AUBURN

ALABAMA LIVESTOCK RESEARCH REPORT

2023

Department of Animal Sciences

Welcome

Almost $450 million worth of cattle and calves were sold in Alabama last year, generating a $2.5 billion impact. Hay and haylage production resulted in a value of $235 million to producers supporting the cattle and horse industries. Over $8 million worth of pork was marketed and $7 million of dairy products was generated in the state. With 47,000 meat goats, the state ranks 8th in goat production. Animal agriculture is alive and well in Alabama.

At a time when the world population continues to grow, there is a greater demand for high-quality animal protein. However, with population growth resulting in more urban sprawl, fewer land resources are available to meet the food and animal production needs. This increases the need for basic and targeted food animal research programs to provide technology that increases the efficiency of food animal production systems.

The Department of Animal Science faculty and staff are answering this call by working synergistically in the development of research and extension programming across the state. Multidisciplinary programs are being developed, implemented, and delivered to meet the needs of stakeholders (both food animal producers and food animal consumers) across the state, region, nation, and world. Thus, it is with great pleasure that we present to you the second edition of the Alabama Livestock Research Report. Building upon the success of our first publication, this edition continues to serve as a comprehensive overview of the research and outreach initiatives developed in the Department of Animal Sciences at Auburn University.

This publication combines reports from faculty, staff, and graduate students on campus and Experiment Stations. We provide in-depth information on our research programs, which incorporate growth biology, reproductive physiology, nutrition, genetics, meat science, animal health, management, and production practices into a comprehensive program. Additionally, we showcase our outreach activities and partnerships with industry stakeholders.

We are proud to say that this report and research are made possible thanks to the financial support of funding agencies, organizations, and stakeholders who believe in the value of our work. Thank you to the faculty, students, and staff that contributed to this publication. 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 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.

Sincerely,

L. Wayne Greene
Professor and Head
Department of Animal Sciences
210 Upchurch Hall, Auburn Univ, AL 36849
greenlw@auburn.edu

CONTENT

Beef Production Systems

Survey of Producer Perspectives: Weaning and Backgrounding Management Practices used by Alabama Beef Cattle Operations **1**

Extended Effects of Sequential Weaning and Backgrounding Management in Beef Calves **6**

Fall Management of Bahiagrass With or Without Nitrogen Fertilization as a Short-term Stockpiling Forage Option **10**

In situ Digestibility and Nutritive Value of Cool-Season Annual Grass Baleage **13**

Parsing the Effects of Bag Type and Length of Incubation on In Situ Digestibility Results **19**

Grazing Cover Crops in a Cotton-Peanut Rotation in the Wiregrass Region of Alabama **25**

Can In Vitro or In Situ Disappearance Assays Accurately Predict In Vivo Digestibility of Bermudagrass? **30**

Cell Wall Digestibility of Four Bermudagrass Cultivars in Beef Heifers **34**

Variation in Nutritive Value of Whole Cottonseed from the Regional Breeding Testing Network **38**

Extent of Use of Supplemental Feeding in Beef Cattle Operations Across the Southeast: A Preliminary Report of the Regional Survey **42**

A Comparison of In Vitro Methodologies for Determination of Forage Digestibility **47**

Targeting Candidate Genes for Pregnancy in Beef Cows **53**

Molecular Targets Differing at Weaning in Beef Heifers with Varying Reproductive Outcomes **59**

Developmental Expression of Myogenic Regulatory Genes in Bovine Myoblast Cultures and Fetal Skeletal Muscle **64**

Meat Sciences

Extended Storage of Beef Steaks Using Thermoforming Vacuum Packaging **69**

Influence of Sous Vide Cooking on Ground Beef Patties **74**

Non-Ruminant Nutrition

Protected Benzoic Acid Supplementation Marginally Increases Growth Performance in the Starter Pigs **80**

Benzoic Acid Reduces Growth Performance in Starter Pigs Fed Low Protein Diets **85**

Extension & Outreach

Bolstering the Social Licensure of Animal Agriculture - Creation of More Effective Communication and Literacy Ecosystems **89**

Livestock and Forages Extension Success Stories: Programs for Alabama Stakeholders **103**

Survey of Producer Perspectives: Weaning and Backgrounding Management Practices Used by Alabama Beef Cattle Operations

S. Maggie Justice [1], M. Kim Mullenix [2,*], D. Alex Tigue [3], S. Leanne Dillard [2], W. Brandon Smith [2], Michelle F. Elmore [2], Max Runge [4], and K. Kelley [5]

[1] Division of Agriculture, University of Arkansas, Little Rock, AR; mjustice@uada.edu;
[2] Department of Animal Sciences, Auburn University;
[3] Cargill Animal Nutrition, Somerset, KY, USA;
[4] Department of Agricultural Economics and Rural Sociology, Auburn University, AL, USA;
[5] Alabama Cooperative Extension System Farm and Agribusiness Management Team, Brewton, AL, USA.
* Correspondence: mullemk@auburn.edu

TAKE HOME MESSAGE

Beef calf management strategies used during the weaning and post-weaning period can have extended effects on growth performance and health in all sectors of the production chain. Understanding post-weaning management strategy impacts on calf performance and health can help producers add value to their operations and further strengthens the viability of the beef supply chain. From an educational perspective, it is important for Extension and industry professionals to understand how post-weaning management strategies are used by beef operations in the Southeast US and the potential success of those practices. The objective of this set of studies was to determine the extent of use of calf management strategies in Alabama cow-calf operations and Extension strategies for increasing practice adoption.

SUMMARY

An online survey with 24 total questions was developed and distributed to cattle producers in the state of Alabama. Questions addressed if producers do or do not use managed weaning and backgrounding strategies. A total of 214 complete responses were received with 94% of respondents considering their operation to be a cow-calf operation. Key challenges producers face in their operations who practice managed weaning and backgrounding strategies included input costs, land availability, and market predictability. Developing demonstration data models to address cost-benefits of weaning and backgrounding may help producers evaluate areas of challenges identified in this survey. Extension educators can apply this data to create resources and programs centered around backgrounding cattle to help improve overall understanding related to calf management post-weaning.

1. INTRODUCTION

Cattle production in the state of Alabama represents a USD 2.5 billion industry (USDA, 2021). The Southeast US beef cattle industry is dominated by cow-calf operations, which operate under individualized management strategies rather than an integrated production chain. With the number of different management strategies that a producer can utilize during these stages of production, periodic characterization of on-farm management practices used by Southeast U.S. cattle producers who do or do not use managed weaning and backgrounding practices may help Extension professionals develop or refine educational strategies for improving adoption. **The**

objective of this study was to determine perceptions, on-farm applications, and potential barriers of adoption of beef calf weaning and backgrounding among Alabama producers.

2. PROCEDURES

A web-based survey was conducted with Alabama beef producers to determine the extent of use of various calf management practices pre- and post-weaning. This survey was approved through the Auburn University Institutional Review Board under Protocol # 22-066. The survey contained 24 total questions and was distributed in March in 2022. Questions focused on management strategies on calves pre- and post- weaning including nutrition and marketing decisions. Several questions focused on the barriers some producers might experience in adopting better management practices. However, total length of the survey for each participant was dependent on the type of answer to specific questions. For example, at question 10, participants were asked if they background their calves following their weaning period. Participants who selected no had three more questions before their survey ended. Participants that stated they background in most or some years had eleven more questions to answer.

The survey was distributed through Qualtrics™ software. Collaboration with state commodity groups (Alabama Beef Cattle Improvement Association, Alabama Cattlemen's Association, Alabama Farmers Federation) and state Extension personnel (County Extension Coordinators and Regional Extension Agents) helped distribute the survey through an online survey response link. These groups sent producer listservs an email with a direct link to the survey. Survey data was also collected through solicitation of responses at in-person commodity group events (n = 2 during March 2022). The survey link was also shared through the Alabama Cooperative Extension System website and affiliated social media pages (Alabama Beef Systems Extension Program and Alabama Forage Focus Program Facebook pages). A total of 214 responses were received by the end of the survey deadline.

3. RESULTS & DISCUSSION

3.1. Demographics of Respondents and Calving Season Distribution

The 214 survey respondents represented beef cattle operations from across the state of Alabama. Participants were asked to classify their operation as: commercial cow-calf, purebred cow-calf, commercial and purebred cow-calf, stocker/backgrounder, cow-calf, and stocker or other. The results were as follows:

- 52% cow-calf operation;
- 11% purebred cow-calf system;
- 17% both purebred and commercial cow-calf operation;
- 13% both cow-calf and stocker operation;
- 6% stocker operations.

Forty six percent of respondents indicated they had a smaller size herd of 50 head or less that calve each year (22% at 25 or less and 24% at 26 to 50 head). A herd size of 51 to 100 head represented 28% of respondents, whereas the remaining operations (26% of respondents) had 101 head or greater. These results follow similar trends reported by McBride and Mathews (2011), where beef cattle production in the Southeast US tend to be centered around smaller cow-calf herds that are considered secondary income for farmers. Almost half of respondents (47%) reported a

calving season in the fall (October to December). Twenty-three percent of participants had a winter (January to March) calving season, and 19% as a spring season (March to May). There was an 11% response for no defined calving season among producers.

3.2. Ranking of Management Considerations

With a diverse landscape of farm size and structure in the cow-calf sector in mind, respondents were then asked to rank a series of calf production management considerations and challenges from most-to-least relevant as they applied to their beef cattle operation (Table 1). Table 1 illustrated the top three key challenges producers face are input costs, land availability, and market predictability. Mid-level topics were lack of marketing options, sickness, facilities, and labor. Topics of lesser priority included stress of operation and wildlife. These responses were consistently placed at the bottom of the scale of responses.

Table 1. Ranking of challenges for Alabama beef cattle operations from most relevant to least relevant**.**

-	**Rank (% of responses)**								
Issues	1	2	3	4	5	6	7	8	9
Input costs	88	71	24	4	2	0	0	2	1
Land availability	69	27	17	22	16	8	9	6	18
Market predictability	11	34	57	40	21	13	10	5	1
Marketing options	2	16	29	46	31	24	21	19	4
Sickness	4	9	11	19	49	54	29	14	3
Facilities	9	12	28	22	20	41	28	21	11
Labor	8	22	23	21	25	17	22	47	7
Stress	1	0	3	16	17	20	62	59	14
Wildlife	0	1	0	2	11	15	11	19	13

3.3. Weaning Management Practices

Survey respondents described their weaning management strategy as follows:

- 55% - abrupt weaning
- 385 fenceline weaning
- 5% nose-flap weaning

Abrupt weaning is considered the more traditional form of weaning for cow-calf operations, where there is no transition period following separation of the calf from the dam. Alternative weaning strategies that reduce the stress of separation at weaning, such as fenceline or nose-flap weaning, may improve overall calf performance. This is especially true if producers plan to retain ownership of calves post-weaning through the backgrounding phase. Low-stress weaning may ease this transition period and improve performance early in the backgrounding period (*see Extended Effects of Sequential Weaning and Backgrounding Management in Beef Calves - research report 2, Justice et al*. 2023).

3.4. Backgrounding Practices

61% of survey respondents indicated that they background their calves, while 25% noted that they do in some years but not always. For the remaining 22% who do not practice backgrounding, they were asked to select reasons why they do not use this strategy. Overall, market unpredictability (22%) was the main concern producers face. Facilities, costs of nutritional

management and health, time, and land availability were also identified as reasons for not backgrounding cattle.

56% of producers use rotational grazing within their farm, with warm-season perennial grasses dominating these systems, followed by the use of cool-season annuals. Two-thirds of producers reported that they regularly soil test pastures, but only 31% of respondents conduct a forage analysis annually. Feed supplementation strategies used during the backgrounding phase rely primarily on byproduct feedstuffs, such as soybean hulls, dried distillers grains, 50/50 soyhulls and corn gluten feed, and whole cottonseed, which are hand-fed daily (62% of respondents who background cattle).

Producers who background calves had a high rate of adoption of vaccination (83%) and castration (81%) practices, whereas the use of implants is moderate (37%). While more than 90% of feedyards utilize implants, and they have been shown to be a cost-effective way to increase weight gain in calves (Beck et al., 2014), adoption of implants may be less in part due to awareness, lack of familiarity with their use, or expected marketing outcomes for calves (i.e. natural beef marketing programs where implants are not used).

Marketing strategies for backgrounded calves primarily included direct sales through the local livestock auction. Direct market sales are the second-most frequent method of marketing calves after backgrounding. Producers also utilize local special sales (28%) like board sales or online auctions (9%). Almost 14% of producers stated that they retain ownership of their calves through the feedyard finishing phase.

Results of this survey can be used to develop and Extension educational response towards enhancing weaning and backgrounding management application in Southeast U.S. beef cow-calf operations. While the survey audience represents cattle producers who are actively engaged in industry promotion and development, results highlight key areas where educational focus can be refined. On-farm demonstrations may provide a stepwise approach to help producers make decisions regarding the adoption of weaning and backgrounding management practices. Cost-benefit tools, such as online budgets, may help producers weigh decisions regarding weaning management, and if backgrounding may be profitable within a given year. An on-farm checklist which enables producers to identify areas of improvement may help make steps towards practice adoption or improve current weaning and backgrounding strategies utilized on farm.

Challenges such as lack of market predictability and marketing opportunities can be met with education focused on communication with stockyards prior to selling calves and full understanding of available premiums offered for backgrounded animals. Programmatic partnerships with value-added calf sales in the state and industry professionals may help bring new or value-added information to beef cattle producers with the aim of increasing the use of various technologies or enhancing marketing opportunities for adopted practices.

Funding

The authors would like to thank the Alabama Cattlemen's Association State Beef Checkoff Program for funding this survey. The results help Extension professionals update current resources and tailor information to our producers.

4. REFERENCES

Beck, P., B. Stewart, S. Gadberry, J. Tucker, D. Hubbell, J. Butterbaugh, T. Hess, K. Coffey, and B. Rudolph. 2014. Effect of daily or alternate-day distillers grains supplementation with or without monensin on performance of grazing calves. Prof. Anim. Sci. 30:515–526.

McBride, W. D., and K. Mathews. 2011. The diverse structure and organization of US beef cow-calf farms. USDA-ERS Economic Information Bulletin. (73).

Extended Effects of Sequential Weaning and Backgrounding Management in Beef Calves

S. Maggie Justice [1], M. Kim Mullenix [2,*], D. Alex Tigue [3], S. Leanne Dillard [2], W. Brandon Smith [2], Soren P. Rodning [2], and Manuel F. Chamorro [4]

[1] Division of Agriculture, University of Arkansas, Little Rock, AR, USA; mjustice@uada.edu;
[2] Department of Animal Sciences, Auburn, AL, USA;
[3] Cargill Animal Nutrition, Somerset, KY, USA;
[4] Auburn University College of Veterinary Medicine – Clinical Sciences, Auburn, AL, USA.
* Correspondence: mullemk@auburn.edu

TAKE HOME MESSAGE

Strategic post-weaning management of beef calves may improve performance and health of calves entering into the feedyard phase. The objective of this study was to assess the collective effects of weaning and backgrounding practices have on calf health and performance through the feedyard phase.

SUMMARY

Beef industry in the Southeast US is largely comprised of cow-calf operations, where calves are commingled at weaning and transported several times before arrival to their final production destination. This project explores opportunities to reduce stress at weaning and determine the impacts of collective weaning and backgrounding practices on calf health and performance through the feedyard phase.

1. INTRODUCTION

Beef calf management strategies used during the weaning and post-weaning periods can have extended effects on growth performance and health in all sectors of the production chain. Understanding post-weaning management strategy impacts on calf performance and health can help producers add value to their operations and further strengthens the viability of the beef supply chain. From an educational perspective, it is important for Extension and industry professionals to understand how post-weaning management strategies are used by beef operations in the Southeast U.S. and the potential success of those practices. **The objective of this study was to assess the collective effects of weaning practices have on calf health and performance through the feedyard phase.**

2. PROCEDURES

A two-year study was conducted using 427 steer calves (n = 216, year 1, average initial body weight ~623 lb; n = 213, year 2, average initial body weight ~640 lb) from three Auburn University experiment stations (Black Belt Research and Extension Center, Marion Junction; E.V. Smith Research Center, Shorter; and the Gulf Coast Research and Extension Center, Fairhope).

Calves were assigned to one of three different weaning method groups for a 14-day experimental period: fenceline, nose-flap, or abrupt weaning. Fenceline-weaned calves had nose-to-nose or visual/vocal contact with their dam through the fence post-weaning. Nose-flap weaned

calves received a nose-flap device at the time of weaning, which is designed to prevent suckling. Abruptly weaned calves were separated from their dams, then transported to a central backgrounding farm (E.V. Smith Research Center). Body weights were collected during the 14-day period as a measure of growth performance. Blood samples were collected to measure vaccination and acute phase protein response.

After the weaning period, calves were brought to a centralized farm and began a 60-d backgrounding period where they were randomized according to previous weaning management and body weight to one of three nutritional management strategies in a 3×3 factorial design: cool-season baleage and 1% BW dried distillers' grains (DDGS), bermudagrass hay and 1% BW DDGS, or grazing mixed warm-season annuals and 1% BW DDGS. Body weights and blood samples were again collected throughout the backgrounding period.

3. RESULTS & DISCUSSION

In both years of the study, fenceline weaned calves had the greatest average daily gain at 2.4 lb/day ($P < 0.0001$) and abruptly weaned calves had the lowest average daily gain losing 0.33 lb/day during the 14-d observation period (Figure 1). In Year 1, steers had a significantly greater ($P < 0.0001$) gain across all treatments than calves in Year 2, with Year 1 calves gaining 17 lb more during the weaning period than Year 2 calves. In Year 1, abruptly weaned calves had greater blood levels of haptoglobin (0.084 mg/mL: $P < 0.0001$) than both the fenceline and noseflap weaned calves (0.023 mg/mL; 0.020 mg/mL).

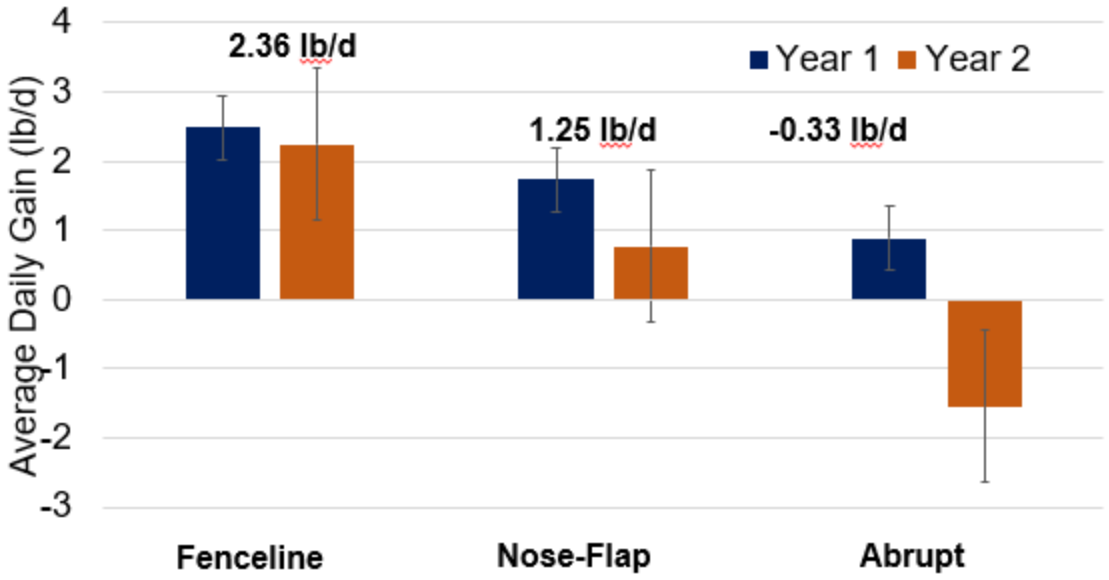


Figure 1. Average daily gain (lb/day) of beef calves during the weaning period (14-d in length, 2-year summary).

During the backgrounding period, fenceline weaned calves had the greatest average daily gain ($P = 0.02$) in the first 30 days regardless of the backgrounding diet type (Figure 2). Calves fed the bermudagrass hay-based diet also had a greater average daily ($P < 0.0001$) than both the grazing and baleage diet groups in the first 30 days of backgrounding. From day 30 to 60 of backgrounding in each year, calves on the hay-based diet had the lowest average daily gain ($P < 0.0001$). Steers on both the warm-season annual grazing and cool-season baleage diets supported greater average

daily gains ($P < 0.0001$) during the last 30-d of the backgrounding period (1.63 lb/day and 1.69 lb/d respectively).

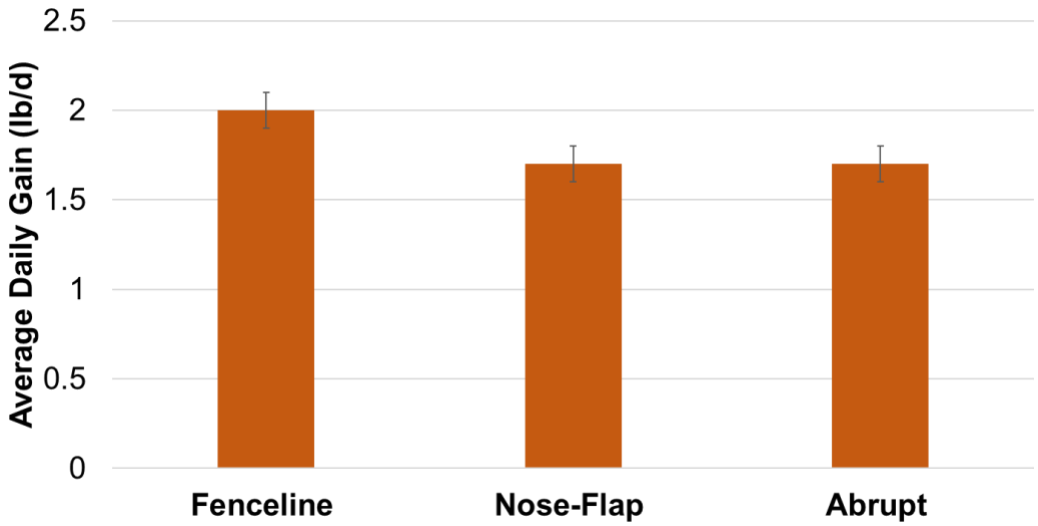


Figure 2. Carryover effects of weaning method (fenceline, nose-flap, or abrupt) on average daily gain during the first 30 days of a 60-day backgrounding period (2-year summary).

Following the backgrounding period, steers were transported to a commercial feedyard in Montezuma, KS, where they remained for the finishing phase. During this phase, performance was tracked through periodic weigh-ins and finally through carcass performance. Weaning method or backgrounding diet did not impact growth performance in the feedyard in this study, and carcass values at harvest were 79% choice or greater (Table 1). This illustrates the effectiveness of various backgrounding strategies, and flexibility in forage-supplement diet choices that producers may utilize before sending cattle to the feedyard finishing phase. Under these nutritional management strategies, cattle transitioned to the feedyard similarly, and produced a quality beef product at harvest.

Results indicate that weaning and backgrounding management strategies may influence calf performance during the transition period into the post-weaning phase. Specifically, the transition from weaning to backgrounding represents a time gap where easing stress results in improved animal performance, especially during the first 30 days. Time of marketing of cattle post-weaning is an important consideration for producers, where the effects of weaning were still prevalent and impacting growth performance up to 45 days post-weaning.

Table 1. Carcass characteristics of beef calves backgrounded on various southeastern U.S. diets

Diet[1]	BD	HD	GD	SEM	*P*
% Choice[2]	87	84	79	13	0.64
Marbling[3]	469.9	474.7	460.6	8.4	0.39
CYG[4]	2.62	2.6	2.62	0.2	0.89
HCW, lb	945	945	941	5.9	0.75
Backfat, in[5]	0.65	0.66	0.64	0.01	0.89
REA, in[2,6]	15.2	15.2	15.1	0.01	0.87

[1] Backgrounding period diets are defined as: BD = cool season annual baleage with 1% of animal body weight per day of dried distillers grains; HD = bermudagrass hay with 1% of animal body weigh per day of dried distillers grains; GD = grazing of warm season annual mixture with 1% of animal body weight per day of dried distillers grains. [2] Percentage of carcasses that were graded USDA Choice or USDA Prime. [3] Marbling Score 300-399 = Slight, 400-499 = Small, 500-599 = Modest degrees of marbling in the *L. dorsi* when observed at the break between the 12th and 13th rib. [4] The unrounded calculated USDA Yield Grade. [5] Thickness of the subcutaneous fat at the break between the 12th and 13th rib, measured in centimeters. [6] Ribeye area is the area of the *L. dorsi* in square centimeters at the break between the 12th and 13th rib of the carcass.

Acknowledgments

Special thanks to the Black Belt Research and Extension Center (Marion Junction), Gulf Coast Research and Extension Center (Fairhope), and EV Smith Research Center (Shorter) for their time, dedication, and expertise in helping our research team manage this project.

Funding

This research was funded by an Alabama Agricultural Experiment Station Interdisciplinary Research Award (2020) and the Alabama Cattlemen's Association State Beef Checkoff Program.

Fall Management of Bahiagrass With or Without Nitrogen Fertilization as a Short-term Stockpiling Forage Option

Michael Agar [1], Josh Elmore [2], Kelly Palmer [2], Anthony Wiggins [2], Ken Kelley [2], S. Leanne Dillard [1], W. Brandon Smith [1], Peyton Zessin [1], and M. Kim Mullenix [1,*]

[1] Auburn University Department of Animal Sciences, Auburn, AL 36849
[2] Alabama Cooperative Extension System, Auburn, AL 36849
*Corresponding author: mullemk@auburn.edu

TAKE HOME MESSAGE

Stockpiling bahiagrass may serve as a short-term grazing crop during the late fall and early winter months in Alabama. For many producers, bahiagrass pastures are reliable and feasible option due to low input management when compared to other forage systems. When targeted fall management is used (clipping or grazing of bahiagrass followed by N fertilization) late in the growing season, it can support moderate forage production and nutritional value during a time when forage quality and production is often minimal.

SUMMARY

Across the two-year period, the average number of production days past the stockpiling interval (6, 8, or 10 weeks of growth) for bahiagrass ranged from 30 to 50 days of production. Forage accumulation ranged from 1,110 to 1,548 lb DM/acre across stockpiling period length and N fertilization strategies. Nitrogen fertilization management increased forage production by an additional 250 to 300 lb DM/acre.

1. INTRODUCTION

Bahiagrass is a perennial warm-season grass that is widely used in Alabama. It can be managed under hay production or grazing and is well-adapted to growing conditions in the southern half of the state. In 2021, the Alabama Beef Checkoff Program funded a 2-year small-plot research study where **we evaluated the optimal time of year to start stockpiling bahiagrass**. From our small plot work, we determined that stockpiled bahiagrass has greater nutritional value than most late-season bahiagrass hay and provides between 1,500 to 2,000 pounds of dry matter per acre in forage available for grazing when stockpiled for a period of 8 to 10 weeks before use.

2. PROCEDURES

This study was established at three locations in the state using private, beef cattle producer farms, and Auburn University outlying research unit farms as research sites: private farm locations include Hope Hull; Bay Minette; and Troy, AL and one site at the Wiregrass Research and Extension Center in Headland, AL. Target date of resetting sites for stockpiling initiation was Sept 15 in fall 2021 and 2022. 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. This

information will be used to help determine the optimum number of weeks for stockpiling bahiagrass, and the number of quality grazing days provided in this system.

Plots were cut to 1-inch stubble height at the end of the stockpiling period for each treatment (6, 8 or 10-weeks). At the 10-week treatment harvest, both the 6- and 8-wk treatments plots were sampled again to determine any additional forage accumulation and nutritive value changes. At the end of the 10-week stockpiling period, remaining forage in all plots were allowed to stand for an additional 30-days to measure changes in forage quality during the fall-winter transition period.

3. RESULTS & DISCUSSION

There was no location × period × N fertilization effect (P = 0.5439) on forage accumulation. Forage accumulation ranged from 1,110 to 1,548 lb DM/acre across stockpiling period length and N fertilization strategies (Table 1). A location × stockpiling period interaction (P < 0.0001) illustrated that stockpiling was feasible for 6 to 8 weeks after initiation in Clanton, but timing of frost significantly reduced available forage at the 10-week interval due to senescence of growth. An N fertilization effect was observed, where N application increased forage accumulation by an additional 250 to 300 lb DM/acre, but there was no stockpiling period × N fertilization interaction (P = 0.2743).

Table 1. Nitrogen fertilization and stockpiling accumulation period length influences on bahiagrass yield (lb DM/acre) at four Alabama locations.

N Strategy	Clanton	Goodway	Headland	Troy	All Locations
0 N	----------lb DM/acre----------				
6 wk	685	1,000	1,380	1,166	1,110
8 wk	722	1,190	1,285	1,222	1,160
10 wk	N/A	1,158	1,917	1,270	1,448
Split N					
6 wk	785	1,405	1,271	1,400	1,276
8 wk	947	1,648	1,218	1,206	1,300
10 wk	N/A	1,370	2,092	1,830	1,764
60 N					
6 wk	809	1,311	1,410	1,548	1,335
8 wk	647	1,740	1,478	1,347	1,397
10 wk	N/A	1,370	1,880	1,395	1,548

Forage CP ranged from 12 to 14% across N fertilization strategies (P = 0.0007), while TDN was an average of 61% at the 6- and 8-week accumulation periods, and 59% at the 10-week interval (P < 0.0001). Forage fiber fractions NDF and ADF did not differ at 6 or 8 weeks of accumulation but increased at the 10-week period. In this study, stockpiled bahiagrass supported moderate forage production during the latter fall months of the year with nutritive value characteristics sufficient to meet the majority of the nutritional needs in beef cow/calf operations.

Across the two-year period, the average number of production days past the stockpiling interval (6, 8, or 10 weeks of growth) ranged from 30 to 50 days of production. In year 2 of the study, fall growing conditions were very dry during the stockpiling, with limited rainfall across all sites followed by an early first killing frost (~ October 31). This shortened the viability of standing bahiagrass to closer to 30 days across sites.

This study demonstrates that while the window of use of bahiagrass when stockpiled may be shorter than that of bermudagrass or tall fescue, it is an option for extending the fall and early winter forage window in the state. This may reduce the onset of hay feeding in beef cattle operations and shorten the transition time to cool-season forage grazing in the state.

Funding

This research was funded by the Alabama Cattlemen's Association State Beef Checkoff Program.

In situ Digestibility and Nutritive Value of Cool-Season Annual Grass Baleage

S. Leanne Dillard [1, 2, *], Kathryn N. Strain [2], Micayla H. West [1], M. Kim Mullenix [1], W. Brandon Smith [1], Eros Francisco [2], and Deidre D. Harmon [3]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA;
[2] Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL, USA;
[3] Department of Animal Sciences, North Carolina State University, North Carolina, USA.
* Correspondence: dillasa@auburn.edu

TAKE HOME MESSAGE

Baleage is a good alternative to dry hay production, requiring less time between mowing and baling. Research is still needed in the Southeast to determine the effects of silage inoculant and different cool-season annual grass species on baleage storage capacity and nutritive value. Therefore, a study was conducted with North Carolina State University to determine the nutritive value of baleage harvested from four common cool-season annual grasses, annual ryegrass, rye, wheat, and triticale. All grass species produced baleage with greater fiber and lower crude protein and total digestible nutrients than reported values. The use of silage inoculant did not provide consistent improvements in baleage nutritive value over non-inoculated baleage. Wheat provided the greatest nutritive value and digestibility, with triticale and annual ryegrass being intermediary. Rye had the greatest fiber and lowest crude protein. Overall, cool-season annual grasses provide high-quality baleage that can be used to provide supplemental feed to beef cattle during periods of low to no forage growth.

SUMMARY

Cool-season annual (CSA) baleage provides reliable forage for beef herds during the winter. The objective of this study was to determine nutritive value and *in situ* digestibility of four CSA grass baleage. Baleage treatments were cereal rye (*Secale cereale*), triticale, (*Triticosecale* Wittmack), wheat (*Triticum aestivum*), and annual ryegrass, (*Lolium multiflorum*). Forages were ensiled in poly-vinyl chloride (PVC) mini silos for 296 days. After ensiling samples were analyzed for nutritive value via near infrared spectroscopy and *in situ* digestibility. Wheat contained the greatest crude protein, water soluble carbohydrates, total digestible nutrients, net energy of maintenance and net energy of gain. The use of silage inoculant had mixed effects on the baleage nutritive value regardless of grass species. Overall, all cool-season annual grasses researched produced high-quality baleage for use in beef cattle systems and can support sufficient animal performance during times of reduced forage production.

1. INTRODUCTION

Feed costs represent the largest portion of on-farm costs accrued for beef cattle operations. As such, the need for reliable and consistent forms of forage preservation are critical for providing high-quality forages for cattle herds across the U.S. Cool-season annual (CSA) grasses provide ample forage quality necessary for both cow-calf and stocker operations and can be strategically used to improve performance of beef cattle during periods of low or no forage production (e.g., winter).

Baleage, or round-bale silage, is a method of preserving surplus fresh forage to be fed at a later date. Baleage is harvested, baled, and stored at a higher moisture content than dry hay (45-60% moisture vs. 10-15% moisture, respectively (Shoup et al., 2022). Storing surplus forage in this manner provides greater flexibility during harvest as variable weather conditions can prevent curing of high-moisture grasses such as annual ryegrass (*Lolium multiflorum*).

Baleage quality relies heavily on successful fermentation during harvest and storage to preserve high-quality fresh forage. During the fermentation process, anaerobic bacteria consume carbohydrates present in the forage to produce organic acids. This creates an acidic environment in which the forage becomes stable for storage. If improper handling occurs at harvest or if oxygen is introduced after storage, spoilage, and decomposition of the baleage can occur (Shoup et al., 2022). **The objective of the current study was to determine which cool-season annual grass produced superior baleage on the basis of forage nutritive value and *in situ* digestibility.**

2. PROCEDURES

2.1 Forage Establishment and Management

Forages were established in a randomized complete block design at North Carolina Department of Agriculture and Consumer Services Mountain Research Station, near Waynesville, North Carolina. In the spring, forage plots were fertilized with 43 lb N/acre. The soil test indicated that the pH, P and K were adequate to meet the nutrient requirements of the forages grown. The CSA used in the experiment were cereal rye (*Secale cereale;* RY), triticale (*Triticosecale* Wittmack; TR), wheat (*Triticum aestivum;* WT), and annual ryegrass (AR). Each of the forage treatments were planted with a cone seeder no-till drill (Hege 1000, Almaco, Nevada, IA). Forages were planted at 105 lb pure live seed (PLS)/acre for WT, RY, and TR and 25 lb PLS/acre for AR.

2.2 Forage Harvest and Ensiling

All forages were harvested at flowering; therefore, the dates of harvest were different for each forage species. A 36A Research Plot Harvester (RCI Engineering, Mayville, Wisconsin) was used to cut the forage at 4 in stubble in 3 ft swaths. Forage was allowed to wilt on woven poly tarps and hand tedded until a microwave DM test indicated 50% DM was reached (12 – 24 h after harvest). Forage was packed in mini silos at 6.0 lb per 4 in diameter polyvinyl chloride (PVC) pipes; then fitted with two rubber end caps and sealed with a hose clamp. Forage was ensiled for 296 ± 1 d before sampling to replicate recommended practices of baleage storage of up to 12 months. After ensiling, forage was oven dried at 131°F for 48 h or until a common weight was reached. Dried forage was sent to Auburn University Ruminant Nutrition Laboratory (Auburn, AL) for determination of nutritive value and in situ digestibility. Dried whole forages were ground to pass through a 0.04 in screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and stored until analyses.

2.3 In situ Digestibility and Nutritive Value

Two ruminally-fistulated beef (crossbred Angus) heifers (approximately 880 lb body weight and 17 month of age) located at Auburn University Beef Teaching Unit located in Auburn, AL were used to determine in situ digestibility of baleage samples. All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee (Protocol Number 2021 – 3988).

The *in situ* procedure was based on adaptations of the nylon bag technique by Vanzant et al. (1998). Acetone-washed fiber filter bags (F57; 25 µ porosity; ANKOM Technology Corp., Macedon, NY) were filled with 0.02 oz of ground forage sample in triplicate per silo per timepoint per heifer. Each time point (except 0 h) was placed in a polyester mesh bag, attached to a stainless-steel chain that was secured to the rumen cannula. Bags were incubated in the ventral rumen sac for the set number of hours (Foster et al., 2011). The time points 2, 4, 8, 12, 24, 48, 72 h were inserted in reverse order and removed simultaneously on June 9, 2022. Time 0 h was not added to the rumen. All rumen bags, including 0 h, were immediately soaked in an ice water bath (15 °F) for 5 minutes to halt microbial activity. All samples were then frozen (32 °F) until further analysis (Vanzant et al., 1998). After thawing, samples were placed into a top-loading washing machine and rinsed following the Vanzant et al. (1998) protocol. Polyester mesh bags containing samples were subjected to gentle cycle with 5 cold-water rinses with 2-minute spin per rinse cycle and 1-minute agitation. Samples were again frozen (32 °F) then transported to the Auburn University Ruminant Nutrition Laboratory (Auburn, AL) for storage pending nutritional analysis. Each forage sample was sent to Cumberland Valley Analytical Services, Inc. (Waynesboro, PA) for near infrared reflectance spectrometry (NIRS) to nutritive value determination.

3. RESULTS & DISCUSSION

Forage species influenced all tested variables [pH, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, crude protein (CP), water soluble carbohydrates (WSC), total digestible nutrients (TDN), net energy (NE) Maintenance, and NE Gain; Table 1). Cereal rye was greater than the other forages in NDF, ADF, and lignin, but had the lowest pH, TDN, WSC, and net energy values. Wheat was least in NDF, ADF, and lignin, and had the greatest CP, TDN, and net energy values.

Table 1. Forage nutritive value of cool-season annual grasses ensiled as baleage.

Item	Annual Ryegrass	Cereal Rye	Triticale	Wheat
	----------% DM basis----------			
pH	5.0b	4.7d	5.1a	4.85c
NDF[1]	56.4c	75.0a	60.7b	51.1d
ADF	38.8b	51.4a	39.8b	32.9c
Lignin	4.7b	7.1a	4.5c	3.9d
CP	6.4a	6.1a	7.2b	8.7c
WSC	4.38b	1.18c	3.36b	9.68a
TDN	51.3b	46.1c	51.8b	56.7a
	----------Mcal/lb----------			
NE Maintenance	0.45b	0.35c	0.46b	0.55a
NE Gain	0.20b	0.001c	0.21b	0.30a

[1] NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein; WSC = water soluble carbohydrates; TDN = total digestible nutrients; NE = net energy; [a,b,c,d] Within a row, means without a common super script differ.

The use of silage inoculant had no effect on NDF, ADF, lignin, or CP of the baleage (Table 2). Inoculated baleage did have a greater pH, which was surprising, however, the results are likely not biologically significant (only 0.1 pH greater). Furthermore, inoculation lowered WSC, TDN, and net energy values for the baleage.

Table 2. Forage nutritive value of cool-season annual grass baleage treated with or without silage inoculant.

Item	Inoculated	Non-inoculated
	----------% DM basis----------	
pH	5.0a	4.9b
NDF1	60.5	61.2
ADF	40.9	40.6
Lignin	5.0	5.1
CP	7.1	7.1
WSC	3.19b	6.10a
TDN	50.8b	52.1a
	----------Mcal/lb----------	
NE Maintenance	0.44b	0.46a
NE Gain	0.82b	0.85a

[1] NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein; WSC = water soluble carbohydrates; TDN = total digestible nutrients; NE = net energy [a,b,c,d] Within a row, means without a common super script differ.

The forage *in situ* digestibility results showed that wheat was highly digestible with the greatest degradable fraction (62% 72 h digestibility; Figure 1). This is likely the result of wheat having the least NDF, ADF, and lignin. Triticale and annual ryegrass were intermediary with a total 72h digestibility of 55-56%. Then rye was the least digestible, being only 48% digestible at 72h, which correlates with its elevated NDF and lignin concentrations observed in this study. The use of silage inoculant did not greatly impact the digestibility of the baleage for the first 40 h (Figure 2). However, the inoculated baleage did have a greater digestibility at 72 h than the non-inoculated; however, the variation in digestibility was not statistically different.

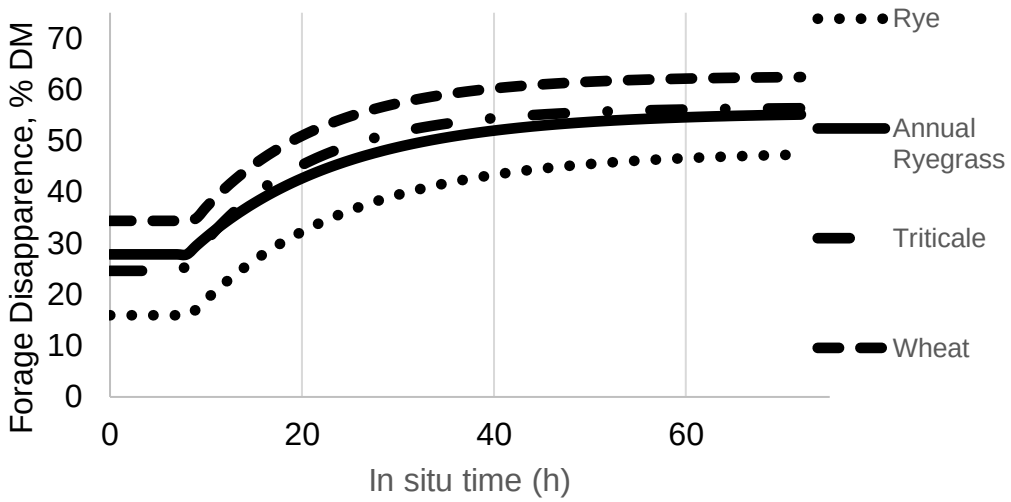


Figure 1. *In situ* dry matter disappearance (% DM basis) of four cool-season grasses (rye, annual ryegrass, triticale, and wheat) ensiled as baleage.

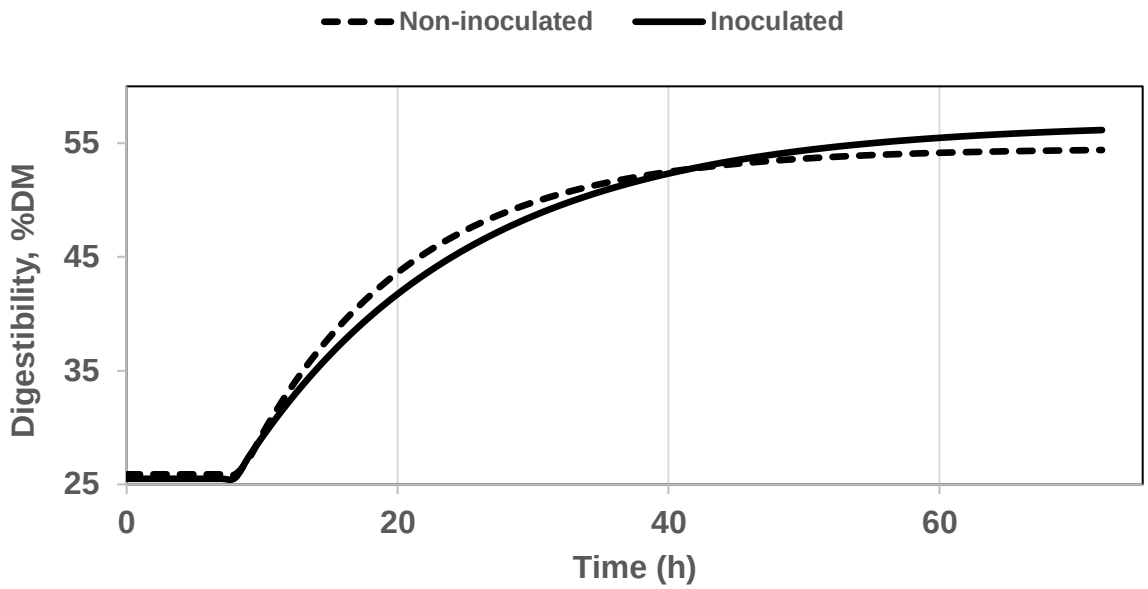


Figure 2. *In situ* dry matter disappearance (% DM basis) of cool-season grasses ensiled as baleage with or without silage inoculant.

Based on the results of this study, wheat has the most potential for baleage production to provide supplemental feed for beef cattle during times of reduced forage growth. While annual ryegrass is the most popular Southern cool-season annual grass (Ball et al., 2015), the nutritive value and digestibility observed in the current study were less than those reported previously (Mullenix et al., 2014). Triticale also showed promise to produce high-quality baleage. However, rye produced the lowest nutritive value and digestibility of all tested forages. The results of this study also validate the need for timely forage harvest, as the quality of all of these cool-season annual grasses is lower than values reported when harvested at the boot stage (Mullenix et al., 2014). Since the forages were harvested post boot stage, it is expected the structural fiber fraction will be higher, the CP lower, and digestibility lower. The use of silage inoculant was inconsistent for both baleage nutritive value and digestibility. Baleage offers a solution to give producers a technique to have more choice and control over forage production during periods of high precipitation. Furthermore, baleage could also offer benefits to cover cropped fields where fencing limits grazing options.

Acknowledgments

We would like to acknowledge the crew at the Mountain Research Station in Waynesville, NC, for harvesting and storage of the samples, as well as the support of the graduate and undergraduate students in the Ruminant Nutrition and Forage Research Laboratory at Auburn University for assisting with the *in situ* study.

Funding

This research was funded by the Southern Sustainable Agriculture Research (SARE) program, grant LS19-310".

4. References

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2015. Southern Forages: Modern Concepts for Forage Crop Management, 5th ed. The International Plant Nutrition Institute (IPNI), Peachtree Corners, GA.

Mullenix, M. K., S. L. Dillard, J. C. Lin, B. E. Gamble, and R. B. Muntifering. 2014. Evaluation of wheat and triticale forage for stocker production in the Gulf Coast region. The Professional Animal Scientist 30(3):296-304. doi: 10.15232/s1080-7446(15)30120-0

Shoup, S. L., R. B. Muntifering, M. K. Mullenix, L. S. Silva, and S. L. Dillard. 2022. In Situ Ruminal Digestion, Fermentation Parameters, and Forage Nutritive Value of Cool-Season Baleage Ensiled under Contrasting Inoculant Strategies. Animals 12(21):2929.

Vanzant, E. S., R. C. Cochran, and E. C. Titgemeyer. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. Journal of Animal Science 76(10):2717-2729. doi: 10.2527/1998.76102717x.

Parsing the Effects of Bag Type and Length of Incubation on *In Situ* Digestibility Results

Aghata E. M. Silva [1,*], Macy L. Rockwell [1], Lauren M. St. Andrew [1], Anna Grace Underwood [1], Abbigail R. Hines [1], Diva N. Rigney [1], and W. Brandon Smith [1]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA.
* Correspondence: ame0066@auburn.edu

TAKE HOME MESSAGE

Making accurate and relevant recommendations for beef cattle feeding, requires true feeding values obtained by analyzing feed and forage nutritive value as well as degradation profiles in the animal. Evaluation of degradation parameters are most optimally generated by direct-feeding animals; however, this is normally cost-prohibitive. *In situ* techniques consist of direct incubation of bags containing feed or forage inside the rumen for a determined number of hours, allowing for the estimation of degradation profiles. However, some researchers have raised concern that differences in bag textiles can inflate the error obtaining on those analysis due to overlooked bag degradation. Therefore, this experiment aimed to examine how different textile bags' integrity changed over time during a series of incubation periods. We found no weight change among textile types up to 192 h of incubation. However, when incubation time is extended, woven textiles (such as the R510) may be preferred.

SUMMARY

While *in situ* techniques are considered to be a vital part of understanding feed and forage utilization in ruminant animals, the physical integrity of *in situ* bags is considered a potential source of error in estimation of feedstuff disappearance. Therefore, the objective of this study was to evaluate the influence of textile type on bag integrity at various lengths of incubation. Bags used in these experiments included the R510 (woven textile, 50 μm porosity), F57, and F58 bags (non-woven textiles with 25 and 10 μm porosity, respectively; ANKOM Technology, Macedon, NY). Bags were filled with 3-mm glass beads to simulate the weight of an incubated feedstuffs, then incubated in each of two ruminally-fistulated heifers for 0, 6, 12, 24, 48, 96, 144, 192, 384, and 576 h. There were no weight differences among unrinsed bags up to 48 h. However, for longer incubation lengths, F57 and F58 bags gained weight relative to R510. After NDF rinsing, there were no weight differences among bags up to 192 h. At longer incubations, the differences in woven and non-woven textiles persists. The R510 bags maintained the greatest tensile strength over time. We concluded that, for standard incubation lengths, all bags have equal efficacy. However, if extended incubation lengths are preferred (such as estimation of indigestible components), the R510 bags are preferable.

1. INTRODUCTION

Accurate and relevant feeding recommendations for cattle require accurate and precise evaluation of the feedstuff or forage in question. Evaluation is assumed to be most accurate when the feedstuff or forage is directly fed to the animal. However, due to the cost- and labor-prohibitive nature of feeding trials, alternative techniques (e.g., *in situ* and *in vitro*) are essential. The conventional *in situ* technique for the determination of ruminal degradability is an animal-based

experiment used to quantify the degradation rate of feedstuffs in the reticulorumen (Mayes et al., 2018). It essentially relies on the use of porous bags made of synthetic fabric filled with feed samples that are suspended in the ruminal contents for different incubation periods. *In situ* techniques generally allow for collection of data that better represents rumen dynamics and interactions that are difficult to characterize or estimate otherwise, such as rate and extension of degradation, the effectively degraded nutrient fraction, and the rumen fill effect of fiber (Sampaio et al., 2009; Valente et al., 2011).

Textiles utilized for feed incubation inside of the rumen vary. Different textiles have been proposed to make the bags used in the *in situ* evaluations such as the R510/R1020 (woven textile bags for concentrate and forage, respectively; Ankom Technologies, Macedon, NY, USA), F57, and F58 bags (non-woven textile bags; Ankom Technologies). Alterations in bag type have been proposed due to an increased concern for particle loss due to bag porosity as a potential source of error when incubating heterogeneous particle sizes for long periods of time (Huhtanen et al., 1994). Further, long incubation periods might impair the physical integrity of bags, which might inflate the error in the analysis even more (Norris et al., 2019). Though this issue has been mentioned in the literature, the interaction of bag type and incubation length on the bag integrity has yet to be explored. Thus, **the objective of this study was to evaluate the effect of bag type and length of incubation on bag integrity during *in situ* incubations**.

2. PROCEDURES

To address our objective, we used a completely randomized design with a 3 × 10 factorial treatment structure. Treatment factors included bag type (R510, F57, or F58 bags with reported porosity of 50, 25 and 10 μm, respectively) and length of incubation (0, 6, 12, 24, 48, 96, 144, 192, 384, and 576 hours). To eliminate the potential confounding effect of feedstuff or forage type, bags were filled with 3-mm borosilicate glass beads at a ratio of 20 mg/cm^2 (Vanzant et al., 1998). Six replicate bags of each textile by time combination were then incubated in 14.2 × 16.5 in polyester bags with 50-μm porosity. These polyester bags were suspended in the rumen of two ruminally-fistulated Angus-cross heifers using the reverse time, all-out technique (Vanzant et al., 1998). Animals were maintained in a dry lot with *ad libitum* access to bermudagrass (*Cynodon dactylon* [L.] Pers.) hay and water. On removal, the polyester bags were separated from the steel chain and rapidly rinsed with cold water, and then placed into an ice water bath for 5 min to stop microbial activity. Bags were rinsed in cold water using the delicate cycle of a household washing machine until the water became clear (approximately 1 h). After rinsing, bags were dried at 221 °F and weighed. Bags were then subjected to neutral detergent fiber (NDF) analysis (Vogel et al., 1999) to remove residual microbial matter, after which they were dried and re-weighed as previously described.

Integrity of each bag was assessed through changes in bag weight and tensile strength. Weight change after incubation and after NDF rinsing was expressed as percent gain or loss. Tensile capacity (tension of rupture) of bags were analyzed on three bags after 0, 96, 144, and 576 h of incubation on length direction (LD), width direction (WD), manufacturer seal (MFS), and manual seal (MNS) at the Alabama Center for Paper and Bioresource Engineering (Ginn College of Engineering, Auburn University, Auburn, AL, USA) using an Instron 5565 tension tester (Instron, Norwood, MA, USA).

3. RESULTS & DISCUSSION

Weight changes after incubation are presented in Figure 1. There was an interaction of bag type and incubation length ($P < 0.01$). Through 48 h of incubation, bag types did not differ ($P < 0.05$). For F57 bags, by 48 h an increase on bag weight was observed linearly overtime ($P < 0.05$; Figure 1). At 96 and 144 h of incubation, F58 gained more weight ($P < 0.05$) than R510 bags, with F57 intermediate. At 192 and 384 h of incubation, both F57 and F58 bags gained more weight ($P < 0.05$) than R510. At 576 h of incubation, all bag types differed ($P < 0.05$). The increase in bag weight at extended lengths of incubation can be associated with the accumulation of ruminal residue in the bag that wasn't removed after rinsing. Therefore, as incubation periods got longer, residue accumulation on the bag increased.

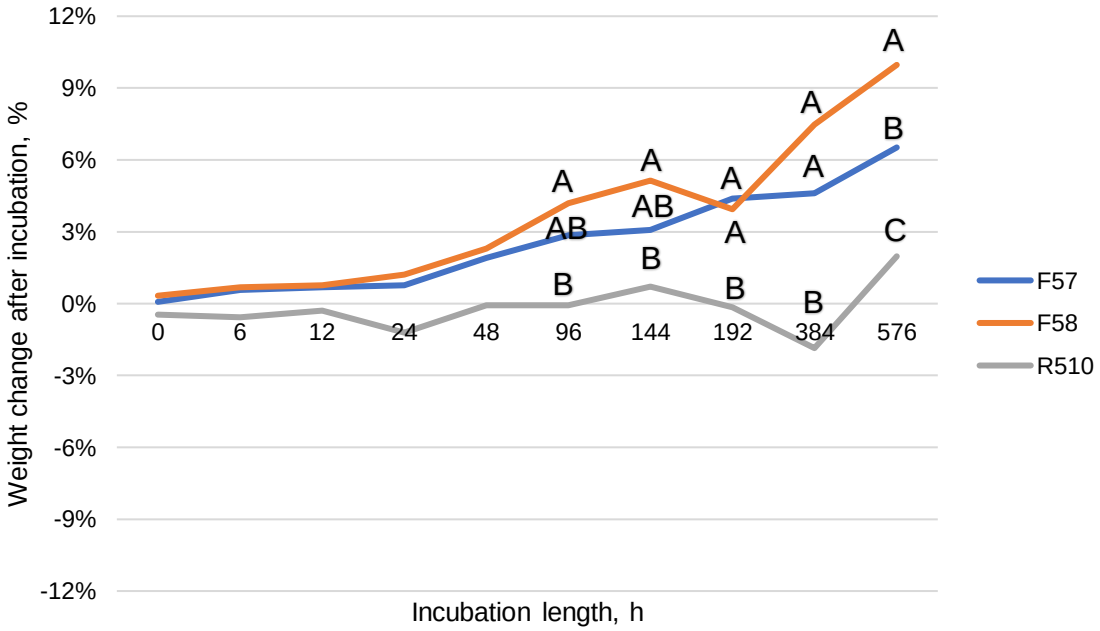


Figure 1. Weight change of *in situ* bag type (R510, F57, or F58) across various lengths of incubation. Lines with differing annotated letters within a timepoint are different ($P < 0.05$).

Understanding that weight gain after incubation was likely associated with residual microbial matter, bags were subjected to an NDF rinse, and weight changes were reassessed (Figure 2). Through 192 h of incubation, bag type did not differ ($P \geq 0.05$). For incubation lengths beyond 192 h (384 and 576 h), R510 bags lost weight while F57 and F58 bags gained weight ($P < 0.05$). Whereas a gain in bag weight following incubation is generally associated with residual microbial matter that is not removed from the bag, a loss in weight signals degradation of the textile material.

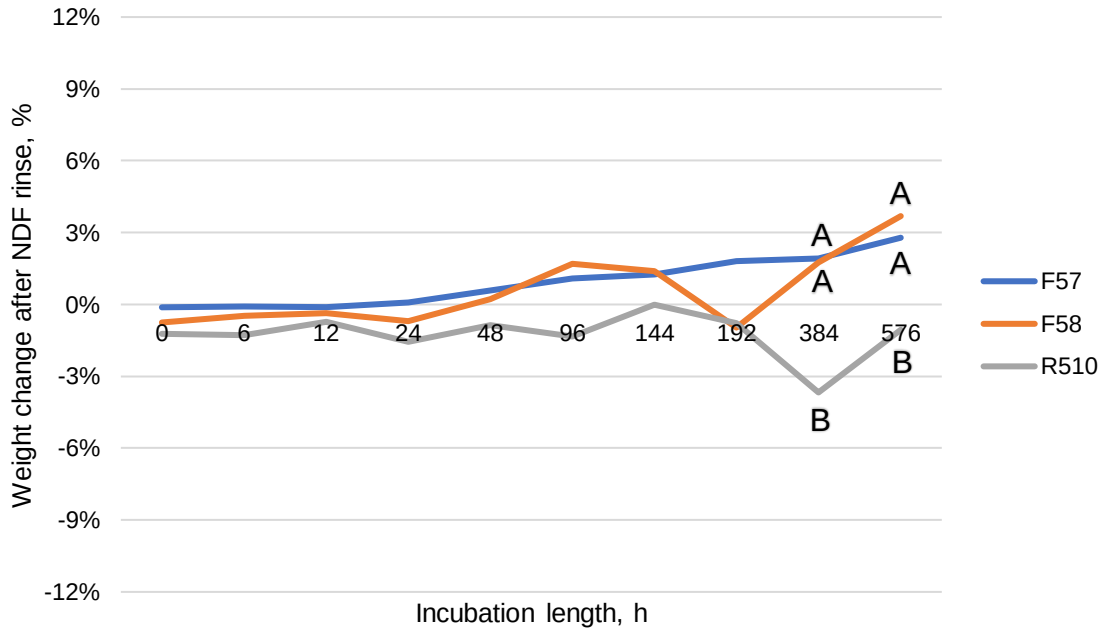


Figure 2. Weight change of *in situ* bag type (R510, F57, or F58) across various lengths of incubation after neutral detergent fiber (NDF) rinsing. Lines with differing annotated letters within a timepoint are different ($P < 0.05$).

In addition to bag weight change, tensile strength of bags at four lengths of incubation was determined. Tensile strength was measured across the bags length (LD), width (WD), manufacturer's seal (MFS), and manual seal (MNS). There was no interaction of bag type and incubation length ($P \geq 0.05$) nor an effect of time ($P \geq 0.09$) on tensile strength for tensile strength across LD or WD. However, R510 bags were stronger ($P < 0.05$) across LD and WD than F57 or F58. There was, however, an interaction ($P < 0.01$) of bag type and incubation length for tensile strength across MFS and MNS (Figure 3). The F58 bags had the strongest ($P < 0.05$) manual seal at all lengths of incubation. The F57 and R510 bags only differed in tensile strength of the manual seal after 576 h.

When bags are subjected to long-term ruminal incubations, the bag textiles might lose resistance due to the tension caused by the continuous ruminal contractions (Valente et al., 2011). Based on our results, we determined that F57 and F58 bags are likely suitable for shorter incubation periods, but R510 bags are preferable for extended incubation lengths.

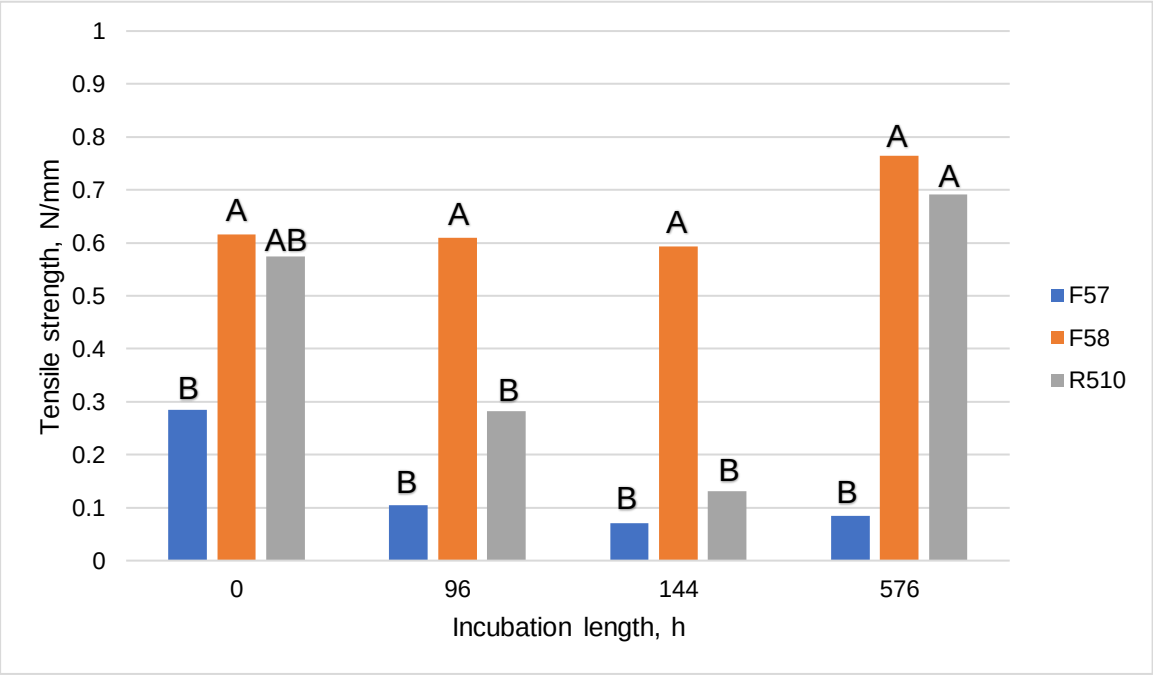


Figure 3. Tensile strength (maximum force per width, N/mm) of the manual seal of *in situ* bag type (R510, F57, or F58) across various lengths of incubation. Bars with differing annotated letters within a timepoint are different ($P < 0.05$).

Funding

This project was financially supported by the Agricultural Research Service, U.S. Department of Agriculture, under Agreement No. 58-6010-1-005.

4. References

Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Tech. 48(3):211-227. doi: 10.1016/0377-8401(94)90173-2.

Mayes, R. W., J. H. Pagella, F. J. Pérez-Barbería, and E. R. Ørskov. 2018. The development of an intraruminal nylon bag technique using non-fistulated animals to assess the rumen degradability of dietary plant materials. animal 12(1):54-65. doi: 10.1017/S1751731117001203.

Norris, A. B., L. O. Tedeschi, and J. P. Muir. 2019. Assessment of in situ techniques to determine indigestible components in the feed and feces of cattle receiving supplemental condensed tannins1. J. Anim. Sci. 97(12):5016-5026. doi: 10.1093/jas/skz329.

Sampaio, C. B., E. Detmann, I. Lazzarini, M. A. d. Souza, M. F. Paulino, and S. d. C. Valadares Filho. 2009. Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. Revista Brasileira de Zootecnia 38.

Valente, T. N. P., E. Detmann, S. d. C. Valadares Filho, M. d. Cunha, A. C. d. Queiroz, and C. B. Sampaio. 2011. In situ estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. Revista Brasileira de Zootecnia 40doi: 10.1590/S1516-35982011000300027.

Vanzant, E. S., R. C. Cochran, and E. C. Titgemeyer. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76(10):2717-2729. doi: 10.2527/1998.76102717x.

Vogel, K. P., J. F. Pedersen, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39:276-279. doi: 10.2135/cropsci1999.0011183X003900010042x.

Grazing Cover Crops in a Cotton-Peanut Rotation in the Wiregrass Region of Alabama

Jose Pereira [1], Russell Carrell [1], M. Kim Mullenix [1], Brandon Smith [1], and S. Leanne Dillard [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA.
* Correspondence: dilasa@auburn.edu

TAKE HOME MESSAGE

Integration of crop and livestock systems can be a valuable management strategy. The incorporation of grazing livestock into cover crops allows producers the opportunity to diversify their economic return, while still maintaining the environmental benefits that cover crops provide within the system. The objective of this study was to evaluate the forage production and animal performance of steers grazing cool-season annual cover crops with differing cattle removal dates. There were no differences for neutral detergent fiber or acid detergent fiber, but crude protein was different among treatments. Furthermore, stocker average daily gain did not differ among treatments. However, the steer grazing days per acre were different, with February removal being the lowest and March and April removal being similar. This current study indicates that cover crops are useful as a potential grazing source for short periods of time. Further research is needed to determine what method of forage utilization is most efficient for production of cattle without negatively influencing the primary agronomic purpose of cover crops.

SUMMARY

Environmental concerns in monoculture systems, such as soil erosion and nutrient runoff, have led to a revitalization of cover crops and renewed interest in their role in ecosystem sustainability. The integration of crop and livestock systems can be a valuable management strategy because the introduction of grazing livestock to cover crops allows producers the opportunity to diversify their economic return, while still maintaining the environmental benefits that cover crops provide within the system. In the current study, the cover crop grazing pastures system consisted of a cover crop mixture including rye (*Secale cereale*), oat (*Avena sativa*), crimson clover (*Trifolium incarnatum*), and 'T-raptor' hybrid brassica (*Brassica napus* × *B. rapa*). The treatments were based on four cattle removal dates that were (1) no grazing (CON); (2) February removal (FEB); (3) March removal (MAR); or (4) April removal (APR). Three Angus crossbred testers steers were used by pasture, in a total of 12 pastures (1.53 acre each). The animal performance was determined by average daily gain and nutritive value by estimation of crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). The results of NDF and ADF showed no difference among treatments, but treatments were different in CP. The average daily gain did not differ among treatments; however, for steer grazing days, FEB was lower than MAR and APR. This research indicates that cover crops can be successfully grazed and provide positive animal gains even during short grazing seasons.

1. INTRODUCTION

Many studies have shown over time that monoculture crop management creates many negative environmental impacts including increased pest infestation, nutrient runoff, increased incidence of plant disease, and herbicide-resistant weeds. These negative effects have led to production settings with potential for severe crop losses, which has led to introduction of plant diversity back into cropping systems to mitigate these problems. One effective way to introduce plant diversity is by using multi-species cover crops during the fallow season.

Cover crops can provide many production advantages for producers through mitigation of environmental risks such as soil erosion, nutrient leaching, and pest and weed encroachment. These results from multiple plant-soil interactions greatly benefit overall health and structure of cropland soils. Consequently, their purpose is to provide support for the following cash crops and are therefore planted during the off-season of the cash crops. Cover crops often include plant species that are also considered common forage crops that are highly productive during a short window and provide one or more ecological benefits to the soil profile.

To provide ecosystem services, cover crops must produce adequate aboveground biomass to protect soil from climatic events such as rain or drought. This plant biomass could potentially serve as a secondary grazing crop for livestock such as beef cattle. Cover crops can be monocultures of small grains, legumes (clovers), or forbs (brassicas), but they often include a combination of these three forage families. The use of these species for a cover-crop mixture increases the positive impact the cover crop has on the soil and the environment. These forages are great tools for farmers in the Southeast, as they can extend the grazing season well into the winter and early spring when properly managed.

Cover crops provide biogeochemical and ecological controls that contribute to the long-term viability of cropping systems. The introduction of grazing livestock to cover crops allows producers the opportunity to diversify their economic return, while still maintaining the environmental benefits that cover crops provide within the system. Although forages used in cover crop mixtures can serve as effective grazing crops, it is not well understood how specific plant species will respond to grazing pressure and subsequent potential beef production per acre using these mixtures. It is unknown if cover crops can withstand grazing pressure throughout the grazing season or if they require early removal of cattle to allow for adequate regrowth to provide ecosystem services to the system for crop production. Further investigation is needed to determine if cool-season cover crops can be a viable grazing source for stocker cattle, while still maintaining their role in providing ecological stability.

2. PROCEDURES

A 4-year grazing experiment was performed during the spring of 2019 through 2022 at the Wiregrass Research and Extension Center located in Headland, AL. Two crop rotations were presented in the area during the crop season, which were annual peanut (*Arachis hypogaea*) and cotton (*Gossypium hirsutum*). The pastures were planted with a four-species cover-crop mixture throughout the study. The cover crop mixture consisted of 'FL401' cereal rye (*Secale cereale;* Melton Seed, Dade City, FL) 'Cosaque' oat (*Avena sativa*; Petcher Seed, Fruitdale, AL), 'AU Sunrise' crimson clover (*Trifolium incarnatum;* Petcher seed, Fruitdale, AL), and 'T-raptor' brassica (*Brassica napus* × *B. rapa*; Southeast Agriseed, Rome, GA). The species in the forage mixture were kept

consistent year to year. In this study, the pastures were considered grazed and non-grazed. In each year, grazing pastures were fertilized with 75.1 lb N/acre and non-grazed pastures fertilized with 38.1 lb N/acre in the form of ammonium sulfate in December prior to grazing in January.

Grazing pastures were considered experimental units and cattle removal dates as treatments. The treatments consisted of non-grazed (CON); and three different removal strategies: February removal (FEB); March removal (MAR); or April removal (APR). Each treatment was replicated three times resulting in 12 pastures, 1.53 acres per pasture. To determine the animal performance, three Angus crossbred (7 months in age; initial body weight (BW) 586 ± 22 lb) steers were used as testers in this experiment to calculate initial BW, final BW, and average daily gain (ADG). The ADG was measured by subtracting the final weight by the initial weight and dividing it by the number of days on pasture. Steers had access to *ad libitum* commercial mineral mix (Mag Plus beef Mineral/salt, Southern States Cooperative Inc., Richmond, VA) and clean water. Grazing was initiated in January of each year and the stocking density adjustments were made every 2 weeks according to mean forage biomass and steer BW. In all four years, a target forage allowance was maintained using the put-and-take method and was considered a forage allowance of 1 lb DM per 1 lb BW during the grazing.

To estimate forage biomass and nutritive value four random samples were collected, every two weeks, from each pasture. Samples were clipped to approximately 2-in within a 155-in^2 quadrat, placed in cloth bags, and transported to the Auburn University Ruminant Nutrition Laboratory (Auburn, AL). Forage biomass samples were dried at 140°F in a forced-air oven for 48 h or until reached a constant weight. To obtain nutritive value, forage samples were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass a 2-mm screen. Crude protein (CP) and dry matter (DM) were analyzed using procedures of AOAC International (1995). For neutral detergent fiber (NDF) and acid detergent fiber (ADF) were used the methodology describe by Van Soest et al. (1994).

3. RESULTS & DISCUSSION

Over the four years of the study, crude protein concentration of cover crops pastures was different among treatments. The treatment MAR was 13% greater than CON pastures (Table 1). For NDF and ADF, no difference was detected among treatments and average 51.5% and 30.7 %, respectively.

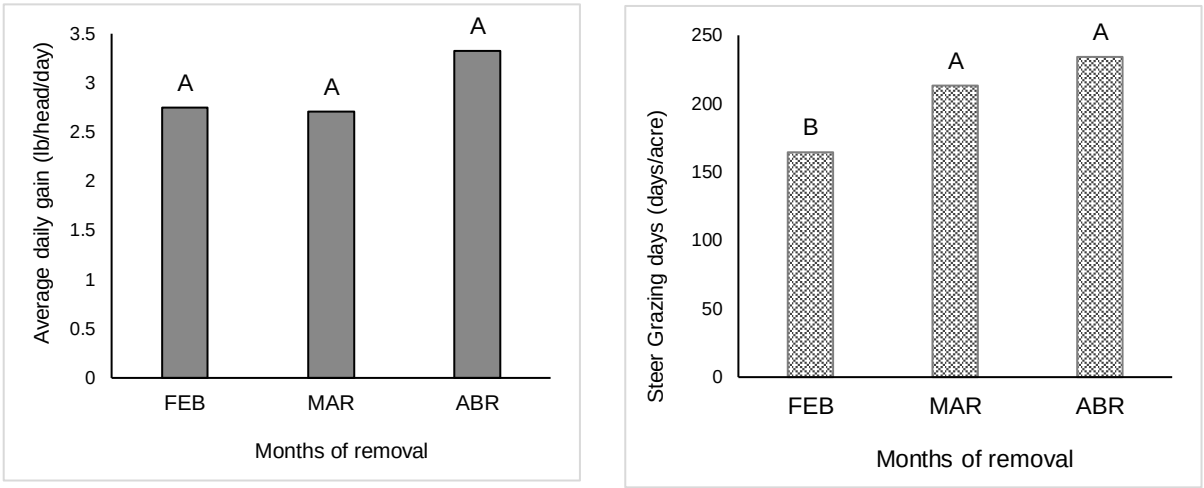
Table 1. Nutritive value of cover crops pastures consisting of cereal rye, oat, crimson clover, and brassica under different grazing removal dates from 2019 to 2022.

Item	CON	FEB	MAR	APR	SE[1]	*P*-Value
CP, %	11.8b	12.3ab	13.4a	12.6ab	0.48	0.02
NDF, %	50.80	51.56	51.08	52.46	3.8	0.55
ADF, %	29.73	30.8	30.65	31.54	1.79	0.08

[1]SE= Standard error; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber. CON = Control; FEB= February cattle removal; MAR= March removal of cattle; APR = April removal of cattle Within a row, values without a common superscript differ ($P < 0.05$).

A lack of difference among treatments can be attributed to increased maturity of cereal rye in all pastures prior to initiation of grazing in January of each year. Similarity in nutritive value among treatments paired with poor biomass regrowth indicate that plants within the mixture may have already initiated maturation and reproduction (Chaves et al., 2006). Cereal rye in all years of the study reached the boot stage of production prior to initiation of grazing in January. As a proportion of total forage biomass, cereal rye dominated forage stands in all years of the study throughout the grazing season. Because aboveground biomass accumulation of cereal rye was greatly reduced later in the growing season, the dominance of cereal rye in the forage stand throughout the entire growing season was likely the result of animal selection.

The individual animal performance presented no significant difference among treatments where the ADG of FEB, MAR and APR averaged 2.9 lb/head/day over the four years of grazing trial (Figure 1a). However, steer grazing days per acre were different among treatments. The treatment MAR did not differ from APR, but both were greater than FEB.



(**a**) (**b**)

Figure 1. Average daily gain (**a**) and steer grazing days per acre (**b**) of steers in cover crop pastures with three removal dates [February (FEB), March (MAR), and April (APR)] during four years of the grazing trial.

Results for steer ADG of this study were in concordance to those reported in previous studies. The ADG were similar to those reported by Jaramillo et al. (2021), which used a blend of clovers and rye and by Dubeux et al. (2016) that used mixtures of various small grains including FL401 cereal rye in combination with annual ryegrass (*Lolium multiflorum*), both in North Florida. The ADG of this trial were slightly greater than ADG in Beck et al. (2014), although spring ADG values were greater than those reported in the current study. Inclusion of winter ADG results in values that are approximately 33% less than those found in the current study. This approach better matches the design of the current trial, but inclusion of brassicas and crimson clover must be considered when comparing current results with studies grazing forage stands consisting of grass monocultures. These results show that the different removal days did not affect the ADG, which means that cover crops are useful as a potential grazing source for short periods of time. Inclusion of different forage species and varieties could potentially extend grazing, but the benefits of different cover crop mixtures on soil and cash crop productivity must be considered as well.

Funding

This research was funded by the USDA NRCS Alabama contract #NR184101XXXXC006.

4. References

Beck, P., T. Hess, D. Hubbell, G. Hufstedler, B. Fieser, and J. Caldwell. (2014). Additive effects of growth promoting technologies on performance of grazing steers and economics of the wheat pasture enterprise. Journal of Animal Science 92(3):1219-1227. https://doi.org/10.2527/jas.2013-7203

Chaves, A. V., G. C. Waghorn, I. M. Brookes, and D. R. Woodfield. (2006). Effect of maturation and initial harvest dates on the nutritive characteristics of ryegrass (Lolium perenne L.). Animal Feed Science and Technology 127(3-4):293-318. https://doi.org/10.1016/j.anifeedsci.2005.08.015

Dubeux Jr, J. C., N. DiLorenzo, A. Blount, C. Mackowiak, E. R. Santos, H. M. Silva, M. Ruiz-Moreno, and T. Schulmeister. (2016). Animal performance and pasture characteristics on cool-season annual grass mixtures in north Florida. Crop Science 56(5):2841-2852. https://doi.org/10.2135/cropsci2016.03.0141

Jaramillo, D. M., Dubeux, J. C., Sollenberger, L. E., Vendramini, J. M., Mackowiak, C., DiLorenzo, N., ... & Ruiz-Moreno, M. (2021). Water footprint, herbage, and livestock responses for nitrogen-fertilized grass and grass–legume grazing systems. Crop Science, 61(5), 3844-3858. https://doi.org/10.1002/csc2.20568

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Ithaca, NY. Cornell University Press.

Can *In Vitro* or *In Situ* Disappearance Assays Accurately Predict *In Vivo* Digestibility of Bermudagrass?

Abbigail R. Hines [1], Diva N. Rigney [1], Anna G. Underwood [1], Werner G.Bergen [1], Todd R. Callaway [2], M. Kimberly Mullenix [1], S. Leanne Dillard [1], and W. Brandon Smith [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL;
[2] Department of Animal and Dairy Sciences, University of Georgia, Athens, GA.
* Correspondence: wbs0001@auburn.edu

TAKE HOME MESSAGE

In nutrition labs across the country and the world, scientists use *in vitro* (IV; laboratory-based techniques) or *in situ* (IS; compartmentalized live-animal techniques) to rank and screen feedstuffs and forages. However, across the literature, there is a lack of information identifying which laboratory methods best represent digestibility within the animal (*in vivo*). Therefore, the objective of our study was to determine the predictive value of IV and IS estimates of disappearance to relative to *in vivo* dry matter digestibility (DMD). In our experiment, neither IV nor IS techniques accurately estimated *in vivo* measurements. Thus, we may conclude that, while both IV and IS methods are valuable tools to screen forages in preliminary experiments, neither of these methods is suitable for prediction of *in vivo* digestibility.

SUMMARY

Through the years, much time and effort has been dedicated to the characterization of digestibility of forages through laboratory and animal-assisted techniques (*in vitro* [IV] and *in situ* [IS], respectively). However, there remains a disconnect between the values obtained in these assays and the observations made in live animal feeding or grazing experiments. Therefore, our objective was to ascertain whether IV or IS estimates of forage digestibility could reasonably predict *in vivo* observations. In an *in vivo* experiment, four ruminally-fistulated heifers were randomly assigned to one of four bermudagrass (*Cynodon dactylon* [L.] Pers.) cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-day periods. Rumen fluid was collected for an accompanying IV experiment to evaluate the interaction of bermudagrass cultivar [previously described] and digestibility method (Tilley and Terry (1963) [TT] or Goering and Van Soest (1970) [GVS]). An accompanying IS experiment was conducted with three treatment factors (*in vivo* diet [previous described], bermudagrass cultivar [previously described], and incubation timepoint [n = 19]). None of the IV or IS digestibility estimates were correlated with *in vivo* dry matter digestibility (DMD; $P \geq 0.18$). Though not significant, the best model through linear regression was the TT IV estimate ($r^2 = 0.09$; $P = 0.26$). Thus, we may conclude that, while both IV and IS methods are valuable tools to screen forages in preliminary experiments, neither of these methods are suitable for prediction of *in vivo* digestibility.

1. INTRODUCTION

Bermudagrass (*Cynodon dactylon* [L.] Pers.) is the predominant warm-season, perennial grass found in the Southeastern US, accounting for approximately 34 million acres (Vendramini et al., 2019). Since 'Coastal' was released as the first commercially available bermudagrass cultivar in

1943, plant breeders have developed a multitude of cultivars with improved characteristics such as yield, digestibility, and nutritive value (Taliaferro et al., 2004). With the successive release of new bermudagrass cultivars, researchers often seek to rank their performance through a variety of techniques. Such techniques include laboratory-based (*in vitro* [IV]) and animal-assisted (*in situ* [IS]) methods, as well as live animal feeding trials (*in vivo*) for estimating dry matter digestibility (DMD). Most new cultivars are ranked relative to older entries using IVDMD as the indicator (Smith, 2017). Estimates from IV and IS experiments act as indicators of digestibility potential in the animal. However, variations between animals, methodology, forage type, etc. can alter the results of an experiment. Though some efforts have been reported sporadically (Goldman et al., 1987; De Boever et al., 1988), there is no definitive established relationship between IV or IS estimates and direct *in vivo* measurement of DMD. Therefore, **the objective of this experiment was to evaluate the predictive nature of IV and IS estimates in calculation *in vivo* DMD.**

2. PROCEDURES

To obtain digestibility estimates, our experiment was conducted as a Latin square design. Four ruminally-fistulated heifers were assigned to 9.8 × 9.6 ft pens and randomly allocated to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]; Figure 1) for four 30-d *in vivo* periods (21 d for adaptation to diet and facilities and 9 d of sample collection). Beginning on d 14, hay and refusal (ort) samples from each heifer were collected until the end of each period. Total fecal samples were collected from d 21 to 25. Hay, ort, and fecal samples were ground and composited based on an assumed 48-h passage rate. Samples were assayed for dry matter (DM), and *in vivo* digestibility coefficients were calculated as intake minus output expressed as a proportion of intake.

	Period 1	Period 2	Period 3	Period 4	Period 5
Heifer 1	Coastal	Tifton 85	Tifton 44	Russell	Coastal
Heifer 2	Russell	Coastal	Tifton 85	Tifton 44	Russell
Heifer 3	Tifton 44	Russell	Coastal	Tifton 85	Tifton 44
Heifer 4	Tifton 85	Tifton 44	Russell	Coastal	Tifton 85

Figure 1. Experimental layout (Latin square) used in the evaluation of bermudagrass cultivar effects on *in vivo* digestibility and metabolism.

On d 28 of each period, rumen fluid was collected 4 h post-feeding for an accompanying IV experiment. This experiment was conducted as a completely randomized design with a 4 × 2 factorial treatment structure. Factors included bermudagrass cultivar [previously described] and digestibility method (Tilley and Terry (1963) [TT] or Goering and Van Soest (1970) [GVS]).

On day 31 of *in vivo* periods 3 and 5, an accompanying IS experiment was conducted. This experiment was conducted as a randomized complete block design with a 4 × 4 × 19 factorial treatment structure. Treatment factors included *in vivo* diet (previous described), bermudagrass cultivar (previously described), and incubation timepoint (0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, 120, 144, and 168 h).

To assess the association of laboratory-based and animal-assisted techniques with live animal observations, correlations were computed between *in vivo* DMD and each of the IV (TT and GVS)

and IS (24, 48, and 72 h) methods. Linear and stepwise regression were used to determine the predictive value of linear combinations of the measurements.

3. RESULTS & DISCUSSION

The results for this study showed no correlation ($P \geq 0.18$) between IV or IS DMD values with *in vivo* DMD (Table 1). Similarly, here was no linear regression of any IV and IS DMD method against *in vivo* DMD that demonstrated significance ($P \geq 0.18$). Of the regression models tested, the best predictor model (based on AIC) was TT IVDMD (r^2 = 0.09; P = 0.26; Table 2). Stepwise regression did not reveal any linear combination of IV or IS DMD that improved prediction beyond single predictor models.

Table 1. Correlations and regression coefficients of *in vitro* and *in situ* disappearance techniques with *in vivo* dry matter digestibility.

	Correlation		Linear Regression		
Method[1]	r	P-value	r^2	AIC[2]	P-value
TT	-0.29	0.26	0.09	-63.30	0.26
GVS	0.22	0.41	0.05	-62.60	0.41
IS-24	0.42	0.28	0.20	-26.85	0.27
IS-48	0.35	0.38	0.14	-26.23	0.37
IS-72	0.50	0.18	0.28	-27.69	0.17

[1] TT = Tilley and Terry (1963) *in vitro* digestibility; GVS = Goering and Van Soest (1970) *in vitro* digestibility; IS-24 = *in situ* disappearance at 24 h of incubation; IS-48 = *in situ* disappearance at 48 h of incubation; IS-72 = *in situ* disappearance at 72 h of incubation; [2] Akaike information criterion.

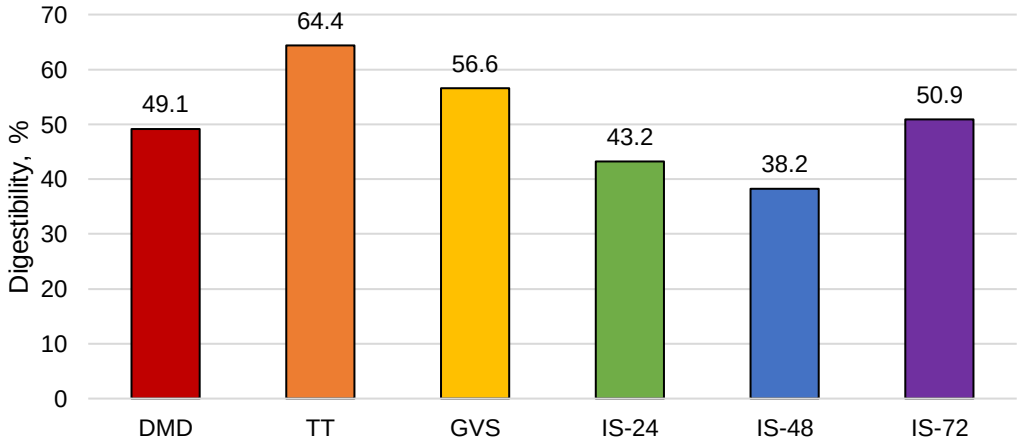


Figure 2. Dry matter digestibility (DMD) of bermudagrass (average across four cultivars) as determined by *in vivo*, *in vitro* (Tilley and Terry (1963) [TT] or Goering and Van Soest (1970) [GVS]), or *in situ* (IS at 24 [IS-24], 48 [IS-48], or 72 [IS-72] h of incubation) techniques.

All the methods addressed in this study are screening tools to evaluate forages and provide valid digestibility estimates (Figure 2). Our results indicated that the TT method was the best predictor of *in vivo* DMD based on fit statistics, but it did not meet the threshold of significance. *In vitro* techniques are great tools in screening forages and ranking their nutritional value for our livestock (García-Rodríguez et al., 2019). Additionally, compared to *in vivo* or IS experiments, IV methods are more cost effective while providing more precise control of experimental conditions,

especially when evaluating larger sets of forages in a relatively shorter timeframe (López, 2005). However, these methods often overlook or may not fully replicate certain aspects of the animal, such as fluctuations in rumen microbial population, accurate simulation of mixing within the gut, and animal to animal variations. While both IV and IS experiments remain viable tools to screen forages and make relative comparisons, results from this experiment indicate that neither of these methods is suitable for prediction of *in vivo* performance.

Acknowledgments

The authors would like to acknowledge all faculty and student members of the Auburn University Beef and Forage Nutrition Research Laboratory that assisted in collections and laboratory work throughout this project.

Funding

This research was funded, in part, by the Alabama Cattlemen's Association through the Beef Checkoff program. Additional funding was received from the Agricultural Research Service, U.S. Department of Agriculture, under Agreement No. 58-6010-1-005.

4. References

De Boever, J. L., B. G. Cottyn, J. I. Andries, F. X. Buysse, and J. M. Vanacker. 1988. The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. Anim. Feed Sci. Tech. 19(3):247-260. doi: 10.1016/0377-8401(88)90088-0

García-Rodríguez, J., M. J. Ranilla, J. France, H. Alaiz-Moretón, M. D. Carro, and S. López. 2019. Chemical Composition, *In vitro* Digestibility and Rumen Fermentation Kinetics of Agro-Industrial By-Products. Animals 9(11):861.

Goering, H. H., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agriculture Handbook 379. United States Department of Agriculture, Washington, DC, USA.

Goldman, A., A. Genizi, A. Yulzari, and N. G. Seligman. 1987. Improving the reliability of the two-stage *in vitro* assay for ruminant feed digestibility by calibration against *in vivo* data from a wide range of sources. Anim. Feed Sci. Tech. 18(3):233-245. doi: 10.1016/0377-8401(87)90074-5

López, S. 2005. *In vitro* and *in situ* techniques for estimating digestibility. In: J. Dijkstra, J. M. Forbes and J. France, editors, Quantitative aspects of ruminant digestion and metabolism. CABI International. p. 87–121.

Smith, W. B. 2017. Impact of dried distillers' grains with solubles supplementation of cattle while grazing bermudagrass on the plant-animal interface. Dissertation, Texas A&M University, College Station, TX, USA.

Taliaferro, C. M., F. M. Rouquette Jr., and P. Mislevy. 2004. Bermudagrass and Stargrass, Warm-Season (C4) Grasses. American Society of Agronomy, Madison, WI, USA. p. 417-475.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci. 18(2):104-111. doi: 10.1111/j.1365-2494.1963.tb00335.x.

Vendramini, J., J. Dubeux Jr, and E. Rios. 2019. 'Mislevy' bermudagrass The Florida Cattleman and Livestock Journal.

Cell Wall Digestibility of Four Bermudagrass Cultivars in Beef Heifers

Abbigail R. Hines [1], Diva N. Rigney [1], Anna G. Underwood [1], Werner G. Bergen [1], Todd R. Callaway [2], M. Kimberly Mullenix [1], S. Leanne Dillard [1], and W. Brandon Smith [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA.
[2] Department of Animal and Dairy Sciences, University of Georgia, Athens, GA, USA.
* Correspondence: wbs0001@auburn.edu

TAKE HOME MESSAGE

With the growing diversity of bermudagrass offerings for pastures and hay fields, there is a need to identify the nutritional differences across common cultivars used across the southeastern US. Therefore, our objective was to evaluate the plant cell wall digestibility from heifers consuming four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]). Across four experimental periods, we found no differences among cultivars for dry matter digestibility or the digestibility of any cell wall fractions. These results suggest that, despite contrary indications *in vitro*, physical, and physiological differences in cultivar do not significantly impact the cell wall digestibility of bermudagrass *in vivo*.

SUMMARY

Since the development of the first commercial bermudagrass (*Cynodon dactylon* [L.] Pers.) cultivar, 'Coastal', in 1943, bermudagrass breeders have made advancements in traits such as yield, digestibility, and nutritive value. However, few experiments have sought to document a comprehensive comparison of cultivars in direct animal feeding experiments. Therefore, our objective was to evaluate the plant cell wall digestibility by beef heifers consuming four commonly available bermudagrass cultivars. Four ruminally-fistulated heifers were randomly assigned to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d periods. Hay, feed refusals, and feces were collected across five days in each period for digestibility estimates. There were no differences among cultivars for digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber, or acid detergent lignin ($P \geq 0.20$). Results are interpreted to mean that, despite previous reports of *in vitro* experiments to the contrary, physical and physiological differences in cultivar do not significantly impact the cell wall digestibility of bermudagrass *in vivo*.

1. INTRODUCTION

When evaluating forages for nutritional management decisions in beef systems, it is necessary to understand to what degree cattle will benefit from the forages they are consuming. Among the most influential factors that can affect the utilization of a forage include the type and abundance of carbohydrates in the plant cell wall. The most common way to express these carbohydrates is by using the detergent fiber system (Goering and Van Soest, 1970). Neutral detergent fiber (NDF) measures the forage's fiber fractions, including hemicellulose, cellulose, and

lignin, while acid detergent fiber (ADF) excludes hemicellulose, and acid detergent lignin (ADL) excludes all but lignin.

Bermudagrass (*Cynodon dactylon* [L.] Pers.) is the predominant warm-season, perennial grass found in the southeastern US, accounting for approximately 34 million acres of pasture and hayland (Vendramini et al., 2019). Since "Coastal" was released as the first commercially available bermudagrass cultivar in 1943, the development of more genetically diverse cultivars has allowed producers to take advantage of improved adaptations for the improvement of characteristics such as yield, digestibility, and nutritive value (Taliaferro et al., 2004). These genetic improvements have led to vast agronomic yield differences (Taliaferro et al., 2004; Smith, 2017). Much of the distinction in *in vitro* forage digestibility has been linked to cell wall constituents (Mandebvu et al., 1999). However, the literature is lacking when it comes to *in vivo* metabolism data to substantiate these *in vitro* observations. Therefore, **the objective of our study was to evaluate the plant cell wall digestibility of four bermudagrass cultivars in beef heifers.**

2. PROCEDURES

Our experiment was conducted as a Latin square design. Four ruminally-fistulated heifers were assigned to 9.8 × 9.6 ft pens and randomly allocated to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]; Figure 1) for four 30-d periods (21 d for adaptation to diet and facilities and 9 d of sample collection).

	Period 1	Period 2	Period 3	Period 4	Period 5
Heifer 1	Coastal	Tifton 85	Tifton 44	Russell	Coastal
Heifer 2	Russell	Coastal	Tifton 85	Tifton 44	Russell
Heifer 3	Tifton 44	Russell	Coastal	Tifton 85	Tifton 44
Heifer 4	Tifton 85	Tifton 44	Russell	Coastal	Tifton 85

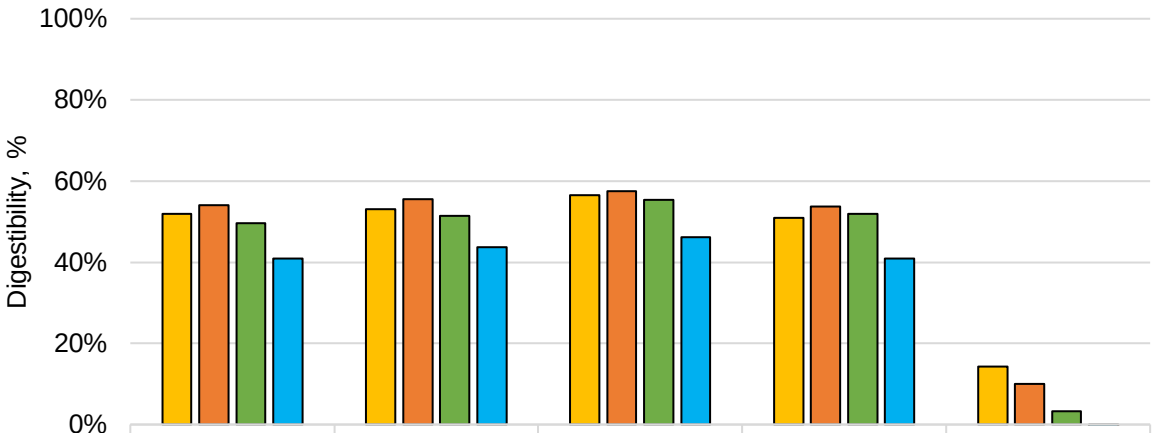
Figure 1. Experimental layout (Latin square) used in the evaluation of bermudagrass cultivar effects on *in vivo* digestibility and metabolism.

Beginning on day 14, hay and refusal (ort) samples from each heifer were collected until the end of each period. Total fecal samples were collected from d 21 to 25. Hay, ort, and fecal samples were ground and composited based on an assumed 48-h passage rate. Samples were assayed for dry matter (DM), organic matter (OM), NDF, ADF, and ADL. Digestibility coefficients were calculated as intake minus output expressed as a proportion of intake.

3. RESULTS & DISCUSSION

There were no differences among cultivars for digestibility of DM (DMD), OM (OMD), NDF (NDFD), ADF (ADFD), or ADL (ADLD; $P \geq 0.20$; Figure 2). Previous studies found that improved cultivars, such as T44, contain decreased ADF and ADL fractions and increased digestibility compared to other improved bermudagrass varieties (Burton and Monson, 1988). These improved varieties typically show higher digestible fractions compared to COS due to the lower amounts of lignin and increased concentrations of soluble sugars. Other reports have shown the *in vitro* DM digestibility (IVDMD) was greater from T85, T44, and RUS (64.6, 61.9, and 60.5 %, respectively) than from COS (53.0 %) (Hines et al., 2023). However, *in vitro* digestibility estimates may not fully represent the digestive capability within the animal. In our current study, all forages were sourced

from southeastern (Alabama, Georgia, or Florida) producers as pure stand hay within the same season from the second cutting (to minimize any effect of maturity or season). Nevertheless, the hay that we obtained could differ from other intensively managed stands. Based on our findings, we may conclude that physical and physiological differences in cultivars do not significantly impact the cell wall digestibility of bermudagrass *in vivo*.



	DMD	OMD	NDFD	ADFD	ADLD
COS	51.96%	53.13%	56.56%	50.94%	14.33%
RUS	54.04%	55.45%	57.47%	53.75%	9.95%
T44	49.68%	51.37%	55.29%	51.86%	3.23%
T85	40.87%	43.78%	46.10%	40.85%	-16.60%

Figure 2. Digestibility of dry matter (DMD), organic matter (OMD), neutral detergent fiber (NDFD), acid detergent fiber (ADFD), and acid detergent lignin (ADLD) from four bermudagrass cultivars by ruminally-fistulated beef heifers.

Acknowledgments

The authors would like to acknowledge all faculty and student members of the Auburn University Beef and Forage Nutrition Research Laboratory that assisted in collections and laboratory work throughout this project.

Funding

This research was funded, in part, by the Alabama Cattlemen's Association through the Beef Checkoff program. Additional funding was received from the Agricultural Research Service, U.S. Department of Agriculture, under Agreement No. 58-6010-1-005.

4. References

Burton, G. W., and W. G. Monson. 1988. Registration of 'Tifton 78' Bermudagrass. Crop Sci. 28(1): crop.sci. doi: 10.2135/cropsci1988.0011183X002800010047x.

Goering, H. H., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agriculture Handbook 379. United States Department of Agriculture, Washington, DC, USA.

Hines, A. R., W. G. Bergen, M. K. Mullenix, S. L. Dillard, T. R. Callaway, and W. B. Smith. 2023. P27 In vitro digestibility comparison of four bermudagrass cultivars. Animal - Science Proceedings 14(4):619-620.

Mandebvu, P., J. W. West, M. A. Froetschel, R. D. Hatfield, R. N. Gates, and G. M. Hill. 1999. Effect of enzyme or microbial treatment of bermudagrass forages before ensiling on cell wall composition, end products of silage fermentation and in situ digestion kinetics. Anim. Feed Sci. Tech. 77(3):317-329. doi: 10.1016/S0377-8401(98)00247-8

Smith, W. B. 2017. Impact of dried distillers' grains with solubles supplementation of cattle while grazing bermudagrass on the plant-animal interface. Dissertation, Texas A&M University, College Station, TX, USA.

Taliaferro, C. M., F. M. Rouquette Jr., and P. Mislevy. 2004. Bermudagrass and Stargrass, Warm-Season (C4) Grasses. American Society of Agronomy, Madison, WI, USA. p. 417-475.

Vendramini, J., J. Dubeux Jr, and E. Rios. 2019. 'Mislevy' bermudagrass The Florida Cattleman and Livestock Journal.

Variation in Nutritive Value of Whole Cottonseed from the Regional Breeding Testing Network

Diva N. Rigney [1], Macy L. Rockwell [1], Anna Grace Underwood [1], Jenny C. Koebernick [2], M. Kimberly Mullenix [1], and W. Brandon Smith [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA;
[2] Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL, USA.
* Correspondence: wbs0001@auburn.edu

TAKE HOME MESSAGE

With the acreage of cotton grown in Alabama, whole cottonseed represents a nutritionally and economically viable supplement for beef cattle. Based on "book values," whole cottonseed provides 90% total digestible nutrients, 52% neutral detergent fiber, 24% crude protein, and 18% crude fat. However, recent work at Auburn University has suggested that there may be a high degree of variability in these values, especially when southeastern varieties are considered. The objective of this study was to assess the variability in nutritive value of whole cottonseed from diverse lines. Crude protein closely matched previously reported values and demonstrated the least variability of any nutrient. Crude fat was both greater and demonstrated a higher degree of variability. Our data suggest that the nutritive value of whole cottonseed can vary greatly depending on the variety. This work will serve as a basis for the identification of genetic components that control these factors.

SUMMARY

Whole cottonseed represents a nutritionally and economically viable supplemental feedstuff for beef cattle. However, recent work suggests that the nutritive value of this feedstuff is highly dependent on growing location and cultivar. The Regional Breeders Testing Network (RBTN) offers a multiple environment trial for cotton breeders to evaluate germplasm. Such a trial offers the opportunity to assess cultivar variability with respect to nutritive value. Thus, the objective of our study was to document the variability in nutritive value of whole cottonseed from diverse lines in the 2023 RBTN trials. For this study, seed from 20 RBTN cotton varieties were obtained for nutritive value analysis. Seed were assayed for detergent fiber fractions, crude protein, crude fat, and *in vitro* true digestibility. In our study, neutral detergent fiber (total cell wall content) was less than previously reported book values (45.8% vs. 51.6%). Crude protein was consistent with book values (25.0% vs. 24.4%) and had the lowest degree of variability among the nutrients tested. However, crude fat (the primary energy source in whole cottonseed) in our samples was greater than values previously reported (20.2% vs. 17.5%) and had a high degree of variability. Our data suggest that the nutritive value of whole cottonseed can vary greatly depending on the variety. This work will serve as a basis for the identification of genetic components that control these factors.

1. INTRODUCTION

Cotton is the most widely produced row crop in the state of Alabama (ALFA, 2021), representing 376,000 acres of production (Price, 2014). As such, whole cottonseed is a widely available feedstuff to support the state's beef cattle industry. Whole cottonseed represents a

nutritionally and economically viable supplemental feedstuff for beef cattle (Jacobs et al., 2019). Based on "book values," whole cottonseed provides approximately 90% total digestible nutrients, 52% neutral detergent fiber, 24% crude protein, and 18% crude fat (NRC, 2000). However, recent work suggests that the nutritive value of this feedstuff is highly dependent on growing location and cultivar (Jacobs, 2021; Jacobs et al., 2021).

The Regional Breeders Testing Network (RBTN) has provided a multi-environment trial (MET) for germplasm lines for over 15 years. Before the RBTN began, cotton breeders were without a MET because of limitations of resources. With the incorporation of the RBTN, breeders can evaluate germplasm yield and fiber quality under stress (Cotton Inc, 2023). While the RBTN MET was designed to evaluate yield and quality characteristics of cotton from an agronomic perspective, there remains potential to use these samples as a barometer for characteristics of whole cottonseed available as a feedstuff. Therefore, **the objective of our experiment was to document the variability in nutritive value of whole cottonseed from diverse lines in the 2023 RBTN trials.**

2. PROCEDURES

For this study, seed were obtained from 20 lines in the 2023 RBTN MET. Residual lint was removed from seed using 98% sulfuric acid (for ease of laboratory assays). After lint removal, seeds were ground using a coffee grinder prior to being subjected to nutritive value assays in the Auburn University Beef-Forage Nutrition Laboratories. Fiber fractions (neutral detergent fiber [NDF] and acid detergent fiber [ADF]) were assayed sequentially according to the procedures of Vogel et al. (1999) using ANKOM^{2000} and ANKOM^{DELTA} Fiber Analyzers. Acid detergent lignin (ADL) was assayed on the ADF residues according to the procedure of AOAC (2000). Crude protein (CP) was measured using the Kjeldahl method (AOAC, 2000). *In vitro* true digestibility (IVTD) was measured using the ANKOM Daisy^{II} incubator (Vogel et al., 1999). A subsample of each ground sample was submitted to a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA, USA) for determination of crude fat, which was measured via the ether extract method.

3. RESULTS & DISCUSSION

Results from the nutritive value assays of the RBTN cotton lines are presented in Figure 1. In the current study, the NDF averaged was 46.7% (34.8 – 51.6%), ADF averaged 33.7% (25.5 – 36.2), and ADL averaged 13.4% (10.4 – 18.7%). Our observed NDF was less than that documented by NRC (2000) (51.6%). Similarly, Bertrand et al. (2005) found that ground cottonseed had NDF values ranging from 51.8 – 52.6% and ADF values ranging from 39.1 – 39.6%, both of which were at the upper end of our observed measurements.

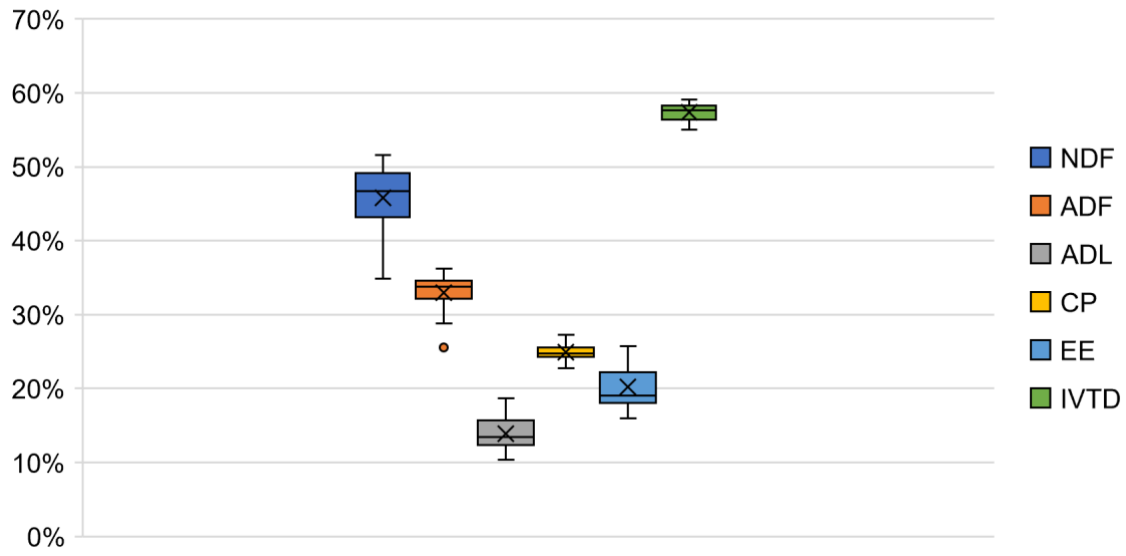


Figure 1. Histogram of variability in nutritive value (NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein; EE = ether extract [crude fat]; IVTD = *in vitro* true digestibility) of cottonseed from the 2023 Regional Breeders Testing Network multi-environment trial.

Crude protein in our RBTN evaluation (24.7%) was in relative agreement with book values (24.4%) (NRC, 2000). Likewise, Bertrand et al. (2005) found CP concentrations ranging from 23.7 – 24.5%. Jacobs (2021), in evaluating 98 lines in the Auburn University Cotton Breeding Program, documented CP of 23.4% and degradable intake protein of 52.9%. It should also be noted that we observed the smallest range (4.5%) and coefficient of variation (CV; 4.6%) among all measures of nutritive value.

Recently, fat has become a point of interest in feeding whole cottonseed to beef cattle (Underwood et al., 2023). This is because fat, the primary energy source of the feedstuff, has been linked to decreased fiber digestibility with increasing dietary inclusion. In our study, crude fat (ether extract) averaged 20.2% (16.0 – 25.7%). This far exceeds the values report by Bertrand et al. (2005) (15.7 – 17.4%) and NRC (2000) (17.5%). There was also considerable variability in these values (CV = 13.1%) relative to other measures of nutritive value.

Ultimately, the most valuable measure of any feedstuff is its utilization by the animal. In our experiment, the average IVTD was 57.4% (55.0 – 59.1%). Bertrand et al. (2005) found digestibility values ranging from 58.3 – 58.6%.

Funding

This research was funded by the Alabama Cotton Commission.

4. References

ALFA. 2021. Alabama Farm Facts. Alabama Farmers Federation, Montgomery, AL, USA.

AOAC. 2000. Official method of analysis of the Association of Official Analytical Chemists International. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bertrand, J. A., T. Q. Sudduth, A. Condon, T. C. Jenkins, and M. C. Calhoun. 2005. Nutrient Content of Whole Cottonseed. J. Dairy Sci. 88(4):1470-1477. doi: 10.3168/jds.S0022-0302(05)72815-0

Cotton Inc. 2023. Regional Breeders Testing Network. (Accessed November 30 2023).

Jacobs, J. L. 2021. Cotton byproducts assessment for improving beef cattle feeding recommendations. Thesis, Auburn University, Auburn, AL, USA.

Jacobs, L., K. Mullenix, and S. Brown. 2019. Whole cottonseed use in beef cattle. Alabama Cooperative Extension System ANR-2608

Jacobs, L. L., K. K. Mullenix, R. B. Muntifering, S. L. L. Dillard, and R. C. Carrell. 2021. Evaluation of Whole Cottonseed Intake and in Situ digestibility in Beef Cattle. J. Anim. Sci. 99(Supplement_2):11-11. doi: 10.1093/jas/skab096.018

NRC. 2000. Nutrient Requirements of Beef Cattle: Update 2000. 7th Revised ed. The National Academies Press, Washington, DC, USA.

Price, C. 2014. Get to Know Alabama Agriculture. https://www.usda.gov/media/blog/2014/05/29/get-know-alabama-agriculture (Accessed December 12 2023).

Underwood, A. G., S. Martin, W. Greene, and W. B. Smith. 2023. 129 Case Study: Passage Dynamics of Whole Cottonseed in Beef Cattle. J. Anim. Sci. 101(Supplement_1):87-87. doi: 10.1093/jas/skad068.103

Vogel, K. P., J. F. Pedersen, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39:276-279. doi: 10.2135/cropsci1999.0011183X003900010042x.

Extent of Use of Supplemental Feeding in Beef Cattle Operations Across the Southeast: A Preliminary Report of the Regional Survey

Lauren M. St. Andrew [1], S. Leanne Dillard [1], M. Kimberly Mullenix [1], and W. Brandon Smith [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA.
* Correspondence: wbs0001@auburn.edu

TAKE HOME MESSAGE

In ongoing efforts to make recommendations for environmentally and economically sustainable beef production practices, supplemental feeding programs have long been staples of production and continue to garner research interest. However, there are a lack of data surrounding the extent of use of supplemental feeding programs in the Southeast as well as which feedstuffs are chosen when these practices are put into place. Thus, the objective of this study was to better understand supplemental feeding practices and choices among beef producers in the southeastern US. We found that the majority (87.6%) of respondents are using some supplemental feeding strategy, roughly evenly split among those choosing to use commodity of byproduct feedstuffs. The leading commodity used was corn (61.4%), while common byproducts included whole cottonseed, corn gluten feed, and soybean hulls (17.1, 16.6, and 14.4%, respectively). These data will aid in developing upcoming research projects for targeted beef cattle nutrition programs.

SUMMARY

With fertilizer and other input costs on the rise, researchers and producers, alike, are seeking alternative management strategies for profitable and sustainable beef production. Studies suggest that supplemental feed provided to grazing cattle may be used to offset fertilizer inputs on the pasture ecosystem. However, there is a lack of knowledge on the extent of supplemental feeding programs in the southeastern US and which feeds may be used in these programs. Thus, the objective of this study was to better understand supplemental feeding practices and choices among beef producers in the southeastern US. To address this objective, a 24-question survey was distributed through Extension and commodity organizations to beef producers across the Southeast. This survey received 116 complete responses. Most respondents operated cow-calf production systems (65.6%) on either tall fescue or bermudagrass pastures (32.7 and 28.0%, respectively). Approximately 88% of these producers employed some type of supplemental feeding strategies, but there is no clear decision on whether commodity or byproduct feedstuffs were preferred. The leading commodity used was corn (61.4%), while common byproducts included whole cottonseed, corn gluten feed, and soybean hulls (17.1, 16.6, and 14.4%, respectively). With this understanding, results will be used to formulate feed choices in upcoming research projects directed at supplemental feeding to reduce fertilizer inputs as part of the pasture ecosystem.

1. INTRODUCTION

Recent agricultural fertilizer prices a hit staggering levels, recording 66% year-over-year increases and tying records set in the last American recession (Good, 2022). Grazed feed accounts for $132.50/head in the United States (23% of operating costs) (ERS, 2022). With record-setting

input costs, many producers begin to ask if fertilization of pastures, especially, is necessary to support production (Johnson, 2022). While current recommendations state that fertilization of pastureland is still advantageous to purchasing or producing hay so long as input costs remain under $2.50/lb (Johnson, 2022), these recommendations do not take into account potential benefits to the system of feeding hay or supplemental byproducts. Thus, there remains opportunity for alternative management of nutrient flows in grazing systems. Cattle on pasture are commonly offered supplemental feed to offset low forage availability or forages with low nutritive value (Horn and McCollum, 1987; Smith, 2017). Past research has indicated that supplemental feeding of grazing cattle may be a viable option to augment or replace pasture fertilization. However, there is a gap in knowledge at the current time as to how many operations are making use of supplemental feeding in and which feedstuffs are being used. Thus, **the objective of our study was to determine the extent of use of supplemental feeding in beef cattle operations across the southeastern United States.**

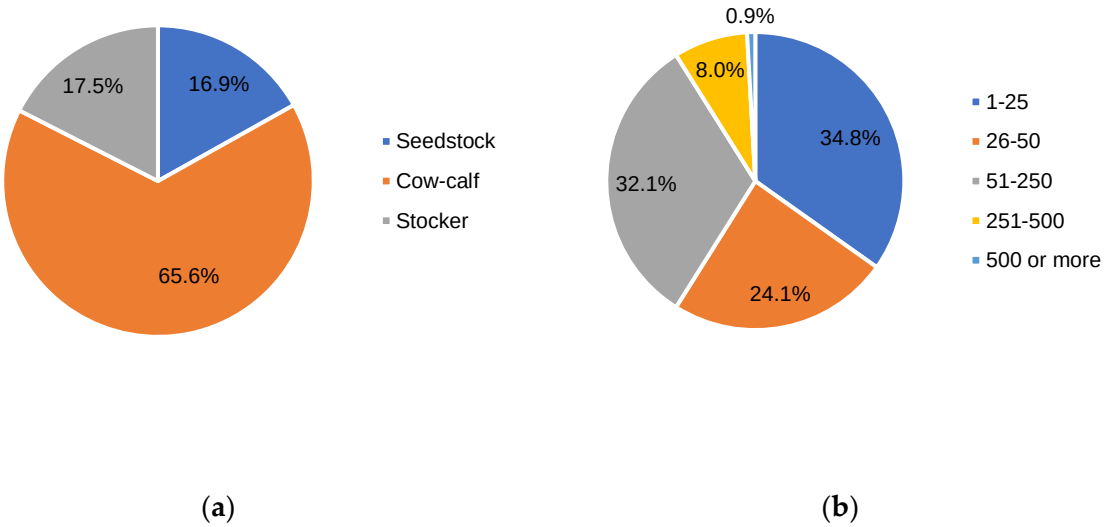
2. PROCEDURES

A survey instrument was developed by the Beef-Forage Nutrition Research Team at Auburn University. The survey was evaluated for content validity by two subject-matter experts prior to distribution. After validation and approval by the Institutional Review Board (Protocol 23-360 EX 2307), the electronic survey was distributed via a link posted to the social media accounts of the Alabama Beef Systems Extension Team. Subsequently, the survey was shared with state cattlemen's associations in all southeastern states (defined as Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Oklahoma, Tennessee, Texas, and Virginia). The survey consisted of 24 questions designed to ascertain the size of the cattle operation, what forages were being used for grazing, whether or not supplemental feeding strategies were being used, and, if so, what feedstuffs were being used. Respondents were also asked to submit a feed sample for nutritive value testing (to create a databank of supplemental feeds).

3. RESULTS & DISCUSSION

Our survey was launched on August 10, 2023. As of December 15, 2023, there were 142 respondents. Of these respondents, 116 owned or managed cattle in the Southeast and were able to complete the survey.

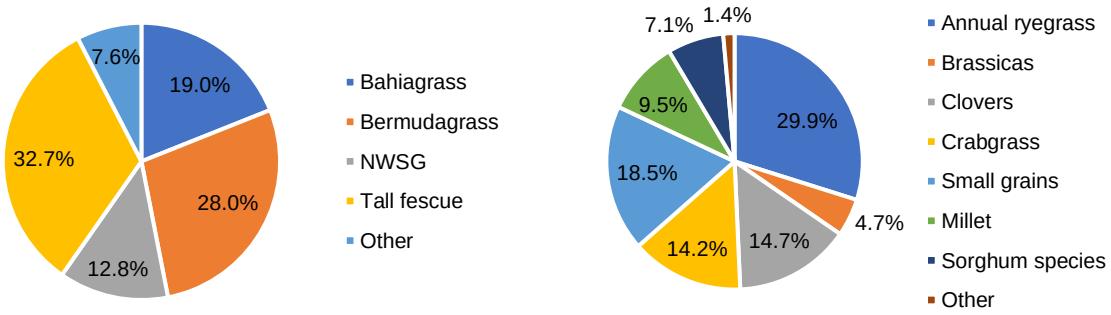
Distributions of operation type and size are presented in Figure 1. Most respondents owned or managed cow-calf operations (65.6%). These operations tended to have less than 250 animals (91.0%), with the majority having less than 25 animals (34.8%). If the respondent owned or managed stocker cattle (17.5%), the operation typically had between 51 and 250 animals (46.4%; data not shown).



(**a**) (**b**)

Figure 1. Distribution of (**a**) operation type and (**b**) operation size (breeding animals) from respondents in a regional survey regarding supplemental feeding strategies in beef cattle operations.

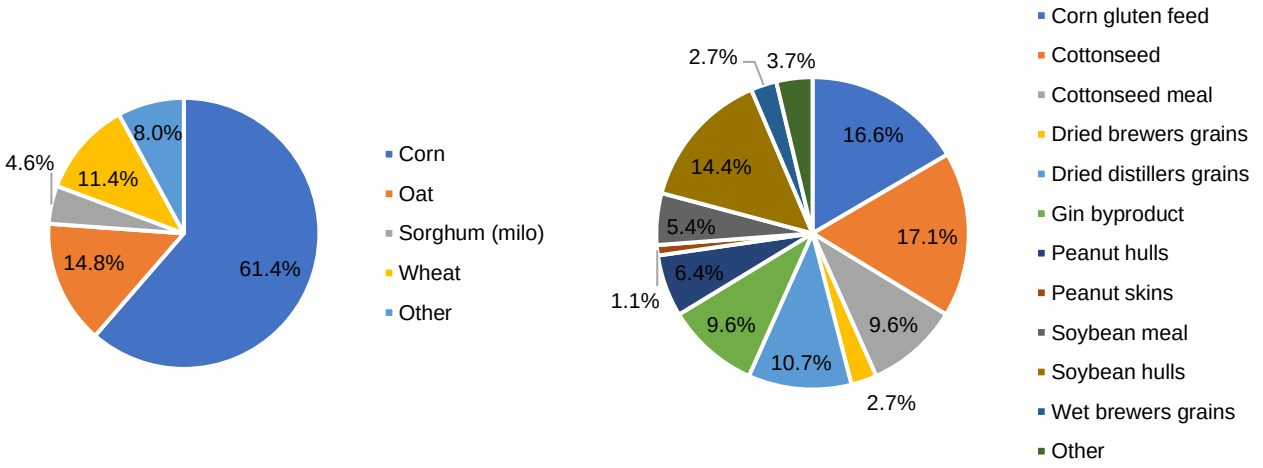
Forage species used in southeastern beef cattle operations are presented in Figure 2. The most widely used perennial forage species was tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.; 32.7%), followed by bermudagrass (*Cynodon dactylon* [L.] Pers.; 28.0%). This is expected, given that bermudagrass, the predominant warm-season perennial grass, accounts for approximately 34.6 million acres in the United States (Vendramini et al., 2019), while tall fescue accounts for approximately 37 million acres (Rogers and Locke, 2013). Annual forages were used by 72.3% of the respondents, with the largest group using annual ryegrass (*Lolium perenne* L. ssp. *multiflorum* [Lam.] Husnot; 29.9%).



(**a**) (**b**)

Figure 2. Distribution of (**a**) perennial and (**b**) annual forage species used by respondents in a regional survey regarding supplemental feeding strategies in beef cattle operations.

Respondents overwhelmingly reported using supplemental feeding strategies in their operation (87.6%), but they were evenly divided on whether they chose commodity or byproduct feedstuffs (49.6 vs. 50.4%, respectively). Of those that chose to use commodity feedstuffs as supplement, 61.4% used corn as the primary feedstuffs (Figure 3). The use of byproduct feedstuffs was more varied, with whole cottonseed, corn gluten feed, and soybean hulls representing the most widely used (17.1, 16.6, and 14.4%, respectively; Figure 3). These byproducts were mainly sourced in bulk from a feed supply vendor (47.7%) as a part of commercial blends (38.0%) that are then stored in bulk bins (42.2%). Of those respondents that stated they did not use any type of supplemental feeding strategy, most cited cost, or efficiency as their reason.



(**a**) (**b**)

Figure 3. Distribution of (**a**) commodity and (**b**) byproduct feedstuffs used by respondents in a regional survey regarding supplemental feeding strategies in beef cattle operations.

Funding

This research was funded by the Alabama Agricultural Experiment Station as part of the Agricultural Research Enhancement, Exploration, and Development program. Additional funding came from Beef Checkoff funds through the Alabama Cattlemen's Association.

4. References

ERS. 2022. Cow-calf production costs and returns, per cow. In: J. Gillespie (ed.). Economic Research Service, United States Department of Agriculture, Washington, DC, USA.

Good, K. 2022. Fertilizer Prices, Higher Still- Producers World-Wide Adjust; U.S. Faces Railway Issues. Farm Policy News

Horn, G. W., and F. T. McCollum. 1987. Energy supplementation of grazing ruminants. In: Grazing livestock nutrition conference. p 125-136.

Johnson, M. Z. 2022. Should I Fertilize My Bermudagrass Pasture This Year? Drovers. Farm Journal, Inc., Lenexa, KS, USA.

Rogers, J. K., and J. M. Locke. 2013. Tall fescue: History, application, establishment and management. In: T. S. R. N. Foundation (ed.) No. NF-F0-13-03. The Samuel Roberts Noble Foundation, Ardmore, OK, USA.

Smith, W. B. 2017. Impact of dried distillers' grains with solubles supplementation of cattle while grazing bermudagrass on the plant-animal interface. Dissertation, Texas A&M University, College Station, TX, USA.

Vendramini, J., J. Dubeux Jr, and E. Rios. 2019. 'Mislevy' bermudagrass The Florida Cattleman and Livestock Journal.

A Comparison of *In Vitro* Methodologies for Determination of Forage Digestibility

Abigail E. Boggier [1], Abbigail R. Hines [1], Aghata E. Moreira da Silva [1], and S. Leanne Dillard [1], M. Kimberly Mullenix [1], and W. Brandon Smith [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA.
* Correspondence: wbs0001@auburn.edu

TAKE HOME MESSAGE

As beef cattle nutritionist, our primary responsibility to the stakeholder is to make accurate and relevant recommendations on feeding. Given the cost-prohibitive nature of direct feeding trials for every feedstuff/forage combination, *in vitro* assays are commonly used to provide an estimate of digestibility. However, multiple *in vitro* procedures exist, and their results do not always agree. Thus, our objective was to determine the effect of methodology on forage digestibility estimates. In the first experiment using diet-adapted rumen fluid, we found that the classic Tilley and Terry (1963) method provided estimates greater than the Goering and Van Soest (1970) method. In the follow-up experiment, however, we found no difference between the methodologies. These results suggest that the rumen fluid collected may have more influence than the methodology. These data will serve as a basis for several follow-up studies to determine the ideal methodology for making feedstuff and forage recommendations.

SUMMARY

In vitro (IV) assays are a staple in the toolbox of all beef cattle nutritionists. These procedures provide estimates of feedstuff digestibility when live animal feeding trials are unavailable. However, there are multiple published methods for conducting IV digestibility assays. Further, few have investigated the possibility of innate differences of the methodology on estimates of digestibility. Thus, our objective was to determine the effect of IV methodology (or components thereof) on digestibility estimates of a common southeastern forage. In our first experiment, ruminally-fistulated heifers (n = 4) that were assigned randomly to one of four bermudagrass (*Cynodon dactylon* [L.] Pers.) cultivars for four 30-day *in vivo* periods in a metabolism trial. On d 28 of each period, rumen fluid was collected from each heifer for use in the IV experiment. The Tilley and Terry (1963) method yielded greater ($P < 0.05$) estimates of IV dry matter digestibility (IVDMD) than did the Goering and Van Soest (1970) method. A second experiment was conducted in which buffer and method were treated as a factorial arrangement using an unadapted rumen fluid. There was no interaction of buffer and method ($P = 0.19$), nor were there differences among buffer type ($P = 0.50$) or IV method ($P = 0.78$). Results are interpreted to mean that rumen fluid characteristics may play a larger role in IVDMD estimation. These data will serve as the basis for several follow-up experiments to determine the causes of methodology discrepancies and the ideal method for estimation of digestibility.

1. INTRODUCTION

Accurate and relevant feeding recommendations for beef cattle require precise evaluation of feedstuffs or forages available to the producer. The most accurate method of evaluation is direct feeding trials. However, these studies are often cost- and labor-prohibitive. Thus, alternative

techniques (e.g., *in situ* [IS] and *in vitro* [IV]) are essential to ruminant nutritionists. There are multiple IV methodologies available for estimation of feedstuff and forage digestibility. Tilley and Terry (1963) proposed a technique (TT) in which feed sample is first incubated with strained rumen fluid and a "synthetic saliva" (McDougall, 1948) for 48 hours, after which it is centrifuged, then digested with a pepsin and hydrochloric acid solution for 48 h (representing post-ruminal digestion). This method has been noted for its accuracy and strong correlation to *in vivo* digestibility measurements (Goldman et al., 1987; De Boever et al., 1988). Goering and Van Soest (1970) proposed an improvement on this method (GVS) in which a feed sample would be incubated with blended and strained rumen fluid (to achieve a greater population of cellulolytic bacteria) and a two-part buffer for 48 h. After incubation, samples would then be subjected to the neutral detergent fiber (NDF) procedure to remove residual microbial matter. ANKOM Technology Corporation (Macedon, NY, USA) amended this method in development of the Daisy^{II} incubator for batch culture evaluation. The amended methodology, though, has been shown to underestimate IV dry matter digestibility (IVDMD) of grass samples (Damiran et al., 2008). To date, the choice of which method to follow for estimation of feed or forage digestibility has been a factor of researcher preference. However, it is known that each method may yield different results. Thus, **the objective of our experiments was to determine the effect of IV methodology (or components thereof) on digestibility estimates of southeastern grasses**.

2. PROCEDURES

2.1 Experiment 1

In our initial study, the IV experiment was a companion project to an *in vivo* metabolism experiment evaluating bermudagrass (*Cynodon dactylon* [L.] Pers.). 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 day 28 of each period, rumen fluid was collected 4 h post-feeding and transported to the lab to be processed. The accompanying *in vitro* experiment was conducted as a hierarchical addition to the *in vivo* Latin square design using a completely randomized design with a 4 × 2 factorial treatment structure (Figure 1). Samples of each bermudagrass were weighed into duplicated 125 mL Erlenmeyer flasks and subjected to either the (Tilley and Terry, 1963) [TT] or (Goering and Van Soest, 1970) [GVS] method.

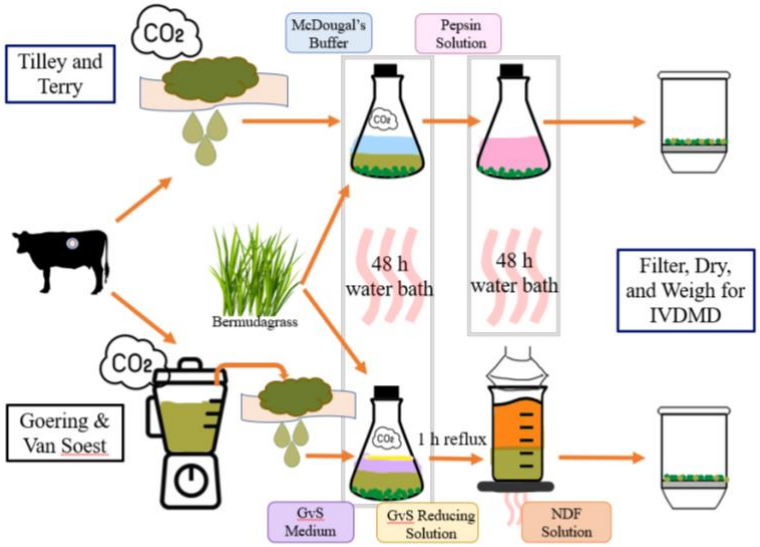


Figure 1. Schematic representation of the comparison of two in vitro digestibility methodologies (Tilley and Terry [TT] or Goering and Van Soest [GVS]).

2.2 Experiment 2

Following analysis of results from Exp. 1, a second experiment was designed to isolate the cause of differences in IVDMD estimates based on method. Our second experiment utilized a randomized complete block design with a 2 × 2 × 4 factorial treatment structure. Duplicate samples of four bermudagrass cultivars (Coastal, Russell, Tifton 44, Tifton 85) were placed in 125 mL Erlenmeyer flasks and incubated according to the assigned IV method. Approximately 1,000 mL of rumen content (solid and liquid fractions) were collected from two ruminally-fistulated heifers in each of two periods (blocks). Heifers were not adapted to any specific dietary treatment (a deviation from Exp. 1). The samples were separated into two 500 mL aliquots; the first aliquot was subjected to straining according to the TT method, and the second aliquot was subjected to blending and straining according to the GVS method. Each aliquot was then subdivided with half receiving the methodologically prescribed buffer solution (McDougall (1948) for TT; two-part for GVS), and the other half receiving the opposite buffer.

3. RESULTS & DISCUSSION

In Exp. 1, there was no interaction of bermudagrass cultivar and IV method (P = 0.19). However, there was an effect of cultivar (P < 0.01; Figure 2) on IVDMD. The IVDMD was greater from T85, T44, and RUS (64.6, 61.9, and 60.5%, respectively) than from COS (53.0%). These data are consistent with previous reports. Tifton 44 has shown decreased cell wall constituents and increased digestibility (Burton and Monson, 1988). Tifton 85 has shown higher digestible fraction than Coastal due to decreased lignin and increased concentrations of neutral sugars (Burton et al., 1993; Mandebvu et al., 1998).

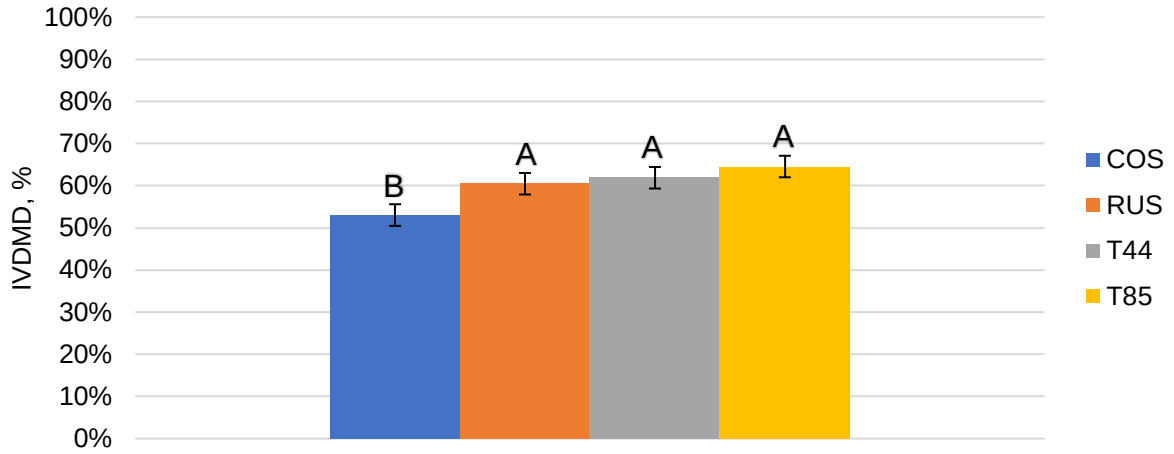


Figure 2. *In vitro* dry matter digestibility (IVDMD) of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) averaged across in vitro methodology (Tilley and Terry (1963) [TT] or Goering and Van Soest (1970) [GVS]). Bars with differing annotated letters are different ($P < 0.05$).

There was also an effect of *in vitro* methodology ($P < 0.01$; Figure 3) on IVDMD. Samples subjected to TT had greater IVDMD (64.4%) compared to GVS (55.6%). Similarly, Damiran et al. (2008) found that GVS underestimated IVDMD in grasses relative to TT. The TT procedure has also been noted for its accuracy and strong correlation to in vivo digestibility (Goldman et al., 1987; De Boever et al., 1988).

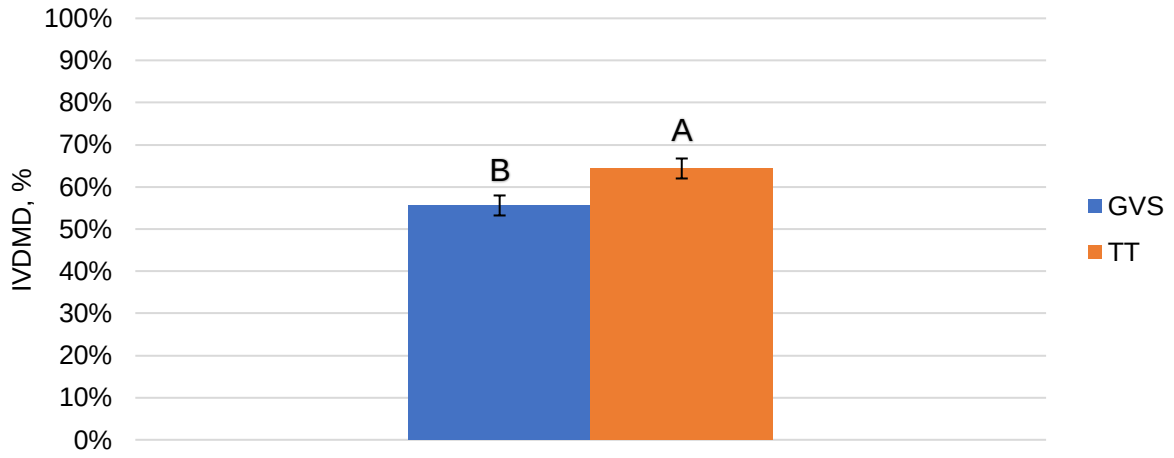


Figure 3. *In vitro* dry matter digestibility (IVDMD) of bermudagrass as influenced by in vitro methodology (Tilley and Terry (1963) [TT] or Goering and Van Soest (1970) [GVS]) averaged across four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]). Bars with differing annotated letters are different ($P < 0.05$).

In Exp. 2, there was no interaction of method and buffer ($P = 0.19$), nor was there an effect of method ($P = 0.78$) or buffer solution (P = 0.50) alone (Figure 4). Given that the rumen fluid used in this experiment was not adapted to the forages being incubated, it is possible that this masked any procedural effects in the experiment. The contrasting outcomes observed in two experiments comparing *in vitro* dry matter digestibility IVDMD

methods underscore the need for further research to elucidate the discrepancies and establish a more comprehensive understanding of two prominent methods.

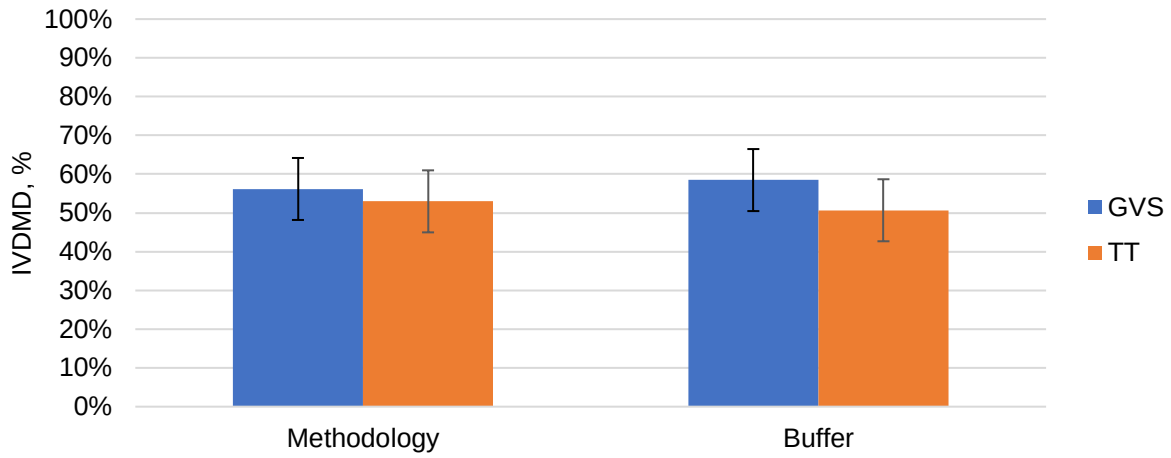


Figure 4. *In vitro* dry matter digestibility of bermudagrass *in vitro* methodology or buffer solution (Tilley and Terry (1963) [TT] or Goering and Van Soest (1970) [GVS]).

Funding

This project was financially supported by the Agricultural Research Service, U.S. Department of Agriculture, under Agreement No. 58-6010-1-005.

4. References

Burton, G. W., R. N. Gates, and G. M. Hill. 1993. Registration of 'Tifton 85' Bermudagrass. Crop Sci. 33(3):cropsci1993.0011183X003300030045x. doi: https://doi.org/10.2135/cropsci1993.0011183X003300030045x

Burton, G. W., and W. G. Monson. 1988. Registration of 'Tifton 78' Bermudagrass. Crop Sci. 28(1):cropsci1988.0011183X002800010047x. doi: https://doi.org/10.2135/cropsci1988.0011183X002800010047x

Damiran, D., T. DelCurto, D. W. Bohnert, and S. L. Findholt. 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. Anim. Feed Sci. Tech. 141(1):15-35. doi: 10.1016/j.anifeedsci.2007.04.007

De Boever, J. L., B. G. Cottyn, J. I. Andries, F. X. Buysse, and J. M. Vanacker. 1988. The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. Anim. Feed Sci. Tech. 19(3):247-260. doi: 10.1016/0377-8401(88)90088-0

Goering, H. H., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agriculture Handbook 379. United States Department of Agriculture, Washington, DC, USA.

Goldman, A., A. Genizi, A. Yulzari, and N. G. Seligman. 1987. Improving the reliability of the two-stage in vitro assay for ruminant feed digestibility by calibration against in vivo data from a wide range of sources. Anim. Feed Sci. Tech. 18(3):233-245. doi: 10.1016/0377-8401(87)90074-5

Mandebvu, P., J. W. West, R. N. Gates, and G. M. Hill. 1998. In vitro digestion kinetics of neutral detergent fiber extracted from Tifton 85 and Coastal bermudagrasses. Anim. Feed Sci. Tech. 73(3):263-269. doi: 10.1016/S0377-8401(98)00155-2

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43(1):99-109.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18(2):104-111. doi: 10.1111/j.1365-2494.1963.tb00335.x

Targeting Candidate Genes for Pregnancy in Beef Cows

Nicholas C. Kertz [1], Juliana Afonso [2], Paul W. Dyce [1], Priyanka Banerjee [1], and Wellison J. S. Diniz [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL 36849, USA;
[2] Embrapa Pecuária Sudeste, Rodovia Washington Luiz, Fazenda Canchim, São Carlos, SP 339, BR.
* Correspondence: wzd0027@auburn.edu

TAKE HOME MESSAGE

Pregnancy is one of the most important factors that affects the profitability and sustainability on any beef cattle operation. Here, we conducted a comprehensive eQTL (expression quantitative trait loci) analysis to unveil the genetic regulation of uterine luminal epithelial cells from beef cows that became pregnant or not. These eQTLs were found distributed across all chromosomes and overlapped reproduction-related QTLs (quantitative trait loci). Additionally, we identified transcription factors and non-coding RNAs regulating gene expression that were affected by eQTLs. Functional over-representation analysis highlighted pathways related to metabolism, immune response, and hormone signaling, emphasizing their importance in successful pregnancy establishment. While further research is needed to validate these findings and understand the mechanisms involved, our study provides insights into the genetic basis of reproductive processes in cattle.

SUMMARY

Despite the collective efforts to understand the complex regulation of reproductive traits, no causative genes and/or mutations have been reported yet. By combining genomics and transcriptomics data in one study, potential regulatory mechanisms could be discovered, providing opportunities to better understand the genetic factors controlling fertility. Herein, we identified regulatory variants from transcriptomic data associated with gene expression regulation in the uterine luminal epithelial cells of beef cows. We identified 4,676 cis and 7,682 trans eQTLs (expression quantitative trait loci) affecting the expression of 1,120 and 2,503 genes, respectively (FDR < 0.05). These variants affected the expression of transcription factor coding genes (71 cis and 193 trans eQTLs) and genes previously reported as differentially expressed between pregnant and non-pregnant cows. Functional over-representation analysis highlighted pathways related to metabolism, immune response, and hormone signaling (estrogen and GnRH) affected by eQTL-regulated genes (p-value < 0.01). Furthermore, eQTLs overlapped with QTL regions previously found for 13 reproduction-related traits (FDR < 0.05). Our study provides novel insights into the genetic basis of reproductive processes in cattle. Further investigation is required to pinpoint the mechanisms that control the expression of uterine genes in beef cattle.

1. INTRODUCTION

Female fertility and reproductive success are the main drivers of economic and production efficiency in cattle operations. While management practices and the adoption of new reproductive technologies have addressed the declining trend in fertility, its genetic progress has been limited (Fleming et al., 2019). There is significant genetic variation associated with fertility traits; however, their improvement has been challenged due to their complex and multi-factorial nature (Veerkamp and Beerda, 2007; Spencer, 2013). To address this challenge, efforts from breeding programs

worldwide have focused on identifying novel fertility phenotypes (Alexandre et al., 2023; Martins et al., 2022). Similarly, the emergence of omics technologies and genomic selection have opened new avenues to increase reproductive efficiency (Veerkamp and Beerda, 2007). Genome-wide association studies have been extensively used to understand the complex genetic basis of cow fertility. Although promising candidate genes have been reported, most variants identified have a small effect size on traits and do not code for proteins. Considering all the complex biological processes that impact fertility, further advances will rely on holistic approaches to identify key genomic factors and their causal relationships. Among these approaches, expression quantitative trait loci (eQTLs) effectively integrate genetic variants controlling gene expression genome-wide.

Here, **we used a genomics-transcriptomics approach to identify regulatory variants associated with pregnancy status and gene expression regulation in beef cows**. We first identified SNPs (single nucleotide polymorphisms) affecting the expression of genes from uterine luminal epithelial cells. Next, we investigated if the SNPs were associated with pregnancy outcomes, affecting differentially expressed genes (DEGs), and/or located in known QTL regions for reproduction traits. Lastly, we identified enriched pathways and biological processes that underlie the eQTL-modulated genes.

2. PROCEDURES

We used publicly available transcriptomic data collected from an experiment performed and published by (Martins et al., 2022). These authors investigated the uterine luminal epithelial profile of 43 multiparous Angus-Brahman cows. Briefly, uterine luminal epithelial cells were collected from estrous synchronized recipient cows three days before embryo transfer using a cytological brush. Pregnancy was diagnosed on day 30 after embryo transfer (25 pregnant – P; and 18 non-pregnant – NP) through transrectal ultrasonography.

RNA sequencing data was downloaded from the public database and put through a quality check. After quality check, we mapped the cleaned reads to the *Bos taurus* reference genome. Non or low-expressed genes were filtered out. The remaining genes, after filtering, were normalized for more accurate comparison between samples.

Genetic variants for each sample were identified and recorded from the transcriptomic data. All files were then merged and were jointly genotyped. Only biallelic SNPs were kept and sexual chromosomes were filtered out. A single file with 43 samples and all genotypes was generated and used for further analysis. In the integrative analysis with genomic and transcriptomic data, we included block as a covariate in the model, representing the two rounds of embryo transfer (Martins et al., 2022). We performed two separate tests for each gene-SNP to identify cis (SNPs affecting a nearby gene, within 1 MB) and trans eQTLs (SNPs within more than 1 Mb of the associated gene). The p-values were adjusted by computing the False Discovery Rate (FDR). eQTLs with an FDR < 0.05 were considered significant.

eQTLs can also affect gene expression by regulating transcription factors (TFs). Thus, we investigated if genes encoding TFs were affected by eQTLs. To this end, we downloaded 1,396 TFs from a TF database and overlapped them with the list of genes affected by cis and trans eQTLs. Additionally, we investigated if genes and TF-coding genes were differentially expressed in the uterine luminal epithelial cells. Therefore, we retrieved the list of differentially expressed genes associated with pregnancy outcomes reported by Martins et al. (2022). Lastly, we searched for

regions in the genome over-represented by DEGs. We performed a test within each window to determine if the number of genes was significantly over-represented (FDR < 0.05).

To investigate if eQTLs were overlapping with previous reproduction-related traits, known QTLs were downloaded from a database and compared to our eQTLs that we found. Significant results were taken when FDR < 0.05. To identify biological processes and pathways underlying genes affected by eQTLs, we performed a functional over-representation analysis (ORA) using InnateDB. We examined the genes regulated by cis and trans eQTLs separately. Common genes targeted by both cis and trans eQTLs were analyzed as well. Significant results were retrieved considering a p-value < 0.01. Furthermore, we analyzed the DEG list and identified pathways containing eQTL-affected genes.

3. RESULTS & DISCUSSION

From the 43 samples analyzed here, and after quality control, a total of 15,029 genes expressed and 203,404 unique variants in uterine epithelial cells were used for further analysis. Most variants were harbored in Chr 19, 18, and 3 (12,297, 11,733, and 11,547 SNPs, respectively). We also predicted the functional consequences of detected SNPs. The most severe consequences showed that variants were mainly harbored in genomic regions that did not directly code for proteins. Although most variants were classified as tolerated (70.6%), we found 12.6% that have a detrimental effect.

The eQTLs calculated from 15,029 genes and 10,879 unique variants were identified by a linear model. We identified 4,676 cis eQTLs (SNPs affecting a nearby gene) consisting of 3,989 unique SNPs affecting the expression of 1,120 genes. Chromosomes 23, 18, and 19 harbored most cis-acting SNPs (347, 339, and 295, respectively). The top five targeted genes included *ENSBTAG00000053827*, *ENSBTAG00000016148*, *AIFM3*, *TMEM69*, and *ENSBTAG00000026758*. Among the cis-regulated genes, we identified 71 coding TF genes affected by at least one SNP.

We then identified 14,680 trans eQTLs (SNPs affecting a faraway gene) corresponding to 7,682 unique SNPs affecting the expression of 2,503 genes. Chromosomes 19, 18, and 25 harbored most trans-acting SNPs (507, 496, and 457, respectively). The top five targeted genes included *ENSBTAG00000007816*, *PPP1R3D*, *ENSBTAG00000052527*, *ENSBTAG00000027075*, and *ENSBTAG00000048353*. The trans eQTLs affected the expression of 193 TFs. The top five TFs included *ZNF420*, *ENSBTAG00000015866*, *HES4*, *ZBTB32*, and *ZKSCAN2*. Lastly, we identified 4,297 eQTLs (consisting of 792 SNPs) acting as cis *and* trans eQTLs and affecting 466 genes.

We performed a SNP-trait association to investigate the effects of SNPs on pregnancy outcomes. Based on the association analysis, we did not find any significant SNP (FDR > 0.05). On the other hand, we identified 18 and nine significantly over-represented QTLs by cis and trans eQTLs, respectively (FDR < 0.05). The top three over-represented traits overlapped by cis eQTLs included non-return rate, luteal activity, and gestation length. From the overlapping trans eQTLs, in addition to non-return rate, top traits included interval to first estrus after calving and interval from first to last insemination (Figure 1).

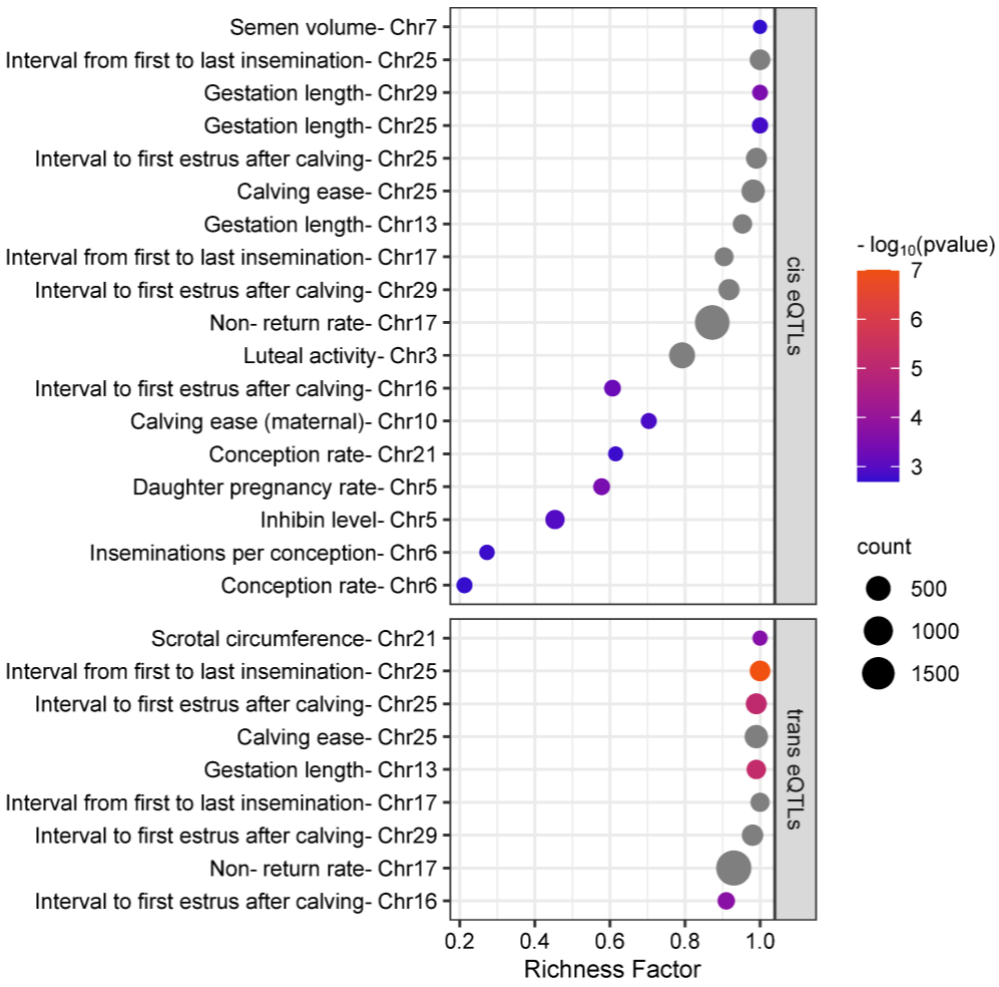


Figure 1. Over-represented reproduction-related traits (QTLs) overlapping cis and trans eQTLs identified from bovine uterine luminal epithelial cells. The x-axis represents the ratio between the number of observed QTLs and the expected number of that QTL, while the y-axis represents the over-represented traits. The color scale indicates the -log10 (p-value), and the dot size indicates the number of observed QTL for that trait. Only significant traits are shown (FDR < 0.05).

To focus on SNPs with potential functional roles in reproduction, we overlapped the 317 DEGs affecting pregnancy outcomes from Martins et al. (2022) with the genes reported here. We identified 32 and 56 DEGs affected by cis and trans eQTLs. Among them, the *ZNF470* and *SNAPC4* TFs were affected by cis, while *PPARG*, *AR*, and *KLF7* were affected by trans eQTLs. Additionally, 13 DEGs were affected by both cis and trans eQTLs. Interestingly, we found 13 regions over-represented by DEGs on chromosomes 18, 19, 22, and 25 (Table 1).

Finally, we performed a functional over-representation analysis to understand the biological processes and pathways affected by the genes targeted by eQTLs. The analysis of cis-targeted genes retrieved eight significant pathways, including amino acid metabolism and catabolism, and metabolism of lipids and lipo-proteins ($p < 0.01$). Secretions from the uterus, like amino acids and lipids, are critical to support embryo survival in early gestation.

Table 1. Enriched chromosomes by differentially expressed genes identified from bovine uterine luminal epithelial cells.

Enriched Chromosomes [a]	Number of Genes	Genes [b]
18	21	***ARHGEF1***, ***CLASRP***, *ENSBTAG00000023367, BICRA, LMTK3, PRR12, NAPSA, ENSBATG00000049577, CD37, ENSBATG00000045880,* ***ENSBTAG00000051367****, ENSBTAG00000053237, VSTM1, ZNF581,* ***KMT5C****, PTPRH,* ***PPP6R1****,* ***TMEM86B****, EPS8L1,* ***SMIM17****, ZNF470*
19	24	*SCARF1, P2RX5, PITPNM3, VMO1, CAMTA2, PLD2, TMEM95, DLG4, KCTD11, NLGN2, SOX15, TMEM88, KDM6B, PIK3R5, GAS7, RARA, ENSBTAG00000024839, STAT5A, CCR10, CNTNAP1, TMEM106A, HIGD1B, FMNL1, ENSBTAG00000055302*
22	5	*SYN2,* ***PPARG****,* ***CHST13****, MGLL, SLC41A3*
25	8	*LAT, CD19, GDPD3, DOC2A, ENSBTAG00000046752, TMEM265,* ***FBXL19****,* ***SEPTIN14***

[a] FDR< 0.05; b Genes affected by cis or trans eQTLs are in orange or red, respectively. Black bolded genes are targeted by both cis and trans eQTLs. Differentially expressed genes were retrieved from Martins et al. (2022).

Immune-related biological processes, affected by the same genes, included antigen processing and presentation of peptide antigen, and immune response. The maternal immune system must be regulated properly to also support early pregnancy success in the first month of gestation. Similarly, the *FOXM1* transcription factor network, ubiquitin-mediated proteolysis, and coregulation of androgen receptor activity were among the over-represented pathways by trans eQTLs affected genes. These pathways have strong roles in hormone signaling and secretion, especially for steroid hormones like estrogen. To support this further, we also identified 50 significant over-represented biological processes (BPs), including embryonic placenta development and response to estrogen.

Genes affected by both cis and trans eQTLs were involved in pathways such as amino acid metabolism and fatty acid degradation ($p < 0.01$). Furthermore, several more immune-related pathways among the over-represented BPs. Lastly, pathways over-represented by DEGs included the estrogen and GnRH signaling pathways and biological processes related to immune response.

Our study provides novel insights into the genetic basis of reproductive processes in cattle. Further investigation is required to pinpoint the mechanisms that control the expression of uterine genes in beef cattle.

Funding

This project was partially supported by the Agricultural Research Service, U.S. Department of Agriculture, under agreement no. 58-6010-1-005, by the Foundation for Food and Agriculture Research—grant no. FF-NIA19-0000000048, and the Alabama Agricultural Experiment Station—Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

4. References

Fleming, A., C.F. Baes, A.A.A. Martin, T.C.S. Chud, F. Malchiodi, L.F. Brito, F. Miglior. 2019. Symposium review: The choice and collection of new relevant phenotypes for fertility selection. J. Dairy Sci. 102:3722–3734. doi:10.3168/jds.2018-15470.

Veerkamp, R.F., B. Beerda. 2007. Genetics and genomics to improve fertility in high producing dairy cows. Theriogenology. 68:S266–S273. doi:10.1016/j.theriogenology.2007.04.034.

Spencer, T.E. 2013. Early pregnancy: Concepts, challenges, and potential solutions. Anim. Front. 3:48–55. doi:10.2527/af.2013-0033.

Alexandre, P.A., L.R. Porto-Neto, B.C. Hine, A.M. Samaraweera, A.I. Byrne, A.B. Ingham, C.J. Duff, A. Reverter. 2023. Development of female fertility indicator traits for the Angus HeiferSELECT genomic tool. In Proceedings of the Proc. Assoc. Advmt. Anim. Breed. Genet. 67–70.

Martins, T., M. Sponchiado, F.A.C.C. Silva, E. Estrada-Cortés, P.J. Hansen, F. Peñagaricano, M. Binelli. 2022. Progesterone-dependent and progesterone-independent modulation of luminal epithelial transcription to support pregnancy in cattle. Physiol. Genomics. 54:71–85. doi:10.1152/physiolgenomics.00108.2021.

Molecular Targets Differing at Weaning in Beef Heifers with Varying Reproductive Outcomes

Priyanka Banerjee [1], Wellison Diniz [1], Soren Rodning [1] and Paul Dyce [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL 36849, USA.
* Correspondence: pwd0003@auburn.edu

TAKE HOME MESSAGE

Sub-fertility in beef heifers leads to inefficient production and cost producers' money. While many studies have utilized omics technologies to study cattle fertility, very few have studied if molecular differences are detectable at the time of weaning using these powerful technologies. We are hoping to develop tools that can help producers make timely decisions on replacement heifers to minimize sunk input costs. Through these studies, we have identified unique molecular (mRNA and miRNA) profiles that differ based on heifer reproductive outcomes. This information has the potential to be used as a fertility-based heifer selection tool and may provide a better understanding of the causes of heifer sub-fertility.

SUMMARY

Alabama cow-calf producers must expend significant resources developing replacement heifers. The discovery of sub-fertile heifers following the first breeding season results in an inability to recover all the sunk costs. Omics technologies allow us to generate molecular profiles with the potential to identify biological differences that are not possible to detect using traditional phenotypic parameters. Therefore, we generated mRNA and miRNA profiles from peripheral white blood cells (PWBC), collected at weaning, from heifers that ultimately are high- or low-performing during their first breeding season. To do this, Angus-Simmental crossbred heifers were put through an estrous synchronization (ES) artificial insemination (AI) program, followed by three natural breeding opportunities. We collected blood at weaning (~238 days after birth), isolated the PWBC, and stored it until pregnancy checking. Following pregnancy checking, we categorized the heifers into high-performing (pregnant by AI) and low-performing (open) groups. We identified 16 miRNA and 92 mRNA candidates at different levels from low and high-performing heifers. More information on these candidate markers may lead to new therapeutic targets underlying fertility in beef heifers.

1. INTRODUCTION

Selecting replacement heifers is one of the most important decisions cow-calf producers make. Heifer sub-fertility is a major contributor to inefficiencies in beef cow-calf production. Management practices, including using traditional phenotypic parameters, have minimized the number of sub-fertile heifers, but a significant number of heifers still fail during their first breeding season (Dickinson et al., 2019). Due to the multifaceted nature of reproduction, many factors, including nutrition, health management, reproductive timing, and genetic background, play a role. For this reason, we approach the problem systematically by using global omics technologies. This approach has the potential to help us identify sub-fertile heifers and the potential genetic pathways responsible.

Traditional selection methods, including age, body condition score, reproductive tract score, and pelvic measurements, are effective at limiting the number of sub-fertile heifers developed as replacements by Alabama cow-calf producers. Our lab is interested in identifying the heifers that meet traditional methods but still fail to become pregnant during their first breeding season. Omics technologies have the potential to help us identify sub-fertility in heifers that outwardly do not have detectable problems.

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 *six* differentially expressed *genes* in peripheral white blood cells that were associated with pregnancy outcomes in beef heifers when collected at the time of AI (Dickinson et al., 2018). While promising and potentially valuable for detecting sub-fertile heifers, the timing is problematic from a producer's standpoint. It would be much more valuable to know the fertility potential of replacement heifers at an early stage of heifer development, such as "at weaning." In this study, **we aimed to compare mRNA and miRNA expression profiles generated from heifers with differing reproductive potentials at weaning.**

2. PROCEDURES

2.1. Animal Use

All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee (IACUC). Heifers included in this study were born and housed at a single Research and Extension Center 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), with access to soyhull/corn-gluten supplementation *ad libitum*, from weaning to pregnancy checking with free-choice ryegrass hay available. Angus-Simmental heifers underwent an estrus synchronization and fixed-time artificial insemination program (7-day CO-Synch + CIDR). Heifers were 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 during artificial insemination (AI). Fourteen days following AI, heifers were exposed to a proven fertile sire for three consecutive estrous cycles. All bulls used passed a standard BSE (Breeding Soundness Exam) with semen quality having < 10% abnormality, and all were cleared for any reproductive issues before the breeding season. All animals selected for transcript profiling had ideal body condition scores (5-6) and reproductive tract scores of ≥4.

2.2. Pregnancy Determination

Pregnancy was determined at 75 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. Seven heifers were selected for mRNA and miRNA profiling from each low and high-performing group.

2.3. Sample Processing and RNA Library Preparation

Blood samples were processed as previously described to isolate the PWBC from each selected heifer. RNA was isolated from the PWBC samples using Trizol reagent (Invitrogen)

following the manufacturer's protocol. Isolated RNA was further processed, and DNase treated using an RNA clean and concentrator kit (Zymo Research) and quality checked on Agilent Bioanalyzer and the RNA 6000 Nano kit (Agilent). For miRNA, the quantity of small RNA was assessed on Agilent Bioanalyzer using a Small RNA kit (Agilent). Only samples with RNA integrity values > 6 were used in this study. For mRNA, library preparation and sequencing were performed on the Nova-Seq platform at Discovery Life Sciences (Hudson Alpha Institute of Biotechnology, Huntsville, AL, USA). For miRNA, libraries were prepared using the protocols of the NEXTflex small RNA-seq Kit v3 (Perkin Elmer). Libraries were sequenced in the NextSeq 500 at Discovery Life Sciences (Hudson Alpha Institute of Biotechnology, Huntsville, AL, USA).

2.4. Data Analysis

The mRNA and miRNA datasets were subjected to an in-house bioinformatics workflow for statistical analysis. The differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMIs) were identified using DESeq2 v1.26.0. The contribution of the phenotypes, such as weaning age, weight, birth weight, and RIN values, to the total gene expression, was checked with ANOVA and principal component analysis prior to differential expression analysis. The pregnancy status (pregnant or non-pregnant) was considered for the design model used on DESeq2. The DEGs within a certain threshold ($padj \leq 0.05$) and DEMIs ($p\text{-}value \leq 0.05$) and absolute (log2 fold change) ≥ 0.5 were considered significant. DEGs and DEMIs were up or downregulated based on the log2 fold change direction of the differentially expressed genes in the low-performing heifer group.

3. RESULTS & DISCUSSION

The timely selection of fertile replacement heifers is critical to sustainable, efficient beef production. Improved understanding of systematic differences between high and low-performing heifers at an early age may help elucidate potential causes of fertility issues. Moreover, it may lead to the development of a reliable selection method for high performing heifers at the earliest. Therefore, we focused on the systemic PWBC population for mRNA and miRNA transcript analysis. The PWBC population is particularly attractive to develop biomarkers of fertility as it represents a relatively non-invasive tissue source. While previous findings in our lab supported using mRNA at the time of AI to discriminate between fertile and sub-fertile heifers, we wanted to determine if those or other differences were present earlier in the heifer's life. Detecting fertility potential earlier would allow Alabama beef producers to make earlier decisions, preventing the costly development of sub-fertile heifers.

In this study, we identified 92 DEGs between the high-performing and low-performing groups (Table 1). Although some identified genes were previously reported in the literature, we found novel candidates that need to be validated in a larger cohort.

Table 1. Top differentially expressed genes identified in high and low-performing heifers. The top genes up and downregulated are reported taking the low performing group as the reference.

Ensembl_ID	Gene Symbol	Status	log2 Fold Change	p-value
ENSBTAG00000039347	*CLEC4D*	Down	-2.21	7.37E-07
ENSBTAG00000018869	*IGSF6*	Down	-2.18	9.37E-09
ENSBTAG00000010328	*KCNK17*	Down	-2.09	1.78E-07
ENSBTAG00000004680	*SLC13A5*	Down	-2.05	6.51E-08
ENSBTAG00000015520	*SLC11A1*	Down	-1.94	1.87E-11
ENSBTAG00000005586	*GATM*	Up	1.63	1.01E-07
ENSBTAG00000020704	*RAMP3*	Up	1.75	1.43E-08
ENSBTAG00000030814	*TFF2*	Up	1.92	3.98E-07
ENSBTAG00000038844	*ANKRD35*	Up	2.10	2.01E-06
ENSBTAG00000011137	*MORN4*	Up	2.54	2.77E-11

There is evidence suggesting miRNAs play critical roles in many essential biological processes, including cell proliferation, apoptosis, and epigenetic regulation. Due to their important role in many regulatory functions, they have the potential to contribute to phenotypic variation, including fertility. In this study, we identified 16 DEMIs in the PWBC of beef heifers at weaning (Table 2).

Table 2. Differentially expressed miRNAs identified in high and low-performing heifers. The miRNAs up and downregulated are reported taking the low performing group as the reference.

miRNA	log2 Fold Change	Status	p-value
bta-miR-92b	-0.977404	Down	1.57E-05
bta-miR-2478	-0.800791	Down	0.00016075
bta-miR-874	-0.728053	Down	0.00081301
bta-miR-574	-0.808561	Down	0.00227153
bta-miR-450b	-1.462573	Down	0.04059786
bta-miR-2419-5p	-1.1765	Down	0.0047849
bta-let-7b	-0.729854	Down	0.0059119
bta-miR-6119-3p	-0.664261	Down	0.03458604
bta-miR-1260b	-0.635942	Down	0.00449088
bta-miR-1306	-0.602922	Down	0.03235006
bta-let-7a-5p	-0.50857	Down	0.0036584
bta-miR-1839	0.524835	Up	0.00445257
bta-miR-140	0.539173	Up	0.0395113
bta-miR-2332	1.231525	Up	0.03122294
bta-miR-677	1.377784	Up	0.00371643
bta-miR-1434-3p	2.948152	Up	0.01394242

Collectively, we identified 92 genes and 16 miRNAs at different levels in the PWBC of heifers with differing reproductive potentials at weaning. As miRNA targets alter the expression of mRNA targets, we compared the genes targeted by differentially expressed miRNAs to our

identified differentially expressed genes from mRNA profiles. Interestingly, we found five miRNAs (bta-miR-92b, bta-miR-2419-5p, bta-miR-1260b, bta-miR-1839, and bta-let-7a-5p) targeting differentially expressed genes identified in the PWBC of the same beef heifers. Among them, bta-miR-92b is the most significant miRNA downregulated in the low-performing group. In a study reported in cattle, miR-92b (downregulated expression) was associated with endometritis, an inflammatory response in the endometrium that causes reproductive disorder. Bta-miR-1260b was significantly downregulated in the low-performing group. In previous studies, miR-1260 has been associated with infertility in humans. We identified *CLEC4D* as predicted genes targeted by bta-miR-1260b. In previous studies, *CLEC4* was found to play important roles in immunity and homeostasis. The significance of these findings and the potential relationship between the miRNA and mRNA identified is a focus for ongoing research in the lab.

The current study identified molecular differences between reproductively low- and high-performing heifers. We investigated the miRNA and mRNA expression profiles in the PWBC of heifers at weaning. We found subsets of both miRNA and mRNA that were expressed at different levels depending on the heifer's reproductive potential. The timing of our project is attractive to cow-calf producers as it would allow them to make early decisions when selecting replacement heifers. Validation of these results in more samples and at different time points would help to establish a framework for future fertility prediction using molecular biomarkers that could be practical for on-farm use and improve the reproductive efficiency of cow-calf operations.

Acknowledgments

The authors would like to thank the staff at the Alabama Research and Extension Centers for their support.

Funding

This research was supported by the Alabama Cattlemen's Association, grant no. FF-NIA19-0000000048 from the Foundation for Food and Agriculture Research and by the Alabama Agricultural Experiment Station. The research was also supported by USDA—Agricultural Research Service—USDA-58-6010-1-005.

4. References

Dickinson, S. E., M. F. Elmore, L. Kriese-Anderson, J. B. Elmore, B. N. Walker, P. W. Dyce, S. P. Rodning, and F. H. Biase. 2019. Evaluation of age, weaning weight, body condition score, and reproductive tract score in pre-selected beef heifers relative to reproductive potential. J. Anim. Sci. Biotechnol. 10:18. doi:10.1186/s40104-019-0329-6.

Dickinson, S. E., B. A. Griffin, M. F. Elmore, L. Kriese-Anderson, J. B. Elmore, P. W. Dyce, S. P. Rodning, and F. H. Biase. 2018. Transcriptome profiles in peripheral white blood cells at the time of artificial insemination discriminate beef heifers with different fertility potential. BMC Genomics. 19:129. doi:10.1186/s12864-018-4505-4.

Developmental Expression of Myogenic Regulatory Genes in Bovine Myoblast Cultures and Fetal Skeletal Muscle

Donald R. Mulvaney [1,*], Gabriella F. Johnson [1], Russell L. Kelley [2], Gaston F. Alfaro [1], Mitchell T. Gore [3], Gerson N. Scheuermann [4], Sara P. Lino [1], and Charles H. Rahe [5]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA;
[2] Royal Canin Discover Team, Science Lead – PHNC, Service & Working Dog Research Manager, Lewisburg, OH, USA;
[3] Integrated DNA Technologies, Inc., Coralville, IO, USA;
[4] Núcleo Temático de Produção de Aves, Embrapa Suínos e Aves, Concórdia, Santa Catarina, BR;
[5] Texas State University, San Marcos, TX, USA.
* Correspondence: mulvadr@auburn.edu

TAKE HOME MESSAGE

The objectives of this study were to determine the effects of selected growth factors on the expression of the myogenic regulatory factors (MRFs) in cultured primary myoblasts and to determine the level of expression of the MRF genes, *Myf-5, MyoD, myogenin, and MRF4* in embryonic and fetal bovine skeletal muscle to reveal windows of developmental sensitivity. Myogenic sensitivity to growth factors plays an important role in skeletal muscle proliferation and differentiation during gestation. The findings bring light on temporal and growth factor induced myogenic regulatory factors (MRF) and MHC genes on cultures bovine myoblasts. In addition, this shows the MRF factors expression pattern in developing bovine fetal muscle and the development link among them.

SUMMARY

Little information on the role of skeletal muscle specific MRF in myogenic development is available in the bovine species. In this study, the expression of MRFs was determined first using bovine myoblasts cultured in two levels of fetal bovine serum (FBS) and different growth factor (GF) supplementation, and subsequently using bovine skeletal muscle from fetuses with ages 40 to 270 gestation-days. MRF expression in cultured myoblasts was affected by culture time, FBS level and growth factor supplementation. Developmental expression pattern observed *in vitro* was *Myf-5* and *MyoD* followed by *myogenin*. Insulin Growth Factor-1 (IGF-I) supplementation enhanced *Myf-5* and *MyoD* expression at 24 and 48h culture times, respectively. Fibroblast growth factor (FGF) supplementation decreased both MRFs' transcript abundance and myogenic differentiation. *Myogenin* expression paralleled differentiation. *MRF4* expression increased with crown-rump length (CRL), peaking in the 72.5 cm fetuses. *Myf-5*, *MyoD*, and *myogenin* showed bi-phasic expression, the age corresponding to highest levels differing for each gene: *Myf-5* at 14 and 42 cm, *MyoD* at 30 and 46 cm and *myogenin* at 42 and 56 cm CRL. This study showed that MRFs are developmentally regulated and are expression sensitive to GFs in the bovine species. The expression of MRFs in developing bovine fetal muscle provided insight into developmental windows in bovine muscle development.

1. INTRODUCTION

Phenotypic and genotypic variation in muscularity within the bovine species is extensive. Muscularity linear type traits have been documented as moderate to highly heritable in beef cattle and are known to be correlated to growth, carcass, and other economic traits. Fetal muscle development differs in cattle that have different postnatal growth patterns by as early as 100 days of gestation and differences in fetal muscle growth are related to differences in hyperplasia (Gore et al., 1994) and genotype (Gore et al., 1995). Therefore, increasing the number of muscle progenitor cells and myofibers can be an important yet unemployed strategy for increasing muscle mass. Considering that proliferative and differentiative processes of myogenic cells are sensitive to altered intrauterine environments (Kelley et al., 1995), shifts in the myogenic pathway during critical windows of embryonic development could lead to enhanced muscle mass in the postnatal animal. However, in domestic livestock species, especially cattle, little is known about the myogenic circuitry or windows of development that may be sensitive to perturbation.

The most important MRFs in myogenesis are in the basic helix-loop-helix (bHLH) gene family (*MyoD*, *myogenin*, *MRF4,* and *Myf-5*), which have been shown to play essential roles in coupling of cell-cycle and muscle differentiation pathways as well as quantity and quality of final meat products. Contributing to the complexity of the myogenic circuitry is a cornucopia of growth factors including the IGF, FGF and Transforming Growth Factor β(TGF-β) families. There is evidence that induced over expression of IGF-I stimulates myoblast proliferation and differentiation and leads to myofiber hypertrophy (Florini et al., 1996). Fibroblast growth factors and their receptors (FGFRs) are ligands and transmembrane proteins, respectively; and they compose an intricate family of signaling molecules that play essential roles in embryo development such as body axes formation, tissue patterning, morphogenesis, among others.

Transforming growth factor beta (TGF-β) is a signal cytokine that regulates different processes in cellular hemostasis, and its responses are based on the ligand concentration. On the one side, TGF-β depletion affects Smad signal kinetics, which are signal transducers for TGF-β superfamily (Zi et al., 2012). On the other side, TGF-β supplementation in cell cultures generally reduces cell proliferation and specifically, induces differentiation in some culture conditions. Knocking out of the GDF-8 gene (or myostatin, a member of the TGF-β super family) resulted in up to two-fold increase in muscle mass in mice (McPherron et al., 1997), mainly due to hyperplasia.

Further research is needed to understand myogenic metabolism or critical windows that would affect future muscle development. Additionally, despite the profound effect of the bHLH gene expression on myogenesis, virtually little is known about their pattern of expression in bovine species, and little information exists for the effects of the referred growth factors on bovine myoblast cells. Therefore, the objectives of this study were to determine the effects of selected growth factors on the expression of the myogenic regulatory factors (MRFs) in cultured primary myoblasts and to determine the level of expression of the MRF genes, *Myf-5, MyoD, myogenin, and MRF4* in embryonic and fetal bovine skeletal muscle to reveal windows of developmental sensitivity.

2. PROCEDURES

For this research, two cell culture studies and an *in vivo* study were performed. All muscle tissue samples were obtained from fetuses originating from pregnant beef cows harvested at S&C

Processors in Montgomery, Alabama. Workers at a dedicated workstation harvested fetal bovine serum. Researchers took the exsanguinated fetuses for measurement of crown-rump lengths (CRL) and tissue extraction.

The cell culture study I evaluated proliferative responses of the myoblasts to eight different culture media treatments. Myoblasts were plated with 6 wells being randomly assigned to each treatment. Prior to the addition of treatment medium, all cells were allowed to recover from the plating process for 12 hours. Following a 24-hour culture, treatment medium was removed, and cells were fixed and stained with the addition of 1 mL methanol containing 5 μg/mL Giesma dye. Cell proliferation was determined by the established procedure of counting the number of nuclei/well (Allen et al., 1984a) using light microscopy.

Regarding the cell culture study II, the effects of growth factors on selected cell characteristics were evaluated. To this end, myoblasts were isolated, propagated and seed plated in four different culture media. Cultures were used to determine proliferation, spontaneous fusion, inducible fusion and for gene expression analysis. Multinucleated cells were enumerated and divided by the total number of cells (mononucleated & multinucleated) to determine percentage fusion rate.

The *in vivo* study consisted of eight treatments based on different CRL: 9.2, 14.5, 30.3, 42.1, 46.3, 56.0, 68.2, 72.5 cm. The CRL groups were considered to reflect a wide range of developmental age, and each group was formed by five to eight fetuses. *Semitendinosus* (ST) muscles (left and right rear-legs) were excised from harvested the bovine fetuses, placed in a guanidine thiocyanate solution, homogenized, and stores at -80 ^{o}C.

RNA was isolated from cultured cells. Total RNA was directly spotted or transferred. Membranes were hybridized to cDNA probes for *myf-5*, *MyoD*, *myogenin*, and *MRF4*. Statistical analysis using GLM procedure of SAS, including orthogonal contrasts and Tukey's multi-range test.

3. RESULTS & DISCUSSION

After effective concentrations of the growth factors were identified, the system was used to assess MRF gene expression *in vitro* and enable comparison of these observations with *in vivo* analyses.

In Study I, myoblasts cultured in 10% FBS-DMEM (control, CTRL), 5% FBS-DMEM + IGF-I (10 or 100 ng/mL), and 5% FBS-DMEM + FGF (10 or 100 ng/mL) showed higher proliferation ($P < 0.05$) compared to those cultured in 5% FBS-DMEM or 5% FBS-DMEM + TGF- β (.01 or .1 ng/mL). No differences were observed between 5% FBS-DMEM and 5% FBS-DMEM + TFG-β (.01 or .1 ng/mL). Also, no differences were detected among the CTRL, 5% FBS-DMEM + IGF-I (10 or 100 ng/mL), and 5% FBS-DMEM + FGF (10 ng/mL). However, IGF-I (100 ng/mL) cultures approached significant proliferative enhancement ($P = 0.06$) when compared to CTRL cultures. Cells in 5% FBS-DMEM + FGF (100 ng/mL) had enhanced proliferation when compared to CTRL or 5% FBS-DMEM treatments ($P < 0.05$).

Results of the *in vitro* study II were similar to the findings from study I, now with significant proliferation differences ($P < 0.05$) between the CTRL and both 5% FBS-DMEM + IGF or FGF cultures at 24 or 72 h with regard to total *nuclei*. There was a decreased proliferative response with the 5% FBS-DMEM treatment relative to 10% FBS or GFs, and as expected, there was a significant increase in cell number over time (24 vs 72 h; $P < 0.01$). In this study, the results from IGF-I

supplemented primary bovine myoblast cultures demonstrated that IGF-I was effective in regulating proliferation, differentiation and MRF expression

In the *in vivo* study, the data were generated from fetuses ranging from 9.0 to 72.5 cm in CRL, spanning gestational ages from about mid first trimester (40 days) to late third trimester (270+ days) in the bovine species. These findings suggest that MRF expression in fetal bovine muscle is developmentally regulated and that their expression patterns are related. *Myf-5* expression in fetal bovine muscle was found to be expressed in a bi-phasic pattern with highest expression levels observed at 14.5 and 42 cm CRL. Following both peak points of *Myf-5* expression, a peak expression level of *MyoD* was observed, which was also bi-phasic with peak levels observed at 30 and 46 cm CRL. The following of *Myf-5* peak expression by *MyoD* peak expression suggest that the genes are developmentally linked in cattle as they have been shown to have redundant roles in other species, based on the knock-out mice experiments (Arnold and Braun, 2000). The relationship of *myogenin* to *MyoD* expression closely resembles the relationship of *MyoD* to *Myf-5* expression, except for the change in developmental window, with each peak expression period of *MyoD* being followed by a peak expression level of *myogenin*. This pattern of expression suggests that at least three of the MRFs, *Myf-5, MyoD* and *myogenin* are developmentally linked and have specific roles in myogenic regulation. *Myf-5* and *MyoD* may serve as co-activators of the pathway, their expression serving as the signal for myoblasts to prepare for cell cycle withdrawal. *Myf-5* expression appears to be upstream to the other MRFs. Its presence alone appears to be enough to drive normal myoblast specification, but not differentiation, since its activity is more related to chromatin modification than transcriptional activator (Conerly et al., 2016).

This study reports spontaneous, temporal and GF induced specific MRF and MHC genes in bovine myoblast cultures, and a temporal expression of MRFs *in vivo*. Responses to GFs suggest myogenic sensitivities may be used to influence proliferation and differentiation through altered intrauterine environment. Based on the pattern of MRF expression in developing bovine muscle, *Myf-5* may be a critical gene for regulation of myogenesis and signaling mechanism for expression of other MRFs. The data here presented serve as a foundation for investigations inter-connecting embryological and molecular events of bovine myogenesis. Future studies will expand MRF regulation in bovine and clarify critical molecular events that are responsible for regulating myogenic factors.

Overall and to reiterate, MRF expression in cultured myoblasts was affected by culture time, FBS level and growth factor supplementation for *Myf-5, MyoD*, and *myogenin*. Developmental expression pattern observed in vitro was *Myf-5* and *MyoD* followed by *myogenin*. Insulin Growth Factor-1 (IGF-I) supplementation enhanced *Myf-5* and *MyoD* expression at respective 24 and 48h culture times. Fibroblast growth factor (FGF) supplementation decreased both MRFs' transcript abundance and myogenic differentiation. *Myogenin* expression paralleled differentiation. *In vivo* results revealed a developmental expression pattern like the *in vitro* study. *MRF4* expression increased with crown-rump length (CRL), peaking in the 72.5cm fetuses. *Myf-5, MyoD*, and *myogenin* showed bi-phasic expression, the age corresponding to highest levels differing for each gene: *Myf-5* at 14 and 42 cm, *MyoD* at 30 and 46 cm and *myogenin* at 42 and 56 cm CRL. This study showed that MRFs are developmentally regulated and are expression sensitive to GFs in the bovine species. The expression of MRFs in developing bovine fetal muscle provided insight into developmental windows in bovine muscle development.

Funding

This research was funded by the Alabama Agricultural Experiment Station, grant number ALA00518.

4. References

Allen, R.E., Dodson, M.V., and Luiten, L.S. 1984. Regulation of skeletal muscle satellite cell proliferation by bovine pituitary fibroblast growth factor. Experimental Cell Research 152, 154–160.

Conerly, M.L., Yao, Z., Zhong, J.W., Groudine, M., and Tapscott, S.J. 2016. Distinct activities of Myf5 and MyoD indicate separate roles in skeletal muscle lineage specification and differentiation. Developmental Cell 36, 375–385.

Florini, J.R., Ewton, D.Z., and Roof, S.L. 1991. Insulin-like growth factor-I stimulates terminal myogenic differentiation by induction of myogenin gene expression. Molecular Endocrinology 5, 718–724.

Gore, M.T., Young, R.B., Claeys, M.C., Chromiak, J.A., Rahe, C.H., Marple, D.N., Hough, J.D., Griffin, J.L. and Mulvaney, D.R. 1994. Growth and development of bovine fetuses and neonates representing three genotypes. Journal of Animal Science 72, 2307–2318.

Gore, M.T., Young, R.B., Bird, C.R., Rahe, C.H., Marple, D.N., Griffin, J.L., and Mulvaney, D.R. 1995. Myosin heavy chain gene expression in bovine fetuses and neonates representing genotypes with contrasting patterns of growth. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine 209, 86–91.

Kelley, R.L., Jungst, S.B., Spencer, T.E., Owsley, W.F., Rahe, C.H., and Mulvaney, D.R. 1995. Maternal treatment with somatotropin alters embryonic development and early postnatal growth of pigs. Domestic Animal Endocrinology 12, 83–94.

Lea, R., Papalopulu, N., Amaya, E., and Dorey, K 2009. Temporal and spatial expression of FGF ligands and receptors during Xenopus development. Developmental Dynamics 238, 1467–1479.

McPherron, A.C., Lawler, A.M., and Lee, S.J. 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387, 83–90.

Zi, Z., Chapnick, D.A., and Liu, X. 2012. Dynamics of TGF-β/Smad Signaling. FEBS letters 586, 1921–1928.

Extended Storage of Beef Steaks Using Thermoforming Vacuum Packaging

Gabriela M. Bernardez-Morales [1], Brooks W. Nichols [1], Savannah L. Douglas [1], Aeriel D. Belk [1], Terry D. Brandebourg [1], Tristan M. Reyes [2], and Jason T. Sawyer [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA;
[2] Winpak Ltd., 100 Saulteaux Crescent, Winnipeg, Canada.
* Correspondence: jts0109@auburn.edu

TAKE HOME MESSAGE

Vacuum packaging film thickness will alter the oxygen transmission rate and subsequently influences the fresh surface color characteristics of beef steaks stored for extended periods (> 60 days). However, with improvements in vacuum packaging technologies fresh meat can appear redder through objective measurements. As expected, storage duration was a contributing factor that caused differences in purge loss, cook loss, and WBSF, but additional research is needed to further identify the mechanism of these changes. Future studies should be directed towards assessing the organoleptic traits of steaks stored for extended periods by eliciting consumer and trained panelist input on vacuum-packaged fresh beef steaks.

SUMMARY

Extended storage duration often results in negative quality attributes of fresh or frozen beef steaks. This study focused on evaluating the fresh and cooked meat quality of beef steaks stored using vacuum packaging for 63 days. Steaks 2.54 cm thick were packaged into one of three thermoforming films. Steaks placed in VPA were lighter (L*) and redder (a*) in surface color ($p < 0.05$) as the display period increased, whereas steaks packaged in VPB and VPC became darker. Yellowness values were greater ($p < 0.05$) in steaks using VPC film as the storage period increased. Results indicate that packaging film thickness can alter surface color of beef steaks previously frozen.

1. INTRODUCTION

Packaging is a fundamental part of the food industry that is used to create a product that is not only functional but also convenient for the consumer. Vacuum packaging for fresh meat throughout the various segments of the meat and food industry is increasingly popular in the United States and is under continual innovation (Roernik and Beebe, 2021). There is a need for centralized packaging methods to increase demand for greater quality and safety of meat cuts for the consumer (Velzen and Linnemann, 2008). Using vacuum packaging requires the placement of beef cuts into plastic bags or pouches and evacuating the atmosphere from within the package. Vacuum packaging can increase the storage life of meat and reduce retail losses, enhance distribution, and maintain meat quality (Rojas and Brewer, 2008).

Thermoforming packaging utilizes heat and pressure to mold a pouch inline using plastic film. After filling the pouch with fresh or cooked meat, a second layer is applied by voiding the atmosphere of the package and sealing with heat. Conventional packaging methods for retail use consisting of polyvinyl chloride (PVC) film and an expanded poly-styrene tray have declined in

use by almost 46% (Roernik and Beebe, 2021). Using permeable films for fresh meat, such as PVC, results in greater exposure of the meat surface to detrimental gases, such as oxygen. Plastic films used in thermoforming applications can limit the transmission rate of atmospheric gases to the meat surface and lengthen the stability of surface color on fresh meat products (Reyes, et al., 2022).

Meat quality greatly influences the marketability of beef, and research continues to highlight surface color as a factor that consumers continue to use in determining freshness and safety at the time of purchase (Rojas and Brewer, 2008). Consumers associate and prefer the bright cherry-red color of fresh beef, in contrast to a purplish-red color linked to vacuum-packaged meat as an indicator of wholesomeness (Faustman and Cassens, 1990). Therefore, **the objective of this study was to evaluate the influence of thermoforming vacuum packaging on the fresh and cooked characteristics of beef steaks after wet aging for 21 days.**

2. PROCEDURES

2.1. Muscle Fabrication

Beef boneless ribeye rolls (Institutional Meat Purchasing Specifications No. 112A) were purchased from a commercial meat processor and transported to the Auburn University Lambert-Powell Meat Laboratory and placed in refrigerated (2 °C ± 1.25 °C) storage (Model LEH0630, Larkin, Stone Mountain, GA, USA). Following 21 days of wet aging, ribeye rolls were removed from their individual vacuum packaging and fabricated. Ribeye rolls (n = 20) were cut to obtain 12 beef steaks 2.54 cm thick using a BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, Ohio, USA). Steaks from each ribeye roll were allocated randomly to one of three packaging treatments. On days 0, 7, 14, 21, 28, 35, and 42, steaks were removed from the refrigerated display case and measured for instrumental color.

2.2. Packaging Treatments

After cutting, beef steaks (n = 80/treatment) were allowed to bloom to simulate an industry steak cutting application for 30 min at 2 °C (± 1.25 °C). After bloom time, each steak was packaged individually into an assigned packaging film using a Variovac Optimus system (OL0924, Variovac, Zarrentin am Schaalsee, Germany). Beef steaks were placed in one of three different thermoforming packaging films: VPA, VPB, or VPC, and sealed with a non-forming layer (NFL) using commercial packaging guidelines (WINPAK, Winnipeg, MB, Canada). Packaging film components, oxygen transmission (OTR), and vapor transmission rates (VPR) of the packaging treatments are presented in Table 1.

Table 1. Vacuum packaging specifications for thermoforming films.

Trt.[3]	Components	OTR[1]	VPR[2]
VPA	250µ nylon/EVOH/enhanced polyethylene coextrusion	0.1 cc/sq. m/24 h	2.5 g/sq. m/24 h
VPB	250µ nylon/EVOH/enhanced polyethylene coextrusion	0.1 cc/sq. m/24 h	2.0 g/sq. m/24 h
VPC	125µ nylon/EVOH/enhanced/polyethylene coextrusion	0.6 cc/sq. m/24 h	4.9 g/sq. m/24 h
NFL[4]	110µ nylon/EVOH/enhanced/polyethylene coextrusion	0.7 cc/sq. m/24 h	6.0 g/sq. m/24 h

[1]OTR: Oxygen transmission rates. [2]VPR: Vapor transmission rates. [3]Packaging treatments defined as (VPA, VPB, VPC). [4] NFL (Non-forming film).

2.3. Simulated Storage Periods

Initially, packaged steaks were stored frozen in the absence of light at −20 °C (± 1.50 °C) for seven days to simulate frozen distribution from manufacturer to retailer at the Auburn University Lambert-Powell Meat Laboratory. Steaks were placed into cardboard boxes, sealed, and stored in a blast freezer (Model LHE6950, Larkin, Stone Mountain, GA, USA). After frozen dark storage, steaks were placed into a refrigerated, multi-deck, lighted display case Avantco (Model 178GDC49HCB, Turbo Air Inc., Long Beach, CA, USA) operating at 3.0 °C ± 1.5 °C. Thawed steaks were displayed under constant light for 42 days. The lighting within the retail case consisted of cool LED strips (TOM-600-12-v4- 3, Philips Xitanium 40 W–75 W, Korea) with a lighting intensity of 2297 lux (ILT10C, International Light Technologies, Peabody, MA, USA).

2.4. Instrumental Color

Instrumental color readings were measured with a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA) according to American Meat Science Association (AMSA) Meat Color Measurement Guidelines.

2.5. Statistical Analysis

The current study was conducted and analyzed as a completely randomized design. Data were analyzed using the GLIMMIX model procedures of SAS (version 9.2; SAS Inst. Cary, NC, USA). Treatment served as the fixed effect, and replication as the lone random effect for meat characteristics instrumental color. Least square means were generated, and significant ($\alpha = 0.05$) F-values were separated using a pair-wise t-test (PDIFF option).

3. RESULTS & DISCUSSION

Sub-primals in this experiment were wet aged for 21 days before being fabricated into steaks and displayed in multi-deck cases for 42 days. Limited research on extended storage (>60 days) of fresh beef is available in the literature; therefore, this was a novel opportunity to evaluate changes in fresh meat color over long storage periods. Anticipating large changes in myoglobin state in these long-stored meat products, instrumental color readings were used to measure the surface color changes between different pigment forms (Hunt; Sorheim, and Slinde, 2008). There was an interactive impact ($p < 0.05$) of the packaging method and storage period on the fresh surface color (Table 2).

From day 0 to 42, steaks packaged in VPC packaging film were darker ($p < 0.05$) than beef steaks packaged using VPA or VPB (Table 2). Regardless of packaging treatment, L* values initially increased ($p < 0.05$) through day 21. However, as the duration of storage increased, steaks in VPC became darker. Lightness is a characteristic of fresh meat as it blooms, and during lighted display and limited oxygen conditions, oxymyoglobin formation can be altered. An increase in L* using VPA and VPB films is likely the result of film thickness limiting oxygenation of myoglobin and mitochondria resulting in more light scattering on the surface of the steak. Similar changes in lightness were reported in previous studies using vacuum-packaged ground beef over a 14-day simulated display period. However, previous literature on the storage of vacuum-packaged whole-muscle cuts after extended wet aging and subsequent fresh storage is limited.

Table 2. Interactive impact of packaging method × day on surface color (L*, a*, b*) values during 42 days of refrigerated storage.

Day	Packaging Treatment[1]								
	Lightness (L*)			Redness (a*)			Yellowness (b*)		
	VPA	VPB	VPC	VPA	VPB	VPC	VPA	VPB	VPC
0	37.76^{f}	37.94^{f}	35.72^{g}	12.23^{jk}	11.72^{k}	13.09^{j}	9.04^{i}	8.92^{i}	9.99^{h}
7	42.25^{de}	45.53^{cde}	42.35^{cde}	20.72^{i}	21.49^{hi}	22.38^{g}	10.82^{g}	10.95^{g}	11.90^{f}
14	44.75^{ab}	46.29^{a}	45.10^{ab}	22.91^{fg}	22.30^{gh}	24.16^{e}	11.67^{f}	11.39^{fg}	12.98^{e}
21	46.23^{a}	45.78^{a}	44.79^{ab}	23.37^{ef}	23.58^{ef}	24.20^{e}	11.64^{f}	11.83^{f}	13.80^{d}
28	41.38^{bc}	41.03^{e}	38.44^{f}	28.15^{a}	27.91^{a}	27.52^{ab}	14.92^{bc}	15.09^{b}	17.79^{a}
35	42.79^{cd}	42.52^{cde}	38.34^{f}	27.33^{ab}	26.68^{bc}	25.24^{d}	14.25^{d}	14.25^{cd}	17.77^{a}
42	43.77^{bc}	42.97^{cd}	37.15^{fg}	26.40^{c}	25.83^{cd}	23.52^{ef}	13.76^{d}	13.70^{d}	17.64^{a}
SEM	0.577			0.315			0.243		

[1]Packaging treatments: VPA (250µ nylon/EVOH/enhanced polyethylene coextrusion), VPB (250µ nylon/EVOH/enhanced polyethylene coextrusion), and VPC (125µ nylon/EVOH/enhanced/polyethylene coextrusion). 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). $^{a–k}$ Mean values within a color measurement lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

An interaction between packaging film and storage day for objective redness values occurred (Table 2). Steaks were redder ($p < 0.05$) after day 35 of storage when using VPA and VPB consisting of greater barrier properties and a concentration of OMB on the sur- face of the steaks. However, there were some similarities ($p > 0.05$) among packaging films for redness values from day 0 to 28 of the storage period. Steaks packaged in VPC were less red ($p < 0.05$) and became more yellow ($p < 0.05$) as storage time increased. Historical values of surface color have been established. Current results enhance the foundation of surface color knowledge on vacuum packaged beef steaks.

Acknowledgments

The authors wish to express their sincerest appreciation to WINPAK for support in providing packaging films and the Variovac packaging machine technology to conduct shelf-life focused investigations.

Funding

Partial funding of this project was supported by Agricultural Research Service, U.S. Department of Agriculture under the Agreement No. 58-6010-1-005 and the Alabama Cattlemen's Association.

4. References

Faustman, C., R.G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: A Review. J. Muscle Foods, 1, 217-243.

Hunt, M.C., Sorheim, O.,Slinde, E. 2008. Color and Heat Denaturation of Myoglobin Forms in Ground Beef.J. Food Sci. 2008, 64, 847–851.

Reyes, T.M., M.P. Wagoner, V.E. Zorn, M.M. Coursen, B.S. Wilborn, T. Bonner, T.D. Brandebourg, S.P. Rodning, J.T. Sawyer. 2022. Vacuum packaging can extend fresh color characteristics of beef steaks during simulated display conditions. Foods, 11, 520.

Roernik, A.M., S. Beebe. 2021. Cryovac Brand National Meat Case Study – A snapshot of the meat case today. In Sealed Air; North American Meat Institute: Charlotte, NC USA.

Rojas, M.C., M.S. Brewer. 2008. Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. J. Food Qual. 31, 173-188

Thoden van Velzen, E. U., A. R. Linnemann. 2008. Modified atmosphere packaging of fresh meats-Sudden partial adaptation caused an increase in sustainability of Dutch supply chains of fresh meats. Packag. Technol. Sci. 21, 37-46.

Influence of Sous Vide Cooking on Ground Beef Patties

Savannah L. Douglas [1], Gabriela M. Bernardez-Morales [1], Brooks W. Nichols [1], Aeriel D. Belk [1], Tristan M. Reyes [2], Jase J. Ball [1], and Jason T. Sawyer [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA;
[2] Winpak Ltd., 100 Saulteaux Crescent, Winnipeg, Canada.
* Correspondence: jts0109@auburn.edu

TAKE HOME MESSAGE

Meat cookery can alter the subjective and objective quality factors of meat products. Applying new technologies such as sous vide cooking may provide a deeper understanding on influencers of meat cookery. Ground beef patties were evaluated and sous vide cooking time does change the objective parameters of cooked patties linked to sensory taste traits.

SUMMARY

With rising consumer demand for fast-food options, quick-service restaurants are constantly developing new menu items to attract consumers. Sous vide cookery has become popular for the in-home and fine dining consumer but has not been considered the first cooking option for quick service applications. Therefore, ground beef patties were manufactured to measure the influence of sous vide cooking time on the patty characteristics of moisture, color, and objective tenderness. Patties were randomly assigned a sous vide cooking time of 30, 60, or 90 min and then grilled to an internal temperature of 71.1 °C. Patties sous vide cooked for 30 min exhibited the greatest ($p < 0.05$) cook loss, Allo–Kramer Shear Force (AKSF) and were darker (L*) than patties sous vide cooked for 60 or 90 min. Additionally, neither internal redness, calculated spectral values of chroma, hue angle, or red-to-brown differed ($p > 0.05$) regardless of sous vide cooking time. Sous vide cooking duration prior to grilling the ground beef patties altered the moisture, color, and objective texture characteristics of ground beef patties.

1. INTRODUCTION

Evaluating the cookery methods of meat products is necessary to understand the changes that occur in meat quality because of cooking, as differences caused by cookery may alter consumer perceptions. Very little is known about the influence of sous vide cooking on ground meat. However, within the United States, the fast-food industry is very popular, and quick-service restaurants often face challenges to providing new items for consumers using ground meat. During the pandemic, a fast-food restaurant focused on menu creativity to enhance consumer demand for dining that incorporated sous vide cooking methods (Restaurant Business, 2023). Sous vide is a cooking technique using low temperatures and longer cooking times and has become popular in commercial applications (Chotigavin et al., 2023). It is estimated that by 2028, the sous vide market will reach USD 10.2 billion with an annualized growth rate of 5.3% (sous vide machine market, 2023). Unlike traditional cooking methods, sous vide cooks the product in a controlled temperature water bath environment inside a heat-stable vacuum-sealed pouch (Schellekens, 1996). In addition, sous vide allows professional chefs or home cooks to use cuts of meats

considered undesirable and turn them into something that becomes a moist, tender, and flavorful dish (Baldwin, 2012). In the fast-food industry, sous vide allows restaurants to hold meat at cooked temperatures, reducing cooking times. Sous vide cooking has several benefits: creating a uniform and desired texture, retaining a desirable color, preventing moisture or flavor losses, and prohibiting cross-contamination in storage. Although sous vide can provide many desirable traits for consumers, it does have the potential to reduce consumer acceptability based on appearance.

Recommendations for using sous vide cookery are often based on variations in the objective tenderness ratings of lower-valued whole muscle cuts of beef or pork. However, little is known about the impact that sous vide has on ground meat characteristics. Sous vide products can exhibit the desired palatability and have been reported to extend shelf life by inhibiting the growth of bacteria and lipid oxidation (Schellekens, 1996). Using sous vide in a way to increase storage duration is possible. Heating fresh meat products at low temperatures for long times can reduce the quantity of vegetative cells (Schellekens, 1996). Reducing microbial organisms in any quantity is well-documented to support the enhanced storing of meat products using a variety of aerobic or anaerobic packaging materials. Additional factors in the combination of sous vide cooking may include water activity, storage temperature, packaging materials, and irradiation to enhance the storage ability of meat and food products to obtain longer storage durations (Schellekens, 1996). Currently, there are no specific guidelines or research practices for using sous vide as a cooking method for ground meat. Therefore, **the objective of this study was to evaluate the influence of sous vide cooking time on the cooked characteristics of color, moisture, and objective texture in ground beef patties.**

2. PROCEDURES

2.1. Raw Materials

Six crossbred (Brangus) cattle were harvested by the Auburn University Lambert- Powell Meat Laboratory (Auburn, AL, USA). Cattle were harvested using commercial meat processing techniques for USDA humane slaughter. Carcasses were chilled at 2 °C (1.25 °C) for 24 h prior to fabrication. After chilling, carcasses were fabricated into wholesale subprimals using fresh beef USDA institutional meat purchase specifications (IMPS). For this study fresh beef (n = 12) shoulder clods (IMPS 114) and (n = 12) chuck eye rolls (IMPS 116D) were removed, and subcutaneous fat was trimmed to not exceed 0.635 cm thick. Combined subprimals totaling 140 kg were coarse ground once through a 9.525 mm plate (SPECO 400, Shiller Park, IL, USA) using a commercial meat grinder (Model AFMG-48, The Biro Manufacturing Company, Marblehead, OH, USA). Coarse ground beef was then ground once through a 3.18 mm plate (SPECO 400, Shiller Park, IL, USA) with a bone eliminator attached (SPECO 400, Shiller Park, IL, USA). After final grinding, the ground beef was formed into 151 g patties using a food portioning machine (Hollymatic Corporation Model 54, Countryside, IL, USA). Formed patties were placed on trays lined with freezer paper (Kold-Lok KL18, Dixie Consumer Products LLC, Atlanta, GA, USA) and crust-frozen for 45 min at 22.2 °C to facilitate packaging. Crust frozen ground beef patties were packaged individually into thermoforming vacuum packaging using a Reiser roll-stock packaging machine (Optimus OL0924, Variovac, Zarrentin, Germany). A total of 225 patties were portioned, packaged, and randomly assigned to a time interval of 30, 60, or 90 min (n = 75/sous vide duration). Patties were sealed in a forming layer with an oxygen transmission rate of 0.8 cc/sq. m/24 h, and a non-

forming layer with an oxygen transmission rate of 1.0 cc/sq. m/24 h (WINPAK Ltd., Winipeg, MB, Canada).

2.2. Cookery Method and Cook Time

At the time of cooking, patties were thawed for 12 h at 2 °C. Using a circulating temperature control sous vide heating element (Model AN400-US00, Anova Culinary, San Francisco, CA, USA) in a 65-qt water bath, the water was heated until reaching 60 °C. Vacuum-packaged patties were placed into the water bath and submerged. Once the cooking time of 30, 60, or 90 min was complete, patties were removed from the water bath, packaging was removed, and the patty was blotted dry with a paper towel and weighed on a calibrated scale (Model PB3002-S, Mettler Toledo, Columbus, OH, USA). A commercial grill pre-heated to 148.8 °C was used to mimic an industry grilling method (Model XPE12, Garland Commercial Ranges, Mississauga, ON, Canada) following the use of sous vide. Each patty was cooked until reaching an internal temperature of 71 °C using a thermometer (Therma K-Plus, American Fork, UT, USA). The time each patty was grilled was recorded in seconds. After removal from the clamshell grill, patties were weighed again on the calibrated scale to obtain final cooked weights.

2.3. Instrumental Color

Once cooled, patties were sliced horizontally through the geometric center of the patty and scanned for internal cooked color using a HunterLab MiniScan EZ colorimeter (Model 45/0 LAV, Hunter Associates Laboratory Inc., Reston, WV, USA). Before data collection, the colorimeter was calibrated using a black and white tile per the manufacturer guidelines for accuracy. Instrumental color values were determined from the mean of three readings on the internal surface of each ground beef patty using illuminant A, with an aperture of 31.8 mm, and a 10° observer to measure the lightness (L*), redness (a*) and yellowness (b*) of each ground beef patty. In addition, hue angle was calculated using the following: $\tan - 1\ (b^*/a^*)$, 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.

2.4. Allo-Kramer Shear Force

Using the 5-Blade-Allo-Kramer attachment (AKSF) with a texture analyzer (Model TA-XT Icon, Texture Technologies Corp., New York, NY, USA) the objective tenderness of each patty was measured (n = 225; 75/treatment). Patties were cooked and cooled according to the procedures described above. After cooling to room temperature 23.3 °C, at a load cell of 500 N and a speed of 3 mm/s, each patty was cut into a 6 × 9 cm square. Each sample was sheared once, and the maximum peak force recorded during analysis was reported in newtons (N) of shear force.

2.5. Statistical Analysis

Data was analyzed using the GLIMMIX model procedure of SAS (version 9.2; SAS Inst., Cary, NC, USA). Least squares means were computed for all variables. When significant ($p \leq 0.05$) F-values were observed, least squares means were separated using pair-wise t-tests (PDIFF option). This experiment had a completely randomized design with each experimental unit (patty) assigned to treatment group times of 30, 60, 90 min at random.

3. RESULTS & DISCUSSION

A concern with using sous vide is the lack of Maillard reaction that occurs on the surface of the meat products. To simulate a commercial cooking process after sous vide, patties were cooked to their specific treatment group time (30, 60, 90 min), then patties were transferred to a clamshell grill to reach an internal temperature of 71.1 °C. The cook time after sous vide was recorded in seconds (Table 1). Expectedly, the cook time on the clamshell grill did not decrease with the increase of time in the water bath as the patties were all cooked at the same temperature before grilling occurred ($p = 0.9868$).

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

	TRT [1]				
	30MIN	**60MIN**	**90MIN**	**SEM ***	**p-Value**
Cook loss (%)	21.23 [a]	17.33 [b]	15.35 [c]	0.469	0.0001
Grill time (s)	38.00	37.50	37.50	0.096	0.9868
AKSF (N)	212.6 [a]	199.6 [b]	183.6 [c]	0.028	0.0001
Lightness (L*) [2]	58.08 [b]	60.41 [a]	59.98 [a]	0.327	0.0001
Redness (a*) [2]	15.93	16.71	16.77	0.417	0.2878
Yellowness (b*)[2]	18.97 [b]	20.08 [a]	19.97 [a]	0.201	0.0001
Chroma (C*) [3]	50.88	38.00	24.96	0.792	0.7885
Hue Angle (°) 4	50.71	38.00	26.21	3.567	0.9869
RTB [5]	50.35 [b]	38.50 [a]	26.14 [a]	0.548	0.0414

[1]Packaging treatments define sous vide cooking duration for patties. [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). a–c Mean values within a row lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

Since meat consists of 75% water, there can be a significant change in the overall weight of the product after cooking, especially when using a two-step cooking method such as sous vide. Measurements were calculated based on the total amount of weight lost after sous vide and grilling. The calculated loss of moisture in the current study does not represent the moisture lost only during water bath cooking, nor the moisture loss after only grilling, cook loss was recorded as the combined moisture loss of sous vide and grilling. The cook loss after grilling decreased with the increased initial cooking time of sous vide. The cook loss in the 30 min test group was greater than the cook loss for the 60 min and 90 min ($p = 0.0001$) test groups (Table 1). The decreasing moisture loss suggests that longer cooking times of a ground meat patty in a water bath may alter the total moisture loss that occurs during the final cooking method.

Even after being cooked to 71.1 °C on the clamshell grill, each patty still appeared reddish/pink on the internal surface. This could pose an issue in the industry for consumers that will not eat a burger perceived to be "undercooked". The b* values represent the change in yellowness. Samples exhibited higher values in the 60 and 90 min sous vide treatment time ($p = 0.0846$) when comparing the 30 min treatment time to 60 min ($p = 0.0001$) and 90 min ($p = 0.0006$). Overall, L* values were significant for this study ($p = 0.0001$). The L* values represent the amount of light detected and values displayed; the 60 min treatment group had the highest value compared

to the 30 min (p = 0.0001) and the 90 min treatment groups (p = 0.3510). The a* values indicate the amount of redness with a larger value indicating a redder color. This often has a greater appeal to consumers when purchasing fresh beef but not necessarily for cooked beef. With the increase in sous vide cooking time, numerically, the a* values of the internal surface of the cooked patties increased. Regardless, the objective color measurement of redness (a*) was not different across the sous vide cooking times suggesting that the degree of doneness as perceived by consumers would not be altered for sous vide patties (Table 1).

In addition, spectral values were calculated from the CIE measurement of L*, a*, and b*. Color measurements were taken from cooked meat patties at three different locations after slicing patties through the geometric center. There was no interaction between any of the cooking test times and hue angle or chroma ($p > 0.05$) suggesting that sous vide does not enhance or negatively affect the internal cooked color of the patties. However, there was a significant difference in the reflectance ratio of 630 nm:580 nm and this value represents a change in color from red to brown. The 60 min patties were found to have a higher RTB value indicating that the 60 min test group had the largest amount of change from red to brown. This is evidence that the sous vide cook time does not influence the vividness or the change in color of the ground beef patties. It does, however, exhibit the change in color from red to brown.

One of the perceived benefits to the sous vide cooking method is the potential for improving the overall tenderness of a cooked product. Objective tenderness was measured via Allo–Kramer Shear Force (AKSF) and reported in newtons (N) of force. Sheaf force values were greatest in patties sous vide cooked for 30 min compared to patties cooked for 60 min (p = 0.0081) or patties cooked for 90 min ($p < 0.0001$). The least amount of shear force was recorded on the patties sous vide cooked for 90 min (Table 1). As sous vide cooking time increased, the amount of Allo–Kramer force required to shear through the patty declined.

Sous vide cooking time does alter the quality attributes of ground beef patties that include objective texture and internal cooked color. Patties with a longer cooking time exhibited less change in cooked color as the 90 min treatment group exhibited the highest redness value. However, cooking patties in sous vide for longer than 30 min had no effect on the internal cooked color. Additional research is needed to further identify the sensory taste impacts on ground beef using sous vide as the primary cooking method compared to other cookery methods. Furthermore, the evaluation of moisture losses using a two- step cooking method, such as sous vide and grilling ground meat patties, is necessary to improve our foundational knowledge of meat cookery.

Acknowledgments

The authors wish to express their sincerest appreciation to WINPAK for support in providing packaging films and the Variovac packaging machine technology to conduct shelf-life focused investigations.

Funding

Partial funding of this project was supported by Agricultural Research Service, U.S. Department of Agriculture under the Agreement No. 58-6010-1-005 and the Alabama Cattlemen's Association.

4. References

Baldwin, D. Sous vide cooking: A review. Int. J. Gastron. Food Sci. 2012, 1, 15–30.

Chotigavin, N.; Kerr, W.L.; Klaypradit, W.; Kerdpiboon, S. Novel sous-vide pressure technique affecting properties of local beef muscle. LWT Food Sci. Technol. 2023, 175, 114439.

Faustman, C., R.G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: A Review. J. Muscle Foods, 1, 217-243.

Restaurant Business. Available online: https://www.restaurantbusinessonline.com/food/how-arbys-spent-two-years- perfecting-its-first-burger (accessed on 15 March 2023).

Sous Vide Machine Market. Available online: https://www.adroitmarketresearch.com/industry-reports/sous-vide-machine- market (accessed on 26 July 2023).

Schellekens, M. New research issues in sous-vide cooking. Trends Food Sci. Technol. 1996, 7, 256–262.

Protected Benzoic Acid Supplementation Marginally Increases Growth Performance in the Starter Pigs

Alex Outlaw [1], Alexandra Gachman [1], and Marko Rudar [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA.
* Correspondence: mzr0097@auburn.edu

TAKE HOME MESSAGE

Nutritional, environmental, pathogenic, and social stressors at weaning contribute to post-weaning growth lag in pigs. Failure to optimally gain body weight increases feed costs in the nursery and days to market, increasing costs to producers. The objective of this work was to determine if supplementing protected benzoic acid in the diet will increase growth performance, digestibility, and gut architecture compared to free benzoic acid and an antibiotic growth promoter in starter pigs. We found that protected benzoic acid marginally increased average daily gain and average daily feed intake in starter pigs.

SUMMARY

At weaning, nutritional, environmental, pathogenic, and social stressors contribute to the post-weaning growth lag. Organic acids, including benzoic acid, are a common feed additive and alternative to antibiotic growth promoters. The objective of this study is to determine if protected benzoic acid increases growth performance, nutrient digestibility, and gut architecture in newly weaned pigs. A total of 192 pigs were assigned to one of four treatment diets (eight pens per diet and six pigs per pen): 1) negative control (NC); 2) free benzoic acid (BZA, 0.6%); 3) protected benzoic acid (BC50, 0.2%); and 4) antibiotic growth promoter (AGP; carbadox, 50 ppm). Treatment diets were fed over two periods (period 1, 7 d; period 2, 14 d) for a total of 21 d. Pen weights and feed intake were measured to determine average daily gain (ADG) and average daily feed intake (ADFI). At the end of each period, fecal samples were collected to measure apparent total tract nutrient digestibility and one pig per pen was euthanized to measure gut morphometry of jejunum and ileum. The AGP group consistently had the greatest ADG, ADFI, and nutrient digestibility among groups. The ADG and ADFI in the BC50 group were intermediate between the NC and BZA groups and the AGP group in period 2. Gut architecture was not different among groups. In summary, protected benzoic acid marginally increased growth performance comparable to the AGP group in starter pigs, suggesting potential as an antibiotic alternative.

1. INTRODUCTION

Weaning pigs from the sow is one of the most stressful events to occur in pig production. During this time, pigs will commonly exhibit low and inconsistent feed intake, altered intestinal architecture, episodes of diarrhea, and increases in morbidity and mortality that all contribute to reduced starter pig growth performance (Moeser et al., 2017; Pluske et al., 2018). This post-weaning growth lag of pigs either losing or maintaining weight in the first 7 to 10 days after weaning necessitates approximately 10 additional days to reach market weight (Collins et al., 2017). Despite improvements in technology and management strategies in the nursery, the post-weaning growth check continues to burden pork producers.

The use of antibiotic growth promotors in nursery diets for starter pigs is highly effective at limiting weaning-induced reductions in growth performance. However, the subtherapeutic use of antibiotics as growth promotants is currently banned in the U.S., Canada, and the E.U. Moreover, the E.U. has phased out zinc oxide due to concerns about environmental zinc accumulation and development of antimicrobial resistance. Thus, other nutritional strategies are essential to address post-weaning growth lag in pigs. Organic acids can be used as antibiotic alternatives and are thought to promote pig performance by increasing gastric acidification and protein digestion. Among organic acids, benzoic acid is reported to increase growth performance, nutrient digestibility, and improve gut health (Choi et al., 2023; Kiarie et al., 2018). The absorption of most organic acids occurs in the stomach and proximal small intestine; however, delaying the release of benzoic acid allows it to exert its effects further in the gut. **The objective of this study is to determine if protected benzoic acid increases growth performance, nutrient digestibility, and gut architecture in newly weaned pigs.**

2. PROCEDURES

Starter pigs (Yorkshire × Duroc and Yorkshire × Hampshire cross) were obtained from the Swine Research and Education Center at Auburn University. A total of 192 pigs (initial body weight, 19.2 ± 2.3 lb) were weaned at 28 ± 1 day of age. Pigs were divided into one of four treatment diets (n = 8 pens per diet; 6 pigs per pen): 1) negative control (NC); 2) free benzoic acid (BZA, 0.6%); 3) protected benzoic acid (Benzocal-50, Guangzhou Insighter Biotechnology, Co. Ltd., Guangzhou, China; BC50, 0.2%); and 4) antibiotic growth promoter (AGP; Carbadox, 50 ppm). Treatment diets were fed over two periods (period 1, 7 d; period 2, 14 d) for a total of 21 d. The experimental diets met or exceeded estimated animal requirements for all nutrients for 22 to 55 lb pigs (NRC, 2012). Each treatment diet was prepared from a common basal diet per period 1 (metabolizable energy, 3427 kcal/kg; crude protein, 21.4%; standardized ileal digestible lysine, 1.40%) and period 2 (metabolizable energy, 3372 kcal/kg; crude protein, 21.6%; standardized ileal digestible lysine, 1.28%). Each additive was mixed with ground corn up to the calculated content of corn in each diet and the additive-corn mix (or corn only for the NC diets) were blended with the corresponding amount of each basal diet.

Individual pig weights were measured at the start of the study and every week after for 3 weeks. Feed addition and removal due to spoilage was recorded daily and feed remaining at the end of each week was weighed and recorded weekly. Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain-to-feed ratio, G:F) was calculated per pen and reported over two periods period 1 (day 1 to day 7), period two (day 8 to day 21), and overall (both periods one and two).

At the end of each period, one pig closest to the pen mean was euthanized to collect jejunum and ileum tissue for gut morphometry. Gut morphometric analysis was performed on formalin-fixed jejunum and ileum samples. Villus height (VH) and crypt depth (CD) were determined in approximately ten well oriented, intact, and symmetrical villi and adjacent crypts per jejunum and ileum using QuPath image analysis software (version 0.4.3).

Titanium dioxide was included in each diet as an indigestible marker to determine apparent total tract digestibility (ATTD) of organic matter (OM), gross energy (GE), and crude protein (CP). Fresh fecal samples were collected from at least three pigs per pen during the last 2 days of each

period, pooled, and frozen at -20 °C before analysis. Fecal samples were mixed, dried, and ground until titanium analysis.

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 pen within block was considered a random effect. Data are presented as least-squares means ± standard error of the mean. Differences among diets were determined with a Tukey honest significant difference (HSD) test. Results are considered significant at $P < 0.05$ and a trend at $P < 0.10$.

3. RESULTS & DISCUSSION

The AGP group had greater ADG and ADFI than the NC, BZA, and BC50 groups (Table 1; $P < 0.05$) in period 1. Feed efficiency was similar among all groups in period 1. Period 2 ADG was greatest in the AGP group, intermediate in the BC50 group, and lowest in the BZA and NC groups ($P < 0.05$), whereas period 2 ADFI was greatest in the AGP group, intermediate in BZA and BC50 groups, and lowest in the NC group ($P < 0.01$). Despite greater ADG in the AGP group in period 2, it was not significantly different from the BC50 group ($P = 0.16$). Feed efficiency was similar among AGP, BC50, and NC groups but was significantly lower in the BZA group in period 2 ($P < 0.05$). There was no immediate effect of supplementing free or protected benzoic acid on growth performance in the first week after weaning. Free benzoic acid supplementation did not improve growth performance in this study compared to the NC group, whereas it appeared that the addition of protected benzoic acid marginally increased growth performance during period 2 compared to both the NC and BZA groups.

Table 1. Growth performance of pigs fed starter diets containing no additive (NC), benzoic acid (BZA, 0.60%), Benzocal-50 (BC50, 0.20%), and antibiotic growth promoter (AGP; carbadox, 50 ppm).

	Treatment					
Item	NC	BZA	BC50	AGP	SEM[1]	*P*-value
Initial BW, lb	18.81	20.08	18.85	19.20	0.90	0.74
Day 7 BW, lb	21.54	22.95	21.76	23.10	0.90	0.52
Day 21 BW, lb	37.79	39.26	39.20	42.20	1.34	0.15
Period 1						
ADG, lb/d	0.39[b]	0.39[b]	0.41[b]	0.56[a]	0.04	0.01
ADFI, lb/d	0.65[b]	0.68[b]	0.66[b]	0.82[a]	0.05	0.01
G:F, lb/lb	0.60	0.58	0.60	0.68	0.01	0.33
Period 2						
ADG, lb/d	1.18[b]	1.18[b]	1.24[ab]	1.37[a]	0.07	0.01
ADFI, lb/d	1.77[b]	1.89[ab]	1.87[ab]	2.07[a]	0.06	0.01
G:F, lb/lb	0.67[a]	0.62[b]	0.66[a]	0.66[a]	0.01	0.04

[1] Maximum value for the standard error of the mean. [a, b] Means without a common superscript differ after Tukey HSD test, $P < 0.05$.

The ATTD of OM and GE was highest in the AGP group, intermediate in BZA group, and lowest in NC and BC50 groups (Table 2; $P < 0.01$). The ATTD of CP was greatest in the AGP group, intermediate in the NC and BZA groups, and lowest in the BC50 group. The AGP group had the greatest ATTD of OM, GE, and CP throughout the study supporting the observed increase growth

performance compared to the NC group. The BZA group had greater ATTD in OM, GE, and CP than the BC50 group. Less stomach acidification from both the delayed release of protected benzoic acid and lower absolute amount of benzoic acid could explain the lower observed ATTD coefficients, especially for CP, in the BC50 group. Despite this difference, however, greater nutrient ATTD did not translate to increased growth performance in the BZA group.

Table 2. Apparent total tract digestibility (ATTD) of organic matter (OM), gross energy (GE), and crude protein (CP) in pigs fed starter diets containing no additive (NC), benzoic acid (BZA, 0.60%), Benzocal-50 (BC50, 0.20%), and antibiotic growth promoter (AGP; carbadox, 50 ppm).

	Treatment				Period			*P*-value[1]	
Item	NC	BZA	BC50	AGP	1	2	SEM[1]	Treatment	Period
ATTD OM, %	81.6[b]	82.6[ab]	80.9[b]	84.4[a]	83.1	81.6	0.9	0.004	0.007
ATTD GE, %	79.0[b]	80.1[ab]	78.2[b]	82.3[a]	80.1	79.7	0.9	0.001	0.41
ATTD CP, %	74.0[b]	75.7[ab]	71.4[c]	78.4[a]	73.1	76.6	2.1	< 0.001	< 0.001

[1] No treatment × period was identified ($P > 0.10$). [2] Maximum value for the standard error of the mean. [a, b] Means without a common superscript differ after Tukey HSD test, $P < 0.05$.

Jejunum and ileum villus height and crypt depth are commonly used as an indicator of gut health and reflect absorptive and digestive capacity of the small intestine. There was no difference among groups in VH, CD, or villus height to crypt depth ratio (VH:CD; Table 3; $P > 0.10$). Both VH and CD increased from period 1 to period 2 ($P < 0.05$), reflecting intestinal recovery from weaning stress. The improved growth performance and digestibility of the AGP group as compared to the NC group was not a consequence of improved gut architecture. Full results from this study are reported in Outlaw et al. (2023).

Table 3. Jejunum and ileum villus height (VH), crypt depth (CD), and VH:CD ratio in pigs fed starter diets containing no additive (NC), benzoic acid (BZA, 0.60%), Benzocal-50 (BC50, 0.20%), and antibiotic growth promoter (AGP; carbadox, 50 ppm).

	Treatment				Period			*P*-value[1]	
Item	NC	BZA	BC50	AGP	1	2	SEM[1]	Treatment	Period
Jejunum									
VH, μm	478	477	538	502	461	536	37	0.28	0.003
CD, μm	227	229	236	213	210	243	18	0.17	< 0.01
VH:CD	2.14	2.09	2.32	2.37	2.24	2.21	0.14	0.29	0.77
Ileum									
VH, μm	326	324	354	320	292	369	37	0.2	< 0.01
CD, μm	211	210	205	189	187	221	7	0.11	< 0.01
VH:CD	1.54	1.57	1.74	1.72	1.58	1.71	0.17	0.2	0.09

[1] No treatment × period was identified ($P > 0.10$). [2] Maximum value for the standard error of the mean.[a, b] Means without a common superscript differ after Tukey HSD test, $P < 0.05$.

In summary, protected benzoic acid supplementation marginally enhanced starter pig growth performance later in the nursery period compared to no additive or free benzoic acid supplementation, but not the same extent as antibiotic growth promoters. Increased total tract nutrient digestibility, but not gut architecture, in pigs fed antibiotic growth promoters could contribute to the observed differences in growth performance. These findings have implications

for improving starter pig health and pork production while reducing reliance on antibiotics and zinc oxide.

Funding

This research was funded by the Guangzhou Insighter Biotechnology, Co. Ltd. (Guangzhou, China).

4. References

Choi, H., Y. Chen, F. Longo, and S. W. Kim. 2023. Comparative effects of benzoic acid and sodium benzoate in diets for nursery pigs on growth performance and acidification of digesta and urine. J Anim Sci 101:skad116. doi:10.1093/jas/skad116

Collins, C. L., J. R. Pluske, R. S. Morrison, T. N. McDonald, R. J. Smits, D. J. Henman, I. Stensland, and F. R. Dunshea. 2017. Post-weaning and whole-of-life performance of pigs is determined by live weight at weaning and the complexity of the diet fed after weaning. Anim Nutr 3(4):372-379. doi: 10.1016/j.aninu.2017.01.001

Kiarie, E., C. Voth, D. Wey, C. Zhu, P. Vingerhoeds, S. Borucki, and E. J. Squires. 2018. Comparative efficacy of antibiotic growth promoter and benzoic acid on growth performance, nutrient utilization, and indices of gut health in nursery pigs fed corn–soybean meal diet. Canadian Journal of Animal Science 98(4):868-874. doi: 10.1139/cjas-2018-0056

Moeser, A. J., C. S. Pohl, and M. Rajput. 2017. Weaning stress and gastrointestinal barrier development: implications for lifelong gut health in pigs. Anim Nutr 3:313–321. doi:10.1016/j. aninu.2017.06.003

National Research Council. 2012. Nutrient Requirements of Swine (11th Revised Edition). National Academies, Washington, DC.

Outlaw, A., A. Gachman, H. Kim, X. Xu, Z. Tan, Z. Qin, X. Peng, and M. Rudar. 2023. Evaluation of protected benzoic acid on growth performance, nutrient digestibility, and gut health indices in starter pigs. Transl Anim Sci 7(1):txad111. doi: 10.1093/tas/txad111

Pluske, J. R., D. L. Turpin, and J. C. Kim. 2018. Gastrointestinal tract (gut) health in the young pig. Anim Nutr 4(2):187-196. doi: 10.1016/j.aninu.2017.12.004

Benzoic Acid Reduces Growth Performance in Starter Pigs Fed Low Protein Diets

Rodrigo Wever [1], Abigail Crosby [2], Alex Outlaw [2], and Marko Rudar [2,*]

[1] Department of Agricultural Sciences and Production, Zamorano University, Honduras;
[2] Department of Animal Sciences, Auburn University, Auburn, AL, USA.
* Correspondence: mzr0097@auburn.edu

TAKE HOME MESSAGE

Adding benzoic acid to nursery diets at or marginally below the estimated total crude protein requirement of starter pigs reduces body weight gain and feed efficiency without affected feed intake by reducing glycine available for lean protein accretion. These findings caution against supplementing benzoic acid to low protein diets without also supplementing additional protein.

SUMMARY

Swine diets oversupply protein because diets containing protein at the estimated protein requirement of pigs often reduce growth performance. The content of glycine, a non-essential amino acid, in corn and soybean meal is low compared to glycine content in lean protein. Glycine is also needed for excreting benzoic acid, a feed additive that can replace antibiotic growth promoters, in urine. The objective of this study was to determine the extent to which benzoic acid reduces growth performance in starter pigs fed diets limited in protein. Three treatment diets were fed to starter pigs for 28 d: 1) high crude protein (HCP; n = 9 pens); 2) low crude protein (LCP; n = 9 pens); and 3) LCP + benzoic acid (LCP+BA; n = 9 pens). While feed intake was not different among groups, body weight gain and feed efficiency were lower in the LCP+BA group than in the HCP and LCP groups. These findings indicate that the addition of benzoic acid to diets at or marginally below the estimated total crude protein requirement reduces growth performance in starter pigs. Maximizing growth performance in pigs fed low protein diets increases profitability, lowers environmental nitrogen excretion, and enhances sustainability of pork production.

1. INTRODUCTION

Swine diets are typically formulated to oversupply protein because diets containing protein at or marginally above the estimated crude protein (CP) requirement of the pig can reduce growth performance (Rocha et al., 2022). This implies that either the protein requirement of the pig is underestimated or that the optimal amino acid profile of protein above the requirement for dietary essential amino acids (EAA), but below the requirement for total CP, is poorly defined (NRC, 2012). Protein (i.e., nitrogen) is an expensive nutrient and the cost of soybean meal and specialty proteins exceeds that of corn. Thus, overfeeding protein in the nursery leads to higher feed costs, inefficient nitrogen utilization, and greater nitrogen excretion. Moreover, overfeeding protein to newly weaned pigs with a gastrointestinal tract insufficiently developed to digest and absorb dietary protein could increase the fermentation of undigested protein in the hindgut that could lead to post-weaning diarrhea (Heo et al., 2013). Therefore, there is a need to delineate the interaction

among total protein intake, the amino acid profile of protein above the requirement for dietary EAA but below the requirement for total protein, and growth performance in starter pigs.

Glycine (Gly) is a dietary non-essential amino acid (NEAA) that is needed for protein synthesis, one-carbon metabolism, and creatine, glutathione, and heme production (Wu, 2014). While nitrogen derived from most amino acids is considered freely available for the endogenous synthesis of Gly, this is likely inaccurate. Instead, the efficiency of using amino acid-derived nitrogen for Gly synthesis may depend on its specific source; in other words, this efficiency could vary from one amino acid to another. This has implications in formulating diets comprised mainly of corn, soybean meal, and milk products that all contain low levels of Gly relative to the level of Gly needed for lean protein accretion (Mahan and Shields, 1998). Benzoic acid, a common feed additive in swine diets used to replace antibiotic growth promoters in nursery diets, is excreted in urine as its Gly conjugate, hippuric acid (Kristensen et al., 2009).

We hypothesize that adding benzoic acid to nursery diets will reduce growth performance in starter pigs fed diets limited in total CP by reducing Gly availability for lean protein accretion. Thus, **the objective of this study was to determine the extent to which benzoic acid reduces growth performance in starter pigs fed diets limited in protein.** Understanding the optimal amino acid composition of the protein fraction above the requirement for dietary EAA but below the requirement for total CP will help guide how low protein diets for starter pigs can be formulated that save on ingredient costs, reduce nitrogen excretion, and mitigate the typical reduction in growth performance.

2. PROCEDURES

A total of 135 pigs (Yorkshire × Duroc and Yorkshire × Hampshire) were weaned at 28 ± 1 d age and assigned to mixed-sex pens (5 pigs per pen; 27 total pens) over two experimental blocks. Pigs were assigned to pens according to body weight (BW) and sex. Pens were separated by powder-coated vertical steel rods and equipped with a single-sided five-hole nursery feeder and cup waterer. Each pen provided 32 ft^2 area on snap-joint polypropylene flooring.

Pigs were fed a commercial starter diet for 4 d after weaning before introducing one of three treatment diets: 1) high crude protein (HCP; 3415 kcal metabolizable energy [ME]/kg, 19.75% CP; n = 9 pens); 2) low crude protein (LCP; 3498 kcal ME/kg,15.75% CP; n = 9 pens); and 3) LCP + benzoic acid (LCP+BA; 3466 kcal ME/kg, 15.75% CP; n = 9 pens). Diets were based on corn, corn starch, dried whey, and soybean meal. The HCP diet met or exceeded the estimated total CP, EAA, and standardized ileal digestible lysine to metabolizable energy ratio (SID Lys: ME) of starter pigs from 22 lb to 55 lb (NRC, 2012). The LCP and LCP+BA diets met the EAA and SID Lys: ME of starter pigs from 22 lb to 55 lb but provided protein at 85% of the estimated total CP requirement. The LCP+BA diet included benzoic acid at an amount isomolar to the amount of Gly in the LCP diet (0.9%). All diets met or exceeded estimated starter pig requirements for vitamins and minerals.

Pig body weight was measured at the start of the study and weekly thereafter for four weeks. Feed addition was weighed and recorded daily; at the end of each week, remaining feed in each feeder was removed, weighed, and recorded. At the start of the study and every two weeks thereafter, whole blood was collected by jugular venipuncture after a 2-h fast into serum vacutainers, allowed to clot for 30 min at room temperature, and centrifuged at 3000 rpm for 20 min. Serum was separated from hematocrit and stored at -20°C. Serum was analyzed for plasma amino acid concentrations by high performance liquid chromatography with fluorescence

detection following deproteinization and derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate.

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 pen within block was considered a random effect. Data are presented as least-squares means ± standard error of the mean. Differences among diets were determined with a Tukey honest significant difference (HSD) test. Results are considered significant at $P < 0.05$ and a trend at $P < 0.10$.

3. RESULTS & DISCUSSION

Initial BW was not different among groups (Table 1; $P > 0.10$). Average daily gain (ADG) was greatest in the HCP group, intermediate in the LCP group, and lowest in the LCP+BA group ($P = 0.001$) that corresponded to differences in final BW among groups ($P = 0.001$). While average daily feed intake (ADFI) was not different among groups ($P > 0.10$), feed efficiency (gain-to-feed ratio, G:F) was approximately 8% lower in the LCP+BA group compared to the HCP and LCP groups ($P < 0.001$).

Table 1. Effect of treatment diet on starter pig growth performance.

Item	Treatment[1]			SEM[2]	*P*-value
	HCP	LCP	LCP+BA		
n	9	9	9		
Initial BW, lb	20.4	20.7	20.8	0.7	0.92
Final BW, lb	56.6[a]	54.2[ab]	51.9[b]	0.8	0.001
ADG, lb/d	1.28[a]	1.20[ab]	1.12[b]	0.03	0.002
ADFI, lb/d	2.20	2.09	2.09	0.05	0.26
G:F, lb/lb	0.58[a]	0.57[b]	0.53[b]	0.01	< 0.001

[1] HCP, high crude protein; LCP, low crude protein; LCP+BA, low crude protein + benzoic acid. [2] SEM, standard error of the mean. [a, b] Means without a common superscript differ after Tukey HSD test, $P < 0.05$.

Plasma Gly concentration was approximately 20% lower in the LCP+BA group compared to the LCP group (Table 2; $P = 0.015$), indicating that a fraction of Gly flux is directed toward hippuric acid formation in the liver. Although total NEAA concentrations were not affected by diet, total EAA concentrations were 19% and 34% greater in the LCP and LCP+BA diets than the HCP diet, respectively ($P = 0.009$). Plasma lysine (Lys) concentrations were similarly 26% and 55% greater in the LCP and LCP+BA diets than the HCP diet, respectively ($P = 0.001$).

Table 2. Effect of treatment diet on select plasma amino acid concentrations (μmol/L).

Item	Treatment[1]			SEM[2]	*P*-value
	HCP	LCP	LCP+BA		
n	5	5	5		
Gly	610[ab]	637[a]	506[b]	32	0.015
Lys	119[b]	150[ab]	184[a]	11	0.001
EAA	715[b]	852[ab]	955[a]	52	0.009
NEAA	1731	1856	1652	78	0.19

[1] HCP, high crude protein; LCP, low crude protein; LCP+BA, low crude protein + benzoic acid. [2] SEM, standard error of the mean. [a, b] Means without a common superscript differ after Tukey HSD test, $P < 0.05$.

Collectively, these findings indicate that the addition of benzoic acid to diets at or marginally below the estimated total CP requirement reduces body weight gain and feed efficiency in starter

pigs. Moreover, the observed reduction in growth performance with benzoic acid exceeds that achieved by CP restriction alone. The reduction in plasma Gly concentration implies that benzoic acid depletes Gly, and thus total nitrogen, available for lean protein accretion. The increase in plasma EAA concentrations in pigs fed diets limited in CP further suggests that EAA were not utilized to the same extent in the LCP and LCP+BA groups compared to the HCP group for lean protein accretion despite similar EAA intake. Benzoic acid exacerbated the low crude protein-induced increase in plasma Lys and EAA concentrations.

While there is some evidence suggesting that feeding low CP diets in the nursery promotes gut health and mitigates post-weaning diarrhea, this work cautions against supplementing benzoic acid in these diets without also supplementing additional nitrogen. However, the optimal source of the additional nitrogen is not clear. Future work will determine the efficiency that nitrogen derived from specific amino acids (e.g., Lys) can support endogenous Gly production in starter pigs fed low CP diets. Maximizing growth performance and whole-body nitrogen retention in pigs fed low protein diets will help reduce feed costs, increase profitability, mitigate environmental nitrogen excretion, and enhance sustainability of pork producers across the United States.

Funding

This research was funded by the USDA National Institute of Food and Agriculture Hatch project 1025705.

4. References

Heo, J. M., F. O. Opapeju, J. R. Pluske, J. C. Kim, D. J. Hampson, and C. M. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J Anim Physiol Anim Nutr (Berl) 97(2):207-237. doi: 10.1111/j.1439-0396.2012.01284.x

Kristensen, N. B., J. V. Norgaard, S. Wamberg, M. Engbaek, J. A. Fernandez, H. D. Zacho, and H. D. Poulsen. 2009. Absorption and metabolism of benzoic acid in growing pigs. J Anim Sci 87(9):2815-2822. doi: 10.2527/jas.2009-2003

Mahan, D. C., and R. G. Shields, Jr. 1998. Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. J Anim Sci 76(2):513-521. doi: 10.2527/1998.762513x

National Research Council. 2012. Nutrient Requirements of Swine (11th Revised Edition). National Academies, Washington, DC.

Rocha, G. C., M. E. Duarte, and S. W. Kim. 2022. Advances, Implications, and Limitations of Low-Crude-Protein Diets in Pig Production. Animals (Basel) 12(24)doi: 10.3390/ani12243478

Wu, G. 2014. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. J Anim Sci Biotechnol 5(1):34. doi: 10.1186/2049-1891-5-34

Bolstering the Social Licensure of Animal Agriculture - Creation of More Effective Communication and Literacy Ecosystems

Gabriella F. Johnson [1], Karen A. Hiltbrand [2], Katie E. Corbitt [1], David S. Martin [3], Jason T. Sawyer [1], Soren P. Rodning [1], and Donald R. Mulvaney [1,*]

[1] Department of Animal Sciences, Auburn University, Auburn, AL, USA;
[2] Marketing Coordinator, Select Sires, Plain City, Ohio, USA;
[3] Department of Hospitality Management, Auburn University, Auburn, USA.
*Correspondence: mulvadr@auburn.edu

TAKE HOME MESSAGE

Segments of society are questioning the welfare of animals in production, whether types of meat consumption have a negative impact on personal health, and the environmental sustainability of producing livestock. Because most Americans are three or more generations removed from production agriculture, they have minimal incentive to question the unsubstantiated statements purported by anti-animal agriculture groups. A GAP has emerged and is widening between those in production agriculture and those in a non-ag category. With growing evidence, it is purported that lack of effective communication will allow continued erosion of ag literacy and social licensure of many aspects of livestock production agriculture. Communicating about science 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. Our communication, leadership and influence-science research program examines how social media channels, as well as video construction can be harnessed to more effectively communicate publicly about animal agricultural production to influence perception. Quantitative and qualitative results from our research provides indication toward tribal views by assessing how the public engages in daily posts of information and misinformation about animal agriculture and provide indication of how and where the industry could design meaningful messaging for improved perceptions.

SUMMARY

The level of trust/distrust among participants in our research was evaluated using continuous response measurement (CRM) instrumentation to identify critical moments of trust and distrust while viewing information. It was found that the lower the consumers' knowledge of the food industry, the more likely they would trust misinformation ($p < 0.05$) and become misinformed. Another mixed-methods study involving consumers viewing video segments revealed more trust in a video clip which included misinformation. Results show limited opportunity to revert consumers' perceptions about the beef once they have engaged in misinformation. In other experiments, multiple surveys were used to scope current agricultural knowledge amongst higher education individuals; sentiment of instructor discussions that spontaneously address agriculture; credibility judgements of students and their past instructors; narratives of misinformation, biases, and other phenomena graduate students have faced academically; and how professional development workshops can train future educators in areas of scientific and agricultural literacy.

Abstracted findings across multiple studies conclude that many factors influence knowledge of animal agriculture and food production. To mitigate harmful effects of negative media, oral messages, and other methods of communication, continuing education of agriculture into higher education is a necessary powerful, proactive mechanism to consider. Our research to-date clearly demonstrates impacts of animal agriculture communication messaging mediated through popular channels and modalities within the information ecosystem can effectively influence shifts in perceptions about specific areas of concern about animal agriculture.

1. INTRODUCTION

The National Academy of Sciences (NAS) 'Critical Role of Animal Science Research in Food Security and Sustainability' identified areas of research and development, technology, and resource needs for research in the field of animal agriculture, both nationally and internationally. With a growing global population, it is clear that animal-derived dietary protein is critically important. The infosphere is filled with anti-animal agriculture messaging. Thinking of "social permission" to conduct our livestock businesses is a continually emerging outcomes (see emergence within European and New Zealand countries; see Dublin Declaration; CA Prop 12; proposed US Agriculture Accountability Act of 2023) and endowing policymakers to take advantage of failed education / communication of our animal ag industry.

Consumers with non-ag backgrounds offer trust challenges and confusion around gathering neutral, factual answers to their concerns and questions. While increasing numbers desire to better understand sustainable connections between animal agriculture and our environment, diet health, animal welfare and production/processing, citizens are frequently inundated by biased voices of citizen journalists as well as marketers and anti-animal agriculture activists. Consumer segments are also skeptical of science and technologies. As detractors who denounce animal agriculture production for contributing to deforestation, environmental and climate change, obesity and cancer with our meat production and products, the concept of a social license to be able to raise animals for food is challenged. **Our research examines strengths, weaknesses opportunities and threats approaches to identify some of the existential challenges but also coalesce data to explore development of a concerted plan for evidence-based, consumer centric messaging/marketing plans for communicating our science and increasing sustainability of animal industry.**

Human perception is a complicated but necessary topic concerning consumer behavior (Ceci, 2023). Consumer behavior studies consumers' choices when searching for, evaluating, purchasing, and using products and services they believe will meet their needs (Wisenbilt & Schiffman, 2019). Amid recent food shortages, increased food prices, and food distribution pains, there has been increased consumer awareness regarding how, why, and where food is produced. This is especially specific to sustainable food production and products. For years, agricultural communicators have discussed, researched, and deployed methods to narrow the communication gap between consumers of agricultural products and food producers (Clemons et al., 2018). One of the many challenges agriculturalists faces is combating the spread of misinformation about agriculture (Van Eenennaam et al., 2021). From video to social media, and on-demand video, consumers experience over 13 hours of media every day (Statista, 2023), meaning agriculture communicators both have an opportunity and a challenge to gain consumers' attention. This is a challenge as the average attention span is roughly 8.25 seconds among audiences (Zauderer, 2023).

Not only are communicators tasked with grabbing consumers' attention but also maintaining it (Zauderer, 2023).

Another dimension of the communication challenge is that the average consumer is more than three generations removed from the farm (Hughes et al., 2017), and for years, agricultural communicators have been trying to understand how agricultural information is best communicated, retained, and understood and, in turn, how this affects consumer buying decisions. Lewis (2018) and Frick, Kahler, and Miller (2018) state, "Agriculture literacy is the awareness and understanding of our food fiber, natural resources, and animal health and its relationship to the public and the environment" (p. 1). Van Eanennaam and Werth (2021) explain that unwarranted fear substantially reduces the ability to produce nutritious food with less environmental impact and increases sustainability.

Agricultural Literacy and being Literate in Agriculture

While agriculture literacy and being agricultural literate sound analogous, the two concepts are different (Lewis, 2018). Agricultural literacy, precisely, does not address an individual's ability to write, read, and communicate (be literate) about a specific topic, which in this case is the topic of agriculture (Clemons et al., 2018). One of the most well-known challenges the agriculture industry faces is the lack of knowledge and connectedness with the public (Settle et al., 2017). While many agriculturists work to communicate to the public, often, their messages get caught in what is referred to as an echo chamber (Ruth et al., 2018). An echo chamber is an individual's intrinsic tendency to seek information that reinforces and confirms their pre-existing beliefs (Ruth et al., 2018). This method can be described as speaking to the choir and is not the circle that the story of agriculture needs to be limited to (Ruth et al., 2019).

In a study conducted by Lewis (2018), it was found that the general population of the United States "was not very agriculturally literate" (p. 47); however, they did understand more in the area of "the Relationship Between Agriculture and the Economy" (p. 60), compared to the construct the "Relationship with Agriculture and Animals" (Lewis et al., 2018, p. 60). Baby boomers had the highest overall agricultural literacy, apart from understanding newer technology related to (Lewis et al., 2018). The generations who possessed the lowest level of agricultural literacy were Generation X and Millennials (Lewis et al., 2018). The two constructs of the pillars of Farm Bureau Ag Literacy Pillars that consumers had the least knowledge about were the "Relationship Between Agriculture and Animals" (p. 62) and the "Relationship Between Agriculture and Lifestyle" pillar (Lewis et al., 2018, p. 62). Results from the Lewis study are significant and indicate that there is much work to do in informing younger generations in those areas of the agriculture industry (Lewis et al., 2018). Agricultural literacy is necessary so consumers understand the food system (Frick et al., 1991) and can be literate about all the following concepts identified by Frick et al. (1991):

- Agriculture's relationship with the environment and sustainability (Frick et al., 1991).
- The processing of agricultural products, which includes food safety and research and development.
- Developing policies regarding agriculture, understanding how the consumer can affect policy, and regulating policies can affect agriculture (Frick et al., 1991).
- To understand agriculture's relationship with natural resources in relation to conservation, stewardship, and symbiosis in the environment (Frick et al., 1991).

- To address society's lack of understanding regarding animal products, precisely consumer concerns, the uses of animal proteins, husbandry, and advancing technologies (Frick et al., 1991).
- To understand the production of plant products in relation to gardens, care of plants, biotechnology, genetics, profit, and society (Frick et al., 1991).
- To understand the economic impact of agriculture from a farm management, micro/macroeconomics perspective, and food costs (Frick et al., 1991).
- To have a working knowledge of how agriculture products are marketed related to public perception (Frick et al., 1991).
- To understand the vastness and processes of the global and domestic distribution of agricultural products (Frick et al., 1991).
- Finally, the global implication of agriculture relating to food economies, hunger, research, global politics, and sociology (Frick et al., 1991).

Consumer Perception of Food and Sustainability

The America Psychology Association (2022) defined perception as: "*The process or result of becoming aware of objects, relationships, and events using the senses, which includes activities such as recognizing, observing, and discriminating*". These activities enable organisms to organize and interpret the stimuli received into meaningful knowledge and act in a coordinated manner.

When faced with a situation, subjects interpret stimuli in a relatable or meaningful way based on their past experiences (Pickens, 2005). The challenges differing perceptions create are that they are highly subjective to every individual (Pickens, 2005). All stimuli uniquely affect different audiences depending on their experiences, environment, and influences (American Psychology Association, 2022).

Attitude is the basis of perception and is made up of three factors: mental status (feeling), a condition (belief), and behavior (Koswat1a et al., 2022; Altman, 2008; Pickens, 2008). Experience and temperament play a significant role in an individual's attitude, and the result of attitude is behavior (Pickens, 2005). Perception comprises four phases, including stimulation, registration, organization, and interpretation (Pickens, 2005). Awareness is an essential factor in perception (Pickens, 2005). Individuals will naturally be more attentive to stimuli corresponding to their preexisting attitudes, beliefs, personalities, and motivations (Pickens, 2005). Perceptual vigilance refers to the mental process by which people achieve their most immediate needs (Pickens, 2005). In contrast, perceptual defense refers to the inclination to avoid stimuli that may cause tension or discomfort (Pickens, 2005). With the average American consumer being three generations removed from agriculture (Hughes et al., 2017), combined with the overload of social media, on-demand video, and resources on the internet, there is an opportunity for misinformation to occur (Ruth et al., 2018).

Perception involves a physical dimension that relates to how received information is transformed into operational information (Koswat1a et al., 2022). The presentation of the data is highly related to how the information was acquired and the source (Koswatta et al., 2022). In the physiological dimension of perception, information received is interpreted through an individual's personal beliefs, values, needs, and interests (Koswatta et al., 2022).

In a study by Koswatta (2022), researchers examined the factors affecting public perception of science, perception formation, and factors contributing to each step in the perception formation process within agricultural communication. Through their review of literature and methods, they

identified four themes: audience beliefs, audience socio-demographics, communication sources, and environment (Koswatta et al., 2022) that contribute to perception formation. The sub-themes that shaped the audiences' beliefs were religious beliefs, political beliefs, trust in science, perceived risks, benefits, and preexisting attitudes (Koswatta et al., 2022). Age, income level, occupation, gender, and knowledge were also said to affect trust in science (Koswatta et al., 2022). The sub-themes that added to the credibility of sources within articles were credibility, scientist trustworthiness, organizational trustworthiness, communications medium, and message characteristic (Koswatta et al., 2022). Finally, the environment theme comprised sub-themes, including exposure to information, type of exposure, social bots, and science-related events (Koswatta et al., 2022). These research observations support the importance of collecting demographic information to explore factors affecting perception and trust.

Consumers buying decisions are the primary controllers of the market (Ruth et al., 2018). The U.S. consumer is becoming further removed from agriculture both experientially, geographically, physically, and educationally (Rumble et al., 2020). This has created an environment for consumers to be easily misinformed and be more concerned with modern agriculture production practices and technologies (Rumble et al., 2020). To address this problem, it is important that agricultural communicators and constituents within the agriculture industry proactively and effectively inform consumers about how their food was raised (Rumble et al., 2020).

Messaging should be customized toward generational affiliation, specifically for college-aged millennials (1982-2004) (Oesterreicher et al., 2018). In a focus group conducted by Oesterreicher et al., (2018), Millennial conversations were based around cattle management, the use of natural resources, the treatment of animals, food safety, and the local economy (Florida) (Oesterreicher et al., 2018).

Shugoll Research (2014) stated that when millennials make buying decisions, they consider "great taste, good value, feeling comfortable, and confidence when preparing the dish, being food they feel good about and having an ideal balance of taste and nutrition" (p 4.). According to Oesterreicher and others (2018) and Shugoll Research (2014), millennials consider trimmed fat and slight marbling while making beef selections, and about one-third consider how cattle are produced and treated. Oesterreicher and others (2018), Shugoll Research (2014), and the Beef Checkoff (2015) said that millennials were said to be "frustrated about the contradictory information about whether or not beef is good for you" (p. 4). Also, millennials associate eating beef with emotions that include excitement, nostalgia, anticipation, and comfort (Oesterreicher et al., 2018; Beef Checkoff, 2015; & Shugoll Research, 2014).

It is essential to understand the scope of the landscape of the current perception of the beef industry as related to sustainability, specifically regarding environmental impact. While difficult to define, sustainability is a common buzzword defined differently by different entities. The United States Department of Agriculture (USDA) defines sustainability as satisfying human needs, enhancing environmental quality, the resource base, and ecosystem services; sustaining the economic viability of agriculture; and improving the quality of life for farmers, ranchers, forest managers, workers, and society (USDA, 2022). Because of the diversity of definitions of sustainability, citizens can be confused about it. The Environmental Protection Agency (EPA) defines sustainability as the fundamental principle that "To pursue sustainability is to create and maintain the conditions under which humans and nature can exist in productive harmony to support present and future generations" (EPA, n.d). Sustainability is a complex, multifaceted, and

often emotionally driven issue, which today is dominated by climate change (Cullman, 2022). Society cares and asks questions to understand improvement opportunities and progress (Cullman, 2022). The three pillars of sustainability include the economic, environmental, and social dimensions pillars. An immense amount of emotion and money is dedicated to making sustainable advancements. For example, "Millennials are set to inherit $24 trillion of wealth in the US alone over the next 15 to 16 years, and 75% believe their investments can influence climate change" (Credit Suisse, 2019, p. 10). When the three pillars of sustainability overlap, they create intersections that produce socio-economic, socio-environment, and eco-environmental areas of sustainability (Cullman, 2022).

The scientific literature ranges from studies that claim consumers are buying into sustainable solutions and others that claim that sustainability does not change their purchasing decisions (Gorynska et al., 2020). In a study by Gorynska (2020), they found that "one-third of their respondents were interested in and actively taking part in searching for information about food consumption and the food market." Consumers were most concerned with a product's health benefits, nutritional benefits, and specific ingredients (Gorynska et al., 2020). The product's origin, price, form of preparation and connection to ecology were also significant to buyers (Gorynska et al., 2020). Consumers thought the most credible information about food was from reports and scientific papers, as well as from family and friends (Gorynska et al., 2020). Word-of-mouth was also an effective communication tool, according to Gorynska's 2020 study. The theory of social attitudes and social communication of theory was also considered (Gorynska et al., 2020). People or groups tend to react to stimuli differently due to their circumstances or values when making sustainable food purchases (Gorynska et al., 2020). Of the study participants, 80% of the respondents had never come across any information concerning sustainable food production values (Gorynska et al., 2020). Of the sample, 12.5% said the information about sustainable food that was most memorable was about environmental issues first, then food waste, shopping planning, change in direction (relating to ecological and personal impact from both a personal and worldly standpoint), human health, consumption about nutritional recommendations and stricter diets (about specific dietary restrictions). This information relating to sustainable food production was more memorable for women than men (Gorynska et al., 2020). According to Gorynska (2020), "the young (18–24 years old) remembered the information about shopping planning and connection between consumption and nutrition recommendations, people aged 25–34 were more interested in environmental issues, the direction of changes and issues of consumption and ecology, while people aged 35–44 were interested in food waste" (p. 12). This study showed that environmental issues and health risks were at the peak of participants' concerns. Still, the authors add that other factors, such as socioeconomic status, education level, and product availability, are essential (Young et al., 2010). Thirty percent of consumers express worries about environmental issues, but only 5% turn those worries into actions (Young et al., 2010). While people claim they are becoming more eco-friendly, there will be no change until action has occurred (Barnett et al., 2010), and until then, there will be little change in the market of sustainable food preferences (Barnett et al., 2010).

In a nationally representative survey paper conducted with 524 randomly sampled individuals utilizing Qualtrics, Settles (2017) reported participants' awareness, knowledge, and trust in 16 agriculture organizations using a 5-point Likert scale (Settle et al., 2017). The results showed that 94.3% of participants were the most aware of the Food and Drug Administration

(FDA), with 14.1% of respondents being least aware of Syngenta (Settle et al., 2017). It is also important to note that only 26.0% of respondents were knowledgeable of Cooperative Extension programs, a well-established federal and state network (established in 1862) focused on providing resources to citizens to educate the public about agriculture (Settle et al., 2017).

Environmentally friendly or green consumers make their purchasing decisions, beginning with environmental issues, assessing human rights, and finally considering animal rights (Wheale & Hinton, 2007). It is essential to clearly understand the consumer landscape to appropriately provide educational resources and products to consumers (Wheale & Hinton, 2007). Europe lacks agricultural breeding technologies due to the amount of scientific misinformation that exists (Smyth & Lassoued, 2019).

Generation Z Population

Generation Z are individuals born between 1997 to now (Thompson, 2022). There is lacking a deficit of research about Generation Z because these individuals are just coming of age to enter the workforce (Meola, 2021). Generation Z is the youngest, largest, and most ethnically diverse generation, accounting for 27% of the United States population (Meola, 2021). Generation Z has the spending opportunity of $143 billion annually, accounting for nearly 40% of the total global consumers in 2020 (Dagnostino, 2001). Knowing that consumers drive the market, studies must be conducted to identify inter-generational buying trends and attitudes, specifically in the agriculture industry.

While there needs to be more specific research on Gen Z about agricultural and food purchasing trends, data is available on the generation's motivations, attitudes, and habits. Many studies have been conducted on millennials' perceptions of the beef industry, but there are few consumer perceptions of Generation Z (Shugoll, 2014).

In a study examining Gen Z communication styles by applying the Hartman and McCambridge assessment tool (Hartman et al., 2011), it was found that Generation Z tends to utilize four primary communication styles, including analytical, driver, amiable, and expressive, based on two dimensions, being assertiveness and responsiveness (Hartman et al., 2011; Humaira Raslie, 2020). It was shown that Gen Z listened more than they talked (Humaira Raslie, 2020). According to Hartman and McCambridge (2011), talking more than listening reflects assertiveness, whereas listening more is a responsive communication style. When working with people on projects, Gen Z is more concerned with what others think and less about getting the task done, implying that individuals can be image focused (Humaira Raslie, 2020). Gen Z participants were better at style-flexing (adapting their communication style to match those who surround them) and were more other-focused (Humaira Raslie, 2020). They are more likely to cooperate during communication, conform, and attempt to relate to their peers (Humaira Raslie, 2020).

Gen Z and Gen Y (millennials) prefer face-to-face communication and visuals for online communication (Humaira Raslie, 2020). Gen Z expects instantaneous feedback when conversing and is low on the assertive scale (Humaira Raslie, 2020). Generation Y and Generation Z have an agreeable communication style, which means they are more receptive than assertive, favor relationship orientation above tasks, and do tasks more slowly and carefully (Humaira Raslie, 2020). Because they tend to comply and collaborate, they may be unable to handle workplace confrontations, which are often unavoidable (Humaira Raslie, 2020). Other studies confirm that when it comes to trust, college students are high on the agreeableness scale (Sriprom et al., 2019).

Generation Z is the first generation to have access to the internet for their entire lifetime (Ho Shin et al., 2021). Due to their connectedness, it is vital that marketers understand their characteristics and can effectively disseminate to digital natives (Ho Shin et al., 2021). Ho Shin (2021) and Krishen (2016) state, "Generation Z is eager to search for information via social network service; they tend to make their decisions based on feedback from others" (p. 4).

Those in Generation Z value social ethics when making purchasing decisions. In a study by Francis and Hoefel (2018), about 80% of Generation Z were reluctant to support a business they felt had unethical practices. The latter observation tells researchers that when communicating with this generation, brands should reflect social responsibility and admirable character (Ho Shin et al., 2021). When communicating with Gen Z, it is essential to treat them as equals as they do not validate an age gap as qualifying them as inferior in the working world (Nguyen, 2021). Knowing that Gen Z prefers to be treated as equal, it is essential to express trust and inclusiveness, not in a way that infers that they lack knowledge (Nguyen, 2021).

Trust in the Science of Agriculture

As defined by Rumble and others (2020), "trust is the fundamental component of all relationships between the public and specific people or groups" (p. 4). Trust alone is a complicated notion, particularly when everyone has a different way of interpreting what and whom they find trustworthy (Rumble et al., 2020). The adoption of new technology in science hinges on the public's trust, public policy, and public perception (Rumble et al., 2020). When the public does not accept science, further advancement can be inhibited, meaning there are fewer opportunities for scientific developments to assist in developing a safe, affordable, and high-quality food supply (Understanding Science, 2020), when social trust is lacking around topics such as climate change, genetically modified food, or environmental issues (National Science Board, 2018), political regulation and market action often become required to monitor practices (Arnot et al., 2016). A lack of trust may result in further government regulation (Understanding Science, 2020).

Trust is an integral component of communicating effectively regarding sharing, comprehending, and narrowing the producer/consumer gap (Settle et al., 2017). In a convergent mixed-methods study by Rumble et al. (2020), trust was analyzed before and after engaging in a conversation via a focus group regarding adopting new technologies to combat citrus greening. Within this study, 76 individuals participated in one of the four-focus groups from around the country (California, Florida, Illinois, and New Jersey). Rumble and others reported (2020) common themes, including "modern science does more harm than good" (p. 9), "belief in scientists and their contribution to science to society" (p. 8), and participants broadly expressed gratitude for the work scientists were doing to solve problems (Rumble et al., 2020). One participant changed their opinion drastically about the protein technology used to combat citrus greening when they recalled that he took a similar medication and could relate to the technology (Rumble et al., 2020). This focus group had significant themes of distrust related to lack of information, skepticism, fear, and benevolence (Rumble et al., in 2020). Lack of knowledge/the unknown was the most common theme among many participants in the four focus groups (Rumble et al., 2020). This information suggests that communicators address all potential questions while delivering knowledge on a particular issue. Rumble (2020) stated that "many participants were skeptical that information was being withheld from them" (p. 11), which stemmed from the fact that the participants had never heard about the specific problem before (Rumble et al. 2020).

Transparency is essential in reducing consumer skepticism (Rumble et al., 2020). When discussing fear, participants mentioned DDT and how it was too late when many people became aware of its poor health repercussions (Rumble et al., in 2020). Trust was hampered when participants felt consumers' best interests were not in mind, which was explicitly prompted by a discussion regarding the financial and economic implications of agriculture technology (Rumble et al., in 2020). Participants shared that they trust educational institutions' research more than corporate research because they are not profit-motivated (Rumble et al., 2020). Even though general trust in science may exist, that does not translate to trust in specific science contexts (Rumble et al., 2020). Without consumer trust, the U.S. agriculture industry is vulnerable to decreased livestock production and crop production, soil and water quality issues, pest issues, and even economic struggles (Geston et al., 2022).

Gross (2021) stated that trust becomes more important when consumers lack knowledge and have uncertainties about food production. When determining consumers' trust in food, they often rely on credible information from their peers or personal sources (Gross, 2021). Kupsala and others (2015) found that women and urban residents have lower levels of trust, and older individuals have more trust in animal production, especially those with a farm background. Age, place of residence, and level of experience are related to the level of knowledge among consumers (Kupsala et al., 2015). Lower levels of trust are less widespread among the older and rural populations (Kupsala et al., 2015).

Misinformation and Disinformation

Eckler and others (2022) define misinformation as "any information that turns out to be false – and poses an inevitable challenge for human cognition and social interaction because it is a consequence of the fact that people frequently err and sometimes lie" (p.1). Disinformation differs as it is intentionally spreading false information (Eckler et al., 2022). The difference between the two types of message distribution defaults on the communicator's intent (Eckler et al., 2022). Aside from the dissemination of incorrect information, Karlova & Fisher (2013) stated that spreading misinformation and disinformation produces "suspicion, fear, worry, anger and decisions" (para. 3), which results in implications of trust. Or inversely, Karlova and Fisher (2013) state that it is becoming more standard to accept information as consistently "true, accurate, and complete" (sentence. 4). Consumers fail to consider the possibility of material being misinformation (Karlova & Fisher, 2013). The social diffusion model of information, misinformation, and disinformation depicts the process and formation of information, misinformation, and disinformation starting with the audiences' environment (Karlova & Fisher, 2013). Information, misinformation, and disinformation, as well as cues of credibility and deception, may all be influenced by social, cultural, and historical factors (Karlova & Fisher, 2013).

Unfortunately, misinformation and disinformation are not new challenges to the twenty-first century. Examples of both misinformation and disinformation over the years have contributed to several antagonistic occurrences that include but are not limited to elections, religious and political oppression, and specific events such as the world's response to the Covid-19 pandemic (Eckler et al., 2022). Unlike in history, today, the digital infrastructure allows for unmatched reach of that incorrect information (Eckler et al., 2022).

Misinformation and disinformation exist in food and beyond (Diekman et al., 2023). Some of the most common issues where misinformation and disinformation live include genetically

modified organisms (GMOs) (Butler-Hortan, 2021; Ryan et al., 2020, p. 15), milk production, animal welfare, and animal protein production (Van Eenennaam, 2022). Newsworthy stories of misinformation and disinformation include lean finely textured beef story and Oprah Winfrey's claim about Bovine Spongiform Encephalopathy (BSE).

2. OBJECTIVES & PROCEDURES

The overarching objective of this research program is to ascertain changes in and status of the social consensus/licensure around animal agriculture and determine how to become more effective at communicating science and technical agriculture production topics. Within the communicating ag issues domain, we:

1. Examine baseline perception and knowledge analysis of views by non-ag audiences.
2. Examine barriers and effectiveness of different modalities for communication of science concepts and animal agriculture topics.
3. Clarify how alternate views of science-based animal agriculture topics and modalities of communication can spread and influence audiences within a non-ag sector.

4. References

Altmann, T. K. (2008). Attitude: A Concept Analysis. Nursing Forum, 43(3), 144-150. https://doi.org/https://doi.org/10.1111/j.1744-6198.2008.00106.x

APA Dictionary of Psychology. (n.d.). American Psychological Association. American Psychological Association, https://dictionary.apa.org/perception

American Psychology Association (n.d) Perception. In American Psychology Association Dictionary of Psychology (E.d). https://dictionary.apa.org/perception

Arnot, C., Vizzier-Thaxton, Y., & Scanes, C. G. (2016). Values, trust, and science–building trust in today's food system in an era of radical transparency. Poultry Science, 95(9), 2219–2224. https://doi.org/10.3382/ps/pew168

Augoustinos, M., Crabb, S., & Shepherd, R. (2010). Genetically modified food in the news: Media representations of the GM debate in the UK. Public Understanding of Science, 19(1), 98–114. https://doi.org/10.1177/0963662508088669

Barnett, C., Cloke, P., Clarke, N., Malpass, A. (2010). The Political Rationalities of Ethical Consumption. Oxford: Wiley.

Beef Board. (2015). 2016-2020 Beef industry long-range plan final report. [Pamphlet]. https://www.beefboard.org/blog/2015 Summer Conference/2016-2020 Beef Industry LRP Final Report - for printing.pdf

Beef Checkoff. (2014). Millennial perceptions of beef production. https://www.beefresearch.org/CMDocs/BeefResearch/BIQ/Millennial Perceptions of Beef Production 1.pdf

Butler-Hortan, M. (2021). College Students' Perceived Ability Versus Actual Ability to Identify Fake News on Agricultural Topics. [unpublished manuscript]. Agricultural Communications, Texas Tech University. https://ttu-ir.tdl.org/bitstream/handle/2346/88708/BUTLER-THESIS-2021.pdf?sequence=1&isAllowed=y

Ceci, L., (2023). Hours of video uploaded to YouTube every minute as of February 2022. Statista. https://bit.ly/3V8am26

Ceci, L. (2023). YouTube – Statistics & Facts. Statista. https://www.statista.com/topics/2019/youtube/#topicOverview

Clemons, C., Lindner, J., Murray, B., Cook, M., Sams, B., & Williams, G. (2018). Spanning the Gap: The Confluence of Agricultural Literacy and Being Agriculturally Literate. Journal of Agricultural Education, 59(4), 238–252. https://doi.org/10.5032/jae.2018.04238

Credit Suisse. (2019). Sustainability Report. Retrieved March 2023, from https://www.credit-suisse.com/ch/en/investment-banking/ibcm/corporate-insights/earning-returns-on-sustainable-terms.html

Dagostino, A. Council Post: Here Is How Gen Z Is Changing the Way We Communicate. (2021). Forbes, Forbes Magazine, https://www.forbes.com/sites/forbescommunicationscouncil/2021/08/09/here-is-how-gen-z-is-changing-the-way-we-communicate/? sh=1ad2e3f41350.2

Definitions: Sustainability and Food Systems. USDA, United States Department of Agriculture, (n.d.), https://www.usda.gov/oce/sustainability/definitions.

Diekman, C., Ryan, C., & Oliver, T. (2023). Misinformation and Disinformation in Food Science and Nutrition: Impact on Practice. The Journal of Nutrition, 153(1), 3-9. 10.1016/j.tjnut.2022.10.001

Getson, J., Church, S., Radulski, B., Sjo¨strand, A., Lu, J., & Prokopy, L. (2022). Understanding scientists' communication challenges at the intersection of climate and agriculture. PLoS ONE, 17(8): e0269927. https://doi.org/10.1371/journal.pone.0269927

Goodwin, J. N., Chiarelli, C., & Irani, T. (2011). Is perception reality? Improving agricultural messages by discovering how consumers perceive messages. Journal of Applied Communications, 95(3), 21–33. https://doi.org/10.4148/1051-0834.1162

Gorynska-Goldman, E. & Gazdecki, M. (2020). Searching for and Perception Of Information By Consumers In The Light Of The Sustainable Consumption Idea — On The Example Of Food Markets. Marketing Of Scientific and Research Organizations, 36(2) 1-18. https://www.sciendo.com/article/10.2478/minib-2020-0013

Gross, S. & Roosen, J. (2020). Effects of information on social trust in farmers regarding animal welfare. International Food and Agribusiness Management Review. 24(1). 10.22434/IFAMR2020.0034

Guttmann, A. (2023). Time spent per day with digital versus traditional media in the United States from 2011 to 2023. Statista. https://www.statista.com/statistics/565628/time-spent-digital-traditional-media-usa/

Ho Shin, J. & Woo Lee, J. (2021). Athlete brand image influence on the behavioral intentions of Generation Z. Social Behavior and Personality an International Journal. https://doi.org/10.2224/sbp.9533vwww.sbp-journal.com

Eckler, U., Lewandowsky, S., Cook, J., Schmid, P., Fazio, L., Brashier, N., Kendeou, Vraga, E., & Amazeen, M. (2022). The psychological drivers of misinformation belief and its resistance to correction. NATURE REVIEW-Psychology, 1, 13-29. https://doi.org/10.1038/s44159-021-00006-yhttps://doi.org/10.1038/ s44159-021-00006-y

Francis, T., & Hoefel, F. (2018). 'True Gen': Generation Z and its implications for companies. McKinsey & Company. https://mck.co/2JzK4F1

Frick, M., Kahler, A., & Miller, W. (1991). A definition and the concepts of agricultural literacy. Journal of Agricultural Education. 32(2), 49–57. 10.5032/jae.1991.02049

Global social networks are ranked by the number of users (2023, January). Statista. https://www.statista.com/statistics/272014/global-social-networks-ranked-by-number-of-users/

Hais, M., & Winograd, M. (2011). Put Millennials first. The Huffington Post. Retrieved from http://www.huffingtonpost.com/michael-hais-and-morley-winograd/put-millennials-first_b_856082.html

Hartman, J., McCambridge, J. (2011). Optimizing Millennials Communications Styles. Business Communication Quarterly. 74(1), 22–24. https://doi.org/10.1177/1080569910395

Holt, J., Rumble, J., Telg, R., and Lamm, A. (2015). The Message or the Channel: An Experimental Design of Consumers' Perceptions of a Local Food Message and the Media Channels Used to Deliver the Information, Journal of Applied Communications, 99(4) https://doi.org/10.4148/1051-0834.1053

Hughes, A., Johnson, T., Edgar, L., Miller, J., & Cox, C. (2017). A Content and Visual Analysis of Promotional Pieces Used in a Communication Campaign for the Arkansas Promotion Board. Journal of Applied Communications, 100(2). https://doi.org/10.4148/1051-0834.102

Karlova, N. & Fisher, K. (2013). A social diffusion model of misinformation and disinformation for understanding human information behavior. Information Research, 18(1) paper 573. http://InformationR.net/ir/18-1/paper573.html

Koswatta, T.; Wingenbach, G.; and Leggette, H. (2022).Factors Influencing Public Perception of Science, Journal of Applied Communications, 106(4). https://doi.org/10.4148/1051-0834.2442

Krishen, A., Berezan, O., Agarwal, S., & Kachroo, P. (2016). The generation of virtual needs: Recipes for satisfaction in social media networking. Journal of Business Research, 69(11), 5248–5254.

Kupsala, S., Vinnari, M., Jokinen, P. & Räsänen, P. (2015). Citizen attitudes to farm animals in Finland: a population-based study. Journal of Agricultural and Environmental Ethics, 28(4): 601–620. https:// doi.org/10.1007/s10806-015-9545-z

LaGrande, L. (2018). Trust in Agricultural Messages: A moment to moment analysis [Master's thesis]. Texas, Tech University, Lubbock, Texas. https://ttu-ir.tdl.org/handle/2346/8267.

Lawson, C., Fischer, L., LaGrande, L., & Opat, K. (2020). Do touch that dial: A guide to continuous response measurement in agricultural communications, Journal of Applied Communications. 104(3). https://doi.org/104148/1051-0834.2333

Lewis, M. (2018). Agricultural Literacy Among the Generations: A National Study. [Unpublished master's thesis]. Tennessee State University, Nashville. https://www.proquest.com/docview/2057991785?parentSessionId=FmBMzbsSMkAAtMB1g30bDET5zirZnkMs0rdBR1CiYFk%3D

McDonald, S. M. (2012). Perception: A Concept Analysis. International Journal of Nursing Knowledge, 23(1), 2–9. https://doi.org/https://doi.org/10.1111/j.2047-3095.2011.01198.x

Nguyen, T. (2021). 5 things you should know to effectively communicate with Gen Z in the workplace. Back to Resources. https://blog.grovehr.com/generation-z-communication-in-the-workplace

Meola, A. (2021). Generation Z News: Latest Characteristics, Research, and Facts. Insider Intelligence, https://www.insiderintelligence.com/insights/generation-z-facts/

National Science Board. (2018). Science and technology: Public attitudes and understanding. https://nsf.gov/statistics/2018/nsb20181/report/sections/science-and-technology-public-attitudes-and-understanding/highlights

Oesterreicher, S., Lundy, L., Rumble, J., & Telg, R. (2018). Collegiate Millennials' Perceptions of Locally Produced Beef, Journal of Applied Communications, 102(4) https://doi.org/10.4148/1051-0834.2226

Petty, R., Brinol, P., & Priester, J. (2009). Mass media attitude change: Implications of the elaboration likelihood model of persuasion. Media effects: Advances in theory and research. 3, 125–164.

Petty, R. E., & Cacioppo, J. T. (1996). Attitudes and persuasion: Classic and contemporary approaches. Boulder: Westview Press

Petty, T., & Cacioppo, J. (1986). The Elaboration Likelihood Model of Persuasion. Advances in Experimental Social Psychology, 19, 123-205.

Pickens, J. (2005). Attitudes and perceptions. In N. Borkowsk (Ed.), Organizational behavior in health care (pp. 43–76). Jones and Bartlett Publishers

Ramanathan, S., & McGill, A. (2007). Consuming with others: Social influences on moment-to-moment and retrospective evaluations of an experience. Journal of Consumer Research, 34(4), 506–524. https://doi.org/10.1086/520074

Randolph, L., Telg, R., Rumble, J.; Galindo, S., and Lindsey, A. (2021). Measuring the Effects of Narrative and Analytical Messages in Video Production, Journal of Applied Communications, 105(1) https://doi.org/10.4148/1051-0834.2361

Stoll, J. (2022). Average daily time spent with video content among adults in the United States from 2019 to 2026, by device. Statista. https://www.statista.com/statistics/420791/daily-video-content-consumption-usa-device/

Research with millennials: Executive summary. (2014). Shugoll Research. http://www.beefissuesquarterly.com/CMDocs/BeefResearch/BIQ/Executive Summary_Revised March 18 2014.pd.

Rumble, J., Wu, Y., Tully, K., Ruth, T., Ellis, J., & Lamm, A. (2020). A Mixed-Methods Comparison of Self-Reported and Conversational Trust in Science, Journal of Applied Communications. 104(4). https://newprairiepress.org/jac/vol100/iss3/10

Rumble, J., Settle, Q., & Irani, T. (2016). Assessing the Content of Online Agricultural Awareness Campaigns, Journal of Applied Communications. 100(3). https://doi.org/10.4148/1051-0834.1233

Ruth, T., Rumble, J., Galindo-Gonzalez, S., Lundy, L., Carter, H., & Folta, K. (2019). Can Anyone Hear Us? An Exploration of Echo Chambers at a Land-Grant University, Journal of Applied Communications. 03(2). https://doi.org/10.4148/1051-0834.2242

Ruth, K., Settle, Q., Rumble, J., & McCarty, K. (2018). Predicting Likelihood to Pay Attention to Agriculture-Related Issues in the News with Demographic. Characteristics. Journal of Agricultural Education, 59(2), 49–63. https://doi.org/10.5032/jae.2018.02049

Ryan, C. D., A. J., Butner, R. & Swarthout, J. T. (2020). Monetizing disinformation in the attention economy: The case of genetically modified organisms (GMOs). European Management Journal, 38(1), 7-18. https://doi.org/10.1016/j.emj.2019.11.002

Wisenblit, J., Schiffman, L. G. (2019). Consumer Behavior. United Kingdom: Pearson Education. 9780134734828, 0134734823

Severin, W., & Tankard, J. (2001). Communication theories: Origins, methods, and uses in the mass media (5th). New York, NY: Addison Wesley Longman, Inc.

Sriprom, C., Rungswang, A., Sukwitthayakul, C., & Chansri, N. (2019). Personality traits of Thai Gen Z undergraduates: Challenges in the EFL classroom? PASAA: Journal of Language Teaching and Learning in Thailand, 57, 165-190

Settle, Q., Rumble, J., McCarty, K., & Ruth, T. (2017). Public Knowledge and Trust of Agricultural and Natural Resources Organizations. Journal of Applied Communications, 101(2). https://doi.org/10.4148/1051-0834.1007

Smyth, S., & Lassoued, R. (2019). Agriculture R&D Implications of the CJEU's Gene-Specific Mutagenesis Ruling. Trends in Biotechnology, 37(4), 337–340. https://doi.org/10.1016/j.tibtech.2018.09.004

Tarpley, T., Fischer, L., Steede, G., Cummins, G., & McCord, A. (2020). How Much Transparency is Too Much? A Moment-to-Moment Analysis of Viewer Comfort in Response to Animal Slaughter Videos, Journal of Applied Communications, 104(2). https://doi.org/10.4148/1051-0834.2302

Understanding Science. (2020). Supporting science. University of California Museum of Paleontology. http://www.understandingscience.org

United States Department of Agriculture (U.S.D.A). Definitions: Sustainability and Food Systems. (n.d.). U.S. Department of Agriculture. https://www.usda.gov/oce/sustainability/definitions

United States Environmental Protection Agency (EPA). Learn About Sustainability (2022). United States Environmental Protection Agency. https://www.epa.gov/sustainability/learn-about-sustainability.

Van Eenennaam, A. & Werth, S. (2021). The animal board invited review: Animal agriculture and alternative meats - learning from past science communication failures. Animal, 15(10), 100360. https://doi.org/https://doi.org/10.1016/j.animal.2021.100360

Van Eenennaam, A. (March 23, 2022). Misinformation in the Agricultural Sciences [Zoom Presentation]. Center of Vet Study. The University of Illinois. https://mediaspace.illinois.edu/media/t/1_k0b1s1mh

Wagner, B., & Petty, R. (2011). The elaboration likelihood model of persuasion: Thoughtful and non-thoughtful social influence. In D. Chadee (Ed.), Theories in social psychology (pp. 96–116). Oxford: Blackwell

Wheale P, Hinton D. (2007). Ethical consumers in search of markets. Business Strategy and the Environment. 16, 302–315. https://doi.org/10.1108/sd.2007.05623jad.005

Young, W., Hwang, K., McDonald, S., Oates, C. J. (2009). Sustainable consumption: Green consumer behavior when purchasing products. Sustainable Development, 18(1), 20–31. https://doi.org/10.1108/sd.2007.05623jad.005

Zauderer, Steven. Average Human Attention Span by Age. (Infographic). In-Home & Clinic-Based ABA Therapy - Cross River Therapy, Cross River Therapy. https://www.crossrivertherapy.com/average-human-attention-span.

Livestock and Forages Extension Success Stories: Programs for Alabama Stakeholders

M. Kim Mullenix *, S. Leanne Dillard, M. F. Elmore, D. Russell, M. K. Stanford, M. L. Marks, J. B. Elmore, K. Palmer, G. Thompson, A. Gotcher, R. Phillips, R. Hudson, A. Wiggins, D. Daniel, K. Kelley, M. Runge, R. Prasad, K. Kesheimer, M. Webb, Z. Brannon, J. Smitherman, B. Crow, K. Crow, C. Heaton, and S. Rodning

Alabama Cooperative Extension System, Auburn University, Auburn, AL 36849
* Correspondence: mullemk@auburn.edu

TAKE HOME MESSAGE

The Alabama Cooperative Extension System Animal Science and Forages Team delivers educational programs in the state to forage-livestock producers. Programs focus on improved management practices. Selected program highlights and team resources for stakeholders are reviewed using multi-year post-program evaluation survey responses.

1. INTRODUCTION

Our team strives to improve the livelihood of forage and livestock farmers through direct education on best management practices, communication, and relationship-building with stakeholders. This benefits Alabama communities through stewardship of animal, land, and water resources and improved food security. Resources provided by our team include one-on-one interaction with Extension agents and specialists on management topics, farm visits, educational programs, livestock sales that emphasize animal performance metrics, field days, online courses, and trainings, and both web-based and print publications. Selected programs are highlighted below using a multi-year program overview of impacts to 1) create awareness about our resources and 2) demonstrate how these programs deliver research-based, scientific information to the public.

2. PROGRAM SUMMARIES

2.1 Management-Focused Education and Field Days

Educational strategies include hands-on learning, one-day conferences, lecture-style topic meetings, and one-on-one farm visits with producers. Our Extension team partners with the Alabama Agricultural Experiment Station outlying research units and private farms to offer field days for producers. Field days provide a seasonal opportunity for producers to see "management practices in action" and are an effective tool for showcasing specific concepts to farmers. Many of our field days focus on forage management strategies to extend the grazing season or optimizing harvest as stored forage, integrated weed management, nutrient and animal management practices. We also highlight current or recently completed livestock and forage research being conducted at Auburn University. Three to six field days are offered annually in varying regions of the state to highlight management specific to the production challenges in this region of the state.

2.2. Quality Assurance Programs

Quality assurance programs are designed to provide education to producers on good animal husbandry and management practices that enhance health and sustainability of livestock production. Key programs offered in the state are the Beef Quality Assurance and Pork Quality Assurance programs. The Beef Quality Assurance program is a partnership with the Alabama Cattlemen's Association, and trains between 1,000 and 3,000 cattle producers and high school agriscience students every year, typically impacting the management of more than 30,000 head of cows, bulls, and calves, demonstrating the reach and application of this program.

2.3. Online Education

Our team provides content to the Alabama Extension website on a variety of topics related to soils, forages, nutrition, herd health, nutrient management, meat science, and economics. The website listed at the end of this publication houses our quarterly newsletter, publications, quick fact sheets, videos, and online courses where producers can learn at their own pace. Since the COVID-19 pandemic, use of the website has increased, and online courses have offered a platform for producers to find tailored information related to their interests. One success story is the Beef Basics Online Course, which was released in January 2016 but gained more traction during the pandemic to present. Since then, 2,055 students have enrolled in the course, with a 22% completion rate. Enrollment is voluntary, and the course generates a certificate of completion for 8 hours of training credit in beef management.

2.4. Value-Added Livestock Marketing

Livestock sales support education of Alabama beef producers to advance the application of best management practices, record keeping, and genetic selection tools to improve operational efficiency and profitability. The Herdbuilder Replacement Female Sale is a marketing program conducted by the Alabama Extension's animal science team and the Alabama Beef Cattle Improvement Association. The sale has two primary goals: to provide high-quality, bred replacement heifers to beef cattle producers and to add value to commercial replacement heifers to enhance profitability. From 1998 to 2021, the Herdbuilder Replacement Female Sale marketed 7,016 heifers for more than $10.1 million in economic impact. Bred heifers and a select group of open heifers have been the primary focus of the sale. In 2022, 320 bred heifers marketed averaged $1,983 per head to impact 58 beef operations directly.

In 2023, the Alabama Beef Cattle Improvement Association (BCIA) held the 51st Annual North Alabama Bull Evaluation Sale at the Cullman Stockyards in Cullman, AL. A survey of Alabama BCIA North Alabama Bull Evaluation participants from 2019–2021 was conducted to determine impacts of this program for their operation. Sixty percent of responses rated bull evaluations very valuable to their operational goals. The most selected areas of value indicated that:

1. evaluation provides an opportunity to evaluate herd genetics to performance standards (17%), and
2. evaluation provides an opportunity to market herd genetics (17%). Participation in the sale increased knowledge of performance measurements in yearling weights and ratios (80%) and foot scores (79%).

Respondents also noted an increase in knowledge of genetic selection tools in evaluation of structural correctness and soundness (46%), expected progeny differences (EPDs; 31%), and selection indices (23%). Overall, 86% of respondents stated that their

participation was beneficial and planned to participate in the future. From 2019-2023, 210 bulls were marketed in the sale ($3,419 average price per head) from 34 livestock operations, resulting in a total economic impact of $718,000.

Read the full team impact report at:

www.aces.edu/go/animalscienceteam

For more information on our programs, visit the following resources from our team:

www.alabamabeefsystems.com

www.alabamaforages.com

www.albcia.com

Check out our Facebook pages:

www.facebook.com/AlabamaForageFocus

www.facebook.com/AlabamaBeefSystemsExtension

www.facebook.com/AlabamaBCIA

Alabama Extension Events Calendar

www.aces.edu/calendar

Funding

Programs described in this manuscript are supported in part by the Alabama State Beef Checkoff Program, the Alabama Beef Cattle Improvement Association, the Southern Risk Management Education Center, the Southern SARE Program, and industry sponsors.


AUBURN
ANIMAL SCIENCES