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Alabama Livestock Research Report

College of Agriculture
DEPARTMENT OF ANIMAL SCIENCES

WELCOME

It is with great pleasure that we welcome you to the first edition of the Alabama Livestock Research Report. Our goal is to provide a comprehensive overview for producers and stakeholders across the state highlighting the research and outreach activities developed in the Department of Animal Sciences at Auburn University.

This publication combines reports from scientists and graduate students on campus and Experiment Stations. Herein we provide in-depth information on our research programs, ranging from animal nutrition and genetics to management and production practices. Additionally, we showcase our outreach activities, such as extension programs and partnerships with industry stakeholders.

We are proud to say that this report and research is made possible thanks to the financial support of funding agencies, organizations, and stakeholders who believe in the value of our work. Likewise, thank you to the scientists, students, and staff that contributed to this publication and have worked tirelessly on these projects. Together, we can continue our mission of improving the lives of livestock producers and enhancing the sustainability of the livestock industry in Alabama and beyond.

We hope that this report will serve as a valuable resource for all those invested in the livestock industry in Alabama. We invite you to explore our findings, learn about our progress, and engage with us as we work together to build a brighter future for our state's agriculture. Should you have any questions about the research reported in this publication, do not hesitate to contact us or any of the authors of the individual reports.

We appreciate your support and all you do for the success of our research programs and the department of Animal Sciences.

Sincerely,

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TAKE HOME MESSAGE

Stockpiling bahiagrass may extend the growing season length by 30 to 45 days based on the first year of a two-year evaluation and provide moderate forage quality for supporting beef operations.

SUMMARY

Bahiagrass is a perennial warm-season grass that is widely used in Alabama. For many producers, bahiagrass pastures are reliable and a feasible option due to the low input management when compared to other forage systems. Identifying alternatives to extend the grazing season using established bahiagrass pastures can provide supplemental forage and fill a production gap in forage-systems in Alabama during the fall. Stockpiling is the practice of allowing forage to accumulate in a pasture for grazing at a later time when growth is limited. Traditionally, most research has focused on stockpiling bermudagrass or tall fescue in Alabama. Our team recently revisited the idea of stockpiling bahiagrass. Bahiagrass is generally not as productive or high quality as well-managed bermudagrass; however, its strong persistence under grazing with relatively low fertilizer inputs, moderate quality and widespread use in Alabama make it a good candidate for stockpiling in beef cow-calf operations. Results from the first year of this study demonstrated that stockpiled bahiagrass can provide up to 1,500 pounds of dry matter per acre during the late fall and early winter months with quality of forage adequate to support fall-calving cows with relatively low levels of feed supplementation.

1. INTRODUCTION

Warm-season perennial grasses such as bermudagrass and bahiagrass are the dominant forage species in the lower half of Alabama. These forages generally have a highly productive, long growing season under the warm summer temperatures and frequent summer rainfall in the state. As fall and early winter approaches, these forages enter a period of dormancy or “fall asleep for the winter” until temperatures rise again the spring. The lack of forage for grazing during winter often requires supplementation in cow-calf operations with stored forages such as hay or baleage and commodity feeds. During times of increasing input costs, strategies to extend the grazing season into this time frame may provide an alternative nutrition strategy to farmers. Stockpiling perennial grasses may support additional grazing days and lengthen the window of use of warm-season perennial grasses in Alabama. **The objectives of this study were to determine the effects of stockpiling initiation**

date and fertilization strategy on bahiagrass forage production and nutritive value during the late fall and early winter months.

2. PROCEDURES

2.1. Experimental Design

A replicated, randomized complete block design study was established to evaluate bahiagrass managed under three stockpiling strategies (6-, 8-, and 10-weeks of accumulation prior to the first anticipated frost date of the year) and three nitrogen (N) fertilization strategies [no N, split application of N (30 lb N/acre at initiation of stockpiling, and 30 lb N/acre after 30 days from first application), or 60 lb N/acre] for two experimental years (fall 2021 and fall 2022). Data from the 2021 experimental year is included in this report.

2.2. Site Management and Data Collection

This study was established at Auburn University Research Stations and private farms at the following locations: Troy, Headland, Goodway, and Clanton, Alabama. Stockpiling was initiated on September 15 for Headland, Troy, and Clanton locations, and September 20 for Goodyway. Data collected during the stockpiling period included: forage mass accumulated during the stockpiling period, forage nutritive value, including digestibility, crude protein, and fiber fractions, and changes in forage quality at 30-days following a frost event.

2.3. Harvest Timing

Forage plots were clipped to 1-inch stubble height at the end of the stockpiling period for each treatment (6, 8 or 10-weeks). At the end of the 10 week stockpiling period, remaining forage in all plots was allowed to stand for an additional 30-days to measure changes in forage quality during the fall-winter transition period.

3. RESULTS & DISCUSSION

Forage mass for bahiagrass stockpiled across these different time periods and under three differing nitrogen fertilization strategies is presented in Table 1. Forage production was an average of 1,450 lb DM/acre in the first year of the evaluation. Timely rainfall following initiation of stockpiling in mid-September supported accumulation of forage during the subsequent weeks when growth traditionally begins to slow.

Average forage nutritional value of stockpiled bahiagrass ranged from 59 to 63% total digestible nutrients and 12 to 15% crude protein across stockpiling periods in this study. This is generally greater quality than most bahiagrass hay produced in Alabama that is tested through the Auburn Soil, Forage, and Water Testing Laboratory, and can meet the needs of a fall calving cow herd with little supplementation.

Based on the forage mass estimates above, if pastures are managed under rotational or strip grazing, bahiagrass may provide 45 to 55 days of grazing. Managed grazing is important to ensure cattle graze the stand more uniformly and do not have as much opportunity to “pick and choose” what they want. If cattle have continuous access to stockpiled forage, they tend to trample the stand more or strip leaves from the stems, reducing the number of possible grazing days.

Table 1. Date of stockpiling initiation and nitrogen fertilization strategy effects on forage mass of stockpiled bahiagrass during the fall-winter 2021 growing season.

Stockpiling Period Length	**Forage Yield (pounds of dry matter/acre)†**
6 weeks	1,499
8 weeks	1,452
10 weeks	1,587
Nitrogen Fertilization Strategy	-
0 lb N per acre	1,323
Split application†	1,597
60 lb N applied at initiation	1,475

†30 lb N per acre applied at the initiation of the stockpiling period and again 30 days later

†Represents average yield across four locations in Alabama.

Relative to stockpiled bermudagrass and tall fescue, bahiagrass provides a shorter window of grazing days. Following a hard frost in late November/early December, stockpiled bahiagrass stands tend to degrade very quickly and are not as palatable to grazing cattle. Bahiagrass will have faster leaf loss and stem breakdown compared to bermudagrass following a killing frost. Despite this window of use, this study demonstrates that bahiagrass may help close the fall forage production gap and reduce early winter hay feeding needs by providing at least an additional month to the grazing season.

Acknowledgments

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Overseeding Bahiagrass Pastures to Extend the Grazing Season and Improve Sustainability in Forage Systems

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TAKE HOME MESSAGE

Overseeding bahiagrass pastures with diverse grass-legume mixtures may extend the forage production season in the winter months. Small grains support grazing earlier in the production season, whereas annual ryegrass and clovers provide mid-to-late spring growth.

SUMMARY

Overseeding warm-season perennial grass pastures with cool-season annual forages may extend the grazing season in the winter months on Alabama beef cow-calf operations. Increasing interest in planting multi-species mixtures of forages in pasture systems led to an evaluation of overseeding bahiagrass with various combinations of small grains, annual ryegrass, and clovers. The objective of the study was to measure forage production and nutritional value of these forage mixtures when planted into dormant bahiagrass in the fall months. The first year of a two-year forage evaluation is reported herein from a study established at the Wiregrass Research and Extension Center in Headland, Alabama. The results of this study suggest that overseeding bahiagrass with cool-season mixtures of small grains, ryegrass and clovers can provide approximately ~ 1,500 pounds of dry matter per acre of grazable forage per grazing event. The extended grazing season provided by the cool-season mixtures overseeded into bahiagrass may reduce feeding costs during the shortage in forage production. The Oat-Wheat-Clover is the mixture with the greatest nutritive value, Oat-Rye-Clover is intermediate, and Rye-Ryegrass-Clover is the mixture with the least. However, all of these mixtures would provide a high-quality forage option for beef cow-calf pairs grazing during this time of the year. When animals not in the growing category are grazing the cool-season mixture overseeded into bahiagrass, any of the mixtures met their nutritional values. More attention must be given when growing cattle are grazing the cool-season mixtures to make more adequate decisions on appropriate supplementation.

1. INTRODUCTION

Warm-season perennial pastures are the base of livestock production systems in Alabama. Warm-season perennial grasses, such as bermudagrass and bahiagrass, are the most common forages grown. The productivity of these forages fluctuates across the year due to the seasonality of forage production. They are generally more

productive from May to October. However, their growth is reduced in late fall and they go dormant throughout the winter. These forages do not regrow until temperatures warm again in the spring. The lack of forage for grazing during the winter often requires supplemental roughage and feedstuffs, increasing annual carrying costs in livestock systems. To overcome the shortage in forage production during this period, overseeding warm-season perennial pastures with cool-season annuals can be used as an alternative to providing forage from late fall and early spring due to their complementary growth period. Because winter feeding is a common concern by Alabama cattle producers, this study aimed to evaluate the effects of overseeding bahiagrass pastures with grass-legume cool season mixtures during the period of dormancy of warm-season forages on forage mass and nutritive value for two consecutive years to extend the grazing season. The **objectives** of this study were to: 1. **Determine forage mass and nutritive value of cool-season forage mixtures overseeded into bahiagrass pastures aiming to extend grazing season on forage-based systems** and 2. **Identify management strategies to increase nitrogen availability for bahiagrass pastures improving sustainability and feasibility of livestock-forage systems.**

2. PROCEDURES

2.1. Experimental Design

A randomized complete block design study was established to evaluate grass-legume mixtures managed under grazing. The mixtures of cool season forages used (Paddocks treatments) include:

1) **OWC:** oats [*Avena sativa*; 55 lb pure live seed (PLS)/acre] + wheat (*Triticum aestivum*; 55 lb PLS/acre) + clover blend [Balansa (*Trifolium michelianum*; 6 lb PLS/acre), red (*Trifolium pratense*; 6.2 lb PLS/acre) and white (*Trifolium repens*; 2 lb PLS/acre) clover]
2) **ORC:** oats + rye (*Secale cereale*; 55 lb PLS/acre) + clovers
3) **RRC:** rye + ryegrass (*Lolium multiflorum*; 17.8 lb PLS/acre) + clovers

2.2. Site Management and Data Collection

The first year (2021) of this study was established at the Wiregrass Research Center in Headland, AL. The bahiagrass field was mowed to 2-inches of stubble height. Sixty days after planting, 34 lb N per acre was applied. Before each harvest event, forage samples were randomly harvested in three sites per paddock to determine forage mass. These samples were subsequently dried and ground to determine forage nutritive value (crude protein, digestibility, and fiber concentrations).

2.3. Harvest Management

Paddocks were mob-grazed for a short period of time (around 4 hours per grazing event) to 4-inches stubble height to determine the influence of grazing on cool-season forage production and regrowth potential. Target pre-grazing height was 10 to 15 inches tall, and animals were removed when the remaining stubble height was 4 inches.

3. RESULTS & DISCUSSION

Forage mass did not differ among treatments and averaged 1,409 pounds of dry matter per acre. Forage mass was greater in the late season of March to April than in the early extended grazing season during January and February (Figure 1). The increased forage mass in the late occurred possibly due to the more favorable weather conditions, hotter and wetter days, from when the animals were removed after grazing during the early extended grazing season until immediately before the late extended grazing season.

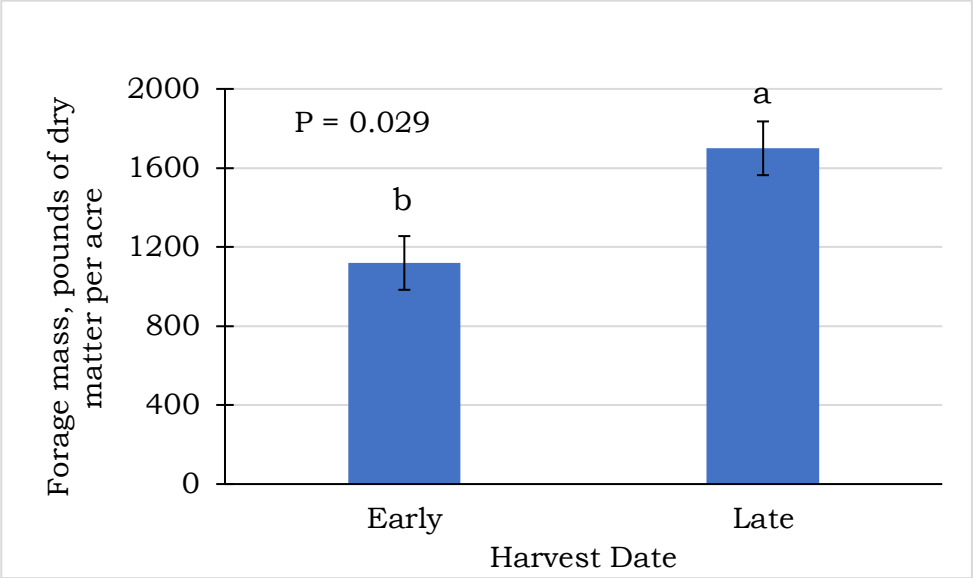

Figure 1. Forage mass of cool-season mixtures overseeded into dormant bahiagrass pastures.

None of the botanical composition components (grass, legumes, or weeds) differed among the cool-season mixtures overseeded nor over time (between early and late winter grazing season). The grass, legume, and weed components averaged 80.7, 0.3, and 19.1% in the botanical composition, respectively. The significantly low percentage of clover in the mixture may be attributed to a delayed fertilization program that favored the growth of the grass component and reduced growth due to shading resulting from the upgrowth of the companion grass in the mixture. Applying nitrogen fertilizer immediately after planting may favor the growth of the legume component at the beginning of the growth period when the grass components are still short.

Regarding the nutritive value of the mixtures, crude protein was greatest for OWC (17%), intermediate for ORC (15%), and least for RRC (13%). Similarly, total digestible nutrients were also different among the cool-season mixtures overseeded, but these changes differed between early and late extended grazing season (Table 1). In the early extended grazing season, when pre-grazing forage height was 5, 11, and 12 inches, for OWC, RRC, and ORC, respectively, total digestible nutrients were similar among all cool-season mixtures. However, in the late extended grazing season, when pre-grazing forage height was 11, 31, and 27 inches for OWC, RRC, and ORC, respectively, total digestible nutrients were greatest for OWC and similar between ORC and RRC (Table 1).

Table 1. Total digestible nutrients of cool-season mixtures overseeded into dormant bahiagrass pastures over the cool season.

Season	Treatment[1]			SEM	P-value
	ORC	OWC	RRC		
	Total digestible nutrients, %			1.3	0.005
Early	82	79	82		
Late	71[b]	79[a]	68[b]		

[1]ORC = Oats-Rye-Clover; OWC = Oats-Wheat-Clover; RRC = Rye-Ryegrass-Clover.

[abc]Means without a common letter are significantly different.

With similar forage mass among all treatments, the decision on which cool-season mixtures overseeded into dormant bahiagrass may be made based on the nutritive value of those mixtures and the category of animal grazing these mixtures. Brood cows in the first months of pregnancy (requirements: 7% CP and 48% TDN) and those in mid-lactation (requirements: 9% CP and 54% TDN) can have their nutritional needs met by grazing any of the three mixtures used in this study. Similarly, cows in peak lactation (60 to 80 days after calving; requirements: 12% CP and 60% TDN) can also have their nutritional needs met by any of the mixtures evaluated. In conclusion, overseeding pastures with mixtures of cool-season grasses and legumes may support winter and spring grazing in beef cattle operations. Number of species in the mixture and seasonality of forage species growth is important in optimizing growing season length. Additional work to improve establishment methods of diverse, cool-season mixtures is being initiated in 2023 to target an earlier grazing window in this system.

Acknowledgments

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Evaluation of Dual-Purpose Wheat Varieties in the Southeastern U.S.

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TAKE HOME MESSAGE

Cool-season annuals, including winter wheat, are nutrient dense forages and can provide suitable total digestible nutrients (TDN) and crude protein (CP) when supplementing a mid- to high quality hay. Dual-purpose wheat is commonly used in the Great Plains but differences in varieties, beef cattle herd composition, and growing season make it underutilized in the Southeast. Research found that timely removal of cattle is an important factor in ensuring economical returns on the grain yield of dual-purpose wheat. Furthermore, variety selection is an important component to determine pre-grazed forage yield and therefore stocking rate. Lactating beef cows require 45 – 55% TDN and 8 – 10% CP, replacement heifers or stocker requirements push upwards of 60 – 70% TDN and 10 – 12% CP. All varieties of winter wheat elevated were able to meet the nutritional requirements of both lactating cows and stocker calves. Varieties of winter wheat currently on the market in the Southeast are viable options for dual-purpose wheat production and can provide an economic advantage versus grazing or grain-production only.

SUMMARY

Dual-purpose wheat (*Triticum aestivum* L.) systems are common in the Southern Great Plains of the U.S. to provide revenue diversification. However, these systems are not often utilized in the Southeast. This study aimed to evaluate forage biomass and grain productivity of four winter wheat varieties managed under a dual-purpose production system. The wheat varieties evaluated were generic feed-type wheat (unknown variety blend, Feed), seed-type wheat (Seed) and two forage-type varieties, 'AGS 2024' and 'Pioneer 26R41'. The experiment was a randomized complete block design (n = 4). Three grazing frequencies were utilized: an ungrazed control (NG), low frequency (LF), and high frequency (HF). Low frequency plots received monthly grazing in January and February while HF treatments received a third grazing in March. Plots were grazed with 20 cow-calf pairs (Bos taurus). Pre-G and Post-G forage biomass was determined using three 0.1m^2-quadrats per plot before and after each grazing event. Forage samples were then dried at 131˚F for 72 h. Data were analyzed using PROC GLIMMIX of SAS (SAS Inst., Cary, NC). Differences were declared at $P < 0.05$. Final biomass was greatest for Pioneer but was not different from AGS or Feed (3,669

lb/acre and 3,571 lb/acre; $P \leq 0.94$). Prior to grazing, AGS forage biomass (2,361 lb/acre) was greater ($P \leq 0.03$) than all other varieties. There was an interaction ($P \leq 0.01$) of variety and grazing frequency for Pre-G forage biomass. Compared with all other varieties, AGS had greater ADF (24.93%; $P < 0.01$) and least TDN (72.49%: $P < 0.01$). Across grazing frequencies, Pioneer had greater final grain yield (3,229 lb/acre; $P < 0.01$) with Seed having the least (1,135 lb/acre; $P < 0.01$). These results indicate that Southeastern wheat varieties can be utilized in a dual-purpose system; however, grazing frequency should be monitored to prevent grain losses.

1. INTRODUCTION

The Southeast relies predominantly on perennial pasture systems [i.e., tall fescue (*Schedonorus arundinaceus*), bermudagrass (*Cynodon dactylon*), and bahiagrass (*Paspalum notatum*)] to meet the nutritional requirements of the beef sector. Therefore, the challenge becomes providing adequate nutrition to lactating and growing animals when warm-season perennial pastures become limiting in the fall. Winter wheat is a cool-season annual that provides adequate biomass and nutritive quality for grazing purposes (Dillard et al., 2018).

This creates a unique opportunity for producers to utilize winter wheat as a both a grazeable forage and grain crop. Depending on producer goals, dual-purpose systems provide greater flexibility in on-farm revenue sources by adding gains to young, growing calves and by providing a marketable grain crop in the late spring. Dependent on market conditions and producer resources, the decision could be made to forego harvesting grain to provide additional forage biomass in the early spring, or grazing can be terminated early as to not incur increased yield losses during periods of increased grain prices.

The objective of the current study was to evaluate common Southeastern winter wheat varieties for agronomic performance under three grazing strategies. To determine the viability of dual-purpose wheat in the Southeast, a study was conducted to evaluate forage biomass, nutritive value, final biomass, leaf area index (LAI), sward height, and final grain yield of four winter wheat varieties.

2. PROCEDURES

This experiment was conducted at the Wiregrass Research and Extension Center (WREC) in Headland, AL, U.S. during the winter of 2019 – 2020 and 2020 – 2021. Treatments consisted of four wheat varieties [AGS 2024 ('AGS'), Pioneer 26R41 ('Pioneer'), Generic Feed-blend wheat ('Feed') and GA-Gore ('Seed')] and three grazing strategies [no-grazing ('NG'), low-frequency grazing ('LF') and high-frequency grazing ('HF')] replicated in four blocks. Low frequency treatments received two grazing events in January and February at 28 d intervals while HF treatments received an additional grazing event in March. Plots were established at 120 lb pure live seed (PLS)/acre using a no-till drill, and fertilized with 120 lb N/acre and 16 lb/acre P and K, respectively.

Forage biomass was determined using three 1.1 ft^2-quadrat samples per plot collected prior to grazing (PreG) and following grazing (PostG). Sward heights were also collected PreG and PostG to determine per-grazing heights and to verify target defoliation height. Forage biomass samples were dried at 131˚F for 72-h until a stable dry-weight was achieved. Subsamples were then ground to pass through a 0.04-inch screen via Wiley Mill (Thomas Scientific, Philadelphia, PA). Leaf area index (LAI) data

were collected during each grazing event and at final grain harvest. Canopy data were collected using an LAI-2200C Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, NE). Final biomass was collected via three 1.1 ft^2-quadrat samples per plot. Grain yield was collected from each plot with a single-plot combine and sampled in-field for test weight and moisture. Forage nutritive value (TDN, CP, NDF, ADF, ADL and ash) was determined via near-infrared spectroscopy (NIRS). NIRS was validated via wet chemistry. Forage nitrate concentration was determined with an Orion™ nitrate ion selective probe (ThermoFisher Scientific, Waltham, MA).

3. RESULTS & DISCUSSION

The observed biomass values support the use of dual-purpose wheat as a viable option to meet DM intake needs for cow-calf pairs in the Southeast. Average forage biomass across all varieties was 1,965 lb/acre which is comparative to results reported from Netthisinghe et al. (2020) and Fieser et al. (2006) (Table 1 and 2). Nutritive value of dual-purpose was able to meet the needs of lactating beef cattle, stocker claves, or replacement heifers without additional supplementation (Table 3). For the current study, variety differences were apparent regarding sward height, LAI and grain yield. Pioneer had the greatest grain yield while obtaining an intermediary sward height and LAI. This is indicative of the potential for Southeastern cultivars to recover after consistent defoliations to produce a marketable grain crop. Netthisinghe et al. (2020) reported grain yield of wheat managed as a dual-purpose crop did not differ from its respective grain-only treatment in both 2017 and 2018 (4,372 vs 4,996 lb/acre and 2,944 vs 3,123 lb/acre, respectively). In comparison with this experiment, similar results were obtained for three of the four varieties evaluated excluding Seed (2,856 lb/acre). Across grazing treatments, this study achieved a grain yield of 2,729 lb/acre under the LF grazing treatment with no differences when compared to the NG treatment (Table 4). These observations support the use of Southeastern wheat varieties under a low frequency grazing treatment for dual-purpose wheat production.

Table 1. Average seasonal forage biomass, sward height, leaf area index (LAI) before and after grazing (Pre-G and Post-G) and end-of-season biomass of four wheat varieties managed as a dual-purpose crop.

		Forage Variety				
	Item	**AGS[1]**	**Feed**	**Pioneer**	**Seed**	**SEM[2]**
Pre-grazed	Biomass, lb/acre	2361[a]	2012[b]	1945[b]	1539[c]	929.1
	Sward Height, in	16.5[a]	12.6[b c]	13.5[b]	11.8[c]	1.67
	Leaf area index	1.90[a]	1.70[a b]	1.47[b c]	1.14[c]	0.206
Post-grazed	Biomass, lb/acre	747[b]	1270[a]	948[a b]	1047[a b]	444.3
	Sward Height, in	5.2[a]	4.9[a]	4.6[a b]	4.3[b]	0.24
	Leaf area index	0.41	0.46	0.50	0.39	0.103
End-of-season	Biomass, lb/acre	3597[a]	3546[a]	3669[a]	1369[b]	618.3

1AGS = AGS 2024; Feed = generic variety blend; Pioneer = Pioneer 26R41; Seed = GA Gore. [2]SEM = Standard error of the mean. [a, b, c] Within rows, means with common superscripts do not differ ($P > 0.05$).

Table 2. Average seasonal forage biomass, sward height, leaf area index (LAI) before and after grazing (Pre-G and Post-G) and end-of-season biomass of dual-purpose wheat managed under three grazing frequencies.

		Grazing Frequency			
	Item	**NG[1]**	**LF**	**HF**	**SEM[2]**
Pre-grazed	Biomass, lb/acre	2677[a]	1590[b]	1626[b]	927.2
	Sward Height, in	15.2[a]	24.7[b]	12.7[b]	1.66
	Leaf area index	1.88[a]	1.34[b]	1.44[b]	0.195
Post-grazed	Biomass, lb/acre	--	1121[a]	885[b]	436.5
	Sward Height, in	--	4.9	4.6	0.18
	Leaf area index	--	0.50	0.38	0.094
End-of-season	Biomass, lb/acre	4826[a]	3165[b]	1144[c]	535.0

[1]NG = no grazing, LF = low frequency, and HF = high frequency. [2]SEM = Standard error of the mean. [a, b, c] Within rows, means with common superscripts do not differ ($P > 0.05$).

Table 3. Nutritive value of four wheat varieties managed as a dual-purpose crop.

	Forage Variety				
Item	**AGS[2]**	**Feed**	**Pioneer**	**Seed**	**SEM[3]**
CP, %[1]	19.3[b]	20.8[a]	20.5[a b]	20.5[a b]	0.51
NDF, %	49.77[a]	47.41[a b]	46.98[b]	46.78[b]	3.112
ADF, %	24.93[a]	22.49[b]	22.85[b]	22.52[b]	1.061
ADL, %	5.62	5.49	5.59	5.52	3.487
Ash, %	5.50	5.38	5.52	5.34	3.050
TDN, %	72.5[b]	75.1[a]	74.7[a]	75.0[a]	1.11
NO_3, ppm	167.8	160.0	163.1	140.0	34.77

[1]CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, TDN = total digestible nutrients, NO_3-N = nitrate-nitrogen, ppm = parts per million. [2]AGS = AGS 2024; Feed = generic variety blend; Pioneer = Pioneer 26R41; Seed = GA Gore. [3]SEM = Standard error of the mean. [a, b]Within a row, means with common superscripts do not differ ($P > 0.05$).

Table 4. Grain yield (lb/acre) of four wheat varieties managed as a dual-purpose crop under three grazing frequencies.

	Grazing Frequency			
Variety	**NG[3]**	**LF**	**HF**	**Mean[4]**
[1]AGS	3456	2917	1038	2470 [b]
Feed	3360	2935	869	2388[b]
Pioneer	4827	3602	1259	3229[a]
Seed	1276	1463	665	1135 [c]
[2]Mean	3230[x]	2729[x]	958[y]	

[1]AGS = AGS 2024; Feed = generic variety blend; Pioneer = Pioneer 26R41; Seed = GA Gore; [2]Standard error of the mean (SEM) = 256.9 lb/acre; [3]NG = no grazing, LF = low frequency, and HF = high frequency. [4]Standard error of the mean (SEM) = 223.9 lb/acre; [a,b,c]Within a column, means with common superscripts do not differ ($P > 0.05$). [x, y]Within a row, means with common superscripts do not differ ($P > 0.05$).

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Methane Production Potential of Heifers Offered Four Bermudagrass Cultivars

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TAKE HOME MESSAGE

The objective of this study was to evaluate *in vitro* methane production from beef cattle as influenced by four bermudagrass cultivars. We found that bermudagrass cultivar did have an influence on potential methane production *in vitro*. These findings have an impact on both the environmental and economic sustainability of southeastern United States beef production systems. From an environmental perspective, not only forage species selection, but also cultivar selection, will be critical in the management of methane emissions from livestock operations. From an economic perspective, the energetic inefficiencies of carbon loss through methane will require intensive nutritional management decisions within forage systems. These data will serve as the foundation for further investigation into ruminal fermentation dynamics of southeastern United States forage systems.

SUMMARY

Though bermudagrass (*Cynodon dactylon* [L.] Pers.) is the predominant warm-season perennial forage supporting the southeastern United States livestock production systems, little is known about its influence on parameters of ruminal metabolism, including carbon loss as methane. Thus, the objective of this study was to evaluate *in vitro* methane production as influenced by four bermudagrass cultivars. Ruminally-fistulated heifers (n = 4) were assigned randomly to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d *in vivo* periods. On d 28 of each period, rumen fluid was collected from each heifer for use in methane production evaluation. Samples of each bermudagrass weighed into duplicate serum bottles and incubated for 0, 2, 4, and 24 h. Following incubation, headspace samples were assayed for methane concentrations by gas chromatography. After 24 h of incubation, methane concentrations were greater (P < 0.05) from T44 and T85 than from RUS and COS. Results are interpreted to mean that bermudagrass cultivars differ in potential methane production.

1. INTRODUCTION

Greenhouse gas (GHG) emissions have become a major concern of livestock production systems. Enteric fermentation from grazing livestock is implicated in the production of 27% of all methane emissions in the United States (EPA, 2022). The southeastern United States is home to approximately 42% (38.5 million head) of all cattle nationally and approximately 37% (14.5 million head) of cows and heifers that calved from January 2021 to January 2022 managed on forage-based systems (NASS, 2022). In southeastern United States beef production systems, cattle are managed on pasture for the majority of their life cycle (Troxel et al., 2014). The predominant source of enteric methane production in beef cattle is the consumption by cattle of feedstocks dense in cell wall material (e.g., when cattle are grazing) (Pinares-Patiño et al., 2003).

Not only does methane represent an environmental concern, but it is also an energetic loss to the production system (Johnson and Johnson, 1995). This loss in production efficiency has stimulated research on methane mitigation strategies, especially nutritional manipulation. Factors such as forage quality and type can influence enteric methane production (Eugène et al., 2021). Bermudagrass, the predominant warm-season perennial grass in the Southeast, accounts for approximately 34.5 million ac in the United States (Vendramini et al., 2019). Much effort has been devoted to improving these forages for better livestock efficiency (Taliaferro et al., 2004). However, to date, there have been few investigations into the effects of bermudagrass cultivars on ruminal fermentation, especially enteric methane production beef production systems. Thus, **the objective of this study was to evaluate *in vitro* methane production from beef cattle as influenced by four bermudagrass cultivars.**

2. PROCEDURES

The *in vivo* metabolism experiment was conducted as a 4 × 4 Latin square. Ruminally-fistulated heifers (n = 4) were assigned randomly to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d *in vivo* periods (21 d adaptation and 9 d collection). The accompanying *in vitro* methane production experiment was conducted using the design of the *in vivo* experiment with the addition of incubation time (0, 2, 4, or 24 h). Laboratory duplicates served as observational units within the *in vivo* experimental unit.

Samples of the four bermudagrass cultivars were collected from bales in the *in vivo* period. Hay samples were dried and ground prior to incubation. Subsamples (0.1 g) of each bermudagrass, representing the cultivar corresponding to the rumen inoculum were weighed into duplicate serum bottles for each incubation timepoint. Following sample addition, buffer solution (Callaway et al., 1997) was added to each bottle under CO_2, then bottles were sealed and brought to temperature in a gravity convection incubator.

On d 28 of the *in vivo* experiment, whole rumen contents were sampled at 4 h relative to feeding (Goering and Van Soest, 1970) and collected into pre-warmed insulated containers for transport to the Auburn University Ruminant Nutrition Laboratory. Rumen fluid was strained with 4 layers of cheesecloth and added to serum bottles under CO_2. These bottles were sealed and kept in a gravity convection incubator for separation of rumen liquor and feed particles. A volume of rumen liquor from each heifer was drawn using a syringe and injected into the prepared serum

bottles (n = 8 per heifer per period). The 0 h timepoint samples were immediately transferred to a refrigerator until further analysis. Other samples were allowed to incubate for their prescribed time then transferred to a refrigerator to stop fermentation until further analysis. Incubated samples were transported on ice to the Department of Animal and Dairy Sciences at the University of Georgia for methane analysis via gas chromatography.

3. RESULTS & DISCUSSION

After 24 h of incubation, methane concentrations were greater ($P < 0.05$) from T44 and T85 than from RUS and COS (Figure 1). Cultivar differences are likely due to differences in chemical composition (Benchaar et al., 2001). The breeding programs that produced these cultivars were directed toward improved animal performance, often through a reduction in or alteration of cell wall constituents. Burton and Monson (1988) identified T44 as having lower concentrations of cell wall constituents and greater digestibility. Similarly, T85 was identified to be more digestible than COS due to decreased lignin concentrations and increased concentrations of neutral sugars (Burton et al., 1993). Along these lines, Mandebvu et al. (1998) found that *in vitro* digestibility of COS was only 53%, comparable to physiologically mature TIF85.

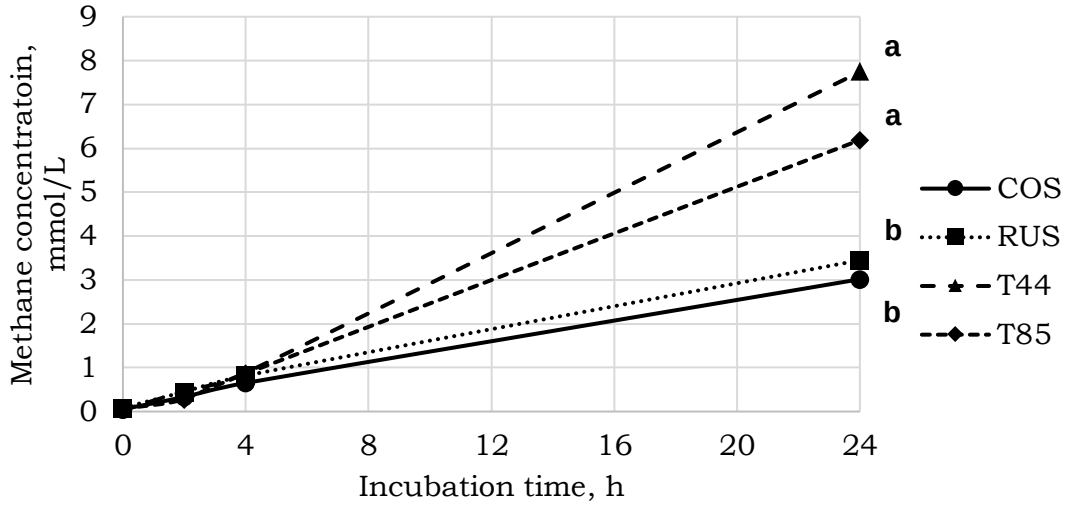

Figure 1. *In vitro* methane production from beef heifers offered one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]).

While there is sufficient evidence to suggest cultivar differences, caution should be used in the interpretation of these data. There are many ways in which to describe methane production. In a bermudagrass supplementation experiment, Smith et al. (2020) found no cultivar differences in methane expressed as total production (in this case, mg/L), but differences arose when methane was expressed relative to chemical constituents of the substrate or relative to digestibility. It could be that, over a longer incubation time, COS and RUS may produce similar total methane as T44 and T85 if their respective rates of fermentation are slower. Thus, the differences observed in the current experiment warrant further investigation into the dynamics of fermentation and relative methane production.

Funding

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In Situ Dry Matter Disappearance Kinetics of Four Bermudagrass Cultivars

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The objective of our study was to evaluate *in situ* digestive kinetics of four bermudagrass (*Cynodon dactylon* [L.] Pers.) cultivars in heifers consuming typical southern forages. We found that bermudagrass cultivar did have an influence on rate and extent of in situ dry matter disappearance. Coastal bermudagrass had the lowest concentration of potentially degradable material and the greatest concentration of undigestible material, rendering it the least effective of the four cultivars tested. Among the other improved cultivars, interpretation of value would depend on the length of ruminal residence time. We interpret the results to indicate that Tifton 44 is the most desirable of the cultivars tested, followed by Tifton 85 and Russell, with Coastal the least desirable. Further, given the lack of visible asymptote, we speculate that bermudagrass likely passes the rumen before full digestion is realized.

SUMMARY

While much effort has been devoted to both the characterization of ruminal fermentation dynamics and the evaluation of bermudagrass production, independently, there is a lack of information regarding ruminal digestive kinetics using beef cattle in southern forage systems. Thus, the objective of our study was to evaluate *in situ* digestive kinetics of four bermudagrass (*Cynodon dactylon* [L.] Pers.) cultivars in heifers consuming typical southern forages. (Coastal, Tifton 44, Tifton 85, and Russell). Ruminally-fistulated heifers (n = 4) were assigned randomly to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d *in vivo* periods (21-d adaptation and 9-d collection). On d 31 of periods 2 and 3, an *in situ* experiment was conducted. There was a lower (P < 0.05) potentially degradable fraction from COS compared with T85, T44, or RUS. Similarly, COS presented the greatest (P < 0.05) undegradable fraction and T85 the least, with RUS and T44 intermediate. There was no effect of cultivar, however, on the rate constant of digestion or lag time. Results are interpreted to mean that T44 is the preferable bermudagrass cultivar, followed by T85 and RUS, while COS is the least favorable.

1. INTRODUCTION

Bermudagrass (*Cynodon dactylon* [L.] Pers.), together with tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.), is one of the two most important forages supporting livestock production in the United States, especially the Southeast. Since Dr. Burton's introduction of 'Coastal' bermudagrass as the first commercial hybrid bermudagrass in 1943 (Myers, 1951), both agronomists and animal scientists have sought to characterize the forage both from the perspective of nutritive value as well as animal performance, often under varying management strategies. However, some 80 years later, we are still trying to ascertain some of the differences that exist and improvements that have arisen from the various breeding programs. Thus, the objective of our study was to evaluate *in situ* digestive kinetics of four bermudagrass cultivars in heifers consuming typical southern forages.

2. PROCEDURES

The *in vivo* metabolism experiment was conducted as a 4 × 4 Latin square. Ruminally-fistulated heifers (n = 4) were assigned randomly to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d *in vivo* periods (21 d adaptation and 9 d collection). On d 31 of periods 2 and 3, an *in situ* experiment was conducted as a randomized complete block design with a 3-factor factorial treatment structure. The first factor (n = 4) was diet consumed by the animal (described above). The second factor (n = 4) was the incubated forage (same cultivars as the *in vivo* experiment). The third factor was incubation timepoint (n = 19; 0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, 120, 144, and 168 h).

The *in situ* degradation was estimated using an adaptation of the nylon bag technique (Vanzant et al., 1998; Norris et al., 2019). Briefly, acetone-washed fiber bags (F57; Ankom Technology, Macedon, NY) were filled in quadruplicate with each of four bermudagrass cultivars undergoing digestive evaluation and heat-sealed. These fiber bags were placed in a nylon-type zipper bag (Norris et al., 2019) and suspended in the ventral rumen. Bags were inserted in reverse order (longest incubation [168 h] first, shortest incubation [0.25 h] last) and removed simultaneously at the end of the incubation period (Vanzant et al., 1998). A separate, unincubated, sample was used to represent the 0-h timepoint for measuring washout. Following removal, samples were immediately submerged in ice water, then rinsed according to the protocol of Vanzant et al. (1998). Following rinsing, half of the samples (2 bags of the quadruplicate) were assayed for NDF, ADF, and ADL (Vogel et al., 1999; AOAC, 2000), and the other half were assayed for CP (AOAC, 2000).

3. RESULTS & DISCUSSION

There was a lower (P < 0.05) potentially degradable fraction from COS (30.0%) compared with T85 (55.1%), T44 (48.1%), or RUS (47.1%; Figure 1). Similarly, COS presented the greatest (P < 0.05) undegradable fraction (37.0%) and T85 the least (15.9%), with RUS and T44 intermediate (25.0 and 21.9%, respectively). There was no effect of bermudagrass cultivar, however, on the rate constant of digestion (0.94%/h; P = 0.26) or lag time (3.2 h; P = 0.38).

Few manuscripts exist that solely compare cultivar differences on ruminal digestive processes. Thus, direct comparison of our data is difficult. The potentially degradable fraction from T85 is similar to the mid-season values reported by Smith

et al. (2017). Mandebvu et al. (1999) reported that T85 had improved digestion constants relative to COS, regardless of plant maturity of ensiling.

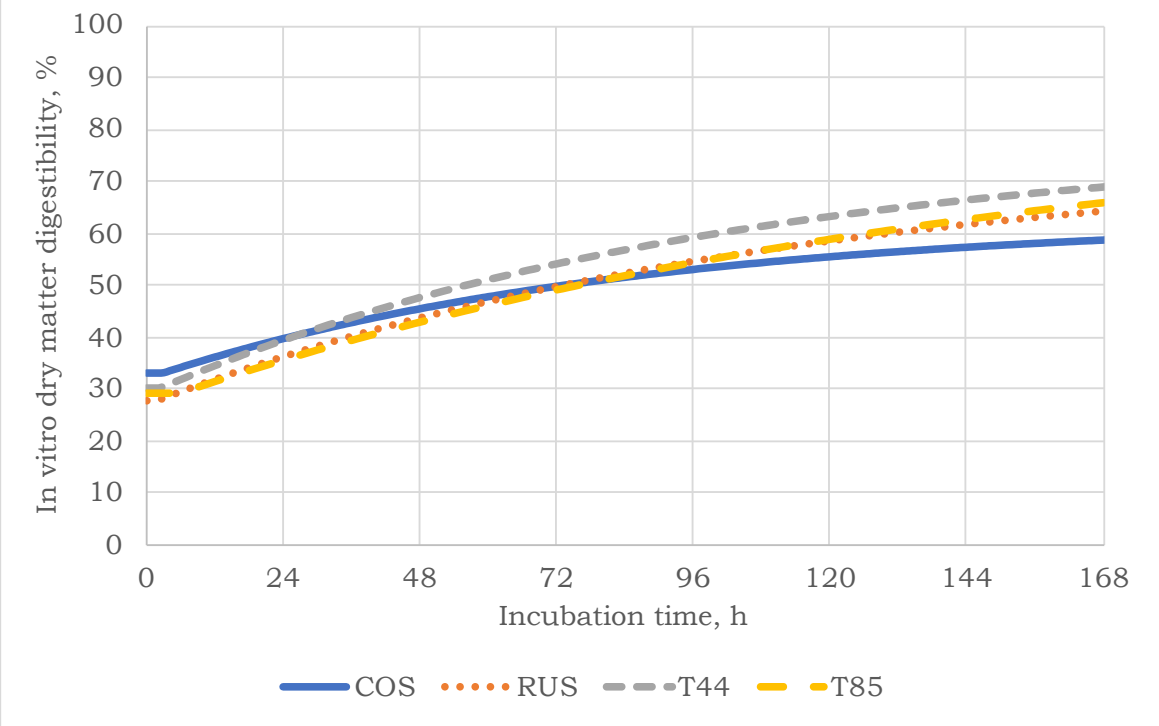

Figure 1. *In vitro* methane production from beef heifers offered one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]).

A visual appraisal of the degradation curve suggests that the asymptote of digestion was not reached at the measured 168 h. Further, given the lack of visible asymptote, we speculate that bermudagrass likely passes the rumen before full digestion is realized.

Funding

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Case Study: Passage Dynamics of Whole Cottonseed in Beef Cattle

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TAKE HOME MESSAGE

The objective of this study was to conduct an observational screening of manure from beef cattle offered near *ad libitum* levels of whole cottonseed (WCS) for passage of undigested seed. We found that a median of 2.5 seeds and 24.5 seed fractions were recovered from each manure sample. While caution should be used in the broad interpretation of these observational results due to sample size and collection procedures, there is sufficient evidence to suggest that high levels of WCS supplementation may seed to decreased digestive efficiency (evidenced by undigested seed in manure). These observations serve as a basis for further investigation in designed experiments to evaluate WCS digestion and passage dynamics.

SUMMARY

Whole cottonseed (WCS) has long been a viable supplement in southern beef production systems, but new cultivars and ginning innovations have renewed interest in its use. Gestating and lactating beef cows have been observed to have adequate or optimum performance under supplementation scenarios as high as 8 lb/head/d. It has been hypothesized that WCS may pass through the gastrointestinal tract without being digested. Thus, our objective was to conduct an observational screening of beef cattle offered near *ad libitum* levels of WCS for passage dynamics. We sampled beef cattle offered an average of 8 lb WCS per head per day. Fresh manure samples (n = 22) were collected at random from the herd. Fecal samples were sorted by wet sieving to recover undigested seed material. Across samples, recovered WCS were approximately normally distributed with a median of 2.5 seed per sample and a range of 0 to 14. Fractional WCS recovered were normally distributed with a median of 24.5 parts per sample and a range of 2 to 76. While caution should be used in broad interpretation of these results, there is sufficient evidence to suggest that high levels of WCS supplementation may lead to decreased digestive efficiency.

1. INTRODUCTION

In 2022, there were 13.7 million acres of cotton planted in the United States (NASS, 2023), from which approximately 5 million tons of cottonseed were produced (ERS, 2022). Whole cottonseed (WCS) is a feedstuff made use of by livestock producers as a supplemental source of both protein (24.4% CP) and fat (Rogers et

al., 2002). Fat, while the primary source of energy in WCS, is also the limiting factor to inclusion of WCS in beef cattle rations (Jacobs, 2021). According to Rogers et al. (2002) and Jacobs (2021), fat intake for beef cattle should be limited to 4% of dietary DM, meaning that beef cattle should be limited to 0.5% BW WCS or 20% of the total diet. For reference, using the standard animal unit of 1,000 lb for a mature cow, WCS should be offered at no more than 5 lb WCS. However, anecdotal evidence has shown that mature cows can overwinter with WCS supplementation up to and in excess of 8 lb/d. Given the lack of performance depression seen as these levels, one may begin to question the role of excess fat in the diet of these cattle. It has been hypothesized that WCS may pass through the gastrointestinal tract without being digested, thereby not interfering with whole diet digestibility. Thus, **our objective was to conduct an observational screening of beef cattle offered near *ad libitum* levels of WCS for passage dynamics.**

2. PROCEDURES

In this case study, we sampled beef cattle (approximately 1000 head) from a farm located in central Alabama. Cattle on this farm were segmented into seven herds based on age and stage of production. Each herd was offered an average of 8 lb WCS per head per day. Across these seven herds, the majority were offered a tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) forage base; the remainder were grazing a bahiagrass (*Paspalum notatum* Flueggé) forage base. Fresh manure samples (n = 22) were collected at random from across the herds. Fecal samples were frozen until further analysis.

After thawing, fecal samples were wet sieving to recover undigested seed material. Counts were conducted to determine whole or fractional seed remaining. To determine nutritive value, WCS (collected from that offered to cattle) and composited samples of undigested WCS and undigested seed fractions were assayed for NDF, ADF, ADL, and CP (Vogel et al., 1999; AOAC, 2000).

3. RESULTS & DISCUSSION

Across samples, we recovered a median of 2.5 whole seeds and 24.5 seed fractions from each manure sample (Table 1). These seeds amounted to approximately 0.3 g (0.01 oz) of DM, or 5% of the total fecal DM. By comparison, Coppock et al. (1985) found that, when offered WCS either with lint attached or acid delinted, dairy cows consumed 6 lb WCS and excreted 0.7 seed per lb of fecal DM, accounting for a passage of 0.4%. In that evaluation, delinting the WCS resulted in increased intake (7 lb), increased excretion (17.5 seeds/lb fecal DM), and an increased passage rate (11.3%) (Coppock et al., 1985).

Though not statistically analyzed, fiber values of the WCS that passed into the feces was similar to that of the unfed WCS (Table 2). However, seed fractions recovered in the feces demonstrated an increase in fiber concentrations. Crude protein, on the other hand, decreased in all seed recovered in the manure.

Table 1. Counts of whole cottonseed (WCS) and seed fractions recovered from the manure of cows offered *ad libitum* access (approximately 8 lb/head/d) to whole cottonseed in the winter of 2021/2022 in central Alabama.

Item	Mean	Median	Minimum	Maximum
WCS	3.3	2.5	0	14
Fractional WCS	31.9	24.5	2	76
Seed weight, g	0.3	0.3	0	1
Seed weight, % DM	5.0	3.2	0.4	23.4

Table 2. Nutritive value of unfed whole cottonseed and fed but undigested whole cottonseed (WCS) and seed fractions recovered from the manure of cows offered *ad libitum* access (approximately 8 lb/head/d) to WCS in the winter of 2021/2022 in central Alabama.

Item[1]	Unfed WCS	Fed, undigested WCS	Fed, undigested seed fractions
NDF, %	57.6	59.5	78.1
ADF, %	43.9	42.6	57.9
ADL, %	12.5	15.6	24.1
CP, %	19.4	15.3	6.8

[1] OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein.

Caution should be used in the broad interpretation of these observational results. The sample size in this case study was small, and assessments were not made in a controlled environment. However, there is sufficient evidence to suggest that high levels of WCS supplementation may seed to decreased digestive efficiency (evidenced by undigested seed in manure). These observations serve as a basis for further investigation in designed experiments to evaluate WCS digestion and passage dynamics.

Funding

This research was funded, in part, by the Alabama Cotton Commission. This project was also financially supported by the Agricultural Research Service, U.S. Department of Agriculture, under Agreement No. 58-6010-1-005.

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Effects of Maternal Selenium Supplementation During Gestation on Newborn Muscle Transcriptome

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TAKE HOME MESSAGE

Selenium (Se) is an essential micromineral that plays a role in immune function, redox status, energy metabolism, and growth in beef cattle. An increasing number of studies have shown that biological processes regulating growth, development, and nutrient utilization are programmed in utero during the fetal phase to adult function. In the current study, we investigated the effects of supranutritional maternal Se-supplementation on the offspring muscle transcriptome profile and the biological pathways differentially affected in response to maternal nutrition. Our findings suggest that supranutritional Se supplementation modulates muscle gene expression leading to beneficial effects during the last third of gestation.

SUMMARY

Selenium plays essential metabolic functions, including energy metabolism regulation and growth. Despite the increasing attention to the role of Se in animal production, the effects of maternal Se-supplementation on fetal programming are still to be examined. Thus, we investigated the effects of supranutritional maternal Se-supplementation on the offspring's muscle transcriptome. To this end, within 12 to 48 h of birth, WB samples and *Longissimus dorsi* (LD) muscle biopsies were collected from 40 calves born to crossbred Angus cows that received, except the control group (CTR), Se-yeast boluses (105 mg of Se/week) at the first (TR1), second (TR2), or third (TR3) trimester of gestation. We identified 3,048 unique differentially expressed genes (DEGs) across all group comparisons (FDR $\leq$ 0.05 and |log2FC| $\geq$ 1.5). Selenoprotein-coding genes (*SELENOO*, *SELENOP*, *GPX1*, and *GPX3*) and myogenic factors (*MYOD1*, *MYOG*, *MYRF5*, and MYRF6) were among the DEGs affected by Se. We found over-represented pathways related to AMPK, insulin resistance, and muscle growth. Our findings suggest a beneficial effect of Se supplementation on muscle development in the last third of gestation as the myogenic factors were upregulated. However, further investigation is still needed. [Please see Diniz et al. (2021) for the details].

1. INTRODUCTION

In ruminant production systems there is potential for periods of inadequate nutrition during pregnancy due to limited quantity or poor forage quality. In particular, some areas lack adequate selenium (Se), which is an essential trace mineral needed for normal growth and health of beef cattle (Mehdi and Dufrasne, 2016). Subclinical Se deficiency has been linked to poor growth and a greater incidence of subclinical diseases. Additionally, inadequate maternal nutrition during critical windows of pregnancy negatively affects fetal development (Reynolds and Caton, 2012). These adverse effects include metabolically compromised offspring with reduced production efficiency (Reynolds et al., 2017). Thus, maternal Se deficiency can compromise the offspring's growth, health, and reproduction.

Our preliminary studies showed significant improvements in production and health outcomes of cattle with supranutritional Se-supplementation. However, the interplay between maternal nutrition, including Se supplementation, and offspring developmental programming, is still to be elucidated. Therefore, **our specific objectives were to investigate the effects of maternal supranutritional organic Se supplementation in different trimesters of pregnancy on whole blood and muscle Se concentration and muscle gene expression profile of newborn calves**.

2. PROCEDURES

During the breeding season, cows were assigned to one of four groups (control and groups 1, 2, and 3 corresponding to the trimester of Se-treatment: CTR, TR1, TR2, and TR3, respectively), using a randomized complete block design. Crossbred Angus cows were bred to one sire using artificial insemination with sexed semen. Cows that did not become pregnant with the sexed semen were bred using several bulls. Except in the CTR group, cows received Se-supplementation during their corresponding pregnancy trimester in the form of three Se-yeast (Phibro Selenium Yeast 2000, Prince Agri Products, Inc., Quincy, IL, USA) boluses per week for 13 weeks, equaling 105 mg Se/wk throughout their treatment trimester. Additionally, cows had free access to a mineral supplement containing 120 mg/kg Se (US FDA regulations) from Na selenite.

Within 12 to 48 h of birth, whole blood (WB) samples and *Longissimus dorsi* (LD) muscle biopsies were collected from 40 calves (10 calves per group). Whole blood and muscle Se concentrations were determined by a commercial laboratory (Utah Veterinary Diagnostic Laboratory, Logan, UT, USA) using an ICP-MS. Statistical analysis of Se concentrations was performed using PROC GLM in SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA). Fixed effects were the treatment group of the dam and the sex of the calf. All tests were two-sided. Statistical significance was declared at $p \leq 0.05$.

Total RNA was isolated from the LD muscle following the manufacturer's protocol. Library preparation and RNA sequencing were performed by Novogene Co. (Nanjing, China). The RNA-Seq data was analyzed to remove low-quality reads, and the gene abundance profile was measured by mapping and counting the reads to a reference genome. In addition, low or non-expressed genes were filtered out. To identify genes that were differentially expressed (DEG) between the groups, we used the CeTF R-package. DEGs were considered significant at an FDR-adjusted *p-value* $\leq$ 0.05 and $|\log2\ FC| \geq 1.5$. To identify the biological processes and KEGG pathways over-represented by the DEGs, we used ShinyGO and WebGestalt. Significant results were

retrieved after *p*-value adjustment using the Benjamini–Hochberg method (FDR $p \leq 0.05$).

3. RESULTS & DISCUSSION

In calves, supranutritional maternal organic Se-supplementation during the first trimester decreased birth weights compared with control but had no impact on birthweights when maternal supplementation was in the second or third trimester. On the other hand, Se supplementation during gestation increased WB-Se concentrations of calves at birth in all three Se-supplemented groups ($p < 0.01$) (Figure 1A). Conversely, muscle Se concentrations were increased only in calves from dams receiving Se supplement in the third trimester of gestation ($p < 0.0001$) (Figure 1B). We conclude that maternal supranutritional supplementation of Se during the latter stages of pregnancy does not impact fetal growth; however, maternal supplementation during the first trimester of pregnancy may decrease birth weights.

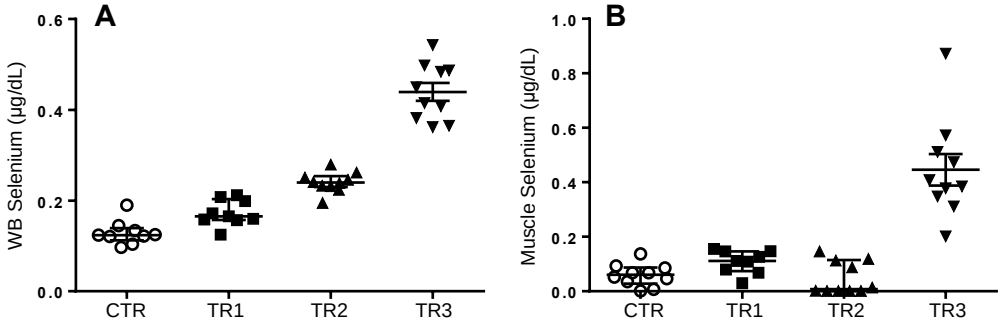

Figure 1. Comparison of selenium concentrations in whole blood (WB) (**A**) and muscle (**B**) at the birth of calves born to dams receiving organic supranutritional Se-yeast supplementation during different trimesters of pregnancy.

The fetal period is critical for muscle development as unbalanced or inadequate maternal nutrition limits myogenesis. Findings in the current study revealed that the effect of supranutritional maternal organic Se supplementation may differ by pregnancy trimester. After quality control of the RNA-Seq data, 12,964 genes were tested for differential expression. We identified 3,048 unique differentially expressed genes (DEGs) across all group comparisons (*FDR* ≤ 0.05 and $|\log2FC| \geq 1.5$) (Figure 2). Selenoprotein-coding genes (*SELENOO*, *SELENOP*, *GPX1*, and *GPX3*) and myogenic factors (*MYOD1*, *MYOG*, *MYRF5*, and *MYRF6*) were among the DEGs affected by Se. Interestingly, in calves born to TR1 cows, genes that act in muscle development (specifically muscle structure-related genes) were downregulated. Conversely, genes involved in muscle function (specifically myosin and actin filament-associated genes) were upregulated in calves from the TR3 group.

The most striking changes in LD muscle transcriptome were independent of the trimester of Se supplementation. These genes were downregulated in the LD muscle transcriptome of newborn calves from Se-supplemented cows. The over-represented pathways were antigen processing and presentation processes (genes mainly from the *BOLA* family).

Findings of the current study revealed that the effect of supranutritional maternal organic Se supplementation may differ by pregnancy trimester. Selenium plays a pivotal role in programming muscle gene expression. Additionally, Se differentially modulates offsprings' muscle gene expression according to the trimester of pregnancy. The results suggest a beneficial effect of Se supplementation during the last third of

gestation as the myogenic factors were upregulated. Further investigation, however, is still needed to confirm this finding and the long-term consequences on offspring muscle development and function.

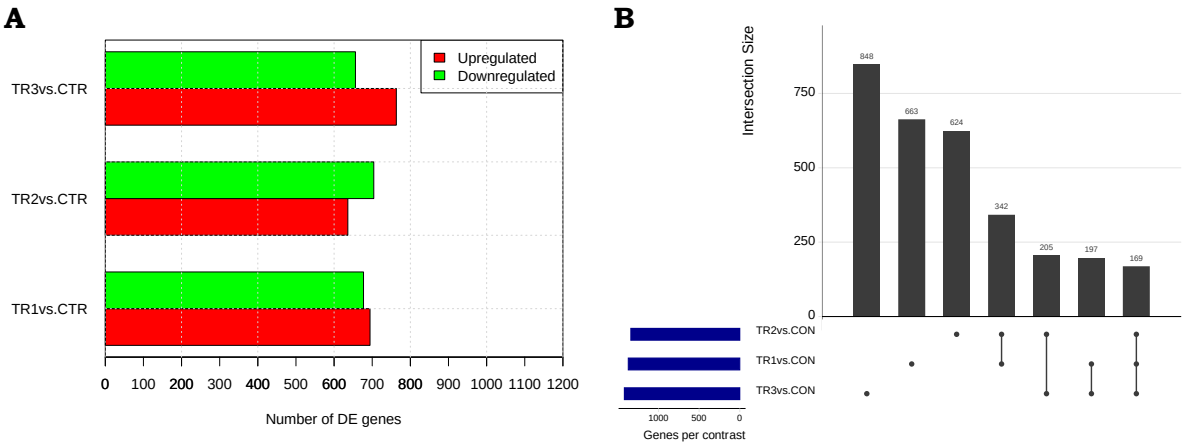

Figure 2. Differential gene expression of *Longissimus dorsi* muscle of calves born to dams fed supranutritional selenium during different trimesters of pregnancy. The number of genes differentially expressed (DEGs) for each contrast (**A**). The UpSet plot represents the intersection between the sets of DEGs from different contrasts (**B**). Each vertical bar shows the number of genes in the intersection. The dot plot reports the set participation in the intersection, and the horizontal bar graph reports the set sizes (total of DEGs).

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Strengthening Alabama Cattlemen by Using the Residual Feed Intake (RFI) Model to Identify Genetic Pathways in the Brain that Regulate Feed Efficiency and Reproductive Outcome in Angus Heifers

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TAKE HOME MESSAGE

Our overall goal is to increase the sustainability of beef production by enhancing the ability of cattlemen to make more money and to better support their families. Sustainably increasing food production is vital to our nation's nutritional security and is critical to human health and wellbeing. Thus, strengthening Alabama cattlemen strengthens America. Cow-calf operators in the Southeast are especially vulnerable to pressures on inputs, given the associated feeding costs and the need to continually improve the efficiency of their seedstock. Our poor understanding of the genetic regulation of feed efficiency is preventing the development of tools that cattlemen need to overcome such challenges. Furthermore, our research suggests that reproductive function and feed efficiency are linked by overlapping regulatory pathways centered in the brain. Thus, we seek to strengthen cattlemen by focusing on the neural regulation of these traits to identify the genetic signature of the "elite heifer," i.e., a highly efficient, highly fertile animal. This knowledge will allow producers to rapidly improve their genetic base with a precision that directly impacts their bottom line by improving feed efficiency while also selecting for optimal reproductive performance.

SUMMARY

Our overall goal is to increase the sustainability of beef production by enhancing the ability of cattlemen to make more money and to better support their families. Sustainably increasing food production is vital to our nation's nutritional security and is critical to human health and wellbeing. Thus, strengthening Alabama cattlemen strengthens America. Cow-calf operators in the Southeast are especially vulnerable to pressures on inputs given the associated costs of feeding and the need to continually improve the efficiency of their seedstock. Our poor understanding of the genetic regulation of feed efficiency is preventing the development of tools that cattlemen need to overcome such challenges. Furthermore, our research suggests that reproductive function and feed efficiency are linked by overlapping regulatory pathways centered in the brain. Thus, we seek to strengthen cattlemen by focusing on the neural regulation of these traits to identify the genetic signature of the "elite heifer", i.e., a highly efficient, highly fertile animal. This knowledge will allow

producers to rapidly improve their genetic base with a precision that directly impacts their bottom line by improving feed efficiency while also selecting for optimal reproductive performance.

1. INTRODUCTION

Beef production in Alabama alone has a $3 billion annual impact on the state's economy which is multiplied severalfold when considering the impact of affiliated satellite industries. Considering total herd production efficiency across the industry, 65% of feed energy is utilized by the reproducing cow herd as opposed to growing cattle reared in feedlots (Nielsen et al., 2013). Cow-calf operators in the Southeast are therefore especially vulnerable to pressures on inputs given the associated costs of feeding and the need to continually improve the efficiency of their seedstock (Archer et al., 2004; BIF, 2010). In order to facilitate the increases in feed efficiency necessary to meet threats to this critical supply chain, new tools and strategies need to be developed to help producers successfully respond to these challenges.

Mechanisms that regulate dry matter (feed) intake influence feed efficiency, a critical aspect impacting the sustainability of beef production. Identifying novel selectable markers and new targets for potential therapeutic interventions to control appetite will facilitate rapid improvements in feed efficiency across all sectors of the cattle industry. The importance of the hypothalamus in controlling feed intake and energy balance suggests this region of the brain may influence feed efficiency in ruminants. Thus, our recent efforts have been directed toward determining associations between RFI status and the expression of genes known to regulate hunger within the hypothalami of cattle. For instance, some neurons in this region express the NPY and AgRP genes which stimulate feeding. Other opposing neurons express the POMC gene, which gives rise to POMC-derived α-MSH that then inhibits feeding behavior. It is of interest to know if the expression of these genes differs between low (efficient) and high (inefficient) RFI cattle. If so, these gene targets might be useful as markers enabling better selection strategies.

Unfortunately, multiple studies suggest that low RFI females tend to conceive later and calve later than high RFI females, most likely attributed to a delay in the first estrus (Arthur et al., 2005; Basarab et al., 2007; Donoghue et al., 2011). Shaffer et al. (2011) observed a 1-unit increase in RFI corresponded to a decrease in age at puberty by 7.5 days with heifers classified as high RFI reaching puberty 13 days earlier than their low RFI counterparts (414 vs. 427 days of age). Likewise, we have observed that low RFI animals exhibit decreased expression of hypothalamic GnRH, the gene that stimulates gonadotropin-induced estrous. It is important to better clarify the relationship between GnRH-producing neurons and those neurons which regulate feeding to avoid unintended negative impact on selecting for RFI upon reproductive performance.

Our research team is well trained and uniquely positioned to study these issues due to 1) the research infrastructure at Auburn University, such as the Calan Gate system at the Auburn Beef Evaluation Center, which allows assessment of residual feed intake (RFI) in growing cattle, 2) the seven research stations across Alabama that house cow-calf herds, and 3) because of the synergistic expertise and research interests of our team members.

2. PROCEDURES

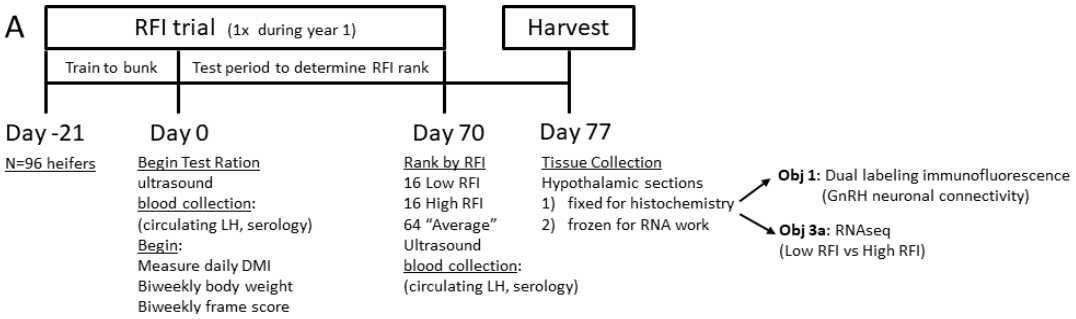

Figure 1. Schematic depicting our experimental approach. **Panel A** illustrates the RFI model workflow to identify Low and High RFI heifers that will be used to examine GnRH neuronal connectivity and to compare genetic pathway differences between Low and High cohorts.

3. RESULTS & DISCUSSION

*3.1. Targeted gene expression studies reveal divergent hypothalamic gene profiles in Low and High RFI individuals while also implicating the gonadotropin axis as a novel pathway influencing feed efficiency in cattle***.**

We initially examined the expression of hypothalamic genes that are known to regulate feeding behavior to determine their role in the divergent feed efficiencies observed between Low and High RFI cattle. Targeted gene expression studies using real-time PCR revealed that mRNA for the hunger suppressing gene, Pro-opiomelanocortin (*POMC*), was 350% higher in efficient steers (Low RFI). Meanwhile, mRNA expression for the hunger-stimulating genes, neuropeptide Y (*NPY*), and relaxin-3 (*RLN3*) was 64% and 62% lower in these more efficient animals relative to their High RFI counterparts (P <0.01; Figure 2). Such a gene expression signature would be predicted to inhibit feeding behavior and indeed efficient (Low RFI) steers exhibit significantly lower dry matter intake compared to their less efficient counterparts. Importantly, these data indicate that Low and High RFI cattle display divergent hypothalamic gene expression profiles implying that gene expression patterns in the arcuate nucleus may regulate feed efficiency and underlie the RFI phenotype.

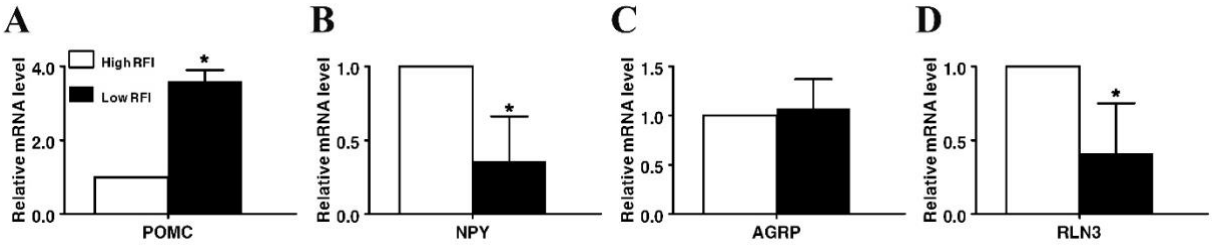

Figure 2. The mRNA expression within the arcuate nucleus of hunger suppressing neuropeptide, (A) Pro-opiomelanocortin (*POMC*), and hunger stimulating neuropeptides (B) neuropeptide-Y (*NPY*), (C) Agouti-related protein (*AGRP*), and (D) relaxin-3(*RLN3*) in efficient (Low RFI) and inefficient (High RFI) steers. Expression was determined by real-time RT-PCR. Values were normalized to *EIF3K* expression. Data is expressed as fold change relative to High RFI steers and calculated according to Pfaffl (2010). Bars denoted by * differ (P < 0.05), n = 14.

In these studies, we also observed a striking reduction in *GnRH* expression in Low RFI steers with levels of *GnRH* mRNA being barely detectable compared to their High RFI counterparts (Figure 3). Importantly, this reduced hypothalamic expression of *GnRH* was accompanied by decreased expressions of *FSHβ* and *LHβ* mRNA in the pituitaries of Low RFI steers, indicating that altered levels of hypothalamic *GnRH* had a functional consequence on the pituitary (Perkins et al., 2014 a, b).

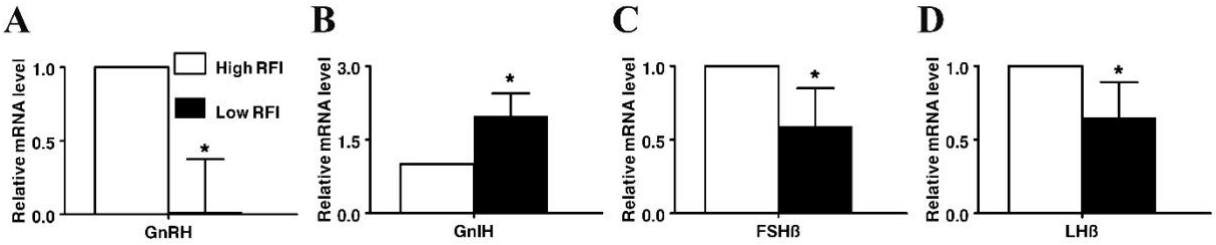

Figure 3. The hypothalamic expression of (**A**) gonadotropin releasing hormone (*GnRH*) and (**B**) gonadotropin inhibiting hormone mRNA and pituitary expression of (**C**) follicle stimulating hormone beta polypeptide (*FSHβ*) and (**D**) luteinizing hormone beta polypeptide (*LHβ*) mRNA in efficient (Low RFI) and inefficient (High RFI) steers. Expression was determined by real-time RT-PCR. Values were normalized to *EIF3K* expression. Data is expressed as fold change relative to High RFI steers and calculated according to Pfaffl (2010). Bars denoted by * differ ($P < 0.05$), n = 14.

Importantly, the suppressed expression of *GnRH* concomitant with elevated *GnIH* in Low RFI steers supports the hypothesis that the gonadotropin axis regulates feed efficiency. This conclusion is also consistent with the emerging role for GnIH as an orexigenic factor which antagonizes GnRH (Johnson et al., 2007; Qi et al., 2009; Clarke et al., 2012). However, these studies and our preliminary data present a conflicting story with regards to the potential impact of the gonadotropin axis upon feed intake. In our RFI studies, animals displaying decreased dry matter intake also displayed lower hypothalamic *GnRH* and higher *GnIH* expression. However, this expression profile was associated with elevated feed intake in rodents, primates and the ovine (Johnson et al., 2007; Qi et al., 2009; Clarke et al., 2012). Nonetheless, these data suggest that manipulating the gonadotropin axis represents a strategy to increase feed efficiency, while these discrepancies within the emerging literature highlight the need to further clarify the role of GnRH in regulating feed intake and efficiency in the bovine.

Dual labeling immunofluorescence demonstrated that a proportion of GnRH-ir cells possessed close oppositions with Kp-ir (42.1%) or GnIH-ir (32.2%) fibers (Figure 4). These proportions did not change between estrus and diestrus animals.

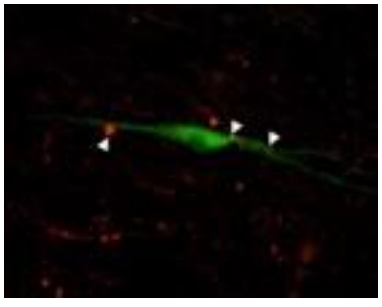

Figure 4. GnRH connectivity in the bovine brain. Representative image of a GnRH-ir cell (green) with Kp-ir fibers (red) in close association (white arrows).

In summary, GnRH and several GnRH-modulating neuropeptides (both inhibitors and stimulators) are co-expressed in the bovine hypothalamus and preoptic area. In addition, these mediators of GnRH release display neuroanatomical connectivity to GnRH neurons. These findings demonstrate that, with the proposed techniques, we

can discern both changes in GnRH protein levels and characterize the connectivity between GnRH and other neuropeptide neurons in the hypothalami of cattle. Our gene data support the hypothesis that gene expression patterns are different in biologically meaningful ways within the hypothalami of Low and High RFI cattle.

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Gene and Metabolite Targets to Predict and Improve Reproductive Outcome in Beef Heifers

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TAKE HOME MESSAGE

Fertility is a complex, multifaceted trait involving several biological systems. Sub-fertility in beef heifers is a major source of production inefficiencies and negatively impacts the profitability of cow-calf production systems. While several studies have looked at beef heifer fertility using single omics systems, we have utilized a multi-omics approach to better identify the biological pathways potentially playing a role. Through this study, we have identified unique genes, metabolites, and gene-metabolite pairs that may provide insight into selecting heifers with a high reproductive potential to serve as replacements. Furthermore, integrating this information may provide a better understanding of the underlying causes of heifer sub-fertility.

SUMMARY

Sub-fertile heifers lead to significant sunk costs for Alabama cow-calf producers. Cutting-edge omics technologies have provided an opportunity to improve reproductive efficiency. For this study, we use transcriptomic (gene) and metabolomic (metabolite) profiles from peripheral white blood cells (PWBC) and plasma of beef heifers. To begin with, Angus-Simmental cross heifers were put through an estrous synchronization (ES) artificial insemination (AI) program, followed by three natural breeding opportunities. We collected blood at the time of AI, separated it into PWBC and blood plasma samples and stored it until pregnancy checking. Following pregnancy checking, we categorized the heifers into high-performing (pregnant by AI) and low-performing (open) groups. Previously, we identified genes and metabolites at different levels in the low and high-performing heifers. But the unanswered question remained if there is any gene-metabolite relationship to identify the varying reproductive outcomes. To address this, the report involved using a multi-staged analysis to integrate the data from six each of low and high-performing heifer groups. Using bioinformatics tools, we identified 17 and 37 candidate genes and 18 and 15 metabolites in the high and low-performing heifers. Furthermore, we identified novel gene-metabolite pairs and pathways associated with each group. A detailed understanding of these novel targets and pathways could lead to new therapeutic targets underlying fertility in bovines.

1. INTRODUCTION

Heifer sub-fertility results in significant inefficiencies in cow-calf production. Technological advancements and improvements in management practices have minimized the proportion of sub-fertile heifers, but a persistent population remains open following their first breeding season (Dickinson et al., 2019). Reproductive success relies on many factors, including breeding strategy, nutritional and health management, and genetic background. Therefore, a systematic approach to improve our understanding may help us identify both sub-fertile heifers and the potential genetic pathways responsible.

Currently, it is recommended that producers use traits, such as body condition score, reproductive tract score, and pelvic measurements, as tools to identify heifers with a high potential for fertility or to remove those with low potential. While these traditional methods are effective, many heifers deemed reproductively satisfactory by these parameters fail to conceive. Therefore, new methods or tools remain valuable in identifying and selecting animals with high fertility potential. Among the options, omics technologies have provided opportunities to understand the genetic mechanisms underlying fertility-related traits in cattle. Recently, transcriptomics and metabolomics have provided promising results as potential fertility biomarkers and have improved our understanding of the underlying causes.

Previous research in our lab and through collaboration have looked at the ability of markers in the blood of heifers to distinguish between high and low-reproductively performing heifers. We found differential expression of *6 genes* in peripheral white blood cells that were associated with pregnancy outcomes in beef heifers (Dickinson et al., 2018). Similarly, analyzing the metabolites present in the blood plasma, we identified 15 at different levels in the high and low-performing heifers (Phillips et al., 2018). While these studies provided insights into heifer fertility, the scope was limited due to their reliance on a single omics technology. The dependence on a single technology disregards the interactions between genes and/or metabolites. Therefore, it is likely that important information can be gained by a more systematic approach that exploits the biological layers and their interactions.

In this study, **our main objective was to unravel the gene expression and metabolomic networks and their interactions to provide insights into the pathways underlying heifer fertility.**

2. PROCEDURES

2.1. Animal Use

All procedures involving animals were approved by the Auburn University Institutional Animal Care and Use Committee (IACUC). Heifers utilized for this study originated from and were housed at a single Research and Extension Center located in Alabama, U.S.A., as part of the Alabama Agricultural Experiment Station. Selected heifers at all locations were placed on pasture (fescue/bermuda grass) from weaning until calving with free-choice ryegrass hay available. All heifers received soyhull/corn-gluten mixture supplementation and trace minerals ad libitum. Angus-Simmental heifers underwent an estrus synchronization and fixed-time artificial insemination program (7-day CO-Synch + CIDR). Heifers were then artificially inseminated with a single straw of semen originating from selected Angus sires 54 ± 2 h following CIDR removal. A second intramuscular injection of 100 μg GnRH was administered at the time of artificial insemination (AI). Fourteen days following

AI, heifers were exposed to an intact sire for three consecutive estrous cycles. Bulls at each research facility were all proven breeders. All bulls passed a standard BSE (Breeding Soundness Exam) with semen quality having <10% abnormality, and all were cleared for any reproductive discrepancies for each breeding season.

2.2. Pregnancy Determination

Pregnancy was determined at 45- and 65-days post AI via transrectal palpation by a trained veterinarian. Heifers were identified as pregnant (AI-Pregnant), pregnant (Bull-Pregnant) or non-pregnant (open) based on the presence or absence of the conceptus. In this study, only samples from heifers remaining open following the AI and natural breeding exposure (open - low performers) and those impregnated through AI (high performers) were analyzed.

2.3. Data Collection

The RNA-Seq and metabolomic datasets previously generated by our group and publicly available were used in the current study. Raw gene counts of 12 samples classified as high or low performers with six animals in each group from an Alabama Research and Extension Center were downloaded from the GEO database (accession number GSE103628) (Dickinson et al., 2018). From the same samples, blood plasma untargeted metabolomic profile was performed on gas chromatography (GC) coupled to time-of-flight mass spectrometry (TOF-MS) (GC-TOF-MS—Agilent 6890 GC). Analytical details were described by Phillips et al. (Phillips et al., 2018).

2.4. Analysis

The RNA-Seq and metabolomic datasets were subjected to an in-house bioinformatics workflow for statistical analysis. The difference in the levels of the genes from RNA-Seq datasets was ascertained using tools such as edgeR and DESeq2. The correlation of the genes and metabolites with the other potential candidates was tested with the partial correlation and information theory (PCIT) approach. The interactions, differential connections of the genes with the other genes and metabolites, and identification of hubs were made through networks constructed with Cytoscape software. The genes and metabolites were analyzed for connectivity gain or loss and biological pathways with respect to reproductive performance. The integration of the targets from gene and metabolite analysis was done using the IntLIM and PCIT approaches.

3. RESULTS & DISCUSSION

Improving our understanding of the genetic basis involved in reproductive outcomes may provide a way for producers to informatively select high-performing replacement heifers in their cow-calf operations. Therefore, we focused on a multilayered approach to how the regulation of upstream gene expression patterns affects downstream metabolic indicators in high and low-performing beef heifers. Although reproduction and fertility involve the regulation of multiple tissues, we focused to measure the expression of genes and the metabolite concentration profiles in the blood for two reasons: (i) The blood is a relatively non-invasive sample to measure molecules for predicting fertility in beef heifers, and (ii) the blood represents a whole organism view into the health and phenotype status of the heifer.

To systematically identify all molecules and their interactions exhibited in the blood for fertility, we used a gene and metabolite co-expression network approach.

This approach identified the correlations and recognized the genes and metabolites playing a role in the reproductive outcome. To accomplish this, we first identified the gene expression differences and retrieved the metabolite differences from our previous study (Phillips et al., 2018). We identified 38 genes and 15 metabolites from the blood plasma at different levels in the high and low-performing beef heifers (Figure 1).

Next, we built co-expression networks and used the identified differential genes and metabolites as features. We identified the hubs and differentially connected genes/metabolites with significant biological roles. We identified 17 and 37 hub genes in the high and low-performing groups, respectively. Further, we identified *TGM2*, *TMEM51*, *TAC3*, *NDRG4*, and *PDGFB* as more connected in the low-performing heifers' network. The low-performing gene network showed a connectivity gain due to the rewiring of major regulators.

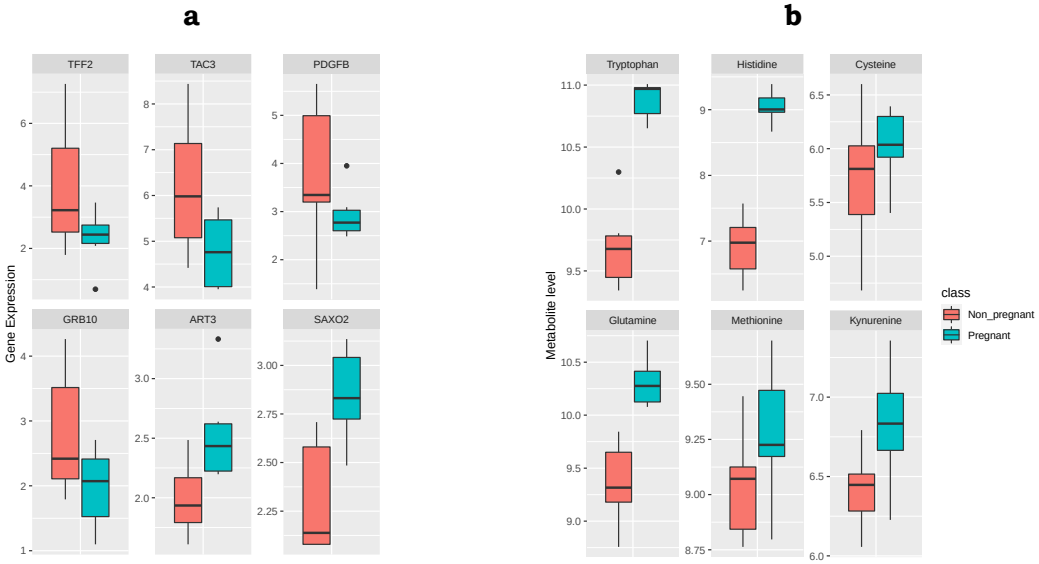

Figure 1. Representative image of **(a)** differentially expressed genes and **(b)** different levels of metabolites in pregnant (high-performing) *vs.* non-pregnant (low-performing) groups.

The metabolomic analysis identified 18 and 15 hub metabolites in the high and low-performing networks. Tryptophan and allantoic acid exhibited a connectivity gain in the low and high-performing groups, respectively. After integrating with the PCIT approach, we identified 1,161 gene-metabolite pairs in the high and 155 pairs in the low-performing groups. The gene–metabolite pairs from the high-performing group, were over-represented by oocyte meiosis, progesterone-mediated oocyte maturation, and the PI3-Akt signaling pathway. In contrast, glyoxylate and dicarboxylate metabolism, alanine, aspartate and glutamate metabolism, and glucagon metabolism were over-represented by the low-performing group. To reduce data dimensionality and retrieve significant gene-metabolite pairs, we overlapped the results of PCIT with IntLIM. We identified significant gene-metabolite pairs, such as tocopherol- a, which was positively correlated in the pregnant group with *ENSBTAG00000009943* and negatively correlated with *EXOSC2*, *TRNAUIAP*, and *SNX12* in the low-performing group. Furthermore, in the low-performing group, we identified tryptophan-*MTMR1* and a-ketoglutarate-*ALAS2* were negatively correlated, while a-ketoglutarate-*SMG8* and putrescine-*HSD17B13* pairs were positively correlated.

Among the differentially expressed genes, we identified *TAC3* (tachykinin precursor 3) as a hub with a network connectivity gain in the low-performing group. The protein encoded by *TAC3* modulates gonadotropin-releasing hormone (GnRH),

which is responsible for the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Thus, the upregulation of *TAC3* identified in blood, and yet the heifers not becoming pregnant, may suggest an alteration in the hormonal and sex-steroid levels. We identified *PDGFB* (platelet-derived growth factor) as differentially expressed and connected with a connectivity gain in the low-performing group. *PDGFB*, identified as a constituent of blood serum and platelets, is essential for female fertility. In our study, *PDGFB* is negatively and differentially correlated with the metabolite hydrocinnamic acid in the low-performing group. Hydrocinnamic acid has antioxidant and anti-inflammatory properties. Phenolic acids, also called polyphenols, have been associated with fertility, development, and fetal health. Collectively, these findings suggest that the over-expression of the *PDGFB* gene and the downregulation of hydrocinnamic acid in the low-performing group triggered the signaling pathways, likely regulating the inflammatory response. Controlled inflammation is necessary for host defense, while an uncontrolled inflammatory response may affect fertility.

We identified the metabolite tryptophan is differentially connected with a connectivity gain in the low-performing group. Tryptophan was negatively correlated with *MTMR1* (myotubularin-related protein 1), a hub gene in the low-performing group. Tryptophan levels are influenced by dietary and hormonal factors. Previous literature shows that, besides fulfilling the demands of maternal proteins, tryptophan is required for fetal growth and development. The need for tryptophan varies as the pregnancy progresses. In early and mid-pregnancy, increased maternal tryptophan availability helps meet the demand for protein synthesis and fetal development in humans. Downregulation of tryptophan could be one of the possible reasons for the infertility of beef heifers in our study.

The current study focused on identifying putative major regulators associated with varying reproductive outcomes. We investigated the differences in the gene or metabolite expression and co-expression in beef heifers. An integrated gene–metabolite analysis approach unveiled the potential gene–metabolite pairs affecting biological processes related to fertility in beef heifers. The genes, metabolites, and interactions identified in the present study over-represented some pathways playing roles in fertility. Some of the genes and metabolites were supported by the previous literature; however, some other targets identified were novel and warrant further detailed studies to evaluate the repeatability of our results in a larger cohort. As the heifers undergo variations during the estrus cycle and pregnancy, it is obvious to expect alterations in genes and metabolite profiles at a particular time point. This means that strategies for applying this information in real-time farming appear to be more complex and require further research. Validation of these results in more samples and different time points would help to establish a framework for future fertility prediction using gene and metabolite biomarker profiles that could be practical for on-farm use and improve reproductive efficiency.

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Metabolite Differences in the Blood Plasma of Beef Heifers with Differing Fertility Potential

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TAKE HOME MESSAGE

Unexplained beef heifer subfertility was investigated through blood plasma metabolomics at weaning and at the time of artificial insemination (AI) to identify potential biomarkers and the dysregulated metabolic pathways leading to subfertility. Metabolites, small molecules present in peripheral circulation, show promise as easily accessible diagnostic factors for health and disease status. This study identified nine metabolites at significantly different levels at weaning and six at AI in reproductively divergent heifers. To identify the metabolite levels and metabolic pathways impacted between weaning and AI, the same subjects were studied in these two time points. Though larger test sets and validation in different classes of animals are required, it may be possible to create diagnostic tests for subfertility in heifers at weaning.

SUMMARY

Subfertility in replacement beef heifers represents major losses for the beef production industry. Plasma metabolomics focuses on the global population of small molecules present in the peripheral circulation and has been suggested as a diagnostic tool for discriminating health/disease status. Metabolomic (metabolite) profiles were generated for 6 high-performing (fertile) and 6 low-performing heifers (subfertile) at weaning and at AI. Whole blood was collected from Angus-Simmental heifers at weaning and at AI (post-estrous synchronization). After pregnancy checking and assigning phenotypes (fertile/subfertile), metabolomic profiles were generated. Plasma profiles between fertile and subfertile cohorts were then queried in three ways: 1) at weaning 2) at AI 3) and significant metabolites from within these time points were compared between weaning and AI to show changes over time. At weaning, nine metabolites were significantly different between fertile and subfertile heifers, whereas at AI we found six metabolites. There was no overlap in significant metabolites between time points. Most metabolite levels changed over time and in most cases, the changes were in the same direction, either increasing or decreasing over time in both phenotypes.

1. INTRODUCTION

Reproductive failure of replacement breeding animals is one of the leading causes of loss to beef production systems in the US. Heifers retained for breeding represent large sinks of resources for producers and upon failure to breed, often fail to be fully recouped at sale. Producers are encouraged to use physical and growth traits to select heifers for breeding, including birth date, age, body condition score (BCS), reproductive tract score (RTS), and dam production history. However, even with these selection criteria in place, there will remain animals that fail to conceive and bear a calf in their first breeding season (Moorey & Biase, 2020).

Metabolites, small molecules present in circulation and tissues, are often the products of or precursors to integral metabolic processes like protein synthesis or hormone synthesis and dysregulation of their levels can be hypothetically traced back to cellular-level dysfunction. Metabolomics is the study of global populations of metabolites, often utilizing high-throughput mass spectrometry and gas chromatography to create 'profiles' of study subjects. As a discipline, metabolomics has shown promise in discriminating disease states from healthy states by showing differences in the populations of these small molecules, not only in studies of fertility but also in studies of metabolic dysfunction, like fatty liver disease in dairy cattle (Zhang et al., 2022). Testing for these altered populations in blood plasma is desirable for its ease of access to researchers, clinicians, and producers.

Previous work by our lab (Phillips et al., 2018) examined metabolomic profiles of 10 subfertile animals and 10 fertile animals at AI and identified fifteen metabolites present at significantly lower levels in subfertile animals when compared to fertile animals. While this study provided interesting results at AI, many producers make decisions on heifer culling much earlier, sometimes as early as weaning. Therefore, **the objective of the present study was to generate metabolomic profiles of fertile and subfertile animals at weaning and AI and examine the changes in the metabolite level over time i.e., from weaning to AI.** This will help to identify time-point differences in the metabolite levels of fertile vs. subfertile beef heifers and to determine the pathways that are potentially dysregulated over time.

2. PROCEDURES

2.1. Animal Care and Use

All experimental procedures involving live animals were approved by the Institutional Animal Care and Use Committee of Auburn University (IACUC). The heifers utilized in this study were born and housed at the Black Belt Research and Extension Center in Marion Junction, AL. Heifers were born in the fall of 2019, weaned in May 2020, and bred by estrous synchronization and fixed-time AI in December 2020. Heifers were bred by a trained technician with one straw of semen from a proven fertile bull. Fourteen days post-AI, heifers were exposed for 60 days to a clean-up bull that had passed a BSE (Breeding Soundness Exam). Heifers were then checked for pregnancy by ultrasound and a trained technician. Animals were classified as pregnant to AI (fertile) or not pregnant (subfertile). Twelve animals were utilized for this study, six fertile heifers that bred to first service AI and six subfertile heifers that either bred late to a bull or not at all, remaining open at the end of the breeding season.

2.2. Sample and Data Collection

Whole blood samples were collected via jugular venipuncture at weaning and AI and blood plasma was isolated for further analysis. Information regarding adjusted birth weight, adjusted weaning weight, adjusted yearling weight, and age at AI were collected from archived information maintained by the staff of the research station. Information regarding BCS on the day of blood sample by and RTS was evaluated 30 days prior to breeding by trained technicians. Plasma samples were submitted to the West Coast Metabolomics Center at UC Davis (Davis, California) for profiling via gas chromatography-mass spectrometry.

2.3. Data Analysis

Metabolomic profiles were analyzed using the online metabolomics toolset MetaboAnalyst 5.0. For profiles compared within time points (i.e., weaning and AI), statistical analysis was constrained to Student t-testing using MetaboAnalyst 5.0 and Prism 5. Prism 5 was used to generate visualizations of metabolite levels. Significance in statistical testing was declared when $p \leq 0.05$.

3. RESULTS & DISCUSSION

There were no significant differences between fertile and subfertile animals when measures of adjusted birth weight (Adj. BW), adjusted weaning weight (Adj. WW), adjusted yearling weight (Adj. YW), age at AI (days), BCS, or RTS were analyzed. Data for growth and maturity measures are shown in Table 1.

Table 1. Average measures (mean ± SD) for adjusted birth weight (lb.), weaning weight (lb), and yearling weights (lb); age at AI (days), body condition score (BCS), and reproductive tract score (RTS).

Phenotype	Adj. BW	Adj. WW	Adj. YW	Age at AI	BCS	RTS
Subfertile	72.5 ± 17.9	581.7 ± 60.9	762.7 ± 110.5	441.2 ± 9.5	5.5 ± 0.6	4.2 ± 1.2
Fertile	70.2 ± 11.3	587 ± 51.7	762.0 ± 74.9	444.2 ± 5.4	4.2 ± 0.8	5.7 ± 0.6
P-value	0.793	0.873	0.990	0.518	0.628	>0.999

At weaning, nine metabolites were present at significantly different levels between fertile and subfertile heifers' metabolomic profiles ($p \leq 0.05$). Lactic acid, maltose, and nicotinamide were present at higher levels in fertile heifers, while arabitol, indole-3-propionic acid, glycolic acid, lyxitol, indole-3-lactate, and palmitoleic acid were present at lower levels in fertile heifers. At AI, six metabolites were present at significantly different levels in fertile and subfertile heifers. Tryptophan, lysine, cystine, histidine, ornithine, and arachidonic acid were all at higher levels in fertile animals when compared to subfertile animals. Relative circulating levels of metabolites are shown in Figure 1 at both time points to show changes over time.

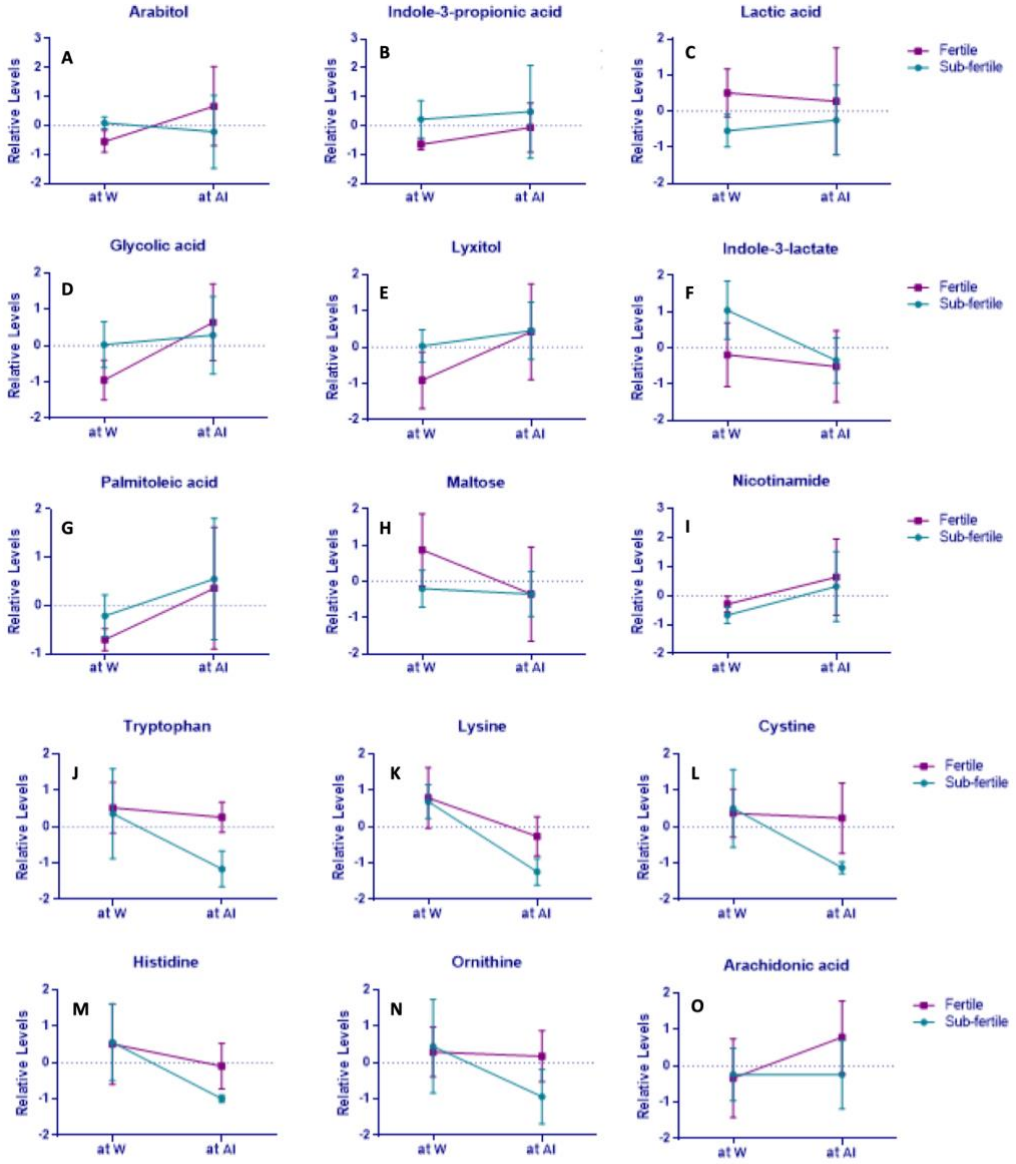

Figure 1. Relative levels of significant metabolites at weaning (Panel A - I) and AI (Panel J - O).

These fifteen metabolites identified at significantly different levels at weaning and AI were then examined across both time points. Arabitol and lactic acid were present at lower levels at weaning in fertile animals and higher levels at AI in fertile animals, with the opposite expression pattern in subfertile animals (i.e., higher at weaning in subfertile animals and lower at AI in subfertile animals). Glycolic acid was lower in fertile animals than in subfertile animals at weaning and higher in fertile animals than subfertile animals at AI. Indole-3-propionic and palmitoleic acid were lower in fertile animals at weaning and AI. Nicotinamide was higher in fertile animals both at weaning and AI. Lyxitol, indole-3-lactate, and maltose showed significant differences at weaning but converged to almost the same measure at AI. Tryptophan, lysine, cystine, histidine, and ornithine showed very similar levels between subfertile and fertile animals at weaning but diverged at AI, falling more drastically in subfertile animals. Interestingly, arachidonic acid also had similar levels at weaning in subfertile and fertile animals. But fertile animals showed an increase in levels at AI while subfertile animals did not show a change in levels. Arachidonic acid

metabolism is closely linked with the production of prostaglandins like prostaglandin F2α, a key factor in estrous cyclicity. The amino acid histidine is closely linked to immune response and inflammation; additionally, a previous study found it present at lower levels in subfertile animals when tested at AI in conjunction with higher inflammatory states (Phillips et al., 2018). In the same study at AI, tryptophan, cystine, ornithine, and lysine were downregulated in subfertile animals. The combination of these results suggests that some dysregulation of amino acid metabolism at AI could contribute to subfertility; however, the molecular mechanisms behind this dysregulation and its effect on reproduction remain to be elucidated.

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Predicting Pregnancy Status in Beef Cows Through Machine-Learning

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TAKE HOME MESSAGE

Female reproductive failure in beef cattle heavily impacts producer economics and production. New technologies, such as machine learning and transcriptomics, are being used to understand better female fertility issues affecting the beef industry. This study involved integrating these technologies on a group of mature recipient cows and searching for genetic predictors of pregnancy outcome (pregnant or open). We identified nine candidate biomarkers that can discriminate pregnancy outcomes before embryo transfer. This project could lead to predictions that aid in the selection of fertile cows and potentially replacement heifers.

SUMMARY

Female reproductive failure is still challenging for the beef industry. Several biological processes responsible for fertility-related traits, such as the establishment of pregnancy and embryo survival, are still unclear. To investigate these processes, we can measure the differences in the abundance of genes that are maybe involved with fertility and, consequently, pregnancy. Herein, we investigated genes that play a potential role (candidate biomarker) in predicting pregnancy status and fertility in crossbred cows. To this end, we analyzed the abundance of genes expressed in the uterine tissue of cows subjected to embryo transfer. Using bioinformatics tools, we investigated the differences in gene abundance between pregnant cows and those that remained open. We identified nine gene biomarkers that could single out pregnancy status in cows. These biomarkers were cooperatively working – co-expressed, with other genes critical for uterine receptivity, including uterine tissue restructuring and embryo development. This study laid out critical biological pathways involved with pregnancy success and provided predictive candidate biomarkers associated with pregnancy outcomes in cows. [Please see Diniz et al., (2022) for the details.]

1. INTRODUCTION

The sustainability of a cow-calf production system relies on the efficiency of reproductive performance per cow. However, a decline in cattle fertility has led to increased reproductive failure (Han and Peñagaricano, 2016; Taylor et al., 2018), which is a challenge for beef producers and a significant cause of economic loss (Mercadante et al., 2020). Fertility is a lowly heritable trait affected by genetic and management factors (Taylor et al., 2018). Despite the limited selection response to traditional selective breeding strategies, reproductive and genomic technologies have provided opportunities to improve reproductive efficiency (Mercadante et al., 2020). Several candidate genes and biological processes have been identified through genome-wide association studies (GWAS) (Ortega, 2018). Likewise, genomic testing and selection, mainly in dairy cattle, has increased the rate of genetic improvement for female fertility (Taylor et al., 2018).

Other approaches, such as transcriptomics and metabolomics, have shed light on the biological processes underlying cattle fertility (Moorey and Biase, 2020). These approaches, however, generate a large amount of data. Lately, machine learning algorithms have provided opportunities to mine these data and strengthen the ability to predict pregnancy outcomes. In this approach, computers use data to identify underlying patterns related to the abundance of genes and make predictions. These genes, however, work cooperatively. Thus, considering multiple gene-gene relationships, a systemic approach can provide a holistic view of female cattle fertility. To this end, gene network approaches have been used to narrow down candidate genes and specific gene interactions.

Our long-term goal is to improve the production efficiency of beef cows by delivering molecular targets for animal selection. In this work, we used a machine learning approach to screen gene expression profiles of uterine samples from recipient cows to predict whether a recipient cow would become pregnant or remain open. Furthermore, we investigated gene-gene relationships and potential regulatory mechanisms involved with pregnancy outcomes and fertility. **Our specific objectives were to identify potential candidate biomarkers that could predict pregnancy outcomes and investigate the biological processes underlying fertility**.

2. PROCEDURES

Public RNA-Seq data from recipient Angus-Brahman crossbred cows (n = 43) were used in this study (Martins et al., 2022). Uterine luminal epithelial cells were sampled three days before embryo transfer from estrous synchronized cows. Pregnancy checks were done by ultrasound 30 days post embryo transfer. Out of the 43 cows, 25 were identified as pregnant and 18 as non-pregnant. From the uterine samples, total RNA was isolated and sequenced. More details on this study can be found in Martins et al., (2022). Once retrieved from the GEO public database, the RNA-Seq data was analyzed to remove low-quality reads, and the gene abundance profile was measured by mapping and counting the reads to a reference genome.

Low or non-expressed genes were filtered out. After gene normalization, we used the BioDiscML software as an automated method to select the best statistical model and identify genes with predictive potential (candidate biomarkers). To determine gene-gene interaction, we implemented a co-expression approach based on the PCIT software. Only genes correlated with the candidate biomarkers ($p \leq 0.05$) were kept

for further analysis. Next, we identified whether the number of connections between genes changed due to the pregnancy status of the cows. These gene lists were then used to retrieve biological processes involved with pregnancy and fertility.

3. RESULTS & DISCUSSION

Fertility is a broad term encompassing several traits related to animal reproduction. Herein, we defined fertility as "the ability to conceive and maintain a pregnancy." Based on our approach, we identified nine genes (*SERPINE3*, *PDCD1*, *FNDC1*, *MRTFA*, *ARHGEF7*, *MEF2B*, *NAA16*, ENSBTAG00000019474, and ENSBTAG00000054585) as potential predictors of pregnancy outcome in cows and are reported here as candidate biomarkers. Figure 1 shows the differences in the expression level of the nine candidates between the pregnant and non-pregnant groups. The five statistical models selected for prediction exhibited an accuracy of 61.54%, except one (accuracy = 53.85%). Our results, however, should be interpreted considering the limited sample size used to train the statistical model. Additionally, many other factors are involved with pregnancy success. Therefore, testing a large herd with a similar approach would validate our findings and improve accuracy.

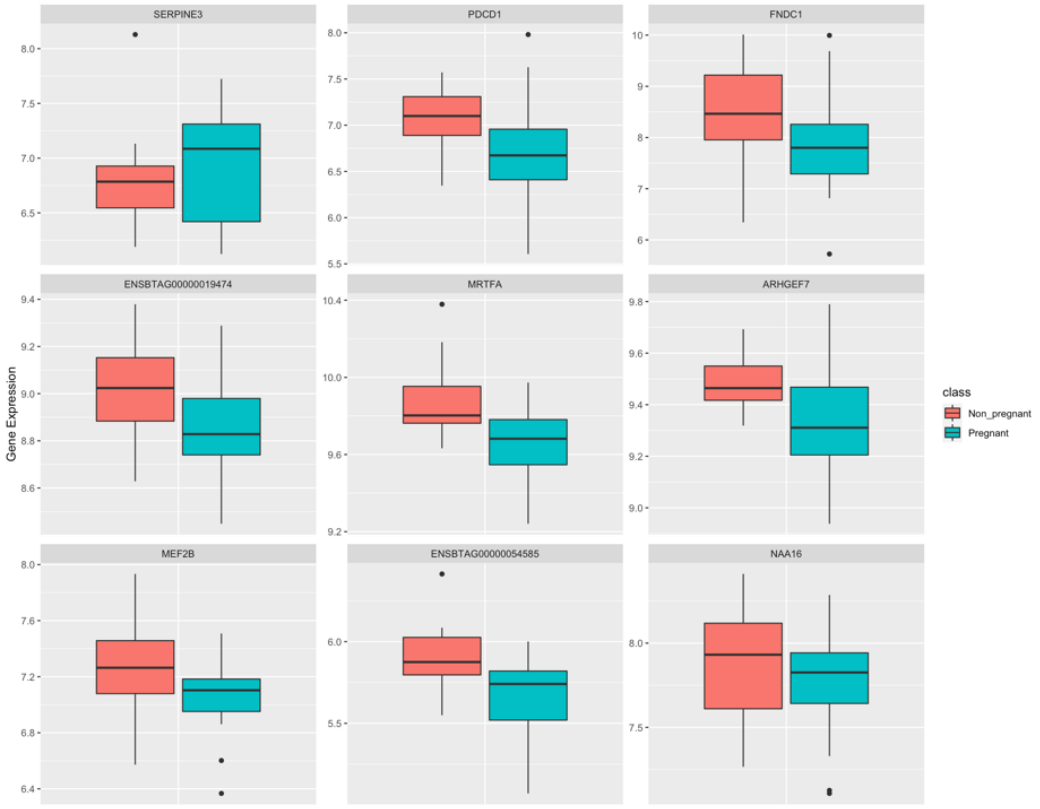

Figure 2. Adjusted gene expression of candidate genes discriminating between pregnant and non-pregnant cows. Horizontal lines within the boxplots represent the average for each group (pregnant and non-pregnant). Black dots represent outliers.

To investigate changes in gene relationships between pregnant and non-pregnant cows, we created co-expression networks from 15,039 genes for each group separately. We used this approach to identify 8,554,787 significantly correlated pairs ($p \leq 0.05$) for pregnant and 7,227,015 for non-pregnant cows, respectively. To mine the data, we kept only gene pairs correlated with the candidate biomarkers (nine genes as identified above). Thus, 5,412 pairs were kept for the pregnant group and

4,204 pairs for the non-pregnant group. By overlapping the gene lists, we identified 1,341 genes that were shared between the groups.

Seven genes were differentially connected – rewired (gaining or losing connections) between pregnant and non-pregnant group networks. The candidate biomarkers identified were more connected in the non-pregnant cows. A significantly increased connectivity in the non-pregnant group networks was identified for the *MEF2B, FNDC1, ENSBTAG00000019474, SERPINE3,* and *MRTFA* genes. Conversely, the genes *NAA16* and *AEHGEF7* were more connected in the network from pregnant cows.

Further, we examined the differentially expressed gene (DEG) list from Martins et al. (2022) to investigate whether these genes were co-expressed with the candidate genes we found. By overlapping the lists, we identified 66 genes that were shared between the pregnant, non-pregnant, and DEG lists. This included *ENSBTAG00000019474, PDCD1,* and *MRTFA*.

Functional analysis of the gene lists was performed to understand biological processes differentially modulated between pregnant and non-pregnant cows. Overlapping genes between the groups (n = 1341) were involved in protein digestion and absorption, ECM-receptor interaction, and focal adhesion. Additionally, we separately analyzed pathways for pregnant (n = 4,382) and non-pregnant (n = 3,166) groups. Unique pathways from pregnant co-expression networks included ribosome, proteasome, and oxidative phosphorylation. Likewise, pathways related to tissue remodeling, such as degradation of the extracellular matrix, collagen formation, ECM proteoglycan, and blood vessel development, were found by co-expressed genes from the non-pregnant group.

We applied a multi-step approach to identify predictive candidate biomarkers and fertility-related co-expressed gene networks. Based on that, we identified nine biologically relevant candidate genes that could predict pregnancy in cows. These genes act in critical biological pathways for uterine receptivity, including endometrial tissue remodeling, focal adhesion, and embryo development. Furthermore, we identified differences in the network connections of biomarker co-expressed genes between pregnant and non-pregnant cows.

In summary, our findings provided new insights into how genes for fertility-related processes interact with one another. We also showed the potential of combining different analytical technologies to focus on candidate genes and shed light on molecular features involved with pregnancy outcomes. Further investigation, however, is still needed to determine the reliability and sensitivity of these genes in larger herds.

Acknowledgments

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The Impact of Antibiotics in Embryo Transfer Flush Media on mRNA Expression of Embryo Health Genes

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TAKE HOME MESSAGE

The use of advanced reproductive technologies is increasing in the cow-calf industry. Embryo transfer is used effectively to rapidly improve herd genetics. Embryos are flushed from high genetic value donor cows, collected from the flush media, then transferred to cost-effective recipients. Media used for embryo flushing commonly contains antibiotics to prevent bacterial infection. However, not much is known of the effects of antibiotics on embryo health. Embryo viability can be ascertained using health-related molecular markers such as *SOD-1*, *TP53* and *BAX*. Therefore, the goal was to determine if there is a significant difference in the expression of health genes between embryos flushed in antibiotic or antibiotic-free flush media. The results suggest that antibiotics in embryo flush media have no effect on mRNA expression of health genes prior to cryopreservation. Further exploration of embryo viability after thawing and transfer to recipient cows to evaluate the long-term effects of flush media on embryo development could improve the success of embryo transfer.

SUMMARY

In beef production, the cow-calf sector is dependent upon reproductive success. Advanced reproductive technologies, such as embryo transfer, can rapidly increase the genetics within a breeding herd. In embryo transfer, embryos are derived from superovulation and artificial insemination then commonly flushed from the donor cow using media which contains antibiotics. Antibiotics are included to prevent bacterial infection of embryos and culture media prior to and during transfer. However, there is little knowledge of the effects of antibiotics present in the flush media on embryo health and viability. We hypothesized that there would be a significant difference in the expression of health-related genes between embryos flushed in antibiotic or antibiotic-free flush media. Embryos were flushed from donor cows in antibiotic or antibiotic-free flush media, and RT-qPCR was performed to evaluate mRNA expression of embryo health genes, *SOD-1*, *TP53*, and *BAX*. The results provided evidence that the incorporation or absence of antibiotics from embryo flush media has no effect on the mRNA expression of the selected genes of interest relating to embryo health. Further exploration of embryo viability results after thawing and transfer to recipient cows to evaluate the long-term effects of flush media on embryo health and development could improve the success of embryo transfer.

1. INTRODUCTION

The cow-calf sector of the beef industry faces many challenges, with infertility and reproductive inefficiency being among the most prevalent and difficult to overcome. Advanced Reproductive Technologies (ART), such as in-vitro fertilization (IVF) and embryo transfer (ET), are tools developed to help producers enhance reproductive efficiency. ET offers an opportunity to increase reproductive success by producing several viable embryos from a single breeding event. ET also allows for rapid improvement of genetics in a breeding herd by expanding sire and dam selection to an international level as cryogenically preserved embryos can be shipped globally (Phillips and Jahnke, 2016). ET has also been shown to overcome challenges to fertility in some cases, such as heat stress, risk of venereal disease, and poor oocyte quality, to name a few. In a multiple ovulation embryo transfer (MOET), donor cows are synchronized into the same stage of the estrous cycle then undergo superovulation in which multiple follicles ovulate at one time. The donor cow is artificially inseminated, and 7 days later, the resulting embryos are flushed from the uterus and uterine horns, collected, and frozen for storage until transfer into a recipient animal. The act of flushing the tract is typically done with media that simulates biological conditions such as Dulbecco's phosphate-buffered saline (DPBS) and includes a surfactant such as Bovine Serum Albumin (BSA) or Fetal Bovine Serum (FBS) which serves to prevent embryos from adhering to the uterine wall or the flush tubing, syringes, filters, and other equipment (Phillips and Jahnke, 2016). The flush media typically includes antibiotics to prevent bacterial infection of the media containing embryos. Previous studies have shown that the presence of antibiotics in embryo culture media can have detrimental effects of development, viability, gene expression, and chromatin integrity (Magli et al., 1996; Liu et al., 2011). However, little is known about the impact of antibiotics present in bovine embryo flush media on the health and viability of the recovered embryos. **The goal of this study was to determine if there is a significant difference in the expression of health-related genes in embryos flushed in antibiotic or antibiotic-free flush media.**

2. PROCEDURES

2.1. Animal Use

All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC). The six *Bos taurus* cows utilized in this study originated from and were housed at the Auburn University College of Veterinary Medicine (AUCVM). Each cow underwent an estrus synchronization, superovulation, and artificial insemination protocol using semen from a bull of proven fertility. 7 days post-artificial insemination, resulting embryos were non-surgically collected through flushing of the uterus and uterine horns. For each animal, one flush was completed with antibiotic-free media while another flush 60 days later was completed with media containing antibiotics. The antibiotic-free flush media contained Dulbecco's phosphate buffered saline (DPBS) combined with 2% fetal bovine serum (FBS). The flush media containing antibiotics was comprised of DPBS, 2% FBS, penicillin (100 U/mL), streptomycin (100 IU/mL), and amphotericin B (0.25 mg/mL).

2.2. Embryo Processing

Flushed embryos were collected in a sterile filter, collection media was used to rinse the filter, and flush and rinse media containing embryos were transferred to a sterile dish. Embryos were then located using a stereoscope and embryo handling pipette. After search and collection, embryos produced by each cow were transferred in groups to sterile cryovials containing 5 uL RNALater (Thermo Fisher Scientific Inc., Waltham, MA, USA) and *snap* frozen in liquid nitrogen. Embryos were then stored at – 80 °C until use.

2.3. RNA Isolation and Quantification and cDNA Synthesis

Total RNA was extracted from embryos using the Arcturus PicoPure RNA Isolation kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Concentration and RNA Integrity Number (RIN) were determined for each sample using the Agilent Bioanalyzer RNA 6000 Pico Kit (Agilent Santa Clara, CA, USA). Only samples with a RIN value greater than 6 were further processed. Complementary DNA (cDNA) was generated from the total RNA using qScript cDNA Supermix (Quanta Biosciences Inc., Beverly, MA, USA) and subsequently diluted 1:5 with nuclease free water.

2.4. mRNA Expression of Embryo Health Targets utilizing RT-qPCR

Primers for the target genes relating to embryo health were designed as well as specificity and efficiency tested including Superoxide Dismutase 1 (*SOD-1*), Tumor Protein 53 (*TP53*), and Bcl-Associated Protein X (*BAX*) (Wrenzycki et al., 2005; Li et al., 2009). Number of samples in antibiotic-free group and antibiotic group were four and five respectively for *SOD*-1 and *TP53*, while four and three, respectively for *BAX*. Primer sequences used for quantitative real time PCR (RT-qPCR) are provided in Table 1. cDNA was combined with PerfeCTa SYBR green supermix (Quanta Biosciences Inc., Beverly, MA, USA) and each forward and reverse primer for the target gene. This reaction mix was used to perform RT-qPCR. The PCR was completed using a Roche LightCycler 480.

2.4. Analysis

Using the delta-delta Ct method, fold change of the mRNA expression was calculated. The expression of the reference gene Histone 2A (*H2A*) present on each plate was used to normalize the expression of target genes between samples (Nino-Soto et al., 2007). Statistical analysis of an unpaired t-test of the delta Ct values was completed using GraphPad Prism v6.01 (GraphPad, San Diego, CA, USA), and the graph of the fold change was made with results reported as mean ± SEM with significance at $p < 0.05$.

3. RESULTS & DISCUSSION

Three target genes of interest related to embryo health were selected for primer design and validation for use in RT-qPCR to compare expression levels between the embryos flushed in antibiotic media and embryos flushed in antibiotic-free media. Primer sequences utilized in this study are given in Table 1.

There was no significant difference between the expression of *SOD-1* between embryos flushed using media with or without antibiotics ($p \geq 0.05$, Figure 1). *SOD-1* is a scavenger of reactive oxygen species and plays a key role in the stress adaptation response of embryos (Wrenzycki et al., 2005). It has been shown that *SOD-1*

expression can be altered by changes in culture environments; however, in this circumstance, the brevity of the period of time in which the embryos were exposed to the antibiotic or antibiotic-free media did not result in differing mRNA expression between the two treatments.

Table 1. Primer sequences for RT-qPCR.

Gene Name	Forward Primer (5'->3')	Reverse Primer (5'->3')
Superoxide Dismutase 1 (SOD-1)	TGTTGGAGACCTGGGCAATG	TTACACCACAGGCCAAACGG
Tumor Protein 53 (TP53)	TCCACAGCCAAAGAAGAAACCAC	TTCCATCCAGAGCATCCTTCAG
Bcl-2 Associated X Protein (BAX)	TTTGCTTCAGGGTTTCATC	CAGCTGCGATCATCCTCT
Histone 2A (H2A)	GTCGTGGCAAGCAAGGAG	GTACTCGGCCGTTAGGTACTC

Similarly, there was no significant difference between the expression of *TP53* in embryos flushed using antibiotic containing or free media ($p > 0.05$, Figure 1). *TP53* is involved in the regulation of cell proliferation, apoptosis, and differentiation in developing embryos (Li et al., 2009). An increase in embryonic expression of *TP53* can result in a slower growth rate or decreased developmental competency due to stress inflicted on the cells of the inner cell mass of the embryo. The antibiotic and antibiotic- free flush media resulted in similar expression levels of *TP53* which can be attributed to a minimal effect of the media treatment on embryo health.

Finally, there was no significant difference between the expression of *BAX* in embryos flushed using antibiotic flush media when compared to embryos flushed using antibiotic-free flush media ($p > 0.05$, Figure 1). *BAX* is a pro-apoptotic gene which regulates programmed cell death during the growth and development of embryos (Wrenzycki et al., 2005). *BAX* expression in embryos has been known to increase when induced by stress, such as changes in environmental factors. The presence or absence of antibiotics in the embryo flush media did not result in a significantly increased level of expression of the pro-apoptotic gene, which is indicative of a minimal impact on embryo health and viability.

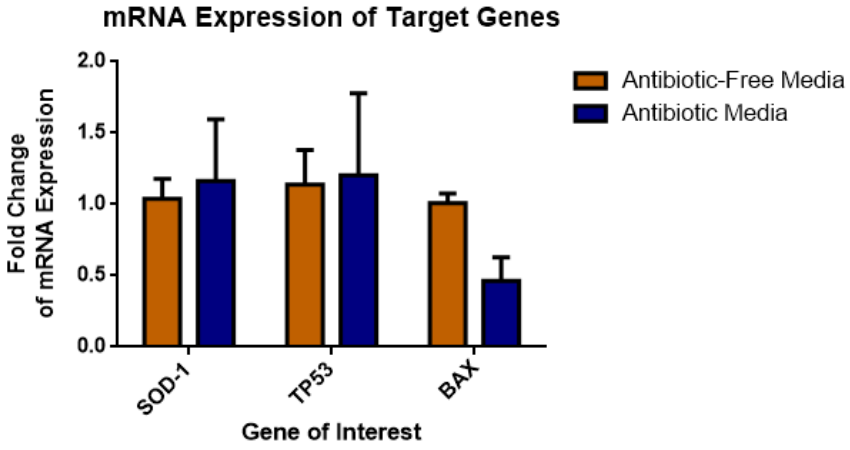

Figure 1. mRNA expression of genes of interest in embryos based on RT-qPCR. Fold change was evaluated for three target genes in both antibiotic flush media embryos and antibiotic-free flush media embryos. Data represented as mean ± SEM in each group. The statistical analysis and graph were constructed using GraphPad Prism v6.01(GraphPad, San Diego, CA).

The lack of significant difference in the gene expression of *SOD-1*, *TP53*, and *BAX* confirms that the presence or absence of antibiotics in embryo flush media has no

effect on embryo health gene transcription levels. However, this study is limited to the expression of only 3 target genes and only their potential effect up to the time of freezing. Through investigating an increased number of embryo health related genes, the response of embryo gene transcription to the use of antibiotic or antibiotic-free flush media could be further characterized. The sample size of this study was limited, and the findings would benefit from an increase in animal number and sample size. As for the limited scope due to the time point the samples were collected, it would be beneficial to further explore embryo viability results after thawing and transfer to recipient cows to evaluate the long-term effects of flush media on embryo health and development. In conclusion, this study provided preliminary evidence to suggest that the inclusion or exclusion of antibiotics from embryo flush media has no effect on the mRNA expression of *SOD-1*, *TP53*, or *BAX*, all of which are indicators of embryo health status.

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Antiviral Drug Discovery Against Bovine Viral Diarrhea Virus

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TAKE HOME MESSAGE

Bovine viral diarrhea virus (BVDV) is causing up to $2.5 billion per year in economic losses, worldwide. In the United States, losses range between $35 and $56 million per million calves born. The ability of BVDV to cross the placenta results in the birth of persistently infected (PI) animals that are shedding large quantities of virus throughout their life. Culling creates financial hardship for the cattle producers that own PI animals. To avoid the associated economic loss, vaccination has been introduced to control the BVDV infection. As the viral antigen is poorly immunogenic, booster doses of the killed vaccine are required to achieve an effective immune response. Thus, there is a clear and unmet need for antiviral medications that can help animals fight off BVDV and shorten the length of viral infection.

SUMMARY

Antiviral therapy is one of the most exciting aspects of virology. It uses basic science to generate effective treatments for serious viral infections. The development of antiviral drugs is very much a work in progress, with active drug discovery programs for many human infecting viruses. The global antiviral therapies market size will reach about $66 billion by 2027. In contrast, the use of antivirals in veterinary medicine is very limited. At present, only one antiviral compound has been licensed for use in veterinary medicine: feline interferon-omega. However, several antivirals licensed for use in human medicine are currently used in therapy for animal diseases. The most severe constraint in the development of antiviral drugs has been the identification of specific viral targets. Using tools of molecular biology and bioinformatics, we have recently identified a segment of RNA genome as an *Achilles*' heel of BVDV virus. This RNA folds into a complex three-dimensional structure, called a pseudoknot, that is indispensable for the synthesis of viral proteins, a critical step in viral reproduction. In the pseudoknot structure, there are many pockets suitable for binding drugs. Our ongoing research is focused on developing drugs that upon binding to the pseudoknot will inactivate virus reproduction

1. INTRODUCTION

Bovine viral diarrhea virus (BVDV) is a member of the genus Pestivirus, family Flaviviridae, that includes the causative agents of economically significant diseases of cattle, pigs, and sheep. The BVDV infections in cattle lead to decreased fertility and milk production, slow fetal growth, diarrhea, respiratory symptoms, reproductive and immunological dysfunction. Two main genotypes of BVDV, namely type 1 of low-virulence and type 2 of high-virulence, have been recognized, and are estimated to cause $20 and $57 million in total annual losses, respectively.

BVDV genome consists of positive-sense single-stranded RNA that codes for one open reading frame (ORF). The coding region is preceded by the distinctly-structured 5' untranslated region (5' UTR), which folds into the Internal Ribosomal Entry Site (IRES)**.** By definition, IRES is the RNA domain that recruit ribosomes to the internal region of mRNAs to initiate translation through a cap-independent pathway. The IRES domains can be found in genomic RNAs of many pathogenic viruses.

Studies have shown that the viral IRESs fold into intricate secondary and tertiary structures. We have already demonstrated BVDV IRES consists of three structurally defined domains connected by a pseudoknot. In our earlier studies, using the comparative structural analyses, we predicted secondary structure of the BVDV IRES pseudoknot (Burks et al., 2011). The presence of RNA pseudoknot in BVDV IRES RNA is supported by compensatory mutations which restore translation. In the most recent study, **we investigated the three-dimensional structure of the pseudoknot to identify potential binding sites for small molecules that can inhibit interaction between BVDV IRES RNA and ribosomes in the infected cells** (Gosavi et al., 2022).

2. PROCEDURES

2.1. Comparative Analysis of BVDV IRES RNA Sequences

BVDV IRES RNA sequences were extracted from the FASTA-formatted Rfam Pestivirus IRES alignment (version 9.1, IRES_Pesti, ID RF00209). Additional sequences were identified in GenBank using keywords and the Entrez search engine. The data were examined using the BioEdit sequence alignment editor (Hall et al., 1999). Sequences were grouped by genotype and sequence similarity, and preliminarily aligned using CLUSTAL. To prove or disprove Watson-Crick and wobble G•U base pairs, the alignment of unique BVDV IRES RNA sequences was examined with the SARSE editor and programs of the RNAdbTools suite (Andersen et al., 2007; Gorodkin et al., 2001). Properties of the alignment were inspected using Jalview.

2.2. Molecular Modeling of IRES RNAs

The sequence and base pair information were entered into the ERNA-3D program to create preliminary coordinates for A-form RNA in helical sections and to calculate the initial conformations of single-stranded regions (Mueller et al., 1995). RNA loops and other structural elements with similarity to known high-resolution structures were identified in the Protein Data Bank (PDB) and the Structural Classification of RNA (SCOR) database followed by incorporating the coordinates into the models. Manual adjustments were made in ERNA-3D.

3. RESULTS & DISCUSSION

A pseudoknot forms when one or more nucleotides in a hairpin loop base pair with nucleotides outside of the loop. Pseudoknots have been known features of many medium-size and large RNA molecules which are involved in translation and other cellular processes. Within the phylogenetically supported secondary structures of the BVDV IRES RNAs a pseudoknot engages sections 3a and 3b together with helices 3.6 and 4. Using site-directed compensatory mutations as well as chemical and enzymatic probing, a similar pseudoknot was shown to exist also in the CSFV IRES RNA (Fletcher and Jackson, 2002; Kolupaeva et al., 2000) and the more distantly related HCV IRES RNA (Kieft et al., 2001; Berry et al., 2010).

Formation of G321:U332 and C322:G331 in the BVDV IRES RNA is only partially supported by covariation analysis (Burks et al., 2011). Chemical footprinting suggest that these two base pairs do not form in the IRES RNA that is not bound to the ribosomes (Gosavi et al., 2022). In contrast, mutagenesis studies indicate that formation both base pairs is critical for the IRES RNA ribosomal functions. These data indicate that due to an inherent flexibility the BVDV pseudoknot can adopt either "open" (inactive) or "closed" (active) conformations. Therefore, we hypothesize that small molecules, which can bind to the pseudoknot and stabilize its "open" structure, will be able to inhibit the synthesis of viral proteins and in that way terminate reproduction of the BVDV virus. The discovery of an alternative pseudoknot base pairing will help in developing strategies for disrupting viral RNA translation.

In summary, our research addresses a major challenge in agriculture and is cost effective considering the potential for significant impact on animal protein production, biosecurity, animal welfare, and environmental protection. Our work extends the long tradition of excellence of BVDV research on the Auburn University campus. Moreover, our project will provide a unique opportunity for Auburn University to be positioned at the cutting edge of the development of novel antiviral agents.

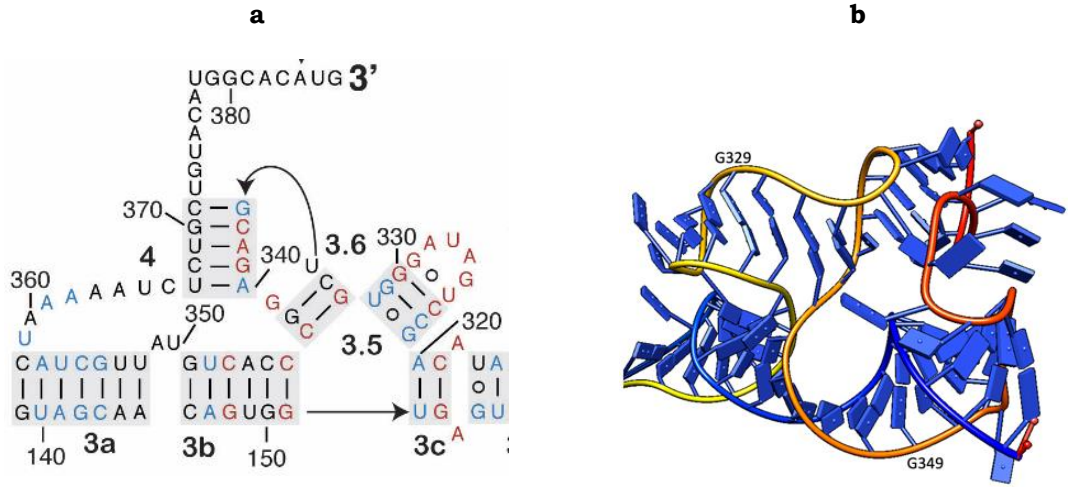

Figure 3. Graphical representations of the BVDV RNA pseudoknot. **a**) Secondary structure: Numbers 3a, 3b, 3c, 3.5, 3,6 and 4 denote helical segments encompassing the pseudoknot structure and its neighborhood. Numbers 140 through 380 denote positions of selected nucleotides in the whole secondary structure of the BVDV-1a strain NADL IRES RNA. **b**) Tertiary structure of the RNA segment depicted in the (a) panel. Positions of nucleotides G329 and G349 are indicated.

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Application of Production Metrics to Evaluate Cow Performance: A Collaboration of the Alabama Beef Cattle Improvement Association and the Alabama Cooperative Extension System

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TAKE HOME MESSAGE

Beef cattle producers engaged in the Alabama Beef Cattle Improvement Association (BCIA) Commercial Record Keeping Program are encouraged to expand records for evaluation of whole herd performance. Collection of cow weight at calf weaning can be used as a metric to estimate cow performance for overall productivity. Five beef cattle operations annually collected individual cow weight at calf weaning across 3 years for a total of 284 cows. Cow age was classified into 3 age range categories. Cow weight was classified into three weight range categories as small, moderate, or large. Cow performance was measured by average calving interval, average calf adjusted weaning weight, and percentage cow weight to calf adjusted weight weaned. Measuring cow weight at calf weaning enhances genetic and management practices. Cow herd productivity can be improved in the application of cow weight metrics to enhance genetic selection for optimal performance within the production environment.

SUMMARY

Performance record keeping within a beef cattle operation is vital for informed management decisions. Performance records reveal current production, identify inefficiencies, and provide information to make improvements. Performance can be evaluated by individual cow metrics such as average calving interval, calf adjusted weaning weight, and percent cow body weight to calf adjusted weight weaned. An average calving interval, or the average number of days from one calving to the next, is ideally 370 days or less. Optimal average calf adjusted weaning weight is established on an individual herd basis. Percent cow body weight to calf adjusted weight weaned evaluates the percentage a cow's body weight being producing in calf weight. Environmental adaptability is significant to reach optimum performance. The production environment is an equal balance of the physical environment, management level, and the biology of the cow. Complementing genetics to the production environment is crucial to reach performance goals. For improved genetic selection, metrics such as cow body weight and its relationship to average calving interval, calf adjusted weaning weight, and percent cow body weight to calf adjusted weight weaned are viable metrics to evaluate a cow's performance within her production environment.

1. INTRODUCTION

The Alabama Beef Cattle Improvement Association (BCIA) is a non-profit organization whose mission is to promote, educate and facilitate the use of beef cattle performance data and record keeping. Alabama BCIA is formally engaged with the Alabama Cooperative Extension System in providing education to beef cattle producers. Alabama BCIA assists its members in use of performance records for herd improvement in production efficiency and quality by providing the Alabama BCIA Commercial Record Keeping Program. Beef cattle producers engaged in this program are encouraged to expand records for evaluation of whole herd performance. The Alabama BCIA Performance Advocate Program was launched in 2019 to provide encouragement for whole herd record keeping and recognize herd efficiency. This program honors data collected in the following areas: breeding, pregnancy percentage, calf adjusted weaning weights and ratios, mature cow weight at weaning for percent of cow weight to calf weaning weight, yearling weights and ratios for replacement heifers and herd health program and treatments.

2. PROCEDURES

Collection of cow body weight at calf weaning can be used as a metric to estimate cow performance for overall productivity. From 2019 to 2021, five beef cattle operations annually collected individual cow weight at calf weaning across these 3 years for a total of 284 cows. Each of the five herds have a fall calving season, where cow and calf weights were annually collected at the same time of year. These herds are of similar breed composition, and are in the west, central and southwest regions of the state. Cow age was classified as 2 to 4 years old, 5 to 8 years old and 9 years and older. Cow weight was classified into three categories as small (1,199 lbs. or less), moderate (1,200 to 1,499 lbs.) or large (1,500 lbs. or greater). Within each age range and size category, cow performance was measured by average calving interval, average calf adjusted weaning weight and percentage cow body weight to adjusted calf weight weaned.

3. RESULTS & DISCUSSION

Overall, each cow age category was evenly represented with 35% 2-to-4 years old, 40% 5-to-8 years old, and 25% 9 years of age and older. A large portion of the cow weights collected represented the moderate weight category at 63%, with 24% in the large weight category and 13% in the small weight category. Average cow weight by weight category resulted in small 1,108 lbs., moderate 1,353 lbs., and large 1,605 lbs. as shown in Figure 1. Average calving interval lengthened as cow weight increased in the 2-to-4 year old age category as displayed in Figure 2. In the 2-to-4 year old age category, average calving interval was 18 days longer between small to moderate categories and 96 days longer between moderate to large categories.

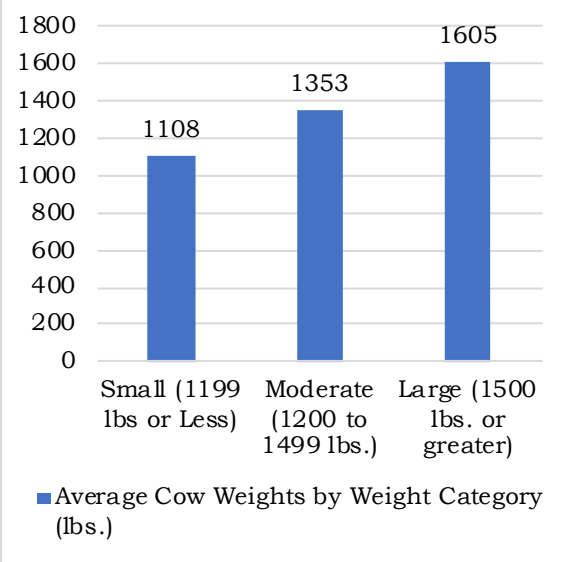

Figure 4. Average cow weight by weight category.

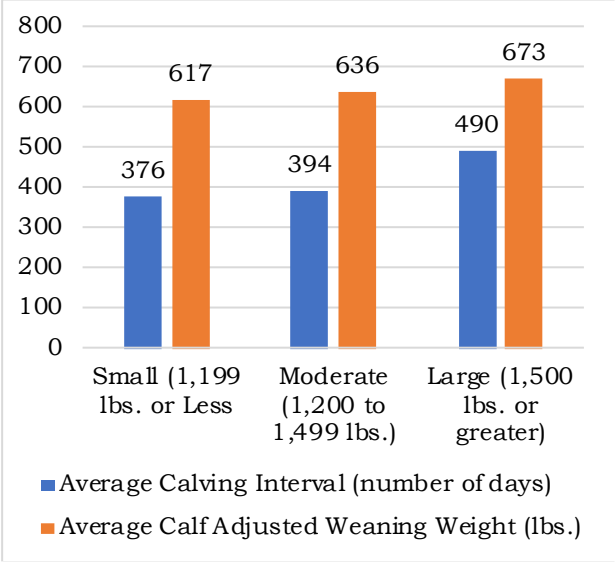

Figure 2. Cow performance of 2 to 4 year old cows by cow weight category.

For cows 5 years of age and older, a modest impact on average calving interval was shown due to an increase in cow weight. Across all ages, a moderate impact was seen on average calf adjusted weaning weight due to cow weight. However, as cow age and cow weight increases, percentage of cow body weight to calf adjusted weight weaned decreases as shown in Figure 3.

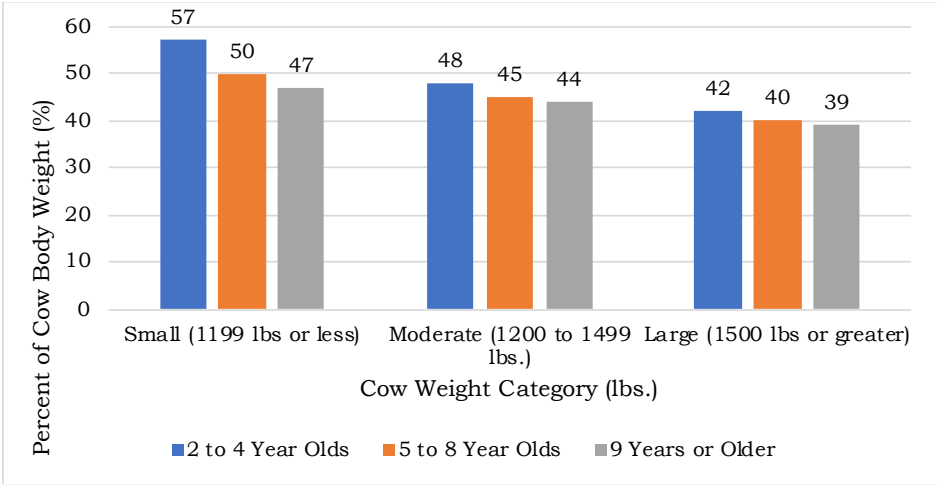

Figure 3. Percent cow weight to average calf adjusted weaning weight weaned.

Measuring cow weight at calf weaning enhances genetic and management practices. Complementing genetics to the production environment is significant to reach optimum productivity. Environmental resources of grazable acres, available forage, grazing systems, and stocking rate should be carefully evaluated. Matching the genetics of the cow herd to the production environment is critical to accomplish performance goals. Cow herd productivity can be improved with genetic selection by evaluating metrics such as cow weight and its relationship to average calving interval, calf adjusted weaning weight, and percent cow body weight to calf adjusted weight weaned. Overall management practices can also be enhanced with the collection of cow body weight in more precise stocking rates, vaccine and medication dosage, economic analysis, and more.

Acknowledgments

The Alabama Beef Cattle Improvement Association (BCIA) provided support in the funding of the Alabama BCIA Commercial Record Keeping Program and access to this data. Support was also received from the Auburn University Black Belt and Gulf Coast Research and Extension Centers and 3 privately owned cow-calf operations in the collection of the data.

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Exploring Means of Communication in Support of AgSTEM and Animal Agriculture

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SUMMARY

A growing anti-meat rhetoric around the world is being propagated through communication messaging and conduits designed to influence non-ag audiences. Communicating factual scientific evidence is critical for an informed citizenry and for public policy decision-making. The Veracis Ag Communication Research Group™ was formed to proactively conduct research in AgSTEM and consumer-industry communication management. Ongoing research projects include: "*Beef Communication within the Digital Pasture: Tools that Impact Consumer Perceptions*"; "*Measuring the Effectiveness of Both Cognitive and Emotional Forms of Instructional Videos Related to the Beef Industry; Instagram as a Tool of Diffusion for the Livestock Industry*"; "*Podcasting as an Extension Tool: Limitations and Opportunities"; "Measuring Agricultural Means of Influence on Young Adults via Instagram*"; "*Anticipating Rate of Engagement by Young Adults with Agricultural Social Media Influencers on Instagram; AgSTEM 360 – Enhancing Science Communication in Higher Education Organizations*"; and "*Agricultural Competencies Effect in Agricultural Communicators Ability to Influence Audiences*". This commentary report will provide an overview and results of the use of video research study. Approaches taken in these research studies involve statistical analysis of surveys directed at predominately next gen and millennial audiences. Our research to-date clearly demonstrates animal agriculture science communication messaging mediated through popular channels and modalities can effectively influence shifts in perceptions about the beef industry. Again, only selected results from the video perceptions study will be presented in this report.

1. INTRODUCTION

Because most Americans are three or more generations removed from production agriculture (American Farm Bureau, 2020), they have minimal incentive to question the unsubstantiated statements supported by anti-animal agriculture groups. Furthermore, only 11% of the population works in agriculture-related jobs, as of 2020, leaving up to 89% of the United States population outside of the field (USDA, 2020). This has divided the public into two anthropologic tribes (Mitchell and Imrie, 2011) of similar ideology: that of agriculture and that of non-agriculture.

The United States population, especially millennials are influenced by resources such as their tribe, lifestyle, and online communities (Biraghi, 2018). A tribe is a group of people who identify together by their similarities (Bolanle, 2019). Fewer citizens watch news networks or receive information from scientific journals; rather,

information about meat is received from other sources such as a tribe member or social media (Specht et al, 2019). These characteristics show either an uneducated and scientifically uninformed consumer or individuals entrenched in their mindset about animal agriculture. These views are established through the application of principles of leadership and influence science in messaging originating and propagated largely from anti-animal agriculture advocates.

Because of a gap of knowledge and understanding, segments of society are questioning the welfare of animals in production, whether or not types of meat consumption have a negative impact on personal health, and the environmental sustainability of producing livestock. With growing evidence, it is purported that lack of effective communication will allow continued erosion of *social licensure* (Bice and Moffat, 2014; Cooney, 2017) of many aspects of livestock production agriculture. Communicating about our science (Hart and Nisbet, 2011) and animal agriculture more effectively to varied public audiences, however, turns out to be difficult. Because there is no single audience for scientific or production agriculture information, and the societal contexts surrounding different aspects of issues of concern can vary considerably. Communication approaches need to be adapted to reflect the circumstances that prevail. Members of non-ag and ag segments or tribes alike need animal production information that is factual, scientifically supported as well as emotionally satisfying. Greene and Greene (2022) offered a perspective of the ramifications of the threats around elimination of the beef industry and called for more pro-active messaging. Consistent with the latter commentary, livestock industry leaders from across the world gathered in October 2022 to hold a summit in the Irish capital of Dublin to explore ways to address anti-meat rhetoric and help attract scientists to communicate their research (Pokinghorne, 2022).

Our research on science communication and influence (Patterson et al., 2008; Stacks, 2017) examines how the social media channels, Twitter and Instagram, as well as uniquely crafted video can be harnessed to more effectively communicate publicly about animal agricultural production to influence perception (Holt et.al., 2015). Quantitative and qualitative research results give indication toward tribal views by assessing how the public engages in daily posts about animal agriculture and provide indication of how and where the Animal Ag industry should design meaningful messaging for improved perceptions (Kim et. al., 2019; Stacks, D. 2017).

2. PROCEDURES

To answer research questions about perception and effects of messages on altering views, quantitative data were typically obtained using IRB exempt surveys prior to and after experiencing a message. In most cases survey participants were from a population of age 18-30 from the United States, who use social media regularly, and a majority of which were members of a nonagricultural audience. In some studies, we utilized students attending Auburn University. In other studies, participants were selected utilizing purposeful sampling that fit the previous description, contacted, and surveyed through the use of Prolific. Study participation was voluntary, and participants had the ability to opt out at any point if they desired to do so. Surveys typically consist of demographic and Likert scale questions. Likert scales allow for the measurement of attitudes and opinions (Privitera & Ahlgrim-Delzell, 2019) and due to the focus of this study pertaining to audience's perceived opinion of their own knowledge as a result of messages. Both pre-message (be it video, written, or social media images) and post-message questions gauged

participant's perceived knowledge and views of agricultural topics. Knowledge of agricultural subjects assessed included a variety of topics germaine to animal agriculture as well as focus areas of diet-nutritional health, animal welfare and sustainability. In some studies, videos or social media posts which differed in their emotional or cognitive (data/information) composition were utilized. Our research data were analyzed to determine any change in perceptions from the pre- and post-messaging utilizing IBM SPSS (Version 28) to calculate descriptive statistics and within-subject data differences through paired samples t-tests (Privitera & Ahlgrim-Delzell, 2019). The alpha significance was set at 0.05 for statistical purposes.

3. RESULTS & DISCUSSION

3.1. Measuring the Effectiveness of both Cognitive and Emotional Forms of Instructional Videos Related to the Beef Industry

Results showed participant's views about the beef industry improved by 82% after watching the cognitive or emotional constructed videos. The emotional video had a greater impact ($p < .05$) on participants perceptions.

With increasing popularity of virtual messaging, there is a need for a more transparent view of the beef industry. Videos are proposed as an effective tool to communicate about specific topics, and there is great opportunity for animal agriculture to implement to increase transparency, communicate to a broader audience, and bridge the knowledge gap between consumers and producers.

In this study, participants were shown two separate videos pertaining to emotional and cognitive aspects related to the beef industry. The main focal points are on the aspect of animal welfare, diet/health of red meat, an environment/sustainability, which was explored through several studies. This study is similar to the studies performed by Rice and others (2020) and Ventura and others (2016) by providing a visual experience. This study focused on providing emotional and cognitive videos as tools in order to engage participants. Crafted by a panel of animal science experts, each video created two different narratives for the participants to connect with. The emotionally charged video had a combination and overlap of stories from actual Alabama beef cattle producers. The producers, all from the same family, shared their experiences, hardships, as well as their motivations to farm beef cattle to create an atmosphere of family values and realism tied to the industry. It was anticipated that these emotionally charged characteristics would be more influential on participant's perceptions toward beef production. The cognitively charged video portrayed facts and statistics about the beef industry and the quality of beef products vectored through an actual practicing extension veterinarian. Dr. Soren Rodning presented the same information as the emotional video in an academic or educational perspective. Both videos had expert-created scripts drafted for use, however, the genuity off-script was used instead because they were seen as most appealing to the projected participants.

Table 1. Example data. Paired sample t-test statistics for participants' responses regarding animal welfare.

Pair of pre and post	Mean Pre	Mean Post	*T*	*p*
I believe beef cattle are treated humanely.	2.78	1.61	19.556	<.001
I believe that it is necessary to treat sick animals. Such treatments could include rest, antibiotics, or medicine.	1.40	1.22	5.228	<.001
I think farmers treat their beef cattle with respect.	2.33	1.56	13.977	<.001
I believe beef cattle deserve to have access to clean water, fresh grass, and healthy feed.	1.29	1.44	-3.381	<.001
I believe farmers treat animals in a way that meets current animal welfare standards.	2.37	1.53	15.499	<.001

[1]Survey of young adult college students about their opinion of welfare of animals prior to and after the viewing of a cognitive and emotionally based videos. n = 326. [2]Mean after viewing the videos. [3]Results creating using a t-test from SPSS. [4]A five-point Likert type scale was used with the response categories: strongly agree (1); somewhat agree (2); neutral (3); somewhat disagree (4); and strongly disagree (5).

It was hypothesized that the intervention of videos will shift opinions optimistically in a positive outlook, especially the emotionally charged video, regarding the beef industry, and the data resulted suggests this is true. In each question subset, animal welfare, diet and health of beef, and environment and sustainability of beef production, significant differences in opinion were recorded post-video intervention. Overall, animal welfare topics demonstrated the highest potential to shift opinion. Results from the diet and health of beef and sustainability of beef production sections were less conclusive than the animal welfare portion, but the results still suggest that video messaging can be an effective tool to explore use of in the future. This study found that people genuinely liked seeing farmers interact with their cattle and gained knowledge. In contrast, Ventura and others (2016) focused on bringing people to an actual dairy, this study shows the participants real life farms.

Video messaging, growing in popularity, has immense potential to alter attitudes toward agricultural topics out of non-agricultural audiences as shown in this study. Both the descriptive statistics and paired samples t-tests results demonstrate this phenomenon. This study found that perceptions regarding animal welfare differed significantly after viewing the videos. Participants perceived the beef industry as a humane, ethical, safe industry with specific understanding that beef cattle are kept to current animal welfare standards. Regarding diet, health, and consumption of beef, participants showed significant shifts in understanding the health benefits of beef. Specifically, participants demonstrated a shift in perception in their confidence that red meat products are healthier than plant-based alternatives. Sustainability of beef, however, demonstrated the least clarity in shifting perceptions of participants. After watching the videos, there were statistically significant perception changes in a negative manner, such as farmers are responsible for current pollution outputs. Considering all of the quantitative measures, qualitative analysis provided greater insight to participant perceptions across the three research areas. Particularly, thematic coding revealed percentages of positive and negative comments addressing the beef industry after viewing the videos. For example, the videos provided a generally

liked transparency of the beef industry, but also scrutinized because the farms presented were not "representative of factory farms." Though negative statements like these were commented throughout hundreds of responses, the videos induced positive outlooks for the beef industry. Thus, reinforcing the concept that well designed video messaging can be an effective tool for promotors and influencers of the industry.

Animal welfare seems to be the biggest concern among college students in the present study as in several others including Edwards-Callaway and Calvo-Lorenz, (2020) and Cardoso and others (2016). Consumer's biggest positive comments were the fact that they perceived beef cattle were being treated humanely by their owners. The most frequent concern was the well-being/welfare of the cattle followed by environmental impact (Cardoso, et al. 2016). Unlike Ventura (2017), after seeing the videos, the participant's behaviors improved in their attitudes towards animal welfare, diet/health of red meat, an environment/sustainability.

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Surface Color Variations of Ground Beef Packaged Using Enhanced, Recycle Ready, or Standard Barrier Vacuum Films

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TAKE HOME MESSAGE

Fresh meat packaging at the retail outlet for consumers remains instrumental in providing a method to preserve and store retail cuts intended for purchase. The meat industry is one of the final stages of production for agriculture that places animal products at the disposal of the consumer. As sustainability remains one of the most focused topics within agriculture, the improvement in methods for investigating these new topics is needed. Although technology for preserving and storing fresh meat products has evolved. The objectives of this study were to evaluate vacuum packaging films and their influence on surface color of ground beef. Vacuum packaging ground beef can protect surface color for longer storage periods and minimize waste that occurs at the retailer or by the consumer.

SUMMARY

With current meat industry efforts focused on improving environmental influencers, adopting sustainable packaging materials may be an easier transition to addressing the sustainability demands of the meat consumer. With the growing popularity of vacuum-packaged meat products, the current study evaluated instrumental surface color on fresh ground beef using vacuum packaging films, recycle-ready film (RRF), standard barrier (STB) and enhanced barrier (ENB). Ground beef packaged using ENB barrier film was lighter (L*), redder (a*) and more vivid (chroma) than all other packaging treatments during the simulated display period ($p < 0.05$). By day 12 of the simulated retail display, the ground beef surface color became lighter (L*), more yellow (b*), less red (a*), less vivid (chroma) and contained greater forms of calculated metmyoglobin, oxymyoglobin ($p < 0.05$). The current results suggest that barrier properties of vacuum packaging film for ground beef are pivotal for extending the surface color during fresh shelf-life conditions.

1. INTRODUCTION

When consumers purchase fresh meat, a primary attribute influencing the consumer's purchasing decision is meat color. As meat is stored in refrigerated conditions following harvest, fabrication, or portioning, surface color variations are dependent on the chemical state of myoglobin. Vacuum packaging is often not the preferred packaging platform of choice within the retail consumer market setting by meat industry retailers due to surface color variations that are presented under vacuum. It is widely known that beef packaged in a vacuum platform displays a purple-red color identified as deoxy-myoglobin. Before vacuum packaging, fresh beef appears as a bright, cherry-red color due to surface exposure to oxygen. The reduction in partial pressure within a vacuum package of fresh meat can result in a shift in color form of myoglobin from a bright red oxygenated color to a purplish red deoxygenated appearance. It is estimated that 74% of consumers utilized color as an important attribute influencing their purchasing intent of meat. Moreover, when consumers are informed on vacuum-packaged beef and its purple-red color, consumers are more likely to purchase vacuum-packaged beef over non-barrier polyvinyl chloride (PVC) packaged beef. Because of the unique fresh characteristics, ground beef is a product that often has a reduced shelf life.

Vacuum packaging has been a resource often used for decades when packaging meat to achieve extended storage periods and meet the fresh and frozen product shelf-life expectations throughout the meat industry. Efforts in previous years have mainly focused on shelf life and surface color of fresh beef. It is evident with the growing trends in use of vacuum packaging, the investigation of recyclable materials and various films used within the vacuum packaging platform is necessary.

Thermoforming vacuum packaging is constructed by the forming of multi-layered films with heat, pressure, and forming duration. Moreover, many combinations of materials exist in the formation of these multi-layer films; some of these materials include amorphous polyethylene terephthalate (A-PET), polyolefins (PO), ethylene vinyl alcohol (EVOH), polyvinylidene di-chloride (PVdC), and Nylon. Although films typically comprised of PO and EVOH have been constructed with intentions of recycling after consumer use, a challenge remains presently that still limits the recycling process of flexible multi-layered films. As technology within the packaging landscape of fresh meat evolves, the investigation of viable options in recycling multi-layer films is still essential to identifying the shelf-life performance of packaging films that could serve as a recycle ready option for the meat industry. Therefore, **the objectives of this study were to evaluate the instrumental changes in surface color of fresh ground beef packaged in enhanced (ENB), standard (STB), and recycle-ready (RRF) vacuum packaging films and stored under simulated retail conditions.**

2. PROCEDURES

2.1. Raw Materials

Coarse ground beef (80:20; lean:fat) packaged in 4.5 kg chubs (DuraChub, WINPAK, Winnipeg, MB, Canada) with an oxygen transmission rate of 0.9 cc/sg. m/24 h) was purchased from a commercial meat processing facility. Fresh, never-frozen chubs were transported under refrigerated conditions 1.5

℃ ± 0.5 ℃ in the absence of light to the Auburn University Lambert Powell meat laboratory. Coarse ground beef was stored in the absence of light at 2.0 ℃ ± 1.0 ℃ for 48 h prior to grinding and packaging. At the time of grinding, coarse ground beef (36 kg) was allocated randomly to 1 of 3 treatments (n = 12 kg/treatment) and ground once using a commercial meat grinder (Model 4346, Hobart Corporation, Troy, OH, USA) through a 3.18 mm plate (SPECO 400, Schiller Park, IL, USA). After grinding, ground beef was portioned into 454 g bricks using a vacuum stuffer (Model-VF608plus, Handtmann, Biberach, Germany).

2.2. Packaging

Ground beef bricks (n = 5/treatment/rep) were placed into a commercial packaging film (WINPAK, Winnipeg, MB, Canada) consisting of an enhanced barrier (175 µm nylon, enhanced EVOH, and polyethylene: ENB), standard barrier (175 µm nylon, EVOH, and Polyethylene: STB), and recycle ready film (175 µm polyolefins and EVOH: RRF). The non-forming film used for all packages was comprised of (75 µm polyester, EVOH, and polyethylene). The oxygen transmission rates (OTR) for each packaging treatment were as follows: ENB (0.2 cc/sq. m/24 h); STB (0.4 cc/sq. m/24 h); RRF (0.5 cc/sq. m/24 h). However, the moisture vapor transmission rates for each treatment were as follows: ENB (3.3 g/sq. m/24 h); STB (3.3 g/sq. m/24 h); RRF (2.8 g/sq. m/24 h). Packages of ground beef bricks were sealed using a Variovac Optimus (OL0924, Variovac, Zarrentin am Schaalsee, Germany). After packaging, ground beef brick packages were individually identified and placed into dark storage at 2.2 °C ± 0.5 °C for 120 h.

2.3. Retail Display

Packaged ground beef was stored in the absence of light at 2.2 °C ± 0.5 °C for 120 h to simulate logistic conditions from manufacturer to retailer. Following dark storage, ground beef packages were placed into a three-tiered, lighted display case Turbo Air (Model 60DXB-N, Turbo Air Inc., Long Beach, CA, USA) operating at 3.0 ± 1.5 °C with three 25 min defrost cycles occurring each day. Shelf-life timeline for measuring surface color began (Day 0) at the time of displaying ground beef packages under constant lighting for simulated retail conditions. The lighting within the retail case consisted of cool LED strips (TOM-600-12-v4-3, Philips Xitanium 40W-75W, Korea) with a lighting intensity of 2297 lux (ILT10C, International Light Technologies, Peabody, MA, USA) on each shelf. Ground beef packages were randomly dispersed throughout the display case shelves and rotated daily to simulated consumer packaging shifting that occurs at the retail counter.

2.4. Instrumental Color

Throughout the 15-day simulated retail display period, instrumental surface color was measured on packages of ground beef (n = 75) with a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA). Prior to surface color readings, the

colorimeter was standardized using a black and white tile covered with the packaging films to confirm instrument accuracy. Surface color readings were captured each day of the simulated display period at 17:00. Instrumental color values were determined from the mean of three readings on the surface of each ground beef through the intact package using illuminant A, with an aperture of 31.8 mm, and a 10◦ observer to measure lightness (L*), redness (a*) and yellowness (b*) of each ground beef package. In addition, hue angle was calculated as follows: tan−1 (b*/a*), with a greater value indicative of the surface color shifting from red to yellow. Chroma (C*) was calculated as: $\sqrt{a^{*2} + b^{*2}}$ where a larger value indicates a more vivid color. Lastly, reflectance values within the spectral range 400 to 700 nm were used to capture the surface color changes from red to brown by calculating the reflectance ratio of 630 nm:580 nm and the relative percentages of deoxymyoglobin (DMB = {[1.395 − ({A572 − A700}/{A525 − A700})]} × 100), metmyoglobin (MMB = {2.375 × [1 − ({A473 − A700}/{A525 − A700})]} × 100), and oxymyoglobin (OMB = DMB − MMB) according to the American Meat Color Measurement Guidelines.

2.5 Statistical Analysis

All data were analyzed as a completely randomized design using the ground beef package as the experimental unit with 25 replications of each treatment. The ANOVA was generated using the GLIMMIX model procedure of SAS (version 9.2; SAS Inst. Inc. Cary, NC, USA) using day of simulated retail display as a repeated measure, with packaging, day, and packaging × day interaction as the fixed effects. Least squares means were generated, and, when significant ($p < 0.05$) F-values were observed, least squares means were separated using pair-wise t-test (PDIFF option).

3. RESULTS & DISCUSSION

There were no ($p > 0.05$) interactive effects for packaging film × day throughout the simulated retail display period on surface color values of vacuum-packaged ground beef. Ground beef displayed using ENB barrier packaging film was lighter ($p < 0.05$) L* than ground beef packaged using STB or RRF (Table 1). Moreover, ground beef packages became lighter ($p < 0.05$) as the duration of storage time increased (Table 2). Similar results for surface lightness (L*) have been noted when using various packaging methods such as vacuum-packaged, overwrapping, or the addition of gasses within the package of fresh ground meat (Rogers et al., 2014). Additionally, fresh meat under lighted display in a limited oxygen pack- age has been reported to impact the formation of oxymyoglobin formation (Greene et al., 1971). Changes that occurred to the surface color lightness (L*) are likely a function of deoxy-myoglobin formation that occurred during the simulated display period. Furthermore, declining changes in lightness are similar with previous studies reporting vacuum-packaged ground beef became darker over a 20-day simulated display period following temperature abuse (Rogers et al., 2014). Ground beef packages were redder and more vivid ($p < 0.05$) when displayed using ENB packaging film, whereas RRF packages were more yellow (b*) and had a greater ($p < 0.05$) hue angle (Table 1). Nonetheless, redness and vividness declined ($p < 0.05$) as storage duration in refrigerated display increased (Table 2). A decrease in redness (a*) values

suggest fresh meat products may be less accepted by consumers, due to the meat product presenting a darker red surface color. Previous studies have evaluated the influence of storage period and packaging method on beef steaks (M. *longissimus dorsi*) Bag˘ datil and Kayaardi (2014). Similar results in vacuum-packaged steaks indicate a* values may decline throughout the storage period (Bag˘ datil and Kayaardi, 2014). Moreover, similar results for vacuum-packaged beef loins have been reported to the current study, indicating chroma values (surface color vividness) will decline as the duration of display increases Strydom and Hope-Jones, (2014).

Table 1. Influence of packaging film on color values of vacuum-packaged ground beef during a simulated retail display.

	TRT [1]			
	ENB	**STB**	**RRF**	**SEM ***
Lightness (L*) [2]	48.94 [a]	48.38 [b]	48.11 [c]	0.044
Redness (a*) [2]	21.02 [a]	18.39 [b]	16.92 [c]	0.096
Yellowness (b*) [2]	13.47 [c]	14.04 [b]	14.64 [a]	0.028
C* [3]	25.02 [a]	23.26 [b]	22.55 [c]	0.067
Hue (°) [4]	32.90 [c]	37.93 [b]	41.58 [a]	0.188
RTB [5]	2.61 [a]	2.17 [b]	1.91 [c]	0.016
MMB (%) [6]	25.22 [c]	33.66 [b]	39.83 [a]	0.321
DMB (%) [6]	66.73 [a]	50.29 [b]	40.68 [c]	0.525
OMB (%) [6]	8.05 [c]	16.05 [b]	19.49 [a]	0.218

[1]Packaging treatments are defined as follows: enhanced EVOH + polyethylene (ENB); nylon + EVOH + polyethylene (STB); and polyolefins + EVOH (RRF). [2]L* Values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color). [3]C* (Chroma) is a measure of total color(larger number indicates a more vivid color). [4]Hue (°) angle represents the change in color from the true red axis (larger number indicates a greater shift from red to yellow). [5]RTB is the reflectance ratio of 630nm ÷ 580nm and represents a change in the color of red to brown (larger value indicates a redder color). [6]Calculated percentages of oxymyoglobin (OMB), deoxymyoglobin (DMB), and metmyoglobin (MMB) using relative spectral values. a–c Mean values within a row lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

Instrumental spectral reflectance data from 400 to 700 nm was used to calculate relative values for the red to brown ratio (630/580 nm), metmyoglobin (MMB), deoxymyoglobin (DMB), and oxymyoglobin (OMB) of ground beef surface color changes. The red to brown ratio for ground beef packaged using ENB barrier packaging film was greater ($p < 0.05$) than ground beef packaged in STB or RRF films (Table 1). Additionally, throughout the duration of the retail display period, the red to brown (630/580 nm) values declined ($p < 0.05$), resulting in a shift from a redder to browner surface color (Table 2). As noted in previous research, red to brown values tend to decline regardless of packaging method (Stivarius et al., 2002). It is plausible the shift in calculated red to brown values is a function of greater metmyoglobin formation over the course of the extended display period. Moreover, relative calculated values of oxymyoglobin captured through instrumental measurements indicated vacuum packages of ground beef in RRF film were greater ($p < 0.05$) than packages of ground beef in STB or ENB packaging films, respectively (Table 1). Surprisingly, calculated values of OMB increased ($p < 0.05$) in vacuum-packaged ground beef as the day of simulated display increased (Table 2). Interestingly, ground beef packaged using RRF films resulted in greater ($p < 0.05$) calculated relative values for MMB and OMB than ground beef packaged in STB or ENB packaging films (Table

1). However, as the time of display in days increased, calculated MMb increased ($p < 0.05$) and DMB values declined (Table 2). Changes recorded in calculated relative values of DMB, MMB, and OMB may be attributed to the oxygen transmission rates of the packaging films. In addition, it has been reported that the ratio of myoglobin forms in fresh meat can be influenced by the available oxygen, oxygen consumption rate, autoxidation of myoglobin or the reducing ability of metmyoglobin (Ledward et al., 1992; Mancini and Hunt 2005). Furthermore, vacuum packaging of fresh meat may result in residual quantities of oxygen that causes the oxidation of DMB and MMB during storage periods. However, it is plausible vacuum packaging resulted in a greater regeneration of NADH which can delay discoloration of fresh meats. Therefore, the addition of continued research evaluating visual surface color of vacuum-packaged fresh meats is necessary.

Table 2. Influence of retail display (d) on color values of vacuum-packaged ground beef.

	Instrumental Value								
	L* [1]	a* [1]	b* [1]	C* [2]	Hue (°) [3]	RTB [4]	MMB (%) [5]	DMB (%) [5]	OMB (%) [5]
Day 0	47.36 ^{g}	22.05 ^{a}	13.35 ^{h,i}	25.86 ^{a}	31.39 ^{g}	3.05 ^{a}	20.81 ^{h}	69.85 ^{a}	9.34 ^{i,j}
Day 1	48.03 ^{f}	21.66 ^{a,b}	13.20 ^{i}	25.42 ^{b}	31.53 ^{g}	2.88 ^{b}	22.06 ^{h}	69.18 ^{a}	8.76 ^{j}
Day 2	48.27 ^{e,f}	21.37 ^{b,c}	13.39 ^{h}	25.28 ^{b,c}	32.26 ^{g}	2.78 ^{b}	22.75 ^{h}	67.05 ^{a}	10.21 ^{g,h,i}
Day 3	48.57 ^{d}	20.80 ^{c,d}	13.71 ^{f,g}	24.99 ^{c,d}	33.63 ^{f}	2.59 ^{c}	25.78 ^{g}	63.31 ^{b}	10.91 ^{g,h}
Day 4	48.60 ^{c,d}	20.61 ^{d}	13.68 ^{g}	24.82 ^{d}	33.82 ^{f}	2.53 ^{c,d}	26.96 ^{f,g}	62.72 ^{b}	10.32 ^{g,h,i}
Day 5	48.98 ^{a}	20.45 ^{d}	13.88 ^{f}	24.80 ^{d}	34.47 ^{f}	2.44 ^{d}	28.33 ^{f}	61.77 ^{b}	9.90 ^{h,i,j}
Day 6	48.96 ^{a,b}	19.78 ^{e}	14.07 ^{e}	24.37 ^{e}	35.84 ^{e}	2.31 ^{e}	30.65 ^{e}	57.79 ^{c}	11.56 ^{g}
Day 7	48.98 ^{a,b}	18.62 ^{f}	14.26 ^{d}	23.58 ^{f}	37.97 ^{d}	2.10 ^{f}	34.78 ^{d}	51.36 ^{d}	13.85 ^{f}
Day 8	48.95 ^{a,b}	18.04 ^{f}	14.35 ^{c,d}	23.18 ^{f,g}	39.07 ^{d}	2.02 ^{f}	36.24 ^{d}	48.82 ^{d}	14.93 ^{f}
Day 9	48.84 ^{a,b,c}	17.42 ^{g}	14.50 ^{a,b,c}	22.80 ^{g}	40.35 ^{c}	1.91 ^{g}	38.36 ^{c}	44.55 ^{e}	17.09 ^{e}
Day 10	48.71 ^{b,c,d}	16.81 ^{h}	14.55 ^{a,b}	22.37 ^{h}	41.47 ^{b,c}	1.85 ^{g,h}	39.69 ^{b,c}	41.90 ^{e,f}	18.41 ^{d,e}
Day 11	48.46 ^{d,e}	16.42 ^{h,i}	14.63 ^{a}	22.13 ^{h}	42.29 ^{a,b}	1.80 ^{h,i}	40.87 ^{b}	39.84 ^{f,g}	19.29 ^{c,d}
Day 12	48.09 ^{f}	15.54 ^{j}	14.34 ^{c,d}	21.26 ^{j}	43.13 ^{a}	1.67 ^{j}	43.50 ^{a}	34.43 ^{h}	22.07 ^{a}
Day 13	48.11 ^{f}	16.04 ^{i,j}	14.45 ^{b,c}	21.69 ^{i}	42.48 ^{a,b}	1.74 ^{i,j}	41.59 ^{a,b}	37.88 ^{g}	20.53 ^{b,c}
Day 14	48.27 ^{e,f}	16.03 ^{i,j}	14.37 ^{c,d}	21.64 ^{i,j}	42.39 ^{a,b}	1.75 ^{i,j}	41.14 ^{b}	38.03 ^{g}	20.83 ^{a,b}
SEM *	0.097	0.214	0.063	0.149	0.419	0.035	0.718	1.174	0.488

[1] L* Values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color). [2] C* (Chroma) is a measure of total color where a larger number indicates a more vivid color. 3 Hue (°) angle) represents the change from the true red axis where a larger number indicates a greater shift from red to yellow. 4 RTB Calculated as 630 nm reflectance/580 nm reflectance which represents a change in the color of red to brown (larger value indicates a redder color). 5 Calculated percentages of oxymyoglobin (OMB), deoxymyoglobin (DMB), and metmyoglobin (MMB) using relative spectral values. a–j Mean values within a column lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

Evaluation of vacuum packaging films for ground beef platforms indicated that ENB film provided a significant packaging solution for sustaining the fresh surface color of ground beef during a simulated retail display. When using ENB packaging films a reduction in surface color variation across the 15-day simulated retail display was noted when compared to STP and RRF packaging films. It is plausible the lack of color stability in ground beef surface color with RRF packaging film may have been impacted by the EVOH layer that exists within the layers of the packaging film. Furthermore, research evaluating RRF film is needed to identify the feasibility of vacuum packaging fresh meat, extension of shelf-life, reduction in lipid oxidation and visual surface color changes that may occur with a packaging film intended to be recycled after consumer use.

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Vacuum Packaging Maintains Fresh Characteristics of Previously Frozen Beef Steaks during Simulated Retail Display

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TAKE HOME MESSAGE

These results align with the idea that in selecting vacuum-packaging film for the storage of beef products, that film's oxygen transmission and rate of vapor transmission are a factor of consideration. During the freeze-thaw cycle of beef steaks, a packaging film's composition can alter surface color and wholesome characteristics. It is plausible that steaks packaged in film possessing reduced oxygen and moisture transmission rates may have a more stable surface color, reduced lipid oxidation, and reduced purge loss. These impacts on the appearance of packaged beef could have implications on consumers' shopping selections at the retail level.

SUMMARY

Our results support the hypothesis that when selecting vacuum-packaging film during the storage of beef products, oxygen transmission and moisture vapor transmission rate of the film should be considered. The potential influence caused by packaging film composition can alter surface color, and wholesome characteristics throughout a freeze–thaw cycle of beef steaks. It is plausible that steaks packaged in film possessing reduced oxygen and moisture transmission rates may have a more stable surface color, reduced lipid oxidation, and hindered aerobic microorganism growth.

1. INTRODUCTION

Meat products are highly perishable and strategies have been explored for decades to extend the fresh shelf life of red meat. Frozen storage is one strategy to extend storage life and reduce quality losses that occurs to fresh meat products. Consumers may consider freezing meat purchases to prolong the interval between purchase and consumption. Freezing meat prior to retail or foodservice use is a technique that has been used to extend storage periods, manage supply chain or facilitate distribution channels. However, despite the benefits, the process of frozen storage often requires a considerable amount of logistical planning that manufacturers or retailers may not find ideal.

During freezing, meat products undergo a physical transformation when water is converted into ice crystals, upon thawing, a transformation to a pre-frozen state occurs. Despite the unavoidable physical changes, it is imperative the frozen-stored and thawed meat will retain the quality attributes consumers associate with fresh

meat. Moisture lost from the muscle during thawing may promote microbial growth as temperature increase causing greater moisture loss from the meat. In addition to microbial growth, other meat characteristics that are affected by freezing and thawing procedures also include: moisture loss, protein denaturation, lipid oxidation, surface color, pH, objective tenderness, and purge loss.

At the point of sale, the visual appearance of beef products represents the most important characteristic influencing consumer purchasing decisions with a characteristic cherry-red color being highly desirable. Vacuum packaging can limit meat surface exposure to oxygen resulting in a darker red surface color. However, when permeability of the packaging film increases, greater concentrations of oxygen can allow the meat surface to possess a bright, cherry-red color.

To date, there have been limited efforts investigating the impact of packaging methods and materials on meat quality has been published. Therefore, **the objective for the current study was to determine the effect of vacuum packaging on shelf-life characteristics of boneless ribeye steaks that have been previously frozen.**

2. PROCEDURES

Beef boneless ribeye rolls (IMPS #122A) were purchased from a commercial meat processor and transported under refrigeration (2 °C) to the Auburn University Lambert Powell Meat Laboratory for processing. Using pack date on each box not exceeding 10 days from the time of packaging, ribeye rolls were selected for steak cutting. Ribeye rolls (n = 18) were fabricated into 2.54-cm-thick steaks (n = 12 steaks/ribeye roll) with a BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, OH, USA). Steaks from each ribeye roll were randomly selected and allocated to one of three packaging treatments.

After cutting, steaks were allowed to bloom to simulate an industry application for 30 min at 2 °C, crust frozen at 23 °C for 45 min, and then packaged with a form and fill packaging machine (Model OL0924, Variovac, Zarrentin, Germany). Steaks were packaged in one of three commercially available packaging films (WINPAK, Winnipeg, MB, Canada) consisting of a high barrier and or low barrier film. The high barrier film (MB) was comprised of 150 µm of nylon, enhanced ethylene-vinyl alcohol (EVOH), and polyethylene. Steaks packaged in low barrier films were constructed with 150 µm polypropylene and polyolefin plastomer (MFS) or a combination of 150 µm polyolefin and polyethylene (MDF). Oxygen transmission (OTR) of the packaging treatments consisted of: MB (0.5 cc/sq. m/24 h); MFS (1100 cc/sq. m/24 h); and MDF (1287 cc/sq. m/24 h). In addition, moisture vapor transmission of each packaging film was measured: MB (3.9 g/sq. m/24 h); MFS (2.9 g/sq. m/24 h); and MDF (3.5 g/sq. m/24 h). Packaged steaks were placed flat on a tray (76.2 cm × 60.96 cm) and stored in a blast freezer (− 23 °C) for 120 min.

Initially, steaks were placed in a two-door, reach-in, commercial freezer (Model AF49EX, Arctic Air, Eden Prairie, MN, USA) for 25 days at 13 °C. Packaged steaks were stored in the absence of light for the duration of the simulated frozen storage period. Temperature during the frozen storage period was monitored using a data recording device (Model-TD2F, Thermoworks, American Fork, UT, USA) with probes placed within the center of each shelf. Throughout the storage period frozen steaks were rotated across all shelves.

Following the 25-day frozen dark storage, packaged steaks were transferred to an LED lighted, refrigerated, 3-tiered, case (Model TOM-60DX-BN, Turbo Air Inc., Long Beach, CA, USA) to simulate a fresh retail setting. Packaged steaks were displayed at 3 ℃ 1.2 °C and data loggers (Model- TD2F, Thermoworks, American Fork, UT, USA) recorded storage temperatures. Continuous lighting intensity (2297 lux) of case shelves was recorded (Model ILT10C, International Light Technologies, Peabody, MA, USA) throughout the fresh display period. During fresh display, steaks were placed across all shelves and rotated on the shelving to simulate consumer movement. On days 0, 7, 10, 15, 20, and 25 steaks were removed from the refrigerated display case and measured for instrumental color, lipid oxidation, purge loss, pH, and spoilage organisms.

Fresh instrumental color readings were measured through the packaging on days 0, 5, 10, 15, 20, and 25 by scanning the surface of each steak through the packaging according to guidelines previously described. Surface color values were collected using a HunterLab MiniScan XE Plus Colorimeter (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA, USA) calibrated against a standard black and white glass tile each day immediately before data collection. The L* (lightness), a* (redness), and b* (yellowness) values of each steak were determined from the average of three readings using Illuminant A10, with a 10◦ observer and a 25 mm diameter aperture and the Commission Internationale de l' Eclariage (CIE L*a*b*) color scale. Chroma (C*) was calculated using the following equation: $\sqrt{a^{*2} + b^{*2}}$ with a more vivid color resulting from a great value. Additionally, hue angle was calculated as: $\tan^{-1}(b^*/a^*)$ where a greater value represents the surface color shifting from red to yellow.

Packages of fresh steaks were sampled for 2-thiobarbituric acid reactive substances (TBARS) as previously described. Steaks were minced into a uniform sample of the entire steak with a hand-held knife. In duplicate, 2 g 0.5 g of each minced steak was pulverized with 8 mL of cold (1 ◦C) of 50 mM phosphate buffer (pH of 7.0 at 4 °C) containing 0.1% ethylenediaminetetraacetic acid (EDTA), 0.1% n-propyl gallate, and 2 mL trichloroacetic acid (Sigma-Aldrich, Saint Louis, MO, USA). Samples were filtered through a Whatmann No. 4 filter paper and duplicate 2-mL aliquots of the clear filtrate were transferred into 10-mL borosilicate tubes, mixed with 2 mL of 0.02 M 2-thiobarbituric acid reagent (BeanTown Chemical, Hudson, NH, USA) and boiled at 100 °C for 20 min. After boiling, tubes were placed into an ice bath for 15 min. Absorbance of each sample was measured at 533 nm with a spectrophotometer (Turner Model-SM110245, Barnstead International, Dubugue, IA, USA) and multiplied using a factor of 12.21 to derive the TBARS value (mg of malonaldehyde/kg of fresh meat). The value of 12.21 was obtained previously from a standard curve using a known malonaldehyde solution measured across multiple absorbencies.

Steaks were removed from their package treatment, blotted dry with a paper towel and weighed on a balance (Model PB3002-S, Mettler Toledo, Columbus, OH, USA). Purge loss calculations is as follows: [(packaged weight − steak weight) ÷ packaged weight × 100)].

Data was analyzed as a completely randomized design with packaged steak serving as the experimental unit using the GLIMMIX model procedure of SAS. Packaging treatment was the lone fixed effect and replication was random effect for instrumental surface color, TBARS, and purge loss. Day of simulated retail display served as a repeated measure. Least square means were generated and when

significant ($p < 0.05$) F-values observed, and least square means separation occurred using the pair-wise t-test (PDIFF option).

3. RESULTS & DISCUSSION

Following frozen dark storage, instrumental color of vacuum-packaged steaks was recorded throughout a 25-day fresh display period. There was no interaction ($p > 0.05$) for packaging method day of display for fresh surface color lightness (L*; values not reported). However, throughout the fresh display there was an interaction ($p < 0.05$) for packaging method day of simulated display for redness (a*), yellowness (b*), chroma (C*) and hue angle (Table 1). Steaks packaged using MB film were redder ($p < 0.05$) from day 7 through 25 of the fresh simulated retail display period. However, steaks packaged in MFS and MDF films were more yellow ($p < 0.05$) initially, but as storage time increased past day 20, yellowness values declined for all packaging methods. This data suggest that the use of MB film may promote a better visual color during retail display post frozen storage compared to MFS and MDF films.

A decline in a* values of thawed meat has been attributed to myoglobin denaturation occurring during colder storage temperatures, but surface redness can increase after thawing when myoglobin is stored in a favorable oxygen binding environment. A similar study conducted examining the relationship between frozen and fresh beef color values reported increased anaerobic refrigerated storage duration can result in a rapid decline of a* values which has also been linked to an increase in lipid oxidation. Our results are consistent with a previous study that evaluated the surface color of meat following a frozen storage period and reported declining b* values throughout a refrigerated storage time after frozen storage (Otremba et al., 1999). Interestingly, duration of storage time may negatively influence the percentage of oxymyoglobin or metmyoglobin causing a detrimental impact on redness values for steaks possessing greater percentages of oxymyoglobin. Nevertheless, it has been reported that increasing oxygen saturation prior to freezing can result in greater oxidation after thawing, and a loss of reducing enzymes through exudate contributing to a deterioration in color stability (Abdallah et al., 1999; Henriott et al., 2020). These results from the current study support the hypothesis that surface color may be negatively altered after storage in frozen and subsequent refrigerated temperatures.

Consistent with observed redness and yellowness values for thawed beef steaks, instrumental surface color was also more ($p < 0.05$) vivid (C*) for steaks packaged in MB from day 7 through day 25 of the fresh display period (Table 1). In contrast, hue angle values for MFS and MDF increased ($p < 0.05$) throughout the entire display period indicative of color shifting from red to yellow for these packaged steaks.

In the current study, packaging steaks using MB film constructed with the lowest OTR rating appeared to confer protection against deterioration throughout the storage periods to thawed beef steaks resulting in greater surface color stability. Color shifting in frozen meat may be caused by physical processes such as drip loss, or when water molecules freeze resulting in a shift of fat, total protein, and water/protein ratio chemical concentrations on the surface layer after thawing. It is plausible that the duration of frozen storage time, packaging materials, and refrigerated storage temperature will affect color stability. Further research is needed to determine the impact of intrinsic and extrinsic factors on meat color stability when storage temperatures are altered. Regardless, these data point to a potential advantage for MB packaging film.

Table 1. The interactive impact of packaging method × day of display on instrumental color values of refrigerated, previously frozen beef ribeye steaks during a simulated retail display.

	Day						
	0	7	10	15	20	25	SEM*
MB[1]							
a*[2]	13.61^{e}	22.11^{a}	20.45^{b}	20.15^{b}	17.52^{c}	15.76^{d}	0.330
b*[2]	13.94^{fg}	13.17^{hi}	13.03^{hi}	13.77^{gh}	13.59^{ghi}	12.88^{hi}	0.251
C*[3]	19.69^{e}	25.79^{a}	24.27^{b}	24.45^{b}	22.25^{d}	20.40^{e}	0.326
Hue (°)[4]	45.87^{d}	30.85^{i}	32.52^{h}	34.41^{g}	38.03^{f}	39.51^{f}	0.716
MFS[1]							
a*[2]	17.21^{c}	10.46^{gh}	10.46^{gh}	10.78^{fgh}	10.03^{hi}	10.17^{ghi}	0.345
b*[2]	16.28^{a}	16.25^{ab}	15.33^{cd}	14.66^{ef}	13.94^{fg}	13.92^{fgh}	0.251
C*[3]	23.76^{bc}	19.94^{e}	18.61^{f}	18.32^{fg}	17.25^{h}	17.29^{gh}	0.344
Hue (°)[4]	43.63^{e}	54.83^{b}	55.85^{b}	53.90^{b}	54.36^{b}	53.69^{b}	0.716
MDF[1]							
a*[2]	17.04^{c}	11.25^{fg}	9.19^{i}	10.29^{gh}	10.69^{fgh}	11.98^{f}	0.345
b*[2]	15.79^{bc}	16.05^{ab}	14.99^{de}	14.34^{fg}	12.99^{hi}	12.75^{i}	0.251
C*[3]	23.31^{c}	19.65^{e}	17.62^{fgh}	17.72^{fgh}	16.94^{h}	17.56^{fgh}	0.344
Hue (°)[4]	43.06^{e}	55.24^{b}	58.58^{a}	54.34^{b}	50.45^{c}	47.06^{d}	0.716

[1] Packaging treatments are defined as: (MB) nylon + enhanced ethylene-vinyl alcohol + polyethylene; (MFS) polypropylene + polyolefin plastomer; and (MDF) polyolefin + polyethylene.
[2] a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color).
[3] Chroma is a measure of total color (larger number indicates a more vivid color).
[4] Hue angle represents the change from the true red axis (larger number indicates a greater shift from red to yellow).
*SEM, standard error of the mean.
[a-i] Mean values within day of display and packaging method lacking common superscripts differ ($p < 0.05$).

There was an interactive effect of packaging method day of display for lipid oxidation on thawed beef steaks (Figure 1). Lipid oxidation increased ($p < 0.05$) in steaks packaged using MDF and MFS films throughout the 25-day simulated retail display. Increases in lipid oxidation values agree with previous findings that evaluated vacuum stored meat products. The reduced lipid oxidation reported in MB- versus MDF, and MFS films is expected as a reduced OTR would reduce exposure of the steak to oxidation throughout the frozen and fresh storage periods. Consistent with this hypothesis, an accelerated rate of lipid oxidation associating with a greater amount of oxygen exposure has been reported across packaging materials. In a study examining minced porcine muscles stored in vacuum packaging, lipid oxidation tended to accelerate after thawing as peroxidation giving rise to rapid secondary lipid oxidation and increased TBARS values were reported (Owen and Lawrie, 1975). In the current study, it appears the MB film confers a greater protection against lipid oxidation than the use of either MDF or MFS films.

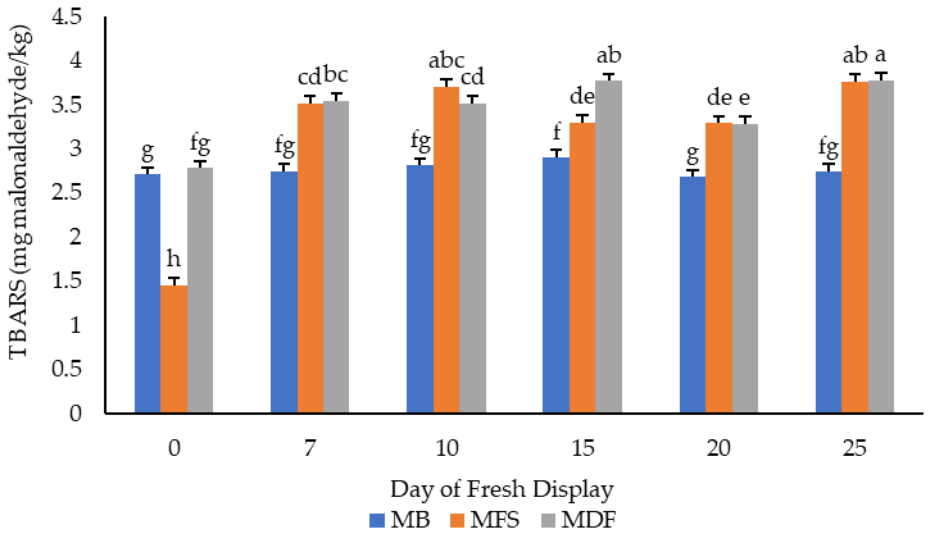

Figure 1. Interactive effect of treatment × day of display for 2-Thiobarbituric acid reactive substances (TBARS) on refrigerated, previously frozen beef ribeye steaks during a simulated retail display. Bars lacking common letters differ ($p \leq 0.05$).

There was no interactive effect for packaging treatment × day on purge loss of packaged steaks (values not reported). Purge loss after frozen storage and throughout fresh, refrigerated storage increased ($p < 0.05$) from day 0 to 25 regardless of packaging (Figure 3). It is plausible that the rise in purge loss could be attributed to the decline in muscle pH that occurred across all packaging treatments causing greater a

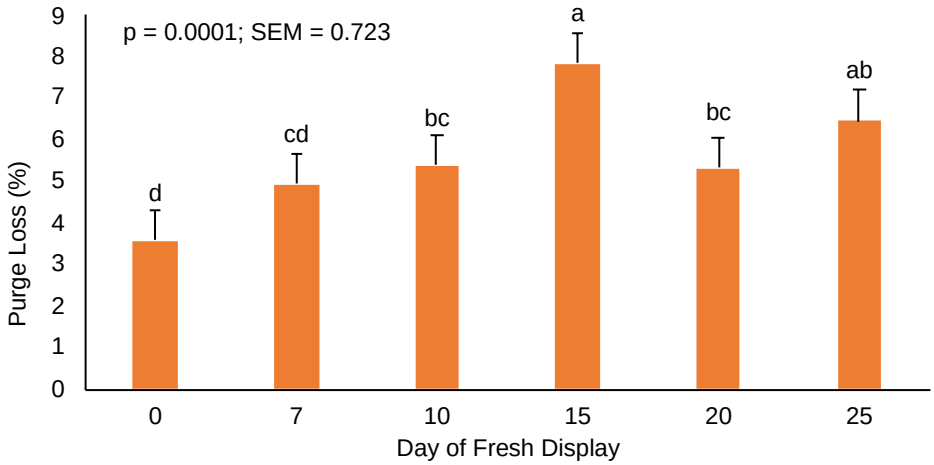

Figure 3. Influence of display day on purge loss (%) of refrigerated, previously frozen, beef ribeye steaks during a simulated retail display. Bars lacking common letters differ ($p \leq 0.05$).

Results presented support the hypothesis that when selecting vacuum packaging film during the cold storage of beef products, oxygen transmission and moisture vapor transmission rate of the film should be considered. The film properties may influence maintenance of surface color, and wholesome characteristics throughout a freeze-thaw cycle of beef steaks. However, to enhance the consumer acceptance of vacuum packaging, additional educational opportunities should be provided to consumers and producers on the various impacts of freezing and thawing of vacuum packaged red meats. Furthermore, additional research should be conducted to

evaluate the sensory profile of meat products during the freeze-thaw cycles when using vacuum packaging films for red meat storage.

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Complexity and Fish Peptide and Enzyme Complex Supplementation of Weanling Pig Diets

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TAKE HOME MESSAGE

Corn-soybean meal (**SBM**) diets are the gold standard for feeding pigs. Young pigs have immature digestive system and are subjected to various stressors at weaning. Providing complex diets containing highly palatable and digestible ingredients is important for their good growth performance. However, providing such diets can be rather costly. Considering the recent development in feed additives and enzymes or enzyme complexes, it might be possible for weanling pigs to extract more energy and nutrients from corn-SBM-based diets. Two studies were conducted to determine the possibility of replacing complex diets with simple, corn-SBM diets by fish peptide and enzyme complex supplementation. Pigs seemed to respond to the diets containing 1.5% fish peptides in the first study. The results of the second study indicated that a complex diet can be replaced with a simply corn-SBM diet supplemented with 1.5% fish peptides and exogenous multienzyme complexes during the phase 1 or day 0 to 14 after weaning.

SUMMARY

The effort was made to determine the optimum inclusion rate of fish peptides in the weanling pig diet in the first study, whereas the possibility of replacing complex diets with simply corn-SBM diets was investigated in the second study. Ninety-six and 48 weanling pigs were used in the first and second studies, respectively, and pigs were assigned to 6 (study 1) and 3 (study 2) diets during the phase 1 (day 0 to 14) and 2 (day 14 to 28). For both studies, the phase 1 and 2 complex positive control (POS) diets containing highly digestible and palatable ingredients were formulated. Simple corn-SBM negative control (NEG) diets were formulated to contain the same lysine as the phase 1 and 2 POS diets in both studies. The NEG diets were supplemented with 0.5, 1.0, 1.5, or 2.0% fish peptides in study 1. Similarly, the phase 1 and 2 NEG diets were formulated to be the same lysine content as the POS diets in study 2, but NEG diets contained 1.5% fish peptides or 1.5% fish peptides and multienzyme complexes. There were no clear effects of dietary treatments on blood metabolites or cytokines in study 1 and metabolites in study 2. Pigs seemed to respond to the diets containing 1.5% fish peptides in study 1 and it was used as the basis for the dietary treatments

in study 2. Based on the results of study 2, it was concluded that a complex diet can be replaced with a simple corn-SBM diet by fish peptide and enzyme complex supplementation during the initial phase or 0 to 14 days after weaning. [Please see Guo et al. (2022) for the details.]

1. INTRODUCTION

Although a corn-soybean meal (**SBM**) diet is the gold standard for feeding pigs, such a diet may not be appropriate for weanling pigs because of, among others, digestive challenges. Weanling pigs are, therefore, fed complex diets that contain many special ingredients that are highly palatable and digestible (Wang et al., 2018), which can be rather costly. There are some potential viable alternative supplements such as fish peptides (Poudel et al., 2020) that can be not only an excellent source of nutrients, but also a bioactive or functional feed additive (Zamora-Sillero, 2018). Such a feed additive may not only enhance the efficiency of energy and nutrient utilization but also animal health because of its antioxidative, antimicrobial, antihypertensive, and other activities, thus, improving the growth performance of pigs. In addition, with the development and availability of various enzymes or enzyme complexes, it might be possible for weanling pigs to extract more energy and nutrients from corn-SBM-based diets more efficiently (Zhang et al., 2014).

Two studies were conducted **to investigate the possibility of replacing typical complex weanling pig diets with semi-complex diets supplemented with some feed additives**. In study 1, the effort was made to determine the optimal inclusion rate of fish peptides based on growth performance, serum metabolite profile, and serum cytokine concentrations. The possibility of replacing complex diets with simple corn-SBM diets by supplementation with fish peptides and multienzyme complexes was investigated in study 2 by assessing the effect of dietary treatments on growth performance and serum metabolite profile.

2. PROCEDURES

A total of 96 pigs in study 1 and 48 pigs in study 2 weaned at 3 to 4 weeks of age were randomly assigned to 6 dietary treatments in study 1 and 3 dietary treatments in study 2 with 4 pens per treatment and 2 gilts and 2 castrated males per pen. In both studies, 2 typical complex, positive control (POS) diets were formulated to contain 1.22 and 1.11% standard ileal digestible lysine for the phase 1 (day 0 to 14) and 2 (day 14 to 28), respectively (Tables 1 and 2). The POS diets for the phase 1 contained various highly digestible and palatable special ingredients, whereas the phase 2 POS diets contained a fewer and less special ingredients.

Two simple corn-SBM, negative control (NEG) diets were formulated to be the same lysine content as the POS diets for the phases 1 and 2 in study 1, and the NEG diets for phases 1 and 2 were supplemented with 0.5, 1.0, 1.5, or 2.0% of fish peptides. Similarly, simple, corn-SBM-based phase 1 and 2 NEG diets were formulated to be the same lysine content as the POS diets in study 2, but the NEG diets contained 1.5% fish peptides or 1.5% fish peptides and multienzyme complexes.

Table 1. Composition of phase 1 (day 0 to 14) and 2 (day 14 to 28) weanling pig diets in the first study[1].

Item	POS	NEG	FP 0.5	FP 1.0	FP 1.5	FP 2.0
Phase 1						
Ingredient (%)						
Corn	45.62	50.91	50.84	50.77	50.70	50.63
Soybean meal (48% CP)	27.34	40.93	40.50	40.08	39.65	39.22
Dried whey	15.00	5.00	5.00	5.00	5.00	5.00
Soy protein concentrate	4.00	-	-	-	-	-
Plasma protein	2.00	-	-	-	-	-
Fish peptides	-	-	0.50	1.00	1.50	2.00
Poultry fat	3.00	-	-	-	-	-
Minerals & vitamins	3.04	3.16	3.16	3.15	3.14	3.14
Calculated composition						
SID Lys (%)	1.22	1.22	1.22	1.22	1.22	1.22
Phase 2						
Ingredient (%)						
Corn	56.28	58.92	58.85	58.79	58.72	58.65
Soybean meal (48% CP)	33.63	37.89	37.46	37.04	36.61	36.18
Dried whey	5.00	-	-	-	-	-
Soy protein concentrate	2.00	-	-	-	-	-
Fish peptides			0.50	1.00	1.50	2.00
Minerals & vitamins			3.19	3.19	3.20	3.21
Calculated composition						
SID Lys (%)			1.11	1.11	1.11	1.11

[1]POS = complex, positive control diet; NEG = simple corn-soybean meal, negative control diet; and FP 0.5, 1.0, 1.5, and 2.0 = NEG diet supplemented with 0.5, 1.0, 1.5, and 2% fish peptides (FP), respectively. CP = crude protein, and SID Lys = standardized ileal digestible lysine.

[2]Fed phase 1 (day 0 to 14) weanling pig diets from 7.9 ± 0.7 to 14.3 ± 1.1 kg and phase 2 (day 14 to 28) weanling pig diets from 14.3 ± 1.1 to 22.0 ± 1.5 kg.

During the fourth week of the study, approximately 5 mL of blood sample was collected from each pig in both studies. Blood samples were analyzed for serum metabolites, including total protein, albumin, globulin, urea N, glucose, cholesterol, and triglyceride concentrations, and cytokines, such as granulocyte-macrophage colony-stimulating factor, interferon-ɣ, various interleukins, and tumor necrosis factor-α, in study 1, and the same metabolites in study 2. The effort was made to estimate the optimum inclusion rate of fish peptides using selected response criteria and statistical and subjective approaches in study 1. The estimated dose was used as the basis for designing dietary treatments for the second study.

3. RESULTS & DISCUSSION

In the study 1 (data not shown), although there were some effects, dietary treatments or fish peptide supplementation seemed to have no clear effects on the growth performance of weanling pigs during the phase 1 or 2, which agreed with the findings reported by Poudel et al. (2020). Similarly, there seemed to be no clear effects of dietary treatments on serum metabolite (Mule et al., 2006; Wang et al., 2018) or cytokine concentrations during week 4 of the study. However, as the dietary fish peptide supplementation increased from 0 to 0.5, 1.0, 1.5, and 2.0 %, pigs tended to respond to the diets containing 1.5% fish peptides in many growth performance during the first 2 weeks after weaning and overall phase and serum metabolite data during week 4 (Figure 1). Therefore, the basis for designing dietary treatments for study 2 was 1.5% fish peptides.

Table 2. Composition of phase 1 (day 0 to 14) and 2 (day 14 to 28) weanling pig diets in study 2[1,2].

Item	POS	NEG	ALL
Phase 1			
Ingredient (%)			
Corn	46.56	54.23	53.60
Soybean meal (48% CP)	26.97	41.02	41.07
Dried whey	15.00	-	-
Fishmeal	4.00	-	-
Plasma protein	2.00	-	-
Fish peptides	-	1.50	1.50
Poultry fat	3.00	-	-
Minerals & vitamins	2.46	3.25	3.34
Calculated composition			
SID Lys (%)	1.22	1.22	1.22
Phase 2			
Ingredient (%)			
Corn	56.75	58.72	58.08
Soybean meal (48% CP)	33.44	36.61	36.65
Dried whey	5.00	-	-
Fishmeal	2.00	-	-
Fish peptides	-	1.50	1.50
Minerals & vitamins	2.81	3.17	3.27
Calculated composition			
SID Lys (%)	1.11	1.11	1.11

[1]POS = complex, positive control diet; NEG = simple corn-soybean meal, negative control diet with 1.5% fish peptides (Peptiva, Vitech Bio-Chem Corp., Orange, CA); and ALL = NEG diet supplemented with multienzyme complexes [Ronozyme Hiphos (GT) 2500 (10,000 FYT phytase/g) and Ronozyme Multigrain (GT) (2,700 U endo-1,4-beta-xylanase, 700 U endo-1,3(4)-beta-glucanase, and 800 U endo-1,4-beta-glucanase/g, and may contain other enzymes such as pectinase, mannanase, cellulase, protease, and amylase; DSM Nutritional Products, Inc., Ames, IA]. CP = crude protein, and SID = standardized ileal digestible.
[2]Fed phase 1 weanling pig diets from 7.8 ± 0.8 to 14.1 ± 1.4 kg and phase 2 weanling pig diets from 14.1 ± 1.4 to 22.7 ± 1.3 kg.

From day 0 to 14 of study 2, the efficiency of feed, lysine, or digestible energy utilization for weight gain was greater in pigs fed the NEG and ALL diets than those fed the POS diet (Table 3), which was a reflection of greater gain to feed, gain to lysine, or gain to digestible energy intake from day 7 to 14 in pigs fed the NEG and ALL diets than those fed the POS diet (data not shown). In addition, pigs fed the ALL diet tended to have greater gain to feed and gain to lysine intake and had greater gain to digestible energy intake than those fed the NEG diet during the first 2 wk (Table 3), which was, again, a reflection of pigs fed the ALL diet to have greater gain to feed, gain to lysine, or gain to digestible energy intake from day 7 to 14 than those fed the NEG diet (data not shown).

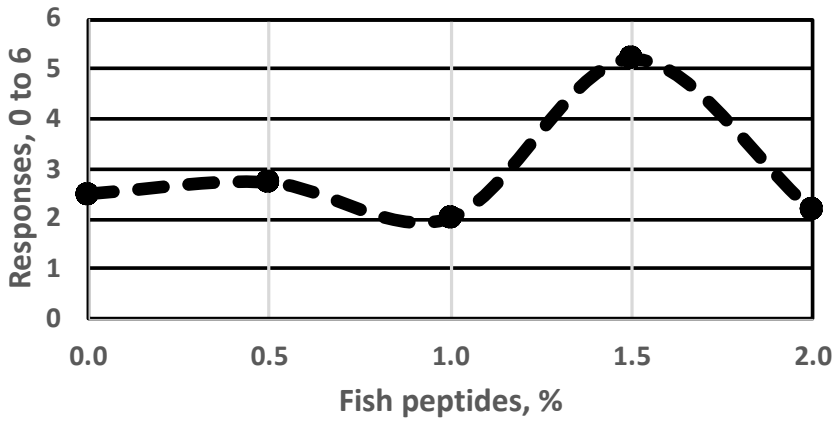

Figure 1. Effect of fish peptide supplementation on growth performance of weanling pigs and their serum metabolites in study 1. Each response criterion was expressed on the scale of 0 (lowest value) to 6 (greatest value), and the dashed line represents the average of 10 response criteria. Weanling pigs were fed phase 1 (day 0 to 14) diets from 7.9 ± 0.7 to 14.3 ± 1.1 kg and phase 2 (day 14 to 28) diets from 14.3 ± 1.1 to 22.0 ± 1.5 kg. [Please see Guo et al. (2022) for the response criteria used for the determining the optimum response to fish peptides.].

Dietary treatments had no effects on the intake of feed, lysine, and digestible energy, or no clear effect on weight gain during the first 2 weeks. Similarly, there seemed to be no clear effect of dietary treatments on growth performance during day 14 to 28 or overall. Pigs fed the NEG and ALL diets had greater serum total protein and globulin concentrations than those fed the POS diets (Table 4). Albumin to globulin ratio tended to be lower in those fed the NEG and ALL diets than those fed the POS diets. There was no clear effect of dietary treatments on serum albumin, urea N, glucose, cholesterol, or triglyceride concentration.

Table 3. Effect of fish peptide and multienzyme complex supplementation on growth performance of weanling pigs during the first 2 weeks of study 2 [1,2,3]

Item	ADFI (g/d)	LysI (g/d)	DEI (Mcal/d)	WG (g/d)	G:F (g/kg)	G:LysI (g/d)	G:DEI (g/Mcal)
Phase 1 (d 0 to 14)							
POS	702	8.52	2.59	192	275	22.6	74
NEG	671	8.16	2.29	202	301	24.8	88
ALL	650	7.90	2.20	218	337	27.7	99
SEM	17	0.21	0.07	7	10	0.8	4
P-value							
POS vs. NEG + ALL	0.406	0.406	0.142	0.147	0.004	0.004	< 0.001
NEG vs. ALL	0.550	0.550	0.113	0.528	0.095	0.095	0.009

[1]POS = complex, positive control diet; NEG = simple corn-soybean meal, negative control diet with 1.5% fish peptides, and ALL = diet supplemented with 1.5% fish peptides and multienzyme complexes; n = 4.
[2]ADFI = average daily feed intake, LysI = standardized ileal digestible Lys intake, DEI = digestible energy intake, WG = weight gain, G:F = gain to feed, G:LysI = gain to LysI, G:DEI = gain to DEI, and SEM = pooled standard error of the mean. d = day.
[3]Fed phase 1 (d 0 to 14) weanling pig diets from 7.8 ± 0.8 to 14.1 ± 1.4 kg and phase 2 (d 14 to 28) weanling pig diets from 14.1 ± 1.4 to 22.7 ± 1.3 kg, even though the data for day 14 to 28 and overall are not presented.

Table 4. Effect of fish peptide and multienzyme complex supplementation on serum metabolites in weanling pigs during the week 4 of study 2 [1,2,3].

Item	TP (g/dL)	Alb (g/dL)	Glob (g/dL)	Alb:Glob	Urea N (mg/dL)	Gluc (mg/dL)	Chol (mg/dL)	TG (mg/dL)
POS	4.31	3.65	0.68	6.04	14.46	119	66.6	49.1
NEG	4.48	3.90	0.58	7.68	14.60	112	66.1	43.8
ALL	4.80	3.73	1.08	3.84	15.41	114	60.9	49.4
SEM	0.07	0.06	0.08	0.77	0.32	2	1.7	2.0
P-value								
POS vs. NEG + ALL	0.002	0.705	0.007	0.063	0.228	0.709	0.168	0.514
NEG vs. ALL	0.166	0.114	0.511	0.347	0.865	0.241	0.919	0.320

[1]POS = complex, positive control diet; NEG = simple corn-soybean meal, negative control diet with 1.5% fish peptides, and ALL = diet supplemented with 1.5% fish peptides and multienzyme complexes; n = 4.

[2]TP = total protein, Alb = albumin, Glob = globulin, Alb:Glob = Alb to Glob ratio, Chol = cholesterol, and TG = triglyceride, and SEM = pooled standard error of the mean. Blood samples were collected during the fourth week of the study.

[3]Fed phase 1 (day 0 to 14) weanling pig diets from 7.8 ± 0.8 to 14.1 ± 1.4 kg and phase 2 (day 14 to 28) weanling pig diets from 14.1 ± 1.4 to 22.7 ± 1.3 kg.

Weaning is the most critical period in the pig's life, and one of the most important weaning stressors is the digestive challenge. Although corn-SBM diets are the gold standard for feeding pigs, the young pig's digestive enzymes are insufficient to utilize corn and SBM efficiently. For that reason, weanling pigs are typically fed complex diets containing various special ingredients such as dried whey, plasma protein, fish meal, soy protein concentrate, and oat groats. Providing such diets containing highly-palatable and highly-digestible ingredients to weanling pigs is very effective in promoting their growth performance (Wang et al., 2018), however, feeding such diets can be rather costly. With the development of feed additives such as fish peptides, which can be not only a great source of nutrients but also considered as a functional feed additive (Zamora-Sillero, 2018), and multienzyme complexes (Zhang et al., 2014), it is possible for weanling pigs to extract energy and nutrients from corn and SBM more efficiently.

The effort to estimate the optimum inclusion rate by using some statistical approaches was not successful in study 1 possibly because of the variations in the data. The subjective evaluation of the selected response criteria, however, indicated that the greatest response to fish peptides seemed to be obtained at 1.5%. In study 2, pigs fed the NEG and ALL diets, which contained 1.5% fish peptides, had greater efficiency of feed, Lys, and DE utilization for WG during the first 2 wk. In addition, pigs fed the ALL diets with 1.5% fish peptides and enzyme complexes tended to have greater gain to feed and gain to lysine intake and had greater gain to digestible energy intake than those fed the NEG diet. The results indicated that it may be possible to replace a complex diet with a simple corn-SBM diet by supplementing the weanling pig diet with fish peptides and enzymes complexes during the phase 1 or from 0 to 14 days after weaning.

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plasma protein, soy protein concentrate, and Zn oxide, respectively. Technical assistance of B. Anderson, R. Britton, the staff at the Auburn University Swine Research and Education Center, and J. Pate and his staff at the Auburn University Poultry Research Farm is gratefully acknowledged.

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Weaning-induced Changes in Liver and Small Intestine Cysteine Utilization in Pigs

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TAKE HOME MESSAGE

Current industry practice of weaning pigs at about 21 days of age disrupts nutrient metabolism and impedes the adequate development and maturation of the gut, which together contribute to post-weaning growth lag. The objective of this work was to determine how weaning-induced anorexia impacts liver and small intestine cysteine metabolism in pigs. We found that despite elevated plasma and liver cysteine concentrations, glutathione concentration declines in the small intestine in weaned pigs. Correction small intestine glutathione, independent of feed intake, may be a potential approach to mitigate post-weaning growth lag in pigs. A cut in this lag of one day alone can amount to savings of about $0.60 per pig in feed costs, or $83,600,000 per year across the U.S., assuming $0.10/lb feed.

SUMMARY

Current industry practice of weaning pigs between three and four weeks of age prevents normal development of the small intestine and, in turn, impairs nutrient utilization and growth. The sulfur amino acids methionine and cysteine are used inefficiently for growth compared to other essential amino acids and, as substrates for one-carbon metabolism and glutathione production, have key roles in maintaining the intestinal lining. The objective of this work was to determine how weaning-induced anorexia impacts liver and small intestine cysteine metabolism in pigs. Pigs were either weaned into the nursery with no access to feed but free access to water (W; n = 9) or remained in the farrowing room with their respective sows (NW; n = 9) for 2 days until euthanasia and tissue collection. We found that, despite elevated cysteine in plasma and liver, cysteine in the small intestine was not affected by weaning. Yet, glutathione declined in the small intestine in, indicating that cysteine is not effectively channeled to glutathione production in this tissue after weaning. Understanding the interaction among weaning, low feed intake, and sulfur amino acid metabolism will help develop new strategies that support gut function and mitigate post-weaning growth lag in pigs.

1. INTRODUCTION

Global demand for high quality animal protein, economics, and sustainability concerns necessitates increases in the efficiency of pork production. However, the immediate post-weaning period is at odds with efficient pork production. Current industry practice of weaning pigs at about 21 days of age disrupts nutrient metabolism and impedes the adequate development and maturation of the gut. The term "gut health" lacks a clear definition but it broadly includes gut morphology, nutrient utilization, the microbiome, mucosal immunity and barrier capacity, and their interactions. Low feed intake is common in newly weaned pigs and is largely responsible for post-weaning growth lag. Indeed, the number of pigs that do not consume any feed within 24 hours of weaning ranges from 30 to 50% and average daily feed intake within the first 4 days of weaning is often less than 0.25 lb/d (Bruininx et al., 2001). However, other factors such as inflammation and infection also contribute to poor gut health and growth performance.

The sulfur amino acids methionine (Met) and cysteine (Cys) are characterized by their comparatively inefficient use for lean protein accretion compared to other essential amino acids (National Research Council, 2012). This is in part due to the high demand for Met in one-carbon metabolism and for Cys as the limiting substrate glutathione (GSH) production, an important antioxidant that also has a role in regulating the turnover of the intestinal lining (Shoveller et al., 2005). Methionine and Cys use, however, is impacted in opposite directions by low feed intake and immune challenge, two key aspects of weaning stress. The relative amount of total sulfur amino acids, Met, and Cys that optimize growth performance and support gut function in weaned pigs with low feed intake is unclear. **The objective of this work was to determine how weaning-induced anorexia affects liver and intestine Cys metabolism in pigs**. Understanding how low feed intake after weaning alters sulfur amino acid metabolism will help refine feeding recommendations for Met and Cys in starter diets and mitigate post-weaning growth lag.

2. PROCEDURES

Yorkshire pigs (initial body weight = 15.2 ± 0.53 lb) were divided in one of two across two blocks at 21 days of age: not weaned (NW; pigs remained on the sow; n = 9) and weaned (W; pigs were weaned, provided no access to feed but free access to water; n = 9). At 23 days of age, pigs were euthanized and blood, liver, jejunum, and ileum tissues were collected, snap-frozen in liquid nitrogen, and stored at – 80°C until analysis. We analyzed plasma and tissue metabolites, including Met, Cys, GSH, and the GSH precursors by high performance liquid chromatography. We also measured the activity of proteins needed for GSH synthesis in liver. Data were analyzed with the generalized linear mixed model procedure of SAS version 9.4 (SAS Institute, Cary, NC); treatment was considered a fixed effect and pig and block were considered random effects. Data are presented as least-squares means ± standard error of the mean. Results are considered significant at $P < 0.05$ and a trend at $P < 0.10$.

3. RESULTS & DISCUSSION

We sought to determine how plasma and jejunum amino thiol concentrations are affected by weaning-induced anorexia. While no feed intake is atypical on commercial farms, there is still a considerable lag between weaning and time to first feed intake. Moreover, total feed intake is normally limited in the first 48 hours after weaning in pigs.

Pig body weight decreased in W pigs to 14.1 ± 0.6 lb, whereas body weight increased in NW pigs to 16.3 ± 0.6 lb over the 2-day period ($P < 0.0001$). Plasma cysteine concentration was 46% higher in W compared to NW pigs (271 versus 192 ± 19 μmol/L; $P < 0.001$). Despite elevated plasma Cys concentration, jejunum and ileum Cys concentrations were not affected by weaning ($P > 0.10$; Figure 1).

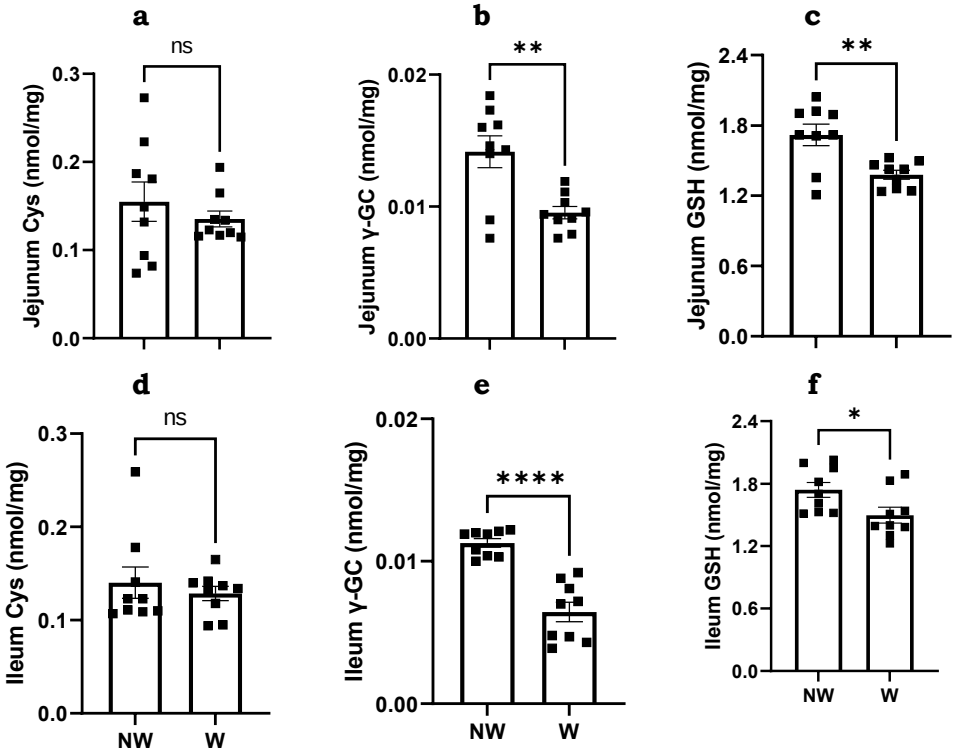

Figure 5. Jejunum Cys (a), γ-GC (b), GSH (c), and ileum Cys (d), γ-GC (e), and GSH (f) concentrations are altered by weaning in pigs. Data from individual pigs are shown; n = 9 per group. NW, not weaned; W, weaned; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$

However, both γ-glutamylcysteine (γ-GC), the immediate substrate for GSH synthesis, and GSH concentrations were lower in both the jejunum and ileum in W compared to NW pigs ($P < 0.05$). Liver Cys was 40% higher W compared to NW pigs ($P < 0.05$), but liver γ-GC was not different between groups ($P > 0.10$). Liver taurine, resulting from the irreversible loss of Cys, was also 52% higher in W compared to NW ($P < 0.01$). Although liver Cys concentrations were higher in W compared to NW pigs, the activity of γ-glutamylcysteine ligase (GCL) and glutathione synthetase (GSS), enzymes needed for GSH production from Cys, were 67% and 25% lower in W compared to NW pigs, respectively ($P < 0.001$; Figure 2).

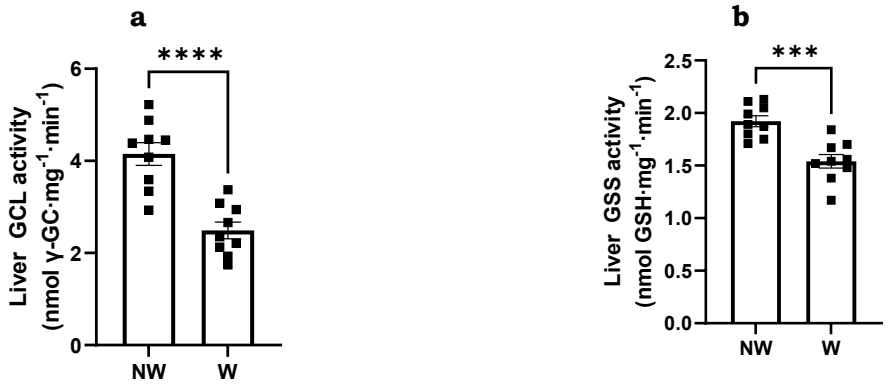

Figure 2. Liver GCL (a) and GSS (b) enzyme activity is decreased in weaned pigs. Data from individual pigs are shown; n = 9 per group. NW, not weaned; W, weaned; ***, $P < 0.001$; ****, $P < 0.0001$.

Collectively, these findings indicate that both intestinal and liver Cys is channeled toward protein synthesis or oxidation at the expense of GSH production in newly weaned pigs with minimal feed intake. Beyond the inadequate supply of nutrients for normal tissue maintenance and growth, the specific depletion in gut GSH levels may contribute to gut dysfunction at weaning by exacerbating oxidative stress, impairing immune function, and affecting the turnover of the intestinal lining. Correction of gut γ-GC and GSH levels, independent of feed intake, could be a potential approach to mitigate weaning stress in pigs. While recent work has indicated that a higher sulfur amino acid levels in the diet is beneficial for nursery pig performance (Capozzalo et al., 2017; Kahindi et al., 2017), complementary mechanistic data will help refine sulfur amino acid needs for nursery pigs and help develop new strategies that support gut health and mitigate post-weaning growth lag. A cut in this lag of one day alone can amount to savings of about $0.60 per pig in feed costs, or $83,600,000 per year across the U.S., assuming $0.10/lb feed. Future work will explore the interaction between increasing feed intake, intestinal and whole-body sulfur amino acid metabolism, gut function, and sulfur amino acid requirements in newly weaned pigs.

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