PRE-WEANING DIETARY MANAGEMENT OF BEEF CALVES: INVESTIGATING THE EFFECTS OF *LACTOBACILLUS ACIDOPHILUS* FERMENTATION PRODUCT SUPPLEMENTATION ON THE GASTROINTESTINAL MICROBIOTA, METABOLOME, GROWTH PERFORMANCE, AND ACUTE PHASE IMMUNE RESPONSE OF ANGUS STEERS

by

TAYLOR RAE KRAUSE

(Under the Direction of T. Dean Pringle)

ABSTRACT

Growing pressure from consumers and legislation to reduce antibiotic usage in cattle production has left producers needing natural alternatives like microbial supplements that are capable of maintaining animal health and performance during times of stress such as the weaning and receiving periods. Therefore, the objective of this research was to investigate the impacts of pre- and post-weaning dietary supplementation of grain, with or without the addition of a *Lactobacillus acidophilus* fermentation product (LAFP) microbial supplement, on beef steer performance, gastrointestinal fermentation, and immune response. Sixty suckling steers (211.4 ± 21.2 kg; 173 ± 17 d) were selected and assigned to 1 of 3 dietary treatments: (1) **CONTROL** – calves were on pasture with dam (2) **CREEP** – same as CONTROL plus given access to *ad libitum* creep feed (3) **LAFP** – same diet as CREEP but with LAFP added. Dietary treatments were administered for 70-d prior to weaning. During this period, the first study was designed to

evaluate the impacts of dietary treatment on growth performance and composition, feed intake, daily activity, and gastrointestinal volatile fatty acid (VFA) profiles of the steers. Results from this study indicated that creep feed supplementation tended to improve steer growth performance over CONTROL. Moreover, the addition of LAFP to the creep feed tended to improve feed efficiency, which could be due in part to the numerically lower feed intake and greater VFA concentrations, especially propionate, reported in the gastrointestinal tracts of LAFP steers compared to CREEP. For the second study, the same steers were weaned and transported to a new facility where they were subjected to a lipopolysaccharide immune challenge on day 4. Overall, results from the second study suggested that while the challenge model employed was successful, as indicated by changes in immune markers over time, there were no significant differences in the responses across the different dietary treatments, indicating that all steers responded similarly to the immune challenge regardless of pre-weaning dietary treatment. In conclusion, this research provides the cattle industry with valuable information regarding the impacts of dietary management and microbial supplementation on steer performance and immune function in response to an immune challenge.

INDEX WORDS: Creep Feed, Immune Challenge, Lipopolysaccharide, Microbial Supplement, Propionate, Volatile Fatty Acids

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December 2022

# DEDICATION

I would like to dedicate this work to my family, especially to those that are no longer with me but are still watching over me. Through them I saw what faith, passion, and true dedication could achieve and it is this vision that drives me each day. To the rest of my family, I cannot thank you enough for your continued support and encouragement. To my parents, Dale and Pat, I can't even begin to find the words to describe how grateful I am for you both. Your unconditional love, understanding, patience, and countless sacrifices do not go unnoticed, and they are the reason I am where I stand today. To my fiancé, Nick, thank you for continuing to believe in me day in and day out (even if I may be wrong), for keeping me grounded when I wander, and for embarking on this journey with me without hesitation. I can't wait to see where life takes us … together … forever together.

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# CHAPTER 1

# INTRODUCTION

Historically, sub-therapeutic levels of antibiotics have been included in livestock feedstuffs as a means to improving animal performance and health (Barton, 2000; Centner, 2008; Papatsiros et al., 2013). This practice was often times employed during the receiving period to alleviate some of the stress and disease challenges encountered by the often recently weaned calves coming into the facility (Finck et al., 2014). Further benefits were then achieved in the feedlot by including sub-therapeutic levels of antibiotics to prevent disease and to promote growth and feed efficiency (Mathews and Johnson, 2013). Such benefits could be seen in a study where two groups of feedlot cattle started at the same weight, but those fed using conventional methods were on feed for 162 days whereas those cattle fed without antibiotics required 212 days to reach the same end weight (Morley et al., 2011).

Nevertheless, consumer concerns regarding the therapeutic, metaphylactic, prophylactic (i.e., sub-therapeutic), and non-therapeutic uses of antibiotics in livestock production have risen over the years with the largest emphasis being on the possible transmission of antibiotic resistance (Krehbiel et al., 2003; Centner, 2008). Unfortunately, research does suggest that repeated and/or long-term exposure of microbes to antibiotics can foster the development of antibiotic resistance (Centner, 2008; Maron et al., 2013). As a result, U.S. consumers along with federal legislators began to advocate for a reduction in antibiotic use, especially the elimination of non-therapeutic

antimicrobials in livestock feeds, similar to the regulations put into effect by the European Union in 2006 (Centner, 2008, 2016). Ultimately, this led to the U.S. Food and Drug Administration (FDA) creating the veterinary feed directive (VFD) in 2015. In brief, the VFD is a legislative document that provides stricter veterinary oversight of antimicrobial usage in food animal production. More specifically, the VFD bans the non-therapeutic (i.e., growth promotion and improved efficiency) use of select over-the-counter antimicrobials in livestock feeds and encourages greater oversight regarding therapeutic uses (i.e., treating, controlling, and preventing disease) (Centner, 2016). In the end, the VFD along with consumer demands have generated a need for antibiotic alternatives capable of maintaining animal health and productivity.

As consumer awareness of livestock production continues to grow, the demand for more "natural" production systems (i.e., no antimicrobials, hormones, growth promotants, or other synthetic supplements) also grows (Ellison et al., 2017; Ovinge et al., 2018). Accordingly, more producers are transitioning into more "natural" management schemes (Wileman et al., 2009), albeit, with some expectations of getting a greater market value since consumers have expressed an increased willingness to pay for such husbandry practices (Umberger et al., 2009; Mathews and Johnson, 2013; Lewis et al., 2017). Nevertheless, this move towards more "natural" production systems has created a need for "natural" alternatives to traditional management practices (i.e., antimicrobials, growth promoters, growth implants, etc.). These natural alternatives need to be capable of maintaining animal health and performance similar to that of traditional production, while also allowing producers to comply with the program's regulations (Wileman et al., 2009; Stackhouse et al., 2012; Mathews and Johnson, 2013; Papatsiros et

al., 2013). In particular, “natural” alternatives should be capable of growth promotion and prophylactic use against pathogens in order to prevent economic burdens greater than those of traditional production (Papatsiros et al., 2013). Some “natural” alternatives that function as non-antibiotic feed additives are prebiotics, probiotics (i.e., direct fed microbials), phytogenics, zeolites, and organic acids (Papatsiros et al., 2013). Early research has shown that microbial dietary supplements (e.g., probiotics, synbiotics, prebiotics) have immune stimulating, growth promoting, gut health enhancing, and antimicrobial producing properties (Miles and Bootwalla, 1991; Yoon and Stern, 1995; Awad et al., 2009; Broadway et al., 2015; Hall et al., 2018; Cangiano et al., 2020), especially when fed during periods of stress (Duff and Galyean, 2007b). While these findings are optimistic, there are a lot of inconsistencies amongst published results due in part to the diversity of concepts being tested and the environments in which they are being tested (Krehbiel et al., 2003; Papatsiros et al., 2013; Seal et al., 2013).

In the end, the need for microbial supplements that can serve as “natural” alternatives to antibiotics is growing. Thus, the overall objective of the current study was to evaluate the effects of supplementation with a *Lactobacillus acidophilus* fermentation product (LAFP) in creep feed on health and performance of beef steers pre- and post-weaning. Specific objectives included 1.) determining if inclusion of LAFP in creep feed prior to weaning can improve nutrient availability and utilization, through alterations in the gut microbiota and metabolites, resulting in improvements in pre-weaning performance 2.) determining if inclusion of LAFP pre- and post-weaning can help to alleviate weaning stress and modulate immune function post-weaning. Immune function, specifically, was evaluated using a lipopolysaccharide (LPS) challenge model which

allowed us to evaluate the capacity of LAFP to alter the acute phase response (APR) from physiological markers to immune mediators to metabolic markers. We hypothesized that LAFP supplementation would improve nutrient availability and utilization, through an altered gut microbiota and subsequent metabolite profile, resulting in improved animal performance pre-weaning. Furthermore, we hypothesized that LAFP supplementation would prime the innate immune system, subsequently altering the APR to an LPS challenge post-weaning.

# CHAPTER 2

# LITERATURE REVIEW

## Overview of Creep Feeding Practices

On cow-calf operations, income is frequently attained by selling calves at weaning. These calves are commonly sold on a weight basis, which means increased weaning weights increases income for these producers. One way these increased weaning weights can be reached is via creep feeding which involves giving suckling calves access to a grain-based supplement (Cremin, 1989; Faulkner et al., 1994; Tarr et al., 1994). This grain-based supplement, or creep feed, has customarily involved unlimited access to a high-starch based supplement; however, some studies have found benefits to limit feeding creep feed and/or using a more fiber-based supplement (Lusby, 1986; Faulkner et al., 1994; Meteer et al., 2013). When comparing high-fiber and high-starch creep feed, the high-starch calves did consume on average 0.24 kg more dry matter on a daily basis, but there were no differences in forage dry matter intake or average daily gain (ADG) between the groups (Faulkner et al., 1994), suggesting that while the high-starch calves were consuming more creep feed, they did not have increased weight gains over those calves on a fiber-based creep feed. When comparing limited versus unlimited access, total dry matter intake increased with increasing creep feed intake and this was accompanied by a 13% improvement in ADG (Faulkner et al., 1994). In general, as creep feed intake increases, forage intake decreases whereas milk intake does not differ (Cremin et al., 1991; Faulkner et al., 1994; Tarr et al., 1994). As a result, calves generally

benefit more from creep feed in situations where they have poor quality and/or low quantities of forage and/or milk (Tarr et al., 1994).

### *Impacts on Performance*

Studies have reported increased weight gains associated with creep feeding (Prichard et al., 1989; Faulkner et al., 1994; Tarr et al., 1994). For instance, Faulkner et al. (1994) described a 39% increase in ADG for limit-fed calves over controls, whereas calves given unlimited access to creep feed had a 52% increase in ADG over controls. When evaluating these same calves for supplemental feed efficiency, however, there were no significant differences between the limited and unlimited calves (Faulkner et al., 1994), suggesting that the increased ADG of the unlimited calves was also accompanied by greater intake, which holds true to the greater total dry matter intake noted previously in *ad libitum* supplemented calves. Other studies have observed that when creep feeding in general, greater weight gains and efficiency can be achieved with longer term supplementation as opposed to shorter-term, due in part to reduced feed efficiency over the first 28 days as the calves acclimate to the supplement (Prichard et al., 1989; Faulkner et al., 1994; Tarr et al., 1994).

### *Impacts on Carcass Characteristics*

When looking at carcass characteristics of previously creep fed calves, one study observed increased backfat measured via ultrasound at weaning (Tarr et al., 1994), whereas another reported no differences on the carcass at weaning (Rouquette et al., 1983). Another study assessed carcass characteristics at slaughter on previously creep fed calves and reported that carcass quality grade linearly increased and backfat thickness

tended to increase linearly with increasing creep feed supplementation, but that other carcass characteristics relating to yield did not differ (Faulkner et al., 1994).

### *Impacts on Rumen Fermentation*

Some growth performance variations, could be attributed in part to alterations in ruminal volatile fatty acid (VFA) concentrations, as VFAs are a major energy source for cattle. Faulkner et al. (1994) and Tarr et al. (1994) both reported increasing total VFA concentrations in the rumens of calves supplemented with increasing levels of creep feed. Specifically, they noted decreased acetate and increased propionate and valerate concentrations with increasing creep feed supplementation (Faulkner et al., 1994; Tarr et al., 1994). It was, therefore, logical that these studies and others observed a decrease in rumen pH with increased creep feed intake (Cremin et al., 1991; Faulkner et al., 1994; Tarr et al., 1994) as an increase in rapidly fermentable substrates would result in a more acidic environment as indicated by the greater total VFA concentrations (Owens and Basalan, 2016).

### *Impacts on Weaning Stress*

Aside from the above-mentioned benefits, the big-picture benefit of creep feeding is rooted in its ability to ease the transition at weaning. Weaning is a normal and necessary management technique that occurs on traditional beef production operations and can be defined as the practice of permanently separating a cow and her calf. Weaning is a stressful event for the calves and often occurs in conjunction with other stressors like transportation and handling (Weary et al., 2008; Enríquez et al., 2011). As a result of these stressors, weaning is often accompanied by a period of poor performance resulting from increased physical activity, reduced nutrient intake, increased vocalizations, and

social restructuring (Enríquez et al., 2011). Along with the poor performance, studies have reported shifts in blood markers like cortisol, catecholamines, acute phase proteins (APP), and leukocytes relating to stimulation of the stress and immune responses (Lefcourt and Elsasser, 1995; Arthington et al., 2003; Carroll and Forsberg, 2007; Carroll et al., 2009a; Enríquez et al., 2011). Unfortunately, periods of acute stress, like weaning, are generally associated with reduced digestive activity (Carroll and Burdick Sanchez, 2014) which can result from a disruption in the gastrointestinal microbiota balance, termed dysbiosis (Sissons, 1989; Carding et al., 2015). One instance in which dysbiosis has been reported to occur is during times of stress, like at weaning, when feed intake is reduced (Sissons, 1989; Gresse et al., 2017; Li et al., 2018). Since creep feeding acclimates the calves to the weaning transition more smoothly by getting them accustomed to grain, their feed intake is often less impacted (Baldwin et al., 2004; Lardy and Maddock, 2007). It is thus possible that creep feeding may help to reduce dysbiosis and the resulting reduced performance around weaning.

**Introduction to the Digestive System of Ruminants**

Ruminants such as cattle have a unique digestive system because they have a four-chambered stomach (i.e., rumen, reticulum, omasum, abomasum) which allows them to ruminate. Ruminating is the action of regurgitating feedstuffs from the rumen for remastication or rechewing. In addition to rumination, ruminal microbial populations assist in the breakdown of plant material (i.e., cellulose, hemicellulose, lignin) into energy substrates usable by the ruminant animal. Ruminal microbial populations along with populations of the lower gastrointestinal tract, are necessary for the successful fermentation of plant material because they produce specialized enzymes (e.g., cellulase)

that mammals are not capable of producing themselves. These microbial enzymes, therefore, allow ruminants to convert indigestible feedstuffs into high quality nutrients like meat and milk via the conversion of the feedstuffs into usable energy for the host. In addition to producing the host's main source of energy, microbial populations in the gastrointestinal tract also provides the host with protein and vitamins for maintenance and growth (Hungate, 1966). More specifically, rumen microbes produce between 70-85% of the energy substrates (i.e., VFA) and 70-100% of the nitrogen (i.e., microbial crude protein) used by the host (Dewhurst et al., 1986; Agricultural and Nutrients, 1992). Consequently, differences in the production and absorption of such compounds, or metabolites, can lead to differences in energy status and nutrient utilization, potentially contributing to differences in host performance (Huntington, 1990; Saleem et al., 2013).

***The Metabolome and Microbiome***

Ultimately, metabolites produced by microbial activity as well as those produced by the host themselves are collectively referred to as the metabolome. The metabolome is defined as the collection of all low molecular weight molecules (i.e., metabolites) that are present in a given living system and capable of participating in metabolic reactions required for maintenance, normal function, and growth of that system (Wilkinson, 2009). A large segment of the metabolome, in ruminants specifically, is produced by the microbial populations, or microbiota, present in a given system. In general, the microbiota collectively refers to all of the living microorganisms (i.e., bacteria, archaea, protozoa, fungi, viruses) present in a given environment. The microbiota are also commonly referred to as the microbiome; however, the term microbiome explicitly refers

to the genetic material of the microbiota, rather than the living functioning microbes themselves.

As mentioned previously, the metabolome in ruminants is reflective of the collective contributions of both the microbiota and the host. Thus, using metabolomics to quantify the metabolic profile of a given sample is one way to describe the functional status of this host-microbe relationship (Fiehn, 2002; Chen et al., 2019). While metabolomics does provide a "snapshot" approach to describing a vastly complex system, taking and pairing multiple "snapshots" across a variety of sample types can still provide a good overview of the collective metabolism taking place throughout the host.

### *The Ruminal Metabolome*

One of the more commonly investigated metabolomes in cattle is the rumen fluid metabolome. While Saleem et al. (2013) provided a very comprehensive study and review of known metabolites in bovine rumen fluid, a large number of molecules remain uncharacterized. Overall, they reported that a large majority of metabolites found in the rumen fluid fall into either phospholipids, short chain fatty acids, VFAs, di- and tri-glycerides, inorganic ions and gases (e.g., sodium, chloride, methane), amino acids, dicarboylic acids, carbohydrates, and cholesterol esters which is supported by the results reported by Gandra et al. (2020) and Ogunade et al. (2020). More specifically, Saleem et al. (2013) identified the 10 most abundant organic metabolites to be acetate, propionate, butyrate, tricarballylic acid, valerate, glucose, isobutyrate, isovalerate, glutamate, and methylamine from highest (55 mM) to lowest (600 µM) concentration, respectively. At the time of publication, they also reported that the metabolites with the lowest concentrations, in the nanomolar range, were from the lysophosphatidylcholine,

phosphatidylcholines, sphingomyelines, and acylcarnitines families; however, these results are subject to change as new compounds are characterized and more precise profiling platforms are used (Goldansaz et al., 2017). Furthermore, the meta-analysis of results by Saleem et al. (2013) revealed that the metabolite concentrations varied by as much as ± 50% across diets, age, etc. which warrants caution when applying these results to specific situations. In the end, Saleem et al. (2013) summarized the presumed origin of the metabolites in the rumen fluid stating that roughly 5% are strictly of microbial origin (e.g., VFAs), 40-45% are of host origin (e.g., sphingolipids), and the remaining 50-55% could be of microbial or host origin (e.g., amino acids).

### *Profiling Platforms*

While Saleem et al. (2013) compiled results from a wide variety of metabolic profiling platforms, it is important to take the platform into account when interpreting results as different platforms capture different metabolites. Some of the more common platforms used in metabolomics research are mass spectrometry (MS)-based techniques and nuclear magnetic resonance (NMR) spectroscopy (Fiehn, 2002; Saleem et al., 2013). In brief, NMR can be defined as a tool to quantify and identify molecules based on their chemical structure whereas MS is a tool to quantify and identify small compounds based on their molecular weight. These different approaches each come with their own advantages and disadvantages which should be taken into account when selecting which platform to use. For instance, NMR is less sensitive (i.e., micromolar range) compared to MS approaches, but is often more reproducible (Saleem et al., 2013). Mass spectrometry, on the other hand, is more sensitive and can detect a broader range of metabolites through the different separation methods (e.g., gas chromatography (GC), liquid chromatography

(LC), time of flight (TOF), etc.) that can be employed prior to the MS quantification step (Chen et al., 2019). For instance, GC-MS is able to capture a range of primary metabolites involved in pathways such as glycolysis, pentose phosphate pathway, and the Krebs cycle (Fiehn, 2016). Fatty acid methyl esters (FAMEs)/GC-MS and inductively coupled plasma (ICP)-MS, on the other hand, can be used to detect lipids and trace minerals in the nanomolar ranges, respectively (Saleem et al., 2013). In the end, the scope of metabolites that can be captured by each platform varies due to instrument sensitivity and biases in the separation methods that can be exploited to target compounds based on their stability, solubility, and volatility (Saleem et al., 2013).

### *Factors Influencing the Metabolome*

Comparative exploration of the metabolome has revealed a strong dependence of the metabolic profiles on the feedstuffs being consumed by the host (Ametaj et al., 2010; Saleem et al., 2013; Brink et al., 2020). These findings are likely due to the influential role that consumed feedstuffs have on the gastrointestinal microbiota composition, which contribute significantly to the metabolic profiles themselves. As a result, the metabolic profiles of a sample are a reflection of the relationship between the microbiota and the feedstuffs they ferment (Saleem et al., 2013). The ability to, therefore, pair information regarding what metabolites are present (i.e., metabolomics data) with information about which microbiota are present (i.e., microbiome data) ultimately allows for a more in depth look into the functionality of the gastrointestinal microbiota (Harrigan and Goodacre, 2003; Chen et al., 2019). Given the ability of the microbiota to alter the metabolome and ultimately host productivity, opportunities to manipulate these microbial populations should continue to be investigated (DiLorenzo, 2011).

### *Development of the Microbiome and Metabolome in Ruminants*

Interestingly, when cattle are born, they do not have a fully functional digestive tract like that of a mature ruminant described above, but rather they are more similar to that of a monogastric; thus, they are termed pre-ruminants. From birth through weaning, the pre-ruminant calves develop into functioning ruminants, but this conversion requires significant rumen growth, from 30% to 70% of the digestive tract, and the establishment of a viable microbial fermentation (Warner et al., 1956; Baldwin et al., 2004). Therefore, the transition from milk to solid feeds not only results in significant anatomic and metabolic changes, but it also results in significant shifts in the microbial populations of the gastrointestinal tract (Baldwin et al., 2004; Cangiano et al., 2020). During these shifts, the gut microbial populations are increasingly susceptible to pressures as they are attempting to become established while also adjusting to dietary fluxes. Accordingly, research suggests that during such vulnerable periods, microbial supplements can be used to help establish and/or maintain gut microbial populations whereas their application is less successful in older animals with well-established populations (Frizzo et al., 2011b; Malmuthuge et al., 2015; Cangiano et al., 2020).

## Introduction to Microbial Supplements

Some of the microbial supplements that can be employed to manipulate the microbiota of the gastrointestinal tract are direct fed microbial (DFM), probiotic, prebiotic, and synbiotic products. The inception of these microbial supplements occurred over a century ago when Metchnikoff (1908) proposed the concept of ingesting fermented foods with “friendly” microbes in order to attain health benefits. Today, microbial supplements are often applied in ruminant nutrition as a preventative of gut

dysfunction (Sissons, 1989). Common applications include in newborn calves and during stressful times like weaning or entering the feedlot, which are often associated with major shifts in the diet (Duff and Galyean, 2007b; Frizzo et al., 2011a; Cangiano et al., 2020). Research has shown that during these vulnerable periods microbial supplements can aid in establishing and maintaining normal gut microbiota, modulate immune function, promote growth, stimulate feed intake, modify rumen fermentation, improve gut and overall animal health (Sissons, 1989; Abe et al., 1995; Chaucheyras-Durand and Durand, 2010; DiLorenzo, 2011; Frizzo et al., 2011b; Uyeno et al., 2015; Hall et al., 2018; Kelsey and Colpoys, 2018; Cangiano et al., 2020).

***Terminology***

Throughout the literature, inconsistencies in terminology have resulted in uncertainty surrounding microbial supplements with regards to differentiating the different mechanisms and modes of action (Yoon and Stern, 1995). For instance, the term probiotic has traditionally been used to describe "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989); however, the term probiotic has also been used to describe anything from viable microbial cultures to enzyme preparations and even culture extracts (Yoon and Stern, 1995). In order to help clarify things, the US FDA coined the term direct-fed microbial, or DFM, to replace the term probiotic (Miles and Bootwalla, 1991), but instead, both terms are now regularly used throughout the literature. According to the US FDA, a DFM is simply "a source of live (viable) naturally-occurring microorganisms" (Yoon and Stern, 1995). Prebiotics, on the other hand, are defined as feed supplements containing indigestible components that benefit the host by supporting the growth and activity of

select beneficial microbes in the host's gastrointestinal tract (Gibson and Roberfroid, 1995). Meanwhile, synbiotics are feed supplements that include both probiotic and prebiotic components, designed to work synergistically to improve gut function via stimulation of beneficial microbial growth while concurrently supplying nutrients to maintain those microbial populations (Hamasalim, 2016). For those products containing live microbes, selection of ideal candidates is largely based on their ability to adhere to epithelial cells, tolerate high concentrations of acids (e.g., bile acids, hydrochloric acid, volatile organic acids) and maintain rapid growth in both the proximal and distal regions of the gastrointestinal tract (Sissons, 1989).

***Product Concepts***

While these selection criteria do limit the microbes that can be utilized in a microbial supplement, there are still a broad range of microbial concepts tested in the literature. Some of these concepts utilize a bacterial approach while others use a yeast-based approach, and some even take a combination of these approaches when it comes to the selection of microbes included in the supplement. Thus, while it is not uncommon to find microbial supplements containing only a single microbial specie, a more common approach has been to supplement multiple species of bacteria and/or yeast that have the ability to work synergistically to achieve a multifactorial response (McAllister et al., 2011). Another concept that is often employed by microbial supplements is the idea of supplementing a microbial specie along with their fermentation products. For example, a *Lactobacillus acidophilus* fermentation product (LAFP) would be created by anaerobically fermenting *L. acidophilus* in a media source, and then packaging it all into a powder or liquid supplement such that the bacteria (can be alive or inactivated), the

media they were grown on as well as metabolites from their fermentation (e.g., organic acids, peptidoglycans, proteins, peptides) are all included in the supplement. A similar fermentation approach is often used with yeast, creating what are called yeast cultures (AAFCO, 2013). While yeast cultures are technically viewed as probiotics, it is worth mentioning that these cultures also contain cell-wall components like β-glucans and oligosaccharides, that act functionally as prebiotics (Cangiano et al., 2020).

When looking at the microbes commonly used in supplements, there are several major contenders. Some of the more common bacterial probiotics used in young ruminants are species from the genera *Lactobacillus, Bifidobacterium, Bacillus, Streptococcus, Pediococcus*, and *Enterococcus* (McAllister et al., 2011; Cangiano et al., 2020), whereas most yeast-based products used in young ruminants come from live *Saccharomyces cerevisiae* or their yeast cultures (Broadway et al., 2015). Furthermore, most probiotics fed to adult ruminants are live yeast-based products from *Aspergillus orizae* and/or *S. cerevisiae* as these can be employed to help regulate rumen pH when consuming highly fermentable diets (Williams et al., 1991). Another microbial supplement design that addresses issues associated with highly fermentable diets is the concept of supplementing lactic acid-producing and lactic-acid utilizing bacteria (Wiryawan and Brooker, 1995). Many probiotics will either supplement lactic acid-producing bacteria as a way to indirectly stimulate lactic acid-utilizing bacteria through increased lactate concentrations, or they will simply supplement lactic acid-utilizing bacteria. Alternatively, some probiotics will supplement both lactic acid-utilizing and producing bacteria. Regardless of the exact mode of action targeted, these supplements all aim to modify lactic acid metabolism in the rumen by increasing lactic acid-utilizing

bacteria. By increasing the populations of lactic acid-utilizing bacteria, the end goal is to shuttle more lactate towards propionate and ultimately glucose (Wiryawan and Brooker, 1995; Yang et al., 2004; McAllister et al., 2011).

### *Probiotic Effects on Animal Performance*

Throughout the literature, responses to microbial supplements in terms of animal growth, efficiency, and overall health have been inconsistent (Finck et al., 2014). These inconsistencies are likely due in part to the wide range of situational applications (e.g., animal age, health status, microbial specie, route of administration, etc.). Cangiano et al. (2020), for instance, summarized nearly 70 studies looking at probiotic and prebiotic applications in young ruminants and found that the majority of responses were either not significant or positive for growth (39/68 and 22/68), feed efficiency (32/70 and 9/70), and health (15/86 and 31/68), respectively. Based on these results, the potential benefits associated with supplementation warrant further investigation (Cangiano et al., 2020).

#### *Lactobacillus – Based Probiotics*

Studies evaluating the supplementation of a *L. acidophilus* probiotic alone or in combination with other *Lactobacillus spp*. have demonstrated a capacity to reduce the incidence of diarrhea and to improve ADG and feed efficiency in young calves (Bechman et al., 1977; Bruce et al., 1979; Ellinger et al., 1980; Beeman, 1985; Abe et al., 1995). When supplementing a LAFP in nursery pigs, however, there were no significant differences in body weight (BW), ADG, average daily feed intake (ADFI), or gain:feed (G:F) compared to the control or antibiotic fed pigs (Acosta et al., 2016). In contrast, other studies feeding a LAFP to nursery pigs reported improved BW, ADG, ADFI, and G:F over both pre- and post-weaning periods (Frank et al., 2012; Bass and Frank, 2017;

Lan et al., 2017). Furthermore, Lee et al. (2016) observed increased final BW, ADG, and ADFI but no significant differences in G:F for weaned pigs supplemented with LAFP compared to controls, suggesting that improvements in weight gain were likely a result of increased feed intake. Another study evaluated the performance effects of LAFP in conjunction with an immune challenge on weaned pigs. In this study, prior to the immune challenge the LAFP supplemented (1kg/metric ton) pigs had significantly greater ADG resulting in a heavier BW (10.05 vs 8.66 kg); however, after the immune challenge, the LAFP pigs had significantly lower ADG (Sanchez et al., 2019). Additionally, prior to the immune challenge G:F did not differ, but following the immune challenge, the LAFP pigs had significantly lower G:F. These results suggest that, following the immune challenge, the LAFP pigs had similar intake to the control pigs but that they had reduced body weight gain leading to poorer efficiency (Sanchez et al., 2019). These results should be interpreted with caution as weight and feed intake data were only obtained for 3 days following the immune challenge.

*Yeast – Based Probiotics*

When compiling the results of studies that evaluated the use of probiotic yeast cultures in young calves there were no negative impacts of supplementation on weight gain, feed efficiency, health, or gut development (Cangiano et al., 2020). Moreover, 3 studies reported positive improvements in weight gain while the other 7 reported no significant differences. For feed efficiency, all 10 studies reported no significant differences. Of the 7 studies that evaluated health parameters, 4 observed positive effects while the remaining 3 saw no significant differences. Lastly, 6 of the studies evaluated gut development with 5 of those reporting positive effects and the other nonsignificant

differences. Other studies looked at yeast culture supplementation in addition to a *Salmonella* challenge and found the supplemented calves to have improved feed intake, ADG, and fecal consistency (Brewer et al., 2014; Harris et al., 2017). In contrast, supplementing a *S. cerevisiae* fermentation product (SCFP) or yeast culture in older calves resulted in no differences in BW and feed intake (Magalhães et al., 2008; Burdick Sanchez et al., 2020).

### *Probiotic Effects on the Microbiome and Metabolome*

#### *Lactobacillus – Based Probiotics*

Unfortunately, research assessing the impacts of probiotics on the gastrointestinal microbiome and subsequent metabolome in livestock production are limited. Some of the early exploration of *L. acidophilus* probiotic supplements in calves demonstrated an ability, albeit using older microbial culturing techniques, to lower coliform counts while increasing *Lactobacillus spp*. (Bechman et al., 1977; Bruce et al., 1979; Ellinger et al., 1980). More recent research has confirmed these findings, as Lan et al. (2017) supplemented a LAFP to pigs and observed a significant decrease in *E. coli* counts and significant increase in *Lactobacillus* counts. Likewise, Fomenky et al. (2018) reported that supplementation of a *L. acidophilus* probiotic to calves prior to weaning significantly reduced the abundances of potentially pathogenic bacterial genera while simultaneously enhancing beneficial bacterial genera. Overall, research suggests that *L. acidophilus* and LAFP probiotics may provide benefits to the host via the stabilization of the gastrointestinal microbiota and overall gut homeostasis (Azevedo et al., 2012; Lan et al., 2017; Fomenky et al., 2018). Additional research has also demonstrated the capacity of LAFP probiotics to improve apparent total tract digestibility of dry matter and nitrogen in

pigs (Lan et al., 2017). Thus, it is plausible that these improvements in digestibility could be attributed in part to the stabilization of the microbiota (Lan et al., 2017). In support of the improved digestion theory, Fomenky et al. (2018) identified altered pathways relating to the cell cycle, bile secretion, proteasome or cAMP signaling pathway, and more via pathways enrichment analysis after supplementing calves with a *L. acidophilus* probiotic.

*Yeast – Based Probiotics*

Similar benefits to those of *Lactobacillus* probiotics were described by Cangiano et al. (2020) in a review of yeast culture supplementation in calves. In general, yeast cultures were found to stimulate microbial activity while also stabilizing the gastrointestinal microbiota. One study in particular reported increased butyrate production and rumen papillae length likely related to the improved *Butyrivibrio* and reduced *Prevotella* abundances in the rumen fluid of calves (Xiao et al., 2016). Unfortunately, Magalhães et al. (2008) found no differences in plasma 3-hydroxybutyrate and glucose concentrations between yeast culture supplemented calves and controls. Studies supplementing SCFP probiotics, on the other hand, have reported increased blood glucose levels with supplementation (Zaworski et al., 2014; Shen et al., 2019) leading some to suggest a possible glucose-sparing effect when supplementing SCFP probiotics (Zaworski et al., 2014; Burdick Sanchez et al., 2020). Given glucose's role as a primary energy source in ruminants (Ndlovu et al., 2007), these elevated blood levels suggest an improved energy status in SCFP supplemented animals.

### *Probiotic Effects on Immune Function*

The gastrointestinal microbiota were once theorized to inertly inhabit the host, but research over the years has clearly demonstrated that the microbiota dynamically interact

with the host to influence the development of their immune system (Cross, 2002; Mazmanian et al., 2005; Peterson et al., 2007; Malmuthuge et al., 2015). One mechanism by which they may interact with the host is simply through the nutrition they provide to the host. Research has shown that the nutritional status (i.e., mineral levels, protein intake, energy status) of an animal impacts the functionality of their immune system (Galyean et al., 1999; Duff and Galyean, 2007b); thus, probiotics could theoretically indirectly impact immune function through alterations in the gastrointestinal microbiota and their subsequent nutrient contributions (i.e., metabolome) (Carroll and Forsberg, 2007). Other theories take a more direct approach, stating that supplementation of probiotics, such as *L. acidophilus* and other *Lactobacillus spp.*, may result in the generation of immune signals with local and systemic stimulation (Pollmann et al., 1980; Cross, 2002; Lee et al., 2016). This probiotic-mediated stimulation of the immune system was observed by Perdigon et al. (1990) which reported increased survival of LAFP supplemented mice when challenged with *Salmonella*. In another study using LAFP, supplemented nursery pigs required fewer antibiotic treatments than controls, with SCFP supplemented pigs being intermediate (2, 38, and 20 injections, respectively) (Frank et al., 2012) which suggests enhanced immune function with probiotic supplementation.

**Introduction into the Immune System**

The immune system has been described by Delves and Roitt (2000) as "an organization of cells and molecules with specialized roles in defending against infection." This organization of cells and molecules (i.e., immune system) can generally be divided into two branches: innate and acquired immunity. The first lines of defense in our immune system are the barriers that are technically part of innate immunity. These

barriers can be physical, chemical, or biological in nature and include things such as the skin, enzymes, and mucus membranes (Delves and Roitt, 2000). Some mechanisms by which microbial supplements benefit the host would fall under barrier-like functions. Some examples of these barrier-like functions would be limiting pathogen attachment to the mucosa, producing antimicrobial substances, and altering nutrient availability and environmental pH (Sissons, 1989; Cross, 2002). If the invader is able to survive and penetrate these barriers, they are then confronted by additional defenses associated with both the innate and acquired immune systems. These two branches of the immune system are fundamentally different in their response which can be reflected in the terminology used to describe them such that the innate system can also be referred to as the nonspecific or natural immunity whereas the acquired system is also known as the specific or adaptive immunity (Delves and Roitt, 2000).

The innate system, ultimately, provides a nonspecific or generic line of defense involving cells and signals that works to prevent and respond quickly to invasion and inflammation. This innate response involves things like APP, complement proteins, and cytokines as well as cells like neutrophils, monocytes, macrophages, basophils, mast cells, eosinophils, and natural killer cells (Delves and Roitt, 2000; Dantzer, 2004), which all fall under the umbrella term of white blood cells (WBC) or leukocytes; however, WBC can also encompass cells like lymphocytes that are part of the acquired immune response. The acquired immune system is adaptive in nature such that it forms a "memory" of invaders that then allow it to produce a more targeted or specific response with repeated exposure. Cells and other compounds involved in the acquired immune response are B and T lymphocytes, antigens, antigen-presenting cells, and

immunoglobulins (i.e., antibodies) (Delves and Roitt, 2000). In the end, the innate and acquired immune responses are not completely discrete, as immune cells and molecules often interact to maintain host health.

### *Inflammation*

The body's inflammatory response, whether stemming from infection or injury, is a defense mechanism to restore the body to homeostasis (Pahwa et al., 2018). Some examples of inflammatory stimuli include bacterial infections, allergens, parasites, foreign bodies, surgical procedures, and even myocardial infarction (Kushner, 1998). Unfortunately, when these inflammatory responses are prolonged or uncontrolled, these same defense mechanisms can result in damage to the host (Pahwa et al., 2018). Due to their differences in response, chronic and acute inflammation are often characterized by the participation of different mediators and cell types (Kushner, 1998; Abdulkhaleq et al., 2018). Some examples of inflammatory mediators include intercellular signaling molecules (i.e., immunoglobulins, coagulation factors, cytokines) and cells like phagocytes, lymphocytes, platelets, endothelial cells, and more (Kushner, 1998). The complexity of the inflammatory response is further demonstrated by the ability of these cells and molecules to either trigger, intensify, maintain, reduce, or resolve inflammation (Gabay and Kushner, 1999). In fact, many of these cells and molecules are actually capable of more than one of the above actions such that they could participate in both the stimulation and resolution of the inflammatory response (Kushner, 1998). Therefore, while molecules involved in the inflammatory response, particularly cytokines, have traditionally been classified as either pro- or anti-inflammatory, research has increasingly demonstrated the ability of some to be both depending on the progression of the

inflammatory response (Kushner, 1998; Cavaillon, 2001). Due to this multifactorial potential, the interpretation of data surrounding these types of molecules must be based on the context in which they were observed. This context includes things like the amount of time relative to insult, but more importantly things like the presence and absence of other mediators (e.g., cytokines) (Sporn, 1997; Cavaillon, 2001).

### *Immune Challenge Model*

One method by which the immune system can be stimulated for research purposes is the administration of an endotoxin like LPS. Since LPS is a part of gram-negative bacterial cell walls, its injection often serves as a means to modeling the inflammation caused by a gram-negative bacterial infection, which ultimately results in the stimulation of various inflammatory mediators (e.g., cytokines, APP, glucocorticoids) (Dantzer, 2001). Fortunately, this response is short-lived, typically resolving within 24 hours, and generally resulting in morbidity without mortality. Therefore, the LPS challenge model serves as a valuable tool to evaluate the effects of a treatment on the innate immune response, in particular, the acute phase response (Burdick Sanchez et al., 2020).

### *Acute Phase Response*

During an infection, or inflammatory event like LPS administration, numerous changes take place throughout the body aside from those occurring at the site of infection (Gabay and Kushner, 1999). The phrase acute phase response (APR) has since been used to describe this set of systemic changes (Kushner, 1982), but this terminology can been misleading since the APR occurs in both acute and chronic inflammation cases (Kushner, 1998; Gabay and Kushner, 1999). The APR is a part of the innate immune system; thus, its response is considered non-specific in nature such that it produces a variety of

generalized responses from behavioral, physiological, biochemical, to even nutritional modifications (Gabay and Kushner, 1999). Some examples of these modifications include exhibiting sickness behavior, increased APP production, a febrile response, anorexia, hypoglycemia, increased circulating WBC levels, and altered blood concentrations of minerals and hormones (Hart, 1988; Fattori et al., 1994; Kushner, 1998; Gabay and Kushner, 1999; Carroll et al., 2009b). In the end, these changes associated with the APR are deviations from normal homeostatic conditions stimulated by endogenous inflammatory molecules, principally cytokines (Kushner, 1998; Gabay and Kushner, 1999).

*Clinical Response*

The first notable change that occurs during the APR is the initiation of sickness behavior by pro-inflammatory cytokines (i.e., TNF-α, IL-6, IL-1 and IFN) and other inflammatory mediators such as lipid-derived mediators (Hart, 1988; Dantzer, 2001). These pro-inflammatory mediators are initially released by macrophages, monocytes, and endothelial cells at the site of infection resulting in localized inflammation; however, this inflammation can be quickly amplified to a systemic response through the production of further pro-inflammatory mediators like APP and additional cytokines (Dantzer, 2001). Moreover, several of these pro-inflammatory cytokines like TNF-α, IL-6, and IL-1 are pyrogenic messengers meaning they act on the thermoregulatory center of the brain to stimulate a febrile response (i.e., fever) (Hart, 1988; Lohuis et al., 1988; Dinarello, 1996; Vybíral et al., 2005). Some of the other sickness behavior that results from systemic stimulation by pro-inflammatory cytokines are reductions in water and feed intake, weakness, sleepiness, lethargy, unresponsiveness to surroundings, reductions in grooming

behavior and social activity (Hart, 1988; Gabay and Kushner, 1999; Dantzer, 2001). Collectively, these sickness behaviors work to redirect energy expenditure towards sustaining the febrile response and supporting the immune system to increase survival chances (Hart, 1988).

*Clinical Response – Timeline*

Following an acute immune stimulus (e.g., LPS), sickness behavior has been observed to increase within 30 minutes for beef calves (Littlejohn et al., 2019). Moreover, a reduction in feed intake generally occurs within an hour, followed by a reduction in social behavior within 2 hours of insult (Dantzer, 2001). Respiration rate (RR) responses are variable, with Carroll et al. (2009b) reporting a peak in RR by 0.5 hours and a return to baseline rates by 2.5 hours following LPS. In contrast, Waldron et al. (2003) observed a more delayed response with peak RR occurring around 1.5 hours with a return to baseline RR by 4 hours following LPS, regardless of LPS dosage. Fever responses typically occur within the first few hours of LPS (Dinarello, 1984). Studies looking at rectal temperatures have consistently reported an acute increase in body temperature within the first hours following LPS injection, then a steady climb to the peak temperature at around 4-6 hours post-LPS, followed by a slow decline over the next 24 hours (Waldron et al., 2003; Carroll et al., 2009b; Finck et al., 2014; Littlejohn et al., 2019; Burdick Sanchez et al., 2020). Similar results have been reported when monitoring vaginal temperatures (Cangiano et al., 2019; Carroll et al., 2021). Other locations like the shoulder, rump, and rumen have been investigated, and tend to increase with LPS injection, but these estimates were not found to be superior to those of rectal temperature (Carroll et al., 2009b). Lastly, when assessing the use of ear skin temperature, one study

reported that it decreased following LPS and that there was a negative correlation between ear and rectal temperature (Carroll et al., 2009b). Thus, this data suggests that ear temperature decreases with increasing body temperature likely due in part to elevated cortisol stimulating vasoconstriction (Kelly et al., 1998). Vasoconstriction shifts blood flow away from the extremities, towards the vital organs, potentially providing a way to conserve heat for the maintenance of the febrile response (Fekety, 1963; Carroll et al., 2009b).

*Cytokine Response*

Cytokines are proteins released by activated immune cells in order to communicate with other cells both locally and systemically (Gabay and Kushner, 1999). For instance, pro-inflammatory cytokines that are released at the site of infection interact with tissue cells and other leukocytes, resulting in the release of more pro-inflammatory cytokines and the subsequent generation of a systemic immune response (Dantzer, 2001; Carroll et al., 2009b). These interactions between cytokines, the cells they are targeting, and the environment in which they interact is complex. Often times cytokines are produced by multiple sources with a range of target cells upon which they can execute multiple functions (Gabay and Kushner, 1999). Environmental influences further complicate the situation as the effects of cytokines on a target cell can be enhanced or inhibited by other molecules (e.g., cytokines, hormones, receptors, antagonists, etc.) (Mackiewicz et al., 1991). It is not uncommon, for instance, for cytokines to regulate the production of at least one other cytokine, often more (Gabay and Kushner, 1999). Thus, additive and inhibitory relationships are often found with various combinations of

cytokines (Mackiewicz et al., 1991) as cells are rarely ever exposed to a single cytokine (Sporn, 1997; Gabay and Kushner, 1999).

Overall, cytokines play several roles in the stimulation of the APR. Possibly the most well-known response during the APR is the increase in APP production in the liver which is stimulated by cytokines such as TNF-α, IL-6, IL-1β, IFN-γ, TGF-β, and potentially even IL-8 (Gauldie et al., 1987; Alsemgeest et al., 1994; Fattori et al., 1994; Godson et al., 1995; Gabay and Kushner, 1999). Of those cytokines, TNF-α, IL-6, and IL-1β, specifically, have been routinely correlated to the febrile response due to their pyrogenic nature (Hart, 1988; Lohuis et al., 1988; Dinarello, 1996; Steiger et al., 1999; Vybíral et al., 2005). These same cytokines, through the stimulation of the APR, have also been associated with reduced feed intake (Johnson, 1997). Ultimately, as cytokines work to protect the host through fever, immune stimulation, and energy repartitioning, they often also elicit undesirable but advantageous reductions in animal performance (Kvidera et al., 2017).

*Specific Cytokine Functions*

While cytokines can be produced by a variety of sources, the initiation of APR is triggered by the release of cytokines from macrophages and monocytes at the site of inflammation (Gabay and Kushner, 1999). Examples of such cytokines released by macrophages and monocytes in response to an LPS stimulus include TNF-α, IL-6, and IL-1β (Gessani et al., 1993; Manderson et al., 2007; Carroll et al., 2009b; Micaroni et al., 2013). Further research suggests that during any inflammatory event, one of the first cytokines to spike in circulation is TNF-α which then promotes other cytokines like IL-6 and IFN-γ to be released (Burdick Sanchez et al., 2020). Tumor necrosis factor is also

known to work closely with, and is even biologically very similar to IL-1 (Dinarello, 1988). The primary role of IL-1 during inflammation has been the stimulation of the febrile response (Dinarello, 1988), but to a lesser extent TNF-α has also demonstrated this ability (Hart, 1988; Dinarello, 1996; Vybíral et al., 2005). Other functions of IL-1 include the up-regulation of IL-6 production (Dinarello, 1988). Moreover, IL-1 along with IL-6 and TNF-α are important regulators of inflammation as they are found to increase APP production in the liver (Gauldie et al., 1987; Kishimoto, 1989; Fattori et al., 1994).

More specifically, IL-6 is believed to be the chief regulator of most of the APPs being produced (Gauldie et al., 1987) and that other cytokines regulate certain APPs (Gabay and Kushner, 1999). Fattori et al. (1994) demonstrated, however, that these roles differ with local versus systemic inflammation. For instance, using a local inflammation model IL-6 is required to stimulate a normal inflammatory response (e.g., APP), but when using a systemic model (i.e., LPS), IL-6 was dispensable because other cytokines (e.g., TNF-α) could maintain a normal inflammatory response without IL-6 present. As described above, IL-6 is traditionally viewed as a pro-inflammatory cytokine, but some researchers have claimed anti-inflammatory properties of IL-6, and the APPs that it regulates, that would lead to the abatement of acute and chronic inflammatory states (Tilg et al., 1997). Some of these anti-inflammatory actions include the inhibition of TNF-α and IL-1 directly and through increased antagonists, as well as an increase in glucocorticoids (Aderka et al., 1989; Tilg et al., 1997) like cortisol that work to suppress inflammation (Chinenov and Rogatsky, 2007). Similarly, IL-13 has been associated with the suppression of macrophage-released cytokines (e.g., TNF-α, IFN-γ), comparable to or

even with more effectiveness than those of anti-inflammatory cytokines like IL-10 and IL-4 (Muchamuel et al., 1997; Baumhofer et al., 1998). These anti-inflammatory properties of IL-13 were demonstrated to increase survival after lethal doses of LPS were given to mice (Muchamuel et al., 1997).

While IFN-γ is not directly produced by monocytes like other pro-inflammatory cytokines, IFN-γ and monocytes still rely heavily on one another to support the immune response. Initially IFN-γ activates monocytes and influences their subsequent differentiation, but once activated the monocytes can produce signals that then promote IFN-γ activity (Gerrard et al., 1989; Delneste et al., 2003; Schroder et al., 2004). IFN-α, on the other hand, is produced by monocytes, macrophages, T cells, and dendritic cells in response to viral and bacterial infections (Akbar et al., 2000). While IFN-α was initially classified as pro-inflammatory, further research has suggested some potentially anti-inflammatory properties (Tilg and Peschel, 1996). Both roles are probably accurate, depending on the context of the inflammatory state.

The cytokine IL-36Ra, previously known as IL-1F5, is believed to be anti-inflammatory due to its ability to inhibit the production of pro-inflammatory cytokines and increase the production of IL-4 which is an anti-inflammatory cytokine itself (Costelloe et al., 2008; Dinarello, 2009; Vigne et al., 2011). Lastly, IL-21 is broadly involved in the immune response through the regulation of both innate and adaptive immune cells (Wang et al., 2006). Production of IL-21 by T cells (Wurster et al., 2002), however, is regulated by itself and other cytokines such as IL-6 and IFN-α (Mehta et al., 2004; Strengell et al., 2004). In the end, the above-mentioned cytokines and their roles in

the immune system is not exhaustive, but merely a brief overview of some of the interactions pertaining to my current research.

*Cytokine Response – Timeline*

In general, pro-inflammatory cytokines are reported to respond within 1-4 hours after an acute immune stimulus like LPS (Carroll et al., 2009a; Carroll et al., 2009b; Carroll et al., 2011; Sanchez et al., 2013; Finck et al., 2014; Sanchez et al., 2018; Littlejohn et al., 2019; Sanchez et al., 2019; Burdick Sanchez et al., 2020). While these response times are relatively consistent throughout the cattle literature, there are some significant variations in the peak concentrations of cytokines observed. These differences (e.g., 0.04-1.3 ng/ml versus 5-120 ng/ml for TNF-α) in concentration are likely due in part to different analytical methods as well as variations in the study designs such as the route of administration, the bacterial serotype used, and the dosage of LPS administered, as these could alter the magnitude of the response (Steiger et al., 1999; Waldron et al., 2003; Kahl and Elsasser, 2006; Carroll et al., 2009a; Carroll et al., 2009b; Rodrigues et al., 2015; Littlejohn et al., 2019).

Throughout the literature, TNF-α has consistently been demonstrated to peak around 1-2 hours with a return to baseline values by 6 hours following LPS (Waldron et al., 2003; Carroll et al., 2009b; Burdick Sanchez et al., 2020). One study did note, however, a biphasic response in TNF-α with a stronger initial peak at 1 hour followed by a weaker peak at 3 hours in beef heifers given LPS (Carroll et al., 2021). Similar to TNF-α, IL-6 has been found to increase within the first few hours following LPS, however, it does not often reach peak values until more like 3-5 hours after, and returns to baseline sometime after 8 hours (Carroll et al., 2009a; Carroll et al., 2009b; Cangiano et al., 2019;

Burdick Sanchez et al., 2020; Carroll et al., 2021). Another study, actually observed a biphasic response for IL-6 such that it initially peaked at 3 hours and then experienced a second peak around 5 hours following LPS (Littlejohn et al., 2019). This biphasic response is comparable, therefore, to the results reported by Carroll et al. (2009b) and Carroll et al. (2021) where they observed continued elevation in IL-6 concentrations following the initial peak. Lastly, IFN-γ and IL-1β have been reported to peak somewhere around 4 hours post-LPS, but the results for IFN-γ depict different patterns of response such that the response can either be a sharp peak, or a steady climb followed by a steady decline (Carroll et al., 2009a; Carroll et al., 2009b; Littlejohn et al., 2019; Carroll et al., 2021). Overall, cytokine responses to LPS are largely consistent in timing across cattle studies with TNF-α peaking within the first few hours followed by IL-6, IFN-γ, and IL-1β between 3-5 hours post-LPS.

*Chemokine Response*

Chemokines are a member of the cytokine family, thus, they can be described as intercellular signaling proteins that work specifically to direct migrating white blood cells to the site of inflammation via chemical signals (i.e., chemotactic) (Cascieri and Springer, 2000). Specifically, chemokines have been found to play an significant role in intercellular communication during bacterial infections (Karlsson et al., 2015) which explains their increased activity following LPS injections. Pro-inflammatory chemokines like CXCL9 (MIG) and CXCL10 (IP-10) have been reported to increase following LPS administration as well as under pro-inflammatory states such as elevated TNF-α and IL-1 (Charo and Ransohoff, 2006; Karlsson et al., 2015; Wakasa et al., 2019). Their production is primarily stimulated by IFN-γ (Metzemaekers et al., 2018). Another pro-

inflammatory chemokine is CCL4 that functions as a chemoattractant for natural killer cells, monocytes, eosinophils, basophils, and lymphocytes following LPS administration (Murphy, 1994; Kitaya et al., 2003; Xu et al., 2019).

*Other Metabolic Marker Responses*

Pro-inflammatory cytokines (i.e., TNF-α, IL-6, IL-1) have been found to increase cortisol production through the activation of the hypothalamus-pituitary-adrenal (HPA) axis (Chrousos, 1995; Chinenov and Rogatsky, 2007). Cortisol then begins working immediately to suppress inflammation by modulating the release of pro-inflammatory cytokines in order to prevent a hyper-inflammatory state (Roth, 1985; Chinenov and Rogatsky, 2007; Carroll et al., 2009b; Cooke et al., 2012; Littlejohn et al., 2019). During the APR, cortisol also functions to increase energy availability for support of the immune response by increasing glucose concentrations through the stimulation of gluconeogenesis in the liver and the release of glucose from glycogen stores (i.e., glycogenolysis) (Long et al., 1940; McGuinness, 2005; Kuo et al., 2015; Kvidera et al., 2017). Unfortunately, the increase in glucose availability is often relatively short-lived as glucose demands of the animal and its immune system are often greater than what can be supplied from a ruminant's limited glycogen stores (McGuinness, 2005; Burdick Sanchez et al., 2014; Kvidera et al., 2016; Kvidera et al., 2017).

*Other Metabolic Marker Responses – Timeline*

During an LPS challenge, cortisol levels are reported to routinely increase within the first hour, but then continue to rise until they peak around 2.5-4.5 hours after the LPS administration, at which point they begin to gradually decline (Carroll et al., 2009a; Carroll et al., 2009b; Carroll et al., 2011; Rodrigues et al., 2015; Littlejohn et al., 2019;

Burdick Sanchez et al., 2020). Across several studies, cortisol concentrations have consistently responded to LPS with peak concentrations ranging between 40-70 ng/ml with baseline values nearing the 10 ng/ml and below mark (Waldron et al., 2003; Carroll et al., 2009b; Carroll et al., 2011; Littlejohn et al., 2019; Burdick Sanchez et al., 2020). These elevated cortisol concentrations then contribute to the subsequent increase in glucose concentrations (McGuinness, 2005; Kuo et al., 2015). Some variations in the amount of time to achieve peak glucose concentrations have been observed, likely due to differences in energy status of the animals; however, in general, glucose has been reported to peak at hyperglycemic levels within 3 hours of LPS administration, followed by a rapid decline, often to sub-basal and even hypoglycemic levels (<40mg/dl) (Waldron et al., 2003; Kvidera et al., 2016; Kvidera et al., 2017; Cangiano et al., 2019).

**Probiotic Effects on the Immune Response**

Although the gastrointestinal tract microbiota were once believed to passively occupy their host, continued investigation has revealed a truly reciprocal relationship exists between the microbiota and the host leading to interactions between them and the host's biological systems like the immune system (Cross, 2002). Due to this interactive relationship, researchers have worked for decades to understand how altering the microbiota through the use of microbial supplements could potentially beneficially alter or support immune responses. As a result, several studies have successfully demonstrated the ability of probiotics to reduce the APR while maintaining or even improving animal performance (Azevedo et al., 2012; Finck et al., 2014; Broadway et al., 2015; Lee et al., 2016; Sanchez et al., 2019; Burdick Sanchez et al., 2020).

### *Clinical Response*

During an LPS immune challenge, Burdick Sanchez et al. (2020) observed reduced sickness behavior scores for SCFP supplemented steers compared to controls at several time points within the first 4 hours following LPS administration, but no differences were seen in respiration rates. These results suggest that while sickness behavior scores overall were relatively low, the SCFP supplemented steers, on average, maintained more normal behavior and less labored breathing, which coincides with the lack of differences noted in the respiration rate data. Several studies have evaluated body temperatures after supplementing a probiotic product and reported varying results likely due in part to different animal species, location that the temperature was measured, and the frequency of measurement (Burdick Sanchez et al., 2020).

When looking at baseline body temperatures, both SCFP and LAFP increased temperature prior to an LPS challenge (Sanchez et al., 2018; Sanchez et al., 2019) whereas yeast products have been found to decrease baseline temperature values (Finck et al., 2014). Following the LPS administration, Burdick Sanchez et al. (2020) reported that SCFP-supplemented steers had greater temperatures essentially from 6 to 24 hours post-LPS. Similarly, Sanchez et al. (2019) found that LAFP-fed pigs had greater temperatures at 10 hours following LPS, but with continued monitoring, they also reported that LAFP-fed pigs had lower temperatures at several points from 24 to 70 hours post-LPS. In contrast, other studies have reported lower temperatures for yeast-based probiotics following the initial peak up to 24 hours (Finck et al., 2014) and even up to several days (Brewer et al., 2014) following an immune stimulus. Nonetheless, some

research has found no difference in body temperature following LPS for SCFP-supplemented versus control pigs (Price et al., 2010; Sanchez et al., 2018).

***Cytokine Response***

Prior to LPS administration, the supplementation of various probiotics including LAFP and SCFP have resulted in no differences in baseline TNF-α values (Sanchez et al., 2018; Sanchez et al., 2019; Burdick Sanchez et al., 2020). Peak values of TNF-α between 1 and 3 hours following LPS administration, however, were lower for SCFP- and LAFP-supplemented animals (Qiao et al., 2015; Sanchez et al., 2019; Burdick Sanchez et al., 2020). The study by Sanchez et al. (2019) did evaluate two dosage levels of LAFP and while the lower dose (1 kg/metric ton) of LAFP resulted in lower TNF-α values as mentioned above, the higher dose (2 kg/metric ton) of LAFP actually resulted in greater TNF-α concentrations during the peak, but the reason for this dichotomy is unclear and warrants further investigation.

Similar to TNF-α, baseline values for IL-6 did not differ due to probiotic supplementation prior to LPS administration (Sanchez et al., 2018; Sanchez et al., 2019; Burdick Sanchez et al., 2020). Following LPS, Qiao et al. (2015) observed no differences in IL-6 concentrations with *L. acidophilus* supplementation; however, studies evaluating LAFP and SCFP have consistently reported at least a tendency for lower IL-6 concentrations in supplemented versus control animals after LPS (Lee et al., 2016; Sanchez et al., 2019; Burdick Sanchez et al., 2020). In contrast, Sanchez et al. (2018) reported greater IL-6 concentrations during the peak for SCFP-supplemented pigs compared to controls during an LPS challenge.

Again, baseline values of IFN-γ were not altered by probiotic supplementation in most studies (Finck et al., 2014; Sanchez et al., 2018; Sanchez et al., 2019), however, Burdick Sanchez et al. (2020) did find a tendency for lower baseline IFN-γ in SCFP supplemented calves. After the immune challenge, *L. acidophilus* and SCFP-supplemented animals had at least a tendency for lower IFN-γ concentrations compared to controls for up to 6 hours post-insult (Qiao et al., 2015; Burdick Sanchez et al., 2020). In contrast, Finck et al. (2014) and Sanchez et al. (2018) reported no differences in IFN-γ concentrations following LPS administration due to yeast-based probiotic supplementation. Overall, these results suggest that yeast and *L. acidophilus* probiotic supplementation, with or without their fermentation products, reduce the pro-inflammatory cytokine response.

***Other Metabolic Marker Responses***

Results for cortisol revealed no differences in pre-LPS concentrations due to probiotic supplementation (Finck et al., 2014; Sanchez et al., 2019; Burdick Sanchez et al., 2020). Responses following LPS, however, have varied throughout the literature. For instance, in steers, Finck et al. (2014) observed a lower cortisol peak and average concentration using yeast-based probiotics, whereas Burdick Sanchez et al. (2020) observed a shift in the cortisol peak such that it occurred earlier and was stronger, but also that is resolved quicker with SCFP supplementation. When supplementing LAFP at two doses in pigs, Sanchez et al. (2019) observed varied responses in cortisol following LPS administration. In the low dose LAFP pigs, they experienced a more delayed but stronger response than controls, whereas the high dose LAFP pigs had a steady, stronger peak that resolved quicker than controls (Sanchez et al., 2019). These differences were

not observed by Lee et al. (2016) as they reported no differences in cortisol response following LPS administration in LAFP-supplemented versus control pigs. Glucose responses are not as regularly investigated and what data is available is conflicting such that one study observed greater glucose levels in SCFP-supplemented calves (Burdick Sanchez et al., 2020) while another observed lower glucose in LAFP-supplemented pigs (Sanchez et al., 2016).

### *Summary of Effects*

In the end, the results reported by Sanchez et al. (2019) confirm the interactions described between pro-inflammatory cytokines, the febrile response, and glucocorticoids in the literature. For instance, they observed a reduction in body temperature as well as lower concentrations of TNF-α and IL-6 which supports the perception that pro-inflammatory cytokines stimulate the febrile response. Furthermore, the greater cortisol concentrations may also explain to some extent the lower TNF-α and IL-6 concentrations reported in those same animals following LPS administration (Sanchez et al., 2019) as cortisol is reported to suppress pro-inflammatory cytokines production (Roth, 1985). Overall, LAFP supplementation resulted in a reduction in body temperature and pro-inflammatory cytokine response suggesting that LAFP may be capable of attenuating the APR. These results are supported by those of Lee et al. (2016) as they also observed a reduction in pro-inflammatory and an increase in anti-inflammatory cytokine levels with LAFP supplementation.

## Conclusions

Over the years, a wide variety of microbial supplement products have been made available to livestock producers for use on their operations. However, the industry has

become increasingly aware over the last decade or so, just how important these microbial supplements may be to maintaining animal health and sustaining livestock production in the future. As a result, new theories and products are being developed every day, yet our understanding of the mechanisms by which they impact the host's performance is still lacking. Research suggests that some benefits of microbial supplements can be achieved through the prevention of dysbiosis in the gastrointestinal tract as well as an altered acute phase immune response. Of particular interest is the use of LAFP supplements which have demonstrated an ability to maintain or even improve animal performance, stabilize the gastrointestinal microbiota, improve digestibility, attenuate the APR, and improve overall animal health. Notably, the majority of this work has been done using a pig model; thus, the question remains whether or not these same benefits would be achieved in beef cattle. Therefore, the current research aims to explore the application of a LAFP supplement in beef calves during a period in which they are vulnerable (i.e., weaning) to performance reductions and disease. In the end, the goal of this research is to provide producers with another tool to proactively maintain or improve performance and health status during the stress associated with weaning in beef cattle, ultimately improving profitability while also complying with natural program requirements.

# CHAPTER 3

# THE IMPACTS OF A *LACTOBACILLUS ACIDOPHILUS* FERMENTATION PRODUCT ENHANCED CREEP FEED ON PRE-WEANING GROWTH PERFORMANCE, FEED INTAKE, DAILY ACTIVITY, AND GASTROINTESTINAL VOLATILE FATTY ACID PROFILES IN ANGUS STEERS[1]

[1] Krause, T.R., J. M. Lourenco, V. E. Ryman, T. R. Callaway, B. D. Heins, J. D. Duggin, and T. D. Pringle. To be submitted to *Journal of Animal Science*.

**Abstract**

The objective of this study was to investigate the effects of *Lactobacillus acidophilus* fermentation product (LAFP) supplementation prior to weaning, via creep feed, on growth performance, feed intake, daily activity, and gastrointestinal volatile fatty acid (VFA) profiles of beef steers. Sixty suckling Angus steers (211.4 ± 21.2 kg; 173 ± 17 d) were randomly assigned to one of the following treatments: (1) CONTROL – calves were on pasture with dam (2) CREEP – calves were on pasture with dam and given access to *ad libitum* creep feed (3) LAFP – same diet as CREEP but with the addition of LAFP at 5 g/hd/d. Dietary treatments were administered for the 70 d prior to weaning. Ultrasound images and digesta samples were collected on d 0 and 55 while weekly feed intake, weekly weights, and daily activity were monitored throughout the study. Body weight tended to be greater ($P = 0.11$) for creep fed versus non-creep fed steers after 70 d. Average daily gain (ADG) tended to be greater ($P = 0.11$) by 0.13 and 0.17 kg/d for the CREEP and LAFP steers, respectively, over the CONTROL. Creep feed intake did not differ ($P = 0.64$) between the CREEP and LAFP steers, however, the LAFP steers ate 0.35 kg/hd/d less, on average, compared to CREEP. Thus, the LAFP steers consumed less creep feed while maintaining similar growth as the CREEP, resulting in a tendency for greater ($P = 0.13$) gain to feed ratios. Ribeye area and intramuscular fat did not differ ($P \geq 0.21$) across treatments but increased ($P < 0.01$) with time whereas backfat thickness was greater ($P < 0.05$) on d 55 in the CREEP and LAFP steers compared to CONTROL. Rumen propionate was greater ($P < 0.05$) in the LAFP steers compared to CREEP and CONTROL. Rumen butyrate, isovalerate, and valerate as well as fecal acetate, propionate, butyrate, and total VFA concentrations were greater ($P < 0.05$) in the LAFP

and CREEP steers versus CONTROL. Total rumen VFA concentrations were greater ($P < 0.05$) in the LAFP steers compared to CONTROL with CREEP being intermediate. The CREEP and LAFP steers spent less ($P < 0.05$) time eating and ruminating, and more time ($P < 0.05$) being inactive and active than the CONTROL. Overall, these results suggest that growth performance tended to improve with creep feed supplementation. Moreover, the addition of LAFP to the creep feed tended to improve efficiency and significantly altered VFA profiles. Thus, further investigation into the impacts of LAFP on gastrointestinal fermentation is warranted.

## Introduction

For decades, sub-therapeutic levels of antibiotics have been added to cattle rations in order to improve health and performance (Centner, 2008; Papatsiros et al., 2013). Traditionally, this practice has been utilized in post-weaning facilities to help alleviate stress, prevent disease, and ultimately improve growth through enhanced feed efficiency (Wileman et al., 2009; Mathews and Johnson, 2013; Finck et al., 2014). Recently, however, consumers' concerns regarding the use of antibiotics in livestock production have been on the rise. At the forefront of these concerns is the transmission of antibiotic resistance and how repeated, long-term exposure to low doses of antibiotics can increase resistance prevalence amongst microbes (Krehbiel et al., 2003; Centner, 2008). Therefore, consumers and legislators began to advocate for the reduction of antibiotic usage in livestock production which resulted in the establishment of the Veterinary Feed Directive (**VFD**) in 2015. In brief, the VFD is a legislative document that bans the use of select antibiotics for non-therapeutic purposes (i.e., growth promotion and increased efficiency) and fosters greater oversight of therapeutic usage (i.e., treatment, control, and prevention of disease) in food animal production (Centner, 2016).

As a result of the VFD and increasing consumer demands for more "natural" production systems (i.e., no antimicrobials, hormones, growth promotants, etc.), the need for "natural" antibiotic alternatives continues to grow. Moreover, these proposed alternatives to traditional management practices (i.e., antibiotics, growth promotants) should be capable of maintaining animal health and performance while also allowing the producer to adhere to the "natural" program's requirements (Wileman et al., 2009; Stackhouse et al., 2012; Mathews and Johnson, 2013; Papatsiros et al., 2013). A group of

"natural" alternatives that have received a lot of attention over the last decade are dietary microbial supplements like probiotics (i.e., direct fed microbials). These microbial supplements are often administered during times of stress to prevent gut dysfunction and have been associated with benefits such as improved growth, increased feed intake and efficiency, stimulation of the immune system, stabilization of the gut microbiota, modified rumen fermentation, enhanced gut and overall animal health (Sissons, 1989; Abe et al., 1995; Yoon and Stern, 1995; Duff and Galyean, 2007b; Awad et al., 2009; Frizzo et al., 2011b; Broadway et al., 2015; Hall et al., 2018; Cangiano et al., 2020).

In particular, studies using *Lactobacillus acidophilus* have reported reduced diarrhea, improved weight gain, stimulation of the immune system, an attenuated acute phase response, stabilization of the gut microbiota, and altered rumen fermentation pathways (Bruce et al., 1979; Abe et al., 1995; Azevedo et al., 2012; Lee et al., 2016; Lan et al., 2017; Fomenky et al., 2018; Sanchez et al., 2019). Further studies have demonstrated that supplementation with *Lactobacillus acidophilus* fermentation products (**LAFP**) results in favorable improvements in performance such as body weight (**BW**), average daily gain (**ADG**), average daily feed intake, and gain:feed (**G:F**) in pigs (Frank et al., 2012; Lee et al., 2016; Lan et al., 2017). While these studies have demonstrated promising results, a lot of this research was done using a pig model, so the question remains whether these same benefits would be achieved in beef cattle.

Therefore, the objective of this study was to evaluate the impacts of supplementing LAFP in creep feed to beef steers on pre-weaning growth performance, composition of gain, feed intake and efficiency, daily activity, and volatile fatty acid (**VFA**) profiles of the rumen fluid and fecal matter. We hypothesized that the LAFP

supplementation would improve steer growth rates through improved feed efficiency and altered VFA profiles.

## Materials and Methods

All animals involved in this study were humanely cared for in accordance with the University of Georgia (**UGA**) Animal Care and Use Committee guidelines (AUP #A2021 12-010-Y1-A0).

### *Animal and Dietary Management*

For this study we used 60 suckling commercial Angus steers (initial BW 211.4 ± 21.2 kg; starting age 173 ± 17 d) that were selected from the UGA beef cattle herd at the Northwest Georgia Research and Education Center in Rome, GA (34$^{o}$ 20 N, 85$^{o}$ 7 W). Briefly, all steers were born around January of 2021, castrated at birth, vaccinated (BoviShield Gold 5 and UltraChoice) in June, and then weaned at approx. 8 mo of age in September. In July, prior to weaning, the steers (approx. 6 mo of age) and their respective dams were identified, stratified by calf weight and age, and then assigned to 1 of 3 dietary treatments (n = 20 steers/treatment). The dietary treatments were offered for the last 70 d (10 weeks: July 9$^{th}$ – Sept 16$^{th}$) prior to weaning and included: (1) pasture with no supplementation (**CONTROL**) (2) pasture plus *ad libitum* creep feed (**CREEP**) (3) pasture plus *ad libitum* creep feed with RumaCell LQ (Pacer Technology, Murtaugh, Idaho) *a Lactobacillus acidophilus* fermentation product (**LAFP**) added at the label-recommended rate of 5 g/hd/d.

Steers were individually weighed on two consecutive days at the beginning (i.e., d 0 and d 1) and end of the feeding trial (i.e., d 69 and d 70) to get an average start and end weight, respectively. Additionally, steers were weighed weekly for the calculation of

ADG. Creep feed was mixed weekly and offered *ad libitum* with refusals being weighed back at the end of the week for the calculation of pen-level feed intake and efficiency. Week 1 feed intake and feed efficiency observations are not reported as the steers were being acclimated to the creep feeders. Both the CREEP and LAFP diets were mixed in the same mixer with CREEP being mixed first, followed by LAFP. Non-study diets were mixed following the LAFP diet to clean the mixer prior to mixing the CREEP diet the following week. The LAFP was a liquid product and was sprayed onto the creep feed during the mixing process, just prior to filling the creep feeder. The composition of the creep feed (as-fed basis) offered to the CREEP and LAFP steers was 27% whole corn, 27% pelletized corn gluten, 26% pelletized soybean hulls, 10% cottonseed hulls, 5% soybean meal, 4% molasses, 1% calcium carbonate, and 2 oz/hd/d of a vitamin and mineral premix. Nutrient analysis of the basal creep feed was evaluated using wet chemistry (Table 3.1).

***Forage Analysis***

Cow-calf pairs were randomly assigned to pastures within their treatments such that there were 2 pastures per treatment with 10 cow-calf pairs/pasture. The pastures were each 6 ha in size and contained a mixture of grasses including tall fescue (*Festuca arundinacea*), bermuda grass (*Cynodon dactylon*), and crabgrass (*Digitaria spp.*). Pastures were monitored for adequate forage availability (Fig. 3.1) and forage quality (Table 3.2) throughout the trial at 21-d increments (i.e., week 0, 3, 6, 9).

Forage availability was evaluated as described by Baxter et al. (2017) using the rising plate meter (**RPM**) method. Briefly, the RPM was placed perpendicular to the ground and then the plate was lowered to the canopy of the forage. This process was

repeated randomly throughout the pasture and then the sum of heights was divided by the number of observations ($n$ = 200 observations per 6 ha pasture) to get an average forage height for each pasture. On week 0 and 6, in addition to the RPM measurements, forage samples were taken for the creation of a regression equation which was then used to estimate forage dry matter (**DM**) availability per ha. Briefly, in each pasture a high, medium, and low area in terms of forage availability were identified. In each of these areas, a RPM measurement for forage height was recorded. A 0.093-$m^2$ quadrant was then placed in the same spot that the RPM measurement was taken to define the sample area. All of the forage mass within the quadrant was then clipped to a uniform stubble height of 2.54-cm, placed in a bag, and dried in an oven at 60°C for 48 hours to determine the DM forage content per ha (kg of DM/ha). A regression equation for forage DM availability was then formed using the calibration heights and their respective DM content. Forage DM/ha throughout the study was calculated using the aforementioned regression equation and the average forage height for each pasture (Fig. 3.1).

Forage quality was determined by collecting grab samples of forage from random locations across the pasture. Once a representative sample of the whole pasture was collected, the forage clippings were mixed by hand and a subsample was placed into a bag. The forage was then dried in an oven at 60°C for 48 hours and subsequently frozen until further analysis. At the end of the trial, dried forages from each collection (i.e., week 0, 3, 6, and 9) were ground and compiled by pasture before being sent to the lab for nutrient analysis (Table 3.2).

### *Activity Data*

All steers were individually fitted with a CowManager (Harmelen, The Netherlands) ear tag sensor beginning on July 16th through weaning. Briefly, the sensors function to continuously monitor ear-surface temperature and animal behavior, classifying it into 1 of 5 behaviors: eating – head down with ears forward, ruminating – rhythmic head bobbing, active – normal movement, highly active – intense movement, or inactive – no movement. The activity is then reported as the number of minutes spent doing each activity within each hour. Within each minute, the activity that occupies the majority of the minute is designated as the sole activity for that minute. For simplicity, the number of minutes per hour for both "active" and "highly active" were summed and reported as one value "active".

Sensors were installed by attaching them to an existing radio frequency identification tag in the right ear. The sensors were then activated, at which point they began wirelessly transmitting data through a router system into a web-based application for storage. Data was then downloaded daily from the software into an Excel file and a summation of minutes spent doing each activity was logged each day. Only days with the full 24 h of observations were analyzed.

### *Carcass Ultrasonography*

Ultrasound images were collected by a field certified (Ultrasound Guidelines Council, UGC) ultrasound technician on d 0 and then again on d 55 to evaluate the composition of gain via carcass characteristics of the steers. Images were collected using an Aloka 500-V ultrasound unit with a 17.2 cm, 3.5 MHz linear probe (Corometrics Medical Systems, Wallingford, CT) placed between the 12th and 13th ribs. Carcass traits

measured were ribeye area (**REA**), 12$^{th}$ rib backfat thickness (**BFT**), and intramuscular fat (**IMF**) content. Images of these carcass traits were interpreted by a UGC-lab certified ultrasound technician using Beef Information Analysis Pro Plus software (Designer Genes USA, Harrison, AR).

***Rumen and Fecal Sample Collection***

Rumen contents and fecal matter samples were collected from the steers on d 0 and d 55 of the study for determination of VFA concentrations. Rumen contents were collected following the procedures described by Lourenco et al. (2019). Briefly, rumen contents were collected individually from each steer by esophageal tubing with a weighted metal perforated probe attached to a vacuum pump. Once the probe was placed in the rumen, an initial sample (approx. 250 mL) was collected and discarded to remove any excess saliva in the tube. A second sample (approx. 250 mL) was then collected, subsampled into a 5-mL screw-cap polypropylene transport tube, and immediately snap frozen by immersion in liquid nitrogen. The frozen tube was then transported back to the lab on dry ice and stored at -80°C until further analysis. Rumen pH was immediately recorded chute-side on the rumen contents using an Oakton portable pH meter (Oakton Instruments, Vernon Hills, IL).

Fecal matter samples were collected from the rectum using palpation. The fecal matter was immediately transferred into a 5-mL tube and snap frozen by immersion in liquid nitrogen. The frozen tube was placed on dry ice for transport back to the lab where it was then placed in a -80°C freezer until further processing.

### *Volatile Fatty Acid Analysis*

Rumen and fecal samples were evaluated for VFA concentrations in duplicate using the procedures described by Lourenco et al. (2020). Briefly, frozen rumen contents were thawed, homogenized via inversion and vortexing, and a 1.5 mL subsample was pipetted into a 2-mL microcentrifuge tube. Similarly, fecal samples were thawed, but then they were solubilized by adding 1 g of fecal matter into 3 mL of water, before being homogenized and subsampled. The subsamples were then centrifuged at 10,000 x *g* for 10 min. After centrifugation, 1 mL of supernatant was removed and combined with 0.2 mL of metaphosphoric acid solution (25% weight/volume) in a new 1.7-mL microcentrifuge tube, vortexed and then placed into a -20°C freezer until analysis.

Frozen samples were thawed, centrifuged at 10,000 x *g* for 10 min, and 1 mL of supernatant was removed and added to 2.4 mL of ethyl acetate along with 0.2 mL of internal standard which was 2-Ethylbutyric acid (Sigma Aldrich, St. Louis, MO). Next, the tube was vortexed for 10 s, left to settle for 5 min, and then 0.5 mL of the top layer was transferred into duplicate glass vials. The samples were analyzed on a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector and a capillary column (Zebron ZB-FFAP; 30 m x 0.32 mm x 0.25 µm; Phenomenex Inc., Torrance, CA). The gas chromatograph was set to inject 1.0 µL using helium as the carrier gas. The injector temperature was 250°C, detector temperature was 350°C, and the column was set to increase from 110°C to 200°C. Duplicate samples with a CV greater than 5% were reanalyzed.

### *Statistical Analysis*

Statistical analyses were performed using Minitab v19.2 (Minitab LLC, State College, PA). A one-way ANOVA was used to analyze overall ADG. All other data were analyzed using a mixed effects model with animal included as a random effect while dietary treatment, time, and their interaction were included as fixed effects. For performance data, initial body weight was included as a covariate when significant. Pairwise comparisons between groups for the activity data were corrected for using Bonferroni's method for multiple comparisons whereas means were separated by Tukey's honest significant difference test for all other data. Results were considered significant at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.15$ for all analyses.

## Results

### *Animal Performance*

There was a tendency for a treatment by time interaction for average BW ($P = 0.11$) such that starting weights were similar but by the end of the feeding trial the CREEP and LAFP steers tended to weigh more, 298 kg and 301 kg respectively, than the CONTROL, 290 kg (Fig. 3.2). Nevertheless, there was a time effect ($P < 0.01$) for BW as a result of the steers gaining weight over the 70-d feeding trial. Over the same 70-d feeding trial, overall ADG across the dietary treatments tended to differ ($P = 0.11$) with the CREEP and LAFP steers having greater ADG, on average, compared to CONTROL (Fig. 3.3).

After the omission of the week 1 feed intake data due to acclimation, the remaining performance data was grouped into three 21-d periods (i.e., week 2-4, week 5-7, and week 8-10) for analysis (Fig. 3.4A-D). Average daily creep feed intake was greater

in the week 5-7 and week 8-10 periods compared to week 2-4 ($P = 0.01$) but did not differ ($P = 0.64$) between CREEP and LAFP steers (Fig. 3.4A). Supplemental G:F tended to differ ($P = 0.13$) between CREEP and LAFP steers such that the LAFP steers consistently had a greater G:F value. There was also a time effect ($P < 0.01$) such that feed efficiency decreased throughout the study for all steers (Fig. 3.4B). There was a significant treatment by time interaction ($P = 0.01$) for average BW across the study (Fig. 3.4C). For week 2-4 (244, 246, and 245 kg), week 5-7 (264, 273, and 272 kg), and week 8-10 (283, 291, and 292 kg) there were no significant differences in BW across the CONTROL, CREEP and LAFP steers, respectively; however, for week 8-10 the BW for the CONTROL steers (283 kg) did not differ significantly from the BW of the CREEP (273 kg) and LAFP (272 kg) steers during week 5-7. For ADG there was a treatment by time interaction ($P = 0.03$) (Fig. 3.4D). In general, the CREEP and LAFP steers had a significant decrease in ADG for week 8-10 compared to previous periods, whereas the CONTROL steers exhibited a steady decline in ADG over the course of the study.

The composition of growth occurring throughout the study is illustrated in Fig. 3.5A-C. There were no effects of dietary treatment ($P \geq 0.21$) on REA or IMF content but there was a time effect ($P < 0.01$) with REA and IMF content both increasing over the first 55 d of the study. For BFT there was a treatment by time interaction ($P = 0.01$) such that all steers had similar BFT at the start of the study, but by d 55 the CREEP (0.55 cm) and LAFP (0.54 cm) steers had significantly greater BFT than the CONTROL steers (0.42 cm).

***Rumen Fermentation***

A treatment by time interaction ($P < 0.01$) was reported for rumen pH (Fig. 3.6). On d 0, prior to starting their respective dietary treatments, there were no significant differences in rumen pH. After 55 d on their dietary treatments, the CONTROL and CREEP steers maintained similar rumen pH values whereas the LAFP steers had significantly lower rumen pH values of 6.4 which is still considered within a normal and healthy range.

Rumen total VFA concentrations, the acetate to propionate ratio (**A:P**), and all individual VFA concentrations, except for caproate, had a treatment by time interaction ($P < 0.01$) (Table 3.3). At the start of the study on d 0, there were no significant differences across dietary treatments with regards to any of the rumen VFA parameters evaluated. The LAFP steers had a significant increase in rumen acetate concentration on d 55 compared to d 0. For propionate, the LAFP steers had significantly greater concentrations in their rumen on d 55 compared to CONTROL and CREEP steers which maintained similar values to those reported for all steers on d 0. The CONTROL and LAFP steers had significantly lower rumen concentrations of isobutyrate on d 55 compared to their d 0 values, whereas the CREEP steers had lower concentrations, but they were not statistically different. On d 55 of the dietary treatments, the CREEP and LAFP steers had significantly greater concentrations of butyrate present in the rumen compared to the CONTROL steers. Both isovalerate and valerate concentrations in the rumen were significantly reduced in the CONTROL steers on d 55 whereas the CREEP and LAFP steers maintained similar values to those reported on d 0. For the A:P, the CONTROL steers had a significantly greater ratio of A:P on d 55 compared to the

CREEP and LAFP steers which had values similar to those reported on d 0. Total VFA concentrations for LAFP steers on d 55 were significantly greater than those of the CONTROL steers with the CREEP steers being intermediate. Caproate concentrations did not differ by dietary treatment ($P = 0.95$) but there was a time effect ($P = 0.03$) resulting from an increase in caproate levels during the study.

***Fecal Volatile Fatty Acid Profiles***

Fecal VFA concentrations are reported in Table 3.4. Significant treatment by time interactions ($P < 0.01$) were noted for the three major VFA (acetate, propionate, and butyrate) as well as for total VFA concentrations. Prior to dietary treatment, all steers had similar acetate, propionate, butyrate, and total VFA concentrations in the feces on d 0. On d 55, however, the CREEP and LAFP steers had significantly greater concentrations of acetate, propionate, butyrate, and total VFA in the feces compared to the CONTROL steers. Isobutyrate tended to differ ($P = 0.07$) while isovalerate did differ ($P = 0.02$) across the dietary treatments such that their concentrations were greater in the feces of the CONTROL followed by CREEP and then LAFP steers. Fecal caproate concentrations decreased over time ($P = 0.01$) but did not differ across treatments ($P = 0.69$). The fecal A:P increased ($P = 0.02$) throughout the study but did not differ based on dietary treatment ($P = 0.98$).

***Activity Data***

The summation of minutes each day that steers spent being inactive, active, ruminating, and eating on average are summarized in Fig. 3.7. For inactive, there was a dietary treatment effect ($P < 0.01$) such that the CREEP and LAFP steers, respectively, spent 88 and 95 minutes more each day being inactive than the CONTROL steers.

Furthermore, a treatment effect was denoted for time spent active ($P < 0.01$) with the CREEP and LAFP steers, respectively, spending 120 and 90 minutes more being active than the CONTROL steers. The CONTROL steers, however, spent 79 and 73 more minutes per day eating ($P < 0.01$) and 128 and 111 more minutes per day ruminating ($P < 0.01$) than the CREEP and LAFP steers, respectively. There was a tendency ($P = 0.09$) for ear surface temperature to differ across dietary treatments with the CONTROL steers having the lowest average daily temperature followed by the LAFP and then CREEP (Fig. 3.8).

## Discussion

### *Diets*

Both creep feed dietary treatments, CREEP and LAFP, were formulated using the same basal creep feed, the only difference being the addition of 5 g/hd/d of the *Lactobacillus acidophilus* fermentation product supplement to the LAFP diet. After compiling the weekly samples, nutrient analysis confirmed only negligible differences in the two nutrient profiles.

Prior to initiation of the study, six pastures of similar size, forage composition, and forage availability were identified and randomly assigned to dietary treatment groups. Throughout the study, forage availability and quality were monitored and while forage availability did increase over time, it was consistent across pastures and was never limiting. Thus, forage availability was deemed adequate for all pastures, however, nutrient analysis did reveal some slight differences in forage quality across dietary treatments. The CREEP pastures had a greater average energy content as indicated by the net energy values compared to the LAFP and CONTROL pastures. Furthermore, the

CREEP and LAFP pastures had a greater average protein content compared to the CONTROL pastures. While these differences in nutrient profiles were not anticipated, it is important to note as they could contribute some variation to animal performance.

***Animal Performance***

Initial BW were the same across treatments, however, after 70 d of dietary treatment, ending BW tended to differ with CONTROL at 290 kg, CREEP at 298 kg, and LAFP at 301 kg. While these differences were around 10 kg/hd, it appears that they were largely due to the addition of the creep feed with no substantial additional benefits from the LAFP. These results are supported by the tendency for overall ADG to differ such that there was an initial benefit of 0.13 kg/d for the CREEP steers with an additional 0.04 kg/d with LAFP supplementation. However, when comparing the creep feed intake over the three periods, it is worth mentioning that the similar weight gain between the CREEP and LAFP steers occurred while the LAFP steers consumed numerically less creep feed (0.35 kg/hd/d) on a regular basis. Thus, the ability of the LAFP steers to gain similar weight while consistently consuming less creep feed resulted in the LAFP steers tending to have improved G:F over each period compared to the CREEP steers. It is also worth noting that the divergence in feed efficiency between the CREEP and LAFP steers was increasing as the study progressed. Therefore, further research investigating the impacts of LAFP supplementation over an extended timeframe may result in greater insight into the trends noted for feed efficiency and weight gain in this study.

While creep feeding has been found to consistently improve weight gain (Prichard et al., 1989; Faulkner et al., 1994; Tarr et al., 1994) the extent to which additional gain is achieved is dependent on the nutritional status of the calves and their dams (Tarr et al.,

1994). For instance, if adequate forage and milk production are available, the weight gain benefits associated with creep feeding are reduced (Tarr et al., 1994; Lardy and Maddock, 2007). Thus, it is possible that the adequate forage availability and quality of the current pastures resulted in enough milk production and a high enough plane of nutrition for all steers such that only slight advantages in weight gain were achieved via creep feeding.

Animal growth, feed intake, feed efficiency and other performance benefits associated with dietary microbial supplements such as probiotics have been largely inconsistent throughout the literature (Ban and Guan, 2021). One meta-analysis evaluating nearly 70 studies where probiotics and prebiotics were fed to young ruminants reported that the majority of responses were positive or did not differ significantly for health (31/68 and 15/68), growth (22/68 and 39/68), and feed efficiency (9/70 and 32/70), respectively (Cangiano et al., 2020). While our results for growth and efficiency were statistically non-significant, they were trending in a positive manner. Moreover, in studies with pigs supplemented with LAFP, positive improvements in weight gain, feed intake, and feed efficiency have been reported (Frank et al., 2012; Lee et al., 2016; Lan et al., 2017).

In terms of the composition of growth, there were no significant differences in REA or IMF content. Nevertheless, the two creep fed groups did have numerically larger REA and greater IMF content which is consistent with the enhanced energy content of the creep feed versus forage alone. Previous research has demonstrated that concentrate based diets, like the creep feed used in this study, often increase muscle and fat deposition. Thus, the increased BFT noted in the two creep fed groups compared to the CONTROL steers was logical given the greater energy content of their diet. These results

are consistent with previous research for creep fed calves at weaning that have reported no significant differences in loin muscle size (Rouquette et al., 1983; Lancaster et al., 2007; Reis et al., 2015) or IMF content (Rouquette et al., 1983) however the results for BFT vary. Similar to our results, Tarr et al. (1994) reported greater BFT with creep feeding while other studies have reported no differences (Rouquette et al., 1983; Lancaster et al., 2007; Reis et al., 2015).

***Volatile Fatty Acid Concentrations***

In general, our results suggest that the millimolar (**mM**) concentration of each VFA in the feces was about half that of their rumen concentration. For instance, acetate concentrations were around 70 mM on average in the rumen versus 35 mM in the feces. While the contributions of fecal VFA to animal performance are not explicitly documented due to difficulty measuring hindgut metabolism, their concentrations are reflective of VFA production occurring in the hindgut and can provide insight into nutrient availability to the animal. Elsden et al. (1946) reported that in the lower gut, VFA concentrations are greatest in the cecum but decline as they reach the rectum (i.e., feces) due to absorption occurring in the lower gut. Hindgut VFA, just like rumen VFA, are readily absorbed into the blood stream, via the gut epithelium, for use by the host (Bergman, 1990). For cattle, roughly 50-70% of energy requirements are met by rumen VFA production while the hindgut contributes up to an additional 10% to the host (Siciliano-Jones and Murphy, 1989).

Moreover, in the present results, the A:P were relatively similar between the rumen and feces (5.2 versus 5.7) indicating that while VFA concentrations were reduced, the relative proportions of these VFA in each location were similar. These results are

supported by previous research which suggests that while VFA proportions and raw concentrations can vary significantly based on the composition of the diet (i.e., acetate:propionate:butyrate of 75:15:10 for grass vs 50:40:10 for grain), those relative proportions are often maintained throughout the digestive tract (Elsden et al., 1946; Bergman, 1990). Interestingly, our results did not reflect a large shift in the proportion of the 3 major VFA as previous research might suggest with the addition of grain to the diet. In fact, rumen concentrations of acetate, propionate and butyrate were roughly 76:13:8 for the CONTROL and 70:15:11 for the two creep fed groups on d 55 which is reflective of the supplemental levels of creep feed consumed as opposed to a shift to a majority grain diet.

When looking at the VFA concentrations across the dietary treatments, it is important to note that all rumen VFA parameters evaluated as well as the 3 major VFA and total VFA concentrations in the feces did not differ significantly at the start of the study when the steers were consuming the same diets. After dietary treatment, rumen concentrations of propionate were greater in the LAFP (17.37 mM) steers compared to the CONTROL (11.28 mM) and CREEP (14.01 mM) steers. Similarly, fecal propionate was significantly greater in the LAFP steers compared to the CONTROL although only numerically greater than the CREEP. Overall, these results suggest that while the creep feed alone does provide some additional propionate production, there is further propionate production achieved with the supplementation of the LAFP in the creep feed. Propionate absorbed through the gut epithelium is often transported to the liver to be used as a substrate for glucose production which is subsequently used by the tissue for energy or deposited in fat (Smith and Crouse, 1984; Bergman, 1990). Regardless, propionate is

viewed as an indicator of the animal's energy status, and thus, the LAFP steers may have had improved energy availability compared to the CREEP and CONTROL steers.

Butyrate and the minor VFA, isovalerate and valerate, were greater in the rumen of the CREEP and LAFP steers compared to CONTROL steers. Additionally, butyrate concentrations were greater in the feces of the CREEP and LAFP steers compared to CONTROL steers. Butyrate is often absorbed and utilized by the gut epithelial cells to maintain gut health and function (Bergman, 1990). Cremin et al. (1991) and Tarr et al. (1994) also reported increases in butyrate, whereas Faulkner et al. (1994) reported a decrease in butyrate with ad libitum creep feed supplementation. In contrast, Cremin et al. (1991) found a decrease in other VFA like valerate and isovalerate, whereas Tarr et al. (1994) found no differences in valerate.

Overall, total rumen VFA concentrations were greater in the LAFP (112.18 mM) steers compared to the CONTROL (88.83 mM) with the CREEP (96.47 mM) being intermediate. The greater total VFA concentrations for CREEP versus CONTROL are consistent with other reports which describe increased VFA concentrations with creep feed supplementation compared to non-supplemented calves (Cremin et al., 1991; Faulkner et al., 1994; Tarr et al., 1994); however, the additional production associated with LAFP supplementation in the current study suggests that there may be alterations in rumen fermentation pathways occurring with the addition of LAFP into the creep feed.

Additionally, while studies have demonstrated that VFA concentrations increase with increasing creep feed consumption (Faulkner et al., 1994; Tarr et al., 1994), the feed intake data from the present study reveals that the LAFP steers ate numerically less creep feed than the CREEP steers on a regular basis and thus this is not a likely explanation for

the LAFP steers having greater total VFA values. Moreover, the increased VFA production along with the reduced feed intake supports the tendency for improved feed efficiency in LAFP steers such that the LAFP steers are more efficient (38 mM of total rumen VFA/kg of creep feed consumed) than the CREEP steers (29 mM of total rumen VFA/kg of creep feed consumed) at converting the feed they consume into energy. Nevertheless, the increased total VFA concentrations in the rumen and feces of the LAFP steers suggest an overall greater energy status of those animals (Bergman, 1990).

The increased VFA concentrations also likely contributed to the significantly lower rumen pH, albeit still healthy pH, of the LAFP steers as increased VFA concentrations are known to lower the pH of the environment (Briggs et al., 1957). Furthermore, lower rumen pH is known to improve VFA absorption (Danielli et al., 1945) which could improve energy availability to the host and contribute to the improved efficiency of the LAFP steers. In the end, the increase in total VFA concentrations for the LAFP were largely due to increased production of the three major VFA rather than one VFA in particular. Additional research into the metabolism of the gastrointestinal VFA is warranted to better understand their impact on animal performance.

***Activity***

The CONTROL steers spent more time eating (379 vs 251 and 268 min/d) and ruminating (352 vs 273 and 279 min/d) than the CREEP and LAFP steers, respectively. These results are logical given that the majority of the CONTROL steers nutrient intake was obtained by grazing as opposed to a combination of grazing and creep feed like that of the CREEP and LAFP steers. Other studies have also reported a reduction in time spent grazing for grain supplemented cattle on pasture (Bodine and Purvis, 2003; Valente

et al., 2013; Martins et al., 2017). Furthermore, the greater time spent ruminating by the CONTROL steers is largely attributable to their presumed increased forage intake (Welch and Smith, 1969). Grazed forages generally have a larger particle size and a slower passage rate, requiring more time spent ruminating before passage down the digestive system occurs (Bartocci et al., 1997; Beauchemin, 2018). With regards to creep feeding, research has found that calves will often maintain milk consumption while replacing forage consumption with creep feed (Faulkner et al., 1994; Tarr et al., 1994). Thus, the CREEP and LAFP steers likely spent less time grazing (i.e., eating) and subsequently ruminating because concentrate rations like the creep feed increase passage rate (Guthrie and Wagner, 1988), are conveniently located in one location for faster mealtimes, and are more energy dense than forages (Waldo, 1986).

Time not spent eating or ruminating is then classified into either inactive or active behavior, where inactive behavior is defined as resting behavior and active behavior denotes any activity that could not be identified under the previous categories. Our results indicated that the CREEP and LAFP steers spent more time being inactive (470 and 477 vs 382 min/d) and active (450 and 420 vs 330 min/d) than the CONTROL steers, respectively. Another study by Valente et al. (2013) saw similar results such that the supplemented calves exhibited more idle time compared to controls. In the end, the CREEP and LAFP steers spent less time eating and ruminating, resulting in more time spent being inactive and active. Overall, these results suggest that the differences in daily activity were largely a result of diet composition, with no notable differences due to LAFP supplementation.

### *Conclusions*

Overall, our findings demonstrated that LAFP supplementation in creep feed prior to weaning has a tendency to positively impact beef steer growth performance and feed efficiency. Furthermore, the LAFP steers consumed 0.35 kg/hd/d less creep feed than the CREEP steers while maintaining similar growth, resulting in improved efficiency that could be due in part to the greater total VFA concentrations noted in both the rumen and feces of the LAFP steers. Moreover, the LAFP steers demonstrated greater butyrate and propionate concentrations which when paired with the lower pH could result in improved absorption leading to enhanced gut health and energy availability to the host. Overall, these results suggest that some alterations in gastrointestinal fermentation may be occurring as a result of LAFP supplementation. Therefore, further investigation into the mechanisms behind the altered fermentation is warranted.

**Table 3.1. Chemical composition[1] of the creep feed[2]**

| Nutrient, % DM | CREEP | LAFP |
|---|---|---|
| DM, % | 88.0 | 87.4 |
| Crude Protein, % | 14.0 | 14.3 |
| Non-Fiber Carbohydrates, % | 42.8 | 41.2 |
| Neutral Detergent Fiber, % | 36.6 | 37.6 |
| Acid Detergent Fiber, % | 19.8 | 21.1 |
| Lignin, % | 3.29 | 3.59 |
| Crude Fat, % | 3.17 | 3.05 |
| Ash, % | 5.85 | 6.46 |
| Calcium, % | 0.63 | 0.63 |
| Phosphorus, % | 0.53 | 0.58 |
| Potassium, % | 1.24 | 1.35 |
| Magnesium, % | 0.37 | 0.39 |
| Sodium, % | 0.16 | 0.17 |
| Iron, ppm | 247 | 216 |
| Manganese, ppm | 51 | 54 |
| Zinc, ppm | 72 | 78 |
| Copper, ppm | 18 | 23 |
| $NE_m$, mcal/kg | 1.79 | 1.76 |
| $NE_g$, mcal/kg | 1.17 | 1.15 |

[1] Analyzed by Cumberland Valley Analytical Services (Waynesboro, PA)
[2] DM = dry matter, ppm = parts per million, $NE_m$ = net energy for maintenance, $NE_g$ = net energy for gain, mcal/kg = megacalories per kilogram

**Table 3.2. Forage quality analysis[1] for each pasture used in the study[2]**

| Nutrients, % DM | CONTROL | CREEP | LAFP |
|---|---|---|---|
| Crude Protein, % | 14.6 | 16.0 | 15.9 |
| Non-Fiber Carbohydrates, % | 9.1 | 11.4 | 11.6 |
| Acid Detergent Fiber, % | 35.1 | 37.2 | 35.1 |
| Neutral Detergent Fiber, % | 72.6 | 65.6 | 67.0 |
| Lignin, % | 5.10 | 4.59 | 4.50 |
| Crude Fat, % | 3.28 | 4.39 | 3.47 |
| Ash, % | 8.04 | 8.42 | 8.00 |
| $NE_m$, mcal/kg | 1.24 | 1.36 | 1.28 |
| $NE_g$, mcal/kg | 0.68 | 0.77 | 0.72 |

[1] Analyzed by Cumberland Valley Analytical Services (Waynesboro, PA)
[2] DM = dry matter, $NE_m$ = net energy for maintenance, $NE_g$ = net energy for gain, mcal/kg = megacalories per kilogram

**Table 3.3. Effects of pre-weaning dietary treatment on rumen volatile fatty acid concentrations[1]**

| Trait | Day 0 | | | Day 55 | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CON | CREEP | LAFP | CON | CREEP | LAFP | | Trt | Time | Trt x Time |
| **Acetate, mM** | 66.51$^{b}$ | 61.71$^{b}$ | 62.79$^{b}$ | 67.69$^{ab}$ | 68.04$^{ab}$ | 78.92$^{a}$ | 2.755 | 0.17 | < 0.01 | < 0.01 |
| **Propionate, mM** | 12.91$^{b}$ | 12.02$^{b}$ | 12.21$^{b}$ | 11.28$^{b}$ | 14.01$^{b}$ | 17.37$^{a}$ | 0.780 | < 0.01 | < 0.01 | < 0.01 |
| **Isobutyrate, mM** | 1.22$^{a}$ | 1.09$^{abc}$ | 1.13$^{ab}$ | 0.75$^{d}$ | 0.94$^{bc}$ | 0.91$^{cd}$ | 0.045 | 0.74 | < 0.01 | < 0.01 |
| **Butyrate, mM** | 8.97$^{bc}$ | 9.29$^{bc}$ | 9.62$^{ab}$ | 6.94$^{c}$ | 10.40$^{ab}$ | 11.83$^{a}$ | 0.577 | < 0.01 | 0.33 | < 0.01 |
| **Isovalerate, mM** | 1.62$^{a}$ | 1.38$^{a}$ | 1.50$^{a}$ | 0.92$^{b}$ | 1.38$^{a}$ | 1.35$^{a}$ | 0.072 | 0.14 | < 0.01 | < 0.01 |
| **Valerate, mM** | 0.78$^{a}$ | 0.62$^{ab}$ | 0.74$^{a}$ | 0.38$^{b}$ | 0.71$^{a}$ | 0.85$^{a}$ | 0.061 | < 0.01 | 0.15 | < 0.01 |
| **Caproate, mM** | 0.81 | 0.77 | 0.76 | 0.88 | 1.00 | 0.95 | 0.106 | 0.95 | 0.03 | 0.64 |
| **A:P** | 5.16$^{b}$ | 5.15$^{b}$ | 5.15$^{b}$ | 6.01$^{a}$ | 5.10$^{b}$ | 4.75$^{b}$ | 0.123 | < 0.01 | 0.19 | < 0.01 |
| **Total VFA, mM** | 92.81$^{b}$ | 86.88$^{b}$ | 88.73$^{b}$ | 88.83$^{b}$ | 96.47$^{ab}$ | 112.18$^{a}$ | 4.002 | 0.07 | < 0.01 | < 0.01 |

[1] CON = control, Trt = treatment, Trt*Time = treatment by time interaction, mM = millimolar, A:P = acetate to propionate ratio, VFA = volatile fatty acid

[a,b] Means that differ ($P \leq 0.05$) within a row are indicated by differing superscripts

**Table 3.4. Effects of pre-weaning dietary treatment on fecal volatile fatty acid concentrations[1]**

| Trait | Day 0 | | | Day 55 | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CON | CREEP | LAFP | CON | CREEP | LAFP | | Trt | Time | Trt x Time |
| **Acetate, mM** | 31.32$^{bc}$ | 32.73$^{b}$ | 34.76$^{ab}$ | 22.70$^{c}$ | 35.21$^{ab}$ | 42.67$^{a}$ | 2.356 | < 0.01 | 0.76 | < 0.01 |
| **Propionate, mM** | 6.12$^{a}$ | 6.04$^{a}$ | 6.29$^{a}$ | 3.90$^{b}$ | 6.06$^{a}$ | 7.55$^{a}$ | 0.487 | < 0.01 | 0.43 | < 0.01 |
| **Isobutyrate, mM** | 1.04 | 0.80 | 0.77 | 0.86 | 0.83 | 0.77 | 0.077 | 0.07 | 0.41 | 0.28 |
| **Butyrate, mM** | 2.03$^{abc}$ | 1.86$^{bc}$ | 1.82$^{c}$ | 1.13$^{c}$ | 2.88$^{ab}$ | 3.00$^{a}$ | 0.256 | < 0.01 | 0.03 | < 0.01 |
| **Isovalerate, mM** | 0.88 | 0.60 | 0.52 | 0.66 | 0.59 | 0.53 | 0.093 | 0.02 | 0.33 | 0.36 |
| **Valerate, mM** | 0.73 | 0.67 | 0.65 | 0.68 | 0.72 | 0.69 | 0.087 | 0.92 | 0.86 | 0.81 |
| **Caproate, mM** | 0.32 | 0.14 | 0.23 | 0.00 | 0.00 | 0.00 | 0.107 | 0.69 | 0.01 | 0.69 |
| **A:P** | 5.41 | 5.44 | 5.71 | 5.98 | 5.90 | 5.73 | 0.214 | 0.98 | 0.02 | 0.30 |
| **Total VFA, mM** | 42.44$^{b}$ | 42.83$^{b}$ | 45.04$^{ab}$ | 29.92$^{c}$ | 46.29$^{ab}$ | 55.21$^{a}$ | 2.997 | < 0.01 | 0.88 | < 0.01 |

[1] CON = control, Trt = treatment, Trt*Time = treatment by time interaction, mM = millimolar, A:P = acetate to propionate ratio, VFA = volatile fatty acid

[a,b] Means that differ ($P \leq 0.05$) within a row are indicated by differing superscripts

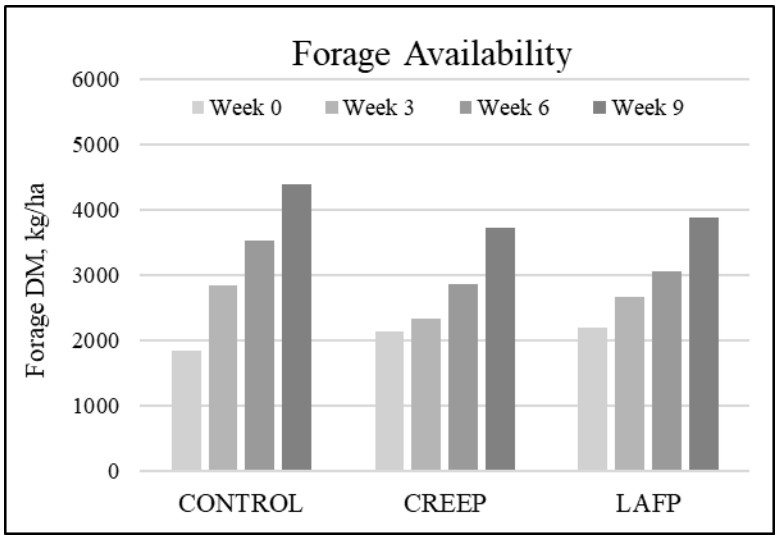


**Fig. 3.1 Forage availability on a dry-matter basis (kg of DM/ha) across the dietary treatments throughout the course of the study. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

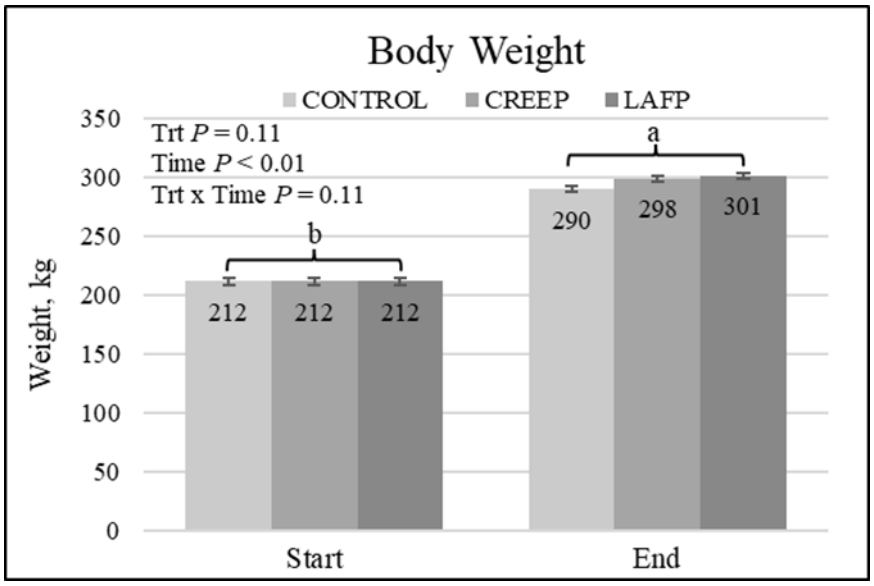


**Fig. 3.2 Starting and ending body weights (kg) reported as the average of weights taken on d 0 and 1 as well as d 69 and 70, respectively. Bars with differing superscripts indicate differences in the means ($P \leq 0.05$). Error bars represent the standard error. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

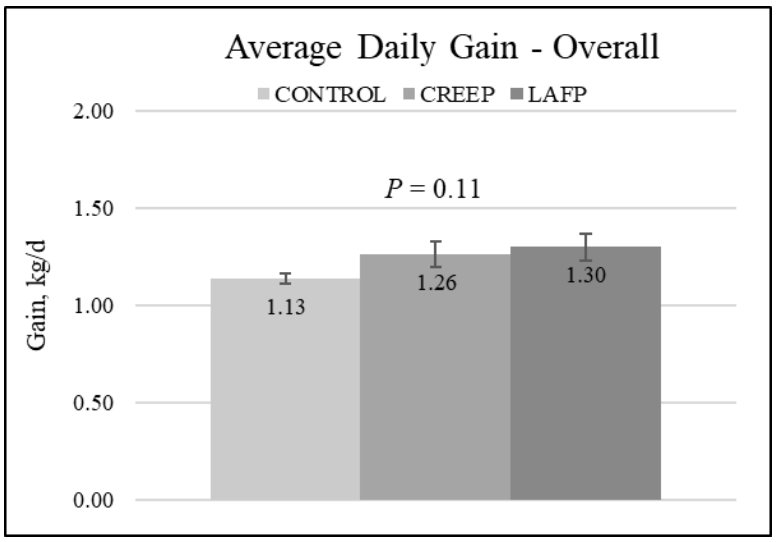


**Fig. 3.3 Average daily gain (kg/d) from d 0 to d 70 across dietary treatments. The *P*-value indicates the significance of the difference between the dietary treatment groups. Error bars represent the standard error. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

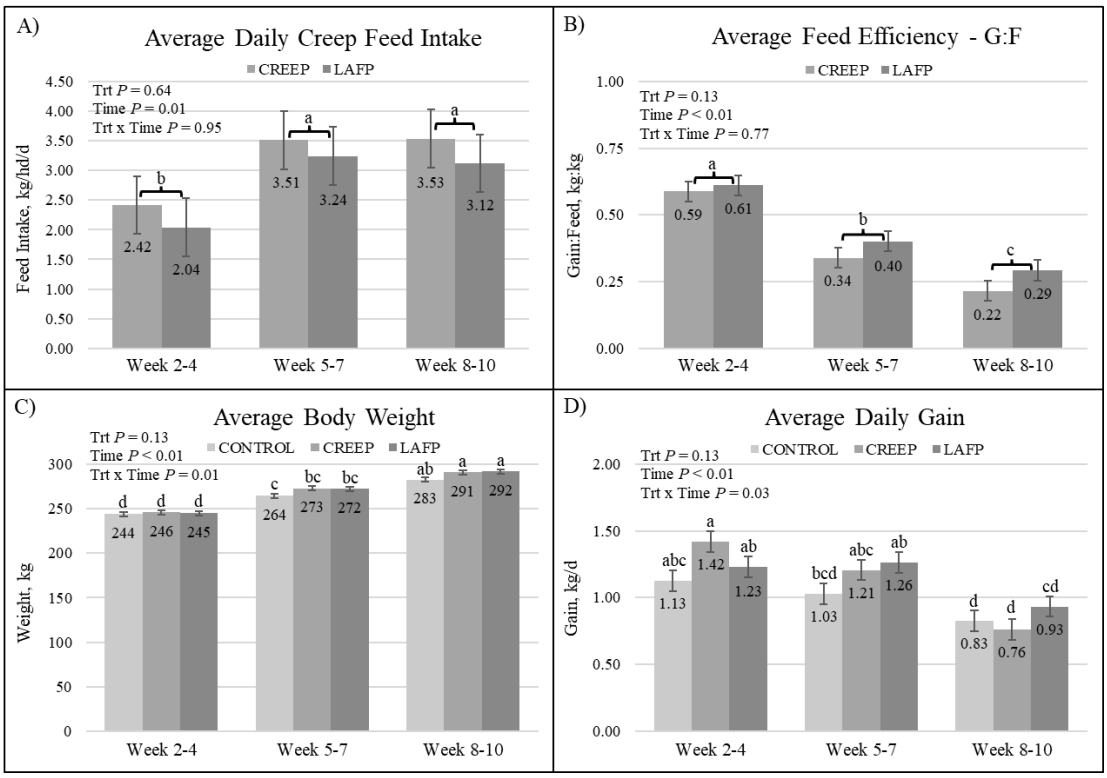


**Fig. 3.4 Animal performance parameters reported across 21-d periods following the omission of the week 1 acclimation period. (A) Average daily creep feed intake per head (kg/hd/d) for the CREEP and LAFP treatment groups (B) Average supplemental feed efficiency for the CREEP and LAFP steers expressed as the gain to feed (G:F) ratio (C) Average weekly body weight (kg) for each dietary treatment (D) Average daily gain (kg/d) for each dietary treatment. Bars with differing superscripts indicate differences in the means ($P \leq 0.05$). Error bars represent the standard error. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

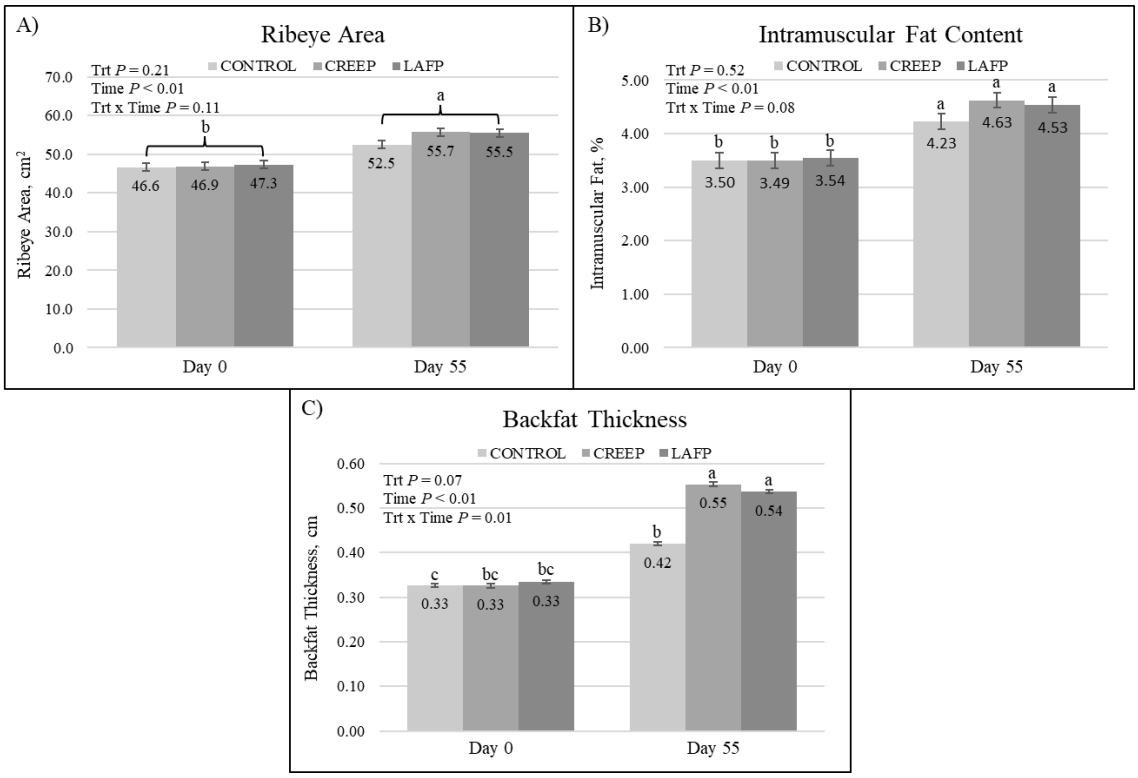


**Fig. 3.5 Carcass characteristics estimated via carcass ultrasonography images taken between the 12th and 13th ribs on d 0 and d 55 of the creep feeding study. Carcass traits evaluated included (A) ribeye area ($cm^2$) (B) intramuscular fat content (%) and (C) backfat thickness (cm). Bars with differing superscripts indicate differences in the means ($P \leq 0.05$). Error bars represent the standard error. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

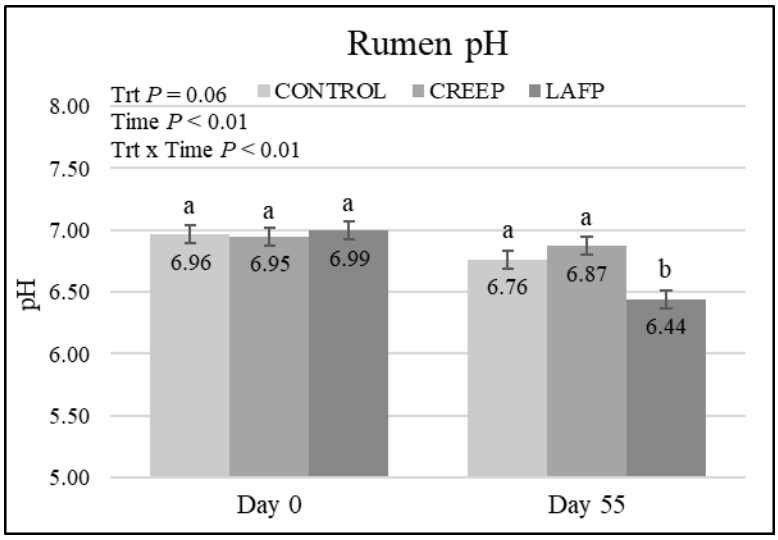


**Fig. 3.6 Rumen pH measured on rumen contents collected via esophageal tubing on d 0 and d 55 of the creep feeding study. Bars with differing superscripts indicate differences in the means ($P \leq 0.05$). Error bars represent the standard error. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

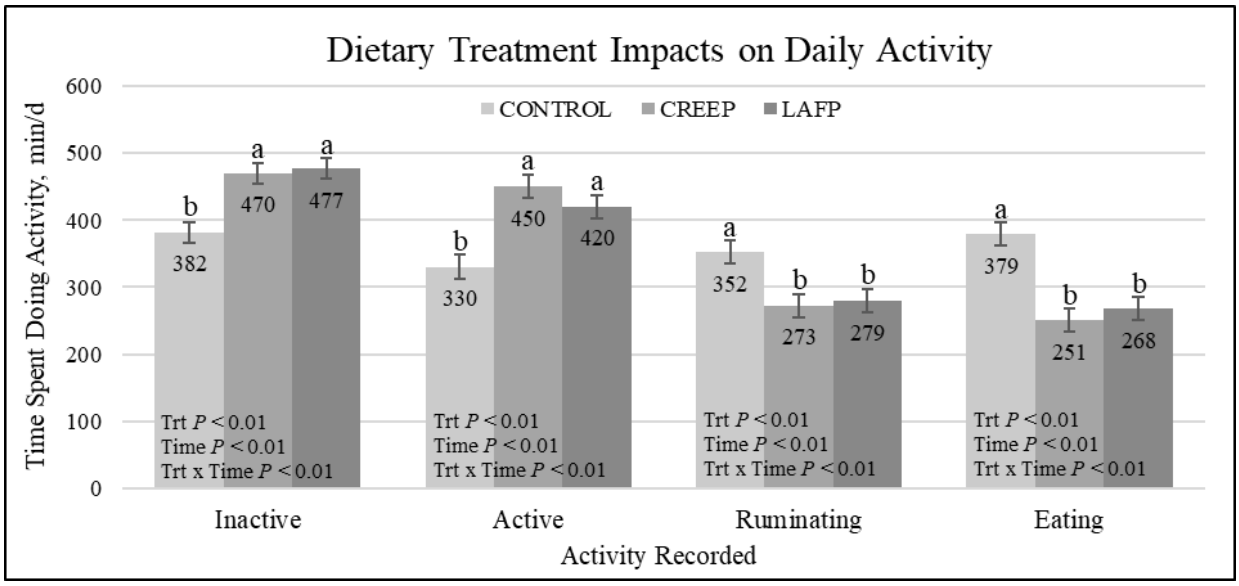


**Fig. 3.7 Daily activity behaviors monitored using CowManager ear tag sensors. Bars with differing superscripts within a reported activity indicate differences in the means ($P \leq 0.05$). Error bars represent the standard error. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

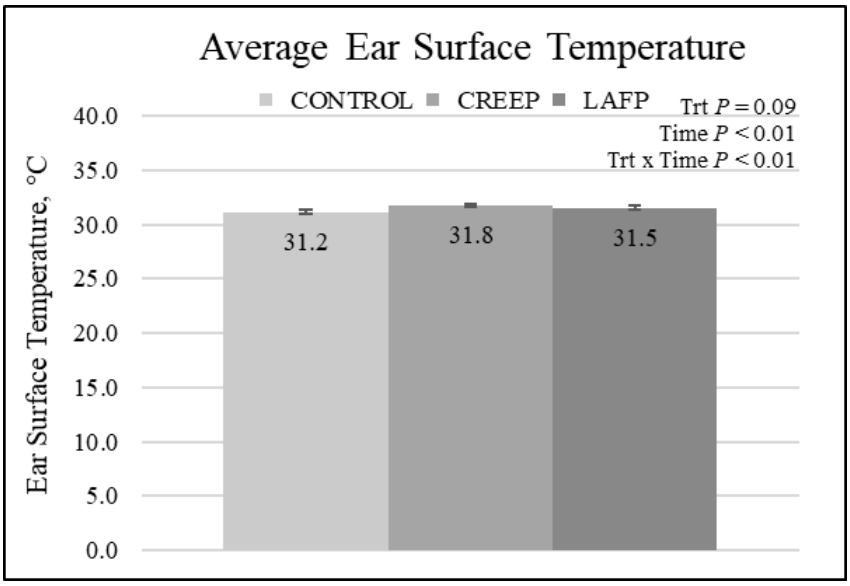


**Fig. 3.8. Average daily ear surface temperature monitored using CowManager ear tag sensors. Bars with differing superscripts indicate differences in the means ($P \leq 0.05$). Error bars represent the standard error. CONTROL = steers on pasture with dam. CREEP = steers on pasture with dam and given creep feed. LAFP = steers on pasture with dam and given creep feed with a *Lactobacillus acidophilus* fermentation product.**

# CHAPTER 4

# ALTERATIONS TO THE ACUTE PHASE IMMUNE RESPONSE OF RECENTLY WEANED ANGUS STEERS FOLLOWING SUPPLEMENTATION WITH A *LACTOBACILLUS ACIDOPHILUS* FERMENTATION PRODUCT PRE- AND POST-WEANING[1]

[1] Krause, T.R., J. M. Lourenco, M. M. Dycus, C. B. Welch, T. R. Callaway, V. E. Ryman, B. D. Heins, and T. D. Pringle. To be submitted to *Journal of Animal Science*.

**Abstract**

Growing pressure from consumers and legislation to reduce antibiotic usage has resulted in an increased interest in probiotic use, especially when administered during times of stress such as the weaning and receiving periods. Therefore, the objective of this study was to evaluate the impacts of pre- and post-weaning supplementation of a *Lactobacillus acidophilus* fermentation product (**LAFP**) on the immune response of newly weaned beef calves challenged with lipopolysaccharide (**LPS**) upon arrival at a post-weaning facility. Steers ($n$ = 60; body weight 211.4 ± 21.2 kg; age 173 ± 17 d) were assigned to one of the following treatments ($n$ = 20 steers/treatment): 1) CONTROL = pasture only 2) CREEP = pasture plus creep feed 3) LAFP = pasture plus creep feed with 5 g/hd/d of a LAFP added. Dietary treatments were administered for 70 days prior to weaning and continued throughout the challenge period post-weaning. A LPS challenge (0.25 µg/kg body weight of *E. coli* O111:B4) was given intravenously on day 4 post-weaning with blood samples collected every hour from -3 h to 6 h as well as at 8 h, 12 h, and 24 h relative to the LPS injection at 0 h. Data were analyzed separately in Pre-LPS (-3 h, -2 h, -1 h, 0 h) and Post-LPS (1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods using a mixed effects model that included dietary treatment, time, and their interaction as fixed effects while animal was included as a random effect. Body weight did not differ ($P \geq 0.45$) across dietary treatments prior to or following the LPS challenge. The only immune response markers that differed across dietary treatments prior to LPS were rectal temperature (**RT**) and respiration rate (**RR**) such that they were greater ($P$ = 0.01) in the CREEP and LAFP compared to CONTROL. Following LPS administration, there were no differences ($P \geq$ 0.15) in RT, cortisol, glucose, IL-6, IFN-γ, IL-1α, IL-21, TNF-α, IL-36Ra, IP-10, or MIG

across dietary treatments. Time effects ($P < 0.01$), however, were noted for RT, RR, cortisol, glucose, IL-6, TNF-α, IL-36Ra, IP-10 and MIG following LPS administration. Overall, these results suggest that the LPS challenge model utilized, successfully modulated immune response markers over time; however, there were no significant differences in any of these responses to LPS across dietary treatments, indicating that all steers responded similarly to the immune challenge regardless of pre- and post-weaning grain supplementation, with or without the addition of LAFP.

## Introduction

The immune system has been previously described by Delves and Roitt (2000) as "an organization of cells and molecules with specialized roles in defending against infection." These cells and molecules are often categorized into two main branches, either innate or acquired immunity, with the former being the body's first line of defense against infection while the latter is the body's "memory" against infection (Delves and Roitt, 2000). Passive immunity, while a part of the acquired branch, are the molecular defenses passed from the dam to the calf via colostrum (Hurley and Theil, 2011). A calf, therefore, has diminishing passive immunity and thus must become increasingly dependent on their own immune function from 4 weeks of age through weaning and into puberty (Chase et al., 2008; Cortese, 2009). Furthermore, the bovine immune system, while fully present at birth, doesn't reach complete maturation until 150 to 240 days of age (Cortese, 2009). Therefore, calves are generally viewed as most susceptible to infection during this transition period from passive immunity to the fully mature immune system as the immune response is present but attenuated and less responsive (Chase et al., 2008; Cortese, 2009). Unfortunately for calves, this lull in immunity often coincides with stressful periods such as weaning and transportation, resulting in compromised immune function during periods of high exposure to new pathogens (Blecha et al., 1984; Phillips et al., 1989; Galyean et al., 1999). As a result, this compromised immune function during periods of stress can lead to economic and productivity losses through greater incidences of morbidity and mortality following weaning (Cernicchiaro et al., 2013).

Traditionally, one attempt to minimize production losses and to improve calf health during stressful events like weaning or entering the feedlot has been to feed

subtherapeutic levels of antibiotics in the feed daily (Barton, 2000; Papatsiros et al., 2013; Finck et al., 2014). Research does suggest, however, that repeated and/or long-term exposure of microbes to antibiotics can encourage the development of antibiotic resistance (Levy and Marshall, 2004; Maron et al., 2013). As a result, consumer concerns regarding antibiotic use in livestock production have risen along with their demands for more "natural" based production schemes (Centner, 2008; Ellison et al., 2017). As producers transition towards more "natural" production systems, the need for "natural" alternatives to traditional management practices (i.e., antibiotics, growth promoters, etc.) grows. Ideally these "natural" alternatives should be able to maintain or improve animal health and performance while allowing producers to adhere to natural program guidelines (Wileman et al., 2009; Stackhouse et al., 2012; Papatsiros et al., 2013).

One potential group of "natural" alternatives that have been around for over a century (Metchnikoff, 1908) but are just now being truly recognized over the last decade for their potential role in animal health are microbial supplements like probiotics (Chaucheyras-Durand and Durand, 2010; Uyeno et al., 2015; Ban and Guan, 2021). Microbial supplements, in general, have been reported to promote growth, modify rumen fermentation, stabilize gastrointestinal microbial populations, modulate immune function, and improve gut and overall animal health (Chaucheyras-Durand and Durand, 2010; Frizzo et al., 2011a; Broadway et al., 2015; Uyeno et al., 2015; Cangiano et al., 2020). While these results are encouraging, inconsistencies throughout the literature are concerning, though likely due in part to the diversity of products (i.e., probiotics, synbiotics, culture extracts, etc.) being tested and their specific application (i.e., species, stage of production, route of administration, etc.).

One microbial supplement of particular interest to the livestock industry is a *Lactobacillus acidophilus* fermentation product (**LAFP**) which contains all components of a *L. acidophilus* anaerobic fermentation including the inactive bacteria itself, the media they were grown on, and the metabolites generated from their fermentation (i.e., organic acids, peptides, enzymes, vitamins, bacteriocins). Studies have found that when supplementing LAFP in pigs, improvements in growth, feed intake, and feed efficiency can be achieved (Frank et al., 2012; Lee et al., 2016; Lan et al., 2017; Sanchez et al., 2019). Further data suggests that LAFP, when fed to pigs prior to an immune challenge, resulted in lower body temperatures, altered cortisol and cytokine patterns as well as reduced circulating white blood cell, neutrophil, and lymphocyte populations (Sanchez et al., 2019). While these results support the use of microbial supplements like LAFP as potential antibiotic alternatives in pigs, the question remains as to their application in ruminants. In this dissertation, Chapter 3 evaluated LAFP supplementation on the performance and gastrointestinal fermentation of suckling beef calves, however, data regarding the impact of LAFP supplementation on the immune response is still lacking.

Therefore, the objective of this study was to evaluate the impacts of LAFP supplementation pre- and post-weaning on the acute phase immune response of beef steers challenged with lipopolysaccharide (**LPS**) shortly after weaning. We hypothesized that steers supplemented with LAFP prior to and following weaning would have a more robust immune response to LPS due to their enhanced energy status prior to the challenge.

## Materials and Methods

All procedures involving live animals were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Georgia (Animal Use Protocol #A2021 08-004-Y1-A0).

### *Animals and Diets*

Animal and dietary management prior to weaning were described by Krause et al., (2022). Briefly, 60 suckling commercial Angus steers (*n* = 60; initial body weight (**BW**) 211.4 ± 21.2 kg; starting age 173 ± 17 d) and their respective dams were identified from the herd at the Northwest Georgia Research and Education Center in Rome, GA (34°20 N, 85°7 W). Prior to weaning in September, steers were weighed and assigned (*n* = 20 steers/treatment) to the following dietary treatments: (1) CONTROL – on pasture with dam but given no supplemental feed (2) CREEP –on pasture with dam and given access to creep feed (3) LAFP – on pasture with dam and given access to creep feed with RumaCell LQ (Pacer Technology, Murtaugh, ID) a *Lactobacillus acidophilus* fermentation product mixed in at 5 g/hd/d. Dietary treatments were offered for at least 70 days prior to weaning at approx. 8 mo of age. Both the CREEP and LAFP steers were given *ad libitum* access to the same basal creep feed (Table 4.1). The basal creep feed was formulated on an as-fed basis to contain 27% whole corn, 27% pelletized corn gluten, 26% pelletized soybean hulls, 10% cottonseed hulls, 5% soybean meal, 4% molasses, 1% calcium carbonate, and 2 oz per head per day of a vitamin and mineral premix. Steers and their dams were randomly assigned within treatments to pastures, such that there were 10 cow/calf pairs per pasture. One CREEP steer died prior to weaning due to unknown causes.

At weaning, approximately 70 days following initiation of dietary treatments, steers were randomly divided into two replicates. On day 70, 29 of the steers (i.e., Rep 1) were removed from their dams and immediately transported to a new facility, while the other 30 steers (i.e., Rep 2) remained with their dam for another week on their respective diets and pastures before being removed and transported. All steers ($n$ = 29/Rep 1 and $n$ = 30/Rep 2) were transported approx. 210 km to Riverbend Farms in Athens, GA (33°55 N, 83°22 W) where they were weighed upon arrival (292.6 ± 28.2 kg) and then sorted into 6 pens ($n$ = 5 steers/pen). Pre-weaning diets and pen groups were maintained at the post-weaning facility, however, the CREEP and LAFP steers were limit fed 4.5 kg/hd/d divided into two feedings at 0800 h and 1800 h.

***LPS Challenge***

After arrival at the post-weaning facility, steers in each rep were allowed to acclimate before being subjected to an immune challenge on their fourth day. On the day of the challenge, steers (Rep 1 BW 293 ± 30 kg and age 247 ± 18 d; Rep 2 BW 299 ± 30 kg and age 252 ± 17 d) were removed from their pens and brought into the working facility where they were held in their respective groups in holding pens. During the challenge, steers were processed through a chute for sample collection, averaging less than 1 min of restraint per trip, and then returned to their holding pen. Body weights were recorded with the first (-3 h Pre-LPS) and last (12 h Post-LPS) collection on challenge day as well as on the day after the challenge (24 h Post-LPS). All steers were given *ad libitum* access to water and hay throughout the day. In addition, the CREEP and LAFP steers were fed their respective rations as indicated above.

Steers were challenged intravenously with 0.25 µg/kg BW LPS from *Escherichia coli* O111:B4 (Sigma Aldrich, St. Louis, MO) at 1000 h on day 4. Blood samples were collected via jugular venipuncture at 1-h increments from -3 h to 6 h, 8 h, 12 h, and 24 h relative to LPS administration (i.e., 0 h). These blood samples were collected into 10-mL red top tubes containing no additive, allowed to clot at room temperature for 30 min and then centrifuged at 2000 *g* for 15 min at 4°C. Serum was then pipetted into ten aliquots of 250 µL and frozen at -80°C until analyzed to measure cortisol, glucose, and cytokine concentrations.

Rectal temperature (**RT**) was recorded at 1-h intervals using a hand-held thermometer as steers were processed through the chute for blood collection. Respiration rates (**RR**) were reported by observing the number of breaths taken in 15 s and then multiplying by 4 to get respirations/min. Sickness behavior scores (**SBS**) were evaluated on a scale of 1 to 5 as described by Sanchez et al. (2013). In brief, a score of 1 indicated that the steer was alert and expressing normal maintenance behaviors, a 3 indicated they were expressing some mild respiratory issues, whereas a score of 5 indicated they were not responsive and required medical intervention. Both RR and SBS were recorded by a blinded and trained observer at 1-h intervals with observations being made in the holding pens immediately following each blood collection.

***Cortisol***

Serum samples were analyzed for cortisol concentrations in duplicates using a commercially available enzyme-linked immunoassay (**ELISA**) kit (Arbor Assays, Ann Arbor, MI) according to the manufacturer's instructions. Standard curves were made using standards provided by the kit with known concentrations of cortisol. Samples with

unknown concentrations were then evaluated against the standard curve to ascertain cortisol concentrations with the limit of detection being 45.4 pg/mL. Duplicate samples with a CV ≥ 10% were rerun. The intra- and inter-assay CV for cortisol were ≤ 13.9% and 8.7%, respectively.

***Glucose***

Serum glucose concentrations were determined in duplicates using a commercially available enzymatic colorimetric assay (FUJIFILM Healthcare Americas Corporation, Lexington, MA). Samples were analyzed on 96-well plates according to the manufacturer's directions for microplate applications. In brief, samples with unknown concentrations were compared against standard curves generated using standards with known concentrations of glucose provided by the kit. Duplicate samples with a CV ≥ 10% were rerun. The intra- and inter-assay CV for glucose were ≤ 9.3% and 2.4%, respectively.

***Cytokines***

Cytokine concentrations were evaluated in serum samples by RayBiotech (Peachtree Corners, GA) testing services using the Quantibody Bovine Cytokine Array 1 kit (RayBiotech, Peachtree Corners, GA) and the RayBio Bovine IL-6 ELISA kit (RayBiotech, Peachtree Corners, GA) according to the manufacturer's protocols. The Quantibody Bovine Cytokine Array 1 used multiplexing technology to simultaneously quantify 10 targets consisting of interferon-alphaA (**IFN-αA**), interferon-gamma (**IFN-γ**), interleukin-13 (**IL-13**), interleukin-1alpha (**IL-1α**), interleukin-36 receptor antagonist (**IL-36Ra)**, interleukin-21 (**IL-21**), IFN-γ induced protein-10 (**IP-10**), monokine induced by IFN-γ (**MIG**), macrophage inflammatory protein-1beta (**MIP-1β**), and tumor necrosis

factor-alpha (**TNF-α**). Target concentrations were measured in quadruplicates with an intra-assay CV of < 20%. All samples were processed in a single run using positive and negative controls which allowed for normalization of the data across arrays. The lower limit of detection for IFN-αA, IL-13, IL-21, IP-10, MIG, and TNF-α was 8.0 pg/mL whereas IFN-γ, IL-1α, IL-36Ra, and MIP-1β was 2.0 pg/mL. Cytokines with over 10% of samples falling outside of their respective standard curve were not reported and include IFN-αA, IL-13, and MIP-1β. Serum interleukin-6 (**IL-6**) concentrations were analyzed in duplicates with the limit of detection being 4.5 pg/mL and the intra- and inter-assay CV being < 11.1% and 3.1%, respectively.

***Statistical Analysis***

During each of the reps, one steer per pen was administered with saline to be used as a reference (data not shown). In addition, any steers that had been previously treated with antibiotics during the pre-weaning phase were removed from the post-weaning immune challenge; thus, the number of steers analyzed in this study was $n = 15$ for CONTROL, $n = 12$ for CREEP, and $n = 15$ for LAFP.

Statistical analyses were performed using Minitab v21.2 (Minitab LLC, State College, PA). Age and BW data were analyzed using a one-way ANOVA. Sickness behavior scores were analyzed using a mixed effects model with dietary treatment, time, the treatment by time interaction, and rep included as fixed effects whereas animal was included as a random effect. All other data were separated into Pre-LPS (i.e., -3, -2, -1, and 0 h) and Post-LPS (i.e., 1, 2, 3, 4, 5, 6, 8, 12, 24 h) periods before analysis, representing baseline values and the response to LPS, respectively. Both Pre-LPS and Post-LPS data sets were analyzed for repeated measures using a mixed effects model

with dietary treatment, time, and their interaction included as fixed effects while animal was included as a random effect and rep was included in the model when significant. Means were separated using Tukey's pairwise comparisons and were presented as mean ± SE. Significance was defined at $P \leq 0.05$ while tendencies were declared at $0.05 < P \leq 0.10$ for all analyses.

## Results

### *Animal Performance*

Steer age upon arrival at the post-weaning facility did not differ ($P = 0.51$) across dietary treatments (Table 4.2). Additionally, steer BW did not differ upon arrival ($P = 0.85$) or throughout the challenge period ($P \geq 0.45$) for the three dietary treatments (Table 4.2).

### *Rectal Temperature*

Prior to LPS administration, there was a difference in baseline RT ($P = 0.01$) across treatments (Fig. 4.1). The CREEP and LAFP steers had greater RT ($P \leq 0.02$) than CONTROL steers (39.37ºC and 39.38ºC vs 38.90ºC, respectively) prior to LPS, but the RT of the CREEP and LAFP steers did not differ ($P = 0.99$) from each other. There was also a time effect ($P < 0.01$) such that all steers exhibited an increase in RT from -3 h to -2 h ($P = 0.01$) and then from -2 h to 0 h ($P < 0.01$) with -1 h being intermediate. Baseline RT also differed across reps ($P < 0.01$) with Rep 1 having greater RT (39.94ºC) than Rep 2 (38.94ºC).

Following LPS administration, there were no significant differences in RT across dietary treatments ($P = 0.15$) but there was a time effect ($P < 0.01$). All steers initially

experienced an elevation in RT from 1 h to 4 h Post-LPS, at which point RT peaked at 4 h, and then decreased from 4 to 5 h ($P = 0.04$) followed by a steady decline through 24 h.

***Sickness Behavior***

There was a treatment by time interaction ($P = 0.03$) for SBS (Fig. 4.2). Mean values, however, did not exceed 1.58 on a scale of 1 to 5 suggesting that only minor deviations from normal behavior were observed. There was also a rep effect for SBS such that scores were greater ($P < 0.01$) for Rep 1 compared to Rep 2 with an average score of 1.21 versus 1.07, respectively.

***Respiration Rate***

Prior to LPS administration, baseline RR differed across dietary treatments ($P = 0.01$) such that CREEP and LAFP steers had greater RR ($P \leq 0.03$) than CONTROL (67 and 65 vs 58 respirations/min, respectively) but did not differ from one another ($P = 0.74$; Fig. 4.3). There was also a time effect ($P < 0.01$) on baseline RR such that they were increased ($P \leq 0.04$) at 0 h compared to -3 h, -2 h, and -1 h, which did not differ from each other ($P \geq 0.59$). Baseline RR differed ($P < 0.01$) across reps with Rep 1 having greater RR on average compared to Rep 2 (67 vs 59 respirations/min, respectively).

Steer RR tended to differ across dietary treatment ($P = 0.09$) following LPS administration, similar to those patterns noted Pre-LPS. A time effect ($P < 0.01$) for RR Post-LPS was also measured such that RR did not differ for 1 h Post-LPS compared to 2 h, 3 h, or 4 h ($P \geq 0.48$), however, there was an increase for 5 h and 6 h ($P < 0.01$) when RR peaked. From 6 h to 8 h RR decreased ($P < 0.01$), followed by a further decrease from 8 h to 12 h ($P < 0.01$) and from 12 h to 24 h ($P < 0.01$). A rep effect ($P < 0.01$) was

observed indicating that RR were greater for Rep 2 versus Rep 1 in response to the LPS dose.

***Cortisol***

There was no dietary treatment effect Pre-LPS ($P = 0.38$) on cortisol concentrations but there was a time effect ($P < 0.01$; Fig. 4.4). Cortisol concentrations were greatest ($P < 0.01$) at -3 h compared to -2 h, -1 h, and 0 h, which did not differ from each other ($P \geq 0.80$).

Similar patterns were noted Post-LPS with no dietary treatment effect ($P = 0.95$) on cortisol concentrations but there was a time effect ($P < 0.01$). All steers experienced peak cortisol concentrations between 1 h and 5 h Post-LPS with a significant decrease in cortisol by 6 h ($P < 0.01$). A further reduction in cortisol was noted between 6 h and 8 h ($P < 0.01$) with a return to near Pre-LPS values between 8 h and 24 h.

***Glucose***

Glucose concentrations did not differ ($P = 0.21$) across dietary treatments prior to LPS administration (Fig. 4.5); however, there was a time effect ($P = 0.05$) with 0 h (69.94 mg/dL) having a lower concentration ($P = 0.05$) than -2 h (72.22 mg/dL), but 0 h did not differ from the other Pre-LPS values ($P \geq 0.85$).

There were no differences ($P = 0.19$) in glucose concentrations in response to LPS across the different dietary treatments. A time effect ($P < 0.01$) was observed such that glucose concentrations peaked between 1-2 h Post-LPS, significantly decreased by 3 h ($P < 0.01$), and then increased between 4-6 h ($P \leq 0.01$) but not completely to Pre-LPS values. A return to near baseline values was observed between 12-24 h ($P < 0.01$)

following LPS administration as glucose values rose to 66.71 mg/dL at 24 h compared to 69.94 mg/dL at 0 h.

***Cytokines***

Prior to LPS administration, there were no treatment or time effects ($P \geq 0.35$) for IL-6 concentrations (Fig. 4.6). In response to LPS administration, there were still no differences ($P = 0.52$) across dietary treatments for IL-6 concentrations but there was a time effect ($P < 0.01$). The concentrations of IL-6 increased following LPS administration from 1 h to 3 h and then peaked between 3 h and 5 h for all steers. From 5 h to 8 h ($P < 0.01$) and from 8 h to 12 h ($P < 0.01$), IL-6 concentrations significantly decreased, returning to near Pre-LPS values by 24 h.

Baseline concentrations of IFN-γ (Fig. 4.7), IL-1α (Fig. 4.8), nor IL-21 (Fig. 4.9) differed across dietary treatments ($P \geq 0.30$) or time ($P \geq 0.48$). Similar patterns were reported in response to LPS with no differences in IFN-γ, IL-1α, or IL-21 concentrations across treatments ($P \geq 0.28$) or time ($P \geq 0.43$).

Concentrations of TNF-α did not differ across dietary treatments ($P = 0.87$) or time ($P = 0.23$) prior to LPS (Fig. 4.10). Following LPS administration, there were no differences across dietary treatments ($P = 0.28$) but there was a time effect ($P < 0.01$). Concentrations of TNF-α peaked between 1-2 h and then decreased by 3 h ($P < 0.01$), followed by a further decrease at 4 h ($P < 0.01$) whose concentrations did not significantly differ from 5 h, 6 h, 8 h, 12 h, or 24 h ($P \geq 0.14$) indicating a return to baseline values.

There were no differences in baseline concentrations of IL-36Ra across dietary treatments ($P = 0.69$) or time ($P = 0.42$; Fig. 4.11). In response to LPS, there was a

tendency for a treatment by time interaction ($P = 0.09$) and a time effect ($P = 0.03$) such that IL-36Ra concentrations were greater ($P = 0.03$) at 1 h compared to 24 h with all other timepoints being intermediate.

Concentrations of IP-10 were similar across dietary treatments prior to ($P = 0.95$) and in response to ($P = 0.93$) LPS (Fig. 4.12). However, there was a time effect regarding IP-10 concentrations for both the Pre-LPS ($P = 0.02$) and Post-LPS ($P < 0.01$) periods. For all steers, baseline IP-10 concentrations increased from -3 h to -1 h ($P \leq 0.05$) and then began to decrease from -1 h to 0 h, however, not significantly ($P = 0.39$). After LPS administration, IP-10 concentrations were increased in all steers from 1 h to 6 h and at 8 h ($P \leq 0.04$) compared to concentrations at 24 h, with 12 h being intermediate.

There was a tendency ($P = 0.09$) for a treatment by time interaction for MIG concentrations prior to LPS (Fig. 4.13). In response to LPS, however, there was no treatment by time interaction ($P = 0.92$) or differences across dietary treatments ($P = 0.75$) only time ($P < 0.01$). Accordingly, MIG concentrations increased from 1 h to 4 h ($P < 0.01$) with peak concentrations occurring between 4 h and 8 h. From 8 h to 12 h ($P < 0.01$) there was a decrease in MIG concentrations followed by a further decrease from 12 h to 24 h ($P < 0.01$) to near baseline values.

## Discussion

### *Animal Performance*

Upon arrival at the post-weaning facility and throughout the challenge period, the steers utilized in this study were similar in terms of age and BW. These lack of differences in BW over the post-weaning period are similar to the lack of differences in BW noted during the 70-day pre-weaning creep feeding trial conducted on these steers

(Krause et al., 2022). It is important to note, however, the short timeframe in which BW was recorded during the post-weaning period. Observations of BW over a 24 h period can be highly variable and thus these results should be interpreted with caution. Overall, all steers, regardless of treatment, lost weight during the challenge period which is likely due in part to feed consumption patterns being altered on challenge day by frequent handling.

In pigs, Sanchez et al. (2019) reported, with caution, similar feed intake but lower weight gains in LAFP supplemented pigs over a 3 day period following an LPS challenge. Prior to the challenge, however, LAFP supplementation had improved BW and feed efficiency compared to controls (Sanchez et al., 2019), which is similar to the results of other studies in LAFP supplemented pigs with reports of improved BW, average daily gain, feed intake and efficiency when fed for 28 days or more (Frank et al., 2012; Qiao et al., 2015; Lan et al., 2017). Nevertheless, extended monitoring regarding the impacts of LAFP supplementation on feed intake and BW prior to and in response to an immune challenge in cattle is lacking and thus requires further investigation.

Research evaluating the impacts of probiotics on animal performance associated with an immune challenge is of great value to the beef industry. Often times, cattle during the receiving period are stressed and experiencing reduced immune function while being introduced into a new environment with new pathogens (Galyean et al., 1999). As a result, these cattle are extremely susceptible to disease challenges and often experience reductions in performance (Duff and Galyean, 2007a). Thus, this study was designed to evaluate the impacts of probiotics on the immune response of newly weaned beef calves using LPS to model a bacterial infection experienced shortly after arrival at a new facility (i.e., day 4). Ultimately, LPS activates the acute phase response of the innate immune

system resulting in a relatively short-term (approx. 24 h) and highly conserved immune response characterized by moderate morbidity without mortality (Burdick Sanchez et al., 2020). The dose used in the current study was chosen based on previous studies using the LPS challenge model as well as steer breed and background were taken into account in order to elicit an adequate response without overstimulating the immune system which would result in the masking of treatment differences (Finck et al., 2014; Littlejohn et al., 2019; Burdick Sanchez et al., 2020).

***Health Indicators***

Throughout the literature, it is well documented that following LPS stimulation, pro-inflammatory cytokines (i.e., TNF-α, IL-1, IL-6) are released. These pro-inflammatory cytokines then signal to the brain to increase body temperature (Lohuis et al., 1988; Dinarello, 1996), creating a febrile response that is temporary but predictable (Carroll et al., 2009b). Moreover, the results of the current study and Carroll et al. (2009b) demonstrated peak RT in response to LPS occurring between 3 h and 5 h; however, other studies have reported a slightly quicker and sharper peak temperature in LPS treated cattle (Sanchez et al., 2013; Finck et al., 2014).

Nevertheless, peak RT values in the current study were similar across treatments suggesting that there would be no differences in dietary treatment with regards to pathogen survival as increased body temperatures have been associated with reduced bacterial growth (Kluger and Rothenburg, 1979; Kluger et al., 1998). The increase in RT noted for all steers prior to LPS administration could be due in part to increasing ambient temperatures and/or natural circadian rhythms that have demonstrated a natural rise in body temperature starting in the early hours of the morning with peak values roughly 12

h later (Aschoff, 1982; Piccione et al., 2002). Given that this experiment was conducted at environmental temperatures, it is possible that the lack of differences noted in peak RT values could have been influenced by elevated ambient temperatures as suggested by Carroll et al. (2021). For instance, environmental temperatures peaked at 23.2ºC around 1400 h or 4 h Post-LPS for Rep 1 whereas during Rep 2 they peaked at 28.3ºC around 1600 h or 6 h Post-LPS. Thus, these peak environmental temperatures do slightly overlap with the timeframe in which RT peaked in the current study.

The CONTROL steers had significantly lower baseline RT compared to CREEP and LAFP steers, but there were no differences in RT across dietary treatments Post-LPS, suggesting that there was a greater increase in RT for the CONTROL steers in response to LPS. Possible explanations for this occurrence will be explored later in the discussion. Other studies have demonstrated variability in RT results such that Burdick Sanchez et al. (2020) reported greater RT in *Saccharomyces cerevisiae* fermentation product (SCFP) supplemented steers whereas Brewer et al. (2014) noted lower RT in SCFP supplemented dairy calves following an immune challenge. In pigs, Sanchez et al. (2018) reported no differences in the temperature response to LPS for pigs supplemented with SCFP. When administering LAFP to pigs, however, Sanchez et al. (2019) indicated that there was less of a change in intraperitoneal temperatures for LAFP compared to controls pigs after LPS. That same study also reported that some differences in body temperature in pigs did not occur until more like 48 h after LPS administration, indicating that LAFP supplementation may be having effects on the immune system outside of the 24 h window associated with the acute phase response which warrants further investigation (Sanchez et al., 2019). In the end, results from these studies demonstrate the importance

of animal model and probiotic strain on the outcome of the febrile response, which solidifies the need to research specific specie-probiotic relationships.

For RR, CONTROL steers had significantly lower baseline RR than CREEP and LAFP steers and this pattern continued as a tendency following LPS administration. Other studies have reported a change in RR over the course of an immune challenge (Carroll et al., 2009b; Burdick Sanchez et al., 2020), as the current research did; however, RR in other studies have peaked more suddenly (approx. 1 h post-challenge) (Carroll et al., 2009b) whereas we observed the increase in RR several hours after the initial challenge. Furthermore, when supplementing steers with SCFP prior to an immune challenge, Burdick Sanchez et al. (2020) reported no differences in RR during the challenge.

In the current study, the patterns noted in RT and RR are biologically logical such that CONTROL steers exhibited lower RT and RR prior to the challenge. A positive correlation between RT and RR has been previously documented, $r = 0.96$ (Gaughan et al., 2000; Li et al., 2020). Nonetheless, all steers in the current study exhibited elevated RR a 1 h Post-LPS, corresponding to the initial spike in RT, cortisol, and TNF-α, with peak RR being observed between 5-6 h Post-LPS. It is plausible that the delayed RR response noted in this study is confounded by ambient temperature. Research suggests that RR generally increase with increasing ambient temperature, albeit their values have been found to lag behind temperature values by up to 2 h (Gaughan et al., 2000). Thus, there could be interactions between the RR peak values and ambient temperature with peak temperatures occurring at 1400 h and 1600 h which corresponds with 4 h and 6 h Post-LPS on the day of the challenge for Rep 1 and 2, respectively. This relationship

between RR, RT, and ambient temperature likely explains the increase in RT and RR noted Pre-LPS for all steers.

In addition to the febrile response, sickness behavior expressed by livestock is considered to be a highly conserved biological response that aims to conserve energy during an infection in order to improve survivability of the host (Hart, 1988). Sickness behavior can be described, in brief, as a set of behavioral and physiological responses that work to shift energy partitioning away from non-essential activities towards those that promote survival, like the febrile response (Dantzer, 2004). In the present study, we reported a mild increase in SBS from 1 h to 6 h, but overall, the SBS observed were very low which indicates that only minor deviations from normal behavior occurred. Relatively low SBS, with average values under 2 on a scale of 1 to 5, were also observed across a similar timeframe in other studies (Sanchez et al., 2013; Burdick Sanchez et al., 2020). These results are likely reflective of the relatively low doses of LPS administered in each of these studies, including the current. It has been noted, however, that SCFP supplementation in weaned beef calves resulted in lower SBS (Burdick Sanchez et al., 2020) while feeding yeast cell wall probiotics to receiving steers has been linked to 2% lower morbidity and 4% lower mortality (Finck et al., 2014). In the current study, SBS generally corresponded with the increased circulation of TNF-α and IL-6, along with increased RT, which is logical as these pro-inflammatory cytokines have been linked to the initiation of sickness behavior and the febrile response (Hart, 1988; Dinarello, 1996; Dantzer, 2001, 2004; Vybíral et al., 2005).

***Cytokines***

While IL-6 is generally associated with increased temperatures, it does not act directly on the thermoregulatory control centers of the brain to induce a fever like TNF-α and IL-1β, but rather through the initial increases in TNF-α and IL-1β that then stimulate IL-6 production to help maintain the febrile response (Dinarello, 1988; Shalaby et al., 1989; Vybíral et al., 2005). Moreover, when injected intravenously, IL-6 alone generated a short-lived hyperthermic response in rabbits, characterized by a reduction in RR coupled with vasoconstriction, overall reducing the dissipation of heat rather than inducing a fever (Vybíral et al., 2005). Thus, it is possible that the 39% and 50% increase in baseline IL-6 concentrations for the CONTROL steers compared to the CREEP and LAFP steers (70.8 vs 50.8 and 47.0 pg/mL), respectively, could be contributing in part to the lower baseline RR noted in the CONTROL steers. Other studies have reported no differences in baseline IL-6 concentrations when supplementing probiotics in the diet (Sanchez et al., 2019; Burdick Sanchez et al., 2020).

Following administration of LPS, IL-6 concentrations in the current study peaked between 3 h and 5 h for all steers, similar to patterns noted in other studies (Carroll et al., 2009b; Sanchez et al., 2019). This peak in IL-6 concentrations coincides with the peak in RT likely due in part to the indirect stimulation of other pro-inflammatory cytokines on the thermoregulatory centers of the brain followed by the subsequent release of IL-6 to help maintain the febrile response as mentioned previously (Dinarello, 1988; Vybíral et al., 2005). This relationship between IL-6 and RT is further supported by Carroll et al. (2009b) which reported a positive correlation of $r = 0.67$ between RT and IL-6 concentrations using a LPS challenge model in cattle. When assessing IL-6

concentrations following probiotic supplementation and LPS administration, results have consistently demonstrated lower concentrations of IL-6 in SCFP steers (Burdick Sanchez et al., 2020) and LAFP pigs (Lee et al., 2016; Sanchez et al., 2019) compared to controls, although Qiao et al. (2015) reported no differences in LAFP pigs. These results are in contrast to the current study which observed no differences across dietary treatments in IL-6 concentrations.

For TNF-α, the current study reported peak values between 1 h and 2 h following LPS which is similar in terms of timeline and peak values reported in other studies (Carroll et al., 2009b; Carroll et al., 2021); however, the baseline values in the current study were much greater than those reported by other studies at nearly 1000 pg/mL and 0 pg/mL, respectively. The reason behind the greater baseline values of TNF-α for all steers in the current study is unclear. When evaluating the impacts of probiotic supplementation on TNF-α response to LPS, results have consistently demonstrated a reduction in TNF-α values for LAFP pigs (Qiao et al., 2015; Lee et al., 2016; Sanchez et al., 2019) and a 3-fold reduction for SCFP steers (Burdick Sanchez et al., 2020) compared to the controls. While not significant, the current study did observe a numerically lower peak in TNF-α for the LAFP steers which corresponded to a slight reduction in IL-6 concentrations around 2-4 h Post-LPS. This is not surprising given the role that TNF-α has in inducing IL-6 circulation following LPS administration (Shalaby et al., 1989). Furthermore, the numerically greater peak TNF-α values for CONTROL steers could contribute some to the greater increase in RT observed in the CONTROL steers, given the ability of TNF-α to induce a fever via the thermoregulatory control centers of the brain (Vybíral et al., 2005).

Concentrations of IL-21, IL-1α, and IFN-γ were similar across dietary treatments. These similar IFN-γ concentrations following probiotic supplementation and LPS administration support the findings of other studies in yeast supplemented cattle (Sanchez et al., 2013; Finck et al., 2014) and LAFP supplemented pigs (Sanchez et al., 2019); however, Qiao et al. (2015) did report a decrease in IFN-γ in response to LPS for LAFP supplemented pigs. Furthermore, there were no differences in IFN-γ concentrations over the course of the LPS challenge in the current study. These results are in contrast to other studies which have reported peaks in IFN-γ concentrations around 2 to 5 h following LPS administration (Carroll et al., 2009b; Sanchez et al., 2019; Burdick Sanchez et al., 2020). Moreover, the concentrations of IFN-γ reported in this study (Pre- and Post-LPS) are much greater than the peak values reported by other cattle studies with 250-450 pg/mL for this study vs 12 pg/mL (Burdick Sanchez et al., 2020), 25 pg/mL (Carroll et al., 2021), and 120 pg/mL (Carroll et al., 2009b) whereas baseline values were under 40 pg/mL for all. It is plausible that some of the discrepancy in concentrations across these studies is due to the LPS dose and analytical method used. However, it is also worth noting the high baseline values of IFN-γ for all steers in the current study as these could be the result of a chronic overproduction and insensitivity mechanism occurring (Cordeiro et al., 2022). This theory requires further investigation, but it could ultimately be contributing to the lack of IFN-γ response to LPS.

Nonetheless, there were responses for both IP-10 and MIG following LPS administration such that IP-10 increased from 1 h to 8 h before dropping off at 12 h and 24 h while MIG peaked between 4 h and 8 h Post-LPS. Under inflammatory conditions such as an LPS challenge, IP-10 and MIG have been reported to increase as they are

released by neutrophils in response to primarily IFN-γ, but also IFN-α, TNF-α, IL-1β, and/or LPS (Gasperini et al., 1999; Karlsson et al., 2015; Metzemaekers et al., 2018). Both, IP-10 and MIG are known to aid in the progression of the immune response via recruitment of adaptive immune cells (Gasperini et al., 1999). In contrast, IL-36Ra is known for its anti-inflammatory properties as it inhibits the LPS-induced release of pro-inflammatory cytokines (van de Veerdonk et al., 2012). While the treatment by time interaction revealed that there were no significant differences in IL-36Ra concentrations between any specific treatment by timepoint comparisons, in general, IL-36Ra concentrations were found to be greater at 1 h compared to 24 h. These results suggest that IL-36Ra may act early in the LPS response to mitigate the chance of a hyperinflammatory reaction. Additionally, there was a slight increase in IP-10 and a greater response in MIG, likely induced in part by cytokines other than IFN-γ, given the lack of an IFN-γ peak response in this study.

***Cortisol***

At the initiation of the challenge period, cortisol values averaged 9.68 ng/mL at -3 h but then dropped to 5.04 ng/mL or less prior to LPS administration. It is likely that the initial increased concentration was due to the removal of the steers from their pens into the working facility, but then diminished as they became acclimated. Nonetheless, research suggests that baseline cortisol levels are around 10 ng/mL under non-stressful conditions while normal handling increases cortisol to 45 ng/mL, and extreme stress is associated with concentrations greater than 70 ng/mL (Grandin, 1997). Thus, with the relatively low values observed in the current study, and the consistency in which the

steers were handled for each collection, there is little reason to believe that the cortisol values reported in this study were influenced by handling of the steers.

Following LPS administration, cortisol values spiked between 0 h and 1 h with peak values occurring around 3 h and a return to baseline values by 8 h Post-LPS for all steers. Other studies have reported similar trends in the cortisol response to LPS, albeit with greater overall cortisol concentrations than those reported here (Carroll et al., 2009b; Burdick Sanchez et al., 2020; Carroll et al., 2021). When evaluating the impact of probiotics on the cortisol response to LPS, cattle supplemented with yeast had lower cortisol concentrations (Sanchez et al., 2013; Finck et al., 2014) whereas LAFP supplemented pigs exhibited no differences (Lee et al., 2016) compared to controls. In Burdick Sanchez et al. (2020), it was noted that SCFP supplemented steers had a cortisol response that was of greater magnitude but shorter duration compared to controls. These findings are important given cortisol's role in the immune response. Initially, cortisol functions in support of a pro-inflammatory response, but upon its continued elevation, cortisol begins working to prevent a hyperinflammatory state (Roth, 1985; Chinenov and Rogatsky, 2007).

***Glucose***

Aside from its role in the up- and down-regulation of the immune response, cortisol also functions to increase energy availability to the immune system so that it can mount an adequate response (Lang et al., 1985). Specifically, cortisol is a glucocorticoid which functions to increase circulating glucose by releasing glycogen stores as well as stimulating gluconeogenesis in the liver (Long et al., 1940; Kuo et al., 2015). After the administration of LPS in the current study, glucose initially increased, peaking between

1-2 h, followed by a significant drop in concentrations by 3 h which was then followed by a slow return to baseline values by 24 h. In general, the initial increase in glucose was likely attributed in part to the increasing cortisol concentrations, which worked to increase circulating glucose via mobilization of glycogen stores and gluconeogenesis. This increase, however, was only temporary as glucose was utilized by the immune response faster than it was being produced, resulting in the reduction in circulating glucose concentrations noted at 3 h. By 4 h, there was some restoration of circulating glucose levels likely due to glycogenolysis and/or gluconeogenesis, but the full return to baseline values did not occur until 12 to 24 h as the energy demands of the immune system (e.g., febrile response) had lessened.

Moreover, research suggests that even a 1ºC increase in body temperature requires 10-13% more metabolizable energy to sustain (Kluger and Rothenburg, 1979; Huntley et al., 2017) which is supported by the high glucose demands of an LPS response reported in cattle by Kvidera et al. (2017). Therefore, even the small differences in baseline RT (approx. 0.47ºC) reported in this study, could be biologically significant from an energy availability standpoint as suggested by Burdick Sanchez et al. (2020). Prior to LPS, glucose concentrations in the current study were numerically greater in the CREEP and LAFP steers compared to the CONTROL. These greater baseline glucose concentrations, likely due in part to the higher energy content of their diet (i.e., creep feed), may have supported the greater RT noted for CREEP and LAFP steers Pre-LPS. Similarly, while there were no significant differences in glucose concentrations across dietary treatments following LPS, the CREEP and LAFP steers did have numerically greater circulating concentrations of glucose at 1 h compared to CONTROL steers (83

and 81 vs 71 mg/dL). It is, therefore, plausible that these lower glucose values at 1 h for the CONTROL steers were not only related to their lower baseline concentrations but also a sign of less abundant glycogen stores readily available for mobilization.

***Conclusions***

Research evaluating the impacts of probiotics on immune function in cattle is lacking, yet it is considered highly valuable information to the industry. Therefore, the present study aimed to examine the effects of LAFP supplementation on the performance and acute phase immune response of recently weaned beef steers using LPS to mimic an acute bacterial infection. Data generated in this study indicated that the LPS challenge model employed was successful given the changes documented in challenge markers (i.e., cytokines, cortisol, glucose, RT) following LPS administration; however, there were no significant differences in any of these responses to LPS across the different dietary treatments indicating that all steers responded similarly to an immune challenge regardless of pre- and post-weaning grain supplementation with or without the addition of LAFP. Nonetheless, this study provides the cattle industry with crucial information regarding the impacts of pre-weaning dietary management and LAFP supplementation on receiving steer performance and immune function.

**Table 4.1. Chemical composition[1] of the creep feed offered pre- and post-weaning to steers[2]**

| Nutrient, % Dry Matter | CREEP | LAFP |
|---|---|---|
| Dry Matter, % | 88.0 | 87.4 |
| Crude Protein, % | 14.0 | 14.3 |
| Non-Fiber Carbohydrates, % | 42.8 | 41.2 |
| Neutral Detergent Fiber, % | 36.6 | 37.6 |
| Acid Detergent Fiber, % | 19.8 | 21.1 |
| Lignin, % | 3.29 | 3.59 |
| Crude Fat, % | 3.17 | 3.05 |
| Ash, % | 5.85 | 6.46 |
| Calcium, % | 0.63 | 0.63 |
| Phosphorus, % | 0.53 | 0.58 |
| Potassium, % | 1.24 | 1.35 |
| Magnesium, % | 0.37 | 0.39 |
| Sodium, % | 0.16 | 0.17 |
| Iron, ppm | 247 | 216 |
| Manganese, ppm | 51 | 54 |
| Zinc, ppm | 72 | 78 |
| Copper, ppm | 18 | 23 |
| $NE_m$, mcal/kg | 1.79 | 1.76 |
| $NE_g$, mcal/kg | 1.17 | 1.15 |

[1] Analyzed by Cumberland Valley Analytical Services (Waynesboro, PA)
[2] ppm = parts per million, $NE_m$ = net energy for maintenance, $NE_g$ = net energy for gain, mcal/kg = megacalories per kilogram

**Table 4.2. Steer weight and age parameters recorded upon arrival (weaning day), prior to (Pre-LPS) and in response to (Post-LPS) an intravenous LPS challenge (0 h) on day 4 post-weaning[1]**

| Trait | CONTROL | CREEP | LAFP | Trt *P*-value |
|---|---|---|---|---|
| Weight, kg | | | | |
| Arrival | 292.6 ± 6.57 | 298.5 ± 9.60 | 292.8 ± 7.59 | 0.85 |
| 3 h Pre-LPS | 291.9 ± 6.34 | 303.3 ± 9.84 | 297.6 ± 8.02 | 0.62 |
| 12 h Post-LPS | 284.5 ± 6.74 | 300.1 ± 10.19 | 293.9 ± 8.95 | 0.45 |
| 24 h Post-LPS | 286.9 ± 6.80 | 301.9 ± 10.59 | 294.4 ± 8.97 | 0.49 |
| Age at Arrival, d | 251 ± 5.7 | 243 ± 4.9 | 245 ± 4.4 | 0.51 |

[1] LPS = Lipopolysaccharide; Trt = Treatment
Results are presented as Mean ± SE

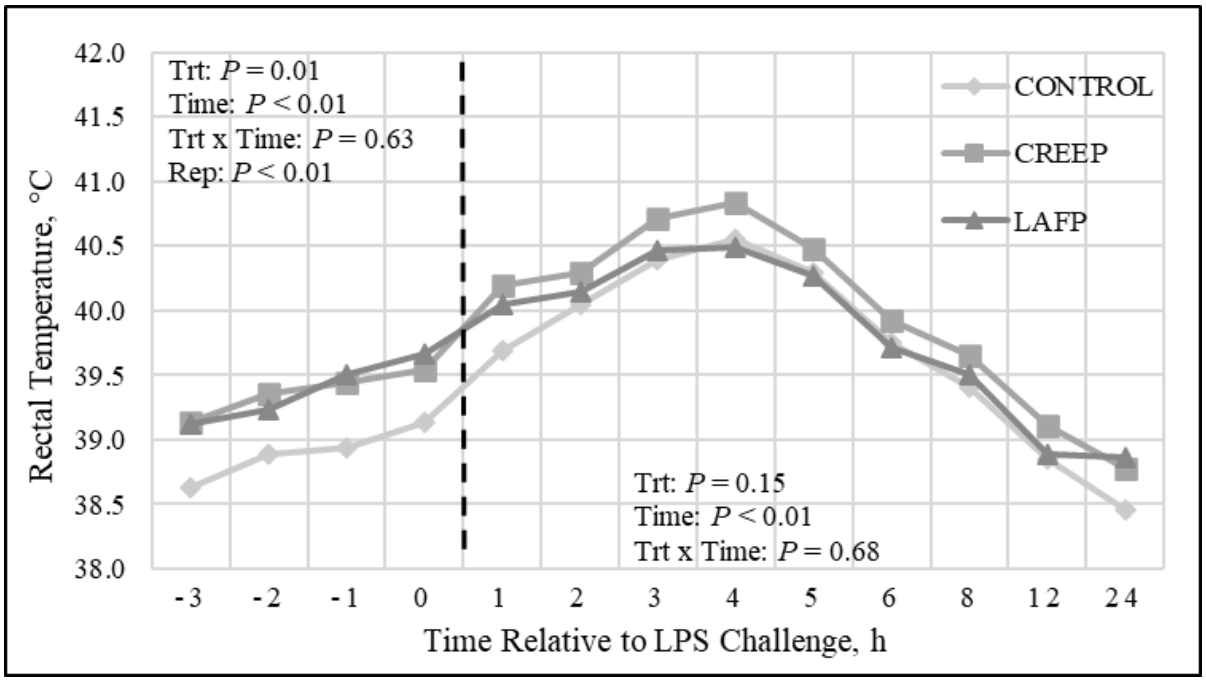


**Fig. 4.1 Effects of pre- and post-weaning dietary treatment on rectal temperature (RT) prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

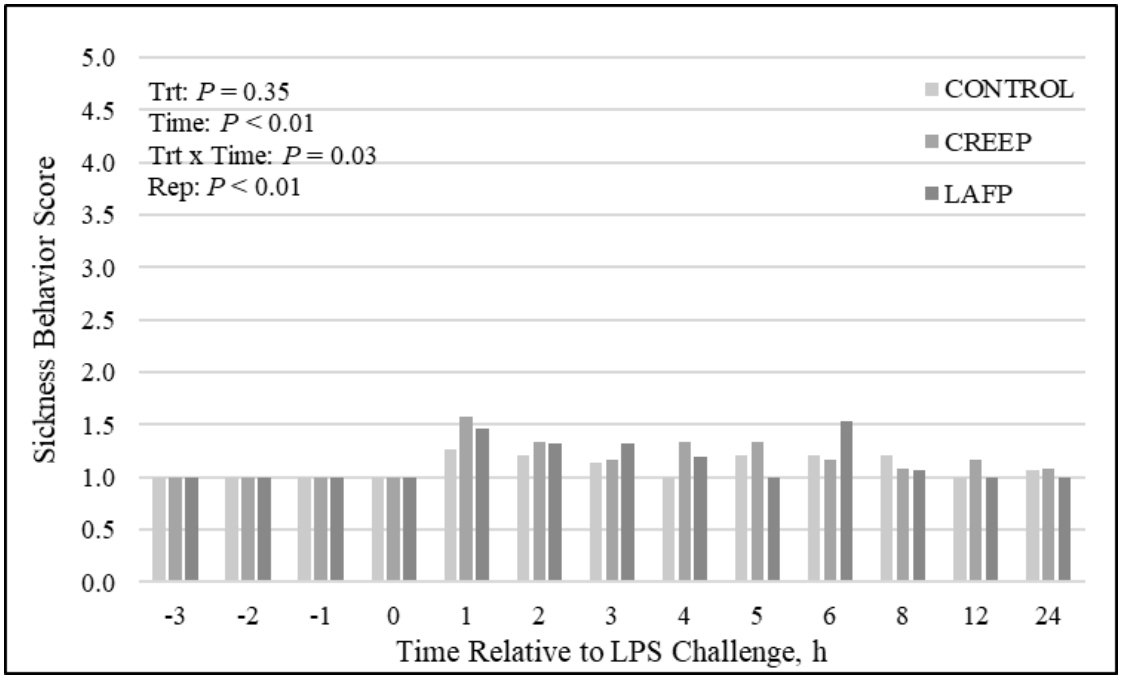


**Fig. 4.2 Effects of pre- and post-weaning dietary treatment on sickness behavior scores (SBS) prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added.**

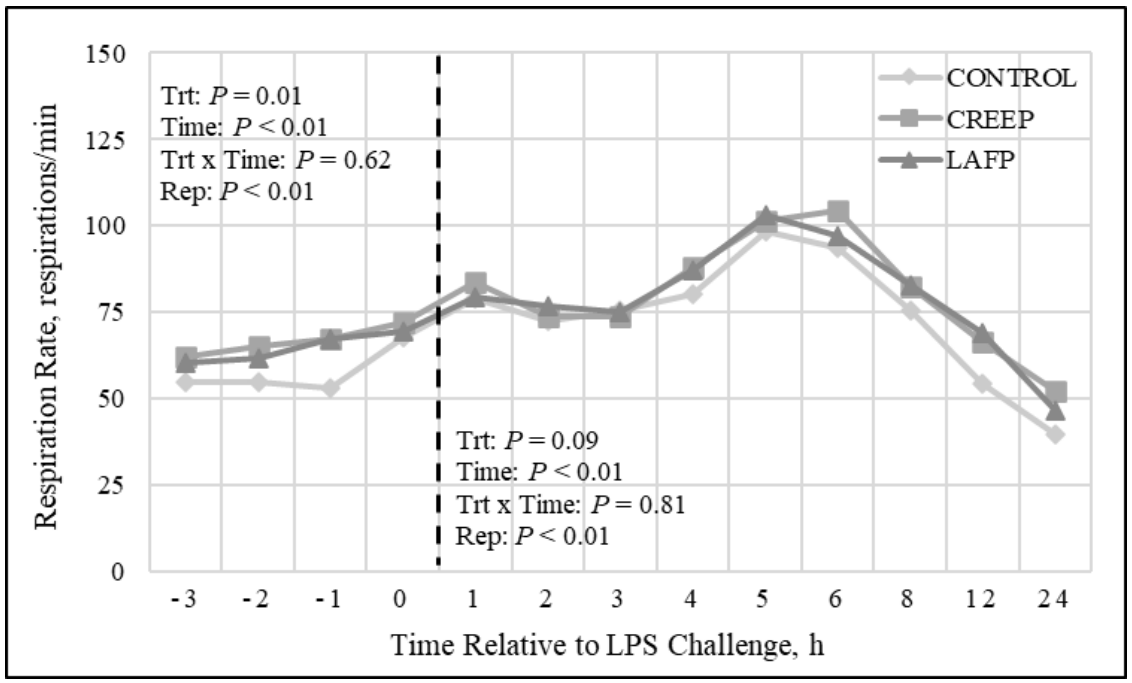


**Fig. 4.3 Effects of pre- and post-weaning dietary treatment on respiration rates (RR) prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

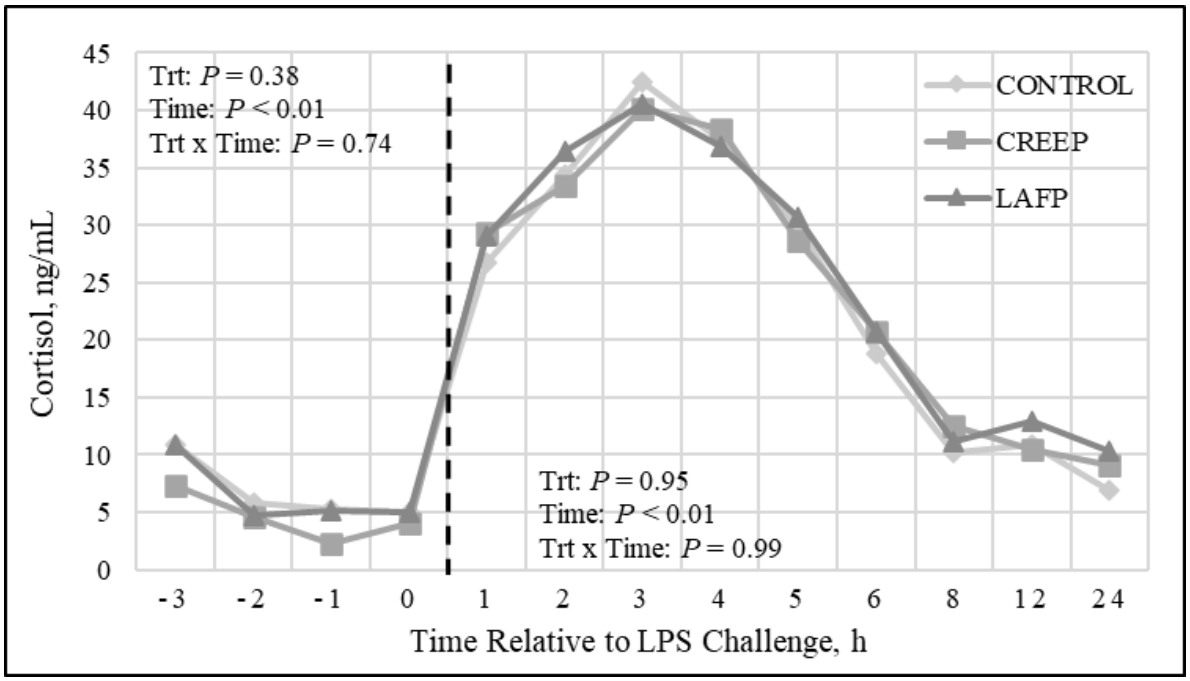


**Fig. 4.4 Effects of pre- and post-weaning dietary treatment on cortisol concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

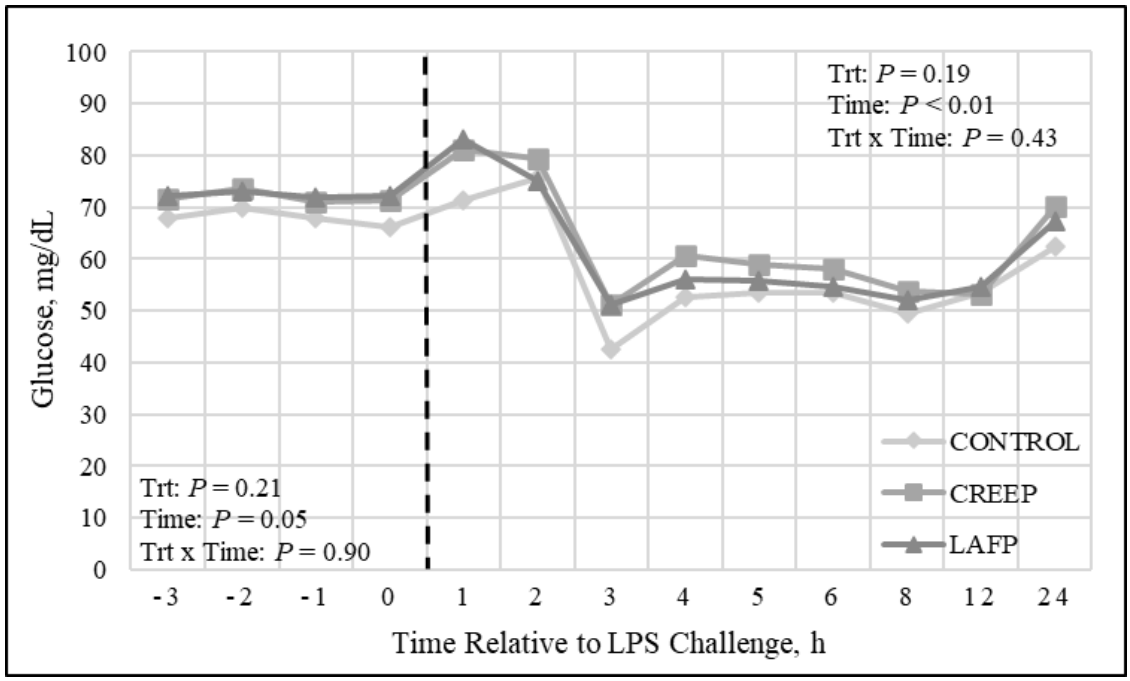


**Fig. 4.5 Effects of pre- and post-weaning dietary treatment on glucose concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

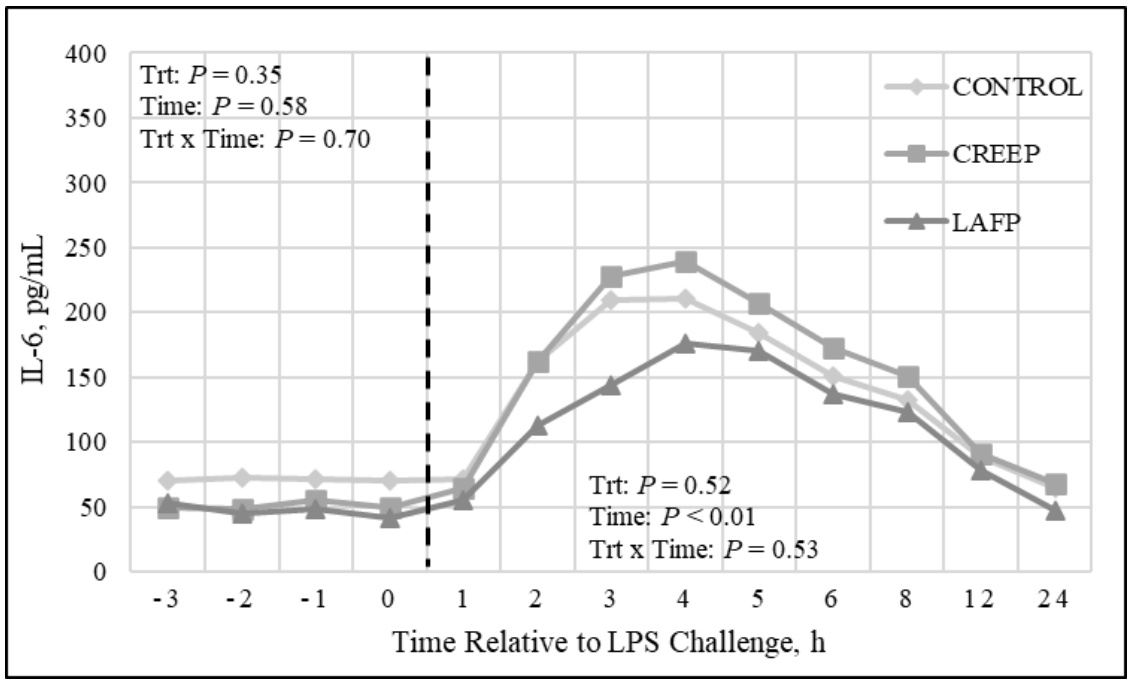


**Fig. 4.6 Effects of pre- and post-weaning dietary treatment on interleukin-6 (IL-6) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

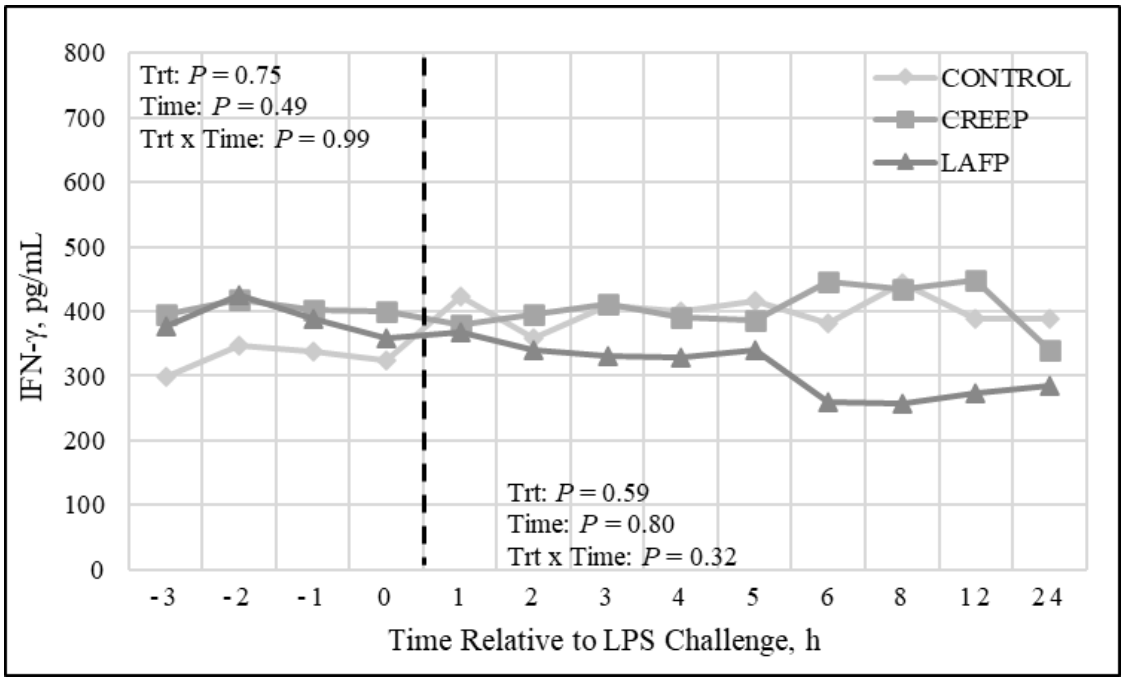


**Fig. 4.7 Effects of pre- and post-weaning dietary treatment on interferon-gamma (IFN-γ) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

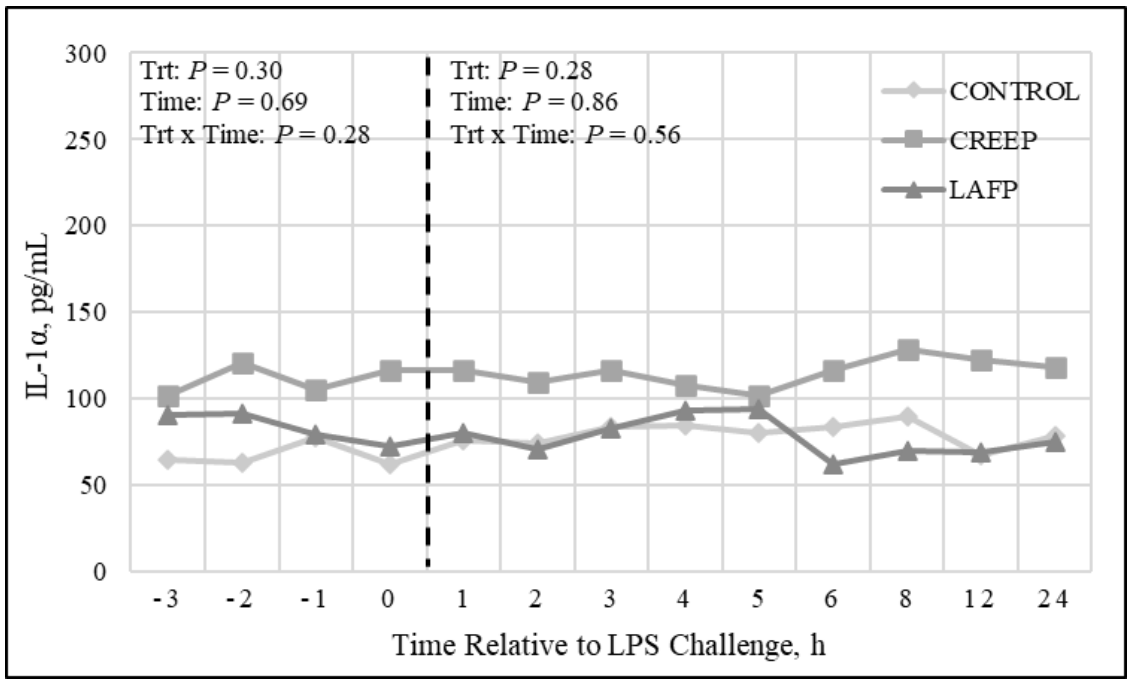


**Fig. 4.8 Effects of pre- and post-weaning dietary treatment on interleukin-1alpha (IL-1α) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

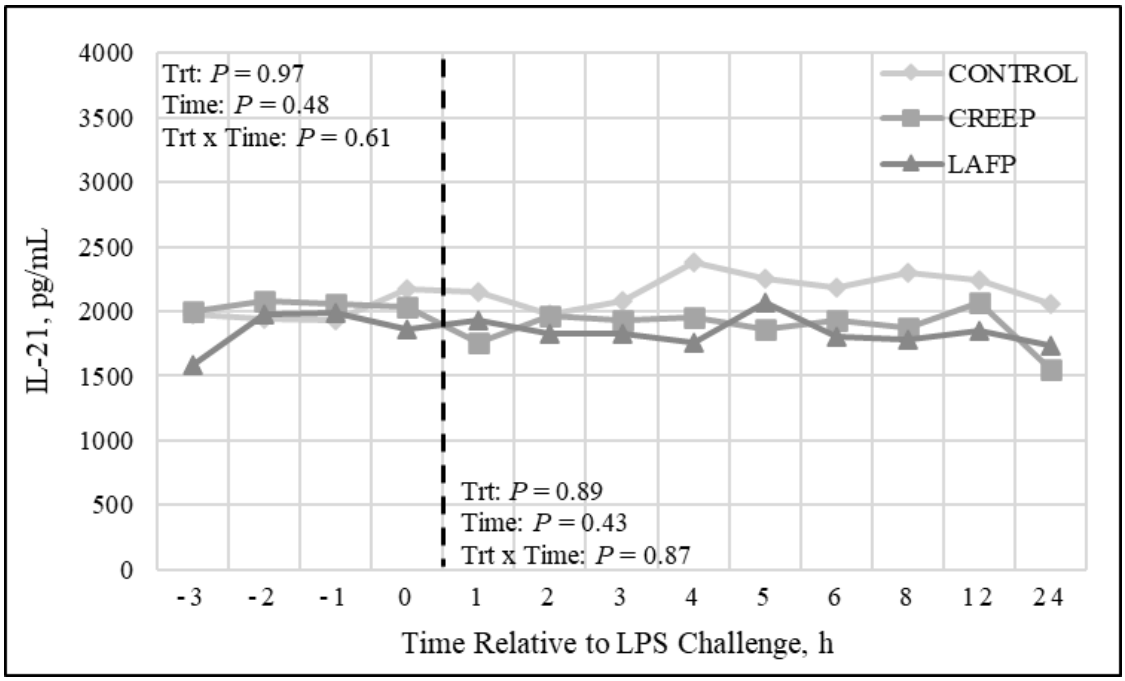


**Fig. 4.9 Effects of pre- and post-weaning dietary treatment on interleukin-21 (IL-21) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

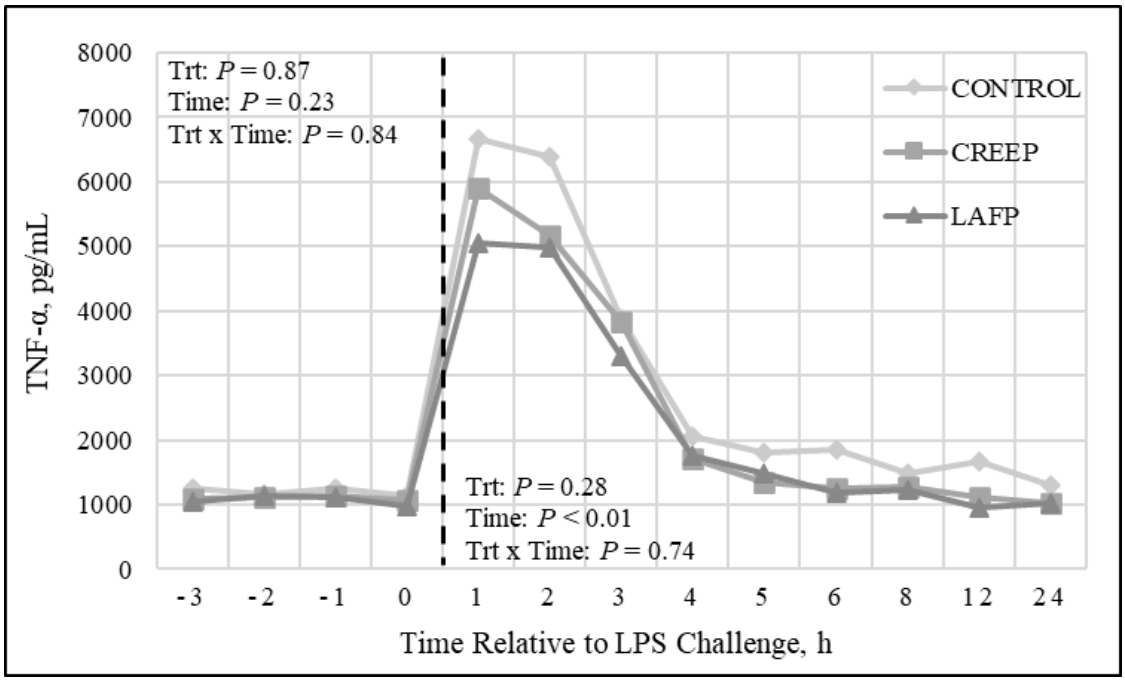


**Fig. 4.10 Effects of pre- and post-weaning dietary treatment on tumor necrosis factor-alpha (TNF-α) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

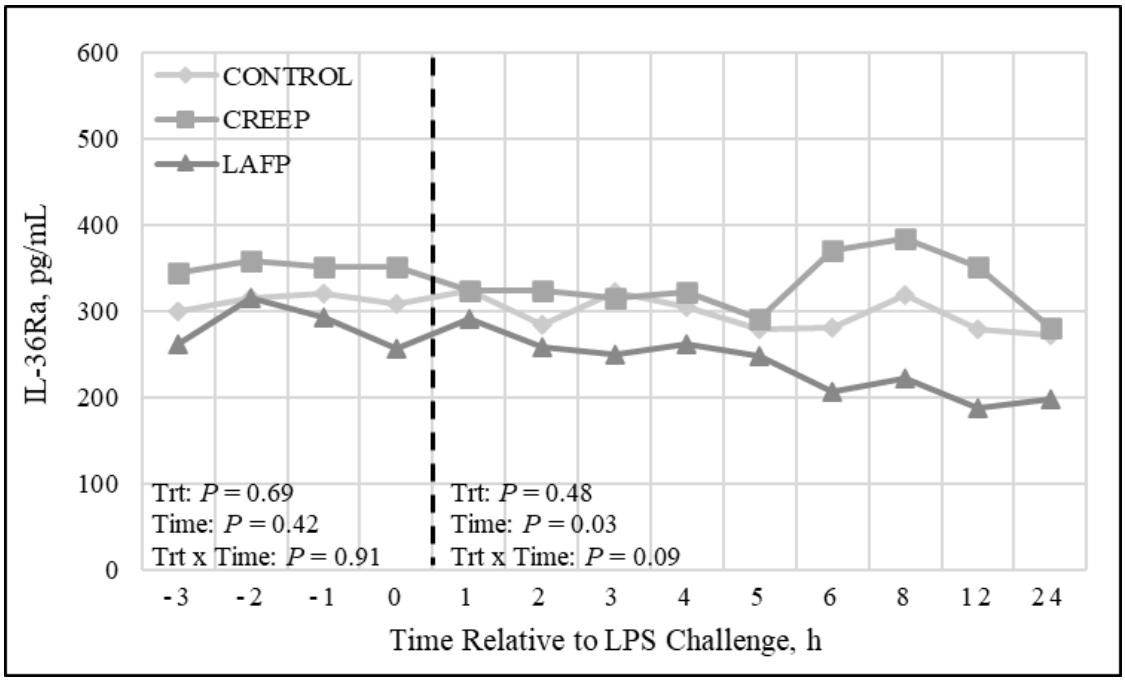


**Fig. 4.11 Effects of pre- and post-weaning dietary treatment on interleukin-36 receptor agonist (IL-36Ra) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

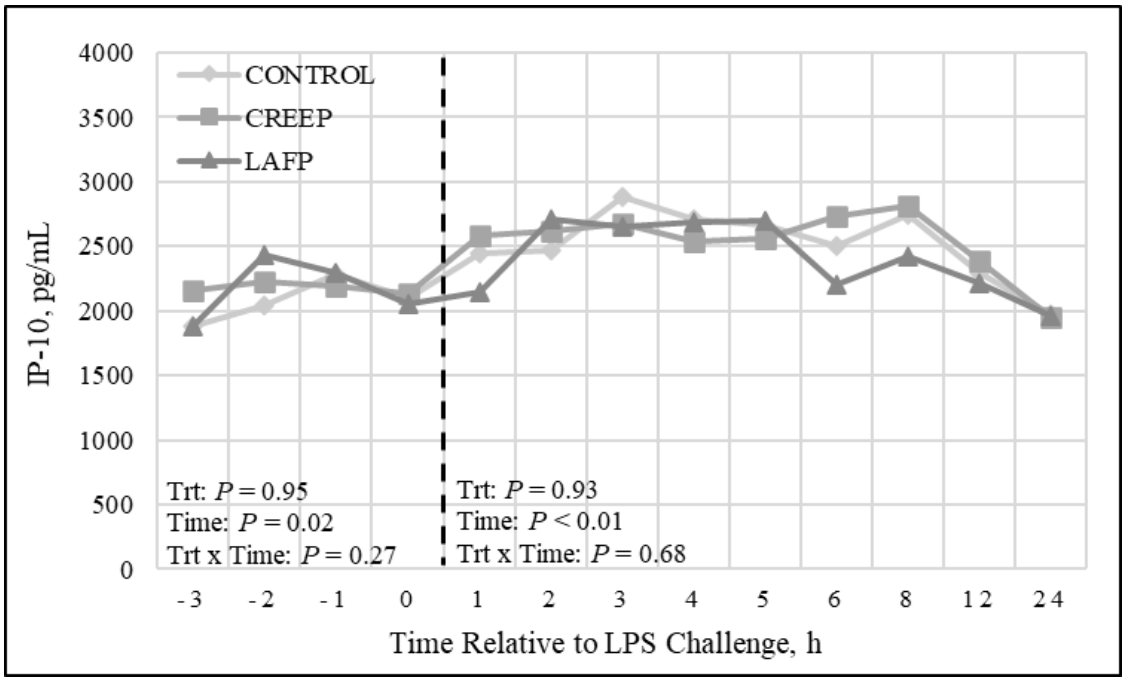


**Fig. 4.12 Effects of pre- and post-weaning dietary treatment on interferon-gamma induced protein-10 (IP-10) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

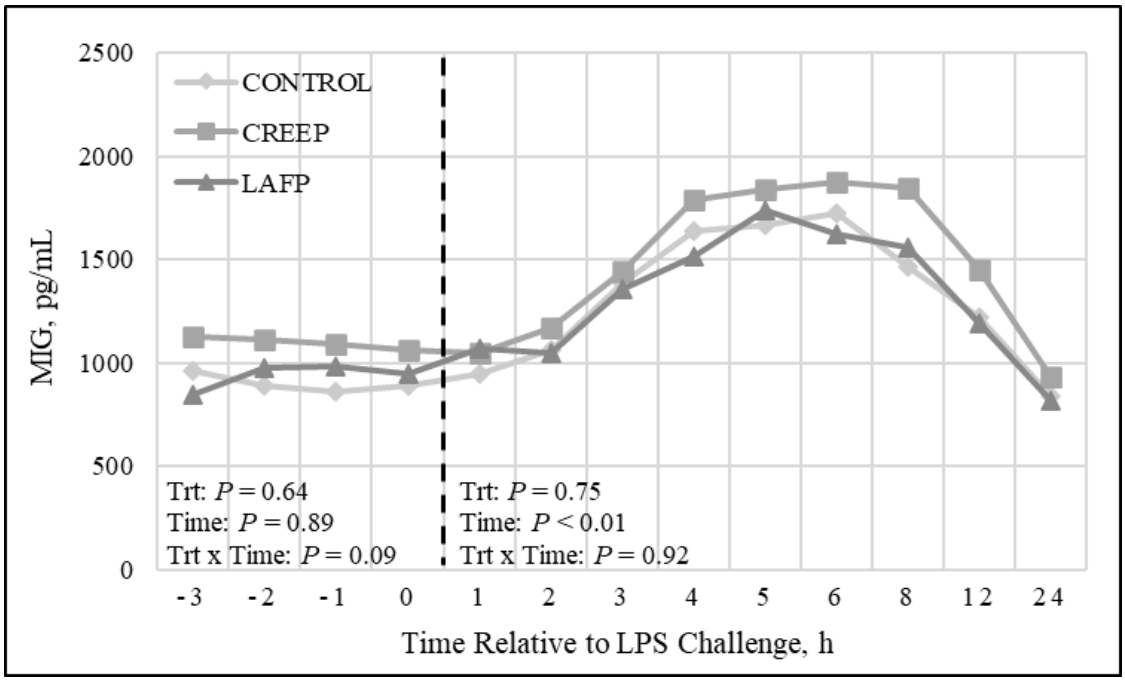


**Fig. 4.13 Effects of pre- and post-weaning dietary treatment on monokine induced by interferon-gamma (MIG) concentrations prior to and in response to an intravenous lipopolysaccharide (LPS) challenge at 0 h on day 4 post-weaning for Angus steers. CONTROL = steers on pasture. CREEP = steers on pasture and given creep feed. LAFP = steers on pasture and given creep feed with 5 g/hd/d of a *Lactobacillus acidophilus* fermentation product added. Data were analyzed separately as Pre-LPS (i.e., -3 h, -2 h, -1 h, 0 h) and Post-LPS (i.e., 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h) periods.**

# CHAPTER 5

# CONCLUSIONS

Due to the increasing concerns of consumers regarding antibiotic use in cattle production, the need for natural alternatives such as microbial supplements continues to grow. Producers are specifically in need of alternatives that can be used to maintain cattle health and performance during times of stress, like the weaning and receiving period, when immune function is often compromised and the cattle are being introduced into new environments with new pathogen challenges. Therefore, the goal of this research was to investigate the feasibility and efficacy of supplementing beef steers with a LAFP microbial supplement prior to weaning as a means to better prepare the steers for weaning stress and a subsequent immune challenge. During the pre-weaning period, steers were evaluated for growth and feed intake performance along with gastrointestinal VFA profiles to explore the impacts of the LAFP on economically important traits for producers and the energy availability of the steers. At weaning, the steers were transported to a new facility and then subjected to an LPS immune challenge four days later. Thus, this part of the study was designed to evaluate the impacts of LAFP supplementation on the immune response of newly weaned beef steers that were challenged with an acute bacterial infection shortly after arrival at a new facility under stressful conditions.

In general, results from the first study revealed a tendency for improved steer growth with creep feed supplementation, but also that creep fed steers had slightly greater

backfat deposition likely due to the greater energy content of their diet and the fact that they spent less time eating and ruminating and more time being inactive. When adding LAFP to the creep feed, there was a tendency for improvements in supplemental feed efficiency for the LAFP steers as they maintained similar growth rates as the CREEP steers while consuming 0.35 kg/hd/d less creep feed, on average. Even while consuming less creep feed, the LAFP steers had greater VFA concentrations, specifically propionate, throughout their gastrointestinal tract which may have contributed to the greater efficiency noted. However, further investigation into the effects of LAFP supplementation on gastrointestinal fermentation pathways to determine the mechanisms by which altered VFA profiles may be impacting animal performance is warranted.

Following weaning, baseline RT and RR values were greater in the creep fed steers compared to the non-creep fed steers, which could be related to the greater energy content of their diet, allowing creep fed steers to maintain a slightly elevated baseline body temperature. During the immune challenge, there were time effects noted for most of the immune markers, indicating that the immune system was stimulated by the LPS; however, there were no significant differences in these responses across the different dietary treatments which indicates that all steers responded similarly to the immune challenge regardless of whether or not they received pre- and post-weaning grain supplementation, with or without the addition of the LAFP microbial supplement.

In the end, these results provide cattle producers with crucial information regarding the impacts of dietary management and microbial supplementation on economically important steer performance traits and immune function in response to an immune challenge. Moreover, these results help to fill some of the gaps in knowledge

regarding the emerging market of natural alternatives and the impacts that they have on cattle production.

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