup>ab</sup>	99 <sup>a</sup>	99 <sup>a</sup>
MSO WITH LECI-TECH	1	% v/v	58.8"			
DuraCor TM	20	fl oz/a	(0.5)	76.3 <sup>ab</sup>	99 <sup>a</sup>	99 <sup>a</sup>
ACTIVATOR 90	0.25	% v/v	62.3ª			
DuraCor TM	20	fl oz/a	<b>67</b> 3	73.8 <sup>b</sup>	99ª	99ª
MSO WITH LECI-TECH	1	% v/v	63ª			
TerraVue	2.13	oz/a	<b>57 5</b> 9	75 <sup>ab</sup>	99ª	99 <sup>a</sup>
ACTIVATOR 90	0.25	% v/v	57.5"			
TerraVue	2.13	oz/a	(1.0)	75 <sup>ab</sup>	99 <sup>a</sup>	99 <sup>a</sup>
MSO WITH LECI-TECH	1	% v/v	61.3ª			
GrazonNext HL	24	fl oz/a	<b>C</b> (1)	83.8 <sup>a</sup>	99 <sup>a</sup>	98.5ª
ACTIVATOR 90	0.25	% v/v	60ª			
2,4-D Amine	24	fl oz/a	5.6.0%	77.5 <sup>ab</sup>	98.5 <sup>ab</sup>	94.5ª
ACTIVATOR 90	0.25	% v/v	56.3ª			
LSD P=.05			8.62	8.86	1.13	2.46
Standard Deviation			5.94	6.11	0.78	1.69
CV			9.94	8.04	0.79	1.72

**Table 1.** Perilla mint control as affected by herbicide application from field study in<br/>Tennessee in 2020.1

<sup>1</sup> Data presented in percent control, with 0 being no plant effect and 100 being complete plant death. Means were separated by Least Significant Difference (LSD) at the 5 % level, and values within a column followed by a different letter are not the same. DAT = Days after treatment.

plant death. Evaluations were made 12, 27, 37 and 63 days after treatment (DAT).

#### **Results and Discussion**

DuraCor <sup>TM</sup> and TerraVue provided excellent control of several broadleaf weeds in this study. Further, DuraCor <sup>TM</sup> at 12 oz/acre provided similar control of perilla mint to 2,4-D and GrazonNext HL at 24 oz/acre. This reduction in use rates without reduction in control of troublesome weeds is a positive for both the producer and environment.

Either surfactant provided good control at all rates, although there was a slight increase at 37 days after treatment to use MSO as compared to NIS, but all treatments were > 95%. Care should be taken to read and follow all label instructions, since herbicides containing aminopyralid have the potential to persist in harvested forage.

While efforts are made in field research to obtain populations that are uniform, there was difficulty in obtaining a field site with perfectly uniform perilla mint plants. This is a common phenomenon given the biology of this plant. Some variation between plots is always present in field work, but the coefficient of variation (CV) in tables 1 and 2 are low enough that the data is valid.

While perilla mint was the focus of this study, other weeds are often present in Tennessee pastures. This study also indicated good control of several other troublesome weed species (Table 2).

**Acknowledgements:** This research was conducted by the TN Agricultural Experiment Station under Hatch Project 526.

Treatment	Rate		% Control at 63 DAT (7/29/2020)			
		Rate	Wingstem	Ladysthumb	clover	
DuraCor TM	12	fl oz/a	QQa	<b>03</b> 5ª	QQa	
ACTIVATOR 90	0.25	% v/v	"	93.5	77	
DuraCor TM	12	fl oz/a	QQa	0 <b>Q</b> a	QQa	
MSO WITH LECI-TECH	1	% v/v	,,,	70	<u> </u>	
DuraCor TM	16	fl oz/a	QQa	0 <b>7</b> a	QQa	
ACTIVATOR 90	0.25	% v/v	,,,	)1		
DuraCor TM	16	fl oz/a	QQa	06 58	QQa	
MSO WITH LECI-TECH	1	% v/v		90.5	22	
DuraCor TM	20	fl oz/a	QQa	98 <sup>a</sup>	99 <sup>a</sup>	
ACTIVATOR 90	0.25	% v/v				
DuraCor TM	20	fl oz/a	QQa	<b>07</b> <sup>a</sup>	99 <sup>a</sup>	
MSO WITH LECI-TECH	1	% v/v	,,,	21		
TerraVue	2.13	oz/a	QQa	<b>98</b> <sup>a</sup>	99ª	
ACTIVATOR 90	0.25	% v/v	,,,	70		
TerraVue	2.13	oz/a	QQa	<b>08</b> 5ª	99ª	
MSO WITH LECI-TECH	1	% v/v	,,,	70.5		
GrazonNext HL	24	fl oz/a	QQa	<b>07</b> <sup>a</sup>	99 <sup>a</sup>	
ACTIVATOR 90	0.25	% v/v		)1		
2,4-D Amine	24	fl oz/a	QQa	80 3 <sup>b</sup>	0 <sup>b</sup>	
ACTIVATOR 90	0.25	% v/v	,,,	00.5		
LSD P=.05				10.23	•	
Standard Deviation			0	7.05	0	
CV			0	7.39	0	

**Table 2.** Control of Wingstem, Ladysthumb and clover 63 days after treatment as affected by herbicide application from field study in Tennessee in 2020.<sup>1</sup>

<sup>1</sup>Data presented in percent control, with 0 being no plant effect and 100 being complete plant death. Means were separated by Least Significant Difference (LSD) at the 5 % level, and values within a column followed by a different letter are not the same.

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### IN PROGRESS: Rezilon Controls Ryegrass in Bermudagrass Fields

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**Take Home Message:** Rezilon (indaziflam) applied in the fall provided good control of ryegrass the next spring, although higher rates were needed for more complete control. A split application of 3 + 3 fl oz/acre of Rezilon in fall and then early spring provided near complete control of ryegrass. Control was more complete than either fall or spring applications of Prowl.

#### Introduction

Bermudagrass grown for high-quality markets, including equine markets needs to be free of annual weeds for high quality hay. Previously, control of annuals was problematic and very difficult. The new product Rezilon (Indaziflam) has recently been introduced for this use in Tennessee.

Rezilon is a liquid formulation that contains 1.67 pounds of indaziflam per gallon of product. Use rates will be 3-5 fl oz / acre in a single application, and no more 6 fl oz / acre in a 12-month period. To control warmseason annual weeds, an application should be made in late winter. When cool-season annual weeds are the target, applications can be made mid-to-late summer. Roundup can be added to control other weeds in the fall application as bermudagrass is dormant or nearing dormancy. Rezilon is a new mode of action in this market and thus will provide a new management strategy for hay and forage producers.

#### **Materials and Methods**

The study was conducted on a wellmanaged hayfield of Vaughn's Number 1 Bermudagrass located in a river bottom on a producer farm in Knox County, TN. Ryegrass pressure was heavy in the study area.

The treatments were replicated four times in a randomized complete block research design. Individual plots were 6.3 feet by 30 feet with running untreated checks of 2 feet on either side. An untreated check plot was included in each replication. Treatment applications were made using a CO<sub>2</sub> powered research backpack sprayer equipped with 8002 flat fan nozzles calibrated to deliver 20 gallons per acre at 3 miles per hour. Applications were made on two dates: a fall application on 10/18/2018 made prior to ryegrass emergence and with adequate soil moisture; and a spring application made on 3/13/2019 to actively growing ryegrass under extremely wet conditions. Over all weather conditions during the study were very wet with mild temperatures.

Visual evaluations of ryegrass control were made using a scale of 0 to 100, with 0 being no effect and 100 being complete plant death. Evaluations were made in March after the fall application and then twice (April and May) after both fall and spring applications. Means were separated by the Least

				CONTROL OF ANNUAL RYEGRASS		
Treatment	Treatment Rate and Timing		3/11/2019	4/30/2019	5/15/2019	
		have used through		144 DAA	194 DAA	209 DAA
				144 DAA	48 DAB	63 DAB
UNTREATED				0°	0°	0 <sup>c</sup>
REZILON	3	fl.oz./ac	FALL	83p	98ª	98 <sup>a</sup>
ROUNDUP POWERMAX	24	fl.oz./ac	FALL	65		
REZILON	3	fl.oz./ac	FALL		98 <sup>a</sup>	98 <sup>a</sup>
ROUND UP POWER MAX	24	fl.oz./ac	FALL	82 <sup>b</sup>		
REZILON	3	fl.oz./ac	SPRING			
REZILON	5	fl.oz./ac	FALL	96 <sup>a</sup>	99ª	99 <sup>a</sup>
ROUND UP POWER MAX	24	fl.oz./ac	FALL	90		
REZILON	7	fl.oz./ac	FALL	08 <sup>a</sup>	99 <sup>a</sup>	99 <sup>a</sup>
ROUND UP POWER MAX	24	fl.oz./ac	FALL	98		
PROWL H2O	4.5	qt./ac	FALL	0°	0 <sup>c</sup>	0 <sup>c</sup>
ROUND UP POWER MAX	24	fl.oz./ac	FALL	0		
REZILON	3	fl.oz./ac	SPRING		53 <sup>b</sup>	43 <sup>b</sup>
ROUND UP POWER MAX	24	fl.oz./ac	SPRING			
REZILON	5	fl.oz./ac	SPRING		74 <sup>ab</sup>	82 <sup>a</sup>
ROUND UP POWER MAX	24	fl.oz./ac	SPRING			
REZILON	7	fl.oz./ac	SPRING		90 <sup>a</sup>	88 <sup>a</sup>
ROUND UP POWER MAX	24	fl.oz./ac	SPRING			
PROWL H2O	4.5	qt./ac	SPRING		53 <sup>b</sup>	33 <sup>b</sup>
ROUND UP POWER MAX	24	fl.oz./ac	SPRING			
LSD P=.05				4.9	16.8	18.4
Standard Deviation				2.9	9.8	10.8
CV				8.76	14.8	17.14

Table 1. Ryegrass control in bermudagrass field as affected by herbicide treatment	nts
applied in Fall 2018 (A application) and Spring 2019 (B application).	

DAA = Days After Application.

LSD = Least Significant Difference, so data within columns with different letters are not the same.

C = coefficient of variation for that column.

--- no data measured since no treatment applied

Significant Difference test at 95% confidence, and standard deviations and coefficient of variations were also determined.

#### **Results and Discussion**

Looking at the fall applications before any further applications the next spring, Rezilon provided much more complete control than Prowl (Table 1). Higher rates (5 or 7) also provided more complete ryegrass control than 3 fl oz. It was interesting to note that later ratings of the same treatments did not differ. As Rezilon needs good rainfall to be activated, could this be a result of getting enough moisture to germinate weeds but not get good activation of the herbicide? We quite often see some germination of weeds we expect to control if applications are made right up to the germination of the seed or if adequate rainfall occurs. Ryegrass typically germinates by mid-October in most years. Spring applications of Rezilon at higher rates provided more ryegrass control than Prowl (~80% compared to 33%). The sequential 3 + 3 application provided good ryegrass control and could also be a treatment option for some growers.

Rezilon appeared able to remain in the field soil surface for extended periods of time, resulting in excellent residual control. Postemergent activity appeared to be minimal, so efforts should be made to apply Rezilon prior to expected weed seed germination. Many of these treatments also included Roundup, which would provide control of any existing weeds, but care should be taken to apply to dormant bermudagrass only. As dormancy approaches in the fall, a possible scenario would be to apply Roundup +Rezilon for control of knotroot foxtail. The Roundup could reduce problems from the perennial foxtail and the Rezilon could control emergence of any seedlings. Preliminary results on this concept show some promise in this difficult-to-control situation.

Rezilon herbicide brings first-of-itskind chemistry to the range and pasture market and can be used on established bahiagrass and bermudagrass fields. Its unique mode of action in this market provides effective, long-lasting control of troublesome weed species, even those that are resistant to other herbicides, such as glyphosate.

Controlling weeds before they emerge is often more effective than controlling them once they have been germinated. Rezilon is a pre-emergence herbicide that contains the active ingredient indaziflam, a long-lasting residual compound that provides extended weed control other products cannot match. This translates into increased quality, better nitrogen utilization, and production from the fields.

Rezilon is labeled for use in warmseason bermudagrass and bahiagrass hayfields. When Rezilon is applied according to label directions, growers could see higher quality forage in their first cutting as a result of controlling annual weed species present. Rezilon should be applied well before weed emergence. Once applied, it can remain on the soil surface for some time with no or minimal degradation from sunlight while waiting on rainfall for activation. Applications can begin as early as August and into the following spring as a result of extended residual weed control. However, in years with good rainfall some growers will have a third cutting after this potential application, and some of the applied material may be removed and thus potentially not available for weed control

### Observations for Using Fence Line Hay Feeders at the Middle Tennessee AgResearch and Education Center in Lewisburg

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**Take Home Message:** Fence line hay feeding systems are an alternative system of feeding hay to beef cattle. Their use has shown to be helpful in time management during feeding, increased safety to both the operator and the cattle, less hay waste, better animal health, less damage to pasture and improved water quality. However, placement, construction and maintenance of these systems are important considerations for these systems.

**Summary:** Observations made at the Middle Tennessee AgResearch and Education Center (MTREC) in Lewisburg at the Beef Heifer Development Center has shown that fence line hay feeders offer the opportunity to reduce the amount of time spent feeding hay, greater safety to operator and cattle, less hay wasted with correct feed panels and pasture damage and field runoff is restricted and managed on smaller portions of the farm.

#### Introduction

Winter feeding takes a lot of time and is the most expensive aspect of beef cattle production. In Tennessee, the conditions for feeding hay during the winter months are often cold, wet and muddy. Traditional systems of hay feeding utilize hay rings or unrolling hay. During wet conditions, hay losses and pasture damage can be extensive from hoof and equipment traffic. There is also the potential risk to water quality via runoff into creeks. Also, it is potentially hazardous for the operator and cattle as multiple trips are made in out of the fields to feed cattle.

In 2018, five different designs of fence line hay feeders were constructed at MTREC to look at their use and construction costs. These designs were based from a demonstration implemented by Eden Shale Farm<sup>1</sup>, a farm jointly managed by the Kentucky Beef Network and the University of Kentucky. The designs and additional information on fence line hay feeders can be found in the University of Kentucky Publication "Fenceline Feeder Systems for Beef Cattle Production and Resource Conservation."<sup>2</sup> The designs varied based on purchased or built feeder panels and the type of feed pad constructed where these feeders were placed.

#### Materials and Methods

The fence line hay feeders were designed at MTREC in Lewisburg to show



**Figure 1.** Fence line hay feeders allow producers to feed beef cattle without entering the field. This reduces environmental damage to the feeding area, increases safety of the cattle and producer, decreases the time spent feeding, improves animal health and reduces hay waste.

different comparisons between feed pad construction, feed panel construction and commonly available materials and resources. Feeder 1 is on a concrete feed pad using metal feed panels, metal posts and a covered roof (Figure 2A). Feeder 2 is an identical setup to Feeder 1 without the covered roof (Figure 2B). Feeder 3 is on a feed pad constructed with geotextile fabric and covered with chert. This feeder uses wooden posts and metal feed panels (Figure 2C). Feeder 4 is on a chert pad and the feeder is made from homemade wooden panels (Figure 2D). Feeder 5 is on a chert pad and the feeder is constructed using two pieces of a 3-piece skirted hay ring (Figure 2E). This last system is actually a 3bale feeding system because purchasing two 3-piece hay rings results in three feeding stations. The cost comparison of the fence line hay feeders that were installed at MTREC in Lewisburg (Table 1).

Table 1. Cost comparisons for fence line hay
feeders installed at MTREC Lewisburg
2018 prices

Feeder	Total Cost	
Feeder 1: Concrete pad with covered	\$2,395	
Feeder 2: Concrete pad with uncovered feeder	\$2,099	
Feeder 3: Chert pad with metal panel feeder	\$1,769	
Feeder 4: Chert pad with homemade wooden feeder	\$1,489	
Feeder 5: Chert pad with hay ring	$$2,406^{*}$	

\*For even comparison to the 2 bale feeders, cost would be \$1604 for 2 feeding stations with this feeder. Two 3-piece hay rings equals 3 feeding stations.

#### **Results and Discussion**

<u>Location</u> - The location where a fence line hay feeder is critical. These feeders should be placed on flat, well-drained areas or ridge tops. Good road access to accommodate bringing hay to the feeder and reducing mud around the feeders is important. Feeders located close to hay storage or other facilities makes time





Figure 2. Images of each feeder type: A) Feeder 1: Concrete pad with covered feeder, B) Feeder 2: Concrete pad with uncovered feeder, C) Feeder 3: Chert pad with metal panel feeder, D) Feeder 4: Chert pad with homemade wooden feeder, and E) Feeder 5: Chert pad with hay ring feeder

management more efficient. Time spent feeding two bales of hay into a fence line hay feeder system next to a field that was utilizing traditional hay rings was compared. Because the operator had to get off the tractor multiple times to open and close gates, cut strings off hay bales and keep cattle from getting out, it took 35% more time to feed in the traditional hay rings versus those built in a fence line. More savings in time occurred when the fence line hay feeders were next to the hay storage and resulted in 57% less time to feed.

As an example of a less than ideal location, one pair of feeders at MTREC were built at the end of an existing catch pen. These set of feeders were located at the bottom of hill and experienced runoff issues. The major issue was that the runoff entered the feeding area from the side of the feeders. This moisture was dammed up by the feeders resulting in conditions that made it difficult to get feed pads cleaned off. Also, more moisture was trapped on the inside of the feeders. It is not ideal for cattle to clean up a wet layer of hay in the bottom of the feeders. The other feeder designs that were located on ridge tops and flat areas have not experienced these issues (Figure 3).

<u>Feed pads</u> - The second critical component for these systems is the construction of the feed pad. These pads are important from an animal welfare standpoint. They allow cattle the opportunity to feed without standing in deep mud. Deep mud can be a prevalent problem when traditional hay rings are used. Research has shown that cattle performance declines by 7% when mud is dewclaw deep and up to 35% when mud is belly deep.<sup>3</sup> Feed pads should be constructed with a 2-5% slope to allow to drain from the



**Figure 3**. Fence line hay feeders should be located on ridge tops or other well-drained areas away from natural water sources. These feeders were incorrectly placed at the bottom of the hill and were acting as dams for the resulting runoff.

pad. When designing the feed pad, gate entry into the feed pad area needs to be considered to facilitate equipment movement for cleaning up around the feeders. Concrete bunkers arranged for manure storage adjacent to the feed pad area could be useful to store manure until it can be spread when favorable weather returns.

At MTREC, one of the feeder designs placed on a concrete pad needed more slope to facilitate more water movement off of the pad. However, mud around this feeder was never deeper than 8 inches throughout any point during the winter. A problem with this particular feeder was equipment access. Gates need to be placed at the feed pad to allow for more efficient clean out in spite of weather or ground conditions. Modifications have been made to remedy this issue.

<u>Inside the Feeders</u> - An issue encountered with all the fence line hay feeders at MTREC is the height of the area inside of the feeders. The inside of the feeders needs to be at least 6 inches higher than the surrounding feed pad. This allows less wicking of moisture by the hay from the feed pad. It also reduces the amount of mud that is kicked up into the feeder as cattle walk up to the feeder. Cattle consume hay better when hay is dry and clean. On concrete pads, that will mean pouring additional concrete in the area inside the feeder. On feed pads constructed on gravel or chert feed pads, it would be necessary to square up the inside of the feeder using pressure treated 2" x 8" lumber and using larger rock to help moisture drain away from hay. The wicking up of moisture from the ground into the bottom of hay bales either during feeding or storage probably contributes to the greatest waste and rejection of hay by cattle. An observation of traditional hay rings that are not frequently moved has shown that cattle will reject hay that may be 12 inches from the ground due to the wicking of moisture. This layer of hay, if left continuously, may actual harbor disease and pathogens that cause health issues for cattle.

Width Spacing - Width spacing of the feeder is important for reducing hay waste. Most of the feeder designs at MTREC are two bale designs that are 8 feet wide and 12 feet long. The reason for this is that it forces the animal into a more natural feeding position as it stretches its neck for hay. Cattle will leave their heads in the feeder and what hay drops from their mouth will be left in the feeder to be consumed later. A couple of the feeders at MTREC were built into an existing fence line and utilized the existing posts. They are narrower than the recommended 8 feet width. Anything narrower than 8 feet results in the hay being pressed close to the panels. There is no room for cattle to put their head into the feeder and what they typically will do is take a bite of hay, take a step back and any hay dropped from the mouth will be lost to trampling.

Feed Panels - Two kinds of feed panels were compared at MTREC. Both of the feed panels were produced by the same company but one design was skirted. It was observed that on windy days that the feeders using unskirted feed panels experienced hay loss that blew out of the feeder. Unskirted feed panels would have hay that was wasted 8 feet or more from the feeder whereas skirted feed panels had very little hay present outside of the feeders. Additionally, there was less mud that was kicked into the feeders using skirted panels. Another consideration for using skirted feed panels is that these feeders are 8 feet wide. As such, there is a 12 to 18-inch swath that is left in the feeder as hay is cleaned up. This is not a problem as this hay is pushed forward with the next bale of hay. However, if the feed panel on the end is unskirted then some of this hay will be pushed out of the feeders. It is recommended to pay the extra money and utilize skirts on feed panels or hay rings for these types of feeders (Figure 4).

Fence line hay feeders provide an opportunity on many beef cattle farms in Tennessee to reduce the time and some of the worry in feeding cattle. These observations made at MTREC has been useful in extension educational programs such Beef Heifer Development School Field Day, Winter Feeding Virtual Field Day and Tennessee Master Beef Producer Programs. In 2020, fence line hay feeders were included in the Tennessee Agricultural Enhancement Program, giving producers a cost-share opportunity to implement this system of feeding beef cattle.

Acknowledgements: Funding for the fence line hay feeding systems constructed at the Middle Tennessee AgResearch and Education Center in Lewisburg was received through the "Increasing the Resilience of Agricultural Production in the Tennessee and Cumberland River Basins Through More Efficient Water Resource Use." Additional help was provided by the Lower Middle Tennessee Priority Team comprised of University of Tennessee Extension Agents from Marshall, Giles, Lawrence, Maury, Hickman, Lewis, Wayne and Moore Counties.



Figure 4. Using skirted feed panels reduces hay waste from mud and wind.

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### Poster Abstracts

Due to rising cases in Covid-19 in our area and the state we have had to make a hard decision to cancel the in-person component of the graduate research posters and competition. We plan to present these posters completely virtually.

The graduate student poster session will be hosted on UTBeef.com as:

- 1) Live Zoom sessions starting at 12 PM, December 15 directly following the meeting, and
- 2) Recorded short videos of the posters

Please visit the UT Beef and Forage Center webpage at the link listed below to view the poster presentations.

Poster Session Link: <u>UTBEEF.COM/Symposium.html</u>

# Effects of Naturally Occurring Bovine Respiratory Disease on Immune Parameters in Stocker Beef Cattle

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Changes in immunological parameters in relation to naturally occurring bovine respiratory disease (BRD) may indicate disease severity and prognosis. Our objective was to determine the temporal changes in cytokines and acute phase proteins in relation to clinical BRD in commercial stocker cattle. Forty newly weaned crossbred beef steers purchased from auction markets were housed at a commercial stocker operation in Crossville, TN in winter 2020. Clinical severity scores (CSS) were collected according to clinical signs of calves and ranged from 0 to 3 (0: healthy, 3: moribund). Calves received antibiotic treatment if CSS was 1 or 2 with a fever >40°C or CSS of 3 regardless of temperature. Blood samples and thoracic ultrasonography scores (TUS; 1: normal to 3: severe) were collected on days 0, 7, 14, and 21. Haptoglobin (HP), IL-8, IL-1β, and TNFa concentrations were determined using commercial ELISA kits. Fixed effects of health status, day of treatment, and their interaction on serological measures were tested, with animals measured within day as random repeated measure ( $\alpha = 0.05$ ). Non-normal responses were logtransformed before performing mixed model analysis of variance (Proc GLIMMIX; SAS 9.4). Association of TUS with health status was tested using Chi-square test (Proc FREQ). Serum HP significantly increased (P= 0.02) in diseased calves (1.9 mg/mL  $\pm$  0.5) compared to healthy (0.7 mg/mL  $\pm$  0.2). Significant day effect (P= 0.02) on TNFa was observed regardless of health status. At d7, TNFa was significantly higher  $(14.4 \text{ ng/mL} \pm 10.1)$  than d21 (9.2 ng/mL  $\pm 7.5$ ). However, d14 concentrations were not different from either d7 or d21. No significant relationship was observed for IL8 and IL1β in between healthy and diseased calves. Health statuses were significantly associated with TUS (P < 0.0001). This study provides insight into immune responses in relation to clinical BRD in commercial stocker calves.

# Influencing Factors of Price of Sport-trained Horses and Ponies Sold at Online Auctions

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Horses and other equids are typically sold in one of three ways: private interactions, face-to-face auctions, and online auctions. Online auctions are becoming more popular in today's industry due to the increasing technology adapted to facilitate online experiences, especially for disciplines and breeds not commonly sold in this format. The objective of the study was to determine factors affecting online auction sale price for horses and ponies trained in sport classified disciplines, including hunt seat, show jumping, dressage, and eventing. Data was collected from online sport horse auctions (n=75, Professional Horse Auctions, LLC). Within each auction, a horse's lot number, sex, breed, registration status, breeding/lineage, level of training were collected. Statistical analysis will include descriptive statistics, and analysis of variance and mean differences. To date, data from 10 auctions have been collected where 480 horses and ponies were listed for sale, resulting in 335 successful sales, 107 unsuccessful listings, 5 horses withdrawn from auction, and 33 with other instructions. The average sale price (n=335) was \$5,531. Based on work from Thompson and Kibler in 2020, many factors influence the price of stock-type horses sold in an online auction format, including the length and detail of notes regarding the horse and the type of pictures provided. In comparison with work completed by Taylor and colleagues in 2006 which assessed live public auctions, individual characteristics that affected sale price included physical and genetic traits, performance record, and age. Assigning an objective value to horses is an industry-wide challenge, thus, the results of this study will give legitimate data to match prices and help sellers to understand trends in the sport horse sector of the equine industry. Further, it is expected that this project will aid in understanding market trends and demand for horses or ponies with specific characteristics.

#### The Effects of Supplemental Protein on Cytokines and Chemokines in the Uterine Environment throughout Beef Heifer Development

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Successful heifer development is highly dependent on nutrition which influences age at puberty and the uterine environment, therefore, impacting reproductive efficiency. Different diets can influence systemic cytokine responses, however, the effect on uterine cytokines has not been evaluated. Our objective was to evaluate the effect of varied levels of supplemented protein on cytokines and chemokines in the uterine environment of developing heifers. Commercial Angus heifers (n = 60) were blocked by body weight, then allocated to a dietary protein supplementation treatment (n = 20 per group, n = 5 per pen): 10% crude protein (CP), 20% CP, or 40% CP. Supplements were provided four times weekly for six months with ad libitum native grass hay, mineral supplement, and water. Body weight, BCS, and blood was taken every 14d. Uterine fluid was collected every 28d to determine concentration of cytokines IL-1a, IL-1β, TNF-a, IFN-γ, IL-10, VEGF-A, IL-17A, and IL-36RA, and chemokines IL-8, MIP-1α, MIP-1β, and MCP-1. The GLIMMIX procedure (SAS 9.4) was used with fixed effects of supplementation, month, and their interaction; random effects of block, interaction of block and supplementation, and heifer within the interaction; and repeated measure of month. Concentrations of all cytokines, except IL-1 $\alpha$ , and chemokines changed throughout development (P < 0.05). The majority of cytokines and chemokines had increased (P< 0.05) concentrations after the first month of supplementation, potentially in response to the initial diet change with supplementation. Monocyte chemoattractant protein-1 concentrations were lower (P < 0.01) in the 40% CP group compared to other treatment groups. Interferon- $\gamma$  and IL-36RA tended (P = 0.06) to be affected by protein supplementation. In conclusion, cytokines and chemokines shift throughout heifer development, potentially leading to differences in future reproductive success. Further research is warranted to determine the overall dietary influence on the development of the optimal uterine environment.

# Case study: On-farm Demonstration for the Impact of Applied Reproductive Technologies

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Demonstrating the implementation of, and results from, applied management technologies on privately held farms is an essential method for Extension educators. On-farm demonstration of reproductive management technologies in beef cow-calf management improves information transfer and stimulates behavioral change for the local community, and its impact can extend across the state and country through presentations and publications. A commercial cow/calf herd, for which the management was being transitioned to a younger family member, was used for this demonstration. Beginning summer 2019, the cow herd was inventoried and evaluated, and a plan was put in place to improve profitability through enhanced reproductive management. The initial evaluation showed an overall pregnancy rate of 75.6%, and a 153d calving interval for cows and breeding age heifers (n = 209). Non-pregnant and un-sound cattle were sold. Estrus synchronization for natural service was utilized in the first breeding season (Fall 2019) of the multi-year demonstration to condense the subsequent calving season. Melengestrol acetate (MGA) was fed for 7 consecutive days and bulls introduced on 7d. Also in year 1, timed artificial insemination was used to breed replacement heifers that were purchased as non-pregnant yearlings. Ultrasonography was used to detect pregnancy and estimate calving date. Year 2 pregnancy rate was 86.3% with a 131d estimated calving interval on 255 cows and heifers, and 94.9% of the pregnancies are expected to calve in the first 90 days. In summary, while it is difficult to eliminate confounding variables and inputs, estrus synchronization for natural service resulted in a year-over-year increase in the proportion of cows calving earlier in the calving season, which will improve marketing potential and profitability. Condensing the calving interval provides opportunity to introduce additional reproductive management practices. This demonstration will continue for five more years with more comprehensive results being incorporated into educational curricula.

#### Rumen and Serum Metabolomes Influenced by Endophyte-infected Tall Fescue Seed and Red Clover Isoflavones in Beef Steers

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Fescue toxicosis can cause negative impacts on the beef cattle industry via reductions in weight gain, muscle development, and reproduction. Recent research has demonstrated that isoflavone supplementation may mitigate fescue toxicosis detrimental effects. The objective of the current study was to evaluate isoflavone supplementation with fescue seed consumption on rumen and serum metabolomes. Thirty-six Angus steers (n = 36) weighing 550 ± 45 lbs were allocated randomly in a 2×2 factorial arrangement of treatments including endophyte-infected (E+) or endophyte-free (E-) tall fescue seed, with (P+) or without (P-) isoflavones. Steers were provided a basal diet with fescue seed for 21 days, while isoflavones were orally administered daily. Following the trial, blood and rumen fluid were collected for metabolite analysis. Metabolites were filtered and extracted, then analyzed by UPLC-MS. The MAVEN program was implemented to identify metabolites for MetaboAnalyst 4.0 and SAS 9.4 statistical analysis. Seven differentially abundant metabolites were identified in serum by isoflavone treatment, and eleven metabolites in the rumen due to seed type (P < 0.05). Pathways affected by treatments were related to amino acid and nucleic acid metabolism in both rumen fluid and serum (P < 0.05). Therefore, metabolism was altered by fescue seed in the rumen, however, isoflavones altered metabolism systemically to potentially mitigate detrimental effects of seed and improve animal performance. This work was supported by the USDA-NIFA Hatch/Multistate Project W4177-TEN00524-Enhancing the Competitiveness and Value of U.S.

# Effects of a Moderate and Aggressive Implant Strategy on the Rumen Microbial Community in Steers

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The effects of growth-promoting implant strategies have been well-defined in research for their ability to impact growth performance in beef cattle. Production-relevant microbiomes in the rumen have also been associated with growth traits. However, the role of implant strategies on the rumen microbiome is not understood. The objective was to determine if varying doses of implant hormones cause gain-associated ruminal microbial community shifts. To assess this, a completely randomized design was used and 336 fallborn steers from the germplasm evaluation population between 450–470 days of age at the U.S. Meat Animal Research Center (Clay Center, NE) were divided into two treatment groups: 1) a moderate implant strategy of Revalor-IS (80 mg trenbolone acetate, 16 mg estradiol) followed by Revalor-S (120 mg trenbolone acetate, 24 mg estradiol) and 2) an aggressive implant strategy of Revalor-IS followed by Revalor-200 (200 mg trenbolone acetate, 20 mg estradiol). Steers were fed the same diet (57.0% dry-rolled corn, 30.0% wet distiller's grains with solubles, 8.0% alfalfa hay, 4.25% supplement, and 0.75% urea, DM basis). Body weights were collected once per month with initial weights of  $439.8 \pm 43.1$  kg. After implants were administered for 84 days, rumen content was collected via orogastric tubing. Samples were sequenced targeting bacterial V1-V3 16S rRNA gene regions, V3-V4 for archaea, and partial 18S rRNA gene of protozoa. Sequences were processed in R utilizing Phyloseq and analyzed with DESeq2 to test differential abundances. Production data between implant strategies were analyzed using a mixed model ANOVA (SASv9.4, Cary, NC). Alpha- and beta-diversity between strategies did not differ for bacteria, archaea, or protozoa (P > 0.05). Average daily gain was different (P = 0.01; 1.72 vs 1.66  $\pm$  0.02 kg, aggressive vs moderate, respectively); however, large microbial community shifts were not associated implant strategy. This work was supported by the USDA-NIFA Hatch/Multistate Project W4177-TEN00524-Enhancing the Competitiveness and Value of U.S. USDA is an equal opportunity provider and employer.

#### The Effects of Fescue Toxicosis on the Transcriptome of Bovine Cumulus-oocytecomplexes

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Tall fescue is a prominent forage found throughout the eastern and pacific northwestern United States. Tall fescue is a commonly used feedstuff because of its adequate nutrition and easy access, but issues arise when the forage is infested with the fungal endophyte, *Neotyphodium coenophialum*. It is estimated that tall fescue makes up 25-40 million acres of pastures in the United States, and roughly 90% of these pastures contain the fungal endophyte. Fescue toxicosis is induced when cattle ingest endophyte infected forage. Fescue toxicosis causes a variety of symptoms such as poor appetite, decreased weight gains, increased rectal temperatures, inability to fully shed, vasoconstriction, and decreased fertility. Cows affected with fescue toxicosis diminished pregnancy rates. Previous research demonstrated poorer day 7 embryo quality and reduced development to the compacted morula stage and beyond. Transfer of control embryos into fescue toxicosis affected cows on day 7 did not yield lower pregnancy rates than non-affected cows, which suggests that pregnancy loss is likely before day 8 and related to poor embryo quality. We hypothesize that reduced embryo quality in cattle experiencing fescue toxicosis is due to poor oocyte quality. To better understand how the oocyte is impacted by fescue toxicosis, we will perform a study with the objective to compare the transcriptome of cumulus-oocyte-complexes collected from fescue toxicosis versus control cows. We will individually feed Jersey cows a total mixed ration either containing endophyte infected fescue seed (FT, n=20) or no seed (control, n=20). We will synchronize follicular growth and collect the cumulus-oocyte complex ~18 hours after the preovulatory gonadotropin surge. We will perform RNAsequencing on single oocytes and associated cumulus cells from FT and control cows to determine differences in gene transcript expression. We anticipate downregulation of genes with important roles in oocyte development in FT oocytes and cumulus cells. This work is/was supported by the USDA National Institute of Food and Agriculture, Hatch project 1022068.

# Relationship Between Beef Cow Body Condition Score and Follicular Fluid Metabolite Profiles

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Body composition has been shown to affect fertility in women and production animals alike. Extremes in body composition reduce fertility and increase reliance on assistive reproductive techniques for pregnancy establishment in women. Similarly, overly thin beef cows tend to experience decreased fertility. A key component of successful pregnancy in both women and cattle is oocyte maturation and acquisition of developmental competence. The oocyte is supported by the surrounding follicle which provides the nutrients and environment needed for it to grow and mature. The follicular fluid metabolome is associated with oocyte developmental capacity, and follicular fluid metabolite content has been demonstrated to vary among body compositions. We hypothesize that altered follicular fluid metabolite profiles contribute to reduced fertility when cattle or women experience extremes in body composition. Therefore, we designed a study with the objective to explore the relationship between body condition score (BCS) and follicular fluid metabolite content in beef cattle. We synchronized ovulation in 163 lactating beef cattle of varying BCS and performed follicle aspirations to collect follicular fluid samples approximately 18 hours after the preovulatory gonadotropin surge. We collected viable follicular fluid samples from 13 cattle with BCS 4 (thin), 37 cattle with BCS 6 (ideal), and 12 cattle with BCS 8 (obese). Follicular fluid samples will be analyzed for a complete metabolome profile and compared amongst body condition scores. We expect to identify differences in the metabolite profiles of key follicular fluid fatty acids that are associated with oocyte developmental competence. This information will provide valuable insight into how low and high body mass compositions may affect beef cow fertility at the level of the preovulatory follicle. Additionally, our results may prove useful as preliminary data related to human fertility, as cattle are an excellent biomedical model for studies related to the follicle and oocyte. This work is/was supported by the USDA National Institute of Food and Agriculture, Hatch project 1022068.

# Establishing an *in vitro* Model of Muscle Stem Cells to Study Growth and Development at the Cellular Level

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Efficient production of lean and healthy muscle is the ultimate goal of the beef industry. Skeletal muscle develops from a population of multipotent stem cells that have the potential to differentiate into both muscle cells and adipocytes. During embryonic and early postnatal development, nutritional and hormonal cues interact to influence the cell fate of this cell population, which ultimately affects body composition and muscle quality. In vitro models of primary muscle stem cells (MSCs) are a valuable tool for identifying factors that affect muscle development and are complementary to in vivo studies. The objective of this work was to establish protocols for isolating, maintaining and differentiating MSCs in vitro, so that this model can be used to identify factors that affect the efficiency and quality of meat production. Protocols were developed using p. major muscle of broiler chicks (3-7 days of age), as a readily available source of muscle samples. However, the stages of myogenic differentiation and the hormonal control of myogenesis are highly conserved between species, which will allow these protocols to be readily adopted for use with bovine tissue samples. Satellite cells were isolated from intact muscle by enzymatic digestion and purified from other cell types based on differential adherence to tissue culture plastic. Various parameters, including the types, concentrations, combinations and timing of digestive enzymes, were tested to arrive at an optimized protocol. Enrichment for SCs in each procedure was evaluated based on the ability to undergo myogenic differentiation and form myotubes in vitro. Candidate protocols were further evaluated by immunofluorescent staining for Pax7, a marker of SCs, and by flow cytometry for CD56, a surface marker for SCs. In conclusion, we have established a model that will be valuable for assessing the impact of nutrients, metabolites and other physiological cues on muscle growth and development.

#### Building a Targeted RNA-seq Panel to Evaluate Muscle Growth and Development

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Efficient beef production relies on a series of coordinated molecular steps that orchestrate the development and growth of skeletal muscle. Muscle fibers comprise the bulk of market cuts, but adipocytes, endothelial cells and other cell types are critical for optimal muscle growth and meat quality. The relative stages of muscle growth can be assessed in muscle biopsies based on the expression of marker genes using methods such as quantitative PCR. However, multiple genes need to be profiled to fully assess development, which makes expression-based measures inefficient and costly for large numbers of samples. Targeted RNA sequencing (T-RNAseq) is a method that fills the gap between QPCR and genome-wide RNAseq by enabling the simultaneous quantitation of relatively large gene sets across multiple samples in a costefficient manner. T-RNAseq differs from genome-wide RNAseq in that quantitation of expression is restricted to a specific set of genes which are selected based on known functions of interest. Targeting is achieved by designing QPCR primers for genes of interest and then using those primers in cDNA amplification prior to sequencing. The process allows the simultaneous quantitation of relatively large gene sets across multiple RNA samples within a single Illumina sequencing lane in a cost-efficient manner. We illustrate here the process used to create, evaluate and implement a T-RNAseq panel that queries multiple aspects of muscle growth, metabolism and composition. The current panel was developed based on the chicken genome, but due to sequence conservation of coding regions across species, it is anticipated that a number of the same primer pairs will query the corresponding orthologs in the bovine genome. After ensuring that the corresponding bovine genes are adequately represented, this panel representing ~ 500 genes can be used to comprehensively profile muscle growth and development in cattle.

#### The Effects of COVID-19 on Equine Ownership and Care

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Impacts of the ongoing COVID-19 pandemic such as supply and demand shifts of goods and services, travel constraints, and limited social interactions have resulted in alterations in many aspects of daily life. Within the equine industry, these impacts may have affected essential care and had other financial implications resulting in increased concern from equine owners and equine facility operators. Thus, our objectives were to determine how the COVID-19 restrictions affected equine essential and non-essential care access, current and future financial stability, and daily activities of equine owners or facility operators. A research survey was administered over a 4-week period to United States residents above 18 years of age (n=767) and was comprised of questions regarding equine ownership status (owner or leaser), care situation (at home or boarded), and facility ownership. Then, participants were asked to classify how the COVID-19 pandemic has impacted essential and non-essential care access, current and future financial stability, and anticipated changes to their current ownership status. Statistical analysis will include analysis of variance and mean comparison using SAS v9.4. The data gathered from this survey will be used to assess the overall impact of the pandemic on boarding, horse ownership, and facility management. In the face of another potential COVID-19 surge and possible shutdown, this data will be used to strengthen and maintain the longevity of the equine industry and develop targeted educational resources. Further, understanding how equine owners anticipate how COVID-19 could impact their ability to financially support their horses in the future may provide an indicator of a future flood of horses for sale in excess of industry demand, or an increase in unwanted or neglected horses due to financial instability of owners.

# Awareness and Implementation of Equine Pasture Best Management Practices within the Southeast

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Pasture management is an important aspect for equine ownership, and if mismanaged can lead to soil erosion, water contamination, and persistent parasitic infection. Utilizing best management practices (BMPs) has positive implications for the environment, pasture quality, and horses themselves; however, knowledge and implementation of pasture BMPs within the Southeast may vary based on an individual's ownership status or facility maintenance experience. In 2013, Privatsky and colleagues examined pasture management in 26 horse farms in Minnesota and Wisconsin, and found only 6 farms rotationally grazed horses, and 1 farm performed soil analysis, illustrating minimal implementation of BMPs in this industry subset. Thus, our objectives are: 1) determine awareness and implementation of pasture BMPs knowledge by horse owners/leasers who board, horse owners who keep their horses at home, and equine facility owners/managers; 2) determine if any BMPs are currently being utilized at equine facilities owned or utilized by survey participants. We hypothesize that equine owners who keep their stock at home and equine facility owners/mangers will display more BMP knowledge and usage than equine owners/leasers who board. An online survey (Qualtrics) will be distributed via email and social media over 6-week period to United States residents over the age of 18 living within the Southeastern region. Survey respondents will answer questions addressing demographics, information sources for pasture management, BMPs knowledge and implementation, and forages used within equine facilities operated by the participant or another individual. Planned analyses include descriptive statistics and frequency analyses. Information generated from this survey will have a direct impact on Extension efforts regarding pasture BMPs by illuminating current BMP awareness and utilization and identifying targeted areas for educational programming through preferred information outlets.

# Effect of Progesterone (P4) on Endometrial Cell Gene Expression in Development of Medias to Improve *in vitro* Produced (IVP) Bovine Embryo Quality

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During early pregnancy, ovarian progesterone (P4) stimulates uterine endometrial cells to secrete molecular factors that support early embryonic development. Studies involving P4 supplementation during early pregnancy in cattle have shown that P4 increases embryo size indirectly, presumably by increasing endometrial cell secretions that promote embryonic growth. Notably, bovine embryo in vitro production (IVP) medias lack endometrial cell secretions of which may partially explain why IVP embryos have reduced developmental potential. Considering the endometrium consists of mostly epithelial (E) and fibroblast (F) cells, we hypothesize that P4 stimulates endometrial E and F cell expression of genes important for early embryonic development and supplementation of IVP bovine embryos with media collected from P4 stimulated endometrial E and F cells will improve IVP embryo development and survival. To test this hypothesis, female bovine reproductive tracts were collected from a local slaughter facility (n=4) and endometrial E and F cells were isolated. The endometrial E and F cells were then treated for 12 h with Roswell Park Memorial Institute (RPMI) medium alone (Control; 0 ng/mL of P4) or RPMI medium containing 1, 15, or 50 ng/mL of P4. Following treatment, endometrial cell mRNA, protein, and media was collected. The endometrial E and F cell mRNA and protein will be used to measure expression of genes considered to be important for bovine embryo development including transporters for amino acids, ions, fatty acids, and monosaccharides such as solute carrier transporter (SLC)1A1, SLC7A1, SLC5A6, SLC25A10, and SLC27A6. The endometrial E and F cell medias will be used to test for a benefit of endometrial cell conditioned media on IVP bovine embryo development, cryopreservation and survival. Importantly, development of technologies that improve IVP bovine embryo quality will reduce early embryonic loss of IVP embryos and improve herd genetics. This work is/was supported by the USDA National Institute of Food and Agriculture, Hatch project 1022068.

# The Effect of Small and Large Ovarian Follicle Fluid on Bovine Oviduct Cell Gene Expression

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During ovarian follicle development, follicular fluid (FF) accumulates around the oocyte and associated cumulus cells (cumulus-oocyte complex; COC). Optimal hormone conditions in cattle promote ovulation, or rupture of a preovulatory follicle during the estrous cycle, releasing the FF and COC into the oviduct. The FF contains a number of molecular factors including steroids and growth factors that support oocyte development. Notably, bovine FF also modifies oviduct cell gene expression and activities. Size of the follicle at ovulation, which would affect concentration of molecular factors within the FF, can impact subsequent embryonic survival. We hypothesize that boyine FF modifies the oviduct environment to support the COC and subsequent embryonic development and the environment created by small follicle fluid (SFF) is suboptimal compared to large follicle fluid (LFF). During a preliminary experiment, female bovine reproductive tracts (n=4) were collected and oviduct cells isolated from the ampulla (A) and isthmus (I) regions. From the ovaries, SFF (follicles < 5 mm in diameter) and LFF (follicles > 5 mm in diameter) were aspirated and used to develop A and I treatments: 1) Roswell Park Memorial Institute (RPMI) medium alone (Control), 2) RPMI medium containing SFF (1:5 total dilution) or 3) RPMI medium containing LFF (1:5 total dilution). The A and I cells were incubated with the treatments for 24 h before cell culture media, mRNA and protein was collected. The cell mRNA and protein will be used to measure expression of valuable oviduct genes with potential to improve COC and embryonic development. The cell culture medias will be used in a follow up study to test their effect on cumulus-oocyte complex and embryo quality. Importantly, a better understanding of the effect of FF on the oviduct environment may lead to technologies that mitigate early embryonic loss in cattle. This work is/was supported by the USDA National Institute of Food and Agriculture, Hatch project 1022068.

# Methods to Investigate the Effects of Meiotic Inhibition and Estradiol Treatment on Oocyte Energy Availability and Early Embryo Development *in vitro*

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As the oocyte is growing within the follicle, it is stockpiling metabolic substrates and increasing its mitochondria number in a process called metabolic maturation. The mitochondria use these metabolic substrates to produce ATP via a process called oxidative phosphorylation. Both the oocyte and early embryo rely on oxidative phosphorylation to produce the ATP necessary to fuel development. Estrogen is present within the follicle and has been shown to improve mitochondrial function in somatic cells. Some assisted reproductive techniques involve removing the oocyte from the follicle early, interrupting metabolic maturation processes and potentially depriving the early embryo of much needed energy producing resources. This could lead to stalled development and early embryo loss. We hypothesize that the addition of a meiotic inhibitor and estradiol to the pre-maturation culture media will allow the oocyte to have more time to become metabolically mature as well as stimulate mitochondrial biogenesis and function. Therefore, we designed a study with the objective to determine the impact of meiotic inhibition and estradiol supplementation on oocyte metabolic maturation and embryo development. Cumulus-oocyte-complexes (COCs) will be aspirated from abattoir-sourced bovine ovaries and divided into four treatment groups: 1. Conventional maturation, 2. Conventional maturation + estradiol, 3. Pre-maturation with meiotic inhibitors followed by conventional maturation, 4. Pre-maturation with meiotic inhibitors + estradiol followed by conventional maturation. We will first collect COCs after conventional maturation for each treatment and quantify ATP concentration and mitochondria number. We expect to see increased ATP levels and mitochondria numbers in the oocytes that were treated with meiotic inhibitors and estradiol. We will then fertilize COCs from all treatments after conventional maturation and determine postfertilization cleavage and blastocyst rates. We expect the hypothesized increase in oocyte ATP levels and mitochondria numbers in the meiotic inhibitor + estradiol group to translate into improved postfertilization development. This work is/was supported by the USDA National Institute of Food and Agriculture, Hatch project 1022068.

# Data Comparison and Propensity to Sell Factors between Stock-type and Sport-type Horses at Online Auction

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Online auctions are becoming a commonplace to sell horses and other equids since the transaction can occur without face-to-face interaction and increases in horse marketability. Typically, online auctions are geared toward a specific classification of horse, equid, or discipline area, and are becoming increasingly popular among stock- and sport-industry classifications. Our objective is to determine which factors impact the likelihood of a horse trained in sport disciplines selling at online auction, and to distinguish differences in online auction outcomes targeted to sell stock horses as compared to sport horses. Data collected from stock (n=51) and sport (n=75) online auction records (Professional Horse Auctions, LLC) will be assessed to determine market specific variations in sale price, along with desired traits including breed or riding association registration, horse's skill level, and physical characteristics. Statistical analysis of stock and sport horse auction data will be completed using analysis of variance and mean comparisons (SAS v9.4). In 2020, Kibler and Thompson determined that stock horses registered with the American Quarter Horse Association are more likely to sell than other breeds, thus it is expected that sport horses of a particular breed association will have a greater likelihood of selling at auction and would hold greater value to prospective buyers. It is expected that sport horses with displayed evidence of advanced training will yield a higher price over stock breeds with a similar training level. Also, it is expected that sport horses taller than 16 hands will have a higher propensity to sell than horses under 16 hands. Results will provide insight as to the most valuable information to include on a horse's sale page, and the potential likelihood of a successful sale due to factors including physical characteristics, skill level, breed, and differences between sale factors within industry sectors.

#### Evaluation of Farm Demographics and Producer Perceptions That Influence Cow-Calf Producer Horn Fly Management Costs in Tennessee and Texas

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The United States livestock industry loses an estimated 1.75 billion dollars annually due to horn flies (*Haematobia irritans* (L.)), making them a widespread and significant pest of livestock. These ectoparasites feed on the blood of their host 20-40 times per day, which can cause blood loss, introduce pathogens, and decrease weight gains and milk production in cattle. Research suggests that proper horn fly management supports increased weight gains in cattle, leading to positive returns for producers; however, little research exists regarding cattle producers' horn fly management decisions and their associated costs. Tennessee and Texas cow-calf producers were surveyed to estimate their horn fly management costs per animal and identify factors influencing this cost. Results indicate that the average horn fly management costs per animal in Tennessee and Texas are \$9.50 and \$12.35, respectively. Tobit regression results indicate that producers who have a college degree, raise Angus cattle, perceive a high intensity of flies to be a problem, agree that horn flies jeopardize cow comfort, or reported having farms with more total acres were associated with lower horn fly management costs per animal. Meanwhile, producers who reported horn flies present on the backs and withers of their cattle, were more likely to spend more on horn fly management per animal. Results indicate that management decisions vary by location and that farm demographics and producer perceptions influence horn fly management costs.



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