POST-WEANING NUTRITIONAL AND MANAGEMENT STRATEGIES TO IMPROVE CATTLE HEALTH AND PERFORMANCE

By

SHANE REYES HERNANDEZ

(Under the Direction of Robert Lawton Stewart Jr.)

ABSTRACT

Three studies were conducted to measure the impact of supplementing increased metabolizable protein during the preconditioning stage, a blend of phytochemicals during a stocker phase, in addition to the use of heat stress abatement strategies on animal performance and health of recently weaned beef cattle in the Southeast United States. Study one found no difference in titer levels, regardless of duration of metabolizable supplementation, study 2 found supplementation of cinnamaldehyde, eugenol, and capsicum is not a good ionophore alternative, and study 3 found blood gas values to be a good short term stress measurement and microbiome analysis as a good long term stress measurement.

INDEX WORDS: Beef Cattle, Feed Additive, Heat stress, Phytochemical, Post-weaning, Weaning stress, Vaccines

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B.S.A, The University of North Georgia 2017

M.S., The University of Georgia

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

Doctor of Philosophy

ATHENS, GEORGIA

2023

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DEDICATION

I dedicate this work to my Papap, Jim Wolf.

You are a model of who I hope to be.

You taught me to be kind, patient, and to always care.

Thank You

ACKNOWLEDGMENTS

No space will allow me to properly acknowledge everyone who has made the last five years possible. Thank you. You are all saints for having the grace and patience required to deal with me.

To my family, this work is only possible with your love and unwavering support. I know five years of graduate school was never the plan, however, you never questioned my choice and enabled me to peruse my dreams.

To Evann, you were a pillar during the challenging times and my mountain tops during the best. But life is not the mountain tops, it's the walking in between and I love you walking next to me.

To the farm crews at all the UGA research locations, thank you for your help and for the tireless work you perform to both manage the research herds and provided an effortless research environment.

To Gina Mckinney and Eliza Lee, thank you for your willingness to work with me and more importantly, to teach me.

To my ADS family, you have made the Rhoades building feel like home.

To the 2nd floor graduate students, your work emboldens me, and I am excited by the future work you all will produce.

To Christina, we have both grown so much over these past five years, however, you have really set the bar for “Exceptional Graduate Student”. Your love and passion for your work has been

inspirational and I am proud to have worked alongside you. Thank you for pushing me to become a better scientist.

To my committee for allowing to purse my interest, regardless of where they may have led me. I would not recommend future graduate students take a similar approach, but I am grateful to you all for letting me grow as a scientist and a member of the agriculture community in my pursuit of this work. The knowledge and experience you all have provided over these past five years is almost incomprehensible. A man from Augusta without an agriculture background to six chapters of work spread across various segments of the beef cattle industry. Thank You.

To Dr. Fluharty, thank you for having an open-door policy. Even amongst your busy schedule, you would find time. I know you smirked every time I knock, thinking to yourself, "what's going on this time?", and to my surprise, you always ask me in to offer wisdom.

To Dr. Stelzleni, I know I was never an official member of your lab, but you always made me feel like I was. From chasing you down in the hallways to ask you a stats questions, or the hours spent in your office talking about science and life. You were always willing to support, challenge, and develop me as a scientist. Your love and passion for the scientific process is something I hope to emulate in my future work.

To Dr. Credille, I know I've said it before, I hope to say it again, and I hope to continue to say it in the future "Look how far we've come". You have always been willing to go out of your way to support me in both my purist to become a veterinarian and a researcher. You never doubted my ability to accomplish my goals. Despite starting with almost no hands-on experience with cattle, I never lacked confidence. Why should I? If Dr. Credille believes I can do it, then shouldn't I too?

To Dr. Callaway, I did not think that ruminant nutrition in 2018 would take us this far. Without your support this work Literally would not be possible. You have always challenged me to think, and provided opportunities to develop professionally, as well as academically. Your passion for developing the next generation of scientist has been a benefit to us all. I can't thank you enough for your time and support throughout these years.

To Dr Stewart, Thank you for everything. These years may not have always been easy, but they've sure have been fun. Your faith in me served as the foundation of all my scientific pursuits. The path we took was not always successful, but you provided the groundwork to learn from both the success and the things we weren't expecting. These years will resonate throughout my work as I continue to improve and develop in this field. I honestly, I do not have enough words or the space to properly thank you for helping me grow as a scientist. Your work serves as a benchmark for where I am and how far I can still work to be. I can't thank God enough for introducing me to Animal Science, and I can't thank you enough for helping me cultivate that passion.

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CHAPTER 1

INTRODUCTION

The old adage “more than the sum of its parts” is never truer than when speaking of the beef cattle industry. Raising beef cattle requires a diverse community of skilled practitioners that work together to provide the beef eating consumer a safe, economical, and high-quality product. While the various segments of the industry work together, there are times in which they do not always work for each other. Discrepancies between segments may cause inefficiencies in the system that can impact the safety, quality, and price of the end-product. For instance, cow-calf producers develop calves destined to be sold to a stocker or feedlot operation. Cow-calf producers, however, primarily get paid on a per pound basis, incentivizing producers to maximize calf growth over herd health. In turn, practices like vaccination are overlooked. In 2009, the USDA reported that less than 30% of all U.S. cattle are vaccinated with any vaccine designed to prevent respiratory disease (USDA APHIS, 2009). This can be a critical oversight as bovine respiratory disease (BRD) is the most common cause of morbidity and/or mortality in the feedlot (Adkins et al., 2020) and represents a $1 to $3 billion dollar loss in potential profits from treatments, loss in feedlot performance, and a decrease in carcass quality. Consequently, calves often endure a rigorous health protocol when they arrive at a stocker or feedlot operation (Snyder et al., 2019). On arrival, calves are typically vaccinated, treated for parasites, and comingled in holding pens until they are placed in a pasture or feeding pen. These protocols can be costly, and moreover, due to the stressful nature of weaning, transportation, comingling, etc. they may be

ineffective at properly mitigating future health challenges. Due to the potential loss in profit, feedlot and stocker producers are willing to pay a price premium for calves that have received a health protocol. One option for the cow-calf producer is to utilize a pre-conditioning phase post-weaning. A pre-conditioning phase is typically a 45 to 60 day period, post-weaning, that is designed to allow the producer to implement various protocols without competing with the additional stress of transportation and comingling with foreign cattle (Lalman & Ward, 2005). Results from Moriel & Arthington (2013) suggest that increasing the proportion of protein in the pre-conditioning diet improved vaccine efficacy. They did not measure, however, how long a producer must increase the protein concentration in the pre-conditioning ration. Moreover Moriel et al. (2015) observed the most critical time point for immune response and appetite depression occurred during the first 14 days post weaning. If it was possible to reduce the protein content in the diet from 60 days to 14 days, while still improving vaccine efficacy, producers would be able to glean the benefits of pre-conditioning programs while reducing the cost of the feeding program.

Stocker producers' goal is to add weight to weaned calves while maintaining a healthy herd. The profitability of a stocker operation is heavily dependent on the cost of feed and efficiently adding weight to their cattle. Many producers often use feed technologies to increase the feed efficiency of their herd. Ionophores are the most commonly used feed technology in the cattle industry (Osweiler, 2011). Ionophores are a class of anti-microbials that disrupt the normal chemo-osmatic function of gram-negative bacteria, ultimately limiting their ability to properly transport ions and eventually experience apoptosis (Bell et al., 2015). In the rumen, many of the protein-degrading bacteria belong to the gram-negative classification (Russell & Houlihan, 2003). When cattle are supplemented with ionophores, rumen available protein degradation is

slowed, increasing protein utilization and improving feed efficiency. Moreover, ionophores provide many additional benefits such as reducing methane, bloat, and can be supplemented to treat coccidia infections (Duffield & Bagg, 2000). Despite their benefits, consumer concerns over antibiotic resistance have increased the prevalence of organic and all-natural marketing streams (Napolitano et al., 2010). Producers who elect to sell their animals through all natural marketing streams can increase profitability of their operations; however, any profit gained may be offset by a decrease in weight gain due to their inability to use feed additives like ionophores (Umberger et al., 2009). While consumers are concerned about antibiotic resistance, USDA reported ionophore usage is not a risk to current human pharmaceutical options (Callaway et. al, 2003). Despite these findings, the European Union banned use of ionophores in 2006 (Florez-Caudrado, 2018) and in the United States, cattle producers are required to obtain a prescription to supplement feed with ionophores (Sneeringer et al., 2019). Due to shifts in the industry, ionophore alternatives need to be researched. Recently, essential oils have been identified as a potential organic feed supplement. Essential oils are a diverse class of plant metabolites, and some oils such as cinnamaldehyde, eugenol, and capsaicin were reported to have antimicrobial properties when measured in ruminal in-vitro studies (Cardoza et al., 2004; Busquet et al., 2006; Calsamiglia et al., 2007). While these studies report improved ruminal efficiency, further live-animal trials are required to measure the impact of essential oils compared to ionophores.

The feedlot is the penultimate stage in the beef cattle's life. The feedlot producer's aim is to develop either a high quality or high quantity product for the abattoir. To accomplish this goal, feedlot cattle are fed a high starch diet designed to provide enough energy for growth and intramuscular fat deposition. Starch diets are critical tool in developing cattle as these diets shift the volatile fatty acid profile, increasing the proportion of propionate produced. Propionate, a

three-carbon chain molecule, is an essential glucogenic compound in cattle as propionate can be used to synthesize glucose, a six-carbon chain molecule, in the liver. While feedlot producers face many challenges, one major concern impacting cattle growth in feedlots in the south is heat stress. In the deep south cattle may suffer heat stress for 9 out of 12 months of the year. Heat stress can cause negative behavioral changes that can impact the profitability of cattle. When suffering from heat stress, cattle will consume less feed to decrease endogenous heat production. Additionally, decreased intake can severely impact the profitability of feedlot cattle with Mitlöhner et al. (2001) reporting an increase in time to reach finishing weight and a negative impact on carcass quality in heat stressed feedlot steers. Current research has sought to identify management strategies that may mitigate the negative effects of heat stress, strategies that include utilizing shade structures, modulating feeding timing, and even utilizing feed technologies to modulate feed consumption. There is currently, however, a lack of research investigating the impact of heat stress and the subsequent behavioral changes, on rumen and fecal microbiome. It is currently unknown whether or not decreased intake may impact microbial function, decreasing efficiency and feed utilization, which would further increase the time to reach finishing weight. Moreover, Edrington et al. (2004) reported an increase in shedding of pathogenic bacteria during the summer months. While the direct relationship between the increase in pathogenic bacteria during the summer months has not been identified, it is important to continue to measure the fecal microbiome in heat stress cattle during the summer months and assess how different heat stress abatement strategies may impact shedding of pathogenic bacteria.

The research in this dissertation focused on post weaning management strategies to improve profitability. The first experiment in this dissertation sought to identify best-use pre-

conditioning diets to maximize vaccine efficacy and reduce feedlot morbidity and mortality. Experiment 1 was a 42 day pre-conditioning study designed to measure if increasing the protein concentration in the diet for 14 days would improve vaccine efficacy similarly to increasing the protein concentration for 42 days. To measure the impact of protein supplementation during a pre-conditioning phase, 48 spring born calves were organized into contemporary pairs based on weights and assigned to pens. Pens were randomly assigned to one of four treatment groups in which calves received either 115% recommended protein or 100% recommended protein. Calves were vaccinated at weaning and again 14 days later. Weights and blood samples were collected on d 0, 3, 7, 14, 21, 28, and 42 to measure the impact of the diet on weight gain, metabolic profile, and their serum titer profile. Results from this study will help identify a more economical pre-conditioning strategy in hopes of increasing industry adoption.

The second experiment investigated a blend of essential oils as an ionophore alternative in an 84-day, 2-year stocker trial measuring the animal performance and in-vivo volatile fatty acid production. For both years, 81 pre-conditioned steer calves were stratified by weight into 9 pens and each pen was randomly assigned one of three treatment groups: 1) No supplementation control, 2) Monensin, 3) Essential oil blend. To assess the effect of both ionophores and the essential oil blend on growth, weights were recorded prior shipping, on arrival (d 0) and every 28 days after. Additionally, rumen fluid was collected on d 0 and 84 to measure in-vivo volatile fatty acid production. This research will add to the continued search for organic alternatives to antibiotics, providing producers greater flexibility in how they manage and market their herd.

The third experiment measured the impact of heat stress on the blood-gas profile, metabolic profile, and rumen and fecal microbiome of feedlot cattle in the Southeast United States. In this experiment 32 steers were randomly assigned to one of four treatment groups:

covered with fans, covered no fans, outside with shade, and outside without shade. Blood gas and metabolic values were measured on d -10, 0, 50, and 85. Additionally feces and rumen fluid were collected on d 0, 50, and 85 for microbiome analysis. This research will further elucidate the physiological response to heat stress in feedlot steers potentially leading to greater sensitivity and specificity in diagnosing heat stress and mitigating the impacts of heat stress. Moreover, the microbiome results from these experiments may highlight possible strategies to mitigate the negative impact of heat stress on feed efficiency. Additionally, this research will increase the body of work identifying relationships between stress in cattle and the shedding of pathogenic bacteria.

The cattle industry is a dynamic system that has worked tirelessly to become ever more sustainable, efficient, and humane and our research goals should be just as dynamic to meet the ever-changing needs of the producers. It is important that current research and best practice recommendations, in any segment of the industry, are made with the utmost consideration of the entire industry. These experiments will provide producers with essential information that may be used to increase the efficiency and profitability of their own operations and better prepare cattle for their next stage in the production cycle. In summary, this research is relevant to the agriculture industry in both practical and theoretical applications in its aim to improve animal health and performance during all production stages of the calf's life.

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CHAPTER 2

THE REVIEW OF THE LITERATURE

Post weaning management is a broad term that encompasses any stage of production post weaning. This includes pre-conditioning, backgrounding, and finishing. Regardless of stage of production, management and nutritional strategies can directly relate to the profitability of an operation. If not managed, stress during the weaning stage can impact overall weight and health of the calf. Protein supplementation during weaning may improve vaccine response and decrease the length of weaning stress. Additionally, utilizing feed technology may provide producers the ability to increase feed efficiency, reducing the cost of feed. Moreover, when finishing steers in the Southeast, heat stress is a major concern. Heat stress may lead to a decrease in feedlot performance and carcass quality, increasing the cost of feed and decreasing the profitability of each animal. To identify management strategies that may be useful in addressing these issues, this literature review is designed in three parts: Protein utilization in cattle, ionophores and alternatives to alternative to ionophores, and heat stress in cattle.

Protein supplementation in cattle

In the livestock industry, feed costs are the largest cost to production (Moore et al., 2009) and it is imperative for producers to manage feed cost by utilizing economical feed rations. While there are many nutritional factors to consider when formulating a feed ration, cattle producers must pay special attention to dietary protein as protein intake can be one of the most expensive components to feed. If a producer does not supply enough protein to meet the protein

requirement, growth may be limited, and profitability may decrease. Furthermore, protein requirements can change depending on the physiological state of the cattle which may increase the protein requirement. If a producer provides protein in excess of requirements, feed cost will be high and excess protein will be eliminated, which can be environmentally harmful.

To formulate an efficient and economical feed ration, it is important to understand how dietary protein levels affect energy utilization (Oldham, 1982). Current literature clearly demonstrates a strong relationship between protein levels and energy efficiency. In general, energy utilization increases linearly as dietary protein levels increase (Schroeder et al., 2008). Dietary protein levels, however, may be affected by dry matter intake, amino acid composition, rumen digestibility, the growth stage of the animal, and the physiological state of the animal. The aim of this review is to discuss the various ways in which dietary protein levels may change, and how different levels of dietary protein may affect energy utilization.

Amino acid requirement

All animals have a dietary requirement for amino acids. There are, however, two classes of amino acids, essential amino acids and non-essential amino acids (Hou et al., 2015). Both non-essential and essential amino acids are required for maintenance functions including protein turnover and metabolism, in addition to being required for growth and immunity (Bergen, 2021). In ruminants, however, not all dietary proteins are directly utilized by the ruminant. In ruminants, dietary amino acids are first digested by the microbial community within the rumen. In turn, the microbial community synthesizes proteins for cell structures and metabolic function (Stern et al., 1979). Microbes washout of the rumen and enter the lower GI where they will be degraded, digested, and absorbed by the ruminant.

Despite the role of microbial crude protein for the production of ammino acid requirements, essential amino acid supplementation can improve overall dietary protein levels. In an experiment using finishing Holstein steers, animals were fed a diet with a low protein to energy ratio and were feed restricted to limit non-essential amino acid production. Furthermore, steers were abomasally infused with 10 g/d of all essential amino acids to ensure proper essential amino acid supply without modifying non-essential amino acid concentrations. Non-essential amino acid was supplemented, again via abomasal infusion. And steers were either: no supplementation or 3 g/d of methionine, and supplemented either 0, 1.3, or 2.6 Mcal/d of gross energy (Schroeder et al., 2006a, Schroeder et al., 2006b). The authors reported a relationship between glucose levels and amino acid utilization, suggesting that energy levels impacted protein utilization when glucose levels are low. Additionally, non-limiting amino acid may act as an additional energy source for the rumen microbiome. Their results highlight the impact of glucose levels on gluconeogenesis, or utilizing protein as an energy source when glucose is low (Ørskov et al., 1999). Furthermore, methionine supplementation decreased plasma concentrations for all other amino acids. Schroeder et al. (2006a) theorized that increased amino acid supplementation increased protein utilization and absorption, and decreased circulation concentrations in plasma.

Protein Intake

Protein intake can have a direct effect on dietary protein levels and energy utilization. Scales et al. (1974) evaluated the effect of protein supplementation in recently weaned calves on a forage-based diet. When protein supplementation was high, calves gained significantly more weight; however, body weight did not increase when protein was either limited or not supplemented at all (Scales et al., 1974). Furthermore, in a study utilizing recently weaned lambs who were fed increasing levels of protein, lambs whose nitrogen intake was limited did not

increase protein retention; and when lambs were fed high nitrogen diets, protein accretion increased (Sañudo & Alfonso, 1998).

Protein intake can also directly influence waste energy. When 2-year-old Brahman steers, weighing 342 kg, were fed four increasing levels of metabolizable protein (MP): normal recommend amounts of MP, 40% more than recommended amount of MP, 80% more than recommended amount of MP, and recommended amount of MP along with ad libitum access of pangola grass (*Digitaria eriantha*) (Chaokaur et al., 2015). There was a linear decrease in methane production and linear increase in average daily gain as MP inclusion increased (Chaokaur et al., 2015). These studies highlight a distinct relationship between protein intake or dietary protein levels and energy utilization. As protein intake increased, energy utilization increased, as less energy was lost in the form of fecal, urinary, or gas energy.

Despite the benefits of feeding protein at levels greater than maintenance, one factor that may influence the impact of dietary protein levels is the specific amino acid composition within the protein sources. These studies demonstrate that increased levels of dietary protein, and specifically amino acids, can provide increased rumen digestible protein, increasing rumen microbial protein synthesis, ultimately increasing animal energy and protein utilization. A producer may be able to take advantage of this effect by supplementing protein or amino acid sources to improve protein utilization, especially when the diet may not supply the recommended amount of protein. The impact of supplementing protein levels above maintenance, however, may be heavily influenced by the digestibility of the protein source.

Rumen Digestibility

In a feed trial utilizing ruminally cannulated yearling steers, Shain et al. (1998) fed 128 steers one of four treatments: 0%, 0.88%, 1.34%, 1.96% of urea on a DM basis to achieve crude protein level of 9.7%, 12.0%, 13.5%, or 15.0%, respectively. At the end of the 81 d feeding trial, they reported a linear increase in animal weight gain and DMI when steers were supplemented with urea, a rapidly degradable nitrogen source. There was, however, no difference between different levels of urea supplementation. These results highlight the interaction between dietary protein in the rumen and energy utilization by the host. Additionally, these results demonstrate the importance of protein digestibility. When protein is highly degradable, it may be metabolized too quickly, limiting availability to both microbes and host. While some nitrogen may be recycled as urea, a majority of excess nitrogen must be eliminated, increasing maintenance cost to eliminate excess nitrogen (Nkrumah et al., 2006; Jennings et al., 2018). In respiration calorimetry experiments, angus steer calves were fed a diet that met the nutritional requirements for growing steers and were either fed the recommended amount of crude protein, 13.8%, or excess protein, 19.5% (Jennings et al., 2018). Increased oxygen consumption and fasting heat production was reported in steers that were fed excess protein compared to steers fed a control diet (Jennings et al., 2018). Although the impact of protein quality, or degradability, on post-ruminal amino acid supply was not analyzed, these results still highlight an increase in maintenance energy when highly digestibly protein is utilized.

Growth and development

Growth and development are factors to consider when discussing the relationship between dietary protein utilization. Protein requirement, energy utilization, or both factors can change drastically depending on the stage of life the animal is in. Two key factors that change throughout the life of a ruminant animal, and all animals in general, are maintenance

requirements and production requirements. During the initial stage of life, increasing protein intake will increase protein accretion (Gerrits et al., 1996; Blome et al., 2003), although this effect may be dependent on total energy supply (Bartlett et al., 2006).

In a comparative slaughter study involving pre-ruminant calves fed an isocaloric diet with increased protein level, researchers observed that calves who were fed low protein levels deposited more fat than calves fed high protein diets (Donnelly & Hutton, 1976). In a metabolic study utilizing newborn 40 kg Holstein calves fed an isocaloric diet, Bloom et al. (2003) observed a linear increase in ADG and protein retention as protein inclusion increased. Additionally, energy deposition did not change as fecal and urinary energy did not differ between treatments. These results suggest that increased protein supplementation during early growth and development may not be wasted, rather increased supplementation increased lean muscle deposition (Bloom et al., 2003). In a follow up study, Holstein calves were fed two different isocaloric diets, 1.25% of body weight and 1.75% of body weight, with increasing levels of crude protein (Barlett et al., 2006). Bartlett et al. (2006) observed similar results to Bloom et al. (2003), in which increasing protein levels linearly increased protein retention and ADG, however, the effect was greater when calves were fed a higher energy diet. Increased weight gain and protein utilization is likely due to increased metabolic function stimulated by increased circulating levels of IGF-1, glucose, and insulin. During early development, increased protein supplementation increases IGF-1, an important growth hormone, stimulating increased protein accretion, increasing lean body weight and ADG over time. Furthermore, during early development, excess protein in the form of amino acids may decrease the energetic cost to synthesize proteins, producing a net increase in circulating glucose and insulin levels (Bloom et al., 2003).

It is important to note that as a ruminant animal ages, dietary protein levels affect energy utilization differently as lipid accretion becomes greater than muscle accretion (Pethick et al., 2001). Increased non-metabolizing tissue will reduce cellular energy production and energy demand for maintenance. As muscle accretion decreases, excess protein will either be converted to fat or eliminated, both of which will affect energy utilization by increasing waste energy.

The relationship between dietary protein and energy utilization is complex and involves many different production factors. When formulating protein into a ration, a producer or nutritionist must consider estimated protein intake, amino acid composition, protein digestibility, and even stage of growth. Depending on the stage of production, providing dietary protein greater than maintenance can potentially increase energy utilization and feed efficiency. Furthermore, utilizing non-limiting amino acid may allow producers to save on feed costs and improve the utilization of essential amino acids. Unfortunately, inclusion of high levels of protein may also increase the energy cost of waste energy and the energy required to eliminate excess waste.

Protein and immunity

Dietary protein levels can directly influence both incidence and severity of morbidity, in addition to the duration of morbidity (Duff & Galyean, 2007). In 1993, Galyean et al. measured the impact of three levels of crude protein supplementation on newly arrived calves' feedlot performance and morbidity. Calves were supplemented with either 12, 14, or 16% crude protein during the 42-d receiving period. Galyean reported a linear increase in ADG as crude protein increased, however, they reported increased morbidity in calves supplemented with 16% crude protein compared to calves supplemented with 14% protein. The morbidity results were not consistent with Fluharty and Loerch (1995), however, who conducted three trials to measure the

protein requirements for new arrival calves. In the first trial, calves were fed a diet containing 12, 14, 16, or 18% crude protein derived from either soybean meal or blood meal. While blood meal improved gain compared to soybean meal, ADG and gain to feed increased linearly with increasing levels of crude protein. They did not report any differences in morbidity across all treatment groups. During trial 2, newly arrived calves were offered 11, 14, 17, 20, 23, or 26% crude protein derived from a blend of soybean meal and blood meal and reported no difference in morbidity across all protein levels. In the third trial newly arrived steers were offered either 12.5% crude protein derived from soybean meal or offered protein derived from five different sources in a step down program in which calves were fed 23% crude protein in the first week, 17% crude protein in the second week, and 12.5% crude protein in the final two weeks of the study. Protein sources included corn gluten meal, ring-dried blood meal, spray-dried blood meal, fish meal, and soybean meal. While morbidity in the step-down program with protein derived from soybean meal was greater than the other treatment groups at 19.4%, there were no differences detected across all treatment groups. Despite the variety of protein sources used in Fluharty and Leorch (1995), some studies still suggest that the protein source may still influence immunology. Protein and energy availability dictate substrate availability for synthesis of proteins responsible in both the innate and adaptive immune response (Bertoni, et al., 2014) Additionally, dietary protein levels are one factor that influences an animal response to stress, and subsequent energy prioritization. Galyean et al. (1993) reported a study conducted by Nissen et al. (1989) in which they measured the impact of increasing levels of metabolizable protein (MP) on newly arrived feedlot calves. Calves were fed diets containing either 5.2, 6.4, 7.4, or 9.5% MP. There was a linear increase in weight gain as MP increased, in addition to

increased circulating levels of cortisol. Moreover, there was a linear decrease in morbidity as MP in the diet increased.

One factor impacting measuring the effect of protein supplementation on morbidity is compounding stress events. Cattle suffer from multiple stressors when they are weaned and transported directly to a feedlot (Duff and Galyean, 2007). Compounding multiple stress events may exacerbate immune challenges, impacting vaccine effectiveness and possibly any benefits of protein supplementation (Richeson et al., 2019). To mitigate compounding stress events, some researchers suggest separating stress events using a pre-conditioning phase. In 2013, Moriel & Arthington measured the impact of 85, 100, and 115% MP in the diet when cattle were vaccinated 7 d post weaning. While performance did not differ between diets, haptoglobin was greatest in steers fed 115% MP on d 14, by d 29 ceruloplasmin concentrations were less than steers supplemented 100 or 85% MP. These results concur with Dai and McMurray (1998) who found that a deficiency in MP may actually cause a decrease in the innate immune response, compromising the animal's ability to respond to additional health challenges. In 2015, Moriel et al. conducted a follow up study to evaluate bovine viral diarrhea 1 and 2 (BVD1 and 2), and bovine rhinotracheitis (IBR) titer levels in response to increasing levels of MP. Similarly, to 2013, calves were vaccinated 7 d post weaning and were fed a diet containing either 85, 100, or 115% MP. In this study, there were linear effects for weight gain, with steers fed 115% MP being the heaviest. Additionally, there were linear effects detected for ceruloplasmin in which steers supplemented 115% MP contained the lowest concentrations and steers fed 85% MP contained the greatest concentrations. Furthermore, BVDV-1b titer levels were greatest in steers fed 115% MP compared to 85 and 100% MP, however *M. haemolytica* leukotoxin titers were greater in calves fed 85% MP compared to 100 and 115% MP. Results between Moriel &

Arthington (2013) and Moriel et al. (2015) were variable and do not demonstrate a clear relationship between dietary protein and humoral response. Another factor that may be impacting pre-conditioning trials, and newly arrived feedlot trials, is pre-weaning calf development. Pre-weaning calf nutrition and development may impact the animal's response to stress and nutritional changes (Galyean et al., 1993). In 2020, Adkins et al. surveyed 588 recently arrived stocker cattle to identify possible relationships between blood metabolites as an indicator for bovine respiratory disease (BVD). Adkins reported that decreased levels of urea and beta hydroxy butyrate at arrival increased the likelihood of a calf to suffer from BVD. Moreover, Adkins concluded that pre-arrival rumen development and nutrition may be a key factor in mitigating respiratory disease.

Ionophores

Regardless of stage in production, feed efficiency heavily influences the profitability of a cattle operation. Rumen dynamics plays a critical role in feed digestion and energy availability. For instance, diets high in forage produce a greater Acetate to Propionate ratio (Aguerre et al., 2013). Acetate is short chain fatty acid and is a component in milk fat production and rumen epithelial tissue synthesis (Forsberg et al., 1984; Gonzalez et al., 2012). Alternatively, diets that contain greater amounts of starch promote greater propionate production with lower acetate to propionate ratios (Gonzalez et al., 2012). Propionate is a primary precursor to glucose, and moreover, improves growth performance and increases muscle development in young calves (Aguerre et al., 2013).

In addition to modulating the gut via the diet, producers may be able to utilize feed technologies to modify rumen fermentation to increase propionate production. Ionophores are a popular feed additive that can be used as a gut modulator, changing rumen fermentation end

products (Wallace et al., 1980). Ionophores modify rumen fermentation via a semi-selective mode of action against gram-negative bacteria. Ionophores diffuse into the cellular membrane of gram-negative bacteria and disrupt normal transportation of ions across the membrane. Eventually the bacteria will lyse due to its inability to maintain homeostasis of important ions like sodium and potassium. In the rumen, bacteria that degrade protein commonly belong to the gram-negative classification. Ionophores reduce the population of protein degrading bacteria, slowing down protein degradation, increasing protein utilization and decreasing nitrogen waste (Tedeschi et al., 2003). Moreover, Horton et al. (1982) reported that ionophore supplementation increases feed efficiency, decreases intake, and improves weight gain. The benefit of ionophores can depend on the diet fed to cattle, with greater effects generally reported in high starch diets compared to forage-based diets. When supplemented in a forage-based diet for 91 days, Packet et al. (2011) reported supplemented steers were 11.9 kg heavier than steers who were not supplemented. Additionally, rumen fluid collected on d 91 from supplemented steers contained greater total VFA values, greater proportion of propionate, and a lower Acetate to Propionate ratio compared to the non-treated group.

Essential oils

Despite any benefits ionophores may provide, future antibiotic use in livestock may be heavily regulated or banned all together (Florez-Caudrado, 2018). While the metaphylactic use of other antibiotics may increase the risk of building antibiotic resistant bacteria populations, ionophores are not commonly used in human medicine, and their use in the animal agriculture industry poses little risk of negatively impacting treatment options for humans (Callaway et al., 2003). While there are recent reports suggesting ionophore supplementation in cattle increases cross-resistance with other classes of antibiotics commonly used to treat illness in cattle, no

research has conclusively identified a concrete link between ionophore resistance promoting cross-resistance to antibiotics relevant to humans (Breidenstein, 2019); Pikkenaat et al., 2022). Regardless, in 2006 the European Union passed a ban on antibiotic use in the livestock industry, and while it was not approved, similar legislation was proposed in the United States (Calsamiglia et al. 2007). Additionally, there is growing consumer demand for products that are produced organically or antibiotic free, opening new marketing streams to cattle producers to increase profitability of their cattle (Napolitano et al., 2010).

Increasing regulatory pressure and consumer demand has driven research into identifying organic alternatives to ionophores as supplement to improve feed efficiency. One possible avenue to organic alternatives is essential oils and tannins extracted from plants (Cardozo et al., 2005). To identify possible interactions with rumen fermentation, Cardozo et al. (2005) used either 0.3, 3, 30, and 300 mg/L of eight different plant extracts in an in-vitro dual flow continuous culture of rumen fluid. Eight plant extracts were evaluated individually and included: eugenol, capsicum (*Capsicum annuum*), anise (*Pimpinella anisum*), cinnamaldehyde (*Cinnamonum cassia*), oregano (*Origanum vulgare*), yucca (*Yucca schidigera*), garlic (*Allium sativa*), and anethole. Capscium supplemented at 0.3 and 3 mg/L increased total VFA, acetate proportion, and decreased propionate proportion compared to the control group. Cinnamaldehyde supplemented at 0.3, 3, or 300 mg/L increased total VFA, propionate proportion, and decreased acetate proportion and acetate to propionate ration. Cardozo et al. (2005) findings concur with Busquet et al (2005) who measured the effect of garlic, monensin, or cinnamon on rumen fluid fermentation in an in-vitro study. In Busquet et al., (2005) rumen fluid was supplemented with: 1.25 mg/L or 12.5 mg/L of monensin, 31.2 mg/L or 312 mg/L of cinnamaldehyde, and either 31.2 mg/L or 312 mg/L of eugonol. While monensin was the most effective at increasing total

VFA production and decreasing the acetate to propionate ratio, garlic supplemented at 312 mg/L and cinnamaldehyde added at 31.2 mg/L increased propionate proportion similarly to monensin. Moreover, garlic and cinnamaldehyde decreased acetate proportions compared to the control. In-vitro results suggest that both garlic and cinnamaldehyde may affect rumen fermentation, especially increasing the propionate proportion of VFA production.

Despite in-vitro results, in-vivo studies have not reported performance benefits from plant extract supplementation. Vakili et al. (2015) supplemented either cinnamaldehyde or thyme to Holstein steers in a 85:15 concentrate to forage diet over a 45 d feeding trial. Regardless of supplement, there were no performance differences detected compared to the control steers, although thyme supplemented steers increased rumen propionate production. Moreover, in-vivo effects on animal performance are inconclusive when using a mix or blend of plant extracts. Tomkins et al. (2015) used a blend of plant extracts, rather than supplementing individual extracts. In this experiment, the extracts used in the blend were: thymol, eugenol, vanillin, limonene, and guaiacol. Steers were supplemented with the plant extract blend at rate of either 1 g/d or 2 g/d or supplemented with monensin at a rate of 60 mg/d or 250 mg/d. Feed intake in steers who were supplemented with monensin at 250 mg/d decreased by 18% compared to all other groups in addition to increasing propionate by 3.4% compared to the control group. The plant extract blend at either level did not differ from the control group for any metric, suggesting that this particular blend does not modify rumen fermentation or improve feed efficiency. Furthermore, Hernandez et al. (2019) compared the effects of monensin and a blend of cinnamaldehyde and eugenol on ultrasound carcass characteristics and performance in stocker cattle fed either a corn-silage diet or a grazing diet. Hernandez reported no differences between monensin, the blend of plant extracts, or control steers during the grazing trial. However, when

steers were offered the corn-silage based diet, monensin supplementation improved weight gain and ADG compared to the plant blend supplemented group, while also increasing ribeye area. These results suggest that this particular blend is not a likely alternative to ionophores during stocker phase utilizing corn silage or a grazing diet.

Heat Stress

Heat stress impacts all stages of cattle production but can severely impact the growth and profitability of finishing beef cattle (St-Pierre et al., 2003). The impact of heat stress on finishing beef cattle is a complex system that is affected by factors such as environment, management, and diet (Lees et al., 2019). Heat stress in cattle occurs when cattle are unable to efficiently dissipate heat, allowing their internal body temperature to rise (Gaughan et al., 2008).

Heat stress in cattle is measured primarily in one of two ways: Temperature humidity index (THI) and Heat load index (HLI). The THI methodology is calculated based on dry bulb temperature, humidity, solar radiation, and wind speed (Thom, 1959). While THI was originally designed to measure discomfort due to heat in both humans and cattle (Bianca, 1962) THI has served as a widely accepted measurement for heat stress in cattle, especially in dairies (Buffington et al., 1981). While THI was designed to measure heat stress, it is most widely used as an indicator for milk production. Bohmanova et al. (2006) collected hourly THI metrics from two different weather stations in dairies located in Athens GA and Phoenix AZ. Milk yield data was collected from 119 herds across both locations which resulted in over 794,388 milk yield measurements. Based on the THI, Bohmanova reported, for unit increase in THI, milk yield declined anywhere from -0.57 and -0.27 kg, although THI was not a useful tool at measuring the impact of heat on milk yield during very humid climates. Despite its historic use, THI fails to

account for additional impacts of heat accumulation such as air flow, solar radiation, and duration of time in stress inducing environments (Gaughan et al., 2008).

To account for additional factors, the heat load index (HLI) model was developed to provide greater flexibility in predicting heat stress in cattle, especially across different environments (Gaughan et al., 2002). There are several stages in the HLI: Thermal neutral (HLI < 70), Hot (77.1 to 86.0), Very hot (86.1 to 96), or extreme (HLI > 96) (Gaughan et al., 2008). Additionally, using HLI provides insight into an animal's ability to dissipate heat by accounting for the amount of time cattle have an HLI value above their threshold; this is known as accumulated heat load units (AHLU).

Acute and chronic heat stress

Regardless of how heat stress is measured, heat stress can impart physiological and behavior changes to limit the negative effects of high internal body temperatures. Initial behavioral responses include shade seeking behavior, increased respiration rate, and decreased dry matter intake (Wolfenson & Roth, 2019). One key factor influencing the thermogenic response to heat stress is the duration of stress, or time in which cattle are unable to dissipate heat (Slimen et al., 2015). During acute, or short-term heat stress, or stress in general, cattle will begin to exhibit behavior changes (Du Preez, 2000). In 2000, Gaughan et al. (2000) conducted an acute heat stress experiment on crossbred feeder cattle. The study was carried out over multiple 24-h cycles in which steers were exposed to various environmental conditions including: temperatures ranges from 24°C to 39°C and THI ranging from 52.5 to 70 classified as thermal neutral and THI ranging from 72.5 to 85.0 classified as hot. During the experiment respiration rate increased 2.8 to 3.3 breaths per minute as THI increased from ranges classified as thermal neutral to THI classified as hot. Increased respiration in cattle is often associated with quick short breaths which

will in turn increase pCO_2, blood pH, and decrease pO_2 (Schneider et al., 1988). Additionally, cattle may attempt to reduce internal temperatures by either sweating, increasing water intake, or both (Mader & Davis, 2004).

If heat stress is prolonged, and cattle are unable to dissipate heat, they will begin to experience chronic heat stress symptoms. In addition to panting or increased respiration, cattle will suffer from decreased intake in order to limit endogenous heat production (Lees et al., 2019). Moreover, to limit damage to critical organs, the circulatory system will undergo vasodilation to move heat away from the body. Increased vasal dilation in addition to dehydration and reduced dry matter intake, impact normal energy availability and delivery to vital organs (Beede & Collier, 1986). Furthermore, Guo et al. (2021) describes the correlation between heat stress and oxidative stress. When Holstein cows are exposed to thermal stressors, an increase in radical oxygen species (ROS) occur, resulting in an imbalance of ROS and antioxidants, leading to excess levels of ROS, negatively impacting milk composition and animal health. Guo et al. (2021) further discusses the perturbations in the inflammatory response of heat stressed Holstein cows. Results indicate damage to the corneum of the rumen epithelium. Spatial alterations of the granulosum and spinosum, as well as increased thickness leading to the translocation of lipopolysaccharides into the circulatory system and decreased nutrient absorption, respectively. The reduction in nutrient absorption, as well as bacteremia will repartition energy sources away from production to maintain animal health. Epithelial dilation is suggested to increase the likelihood of leaky gut and potential sepsis (Mani et al., 2012).

Heat abatement strategies

Ultimately, chronic heat stress can severely negatively impact normal metabolic functions and growth. At best, chronic heat stress can decrease efficiency and reduce growth and

profitability. At worst, chronic heat stress may lead to increased observed mortality in the feed lot (Busby, 1997). To mitigate the impacts of heat stress, producers may implement various heat abatement strategies. One option to producers is the use of shade structures, although non-natural structures can be difficult to install due to cost and labor. A survey by Simroth et al. (2017) reported that, in the high plains region, only 17% of feedlot pens offered shade. Regardless of industry adoption, research supports the use of shade structures to mitigate heat stress. While shade may not affect air temperature or humidity, it can limit direct heat accumulation by limiting direct solar radiation (Mader et al., 1999). Natural shade provided by trees, depending on height, spacing, and limb density, can provide adequate shade and increase time grazing and reduce respiration rates compared to cattle without shade offered (Hawke and Wedderburn, 1994). Even using artificial shade structures, shade coverage creates a linear effect on heat abatement when shade is offered to grazing cattle. Schütz et al. (2011) offered either no shade, 2.4 m^2 shade/cow, or 9.6 m^2 shade/cow to Holstein cattle on pasture. When cows were offered 2.4 m^2 shade, respiration rates decreased compared to cows offered no shade. When cows were offered 9.6 m^2 of shade they spent 50% more time in shade compared to cows who were offered 2.4 m^2 in addition to exhibiting less aggressive behavior. Additionally, respiration rate in steers offered 9.6 m^2 was 11 breaths per minute lower than no shade steers and respiration rate of steers offered 2.4 m^2 of shade 5 breaths per minute lower than no shade. In 2017, Veissier et al. measured the impact of shade, 10.5m^2 per cow, or lack of shade on cortisol, milk yield, and milk composition in lactating dairy cows during the summer months. When HLI reached 85 or greater, shade-seeking behavior increased by 65%. Moreover, compared to cows who were not offered shade, shade structures decreased respiration rates and panting scores. In a feedlot trial measuring the impact of shade on feedlot performance, Blain and Nsahlai (2010) offered one

group of finishing steers 2.87 m^2 of shade and another group no shade with temperatures ranging from 17°C to 38°C. After 11 d, Blain and Nsahlai reported a 1.74% increase in DMI and an increase in hot carcass weights by 8.33 kg. Additionally, in a feedlot study, Mitlöhner et al. (2002) utilized 168 finishing heifers in a feedlot study in which one group was offered a 36.6 m by 4.9 m shade structure or no shade at all. During the feeding period, heifers who were offered shade had increased ADG and DMI, although gain to feed did not differ. Additionally, post slaughter, Mitlöhner reported a 19.6% increase in carcasses grading USDA choice or better. Despite studies reporting improvement in heat stressed feed lot cattle, shade does not always mitigate the negative effects of heat stress.

Another option to producers, albeit more difficult to implement than shade structures, is utilizing fans and sprinkler systems. Fan structures increase ambient air circulation, increasing local heat capacity and heat transfer, reducing heat accumulation in nearby cattle (Garner et al., 1989). In a feedlot study, the use of ceiling fans decreases respiration rates, time spent laying down, and increased rumination time; although ceiling fans did not improve feed lot performance (Margin et al., 2017). When compared to shade, sprinkler systems decreased surface temperatures by 10.4% and decreased temperatures by 12.8% compared to no heat abatement strategy (Schütz et al., 2011). In a lactating dairy study, Turner et al., (1992) measured the impact of sprinkler and fan systems in a temperate, humid climate. Sprinklers and fans were installed above feed bunks and were designed to activate when temperatures exceeded 26.7°C. While milk production was the primary focus, rectal temperatures, ambient temperatures, and relative humidity were also measured. When offered fans, milk production increased by 2.4 kg/d and rectal temperatures decreased by 0.6°C. Despite temperatures averaging 28.1°C with a relative humidity of 69.5%, the cooling system was still able to mitigate the effects of heat stress.

This system decreased the heat load of the cow by improving heat transfer capacity of the surface of the cow by increasing evaporative potential, something that was not possible without the combination of fans and sprinklers. When sprinklers are coupled with fans, an additive effect may occur. Kendall et al. (2006) reported a 7% decrease in respiration rates in dairy cattle who were offered shade and sprinklers, compared to shade or sprinklers alone, and 60% compared to no heat abatement strategy. Moreover, body temperature and stress behavior decreased in cows offered shade and sprinklers compared to the control cows.

When managing heat stress, it is also important to consider the diet of cattle as the high-starch diets like those fed in finishing diets can produce greater amounts of heat compared to diets with lower starch or fat compositions (Morris et al., 2020). The are many ways a feedlot manager can manage heat stress without the use of shade structures and fans. Feed programs that utilize smaller more frequent feeding periods is one strategy. By limiting the amount of feed offered at one time, a manager is limiting the endogenous heat produced by cattle (Renaudeau et al, 2011). Additionally, by increasing the frequency of the feeding period, a manager can still provide similar amounts of energy as if larger meals were fed. Feeding at night is another option that can be utilized instead of or in addition to smaller meals/more frequent feeding period. Feed modulation supplements is another option to managers to mitigate the impact of heat stress in a feedlot. There are many feed modulation supplements on the market with the aim to limit feed intake to reduce the amount of endogenous heat produced. For example, an oil extract from peppers known as capsaicin has been studied as feed modulator in both cattle and pigs. In a lactating dairy cow heat-stress study, An et al. (2022) reported an increase in DMI and milk yield when supplemented with 80 mg of capsaicin over the 30-d study. Furthermore, dairy heat stress studies have reported an increase in non-esterified fatty acid (NEFA) concentrations in plasma

when suffering from heat stress; however, NEFA concentrations were lower in cows when supplemented with 80 mg of capsaicin.

While not always true, heat accumulation is often less at night due to lower black globe temperatures and may mitigate the overall duration of heat stress (Mader et al., 2006). Ideally, cattle are provided enough time during the night to release heat, this may not always be the case, especially in humid subtropical climate experienced during the summer months in the Southeast U.S.

Conclusion

In the cattle industry, feed cost will always be a concern. Regardless of stage of production, reducing feed is critical to improving profitability and decreasing the environmental impact of producing high quality animal protein. Improving efficiency, however, is a broad term and may require a multi-faceted approach across the entire industry to achieve large scale success. Finding strategies to reduce post weaning stress, and to reduce the cost of those strategies, can provide greater efficiency and growth. Utilizing organic feed supplements may help producers regain feed conversion improvements that may be lost with growing movement to limit antibiotic use in livestock production. Reducing the negative effects of heat stress in feedlot cattle reduces the time to reach a target weight, requiring less feed and cost, and strengthening the supply chain of animal protein. The research that follows addresses these issues and includes three experiments that seek to improve vaccine efficacy utilizing protein supplementation, investigate organic ionophore alternatives, and measure the physiological and microbiome response to heat stress abatement strategies.

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CHAPTER 3

POST-WEANING MANAGMENT STRATEGIES: INCREASING VACCINE EFFECTIVENSS IN RECENTLY WEANED BEEF CATTLE

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Abstract

A one year 42-d pre-conditioning study was conducted to measure the impact of differing levels of protein supplementation on vaccine efficacy. On d 0, forty-eight (N = 48) six month old calves (BW = 261 ± 0.6 kg) were weaned and separated into one of four groups: Calves receiving NRC recommended levels of protein during the entire study (**P0**), calves receiving increased protein for the first 7 days and then receiving NRC recommended levels (**P7**), calves receiving increased protein for the first 14 days and then receiving NRC recommended levels (**P14**), calves receiving increased protein for the entire study (**P42**). Diets were isocaloric and only differed in protein concentration. Calves were limit fed to ensure complete consumption of diet and were fed daily in the morning at the same time. Weight and blood were collected on d 0, 3, 7, 14, 21, & 42 to measure performance, serum metabolites, and titers response. At the end of the study, there were no treatment effects for weight gain and ADG ($P > 0.74$). Among metabolites measured, there were treatment by time interactions for only NEFA, BUN, and glucose ($P < 0.02$). There were no treatment effects detected for titers measured ($P > 0.33$) except for BVD2, in which there was a tendency for P7 calves to have a greater BVD2 titer response than P14 calves. There were no differences in BVD2 response for all other treatment groups ($P > 0.15$). Based on these results, increased protein supplementation did not improve vaccine efficacy during the pre-conditioning phase. These results, or lack thereof, could be due to the limit feeding strategy, in which animals were not provided enough energy to support both growth and immune function.

Key words: Post-weaning, preconditioning, vaccine, protein supplementation, metabolizable protein

Introduction

Southeast cow-calf producers account for 12% of all weaned calves in the United States (Short, 2001). Despite their size, Southeast producers face price discrimination in the market when selling weaned calves (Clary et al., 1986). Regional price discrimination is largely due to both the cost of transporting Southeast calves out to a feedlot or stocker operation, along with the perceived cost of treating sick cattle once they arrive at their new location. The most economically impactful illness in the feeding industry is bovine respiratory disease (BRD; Blakebrough-Hall et al., 2020). While veterinary cost can impact profitability, a decrease in animal performance and carcass quality are the most concerning. In a survey conducted by Duff and Gaylen (2007), the cost of treating BRD can cost up to $40/head, however, treating cattle 3 times can cost producers over $290 due to losses in performance. Cattle can be vaccinated to mitigate BRD (Ives et al., 2015), however, only approximately 30% of southeast cow-calf producers vaccinate for any respiratory disease (USDA, 2019). Unfortunately, even when cattle are vaccinated, they are often stressed as vaccination most commonly occurs at weaning or on arrival at a feedlot (Richeson et al., 2019). Vaccination, compounded with stress, may decrease titer levels and decrease the effectiveness of vaccines and protection against illnesses like BRD. One option available to the cow-calf producer is to implement a pre-conditioning phase post weaning. Pre-conditioning is a post-weaning management strategy that is designed to mitigate the negative effects of weaning stress while delivering a more consistent product to buyers. Utilizing a pre-conditioning phase can increase a calf's marketability and lead to increased profit per head for the producer (Blank et al., 2016). Previous research has demonstrated that pre-conditioned calves have significantly improved

immune response when compared to highly stressed and commingled sale barn calves. Moreover, Moriel and Arthington (2013) observed supplementing metabolizable protein during the post-weaning phase can lead to improved animal health and increased vaccine efficacy. Regardless of effectiveness, management practices like pre-conditioning must make economic sense to increase industry wide adoption. These value-added programs can often vary in profitability depending on cattle prices and cost of feed (Blank et al., 2016).

Current research has identified a link between nutrition and vaccination efficacy; however, one major question remains; how long should a producer provide increased supplementation to achieve greater vaccine efficacy? The economic benefit of a pre-conditioning phase may be lost if the cost of providing a greater protein supplementation over a typical 42-45-day period is greater than the added income. Further research is required to determine the best supplementation strategy along with determining the overall economic viability of supplementing calves to increase vaccine efficacy. Therefore, the objective of this study was to measure the impact of increased metabolizable protein supplementation, and duration of supplementation, on titer and metabolic values in calves that were vaccinated at weaning and fed an energy limiting diet.

MATERIALS AND METHODS

All practices and procedures used in this study were examined and approved by the University of Georgia Animal Care and Use Committee prior to the start of the study.

Animal and Diet Management

This study included 48 (n = 48) spring born steers (initial body weight 262 ± 38.7 kg) used in the 48 d post-weaning experiment. Steers were weaned in early September at the Alapaha

Range Grazing Unit (Alapaha, Ga). At weaning, steers were stratified by weight into one of 24 pens (2 steers per pen, N = 24). Pens were housed inside a covered feed barn and provided 3.65 x 5.49 m of space and bedded with sand. Pens were randomly assigned to one of four treatment groups: Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**). At weaning, d 0, cattle were dewormed, vaccinated, and tagged with activity monitors. Calves were dewormed with an orally drenched oxfendazole (Synanthic, Boehringer Ingelheim, Duluth, GA). Calves were also vaccinated against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus type 1 and 2 (BVD1, BVD2), parainfluenza-3 (PI3) via 2 mL subcutaneous injection of Bovi-Shield Gold One Shot (Zoetis Inc., New York, NY). Cattle were also vaccinated against *Clostridium* via a 2 mL subcutaneous injection of Ultrabac 7 (Zoetis Inc., New Your, NY). Fourteen days later, calves were boosted with a 2 mL subcutaneous injection of Bovi-Shield Gold 5 (Zoetis Inc., New York, NY) and Ultrabac 7. Additionally, at weaning, cattle were tagged with CowManager ear tag sensors (Harmelen, Netherlands) to measure effects of treatment on animal behavior post weaning and vaccination. Calves were fed daily, in the morning, and feed was weighed using a hanging scale (Intercomp, Medina, MN). Diets were formulated to be isocaloric, differing only in metabolizable protein content; compositions are presented in Table 3.1. Steers were limit fed at 2% BW, and diets were formulated to achieve an average daily gain of 0.6kg/day. Diets were reformulated every week post weight collection.

Animal Performance, antibodies, and blood metabolites

Unshrunk weights for each steer were collected in the morning prior to feeding on d 0, 3, 7, 14, 21, 28, 35, and 42. Full weights were collected to assess the impact of treatments on weight and average daily gain throughout the 42-d period. Additionally, 20 mL of serum was collected from the coccygeal vein on d 0, 3, 7, 14, 21, and 42. Serum was submitted to the Texas A&M University Veterinary Diagnostic lab for metabolic panel analysis which included: Serum calcium (mg/dL), phosphorus (mg/ dL), magnesium (mEq/L), albumin (g/dL), blood urea nitrogen (mg/dL), glucose (mg/dL), cholesterol (mg/dL), NEFA (mEq/L), sodium (mEq/L), potassium (mEq/L), chloride (mEq/L), sodium:potassium ratio, BHBA (mmol/L and converted into mg/dL), and NEFA:cholesterol. Additionally, serum was submitted to the University of Georgia Veterinary diagnostic lab to be test serum antibody titers against bovine viral diarrhea virus type 1 (BVD1), bovine viral diarrhea virus type 2 (BVD2), and Parainfluenza-3 virus (PI3). Serum titers were reported as the base log 2 of the greatest dilution of serum that proves complete protection of cells (Moriel et al., 2016). For seroconversion analyses, samples with serum neutralization values of < 4 were considered negative and assigned a value of 0, and samples with a neutralization value of ≥ 4 were considered positive and assigned a value of 1. Scores were then used to calculate positive seroconversion (% of steers with positive serum neutralization; Moriel et al., 2015a.)

Activity data

Steers were fitted with CowManager ear tag sensors (Harmelen, Netherland) at weaning. Sensors continuously monitored ear surface temperatures and five different metrics of daily behavior: eating, ruminating, normal movement, highly active, and inactive. Data were recorded in minutes and only days with a full 24 h of observation were analyzed.

Statistical Analysis

Data were analyzed as a stratified randomized design by performing a mixed model with the CRAN package "lme4" using the R statistical programming v4.0.2 (Vienna, Austria) within the intergraded development environment Rstudio V1.3.1073 (Boston, Ma). For all variables pen was defined as the experimental unit, and steer was used as the observational unit to determine differences across the four treatments. For weight gain and metabolic variables, treatment and day was defined as the main effect while and pen (animal) was defined as random effects. Means were separated using the CRAN package "lmertest" and were calculated using Tukey's pairwise comparison test, and results were considered significant at $P \leq 0.05$ and tendencies were considered at $P > 0.05$ and $P \leq 0.10$. For antibody seroconversion, a binomial analysis was conducted using the CRAN package "glmer" with treatment and day defined as the main effects while pen (animal) was defined as the random effect. Means were separated using the CRAN package "emmeans" and were calculated using Tukey's pairwise comparison test, and results were considered significant at $P \leq 0.05$ and tendencies were considered at $P > 0.05$ and $P \leq 0.10$.

Results

Animal performance

Weight and ADG data is reported in Figure 3.1 and 3.2 below.

There were no treatment x day interactions detected for weight gain (P = 0.75), nor were there treatment effects detected either weight gain or ADG (P > 0.84). There were day effects in which steer weights were greater on d 0 than d 7 and 14 (P < 0.01); however, as the study continued steer weights began to increase with no difference between d 0 and d 21, 28, and 35 (P > 0.83). At the end of the study, steers were heavier than steers on d 0 (P < 0.01).

Metabolic parameters

Metabolic parameter data is reported in figure 3.3 and table 3.2 below.

There were treatment x day interactions detected for non-esterified fatty acids (NEFA), blood urea nitrogen (BUN), and glucose ($P < 0.02$). Serum glucose did not differ between treatment groups on d 0 ($P > 0.46$). Between d 0 and d 3, serum glucose in P14 calves increased ($P = 0.05$), although there were no differences from d 0 and 3 for any other treatment group ($P > 0.15$). On d 3, serum glucose in P0 was lower compared to serum glucose in P7 and P42 ($P < 0.02$) while the other three treatment groups did not differ from each other ($P > 0.43$). Serum glucose in P0, P7, and P42 calves increased ($P < 0.01$) from d 3 and 7 while P14 did not differ ($P = 0.12$) between the two days. On d 7, serum glucose did not differ between P7 and P42 calves ($P = 0.67$) and both were greater than P0 calves ($P < 0.05$) but did not differ from P14 calves ($P = 0.51$). Serum glucose in P14 calves tended to be lower than P42 calves ($P = 0.08$) but did not differ from P7 Calves ($P = 0.21$). Between d 7 and 14, serum glucose in P7 and P14 increased ($P < 0.05$) while serum glucose in P0 and P42 did not differ between days ($P > 0.31$). Additionally, on d 14, P0 serum glucose in P0 calves was lower than the other three treatment groups ($P < 0.01$) while the other three treatment groups did not differ from each other ($P > 0.50$). Between d 14 and 21, serum glucose in P7, P14, and P42 ($P < 0.01$) decreased while serum glucose in P0 did not differ ($P = 0.11$) between days. Additionally, on d 21, serum glucose in P0 calves was lower than serum glucose in P7 and P42 calves ($P < 0.05$) but did not differ from serum glucose in P7 calves ($P = 0.24$). Serum glucose in the other three treatment groups did not differ from each other on d 21 ($P > 0.26$). Between d 21 and 42, serum glucose in all treatment groups increased ($P < 0.04$) and on d 42 serum glucose did not differ between treatment groups ($P > 0.23$).

Serum BUN concentrations did not differ between treatment groups ($P > 0.27$) on d 0. Between d 0 and 3, serum BUN increased from d 0 across all treatment groups ($P < 0.01$). Additionally, on d 3, serum BUN was greater in P0 calves than the other tree treatment groups ($P < 0.05$) while serum BUN did not differ between the other treatment groups ($P > 0.69$). Between d 3 and 7, serum BUN decreased in P0, P14, and P42 calves ($P < 0.01$), however, serum BUN did not differ from the two days in P7 calves ($P = 0.34$). Additionally, there were no treatment differences on d 7 ($P > 0.17$). Between d 7 and 14, serum BUN in P42 increased ($P < 0.01$), although there were no differences in the other three treatment groups between the two days ($P > 0.20$). On d 14, serum BUN in P14 calves was lower than serum BUN in P14 ($P < 0.01$) and tended to be lower than serum BUN in P0 calves ($P = 0.10$). Moreover, on d 14, serum BUN did not differ between the other three treatment groups ($P > 0.14$). Between d 14 and 21, serum BUN increased ($P < 0.01$) and there was tendency for serum BUN to increase in P7 calves ($P = 0.08$). There were no differences in serum BUN between d 14 and d 21 in P14 and P42 calves ($P > 0.21$). On d 21, serum BUN in P0 calves was lower than serum BUN in P42 calves ($P = 0.02$) and tended to be lower than P7 and P14 calves ($P = 0.07$ and $P = 0.09$, respectively). Moreover, on d 21, there were no differences in serum BUN between the other three treatment groups ($P > 0.53$). Between d 21 and 42, serum BUN in all four treatment groups did not differ ($P < 0.13$), nor were there treatment differences in serum BUN on d 42 ($P = 0.26$).

Serum NEFA concentrations did not differ between treatment groups on d 0 ($P > 0.16$). Between d 0 and 3, serum NEFA in all four treatment groups did not differ($P > 0.46$) nor were there differences in treatment groups on d 3 ($P > 0.15$). Between d 3 and 7, serum NEFA in all four treatment groups did not differ ($P > 0.14$) nor were there differences in

treatment groups on d 7 ($P > 0.13$). Additionally, between d 7 and 14, serum NEFA in all four treatment groups did not ($P > 0.12$) nor were there differences in treatment groups on d 14 ($P > 0.23$). Between d 14 and 21 serum NEFA did not differ in all four treatment groups ($P > 0.47$). Moreover, on d 21, serum NEFA tended to be greater in P0 calves compared to P42, although serum NEFA did not differ between P0 calves and P7 or P14 calves ($P > 0.23$). Additionally, there were differences in serum NEFA in the other three treatment groups on d 21 ($P > 0.45$). Between d 21 and 42, serum NEFA increased in P7 ($P < 0.01$) while the other three treatment groups did not differ between the two days ($P > 0.12$). Additionally, on d 42, serum NEFA in p7 calves was greater than the other treatment groups ($P < 0.02$) while the other three treatment groups did not differ on d 42 ($P > 0.39$).

There were no treatment x day interactions detected for calcium, phosphorus, magnesium, cholesterol, sodium, potassium, and chloride ($P > 0.18$). There were treatment and day effect for sodium ($P = 0.03$), additionally there was a tendency for treatment to affect chloride ($P = 0.06$). There were no treatment effects measured for any other metabolic variable ($P > 0.19$). For all metabolic variables measured, there were day affects ($P < 0.01$). Serum sodium levels were decreased in P42 calves compared to P7 calves ($P < 0.01$) and tended to be decreased compared to P14 calves ($P = 0.08$). Serum sodium levels did not differ between P42 calves and P0 calves ($P = 0.12$) nor were there differences between the other three treatment groups ($P > 0.12$). Additionally, across all treatment groups, serum sodium was greater on d 0 compared to d 3 but did not differ between d 3 and d 7 nor did it differ between d 7 and d 14 ($P > 0.16$). Moreover, serum sodium levels increased from d 14 to d 21 ($P < 0.01$) but decreased from d 21 to 42 ($P < 0.01$). For serum chloride, across all treatments, d 0 was greater than d 3 ($P < 0.01$) and continued to decrease until d 7 ($P < 0.01$). There were no differences in serum

chloride between d 7 and d 14, however, serum chloride levels increased from d 14 to d 21 ($P < 0.01$) and decreased from d 21 to d 42 ($P < 0.01$). Serum calcium and magnesium, across all treatments, were greater on d 0 than d 3 ($P < 0.02$) and decreased from d 3 to d 7 ($P < 0.01$). Both serum calcium and magnesium continued to decrease from d 7 to d 14 ($P < 0.01$) but increased from d 14 to d 21 ($P < 0.01$). Serum calcium and magnesium levels increased from d 21 to d 42 ($P < 0.01$). Serum potassium levels, across all four treatment groups, increased from d 0 to d 3 ($P < 0.04$), but did not differ from d 3 to d 7 ($P = 0.66$). Serum calcium levels decreased from d 7 to d 14 ($P = 0.03$), increased from d 14 to d 21 ($P < 0.01$) and decreased from d 21 to 42 ($P = 0.04$). Serum phosphorus levels, across all treatment levels, tended to increase from d 0 to d 3 ($P = 0.10$) and did not differ from d 3 to d 7 ($P = 0.67$). Additionally, serum phosphorus levels increased from d 7 to d 14 ($P < 0.01$), increased from d 14 to d 21 ($P < 0.02$), and decreased from d 21 to d 42 ($P < 0.01$). Serum cholesterol levels, across all treatments, decreased from d 0 to d 3 ($P < 0.01$) and decreased from d 3 to d 7 ($P < 0.01$), but did not differ between d 7 and d 14 ($P = 0.36$). Additionally, serum cholesterol levels increased from d 14 to d 21 ($P < 0.01$) and decreased from d 21 to 42 ($P < 0.01$).

Antibody and seroconversion results

Antibody and seroconversion data is reported in figure 3.4 and table 3.3 below.

There were no treatment x day interactions detected for any antibody measured ($P > 0.25$). Additionally, there were no treatment effects detected for BVD1, IBR, PI3, or BRS ($P > 0.33$). There was a tendency for treatment to affect BVD2 antibody results ($P = 0.06$). Moreover, for all antibodies except for BRS, there were time effects detected ($P = 0.43$ and $P < 0.01$, respectively). Serum antibody levels of BVD2 tended to be greater in P7 and P42 calves than P14 calves ($P = 0.06$) but did not differ from P0 calves ($P = 0.25$). Additionally, there

were no difference in serum antibodies between the other three treatment groups ($P > 0.15$). On d 0, all serum antibodies except for BRSV were greater on d 0 than d 7 ($P < 0.02$). Serum antibodies for BVD1 and PI3 increased from d 7 to d 14 and did not differ between d 14 and d 21 ($P = 0.38$). Serum antibodies of BVD2 and IBR did not differ between d 7 and d 14 ($P > 0.23$) and did not differ between d 14 and 21 for IBR antibodies ($P = 1.00$). Serum BVD2 antibodies increased from d 14 and d 21 ($P < 0.01$). All antibodies measured, except for BRSV, increased from d 21 to d 28 ($P < 0.01$) and d 28 to d 42 ($P < 0.04$). There were no treatment x day interactions ($P > 0.27$), treatment effects ($P > 0.64$), nor day effects ($P > 0.64$) for seroconversion percent for all antibodies measured.

Activity results

Activity data is reported in figure 3.5 below.

There were treatment × time interactions detected for non-active behavior ($P < 0.01$). During week 1, there were no difference between treatments ($P > 0.18$). Between week 1 and week 2, all treatments increased the percent of non-active behavior ($P < 0.01$). Additionally, during week 2, P7 calves spent more time being non active than P14 ($P = 0.02$) calves and tended to be less active than P42 ($P = 0.08$), however, P7 did not differ from P0 ($P = 0.21$). All other treatment groups did not differ from each other ($P > 0.23$). Between week 2 and 3, non-active behavior increased in P0 and P7 calves ($P < 0.01$) but did not differ between weeks in P14 and P42 calves ($P > 0.63$). During week 3, there were differences in non-active behavior between treatment groups ($P > 0.17$). Between week 3 and 4, non-active behavior decreased in P0, P14, and P42 calves ($P < 0.05$) but did not differ between weeks in P7 calves ($P = 0.21$). During week 3, there were no difference in non-active behavior between treatment groups ($P < 0.40$). Between week 4 and 5, non-active behavior decreased in all four treatment groups ($P <$

0.01), however, during week 5 non-active behavior did not differ between treatment groups ($P > 0.46$). Between week 5 and 6 there were no differences in non-active behavior in all four treatment groups ($P > 0.19$), nor were there any differences in non-active behavior between treatment groups ($P > 0.28$).

There were treatment × time interactions detected for active behavior ($P < 0.01$). Percent time spent active decreased in all treatment groups in week 1 ($P < 0.01$) but increased in all treatment groups in 2, 3, and 4 ($P < 0.01$). Moreover, there were no differences between treatment groups in week 1, 2 and 3 ($P > 0.14$). During week 4, active behavior tended to increase in P42 compared to P7 calves ($P = 0.09$), although P42 did not differ from the other two treatment groups, nor did the other treatment groups differ from each other ($P > 0.17$). There were no differences in active behavior between week 5 and 6 in all treatment groups ($P > 0.15$) nor were there differences between treatment groups in week 5 and 6 ($P > 0.13$).

There were treatment × time interactions for hi-active behavior ($P < 0.01$), although there were no differences between treatments during all six weeks ($P > 0.14$). Between weeks 1 and 2, hi-active behavior decreased across all four treatment groups ($P < 0.01$). Furthermore, during weeks 2 and 3, hi-active behavior decreased in P0 and P7 ($P < 0.01$) but did not differ in P14 and P42 calves ($P > 0.22$). Between weeks 3 and 4, hi-active behavior increased in P0, P14, and P42 ($P < 0.01$) and tended to increase between the two weeks in P7 calves ($P > 0.09$). Between weeks 4 and 5, hi-active behavior increased across all four treatment groups ($P < 0.01$), although between weeks 5 and 6, hi-active behavior increased in P0, P7, and P42 calves ($P < 0.02$) while hi-activity in P14 calves did not differ between weeks ($P = 0.92$).

There were treatment × time interactions for rumination ($P < 0.01$). During the first three weeks, all treatments were similar ($P > 0.29$), however precent time spent ruminating decreased across all treatments in week 2 and increased in week 3 ($P < 0.01$) Between weeks 3 and 4, rumination increased in P42 calves ($P < 0.01$) but did not differ between the other three treatment groups ($P > 0.13$). During week 4, rumination tended to be greater in P7 calves than P42 calves ($P = 0.09$) but did not differ from the other two treatment groups nor did the other two treatment groups differ from each other ($P > 0.17$). Between weeks 4 and 5, rumination increased across all treatment groups ($P < 0.01$), however, during week 5 there were differences in rumination across all four treatment groups ($P > 0.15$). There were no differences in rumination between weeks 5 and 6 across all four treatment groups ($P > 0.13$), additionally, during week 6 there were no differences in ruminations across all four treatment groups ($P > 0.25$).

There were treatment × time interactions for surface temperature ($P < 0.01$). Surface temperatures decreased in all treatment groups between weeks 1, 2, 3, and 4 ($P < 0.01$) and increased during week 5 to 6($P < 0.01$). Moreover, temperatures did not differ between treatment groups during week 1, 2 and 3 ($P > 0.12$). During week 4, temperatures in P14 calves was greater than P42 calves ($P = .03$) and tended to be greater than P0 calves ($P = 0.10$), although temperatures in P14 calves did not differ between P7 calves nor did the other three treatment groups differ from each other ($P > 0.22$). During week 5, there was a tendency for P14 calves to have greater temperatures than P42 ($P = 0.07$), although P14 calves did not differ from the other two treatment groups nor did the other three treatment groups differ from each other ($P > 0.13$). During week 6, temperatures in P14 calves were greater than P42 calves ($P =$

0.02) and tended to be greater than P0 (P = 0.06) but did not differ from P7 calves (P = 0.12). The other three treatment groups did not differ from each other (P > 0.41).

Discussion

Stress is a dynamic condition that if not managed, can cause serious negative effects on health and performance (Moberg, 2000). During weaning, cattle may experience stress from a variety of sources, which may lead to behavior changes like decreased feed intake. A decrease in energy and protein may impact normal animal maintenance like the normal immune response and antibody production in response to vaccines (Hay et al., 2016). The downward cascade of health is caused by a variety of events that can be compounded by a lack of nutrients due to decreased feed intake. During this acute stage, bioenergetic changes occur, prioritizing glucose to support immune function, rather than growth (Baumgard and Rhodes, 2012). Moreover, when energy and protein intake is low (i.e. during weaning or immune challenges) amino acids are utilized as an energy source via lipolysis and gluconeogenesis; this process limits amino acid availability for antibody production and reduces the vaccine competency (Esposito et al., 2013) of cattle.

One strategy to mitigate the negative effects of weaning and vaccination is to increase the protein content in the diet. In theory, increasing protein in the diet may support both growth and immune function via gluconeogenesis and increased amino acid supply. In 2013, Moriel & Arthington measured the impact of increasing levels of metabolizable protein (MP) on blood plasma metabolites in addition to the APP response during a 42-d preconditioning study. Steers were weaned on d 0 and vaccinated on d 7. During the study, BUN was greatest in steers supplemented with 115% MP, followed by steers supplemented 100% MP while plasma in steers supplemented with 85% MP contained the lowest levels of BUN. Moreover,

the acute phase response increased sharply after the vaccination event, although plasma from steers supplemented with 115% MP contained the greatest haptoglobin levels compared to the other two, while also containing the lower ceruloplasmin compared to the other two. In 2015, a follow up 42-d precondition study was conducted in which steers were vaccinated 14 d post weaning (Moriel et al., 2015). Similar to Moriel & Arthington (2013), in 2015, BUN in steers supplemented with 115% MP were greater than steers supplemented with 100% and 85% protein. Additionally, Moriel et al. (2015) also observed the largest spike in acute phase protein production during the first 7 d post vaccination, however, unlike the 2013 study, did not observe differences in ceruloplasmin production and reported that cortisol in steers supplemented with 100% MP were greater than steers supplemented with 115% and 85% MP only on d 14, after which there were no differences between treatment groups. Additionally, serum antibodies titers for BVD1-b steers fed a diet with 115% MP, although M. *haemolytica* antibody titer were greater in steers supplemented with 85% MP compared to 100% and 115% MP. In both studies, the diets were isocaloric, differing only in MP content, and did not affect weight gain. While both studies did not vaccinate at weaning, results suggest that increasing MP in diet improved titer levels, although APP results were inconclusive in addition to both studies not measuring metabolic variables like glucose or minerals.

In this study, glucose concentrations in serum from P0 calves were lower than P7 and P42 calves on every day measured. While it is not clear why P14 calves did not differ from P0 calves, decreased glucose concentrations can indicate a metabolic and/or immune challenge. Additionally, on d 42, NEFA concentrations were 42% greater in P7 compared to the other three treatment groups. When cattle were challenged with a lipopolysaccharide (LPS) infusion, Kvidera et al. (2017) reported a 100 mg/dL decrease in glucose compared to control cows, in

addition to a 46% decrease in NEFA compared to control cows. While glucose change was not as dramatic in the current study compared to the results observed in Kvidera et al. (2017), the steers in this study were not challenged by LPS. Moreover, BUN only differed on d 3, 14, and 21 in which BUN in P0 calves on d 3 was approximately 2 mg/dL greater than the other three treatment groups. By the end of the study, there were no differences in BUN between groups. Changes in BUN were expected as supplemented MP decreased as P7 and P14 steers were transitioned into a diet with lower MP, however P0 calves containing greater BUN during d 3 and lacking difference between P42 calves and the other three groups was not expected. Additionally, these results were dissimilar to the work in Moriel et al. (2013, 2015) in which a clear separation was observed between the three supplementation groups from the start of the pre-conditioning phase. While unexpected, the difference between the results observed in the current study and previous work may be caused by differences in feeding strategies. Steers in the current study were limit fed to ensure complete consumption of diet. By limit feeding the diet, however, steers may not have received enough energy from the diet to meet the requirement for their targeted growth, requiring increased energy production via gluconeogenesis, reducing available protein supply. Furthermore, antibodies titers may have been affected similarly in which there were no difference in titer levels across all treatment groups. The results in this study concur with antibody results observed in Spore et al., (2018). To improve immunity and growth during the post-weaning phase, Spore et al. (2018) provided increasing levels of energy, 0.99, 1.10, 1.21, and 1.332 Mcal Ne_g / kg DM, however, limit fed the diets at 100%, 95%, 90%, 85% to ensure isocaloric intake of diets. While serum in steers that did not get sick contained greater levels of haptoglobin and titer levels for BVD1 and IBR, there were no diet differences in any metabolic or antibody measured.

In a feedlot study utilizing activity tags to measure health status, Marchesini et al. (2018) examined the relationship of rumination and activity on BRD status in 214 finishing steers. Six days before clinical signs of BRD, using rumination as a predictor had a sensitivity of 0.81 when rumination decreased by at least 9% of daily activity. Rumination is dictated by a variety of factors that include eating behavior and feed quality (Watt et al., 2015; Gentry et al., 2016). In terms of morbidity, however, a decrease in rumination is most likely associated with a decrease in feed intake (Watt et al., 2015). Additionally, Marchesini et al. (2018) reported limited predictive ability when using activity as a sign of morbidity. When activity decreased by 7%, the model sensitivity was 0.44 and when activity decreased by 11% the model selectivity was 0.26. Using activity as a metric of BRD may be challenging, as it is a broad behavior that includes walking, socializing, and eating (Hillman et al., 2005). The use of activity sensors was relatively novel in a study. In the current study, rumination decreased by 50% in all treatment groups between week 1 and 2, which concurs with previous research and reaffirms the negative impact of weaning and vaccination on feed intake. As the current study progressed, rumination increased in all treatment groups, highlighting recovery from the stress and immune event. A lack of difference between treatment groups, however, may also be explained by limit feeding the diet, as groups were offered approximately 2% of body weight and not fed ad libitum. Additionally, active behavior increased in P7 steers, compared to all other groups after d 7. Change in activity could be indicative of improved health and increasing socialization; however, activities such as increased standing and vocalization have been reported to have a positive relationship with stress, both during mastitis and heat stress (Galán et al., 2018). Regardless of lack of treatment differences detected, the use of activity

tags appeared to appropriately model the behavior effects of steers suffering from weaning stress and vaccination.

Conclusion

Mitigating post weaning stress will be a preventive step to limit BRD incidence in feedlots, leading to improved performance, quality, and profitability. Moreover, prevalent use of metaphylaxis has led to increased prevalence of antibiotic resistance bacteria. Due to the benefits of developing healthy cattle pre-arrival at the feedlot, cow-calf producers may earn a premium on conditioned cattle. It is important to note, that pre-conditioning programs must be cost effective in addition to productive. This paper's aim was to evaluate vaccine efficacy via MP supplementation. Additionally, this paper sought to measure how many days of MP supplementation would produce the greatest benefits to titer levels. In this study, serum glucose levels were lower in P0 calves during d 0 to d 14. While not solely indicative of stress or an immune challenge, decreased serum glucose levels may suggest decreased energy availability for growth and maintenance. Furthermore, activity data may be used as an indication of negative impacts of weaning and vaccination stress. Most importantly, there were no differences in titer levels or seroconversion across all treatments. These results do not concur with previous work, however, unlike Moriel & Arthington (2013), this study vaccinated on weaning and limit fed diets which may impact campions to. While separating the weaning and vaccination events should reduce the immune and stress challenge, vaccinating at weaning has a broad application as results may also be applied to instances in which vaccination events are stacked with other stress events such as transportation and comingling. Ultimately, further research is required to identify possible nutritional strategies to mitigate weaning stress and improve vaccination effectiveness.

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Table 3.1 Chemical composition of base diets for steers throughout a 42-d pre-condition trial

	Diet[1]	
Ingredients, % DM	High Protein	Control
Corn silage	29.81	30.33
Rolled Corn	16.73	15.67
Dried Distiller Grains	35.58	35.70
Soy-bean meal	14.24	6.42
Corn Gluten feed	3.63	11.88
DM, %	63	63
NDF, %	25	31
TDN, %	79	76
ME, Mcal/kg	1.26	1.22
NE_m, Mcal/kg	0.84	0.80
NE_g, Mcal/kg	0.55	0.51
CP, %	20.6	17.2
Fat	3.7	3.8

[1] Diets were formulated to achieve either 110% of NRC recommended metabolizable protein levels (High Protein) or 100% of NRC recommended metabolizable protein levels (Control)

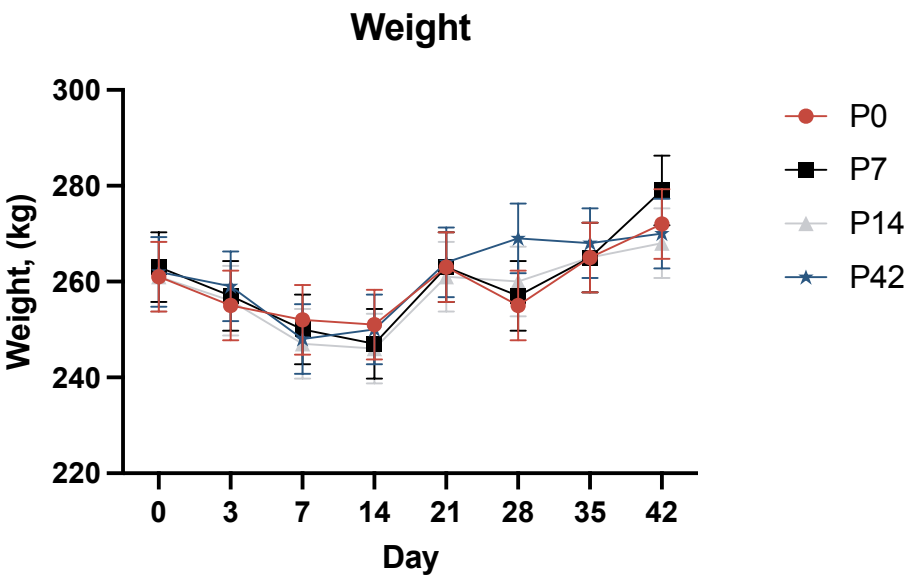

Weight
300
280
260
240
220
Weight, (kg)
0
3
7
14
21
28
35
42
Day
P0
P7
P14
P42

Figure 3.1. Average weight for each treatment at 8 time points. Weights were collected from 48 steers who were paired into pens (n = 24). Each pen was randomly assigned one of four treatments: Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**).

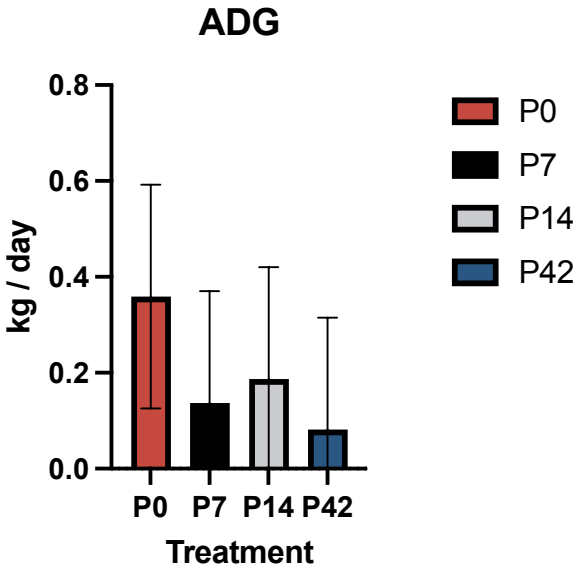
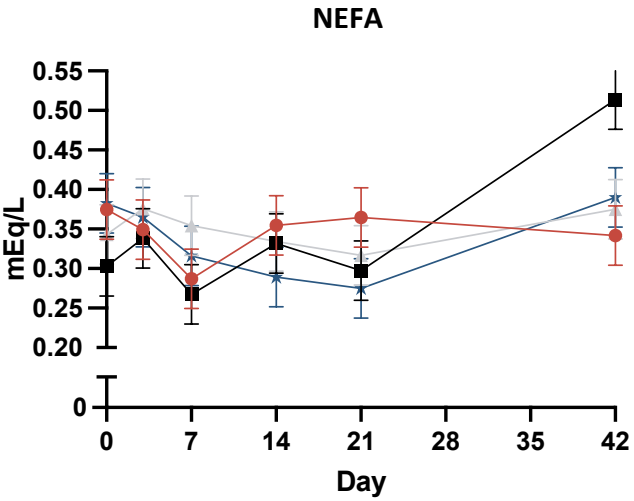
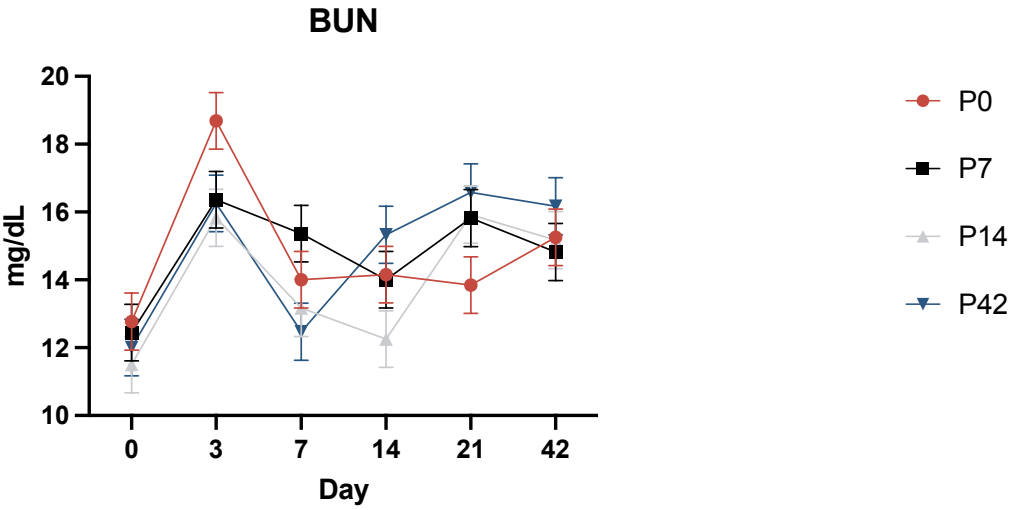

ADG
0.8
0.6
0.4
0.2
0.0
kg / day
P0
P7
P14
P42
P0
P7
P14
P42
Treatment

Figure 3.2. Average daily gain for each treatment over a 42-d preconditioning trial. Weights were collected from 48 steers who were paired into pens (n = 24) across 8 days. Each pen was randomly assigned one of four treatments: Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**).


NEFA
mEq/L
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0
0
7
14
21
28
35
42
Day


BUN
mg/dL
20
18
16
14
12
10
0
3
7
14
21
42
Day
P0
P7
P14
P42

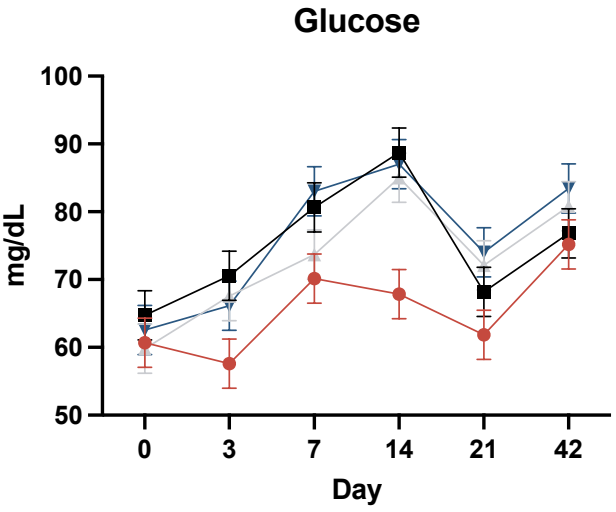

Glucose
mg/dL
100
90
80
70
60
50
0
3
7
14
21
42
Day

Figure 3.3. Average measurements for **a)** non esterified fatty acids (NEFA), **b)** blood urea nitrogen (BUN), **c)** and glucose for each treatment at 6 time points. Serum data were collected from 48 steers who were paired into pens (n = 24). Each pen was randomly assigned one of four treatments: Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**).

Table 3.2. Metabolic profile of steers[1]

	Treatment[2]							
	P0	P7	P14	P42	SE	Treatment	Time	Interaction
Metabolite, mg/dL								
Calcium	9.69	9.80	9.70	9.64	0.10	0.57	< 0.01	0.38
Phosphorus	8.12	7.85	7.87	7.68	0.27	0.41	< 0.01	0.23
Magnesium	1.90	1.86	1.93	1.94	0.04	0.19	< 0.01	0.19
Cholesterol	106.9	114.0	107.9	122.1	7.00	0.29	< 0.01	0.73
Sodium	138.4	139.2	138.5	137.5	0.66	0.03	< 0.01	0.20
Potassium	4.64	4.71	4.63	4.57	0.09	0.47	< 0.01	0.19
Chloride	99.8	97.1	97.0	96.8	0.57	0.06	< 0.01	0.18

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1] Data collected from forty-eight steers, with two steers per pen (n = 24)

[2] Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**).

Table 3.3. Titer seroconversion profile of steers

	Treatment[2]							
	P0	P7	P14	P42	SE	Treatment	Time	Interaction
Seroconversion, %								
BVD1	53.5	55.6	52.8	55.6	1.84	0.79	0.96	0.82
BVD2	43.7	36.1	31.9	38.9	1.42	0.64	0.73	0.27
IBR	15.5	11.1	11.1	15.3	1.07	0.87	0.66	0.90
PI3	66.2	73.6	63.9	69.4	1.30	0.86	0.12	0.64

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1] Data collected from forty-eight steers, with two steers per pen (n = 24)

[2] Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**).

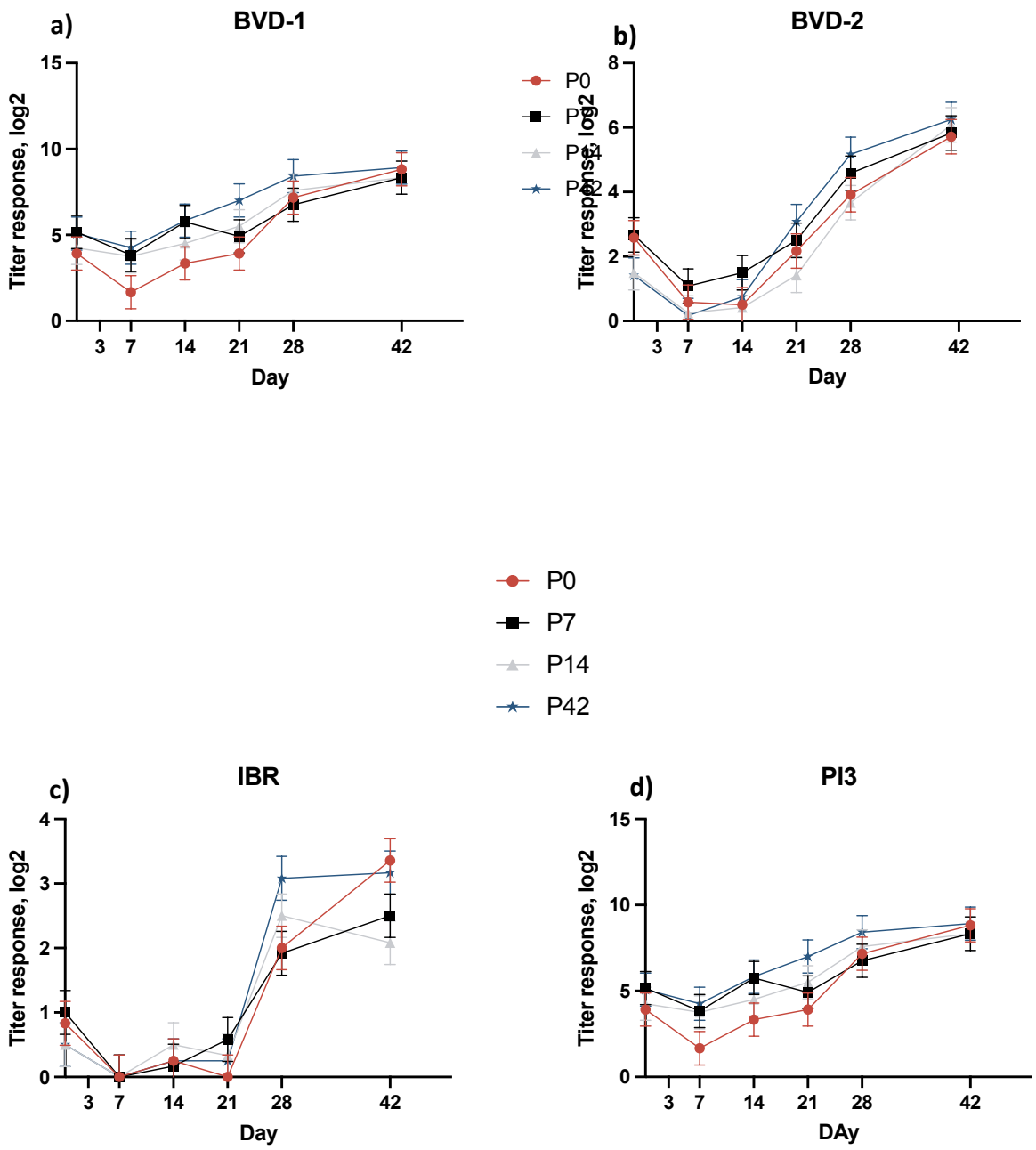
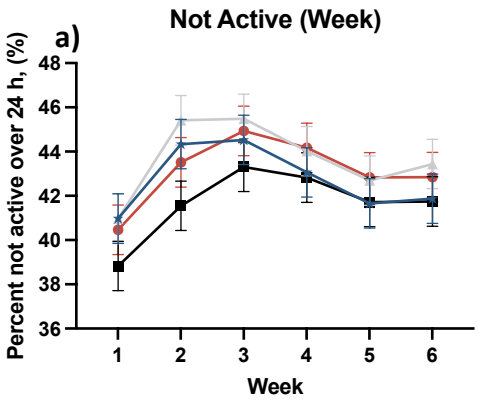
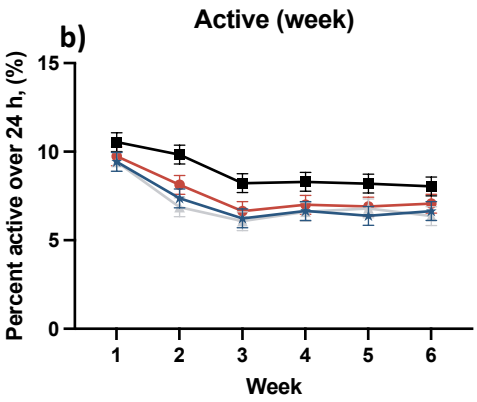
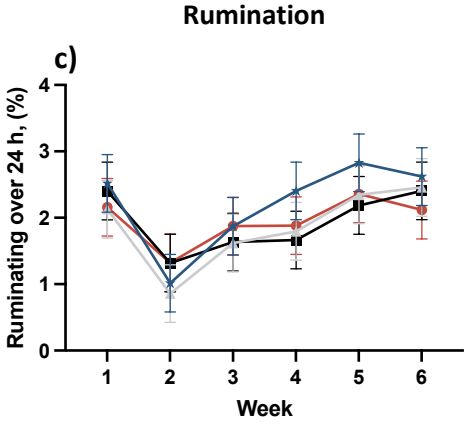
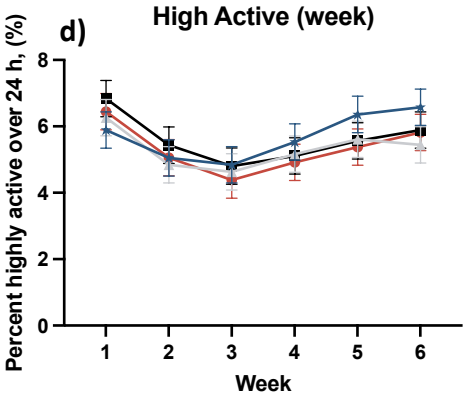

a)
BVD-1
Titer response, log2
Day
b)
BVD-2
Titer response, log2
Day
P0
P7
P14
P42
c)
IBR
Titer response, log2
Day
d)
PI3
Titer response, log2
DAy

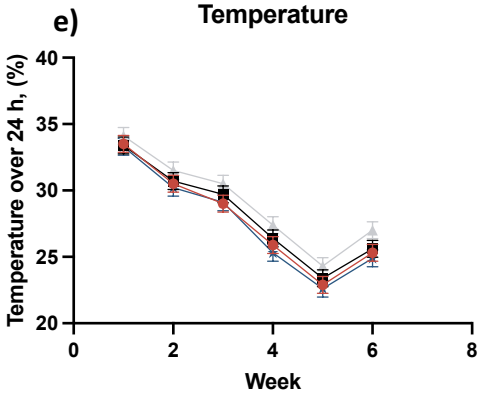
Figure 3.4. Average titer levels for **a)** BVD-1, **b)** BVD-2, **c)** IBR, and **d)** PI3 for each treatment at 6 time points. Serum data were collected from 48 steers who were paired into pens (n = 24). Each pen was randomly assigned one of four treatments: Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**).


a)
Not Active (Week)
Percent not active over 24 h, (%)
48
46
44
42
40
38
36
1
2
3
4
5
6
Week


b)
Active (week)
Percent active over 24 h, (%)
15
10
5
0
1
2
3
4
5
6
Week


c)
Rumination
Ruminating over 24 h, (%)
4
3
2
1
0
1
2
3
4
5
6
Week


d)
High Active (week)
Percent highly active over 24 h, (%)
8
6
4
2
0
1
2
3
4
5
6
Week


e)
Temperature
Temperature over 24 h, (%)
40
35
30
25
20
0
2
4
6
8
Week

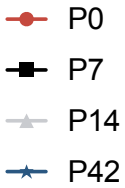

P0
P7
P14
P42

Figure 3.5 . Average activity levels for **a)** non-active, **b)** active, **c)** rumination, **d)** high-active, and **e)** temperature for each treatment at 6 time points. Activity data was continuously collected over the 42-d pre-conditioning trial and only days with data for a 24 h were considered. Data were averaged by week and were collected from 48 steers who were paired into pens (n = 24). Each pen was randomly assigned one of four treatments: Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**).

CHAPTER 4

POST-WEANING NUTRITION STRATEGIES: IN VITRO AND IN VIVO ANALYSIS OF PHYTOCHEMICAL SUPPLEMENTATION ON RUMEN FERMENTATION AND ANIMAL PERFORMANCE IN SOUTHEAST U.S. STOCKER CATTLE

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Abstract

This study was conducted across two studies designed to measure the in vitro and in vivo effects of a proprietary blend of phytochemicals on rumen fermentation and animal performance. In experiment 1, thirty-six 250 mL flasks were used to measure dry matter disappearance at 0, 24, and 48 h (6 flasks per treatment per time point). Additionally, fifteen 250 mL Ankom bottles fitted with gas production modules were used to measure gas production and pH at 0, 4, 8, 12, 24, and 48 h (5 flasks per treatment). In experiment 2, One hundred and sixty steers, across two years, were used in an 84-d stocker trial. Steers were stratified by weight into one of nine pens, and pens were randomly assigned to one of three groups with: no supplementation control (CON), monensin supplementation at a rate of 200mg•hd^{-1}•d^{-1} (MON), and a blend of cinnamaldehyde, eugenol, and capsicum supplemented at a rate of 1g•hd^{-1}•d^{-1} (PCB). All cattle received the same base ration of corn silage-based diet, supplemented with dried distillers' grains at 1.81 kg per head per day. Additionally, rumen fluid was collected from seventy-two steers, thirty-six from each year (12 steers per treatment per year). Samples were collected on d 0 and again at the end of the trial (d 84) to assess differences in VFA profiles. VFA concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate were analyzed by gas chromatography. During experiment 1, gas production was similar between MON and PCB bottles ($P = 0.97$) and both differed from CON bottles ($P < 0.01$). Moreover, dry matter disappearance at 24 h did not differ between MON and PCB supplemented bottles ($P = 0.92$), but disappearance in both bottles were greater than CON bottles ($P < 0.03$). During experiment 1, MON supplemented steers weighed more than CON steers at the end of the study ($P = 0.02$), and

PCB did not differ from the other two groups ($P > 0.20$). Additionally, MON supplemented steers had greater ADG and feed to gain during the whole study compared to PCB and CON steers ($P < 0.01$) and the other two groups did not differ from each other ($P = 0.33$). These results suggest that the phytochemical blend may modulate rumen fermentation, although this effect may be diet dependent or different than ionophores.

Key words: Phytochemical, Ionophore, monensin, garlic, cinnamon, capsicum, secondary plant metabolites, essential oils, organosulfur compounds.

Introduction

Ionophores are a common feed technology in the cattle industry and have been since the approval of monensin in 1975 (Osweiler, 2011). Ionophores, classified as an antibiotic, are also used as a growth promoter, gut modulator, and a prophylactic supplement (Wallace, 1995). These compounds interact with the cellular membrane of gram-positive bacteria, disrupting the normal ion concentration gradient and normal metabolic functions (Bell et al., 2015). Furthermore, the specificity of ionophores for gram-positive bacteria creates a flora shift within the rumen, affecting normal volatile fatty acid production, increasing propionate concentrations while decreasing acetate production (Bell et al., 2015). Additionally, ionophores provide animal performance benefits, decreasing methane production while also increasing average daily gain, feed efficiency, and apparent digestibility (Duffield et al., 2015, Vendramini et al., 2015). Furthermore, ionophores, depending on diet, can mitigate acidosis and limit incidents of bloat (Bell et al., 2015).

Regardless of their benefits, there are many concerns regarding antibiotic use in agriculture. Bacteria can develop resistance to antibiotics, rendering the drug ineffective (McEwen, 2007). Resistance to ionophores, however, has been observed in all cattle operations, regardless if the cattle were supplemented with ionophores or not (Russell, 2003); however, studies found no evidence that ionophore usage is a risk to current human pharmaceuticals options (Callaway, 2003). Despite this, the European Union (**EU**) banned the use of ionophores in 2006 (Florez-Caudrado, 2018), and a similar proposal was considered in the U.S. but was ultimately not included in the Animal Drug Availability Act (Steede et al., 2019) Despite the lack of regulatory limitations, consumer concerns over antibiotic use in agriculture increased the prevalence of organic and all-natural marketing streams. Producers who elect to sell their animals through these marketing streams can increase the profitability of their operations, however, they cannot use antibiotics like ionophores (Umberger et al., 2009). The EU ban, along with consumer awareness, has motivated researchers to look for alternatives to ionophores.

Plant metabolites have historically been utilized as pharmaceuticals due to their antifungal and antimicrobial activity (Voda et al., 2003), and researchers identified these compounds as a likely "ionophore alternative". Phytochemicals such as phenols, terpenes, and organosulfur compounds, are synthesized by plants as protection from environmental challenges like competing plants and scavenging animals (Pagare et al., 2015). Recently, phytochemicals were investigated in livestock operations due their antioxidant activity and possible gut health benefits (Beretta et al., 2017). Like ionophores, phytochemicals modify ruminal fermentation, decrease rumen protein degradation, and reduce methane production (Cardoza et al., 2004). Despite the potential benefits of phytochemicals, there is little research evaluating the benefits of

essential oils and organosulfur compounds in stocker cattle in the southeast. Therefore, the objective of this study was to evaluate the impact of a blend of cinnamaldehyde, eugenol, and capsicum on rumen fermentation and gas production in stocker cattle in the Southeast United States.

MATERIALS AND METHODS

All practices and procedures used in this study were examined and approved by the University of Georgia Animal Care and Use Committee prior to the start of the study.

Experiment 1

Ruminal fluid collection and bottle preparation

Rumen fluid was collected following the procedures outlined in Davis et al. (2021). Briefly, 3 ruminal cannulated steers, housed at the University of Georgia's J. Phil Campbell, Sr. Research Station (Watkinsville, GA), were utilized as rumen fluid donors for collection. From the cannulated steers, rumen contents were strained through paint strainers (Reaves and Company, Durham, NC) into a pre-heated (39°C) one-liter thermos. One liter of ruminal fluid was collected from each steer and transported back to the laboratory. The 3 mL of rumen fluid collected was then pooled into 6 mL of (33% v/v) anoxic media composed of 292 mg of K_2HPO_2, 240 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4000 mg of Na_2CO_3, and 600 mg • L of cysteine • HCl. Two-hundred mL of mixed rumen media were anaerobically transfer to O_2 free, CO_2 flushed bottles. To measure gas production and pH, an Ankom RF gas production system (Ankom technology, Macedon, NY) was used along with gas production modules (RF1X, Ankom technology,

Macedon, NY) that fit 250 mL bottles. Production modules wirelessly measure gas production every 5 min and data were recorded in psi locally to Excel (Microsoft, Redmond, WA) on an adjacent computer. Modules also permit anaerobic collection of fluid via a portal septa. Ankom bottles were prepared in five replicates for each treatment (n = 15). Ankom bottles were placed into the Ankom RF Gas Production system to incubate for 48 h at 39°C and 127 revolutions per minute to continuously measure gas production. Fluid was collected via portal septa at 0, 4, 8, 12, 24, and 48 h to measure changes in pH.

To measure dry matter disappearance, 250 ml Erlenmeyer flasks were prepared in six replicates for each treatment at each time point (n = 36). For each bottle, F57 bags (Ankom technology, Macedon, NY) were fill with 500 mg of dried corn silage that was grinded down to a particle size of 1 mm. Flasks were mixed with O_2 free CO_2 for ten minutes and then filled with inoculum that was prepared as described above, after which the bottles were sealed. Flasks were placed into a water bath at 39°C and incubated for 24 and 48 h.

In vitro Dry matter disappearance, Gas production, and pH analysis

For gas production, VFA, and pH analysis, bottles were incubated at six time points 0 h, 4 h, 8 h, 12 h, 24 h, and 48 h. Gas production was continuously measured throughout the 48-h time period. Bottles were removed at each time point and 10mL of fluid were extracted via syringe from an airtight rubber seal to measure fermentation, and pH (pH 11 Series, Cole-Parmer® Scientific; Vernon Hills, IL). After measuring pH, each fluid sample was immediately frozen at -20°C until further analysis. In vitro dry matter disappearance (IVDMD) was measured at 0 h, 24 h, and 48 h. Bags were removed from each flask and rinsed with cold water until rinse

water appeared clear and dried in an oven at 60°C for 24 h. After drying, bags were weighed, and IVDMD was calculated. Blank bags were utilized and used as a correction factor. To calculate dry matter disappearance, corn silage was oven dried at 65°C overnight. Corn silage was then ground down to 1mm particle size. F57 bags were first weighed without the diet, and then again after 500 mg of dried 1mm corn silage was added. Post inoculation, bags were cleaned, and oven dried at 65°C overnight. Dry weight of bag was recorded, and the original empty bag weight was subtracted to assess remaining substrate weight. Disappearance was then calculated as

$$\frac{Weight\ of\ Substract\ remaing}{Weight\ of\ original\ substrate\ added} * 100 = Disappearance\ \%$$

Experiment 2

Animal and Diet Management

Experiment 2 was conducted over two years and included 162 (n = 81 per year) spring born steers (276 ± 5.6 kg) that were used in the 84-d stocker experiment. Steers were weaned in early September at the Eatonton Beef Research Unit (Eatonton, Ga), vaccinated with Triangle 4, Type II BVD, Ultra Vac 8 (Fort Dodge Animal Health, Overland Park, KS), and dewormed with transdermal ivermectin (Pfizer, New York, NY). In late October, steers were transported to the Georgia Mountain Research and Education Center (Blairsville, GA). On arrival, steers were weighed and stratified by weight into one of nine pens (n = 18, 9 per year). Pens were then randomly assigned one of three treatments: 1) No supplementation control (CON), 2) Monensin (Rumensin, Elanco Animal Health, Greenfield, IN) supplementation at a rate of 200mg • head • day^{-1} (MON), 3) a proprietary blend of cinnamaldehyde, eugenol, and capsicum (Xtract, Pancosma, Rolle, Switzerland) supplementation at a rate of 1g • head • day^{-1} (PCB). During the

84-d experiment, cattle were fed corn silage-based diet supplemented dried distillers grains at 1.8 kg•steer^{-1}•day^{-1}. Both diets' chemical composition and supplemental mineral mix are presented in Table 4.1 and 4.2 below.

Animal Performance and Volatile Fatty Acid analysis

Full weights for each steer were collected in the morning prior feeding on d 0, 28, 56, and 84. Rumen fluid was collected by esophageal tubing from 72 steers (n = 24 per treatment) during both years at the start of the study, and again at the end of the study. Rumen fluid was collected directly into 50 mL conical tubes, transported on ice, and stored at -80°C until further processing. Concentrations of volatile fatty acids (**VFA**) in steers' ruminal fluid were determined in a water-based solution using ethyl acetate extraction as described by Lourenço et al. (2020). Ruminal fluid samples were removed from the freezer and allowed to thaw at 20°C. Five-milliliters of rumen fluid were centrifuged for 10 min at 10,000 ×*g* at 4°C, 2.5 mL of the supernatant was transferred to another centrifuge tube, and samples were frozen overnight in a -20°C freezer. The following day, samples were thawed at room temperature and centrifuged for 1 min at 10,000 ×*g* at 4°C. One milliliter of supernatant was transferred to a vial, mixed with 2 mL of ethyl acetate, vortexed, and left to separate for 5 min. One-half milliliter of the ethyl acetate fraction was then transferred to another vial and analyzed by gas chromatography (Shimadzu GZ-2010 Plus: Shimadzu Corporation, Kyoto, Japan) using a flame ionization detector, a capillary column, and helium as the gas transporter (Zebron ZB-FFAP GC Cap. Column 20m×0.32mm×0.25µm; Phenomenex Inc., Torrance, CA). Column temperature was initially set to 110°C and gradually increased to 200°C. Injector and detector temperatures were

set at 250°C and 350°C, respectively. Individual VFA were identified by comparing peaks to known standards and concentration of VFA were determined using the area under the curve.

Statistical Analysis

For both experiments, data were analyzed as a stratified randomized design by performing a mixed model with the CRAN package "lme4" using the R statistical programming v4.0.2 (Vienna, Austria) within the intergraded development environment Rstudio V1.3.1073 (Boston, Ma).

For experiment 1, pen was defined as the experimental unit, and steer was used as the observational unit to determine differences across the three treatments. For weight gain, treatment was defined as the main effect while pen (animal), and year were defined as random effects. Means were separated using the CRAN package "lmertest". For ADG and feed to gain, treatment was defined as the main effect and pen (animal), year, and blocking factor were used as the random effects. For VFA concentrations, treatment and sample time were considered main effects and animal, pen, and year were used as the random error. Contrasts were calculated using Tukey's pairwise comparison test, and results were considered significant at $P \leq 0.05$ and tendencies were considered at $P > 0.05$ and $P \leq 0.10$

For experiment 2, data were analyzed using an analysis of variance using treatment and sample time as main effects. Contrasts were calculated using Tukey's pairwise comparison test, and results were considered significant at $P \leq 0.05$ and tendencies were considered at $P > 0.05$ and $P \leq 0.10$

RESULTS

Experiment 1

Gas production, dry matter disappearance, and pH

The effect of treatment on gas production, pH, and DM disappearance are presented in Table 4.4 and 4.5, along with Figure 4.1 below, respectively. There was no treatment by time interactionfor gas production, but there was a treatment and time effect ($P < 0.01$). The PCB bottles do not differ from the MON supplemented bottles (P = 0.97), but both treatments produced greater gas on average than CON bottles (P < 0.01). Moreover, gas production increased at every time point (P < 0.03), except between 12 and 24 h, in which there were no differences (P = 1.00).

There was a treatment by time interaction for pH ($P < 0.003$). The pH was greatest in MON supplemented bottles at 4, 12, and 48 h time points (P < 0.05). The PCB supplemented bottles did not differ from CON bottles on 4, 8, and 48 h time points (P > 0.42) and did not differ from MON bottles on 8 and 24 h (P > 0.49). pH in MON bottles did not differ from CON bottles on at 8 h (P = 0.13). There was a treatment by time interaction for DM disappearance ($P = 0.043$). At the 24 h time point, CON supplemented bottles had greater disappearance than PCB and MON supplemented bottles, and PCB and MON bottles did not differ from each other. At the 48-h time point, the treatments did not differ from each other.

Experiment 2

Animal Performance

Animal performance data is presented in Table 4.3 below. For weight gain, there were treatment x day interactions (P = 0.02). On d 0 and 28, there were no differences between the

three treatment groups (P > 0.31). On d 56 and 84, however, steers who were supplemented with MON were heavier (P < 0.01) compared to CON steers; additionally, steers that were supplemented with PCB did not differ from either MON supplemented steers or CON steers (P > 0.11).

During the first period there were tendencies for ADG to be greater in MON supplemented steers than PCB supplemented steers (P < 0.06), although CON calves did not differ between the two groups (P > 0.15). During period 2 and 3, MON supplemented calves had greater ADG than PCB supplemented steers and CON steers (P < 0.01); for both periods, PCB supplemented steers did not differ from CON calves (P > 0.26). Moreover, feed to gain did not differ between the three treatment groups for the first two periods (P > 0.12). During period 3, feed to gain decreased in MON supplemented steers compared to PCB supplemented steers and CON steers (P < 0.05) and the PCB supplemented steers did not differ from CON steers (P = 0.99).

Rumen Fermentation

Rumen fermentation data is presented in Table 4.6 below. There were treatment effects for acetate, butyrate, and valerate, the acetate to propionate (A:P) ratio, and total VFA concentration ($P < 0.025$). Fluid collected from MON supplemented steers had lower acetate, butyrate, and valerate concentrations than steers supplemented with PCB or CON steers ($P < 0.001$). The A:P ration did not differ ($P = 0.649$) between MON supplemented steers and CON steers, however, the A:P ration from MON supplemented steers was lower ($P = 0.028$) than PCB supplemented steers. Additionally, total VFA concentrations in fluid collected from CON steers

was greater ($P = 0.001$) than fluid from MON steers, while neither treatment differed from PCB supplemented steers ($P > 0.145$).

Discussion

The U.N. estimates the global population will exceed 10 billion by 2100 (Adam, 2022) and while the exact rate of growth may be debated (Adam, 2021), one fact is universally accepted, the global population will rise. To meet the demand of a growing population, the livestock industry must increase production resulting in increased land usage and greenhouse gas production. Feed technologies will be critical to mitigate the negative impacts of increased animal protein production. Ionophores are a common feed technology used in beef cattle to improve feed efficiency, decreasing feed cost (Hersom & Thrift, 2018). Ionophores improve feed efficiency by modulating the rumen microbial population, shifting normal rumen fermentation, and improving protein utilization (Chen & Russell, 1991).).

Regardless of the benefits of ionophores, they are classified as an antibiotic and face scrutiny from both regulatory bodies and consumers alike. Currently, prophylactic antibiotic use in the livestock industry is banned in the EU, and in the US antibiotic use is limited to veterinarians' discretion. In response to increasing pressure, researchers have investigated organic alternatives to antibiotics like ionophores. In this study, in vitro analysis of gas production, pH, and DM disappearance concur with previous in vitro studies using a blend of cinnamaldehyde, capsicum, and eugenol. Both PCB supplemented bottles and MON supplemented bottles improved disappearance by 5.1% and 5.5%, respectively, compared to CON bottles. Additionally, while gas production in PCB supplemented bottles did not differ

from MON supplemented bottles, they produced 8.6 psi more gas than CON bottles. However, pH in PCB supplemented bottles and CON supplemented bottles did not differ by 48 h. These results agree with in vitro experiments who have reported the use 7.5 mg/kg DM cinnamaldehyde, capsicum, and eugenol to modify rumen fluid media, suggesting these compounds may have anti-microbial properties (Cardozo et al., 2004; Calsamiglia et al., 2007). Furthermore, in a continuous culture experiment, when cinnamaldehyde, capsicum, and eugenol was supplemented at 500 mg • L^{-1} • D^{-1} cinnamaldehyde tended to decrease digestibility, while capsicum and eugenol increased pH compared to the control cultures (Tager & Krause, 2009; Ye et al., 2018).

In vivo results, however, did not concur with pervious findings. In experiment 2, there were no differences in weight between PCB and CON steers, although MON steers weighed 13 kg more than CON steers on d 58 and 86. Additionally, MON supplementation improved ADG and feed to gain compared to PCB and CON supplemented calves. Live animal study results may change based on diet, intake, and rumen dynamics (Soltan et al., 2018). In an 84 d stocker study, Geraci et al. (2012) fed steers a corn based diet that was either supplemented with a blend of cinnamaldehyde, capsicum, and eugenol or monensin. While supplementation did not impact weight gain or ADG during the first 44 d of the study, ADG during the final 44 days was 0.2 kg/d greater in the phytochemical blend supplemented group. Moreover, in the final 44 d of the study, intake was greater in the phytochemical blend group. Moreover, rumen VFA in MON supplemented steers contained 8.8 and 4.8 mmol/L of acetate less than CON and PCB steers, respectively. There were no changes in propionate, however, across the three treatment groups, leading to a decrease in the A:P ration only in MON supplemented cattle. The difference

between the results in this stocker trial and those in Geraci et al. (2012) could be due to both differences in diet and supplementation rate. In Geraci et al. (2012) steers were supplemented with 266mg • d^{-1} of cinnamaldehyde and eugenol and 133 mg • d^{-1} capsicum, whereas in this study, a proprietary mix of the three phytochemicals were fed at 1g • d^{-1}. Additionally, both in vitro and live animal studies have primarily utilized a corn-based diet. The steers in this study were supplemented with dried distiller grains and the primary diet was corn-silage. While corn silage is a high energy feed source, digestibility can be variable and tends to be lower compared to rolled corn feed (Zhang et al., 2022). Additionally, one factor not considered between phytochemical studies is the encapsulation methodology used between studies. Currently there are over 200 different forms of micro encapsulation that may vary in wall structure, shape, and size (Wei et al., 2022). Differences in encapsulation can affect the bioactivity and bioavailability of the encapsulated compound (Zhao et al., 2020) and differences may ultimately affect study to study inferences.

Conclusion

Ionophores are a powerful tool in the livestock industry that improve animal health and feed efficiency and reduce the cost of feed. When supplemented in an in vitro trial, the blend improved dry matter disappearance similarly to monensin, which highlights possible antimicrobial activity of cinnamaldehyde and eugenol. However, during an 84- d stocker trial, the blend of phytochemicals did not improve efficiency whereas monensin supplementation did improve average daily gain and feed to gain, while decreasing the acetate to propionate ratio. These results suggest that, in a corn-silage based diet, this particular blend of cinnamaldehyde,

capsicum, and eugenol is not an alternative to ionophores. Future research is required to measure different levels of supplement of each phytochemical, along with different microencapsulation methods.

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Table 4.1. Chemical composition of base diets for steers throughout an 84-day stocking period[1]

	Percent DM, %
DM, %	40
NDF, %	42
TDN, %	72
ME, Mcal/kg	1.15
NE_m, Mcal/kg	0.74
NE_g, Mcal/kg	0.47
CP, %	12.6
Fat	4.7

[1] Diet was fed to 84 steers over two years. Treatments include 1) No supplementation control (CON), 2) Monensin supplementation at a rate of 200mg • head • day^{-1} (MON), 3) EPCBact supplementation at a rate of 1g • head • day^{-1} (PCB; PCB; Xtract, Pancosma, Rolle, Switzerland). During the 84 d experiment, cattle were fed corn silage-based diet supplemented dried distillers grains at 1.8 kg•$steer^{-1}$•day^{-1}.

Table 4.2. Mineral composition for steers supplemented with three different mineral pre-mix formulations throughout an 84-day stocking period

	Treatment[1]		
Mineral	Control	PCB	MON
Ca, %	22	22	30.5
P, %	2.50	2.50	0
NaCl, %	20	20	8.9
Mg, %	1.00	1.00	0
Cu, ppm	2,000	2,000	2,000
Se, ppm	26.4	26.4	26.50
Zn, ppm	7,500	7,500	7,500
Vitamin A, IU/kg	136,363	136,364	136,364
Vitamin D_3, IU/kg	9,091	9,091	9,091
Vitamin E, IU/kg	91	91	91
Thiamine, IU/kg	0	0	91
Plant extract blend[2], mg/kg	0	1764	0
Monensin sodium[2], mg/kg	0	0	1058

[1]Background steers were supplemented with one of three treatments: no supplementation (**CON**), cinnamaldehyde, eugenol oil, and capsicum blend (**PCB;** PCB; Xtract, Pancosma, Rolle, Switzerland**),** monensin (**MON;** Rumensin, Elanco Animal Health, Greenfield, IN).

[2] Formulated to provide Monensin supplementation at a rate of 200mg • head • day^{-1} and the phytochemical supplementation at a rate of 1g • head • day^{-1} (PCB).

Table 4.3. Mean performance data presented by treatment. Data collected from calves on d 0, 30, 58, 86.

	Treatment[1]				
	CON	PCB	MON	SE	P-Value
Weight, kg					
d 0	275	277	276	5.64	0.67
d 28	311	312	315	7.31	0.31
d 56	339[b]	346[ab]	352[a]	8.86	0.01
d 84	368[b]	373[ab]	381[a]	10.3	0.02
ADG, kg • day^{-1}					
d 0 to d 28	1.28	1.25	1.44	0.222	0.06
d 0 to d 56	1.15[b]	1.24[b]	1.37[a]	0.055	< 0.01
d 0 to d 84	1.11[b]	1.14[b]	1.26[a]	0.142	< 0.01
Feed to gain, kg of feed • kg of gain^{-1}					
d 0 to d 28	20.0	12.3	10.6	6.65	0.19
d 0 to d 56	13.5	12.4	11.4	1.22	0.12
d 0 to d 84	14.0[a]	14.0[a]	12.6[b]	1.14	0.03

[abc] Means within a row without a common superscript differ (P < 0.05)

[1] Treatments included: Monensin (MON) supplemented at a rate of 200mg • head • day^{-}1, a plant-derived phytochemical mixture including cinnamaldehyde, eugenol, and capsaicin (PCB; Xtract, Pancosma, Rolle, Switzerland) at a rate of 1g • head • day^{-1}, Control (CON) no supplementation

Table 4.4. Effects of 3 different supplements on cumulative gas production measured at 6 different time points over 48 h[1]. Data were collected from fifteen 250 mL Ankom bottles (5 per treatment) fitted with gas production modules (RF1X, Ankom technology, Macedon, NY) that fi 250 mL bottles. Production modules wirelessly measure gas production every 5 min and data[1] were recorded in psi locally to Excel (Microsoft, Redmond, WA) on an adjacent computer.

	Treatment[2]						
	CON	PCB	MON	SE	Treatment	Time	Interaction
Total gas production, psi				7.631	< 0.002	< 0.001	0.770
0 h	0.00	0.00	0.00				
4 h	13.3	15.1	20.5				
8 h	21.0	27.7	29.5				
12 h	25.7	36.5	35.7				
24 h	25.7	36.5	35.7				
48 h	34.1	46.5	41.9				

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1] Data were averaged ± 2 min around hour mark to assess production at each time point.

[2] Treatments included plant metabolite blend (**PCB;** Xtract, Pancosma, Rolle, Switzerland), monensin (**MON**; Rumensin®, Elanco Animal Health, Greenfield, IN), or no supplementation (**CON**)

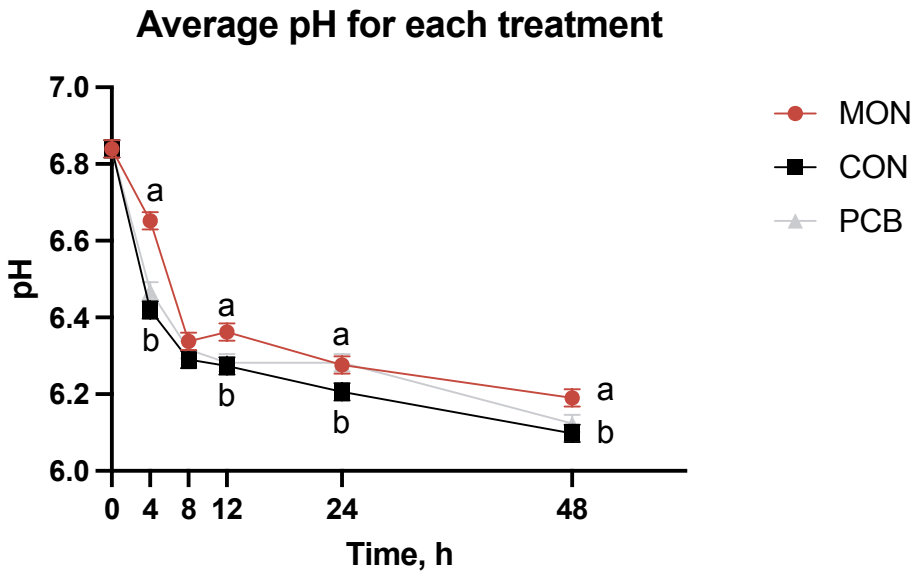
Average pH for each treatment
7.0
6.8
6.6
6.4
6.2
6.0
pH
0
4
8
12
24
48
Time, h
a
b
a
b
a
b
a
b
MON
CON
PCB

Figure 4.1. Average pH for each treatment at the six different time points.
[ab] Means within a row without a common superscript differ ($P < 0.05$).

Table 4.5 Effects of 3 different treatments on in-vitro dry matter disappearance[1]

	Treatment[2]						
	CON	Xtract	MON	SE	Treatment	Time	Interaction
DM disappearance, %				3.40	0.810	0.023	0.043
24h	44.0[a]	38.9[b]	38.5[b]				
48h	41.9	45.0	47.2				

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1] Data collected from fifteen bottles (n = 15), with 5 bottles per treatment.

[2] Treatments included plant metabolite blend (**PCB**), monensin (**MON**; Rumensin®, Elanco Animal Health, Greenfield, IN), or no supplementation (**CON**)

Table 4.6. Volatile fatty acid data[1] presented by treatment and collected from 72 calves across 2 years (36 per year, 12 per treatment)

VFA's, mmol	Control	PCB	MON	SEM	P-Value
Acetate	46.2[a]	42.2[ab]	37.2[b]	3.36	< 0.001
Propionate	10.5	9.10	9.16	0.639	0.083
Butyrate	5.68[a]	5.50[a]	3.84[b]	0.459	< 0.001
Isobutyrate	0.692	0.678	0.601	0.106	0.082
Valerate	0.477[a]	0.447[a]	0.294[b]	0.086	< 0.001
Isovalerate	0.938	0.970	0.802	0.209	0.107
A:P[3]	4.56[ab]	4.85[a]	4.42[b]	0.116	0.025
Total	64.5[a]	59.0[ab]	52.1[b]	5.54	0.001

[ab]Means within a row without a common superscript differ ($P < 0.05$)

[1]Data were collected on d 0 prior to treatment and again on d 84 from

[2] Treatments included: Monensin (MON) supplemented at a rate of 200mg • head • day^{-}1, a plant-derived phytochemical mixture including cinnamaldehyde, eugenol, and capsaicin (PCB; Xtract, Pancosma, Rolle, Switzerland) at a rate of 1g • head • day^{-1}, Control (CON) no supplementation.

[3]A:P calculated by (acetate concentration/propionate concentration) within each treatment group.

CHAPTER 5

POST WEANING MANGMENT STRATEGIES: MEASURING THE IMPACT OF HEAT STRESS ABATEMENT STRATEGIES ON FECAL MICROBIOME AND BLOOD GAS VALUES IN FINISHING STEERS DURING THE SUMMER MONTHS IN THE SOUTHEAST U.S.

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Abstract

This study evaluated the effect of heat stress abatement strategies on the fecal microbiome of finishing beef steers in the Southeastern United states. The study was designed as a completely randomized block using 32 steers (BW 453k ± 4 kg) stratified by weight and randomly assigned to one of four treatment groups: covered with fans (**CWF**), covered no fans (**CNF**), outside with shade (**SHADE**), and outside without shade (**OUT**). All steers were housed outside from June to July and were brought up on a common feedlot ration. After-which steers were placed into treatments on d -10 and covered steers were given 10 days to acclimate to Calan-gate system. During the acclimation phase, both covered and outside steers were brought up at a similar rate. Steers were weighed and fecal samples were collected on d 0, 50, and 85. Arterial blood was collected from the aural artery to measure blood gas values on d -10, 0, 50, and 85. Microbial DNA was extracted from the samples using a combination of mechanical and enzymatic processes, and the DNA sequences were analyzed using QIIME v2.0. Additionally, blood gas values were measured chute side utilizing an Abbott i-STAT Alinity blood analyzer. There was a treatment x day interaction for Actinobacteriota, Bacteroidota, and Patescibacteria phyla. Additionally, there was a day effect for all three phyla ($P < 0.001$), but there was only a treatment effect ($P < 0.001$) for Patescibacteria. There was not an interaction for Fibrobacterota, Firmicutes, or Proteobacteria ($P > 0.226$), but there was a day effect ($P < 0.019$) for all three phyla. There was a treatment effect ($P = 0.036$) for Fibrobacterota, however there was not a treatment effect for Firmicutes and Proteobacteria ($P > 0.394$). Moreover, there was no interaction, treatment, or day effect ($P > 0.39$) for the Firmicutes to Bacteroidota ratio. For blood gas values, there were treatment by time interactions for all variables ($P < 0.05$) except for potassium, glucose, PO2, and HCO3 ($P > 0.06$).

These results suggest that stress abatement strategies may influence the fecal microbiome over time, and they might help better understand how stress affects the microbiome and nutrient digestibility in the lower gut.

KEY WORDS: Heat stress, Cattle, Microbiome, Blood gas

Introduction

The first laboratory confirmed case of coronavirus in the United States was reported in January 2020. Covid-19, and the subsequent shut down of the country, negatively impacted the national economy, limiting supply availability and the active work force. The beef industry was heavily impacted, threatening the sustainability of both the packer industry and cattle producers alike. The extent of covid-19's impact on the beef cattle industry was caused by several factors but can be traced primarily due to the heavy concentration of control that is shared between four major companies (Richards, 2020). As processing plants began to halt or limit production, supply to retail options began to decrease and prices began to rise (Lusk et al., 2021). At the same time, plants reduced purchasing of cattle, increasing supply of finished beef cattle in the market and decreasing the price and profitability of cattle operations. In response to the growing price spread between cattle prices and retail beef prices, cattle producers began to explore regional retail and direct to consumer options (Richards, 2020). While market analysis suggests an eventual normalization of beef price spreads, highlighting the resilience of the beef industry supply chain, consumer adoption to local beef has helped regional processing and direct to consumer marketing become a more viable option to beef cattle producers (Ramsey et al., 2020).

In the southeast United States, heat stress is a major obstacle to efficiently finish beef cattle, especially during the summer months (Sims et al., 2019). During times of stress, beef

cattle may suffer from a decrease in dry matter intake and growth, increasing the cost to develop cattle to slaughter and reducing the carcass quality (St-Pierre et al., 2003). Moreover, animal behavior and physiology can have a direct impact on the rumen and hindgut microbiome (Gonźalez et al., 2012). Specifically, changes in feed intake and immune function in response to heat stress may modify the microbiome, further impacting the efficiency of cattle. While previous research has sought to measure the effectiveness of heat stress abatement strategies on animal performance and carcass quality (Sims et al., 2018), little research has investigated the impact of abatement strategies on blood metabolites and rumen and fecal microbiome in beef cattle. Therefore, the objective of this study was to evaluate the impact of three different heat stress abatement strategies on blood gas values and the fecal microbiome compared to heat stressed steers.

Material and Methods

All procedures and guidelines that involved animals were approved by the University of Georgia Institutional Animal Care and Use Committee (A2019-01-017-Y2-A2).

Animal and Diet Management

This study was conducted in conjunction with Sims et al. (2019) who measured the impact of heat stress abatement strategies on feedlot performance and carcass characteristics. This study utilized thirty-two (N = 32) of the eighty crossbred Angus steers (BW 453k $\pm$ 4 kg; 14 to 15 mo of age). Prior to the study, all steers were comingled in a holding pasture from the months of May to July. Ten days prior to the study, CNF and CWF steers were moved inside the covered barn for acclimation to the Calan gate feeders. On arrival, steers were stratified by weight into four balanced groups and then groups were randomly assigned to one of four

treatments as described in detail in Sims et al (2019). Briefly, the four treatments were: Inside a feeding barn with cooling fans (**CWF**), inside feed barn with no cooling fan (**CNF**), outside drylot with an optional shade structure providing 3.05m × 3.66 m of shade per animal (**SHADE**), or outside drylot with no shade or fan (**OUT**). Additionally, relative humidity, black globe temperatures and wind speed were measured every 30 min for each treatment utilizing two Kestrel meters (5400AG cattle heat stress trackers; KestrelMeters; Boothwyn, PA) equally spaced across pens. Climate data was used to measure heat load index (**HLI**) and accumulated heat load unit (**AHLU**) across the study.

Automated water troughs were placed in all pens to allow *ad libitum* access to water. Both OUT and SHADE steers were pair fed (n = 2 per pen; N = 16). Steers housed inside, CWF and CNF, were individually fed using a Calan Broadbent Feeding System (American Calan, Inc,; Northwood, New Hampshire). Across all four treatments, the same total mixed ration (**TMR**) was fed to all steers, diet composition is presented in Table 5.1. Feed was weighed using a Calan Data Ranger (American Calan, Inc,; Northwood, New Hampshire) for inside covered steers and a floor scale (Prime Scales, PS-IN202; Ontario, CA) for OUT and SHADE steers.

Blood gas Analysis

Whole blood was collected from the aural artery on d 0, 50, and 85 to measure blood gas values. Arterial blood was collected with a blood gas analysis syringe (Smiths Medical, Northfield, IL) and immediately analyzed chute-side using an i-STAT Alinity blood analyzer and the C8+ blood analyzer cartridges (Abbott Core Laboratory Systems, Chicago, IL) that reports whole blood concentrations of: Na (mEq/L), K (mEq/L), ionized calcium (mg/dL), glucose (mg/dL), hematocrit (% fraction), hemoglobin (mmol/L), pH, PCO_2 (kPa), PO_2 (kPa), TCO_2 (mEq/L), HCO_3 (mEq/L), base excess (mEq/L), and sO_2 (%). Base excess is defined as the

amount of acid or base that needs to be added to the whole blood to return pH to 7.4; if base excess is positive, a strong acid is required and if base excess is negative, a strong base is required.

Fecal Collection

Fecal samples were collected from all steers ($N = 32$) on d 0, 50, and 85. Fecal samples were collected aseptically via fecal grab using separate gloves to prevent cross contamination. Fecal samples were placed into a sterile, 15 mL conical tube, and stored on dry ice until being transferred to a -80°C freezer until further analysis.

Fecal DNA Extraction

DNA extraction was performed on fecal samples following the protocol described in Welch et al. (2020) with slight modifications. This procedure used 350 mg of sample placed in 2-mL Lysing Matrix E tubes (MP Biomedicals LLC, Irvine, CA), which were homogenized using a FastPrep 24 Instrument (MP Biomedical LLC, Irvine, CA) to disrupt the cells. Enzymatic inhibition was achieved by using InhibitEX Buffer (QIAGEN, Venlo, Netherlands), and DNA elution and purification were carried out using a spin column and a series of specialized proprietary buffers according to manufacturer's specifications (QIAamp Fast DNA Stool Mini Kit; QIAGEN, Venlo, Netherlands). Determination of DNA concentration and purity in the resulting eluate was performed spectrophotometrically using the Synergy LX Multi-Mode Microplate Reader in conjunction with the Take3 Micro-Volume Plate (BioTek Instruments Inc; Winooski, VT, USA). Samples with a minimum volume of 100 µL and 10 ng/µL of DNA were stored at -20 °C until further analysis.

16S rRNA Gene Sequencing

Following DNA extraction, samples were transported on ice to the Georgia Genomics and Bioinformatics Core (Athens, GA) for library preparation and 16S ribosomal ribonucleic acid (**rRNA**) gene sequencing. The library preparation step included polymerase chain reaction (**PCR**) replications using the forward: S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and reverse: S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) primer pairs, followed by a PCR clean-up using AMPure XP beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA). A second PCR step was then carried out to attach Illumina's indices and sequencing adapters (Nextera XT Index Kit; Illumina Inc., San Diego, CA, USA), followed by another PCR clean-up step using AMPure XP beads. Following this final library clean up, the library was quantified using qPCR, and the nucleotides were sequenced using an Illumina MiSeq instrument and a MiSeq v3 reagent kit (Illumina Inc., San Diego, CA, USA). A well-characterized bacteriophage PhiX genome (PhiX Control v3 Library; Illumina Inc., San Diego, CA, USA) was used as a control for the sequencing runs.

Bioinformatics

The sequencing data was demultiplexed and converted to FASTQ files. Samples were imported into QIIME 2 (Bolyen et. al., 2019). Non-biological nucleotides and sequences were denoised, dereplicated, and chimera-filtered using DADA2 (Callahan etl. al., 2016). A pre-trained Naïve Bayes classifier trained with the SILVA 138 SSU databases (Quast et. al., 2013) was used to assign taxonomies with reads classified by taxon using the fitted classifier (Pedregosa et. al., 2011). Sequencing depth was set at 28,025 sequences per sample.

Statistical Analysis

Statistical programing was conducted using the R statistical programing v4.0.2 (Vienna, Austria) within the intergraded development environment (**IDE**) Rstudio v1.3.1073 (Boston, MA).

Blood gas and Microbiome

Blood gas values and microbiome data were analyzed using a stratified randomized design. Animal was defined as the experimental unit and was used to determine differences across all four treatments. A mixed model was performed using the CRAN package "lme4". Treatment and time were defined as the main effect and animal and pen were included as the random effects. Additionally, animal was included as the repeated measure. Pairwise comparisons were computed using the Tukey's HSD post-hoc test. Differences were considered significant at $P = 0.05$ and tendencies were considered at $P < 0.10$.

Results

Whole blood gas data were presented in Figure 5.2, Figure 5.3, Figure 5.4 below.

Sodium

There were treatment x time interactions for Na (P = 0.01). On d 0, CNF and CWF did not differ (P = 0.90) and both were lower than SHADE and OUT (P < 0.05). Whole blood Na in OUT steers tended to be lower than SHADE steers (P = 0.098). There were no differences between d 0 and 50 for all treatment groups (P > 0.11). On d 50, Na whole blood in CWF tended to be lower than SHADE steers (P = 0.08). There were no other differences detected (P > 0.15). Between d 50 and d 85, there was a tendency for sodium to increase (P = 0.08), however the other three treatment groups did not differ between the two days (P > 0.20). On d 85, Na whole

blood in CNF tended to be lower than OUT steers ($P = 0.09$), but there were no other differences detected ($P > 0.12$).

Potassium

There tended to be treatment x time interactions for whole blood K ($P = 0.08$). On d 0, whole blood K in CWF steers was lower than CNF steers ($P = 0.02$), but greater than OUT and SHADE steers ($P < 0.01$). The other three treatment groups did not differ from each other ($P > 0.12$). Between d 0 and d 50, whole blood K in OUT steers increased ($P = 0.02$) and there were a tendency for whole blood K to decrease in CWF steers ($P = 0.07$). Whole blood K did not differ between the two days in CNF and SHADE steers ($P > 0.93$). On d 50, there were differences between treatments ($P = 0.36$). Between d 50 and 85, there was a tendency for whole blood K decrease ($P = 0.08$), although the other three treatment groups did not differ between the two days ($P > 0.24$). On d 85, whole blood K in OUT steers tended to be lower than CWF and SHADE steers ($P = 0.09$), but there were no other differences detected ($P > 0.19$).

Calcium

There were treatment x time interactions for Ca ($P < 0.01$). On d 0, whole blood Ca in CWF steers was lower than OUT steer ($P = 0.02$), but there were no other differences detected ($P > 0.10$). Between d 0 and d 50, whole blood Ca increased in SHADE steers ($P < 0.01$), and there was a tendency for whole blood Ca to increase in CWF ($P = 0.08$). There were no differences in whole blood Ca in CNF and OUT steers between the two days ($P > 0.14$). On d 50, whole blood Ca was lower in CWF steers than OUT and SHADE ($P < 0.04$) and there were no other differences detected ($P > 0.22$). On d 85, there were no differences detected ($P > 0.20$).

Glucose

There was a tendency for treatment x time interactions for whole blood glucose (P = 0.06). On d 0, whole blood glucose did not differ between OUT and SHADE steers (P = 0.37), nor did it differ between CNF and CWF steers (P = 0.46). Whole blood glucose was lower in CWF steers than OUT and SHADE (P < 0.05) and whole blood glucose was lower in CNF steers than SHADE steers (P = 0.03). There were no differences between CNF and OUT steers (P = 0.20). Between d 0 and d 85, whole blood glucose increased in CNF and CWF steers (P < 0.05); there were no differences in whole blood glucose between the two days in OUT and SHADE (P > 0.60). On d 50, there were no differences in whole blood glucose detected (P > 0.22). Between d 50 and 85, whole blood glucose increased in OUT and SHADE steers (P < 0.02), although it did not differ between days in CNF and CWF steers (P > 0.47). On d 85, whole blood glucose was lower in CWF steers than OUT steers (P = 0.02), but there were no other differences detected (P > 0.13).

Hematocrit

There were treatment x time interactions detected for Hematocrit (P = 0.02). On d 0, hematocrit did not differ between CNF and CWF steers (P = 0.55) nor did it differ between OUT and SHADE steers (P = 0.61). Hematocrit in CNF steers was greater than OUT and SHADE steers (P < 0.04). Additionally, the hematocrit was greater in CWF steers than SHADE steers (P = 0.046) but did not differ from OUT steers (P = 0.13). Between d 0 and 50, whole blood hematocrit increased in SHADE steers (P < 0.01) but tended to decrease in CWF steers (P = 0.07). Whole blood hematocrit did not differ in the other two treatment groups (P > 0.33) between the two days. On d 50, hematocrit tended to be lower in OUT steers than either SHADE or CNF (P < 0.07). There were no other differences detected (P > 0.24). Between d 50 and 85, whole blood hematocrit increased in OUT steers (P < 0.01) but did not differ in the other three

treatment groups between the two days (P > 0.11). On d 85, there were no other differences detected (P > 0.47).

Hemoglobin

There were treatment x time interactions detected from hemoglobin (P = 0.02). On d 0, hemoglobin did not differ between CNF and CWF steers (P = 0.57) nor did it differ between OUT and SHADE steers (P = 0.62). Hemoglobin in CNF steers was greater than both OUT and SHADE steers (P < 0.04). Hemoglobin was greater in CWF than SHADE steers (P = 0.05) but did not differ from OUT steers (P = 0.13). Between d 0 and 50, whole blood hemoglobin increased in SHADE steers (P < 0.01) but tended to decrease in CWF steers (P = 0.07). Whole blood hemoglobin did not differ in the other two treatment groups (P > 0.33) between the two days. On d 50, hemoglobin in CWF steers did not differ between SHADE, CNF, or CWF steers (P > 0.25), and SHADE and CNF tended to be greater than OUT steers (P < 0.08). Hemoglobin in CWF steers did not differ from OUT steers (P = 0.561). Between d 50 and 85, whole blood hemoglobin increased in OUT steers (P < 0.01) but did not differ in the other three treatment groups between the two days (P > 0.11). On d 85, there were no differences detected (P > 0.485).

pH

There were treatment x time interactions detected for whole blood pH (P < 0.01). On d 0, whole blood pH did not differ between CNF and CWF steers (P = 0.73) nor did it differ between OUT and SHADE steers (P = 0.87). Additionally, CNF and CWF steers whole blood pH was lower than both SHADE and OUT steers (P < 0.01). Between d 0 and 50, whole blood pH in CNF and CWF steers increased (P < 0.01), while whole blood pH decreased in SHADE steers (P = 0.03). Whole blood pH did not differ in OUT steers between the two days. There were no

differences in whole blood pH between d 50 and 85 across all four treatments ($P > 0.23$), and there were no treatment differences detected on d 50 and d 85 ($P > 0.19$).

pCO_2

There were treatment x interactions detected for whole blood pCO_2 ($P = 0.011$). On d 0, whole blood pCO_2 did not differ between CNF and CWF steers ($P = 0.63$) nor did it differ between OUT and SHADE steers ($P = 0.50$). Whole blood pCO_2 was greater in CNF steers than OUT and SHADE steers ($P < 0.03$). Additionally, whole blood pCO_2 was greater in CWF steers than OUT steers ($P = 0.02$) and tended to be greater than SHADE steers ($P = 0.09$). Between d 0 and 50, whole blood pCO_2 increased in CNF, CWF, OUT steer ($P < 0.01$), but did not differ in SHADE steers ($P = 0.41$). On d 50, whole blood pCO_2 in SHADE steers was greater than OUT steers ($P = 0.03$) and tended to be greater than CWF steers ($P = 0.08$). There were no other differences detected. Between d 50 and 85, whole blood pCO_2 increased in CNF and OUT steers ($P < 0.02$) and tended to increase in SHADE steers ($P = 0.07$). There were no differences in whole blood pCO_2 between the two days in CWF steers ($P = 0.17$). On d 85, whole blood pCO_2 in SHADE steers tended to be greater than CWF steers ($P = 0.82$), and there were no other differences detected ($P > 0.26$).

pO_2

There were no treatment x time interactions detected for pO_2 ($P = 0.61$), nor were there treatment effects detected ($P = 0.1$). There were time effects detected ($P = 0.01$) in which whole blood pO_2 was lower on d 0 than d 50 ($P = 0.06$) and lower on d 0 than d 85 ($P < 0.01$). Whole blood pO_2 did not differ between d 50 and d 85 ($P = 0.59$).

tCO_2

There were treatment x time interactions detected for tCO_2 ($P = 0.05$). On d 0, whole blood tCO_2 did not differ between CNF and the other the treatment groups ($P > 0.16$) nor did it differ between OUT and SHADE steers ($P = 0.45$). Whole blood tCO_2 was lower in CWF and CNF steers than SHADE steers ($P < 0.03$). Between d 0 and 50, whole blood tCO_2 decreased in all treatment groups ($P < 0.01$). On d 50, whole blood tCO_2 was greater in SHADE steers than OUT steers ($P = 0.02$) but no other differences were detected ($P > 0.19$). Between d 50 and 85, whole blood tCO_2 increased in CNF, OUT, and SHADE ($P < 0.02$), but did not differ in CWF steers. On d 85 whole blood tCO_2 in CWF steers was lower than either OUT or SHADE steers ($P < 0.03$). Additionally, CNF steers tended to have greater tCO_2 than CWF steers ($P = 0.08$). No other differences detected ($P > 0.40$).

HCO_3

There were no treatment x time interactions detected for whole blood HCO_3 ($P = 0.74$), nor were there any treatment effects ($P = 0.81$). There were time effects detected ($P = 0.010$) in which, whole blood $3HCO_3$ was greater on d 0 than d 50 ($P < 0.01$) and tended to be greater on d 0 than d 85 ($P = 0.06$). Whole blood HCO_3 did not differ between d 50 and d 85 ($P = 0.33$).

Base Excess

There were treatment x time interactions detected for Base Excess ($P < 0.01$). On d 0, whole blood base excess did not differ between CNF and CWF ($P = 1.00$) nor did it differ between OUT and SHADE steers ($P = 0.53$). Additionally, the base excess for CNF and CWF steers was greater than both OUT and SHADE ($P < 0.01$). Between d 0 and 50, base excess increased in OUT and SHADE decreased ($P < 0.01$) but did not differ in CNF and CWF steers ($P > 0.12$). Between d 50 and 85, base excess increased in CNF, OUT, and SHADE steers ($P <$

0.01), but did not differ in CWF steers (P = 0.73). On d 50 were no differences detected (P > 0.27), but on d 85, whole blood base excess was lower in CWF than the other three treatment groups (P < 0.02) and the other three groups did not differ from each other (P > 0.23).

Alpha diversity

Alpha diversity data are presented in figure 5.5 below.

There was a treatment x time interaction for Observed features and Shannon diversity index (P < 0.001). For both metrics, on d 0, CWF and CNF steers did not differ from each other but were less than Shade and OUT steers. Shade and Out steers did not differ from each other. Between d 0 and 50, observed features increased in all steers (P < 0.03) and Shannon diversity index increased in CNF, OUT, and SHADE (P < 0.01), but did not differ in CWF steers (P = 0.23). Between d 50 and 85 observed features increased in CNF and CWF steers (P < 0.03) but did not differ between days in OUT and SHADE steers (P > 0.25). Additionally, Shannon diversity index increased in CNF steers (P < 0.01) and tended increase in CWF steers (P = 0.06). Moreover, Shannon diversity index did not differ between the two days in OUT and SHADE steers (P > 0.11). There were no treatment differences on d 50 and d 85.

Family results

Bacterial family data is presented in Figure 5.6 below.

There was a treatment x time interaction for *Atopobiaceae*, *Bifidobacteriaceae*, F082, *Muribaculaceae*, *Saccharimonadaceae*, *Oscillospiraceae,* and *Muribaculaceae* (P < 0.04).

On d 0, F082, *Saccharimonadaceae, Oscillospiraceae*, *Bifidobacteriaceae*, and *Ruminoccaceae* abundance did not differ between feces from CNF and CWF steers (P > 0.46).

Additionally, feces from CNF and CWF were less abundance than OUT and SHADE in F082, *Saccharimonadaceae, Oscillospiraceae*, ($P < 0.01$). Feces from CWF steers contained greater *Ruminoccaceae* and *Bifidobacteriaceae* abundance than feces from OUT steers ($P < 0.04$) and tended to be greater than SHADE steers ($P < 0.09$) Feces from CNF steers contain greater *Bifidobacteriaceae* abundance than OUT and SHADE ($P < 0.004$) and tended to contain greater *Ruminoccaceae* abundance than SHADE steers ($P < 0.09$) and OUT steers ($P = 0.06$). Feces collected from OUT steers contained greater *Saccharimonadaceae* abundance than SHADE steers ($P < 0.01$). Relative abundances of F082, *Ruminoccaceae*, *Bifidobacteriaceae,* and *Oscillospiraceae* did not differ in feces from OUT and SHADE steers ($P > 0.68$). Additionally, there were no differences between treatment groups for *Atopobiaceae* and *Muribaculaceae* ($P > 0.67$).

Between d 0 and 50, *Atopobiaceae, Muribaculaceae,* and *Muribaculaceae* increased in all treatment groups ($P < 0.01$). *Oscillospiraceae* and F082, however, decreased in all treatment groups between the two days ($P < 0.01$). Additionally, *Bifidobacteriaceae* decreased in CNF and CWF ($P < 0.03$) but did not differ in OUT and SHADE steers between the two days ($P > 0.17$). *Saccharimonadaceae* did not differ between the two days in CNF and CWF steers ($P > 0.91$) and decreased in OUT and SHADE steers ($P < 0.01$).

On d 50, there were no differences in *Bifidobacteriaceae*, F082, *Saccharimonadaceae*, and *Oscillospiraceae* abundance ($P > 0.149$). Feces from CNF steers contained greater abundances of *Atopobiaceae* and *Ruminoccaceae* than the other three treatment groups ($P < 0.01$), but *Atopobiaceae* abundance did not differ between the other three treatment groups. Feces collected from CWF steers contained greater *Ruminoccaceae* abundance than OUT and SHADE steers ($P < 0.03$). Feces from OUT and SHADE steers did not differ from each other (P

= 0.99). Furthermore, feces collected from CNF and CWF steers did not differ in *Muribaculaceae* abundance (P = 0.93) and feces from both groups contained lower abundance than OUT and SHADE steers (P < 0.01). Additionally, feces from OUT steers contained greater abundance of *Muribaculaceae* than SHADE steers (P = 0.01).

Between d 50 and 85, *Atopobiaceae* decreased in CNF, CWF, and OUT (P < 0.05), moreover there was a tendency for *Atopobiaceae* to decrease in SHADE steers (P = 0.06). *Muribaculaceae,* between the two days, decreased in OUT and SHADE steers (P < 0.01), but did not differ in CWF and CNF steers (P > 0.20). *Ruminoccaceae* decreased in CNF steers (P = 0.04) but did not differ in the other three treatment groups (P > 0.19). *Bifidobacteriaceae*, F082, *Saccharimonadaceae*, and *Oscillospiraceae* did not differ between days in any treatment groups (P > 0.11).

On d 85, there were no differences in *Bifidobacteriaceae*, F082, *Saccharimonadaceae*, *Muribaculaceae*, and *Oscillospiraceae* abundance (P > 0.15). Feces collected from OUT steers contained greater abundance of *Atopobiaceae* than feces from CWF and SHADE steers and did not differ from CNF steers. Additionally, *Atopobiaceae* abundance did not differ between feces from CNF steers and CWF or SHADE steers. Additionally, feces from CNF steers contained greater abundance of *Ruminoccaceae* than feces from SHADE and OUT steers (P < 0.03) and tended to contain greater abundance than CWF (P = 0.08). Additionally, feces from CWF steers contained greater *Ruminoccaceae* abundance than OUT steers (P = 0.02) and did not differ from the SHADE steers (P = 0.68). Feces from SHADE steers tended to contain greater *Ruminoccaceae* abundance than OUT steers (P = 0.06).

There were no treatment x time interactions detected for *Lachnospiraceae* and *Fibrobacteraceae* abundance (P > 0.28), but there were treatment (P < 0.04) and day effects (P <

0.02) detected. Overall, feces collected from CWF steers contained greater *Lachnospiraceae* abundance than the other three treatment groups ($P < 0.05$) and the other three treatment groups did not differ from each other ($P > 0.30$). Additionally, *Lachnospiraceae* abundance decreased from d 0 to d 50 ($P = 0.02$) and d 0 to d 85 ($P < 0.01$) and tended to decrease from d 50 to d 85 ($P = 0.56$). Moreover, feces collected from CNF steers contained greater abundance of *Fibrobacteraceae* than OUT and SHADE steers ($P < 0.02$) and did not differ from feces collected from CWF steers ($P = 0.27$). There was a tendency for feces collected from CWF steers to contain greater *Fibrobacteraceae* abundance than OUT steers ($P = 0.10$) and feces from SHADE steers did not differ from CWF or OUT steers ($P > 0.21$). Moreover, there were no differences in *Fibrobacteraceae* abundance from d 0 to d 50 ($P = 0.31$), but there was a tendency for abundance to decrease from d 50 to d 85 ($P = 0.07$) and overall abundance decreased from d 0 to d 85 ($P < 0.01$).

Species Results

There was a treatment x time interaction detected for *Bifidobacterium pseudolongum* ($P = 0.05$). On d 0, abundance did not differ between feces from CNF and CWF steers ($P > 0.39$) and both feces contained greater abundance than feces from OUT and SHADE steers ($P < 0.01$). There were no differences on d 50 and d 85 ($P > 0.99$).

Discussion

Heat stress is a serious issue impacting cattle production and profitability. While cattle in some areas in the United States may experience acute heat stress, cattle located in the Southeast US often experience prolonged stress with temperatures that exceed the thermal neutral zone for cattle, 5°C to 20°C, 8 out of 12 months of the year (Brew et al., 2011; NCEI, 2023). Prolonged

heat stress is dangerous as cattle are not able to adequately dissipate heat and can lead to metabolic dysfunction and changes in normal animal bioenergetics (Lees et. al, 2019). In a concurrent study, Sims et al. (2019) reported differences in black globe temperatures with CNF and CWF steers experiencing max temperatures that were 12°C to 15°C cooler than OUT and SHADE steers. Minimum black globe temperatures, however, did not differ between treatments. Furthermore, Sims reported differences in max heat load index (HLI) between the two groups, with HLI for OUT and SHADE steers being ~20 units greater than CNF and CWF steers housed inside the feed barn. Like black globe temperatures, minimum HLI did not differ between treatment groups. Despite all four treatment groups experiencing similar minimum HLI and BGT, accumulated heat load units (AHLU) were extreme in OUT and SHADE steers reaching maximum AHLU levels of 400. Steers housed in the feedlot barn, however, did not accumulate heat load units, reaching a maximum of 2.36 HLU in CNF steers during week two. Results from Sims et al. (2019) confirm that covered housing, with or without fans, drastically reduce heat stress by improving heat dissipation. Reducing AHLU is critical because when heat dissipation is minimal, cattle may try to mitigate serious damage by decreasing endogenous heat production by decreasing dry matter intake which negatively affects efficiency and growth. Additionally, a bioenergetic shift in energy prioritization and utilization occurs, disrupting and changing normal metabolic functions which negatively affects physiological homeostasis and immune function (Corvo et. al, 2021). These changes may be observable in behavioral changes such as decreased dry matter intake, increased water intake, and increased respiration (Kamal et al., 2018). It is important to note, in this study, both CWF and CNF steers were housed outside until d -10 in late July. Pre-trial management complicates analysis of d 0 values as steers in both groups were potentially heat stressed prior to entering their heat stress abatement strategy. Additionally, all

steers were likely adapted to the hot climate prior to entering their treatments, meaning, this study measured the acute and long-term response to heat stress relief instead of identifying differences between heat stressed and non-heat stressed cattle. For instance, respiration rate has often been used as a tool to identify cattle that are suffering from heat stress (Gaughan et al., 2000). Metabolic changes due to heat stress have often been observed in blood values (Schneider et. al, 1988) and in this study, on d 0, blood collected from steers that were housed inside had 5 kPa pCO_2 greater than OUT and SHADE steers. While there is little research in cattle linking respiration rate and acid-base balance, there has been a distinct link identified in humans. In general, a slower respiration rate has been linked with greater pKa pCO_2 values (Hopkins et al., 2022). Due to the pre-trial management, both inside groups were likely suffering from heat stress. While CNF and CWF pCO2 levels suggest a decrease in respiration rate, it may also represent an acute stress response to the acclimation of the heat stress abatement strategies. The acute stress response can be highlighted by the whole blood pH and base excess values observed on d 0. There was 0.06 decrease in pH, in addition to a 3 to a 3.5 mmol/L decrease in base excess, in the blood collected from both CNF and CWF steers compared to OUT and SHADE steers. An accumulation of pCO2 and a decrease in pH and base excess are clinical signs of respiratory acidosis, a more common concern in postnatal calves, and may be a response to the hypoventilation that occurs during the acute acclimation from cattle suffering from heat stress entering heat stress abatement strategy (Bleul, 2009). Furthermore, in this study, the acute stress of acclimation can be further identified in whole blood glucose and insulin values on d 0. There is strong evidence that as energy prioritization shifts and feed intake decreases, physiological and immunological changes begin to occur, impacting circulating glucose and insulin (Wheellock et. al, 2010; O'Brien et. al, 2010). Baumgard and Rhoads (2013) highlight the glucose sparing effect

that is observed in response to immunocompromised cattle. During an immune challenge, glucose transporters downregulate insulin binding proteins, increasing the circulating levels of insulin. Despite a decrease in insulin activity, glucose levels do not rise, likely being utilized as an energy source of increased immune function (Abbas et al., 2020). Alternatively, shifts in the insulin and glucose axis may be a physiological response to reduce endogenous heat production as oxidizing amino acids produces less heat than oxidizing fatty acids (Herbut et al., 2019). While it is not explicitly clear which mechanism influences glucose and insulin in this study, a decrease in glucose and an increase in insulin has been identified as a useful pattern to detect heat stress. Baseline glucose levels may depend on various factors like age, diet, breed, and management conditions, all which make cross study comparisons challenging. For example, Loe et al., (2003) reported glucose levels in clinically healthy crossbred yearling heifers on arrival to a feedlot could range anywhere from 57 mg/dL to 78 mg/dL. In this study, on d 0, it appears that steers from all four treatment groups were between 62.5 mg/dL and 71.9 mg/dL, however, the whole blood in CWF and CNF steers contained between 5 to 9 mg/dL of glucose compared to OUT and SHADE steers. These results suggest that, counterintuitively, the steers who were placed in their heat stress abatement treatment suffered from acute acclimation stress during the early period of the study.

The results from this study highlight the usefulness of blood gas analysis for early stress detection, however it can be a challenge utilizing blood values as a metric of during a prolonged period of stress. Even during the period of acclimation to heat stress, the body will work towards homeostasis, limiting any serious changes in the blood (Schneider et. al, 1988). This study faced similar challenges measuring long term heat stress using blood gas analysis. Regardless of treatment, there were only differences detected for tCO_2, glucose, and potassium on d 85 across

all four treatment groups. These results indicate that all steers over time acclimated to their treatment, limiting the long-term use of blood gas values as a metric of heat stress abatement. One unexplored tool for measuring prolonged heat stress in cattle is measuring the change in rumen and hindgut microbiome. Ruminants derive a majority of their energy from rumen fermentation; however, recent studies suggest that hindgut fermentation may play a critical role in feed efficiency (Welch et. al, 2021). An efficiency study utilizing Nellore cattle found bacterial families such as *Lachnospiraceae* and *Ruminococcaceae* were more abundant in the rumen of more efficient cattle (Lopes et. al, 2021). Acclimation to prolonged heat stress may cause shifts in rumen and hind gut flora which can decrease the abundance of beneficial families of bacteria leading to decreases in efficiency and metabolites beneficial to animal and gut health (Corvo et. al, 2021).

In this study, there was a distinct change in hindgut microbiome flora that occurred during the initial ten-day acclimation period. At the start of the feedlot period OUT and SHADE steers had greater access to forages during the acclimation period, however, CNF and CWF steers' feces contained greater abundances of *Oscillospiraceae*, *Lachnospiraceae*, and *Ruminoccaceae*. Several studies have reported bacterial families such as *Lachnospiraceae*, *Ruminoccaceae*, and *Oscillospiraceae* to be highly abundant in forage-based diets and serve primarily as forage degraders within the rumen and hind gut (Correa et. al, 2021; Zoelzer et. al, 2021; Coates et. al, 2022). Greater abundance of forage degraded can indicate that feed intake of CWF and CNF steers was greater than that of OUT and SHADE. As stated previously, studies found that Lachnospiraceae and Oscillospiraceae were associated with feed efficiency and this study supports those findings. Feces collected on d 0 from OUT and SHADE steers contained greater abundance of F082. The family of F082 was reported to be positively correlated with

methane emission levels (Andrade et. al, 2022). While some methane production is necessary in normal rumen and hindgut fermentation, high levels of methane production represent efficient fermentation and reduced feed conversion (Basarab et. al, 2013). As the study progressed and the steers acclimated to their new conditions, so did their hindgut microbiome as fewer differences were detected on d 50 and d 85. There were prolonged effects detected for the bacterial families *Atopobiaceae* and *Ruminoccaceae*. Both bacterial families are reported to be more abundant in high starch diets and positively correlated with sub-acute ruminal acidosis (Mao et. al, 2012; Plaizier et. al, 2017). While an increase in abundance of these families may be influenced by the high-starch feedlot diet fed in this study, the difference between CWF and CNF steers compared to the OUT and SHADE steers is more likely attributed to the difference in intake as all four treatment groups were fed the exact same diet.

Conclusion

Heat stress is a dynamic symptom that can be defined by both its acute and prolonged impacts on the animal. While not the original intent of this study, blood gas analysis was an effective tool to measure acute physiological changes in heat stressed steers. Sims et al. (2019) confirmed OUT and SHADE steers were in extreme hot climate conditions; the initial blood gas data identified acute physiological changes during the acclimation phase in steers that were housed used the feeding barn. These results highlight the challenge of using whole blood values as a definitive identifier of heat stress, especially in cattle that may have acclimated to their climate. It is often difficult to measure the effects of long-term heat stress due to the natural physiological drive to maintain homeostasis. This study, using next-gen sequencing, was able to detect long term changes in the fecal microbiome. Shifts in the microbiome can be indictive of

dysbiosis and efficiency and can be a powerful tool to measure the long-term effects of heat stress on feedlot performance and health.

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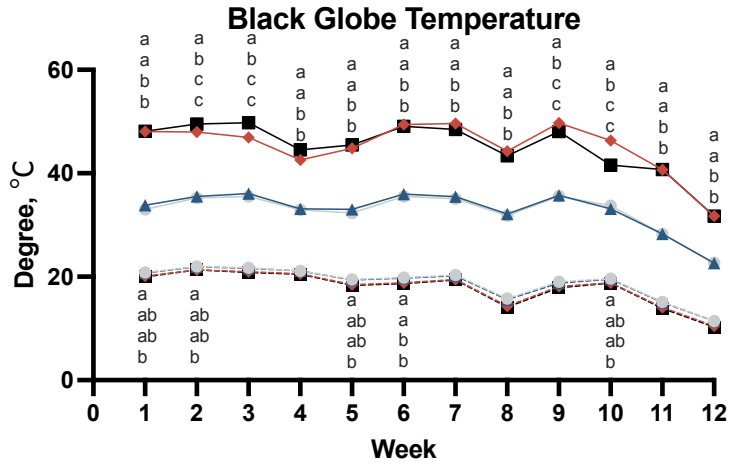
Table 5.1. Composition of diet for feedlot steers.[1]

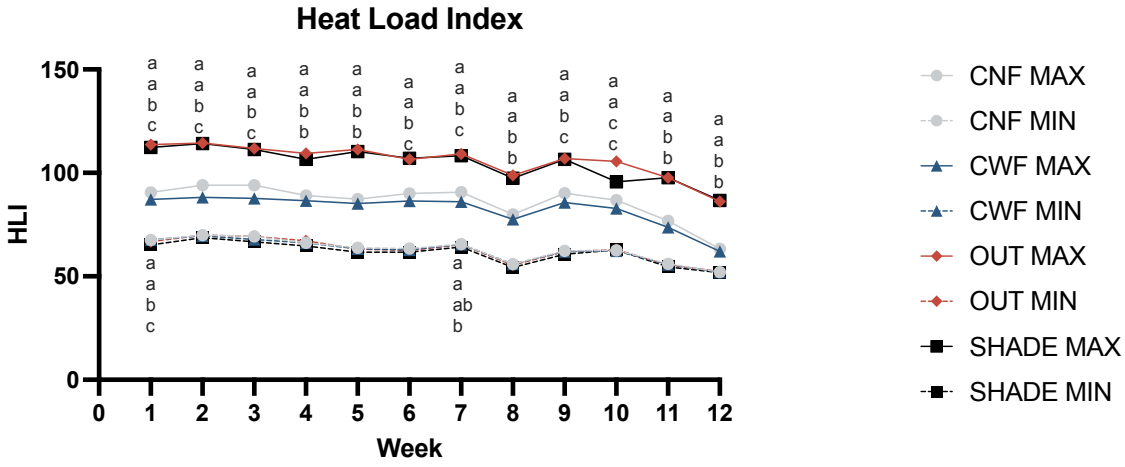
Ingredients	Percent dry matter basis
Ground corn	46.00
Corn distiller grains	24.00
Ground hay	12.98
Soy hulls	5.00
Peanut hulls	5.00
Molasses	2.00
Calcium carbonate	1.50
NaCl	1.00
TM Godfrey's mineral mix[2]	1.25
Sodium bicarbonate	1.00
Ammonium chloride	0.20
Vitamin A, D, & E	0.05
Rumensin 90	0.02
Analyzed nutrient composition, % DM basis	
Dry matter	89.01
Crude protein	13.12
Neutral detergent fiber[3]	26.36
Acid detergent fiber[3]	14.00

[1] Diet composition reported in Sims et al. (2019)

[2]Trace mineral Godfrey's feed; Ca 19.65%, S 8.20%, Mg 0.27%, Co 769.00 mg/kg, Cu 15,361.01 mg/kg, I 1,441.00 mg/kg, Fe 12,030.00 mg/kg, Mn 57,632.00 mg/kg, Se 288.00 mg/kg, Zn 72,000.03 mg/kg.

[3]Calculated using Ankom 2000 Fiber Analyzer.


Black Globe Temperature
Degree, °C
Week


Heat Load Index
HLI
Week
CNF MAX
CNF MIN
CWF MAX
CWF MIN
OUT MAX
OUT MIN
SHADE MAX
SHADE MIN

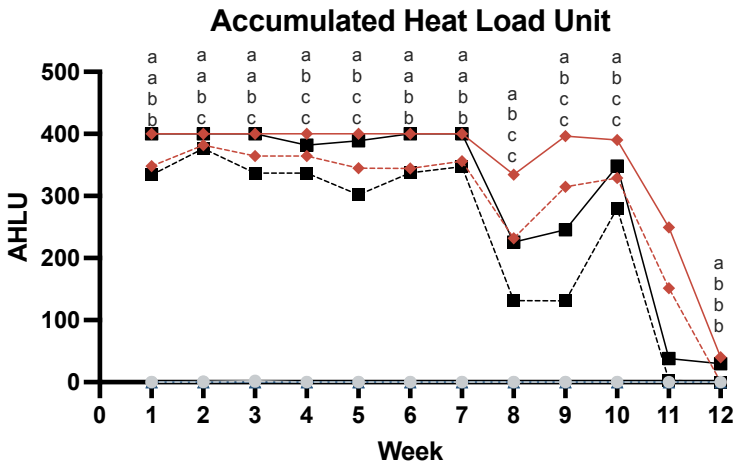

Accumulated Heat Load Unit
AHLU
Week

Figure 5.1. Climate data for **a)** Black globe temperatures (°C), **b)** Heat load index (HLI), **c)** Accumulated heat load unit (AHLU) measured every 30 min for each treatment utilizing two Kestrel meters (5400AG cattle heat stress trackers; KestrelMeters; Boothwyn, PA). Two Kestrel were placed even along pens and feeding barn which housed 32 steers that were assigned to one of four shade abatement strategies: Inside a feeding barn with cooling fans (**CWF**), inside feed barn with covered no cooling fan (**CNF**), outside drylot with an optional shade structure providing 3.05m × 3.66 m of shade per animal (**SHADE**), or outside drylot with no shade or fan (**OUT**). Results are presented treatment × time, however, differences are presented within day. [ab]Treatment differences are significant at $P < 0.05$ on d 0, [cd]Treatment differences are significant at $P < 0.05$ on d 50, [ef]Treatment differences are significant at $P < 0.05$ on d 85

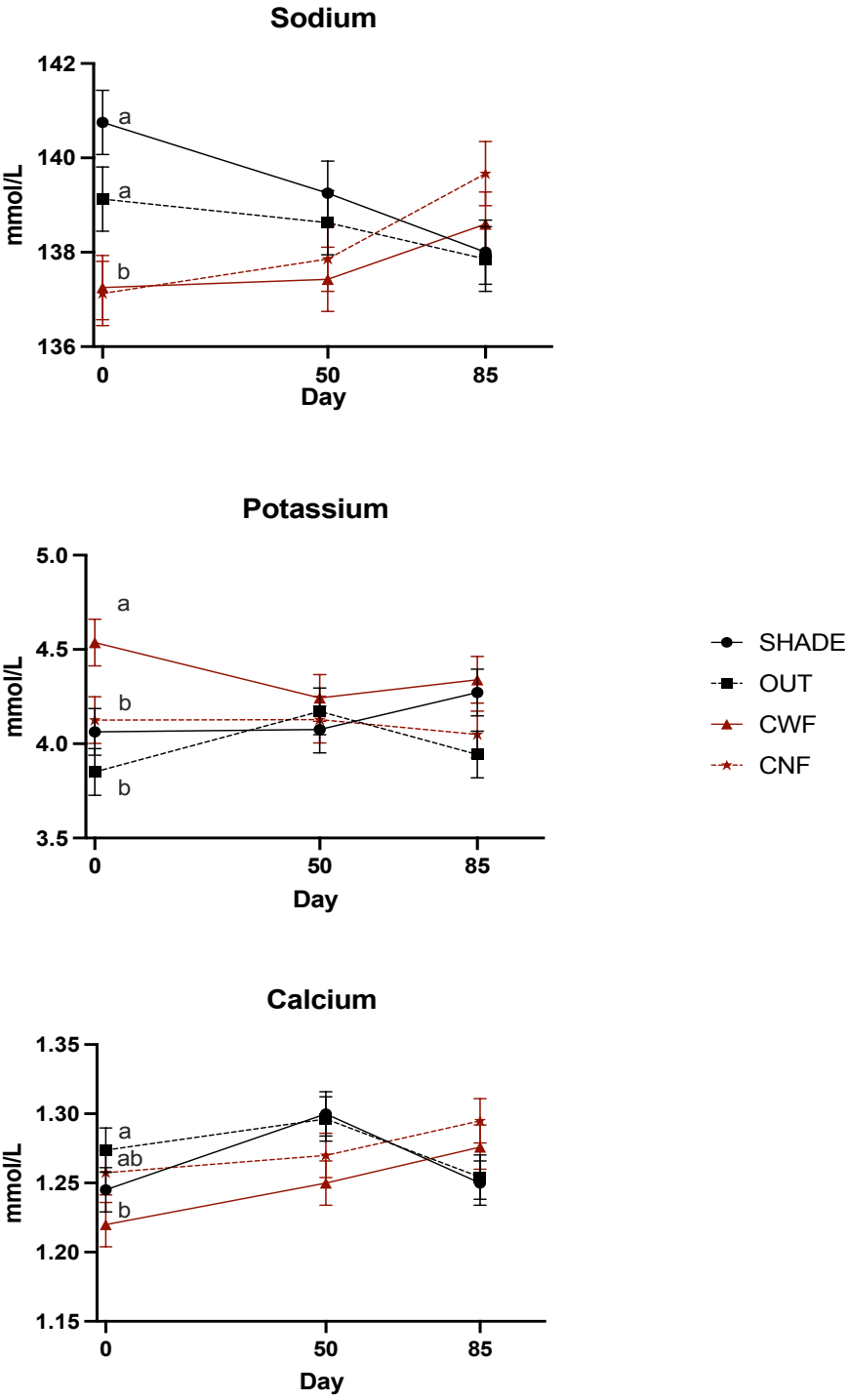

Sodium
142
140
138
136
mmol/L
a
a
b
0
50
85
Day
Potassium
5.0
4.5
4.0
3.5
mmol/L
a
b
b
0
50
85
Day
SHADE
OUT
CWF
CNF
Calcium
1.35
1.30
1.25
1.20
1.15
mmol/L
a
ab
b
0
50
85
Day

Figure 5.2. Whole blood values for **a)** Sodium (Na, mmol/L), **b)** Potassium (K, mmol/L), **c)** Calcium (Ca, mmol/L) measured in fecal samples collected from 32 steers that were assigned to one of four shade abatement strategies: Inside a feeding barn with cooling fans (**CWF**), inside feed barn with covered no cooling fan (**CNF**), outside drylot with an optional shade structure providing 3.05m × 3.66 m of shade per animal (**SHADE**), or outside drylot with no shade or fan (**OUT**). Results are presented treatment × time, however, differences are presented within day. [ab]Treatment differences are significant at $P < 0.05$ on d 0, [cd]Treatment differences are significant at $P < 0.05$ on d 50, [ef]Treatment differences are significant at $P < 0.05$ on d 85

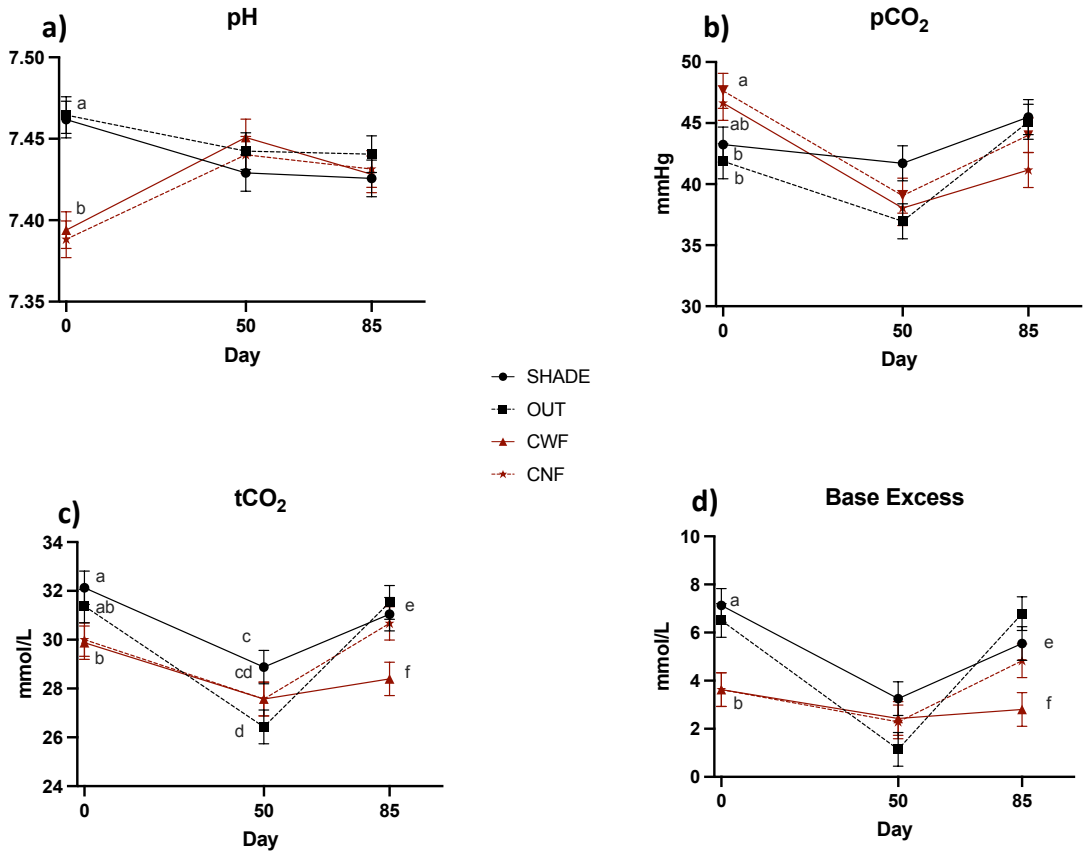

a)
pH
7.50
7.45
7.40
7.35
a
b
0
50
85
Day
b)
pCO2
50
45
40
35
30
mmHg
a
ab
b
b
0
50
85
Day
SHADE
OUT
CWF
CNF
c)
tCO2
34
32
30
28
26
24
mmol/L
a
ab
b
c
cd
d
e
f
0
50
85
Day
d)
Base Excess
10
8
6
4
2
0
mmol/L
a
b
e
f
0
50
85
Day

Figure 5.3. Whole blood values for **a)** pH, **b)** pCO_2 (mmHg), **c)** tCO_2 (mmol/L), **d)** Base excess (mmol/L) measured in fecal samples collected from 32 steers that were assigned to one of four shade abatement strategies: Inside a feeding barn with cooling fans (**CWF**), inside feed barn with covered no cooling fan (**CNF**), outside drylot with an optional shade structure providing 3.05m × 3.66 m of shade per animal (**SHADE**), or outside drylot with no shade or fan (**OUT**). Results are presented treatment × time, however, differences are presented within day. [ab]Treatment differences are significant at $P < 0.05$ on d 0, [cd]Treatment differences are significant at $P < 0.05$ on d 50, [ef]Treatment differences are significant at $P < 0.05$ on d 85

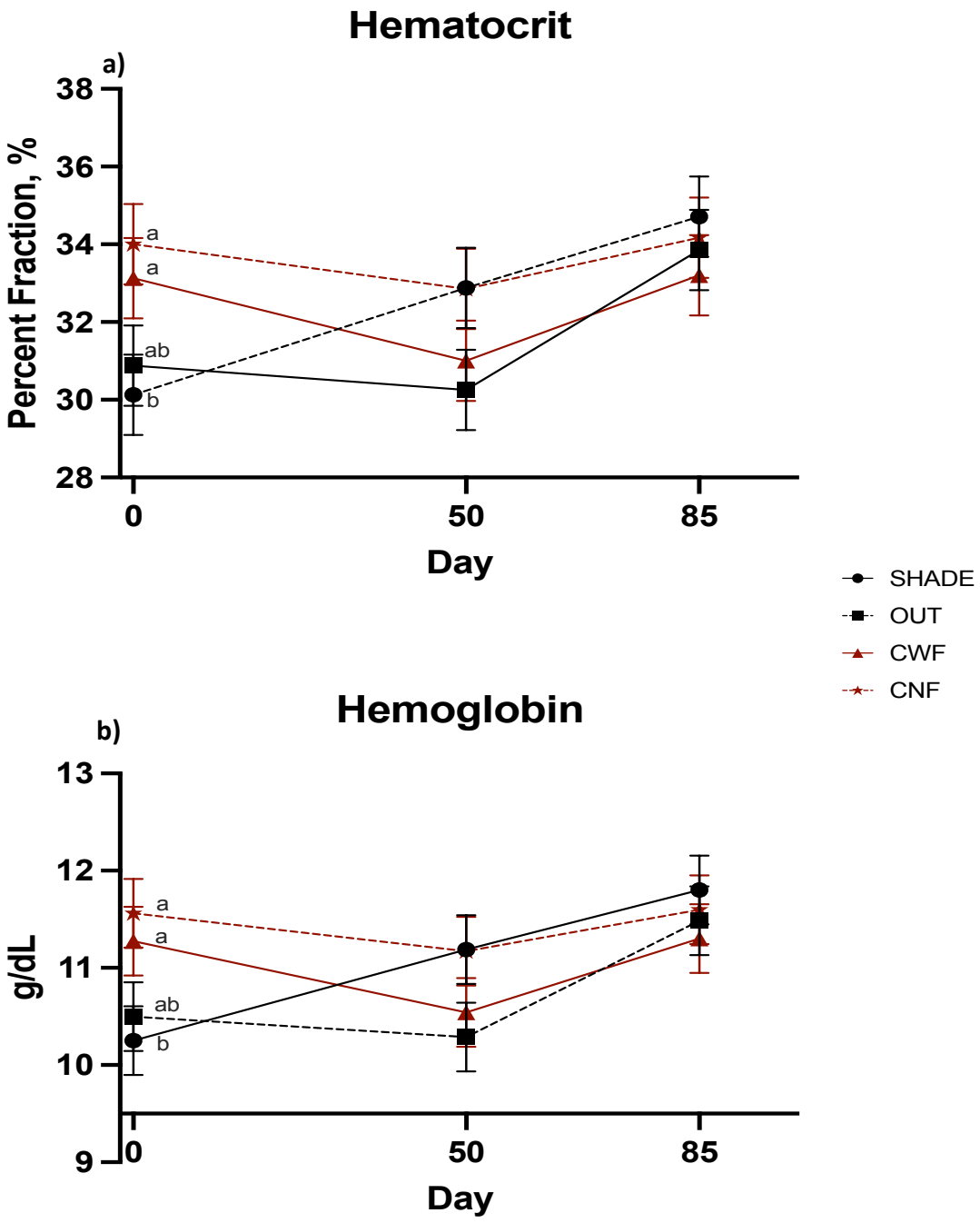

Hematocrit
a)
Percent Fraction, %
38
36
34
32
30
28
a
a
ab
b
0
50
85
Day
SHADE
OUT
CWF
CNF
Hemoglobin
b)
g/dL
13
12
11
10
9
a
a
ab
b
0
50
85
Day

Figure 5.4. Whole blood values for **a)** hematocrit (%) and **b)** hemoglobin (g/dL) measured in fecal samples collected from 32 steers that were assigned to one of four shade abatement strategies: Inside a feeding barn with cooling fans (**CWF**), inside feed barn with covered no cooling fan (**CNF**), outside drylot with an optional shade structure providing 3.05m × 3.66 m of shade per animal (**SHADE**), or outside drylot with no shade or fan (**OUT**). Results are presented treatment × time, however, differences are presented within day. [ab]Treatment differences are significant at $P < 0.05$ on d 0

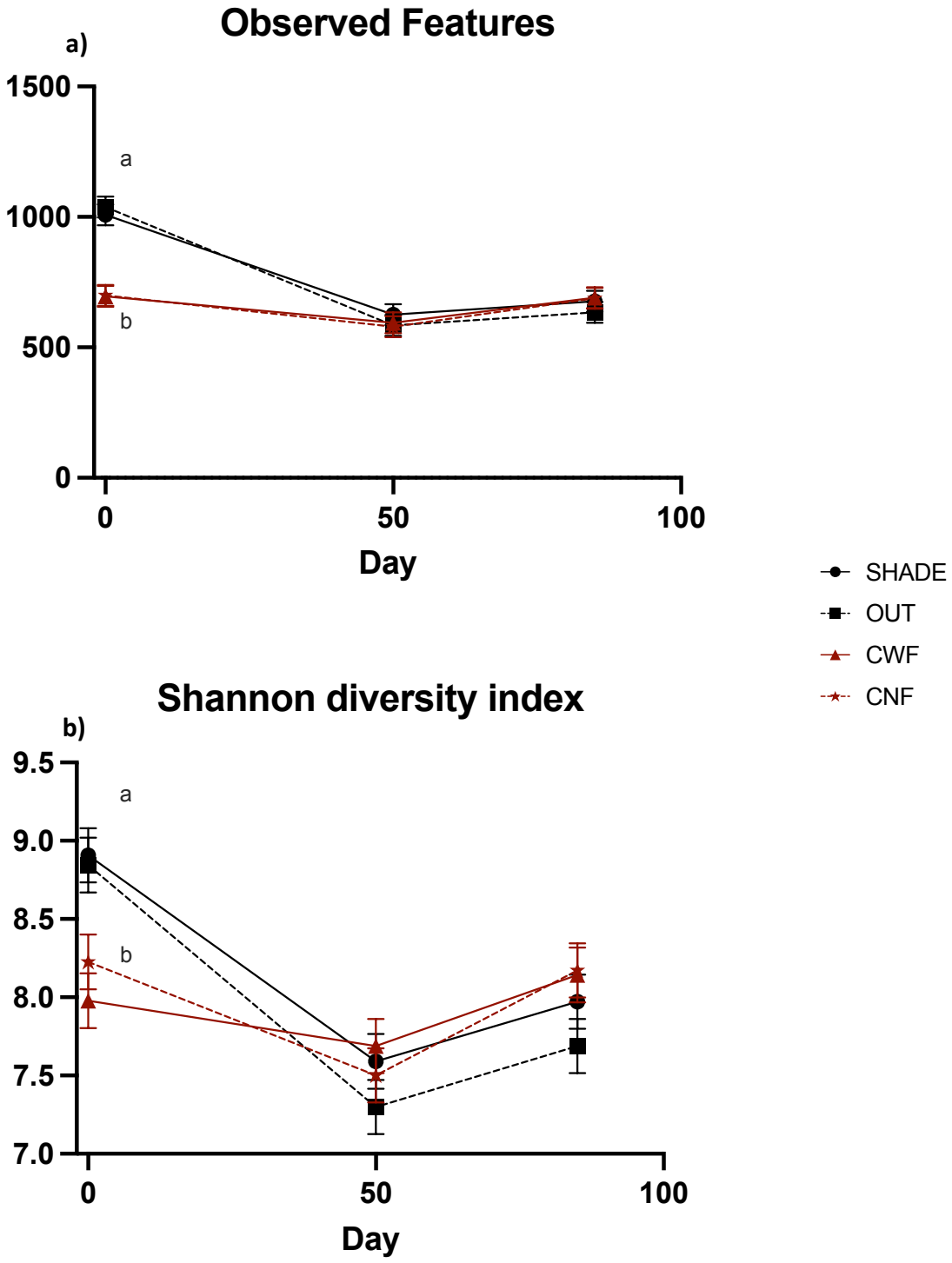

Observed Features
a)
1500
1000
500
0
a
b
0
50
100
Day
SHADE
OUT
CWF
CNF
Shannon diversity index
b)
9.5
9.0
8.5
8.0
7.5
7.0
a
b
0
50
100
Day

Figure 5.5. Alpha diversity metrics, **a)** Observed features and **b)** Shannon diversity index, measured in fecal samples collected from 32 steers that were assigned to one of four shade abatement strategies: Inside a feeding barn with cooling fans (**CWF**), inside feed barn with covered no cooling fan (**CNF**), outside drylot with an optional shade structure providing 3.05m × 3.66 m of shade per animal (**SHADE**), or outside drylot with no shade or fan (**OUT**). Results are presented treatment × time, however, differences are presented within day. [ab]Treatment differences are significant at $P < 0.05$ on d 0

Day		Treatment			
		CNF	CWF	OUT	SHADE
0	Atopobiaceae	0.12	0.23	0.19	0.19
	Bifidobacteriaceae	*1.34	*1.03	◊0.14	◊*0.23
	Fibrobacteraceae	0.21	0.09	0.00	0.00
	Lachnospiraceae	*17.58	◊*17.59	◊13.07	◊11.69
	Muribaculaceae	1.95	1.73	1.23	1.15
	Oscillospiraceae	◊18.53	◊19.41	*25.13	*24.76
	Ruminococcaceae	◊*5.68	*6.04	◊2.93	◊*3.55
	Saccharimonadaceae	◊0.00	◊0.00	*0.23	*0.14
	F082	◊2.78	◊2.95	*6.45	*6.45
50	Atopobiaceae	*13.78	◊7.78	◊4.07	◊6.90
	Bifidobacteriaceae	0.28	0.16	0.45	0.76
	Fibrobacteraceae	0.22	0.21	0.09	0.09
	Lachnospiraceae	15.24	21.81	17.58	17.78
	Muribaculaceae	7.20	7.37	20.85	15.59
	Oscillospiraceae	+11.76	+11.03	*11.00	◊13.31
	Ruminococcaceae	14.78	[illegible]	6.88	6.90
	Saccharimonadaceae	*0.00	*0.00	◊0.00	◊0.00
	F082	0.00	0.03	0.03	0.04
85	Atopobiaceae	6.58	3.14	9.46	2.57
	Bifidobacteriaceae	◊*0.29	◊0.10	*0.21	◊0.28
	Fibrobacteraceae	0.52	0.34	0.09	0.20
	Lachnospiraceae	*19.83	◊*22.52	◊21.52	◊18.47
	Muribaculaceae	5.60	4.95	5.50	7.10
	Oscillospiraceae	12.47	13.91	12.33	14.40
	Ruminococcaceae	11.79	9.25	5.83	8.65
	Saccharimonadaceae	*0.00	◊*0.00	+0.00	+◊0.00
	F082	0.03	0.02	0.05	0.03

Figure 5.6. Changes in relative abundance of bacterial families in steers feces who were either: Inside a feeding barn with cooling fans (**CWF**), inside feed barn with covered no cooling fan (**CNF**), outside drylot with an optional shade structure providing 3.05m × 3.66 m of shade per animal (**SHADE**), or outside drylot with no shade or fan (**OUT**). Relative abundance for *Atopobiaceae*, *Bifidobacteriaceae*, F082, *Muribaculaceae*, *Saccharimonadaceae*, *Oscillospiraceae, Lachnospiraceae, Fibrobacteraceae,* and *Muribaculaceae* measured in fecal samples collected on d 0, 50, 84 from steers. Data is expressed as mean. *◊+Treatment differences are presented within day and are significant at $P < 0.05$

CHAPTER 6

Conclusion

In the southeast U.S. the largest sector of the beef industry is cow-calf operations. There is, due to post-covid changes in the market, increased interest in more regional growth in stocking and finishing operations. There are major regional differences that may influence the growth of stocker or finishing operations in the southeast. In the southeast, feed cost can be greater, in addition to the hot climate. In order to better prepare the Southeast producer for increased production, further research is required to identify management strategies that may improve the efficiency of their systems.

In the first study, 48 recently weaned beef calves were paired by weight into pens (N = 24) and each pen was assigned one of four treatment groups: Calves receiving 100% NRC recommended levels of metabolizable protein (MP) during the entire study (**P0**), calves receiving 110% NRC recommended levels of MP for the first 7 days and then received 100% NRC recommended levels for the remainder of the study (**P7**), calves receiving 110% NRC recommended levels of MP for the first 14 days and then received 100% NRC recommended levels for the remainder of the study (**P14**), calves receiving 110% NRC recommended levels of MP for the entire study (**P42**). Calves were vaccinated at weaning and vaccination booster were administered on d 14. Weights were collected at weaning and again every 7 days, and serum was collected at weaning and again on d 3, 7, 14, 21, and 42. Increased supplementation increased serum glucose levels

during d 3 to d 14, however, after the initial weaning stress phase, there were no differences. Furthermore, supplementation did not improve titer levels or weight gain during the trial.

In the second study, two experiments were conducted to measure the impact of a blend of phytochemicals on fermentation and performance in stocker cattle. Experiment one was conducted over 2 years, 162 recently weaned beef calves were stratified by weight and assigned to one of nine pens. Each pen was then randomly assigned one of three treatments: 1) No supplementation control (CON), 2) Monensin supplementation at a rate of 200mg • head • day^{-1} (MON), 3) Extract supplementation at a rate of 1g • head • day^{-1} (PCB). During the 84 d experiment, cattle were fed corn silage-based diet supplemented dried distillers grains at 1.8 $kg•steer^{-1}•day^{-1}$. Weight was measured on d 0 and again every 28 days. Additionally, rumen fluid was collected on d 0 and d 84 from all steers for both years. In experiment two, an in vitro study was conducted to measure the impact of the three treatments on dry matter disappearance, gas production, and pH. In the in vitro experiment, dry matter disappearance and gas production was similar between PCB and MON supplemented bottles. During the stocker experiment, however, MON supplemented steers gained more per day than PCB and MON supplemented steers while producing lower concentrations of acetate in the rumen.

In the third study, blood gas values and fecal microbiome were analyzed to measure the impact of heat stress abatement strategies on animal physiology. In the study, 32 angus steers were randomly assigned to one of four treatments: covered with fan (CWF), covered no fan (CNF), outside drylot with optional shade (SHADE), or outside drylot with no shade or fan (OUT). Whole blood and fecal samples were

collected on d 0, 50, and 85 to measure blood gas values and fecal microbiome. Differences in blood gas values were observed on d 0, but as steers acclimated, no differences were detected. There were, however, long term differences measured in fecal microbiome, highlighting the effect of increased intake in the CWF and CNF steers compared to OUT steers.

The research in this dissertation highlights the complex relationship between nutrition and animal physiology. While previous research has suggested that increased protein supplementation or phytochemical supplementation should improve animal performance and health, no differences were measured in the first two studies. This work reaffirms the challenges when comparing results between studies, in impact of various environmental and management conditions. Additionally, the third study demonstrates the use of next-gen sequencing as a tool to measure stress, in addition to diet derived changes. Where blood gas values failed to measure differences after steer acclimated to their environment, next-gen sequencing was still able to detect differences between groups.