Evaluation of dietary additives to improve feedlot cattle health and performance

by

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ABSTRACT

The study objective was to determine the effects of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on health, performance, serum chemistry and complete blood count variables, and fecal *Salmonella* spp. count in high-risk beef cattle during a 56-day feedlot receiving period and the subsequent finishing period. Four truckload blocks of crossbred beef bulls (n = 300) and steers (n=84; BW = 220 ± 16.2 kg) were sourced from regional auction markets and assigned randomly to treatments arranged in a 2 × 2 factorial. The generalized complete block design consisted of 3 pen replications per treatment within block, totaling 12 pen replications per treatment (24 pen replications per main effect) with pen as the experimental unit. Treatments were: 1) placebo control (CON); 2) 13 g/animal/d of *Bacillus subtilis* PB6 (CST); 3) 450 ppb DM chromium propionate (CHR); and 4) 13 g/animal/d of *Bacillus subtilis* PB6 and 450 ppb DM chromium propionate (CST + CHR). Treatments were top dressed in feed bunks daily using 0.45 kg/animal/d ground corn carrier immediately following feed delivery. Cattle fed CON received an equivalent amount of ground corn only. Feedlot personnel were blinded to experimental treatments by assignment of color codes to treatment pens and ear tags. Blood samples were collected via jugular venipuncture on days 0, 14, 28, and 56 for analysis of serum chemistry and complete blood count (CBC) variables from a pen subset (n = 2/pen). Additional blood samples were collected for CBC analysis every subsequent 28 d following the end of the receiving period (d 56). Data were analyzed

using mixed models. Repeated measures analysis was used for blood variables and salmonella counts. During the receiving period, DMI was increased ($P \leq 0.03$) for CST during each interim period and overall receiving period DMI was 0.35 kg/animal/d greater for CST ($P = 0.01$). The CST supplemented cattle had greater ($P \leq 0.06$) BW on days 14, 28, and 56. Likewise, ADG was improved for CST from day 0 to 14 ($P = 0.04$) and for the overall receiving period (day 0 to 56; $P = 0.04$). During the finishing period, CHR lowered ($P = 0.02$) final BW and ADG during the finishing period (day 56 to final; $P = 0.01$) and the entire feeding period (day 0 to final; $P = 0.03$). In addition, CHR fed cattle had lower ($P = 0.02$) DMI from d 224 to final. Main effects of both CST ($P = 0.02$) and CHR ($P = 0.03$) were observed to lower the overall percentage of calves treated for bovine respiratory disease (BRD). Morbidity was 28.0 and 39.9% for cattle with and without CST supplementation, respectively. Similarly, BRD morbidity was 28.6 and 39.3% for cattle with and without CHR supplementation, respectively. Cattle fed CHR had greater total leukocyte count ($P = 0.04$) and neutrophil count ($P = 0.02$) during the receiving period. In addition, cattle fed CHR had greater serum aspartate aminotransferase concentration ($P = 0.04$) and less ($P = 0.02$) serum calcium. Day affected ($P \leq 0.03$) all serum chemistry variables evaluated except total CO_2 ($P = 0.34$). During the finishing period, percent monocyte concentration was greatest in CST, with CON and CHR being intermediate and CST + CHR being the least (CST × CHR $P = 0.03$). Day affects during the finishing period were observed for hemoglobin, hematocrit, mean corpuscular volume, platelet count, neutrophil count, lymphocyte count and percent, monocyte count and percent, and neutrophil:lymphocyte ($P \leq 0.04$). Hot carcass weight decreased ($P = 0.01$) in cattle fed CHR. An interaction of CST × CHR existed

such that percentage of YG1 ($P < 0.01$) carcasses was greater in cattle fed CST and CHR, with CST + CHR being intermediate and CON being the least. Further, a CST × CHR interaction was observed among percent YG2 ($P = 0.03$) carcasses where numerically, CON and CST + CHR had a greater percentage. Interaction of CST × CHR ($P = 0.05$) was observed to increase percentage of YG 4 or 5 carcasses among CST and CHR, with CON being intermediate and CST + CHR being the least. Percentage of edible livers tended to increase (CST × CHR; $P = 0.08$) numerically in the CST treatment. Cattle fed CST had lower ($P = 0.07$) overall fecal *Salmonella* spp. count as well as during week 4 ($P = 0.01$). Feed intake and performance outcomes during the receiving period were improved by CST, but not CHR supplementation. However, both CST and CHR supplementation decreased BRD morbidity rate. During the finishing period, performance and HCW was reduced in cattle supplemented with CHR.

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"Maybe that's enlightenment enough – to know that there is no final resting place of the mind, no moment of smug clarity. Perhaps wisdom is realizing how small I am, and unwise, and how far I have yet to go." –Anthony Bourdain

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

REVIEW OF LITERATURE

Probiotics

Classification

A robust and diverse microbial population is the foundation of a healthy ecosystem, as demonstrated by the human gastrointestinal tract, which is populated by approximately 100 trillion microbes across over 1,000 species and 7,000 strains, which collectively weigh 1 to 2 kg and outnumber human body cells 1.3:1 by most recent estimates (Quigley, 2013; Wiley et al., 2017). In comparison, the rumen of a mature dairy cow can house a liquid volume of 60 to 100 liters (Wallace and Newbold, 1992) and the bacterial population is 10^{10} cells per gram of rumen content (Russell, 2002).

As described by Fuller (1989), a probiotic can be defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" and may include live bacteria or yeast, culture extracts, enzyme preparations, or any combination thereof (Krehbiel et al., 2003). In young ruminant animals, probiotics may be fed with the intention of preventing diarrhea or to promote development of digestive function (Wallace and Newbold, 1992). In adult ruminants and specifically beef cattle, the goals of probiotic use are to stabilize fermentation, promote weight gain, improve feed efficiency, reduce acidosis, and/or modify an immune response. Therefore, probiotic use should improve the health and performance of the animal and mitigate shedding of pathogens that may be harmful to animals or humans

(Fuller, 1992a; Wallace and Newbold, 1992; Chaucheyras-Durand and Durand, 2009). Microbially-derived feed additives, including probiotics, which are intended to be fed to livestock are referred to as a direct-fed microbial (DFM). Label and promotional marketing claims of these products are not supported by the United States Food & Drug Administration (FDA); however, the organisms contained within the product are generally recognized as safe (GRAS) by the FDA. For regulatory purposes, DFM may be classified under Section 36, Fermentation Products, or Section 96, Yeast, of The Official Publication of the Association of American Feed Control Officials (AAFCO) and are defined as "feed products that contain only a source of live or naturally occurring microorganisms" (Brashears et al., 2005; FDA, 2015).

History and development

As early as the 1920's, yeast and their associated by-products have been included in ruminant diets as a source of energy and protein (Eckles and Williams, 1925; Wallace and Newbold, 1992). Although living microorganisms have been employed for their fermentation abilities as a method of food preservation for thousands of years, the term 'probiotic' as it relates to dietary supplements for livestock as we are familiar with today did not coalesce in the literature until the mid 1970's (Parker, 1974; Fuller, 1992a). Since the 1980's, many products have been developed and marketed with the intent of enhancing gut function, including yeast, filamentous fungi, bacteria, and stabilized rumen extract products or conditioners. These products may be administered to the animal in a variety of fashions, such as an oral drench, encapsulated bolus, as a feed additive, or top dressed at the time of feed delivery (Wallace and Newbold, 1992). Research by Harris et

al. (1983) evaluated a fungal feed additive (*Aspergillus oryzae)* as an inoculant in silage, demonstrating non-animal use of a probiotic that is common today.

Among these systems, viability of the microbes is of paramount importance to quality assurance and probiotic efficacy. Inconsistencies between product specifications and the final product have been documented. Gilliland and Speck (1977) analyzed the microbial content of probiotic supplements advertising inclusion of *Lactobacilli acidophilus*. Of the 15 probiotic products evaluated, only 7 were found to contain *Lactobacilli* of any species. Of those 7, only 3 contained *L. acidophilus*. Further, microbial metabolism and ability to attach to the gut wall varies even between strains of the same species, which may alter intended results (Fuller, 1992b). In a more modern perspective, companies which manufacture DFM products are able to ensure survivability of the microbes and the resulting colony forming units (CFU) by screening for hardiness when the cultures are grown. For storage, live cultures are freeze dried to reduce water activity. As they are introduced to the GIT of the animal, the cultures rehydrate and resume function. The inclusion rate of cultures within the product are typically over-formulated to provide a two-year shelf life. As a result, said product will contain the specified concentration of CFU, even if some microbial death occurs during storage (Cravey, 2018).

Probiotics in ruminants

The survival of a probiotic organism within the rumen environment is predicated upon its ability to evade the naturally occurring antimicrobial effects of the rumen, such as peristalsis, desquamation, predatory species, and the effects of secretory IgA. In

addition, imbalance may further occur due to overgrowth of pathogenic species or damage to the epithelial lining of the gastrointestinal tract, subsequently initiating an inflammatory response in the host. Organisms can overcome these challenges by associating with the gut wall or by proliferating more rapidly than the pace at which they are flushed out by peristalsis (Drisko et al., 2003).

Therefore, as described by Drisko et al. (2003), to meet the qualifications of a beneficial microbial species the organism must be resistant to the characteristically low pH of the GIT, must remain metabolically active within the gastrointestinal tract, must be able to associate with the wall of the gastrointestinal tract, must display an inhibitory effect against pathogenic bacteria, and must reduce colon pH (Drisko et al., 2003). Further, a probiotic organism developed with the intention of being included as a feed additive for livestock should be time- and heat-stable to remain viable throughout storage and the process of mixing the diet (Cravey, 2018).

Mode of action: general overview

Administration of probiotics may be appropriate in a variety of circumstances in which the host's existing microflora have been disrupted due to stress, disease, administration of antimicrobial drugs, or any combination thereof (McFarland, 2000). When the competency of the existing microbial population is compromised, the host is left susceptible to further infection by way of occupation by pathogenic bacteria, immunosuppression, and decreased metabolic activity (Fuller, 1989; Ohashi and Ushida, 2009).

Replenishing the microbial population by introducing exogenous microorganisms is a complicated task, due to the broad and complex nature of the existing microbial population. The fetal gastrointestinal tract is sterile and begins to develop a microbial load upon initiation of passage through the birth canal. As the neonate encounters its environment and begins feeding, the microbial population of the gut is enhanced and diversified. Therefore, the host's microbial population is a unique result of its immediate environment (Fuller, 1989; McFarland, 2000; Drisko et al., 2003).

Probacterial intervention of the GIT generally fall under the following classifications: 1) probiotics, the introduction of a species to the existing population which is "normal" or naturally occurring within the host, or 2) prebiotics, the introduction of a substrate which is not directly utilized by the host, but rather encourages beneficial host microorganisms to expand their population (Callaway et al., 2004). Within the realm of probiotics, the mode of action varies from species to species. Some species hydrolyze toxins produced by pathogenic bacteria or produce antimicrobial peptides as part of the innate immune response, such as bacteriocins, which limit the ability of pathogenic bacteria to grow and reproduce. Other strains exclude pathogenic species from propagating by competitively populating adhesion sites or dominating the available nutrient supply. Further, similar to that of a prebiotic, some beneficial microbes stimulate the growth of other beneficial microbes by producing necessary nutrients and growth factors (Chaucheyras-Durand and Durand, 2009).

Mode of action: yeast & others

Live yeast and yeast cell wall supplements, such as mannan and β-glucan, are widely available for use in livestock production and have been proposed to modulate immune function and gut motility in feedlot cattle, leading to improved feedlot performance and reduce morbidity and mortality events (Newbold et al., 1995; Callaway and Martin, 1997; Medzhitov and Janeway, 2000; Broadway et al., 2015; Word, 2018). There are many types of yeast DFM products and therefore many varying mechanisms of action. For example, the immunomodulatory effects of β-glucan reportedly vary according to helical conformation, branching, polysaccharide chain length, and molecular weight. Further, live yeast products may bind directly to pathogenic bacteria without binding to epithelial cells, elucidating that they do not disturb host cells directly. Alteration of ruminal fermentation has been frequently reported in the literature as live yeast products and their derivatives provide soluble growth factors such as organic acids, B vitamins, and amino acids. In turn, growth of ruminal bacteria is stimulated, and the fermentative capacity of lactate-utilizing cellulolytic bacteria is enhanced. In some cases, the population of cellulolytic bacteria and/or total rumen population were increased. Greater DMI is also commonly reported (Phillips and VonTungeln, 1985; Cole et al., 1992; Keyser et al., 2007; Finck et al., 2014; Word, 2018). However, improvement among morbidity and mortality has been inconsistent.

Limitations

Direct fed microbials yield a variety of results under the influence of countless different environments in which they inhabit. Incidentally, there is an apparent

inconsistency in response largely due to the variability between test subjects. This may include differences within the rumen environment itself and interactions with the other gut microflora or the host; all of which are heavily influenced by the host's environment. For example, research by Pollman et al. (1980) found the response to dosing DFM to be greater when supplemented to starter pigs compared to grower-finisher pigs. The gut of any animal is an ever-changing environment that constantly undergoes microorganism turnover, whether they are an introduced or indigenous species. Furthermore, although the gross count of any particular species may remain relatively constant throughout the life of the animal, the dominant serotype of the species may change periodically. Therefore, it is unlikely that an exogenous organism would permanently colonize an introduced environment, as was demonstrated by Jonsson (1986) in pigs and Votava et al. (1987) in chickens (Fuller, 1992b).

The brain-gut-microbiota axis describes the extensive communication which occurs between the brain and GIT of a host and its microbiome. Current research compiled in a meta-analysis by Wiley et al. (2017) highlights the importance of the development of this pathway early in life as it is believed to play a role in physiological, emotional, behavioral, and cognitive development. Impairment of early-life gut assembly may negatively impact the host's long-term health and can lead to development of disorders such as inflammatory diseases. As the host and its endogenous microbiome share a symbiotic relationship, the hologenome theory of evolution points to genetic abundance and diversity of these symbionts to be a hallmark feature of thriving higher organisms. Therefore, facilitating and nurturing an optimal gut environment in production

animals can increase their productivity and assist the animal in mitigating the effects of stress and health insults.

The brain-gut-microbiome axis is comprised of the central nervous system (CNS), the neuroendocrine and neuroimmune systems, the autonomic nervous system, the enteric nervous system (ENS), and the intestinal microbes themselves. The gut and brain communicate via several pathways, including activation of sensory fibers of the vagus nerve, neuroimmune and neuroendocrine pathways, microbial metabolites, and microbially derived neurotransmitters. The predominant pathway among these are those associated with the vagus nerve (Wiley et al., 2017). Neurotransmitters and neuromodulators secreted by microbes, such as acetylcholine and catecholamines, can impact brain function and subsequent behavior by modulating neural signaling between the GIT and the ENS (Wall et al., 2014). This influence on behavior is particularly mediated by gamma-aminobutyric acid (GABA) producing bacteria, such as *Lactobacillus* and *Bifidobacterium*, which release large milligram quantities of GABA as part of the stress response (Wiley et al., 2017). As was demonstrated in mice by Asano et al. (2012), bacteria which comprise the typical murine microbiome are capable of producing large quantities of noradrenaline. Some bacteria, such as *Lactobacillus planatarum, Escherichia coli* JCM 5491, and *Bacillus subtilis* PCI 219 are capable of in vivo acetylcholine production (Horiuchi et al., 2003).

Activation of the hypothalamic-pituitary-adrenal (HPA) axis and production of cytokines is also recognized as a pathway for communication among the brain-gut-microbiome axis. Enterochromaffin cells associated with the epithelial lining of the gut lumen and immune cells associated with the lamina propria release signaling molecules

with which the brain communicates. Activation of the HPA axis, autonomic nervous system, and/or the neuroendocrine system initiates indirect communication which can alter gut motility, permeability, and secretion. Gut microbes can directly communicate with the brain via receptor-mediated signaling of epithelial cells and those of the lamina propria (Wiley et al., 2017). When the microbiome of the gut is altered to the point of affecting intestinal permeability via activation of the HPA axis, autonomic nervous system, or neuroendocrine system, the release of lipopolysaccharide (LPS) into the blood stimulates the CNS to increase activity in areas of the brain associated with emotional control, leading to the production of proinflammatory cytokines (Mangiola et al., 2016; Wiley et al., 2017).

Because specific microbial species have preferential substrates, the gut microbiome is heavily influenced by diet and is prone to alimentary stress (Wiley et al., 2017). Destrez et al. (2015) investigated this topic in horses transitioning from a diet high in fiber to another with low-fiber and high-starch. Behavioral cues of this alimentary stress were observed, as well as significant increases of colonic total anaerobic bacteria, lactate-utilizing bacteria, and amylolytic bacteria (Destrez et al., 2015). Changes in diet may not always elicit an observed behavioral response, as was demonstrated by Hammons et al. (2010) in two groups of broilers fed either a corn-soybean diet or a diet high in wheat middlings. Although these two diets did not greatly differ nutritionally, the microbial species of the GIT did significantly adapt and especially among the *Lactobacillus* strain. For example, *Lactobacillus agilis* R5 was the predominant strain found in birds fed the corn-soybean meal diet; however, this strain could not be detected in birds fed the wheat middlings diet. These results demonstrate that even seemingly

diminutive changes in the diet can elicit a marked change in the existing microbiome (Hammons et al., 2010). Therefore, some bacterial DFM are proposed to mitigate alimentary stress during the feedlot dietary adaptation period by modulating fermentation to prevent accumulation of lactate (Krehbiel et al., 2003).

Swanson et al. (2018) conducted a 2 × 2 × 2 factorial study to evaluate how a combination of bacterial DFM affected either a natural or conventional feedlot finishing system where dietary adaptation period varied in length. The authors characterize cattle within the natural finishing system to have received no growth-promoting implant, monensin, or tylosin. Conversely, cattle enrolled in the conventional finishing system were administered a growth-promoting implant and were fed both monensin and tylosin. The greatest improvements in ADG and G:F were attributed to steers fed conventionally. A feeding system × DFM interaction occurred such that conventionally finished steers supplemented with DFM had the greatest ADG and G:F, while the same variables in steers finished in a natural program with supplemental DFM were the least. The biological significance of this finding is unclear, and more research is needed if DFM are to be considered as an alternative to ionophores in cattle finished in a natural feeding program. Liver abscess prevalence and severity was greatest in naturally fed steers given a 14-d dietary adaptation and no DFM supplementation. The researchers suggest that because many of the interactions found in the study occurred between feeding system and DFM supplementation or dietary adaptation period and DFM supplementation, DFM supplementation itself is largely influenced by external management factors (Swanson et al., 2018).

Because the brain-gut-microbiome axis is critically influential among metabolic, nutritional, and immunologic process, research across many disciplines looks towards use of microbiome modulators such as probiotics and prebiotics to provide therapy for or aid in prevention of potential disorders and promote overall wellness. Animal production systems may especially benefit from the use of microbiome modulators, as stress and its associated decrease in valuable production parameters is an unavoidable aspect of the system.

In feedlot heifers supplemented with a combination of live yeast and yeast cell wall (YEAST), Broadway et al. (2016) reported the product to mitigate some negative effects of heat stress (HS) compared to heifers fed a non-supplemented diet (CON). During HS, YEAST heifers had decreased respiration rate, consumed more water, and drank more often than CON heifers. Cortisol concentration tended to increase in CON heifers during HS; however, no difference in glucose or non-esterified fatty acid (NEFA) concentration was observed. No changes in BW or ADG were observed between YEAST heifers and CON.

Due to the increasing demand for antibiotic alternatives, researchers Kayser et al. (2018) evaluated the effect of *Saccharomyces cerevisiae boulardii* on the immune response following a challenge with *Mannheimia haemolytica*, similar to that of an acute response to bovine respiratory disease (BRD). The experiment was conducted as a 2 × 2 factorial, wherein factor 1 was a roughage-based control diet (CON) or the same diet formulated to include 25 g/hd/d *Saccharomyces cerevisiae boulardii* strain CNCM I-1079 (LY), and factor 2 consisted of bronchoselective endoscopic inoculation with either *Mannheimia haemolytica* serotype A1 (MH) or phosphate buffer solution (PBS).

Although not statistically significant, final BW of MH-challenged steers was numerically 14 kg lighter compared to PBS-treated steers. No further dietary or inoculation treatment difference in final BW were observed. Clinical illness score (CIS) was not affected by inoculation or diet, and only one steer was scored a CIS of 3 or greater, resulting in antimicrobial treatment as rectal temperature exceeded 40.5 °C. Rectal temperature was greater overall in steers supplemented with LY when compared to CON steers. Reticulorumen temperature was greater overall in MH-challenged steers compared to PBS-treated steers. On day 0, RUT in MH-challenged steers was greater than in PBS-treated steers and tended to be greater on days 1 and 2. Reticulorumen temperature was not affected by diet nor was a significant diet × inoculation × day interaction detected. Following MH challenge, serum neutrophil concentration sharply increased on day 1 and remained elevated on days 2, 3, 7, and 10 post-challenge compared to PBS-treated steers. Leukocyte concentration was also greater in the MH-challenged steers on days 1 and 2 post-challenge. Serum haptoglobin concentration was 15-fold higher in MH-challenged steers compared to PBS-treated steers on days 1, 2, 3, and 5 post-challenge. Serum cortisol did not differ among inoculation groups; however, overall cortisol concentration was greater in steers supplemented with LY than in those fed the CON diet. On the day of MH challenge, DMI sharply declined in steers receiving the challenge compared to those administered PBS, which continued for 3 days post-challenge. During this time, frequency and duration of bunk visits was also reduced, however did not differ thereafter from PBS-treated steers.

Dosing regimen influences the efficacy of a probiotic supplement. As previously mentioned, there are a variety of ways a probiotic product may be introduced to the host.

Within those methods, a probiotic may be administered continuously or in a single dose. Research conducted by both Cole and Fuller (1984) and Goldin and Gorbach (1984) indicate the positive effect of a probiotic administered to rats and humans is lost when dosing of the product stops, suggesting that a continuous dosing program may be advantageous. In nursery pigs, Brooks and Kim (2017) reported supplemental *B. subtilis* to produce no differences among serum and jejunal mucosa TNF-α, IgG, IgA, protein carbonyl, or malondialdehyde. A study conducted by Elam et al. (2003) included an evaluation of gut integrity in finishing feedlot steers supplemented with either *L. acidophilus*, *Propionibacterium freudenreichii*, or a combination thereof all compared to a non-supplemented control. Steers supplemented with DFM had decreased prevalence of *E. coli* O157:H7 in both feces and on the hide compared to controls. Further, treated steers had decreased lamina propria thickness, indicating lessened inflammatory effects of pathogenic organisms (Elam et al., 2003).

Bacterial DFM in high-risk cattle

Upon feedlot arrival, beef calves have experienced persistent stressors likely over the course of several days, including weaning, transport, dehydration, commingling coupled with pathogen exposure, castration, and nutrient deficit. As a result, microbes in both the rumen and lower gut are altered, leading to decreased growth performance and increased morbidity and mortality. Bacterial DFM have been proposed as an agent to repopulate the microbiome, mitigating these negative effects (Krehbiel et al., 2003).

Morbidity and mortality events associated with BRD result in great economic losses incurred by the producer, through labor and drug expenses as well as negative

impacts on feedlot performance and subsequent carcass merit (Duff and Galyean, 2007). Fulton et al. (2002) reported that cattle treated for BRD once returned $40.64 less than non-treated cohorts, those treated twice returned $58.35 less, and cattle treated three or more times returned $291.93 less. Bovine respiratory disease is a multifactorial complex, influenced by not only viral and bacterial pathogens but a host of pre-weaning and post-weaning factors, including marketing stress, pre-shipment and arrival management, and plane of nutrition pre- and post-weaning (Duff and Galyean, 2007).

High-risk, lightweight cattle newly received to the feedlot are especially prone to morbidity and mortality events associated with BRD and digestive disturbances. Weaning, marketing, and transportation expose cattle to stress over the course of several days, coupled with an unfamiliar environment and pathogen exposure due to commingling. Access to feed and water throughout the marketing process is limited. Consequently, cattle experience immunosuppression and a negative energy balance upon feedlot arrival. Feed intake is concomitantly low during this time, averaging 1.5% of body weight in the first two weeks following arrival. Low nutrient intake further exacerbates immunosuppression (Nagaraja et al., 1998). As observed by Sowell et al. (1998), steers diagnosed with BRD spent 30% less time at the feed bunk than healthy cohorts, with differences in feed intake being most pronounced within the initial 4 days following arrival (Sowell et al., 1998; Sowell et al., 1999). Cattle treated for BRD multiple times during the receiving period will likely not achieve the same carcass value as non-treated cohorts; however, they may reach similar compositional endpoints given additional days on feed to compensate for lost performance early in the feeding period (Wilson et al., 2017).

Bacterial DFM are designed to be a non-antimicrobial alternative to address these health and nutrition challenges in newly received high-risk cattle. Both Krehbiel et al. (2003) and McDonald et al. (2005) propose DFM to improve feedlot health and performance via alteration of the intestinal microflora with potential effects on ruminal fermentation, competitive exclusion of pathogens for nutrients, improved gut permeability, and a superior immune response (Duff and Galyean, 2007).

More DFM dose titration research is warranted as it is currently limited, especially in cattle. Orr et al. (1988) demonstrated a quadratic relationship between ADG and increasing CFU dose of *L. acidophilus*. No difference in feed intake or efficiency was reported among treatments, although ADG was greater in calves fed the two median doses of 2.2×10^6 CFU or 2.2×10^8 CFU compared to the 2.2×10^{10} CFU dose and non-supplemented control. Similar performance improvements were demonstrated by Lee and Botts (1988) in calves supplemented with *S. faecium* compared to non-supplemented cohorts.

In feedlot cattle, classic research has demonstrated lactate-producing and/or lactate-utilizing bacteria improve feed efficiency and ADG alone as well as in combination with *Propionibacteria* species (Swinney-Floyd et al., 1999; Galyean et al., 2000; Rust et al., 2000a, 2000b). In a meta-analysis, Krehbiel et al. (2003) summarized that bacterial DFM returned a 2.5 to 5% increase in ADG and approximately 2% improvement in feed efficiency to feedlot cattle, although effect upon DMI was inconsistent. Of the studies included, HCW was increased by 6 to 7 kg in cattle supplemented with a bacterial DFM.

Bacillus subtilis PB6

Bacillus subtilis is classically characterized such that it is strictly aerobic (Fuller, 1989; Priest, 1989). However, more recent mapping of the genome indicates the species is able to grow and function anaerobically when nitrate is present as an electron acceptor (Kunst et al., 1997). The first gene located in subspecies with the ability to complete glycolysis in anaerobic environments was reported by Glaser et al. (1995) and was identified as *narA*. The authors hypothesized the species evolved to include this trait because soil is the natural habitat of *B. subtilis,* where some anaerobic conditions may occur. Under these conditions, *B. subtilis* first reduces nitrate to nitrite and then further reduces nitrite to ammonia. The glycolytic pathway yields 2 ATP, 2 NADH, and 2 pyruvate. In order to continue completion of glycolysis for their own metabolism, the bacteria utilize the pyruvate molecules to oxidize NADH to NAD, thus resulting in a variety of fermentation byproducts which the host can use for energy (Slonczweski and Foster, 2009). The primary fermentation products of *B. subtilis* include lactate, acetate, acetoin, ethanol, and succinate (Cruz Ramos et al., 2000).

Development of Bacillus subtilis PB6

Bacillus subtilis PB6, confirmed to be *Bacillus* spp. *Bacillus megaterium*, was developed by Lin et al. (2007) by isolating beneficial microbial species from the intestinal tract of chickens and were evaluated for probiotic effects against *Clostridium perfringens*, the organism associated with necrotic enteritis. The concentration of microbes in the nonruminant gastrointestinal tract is least in the duodenum and increases through the cecum (Mead and Adams, 1975). Therefore, isolates were collected from

various portions of the digestive tract. The cells and fermented extracts of *B. subtilis* PB6 were found to be inhibitory against *C. perfringens,* as well as human pathogens *Streptococcus pneumoniae*. These antimicrobial factors were also found to remain stable under high heat, conditions of low pH, and elevated concentrations of bile salts. *Bacillus subtilis* PB6 was found to exhibit anticlostridial factors against *C. perfringens* by limiting production of disease-causing enterotoxins (Lin et al., 2007).

Mode of action

Bacillus subtilis PB6 is a spore-forming microorganism which, upon ingestion and stimulation by low pH and bile salts, begins to germinate. The vegetative cells produce secondary metabolites and lipopeptide surfactants, which initiate cell wall lysis of the target pathogen and results in cell death (Lin et al., 2007). *Bacillus subtilis* PB6 is a naturally occurring microorganism and is stable during processing, when blended with other feedstuffs, and under normal pelleting conditions. For long term storage, it is stable from 5° C to 65° C for 1 year and is stable up to 90° C for 10 minutes for short term processes (Wilson et al., 2019).

Research in livestock & poultry

Teo and Tan (2005) screened a variety of beneficial bacteria isolated from the GIT of healthy chickens for their inhibitory affects against *C. perfringens.* Both strains of *B. subtilis* evaluated, PB3 and PB6, were inhibitory against *C. perfringens* and were found to remain stable in the presence of high heat and bile salts. The bacteriocin produced by *B. megaterium* (PB6), megacin, were further found to inhibit *C. difficile, C.*

jejuni, C. coli, and *S. pneumoniae*. The pinnacle of PB6 antimicrobial activity occurred in broth at pH 6.2 incubated at 37° C. These findings characterize the bacteriocins of *Bacillus* spp. to have a broad range of inhibitory abilities compared to their *Lactobacillus* spp. counterparts, which have a narrow antimicrobial spectrum (Teo and Tan, 2005).

Knap et al. (2007) isolated a composition of *Bacillus* with the unique function of excreting high levels of phytase into the GIT of the animal. Phytase is an enzyme that improves digestibility of phytate, the predominant form of phosphorus found in cereal grains, oil seeds, and legumes. Non-ruminant animals lack this enzyme, resulting in poor phosphorus absorption and eventual excretion in manure. Consequently, there is an additional cost to the producer in supplementing phosphorus to meet the animal's dietary requirements. In addition, excess phosphorus in the environment contributes to eutrophication of surface water sources. For these reasons, researchers developed this DFM to compensate for the lack of endogenous enzyme in non-ruminant animals. Bacillus spores are an advantageous delivery system of enzymes and bacteriocins to the GIT because they are able to pass the acidic gastric barrier and grow within the GIT. Because these abilities can be weakened in the presence of bile salts, researchers screened for *Bacillus* cells that demonstrated bile resistance for up to 20 hours in the presence of 4 and 6 mM of bile salt. The resulting *Bacillus* cells were bile resistant, quickly germinated and proliferated, excreted high amounts of phytase, and inhibited pathogenic bacteria by producing bacteriocins.

Broadway et al. (2017) evaluated the effects of supplemental *B. subtilis* PB6 on weaned Holstein steers facing a challenge with *Salmonella typhimurium* (STM). Under the conditions of this study, researchers found that in calves receiving supplemental PB6,

STM concentrations were significantly reduced in the jejunum, ileum, and transverse colon 48 h post-challenge and were numerically reduced in all gastrointestinal tissues 96 h post-challenge. In addition, calves receiving supplemental PB6 were less likely to sustain a febrile response following the STM challenge.

Wilson et al. (2019) evaluated the effects of supplemental *B. subtilis* PB6 on the clinical health, performance, and carcass characteristics of steers during the feedlot receiving and finishing phases. The 397 crossbred steers were transported from Florida to Stillwater, Oklahoma with an average arrival BW of 257 ± 22.3 kg. Total days on feed was approximately 232 days, including a 60-day receiving period. Experimental DFM treatments were mixed into a supplement of ground corn and wheat middlings and was top-dressed at the time of feeding. No differences were found among BW, ADG, or DMI across both the receiving and finishing period. However, steers supplemented with DFM tended to be more efficient across both feeding periods. Although DFM supplemented steers had a slight numerical decrease in treatment for BRD, there were no observed statistical differences in health outcomes related to BRD. There were no differences in carcass characteristics (Wilson et al., 2019).

Payling et al. (2017) conducted a meta-analysis to further understand the effect of a multi-strain *Bacillus* DFM supplemented in combination with protease on the health and performance of grower pigs. Base diets (CON) across three studies included in the analysis were corn- and soybean meal-based with little variation in composition. The DFM preparation included 5,000 U/kg *B. subtilis* protease alone (CON + Protease) or in combination with three strains of environmentally-derived *Bacillus* formulated such that 1.5×10^5 cfu/g was provided (CON + DFM; CON + Protease + DFM). Main effects of

protease and DFM did not influence growth performance across the 42 d trial when compared to CON. The CON + Protease + DFM treatment was the only treatment to improve BW on d 42 as well as improve overall ADG and G:F. Further, the CON + Protease + DFM treatment was effective in increasing energy digestibility compared to main effects of protease and DFM. For this reason, a tendency to improve apparent total tract digestibility (ATTD) of gross energy (GE) and digestible energy (DE) was observed. Overall, when compared to CON, the CON + Protease + DFM was the only treatment to improve ATTD of DM, N, GE, acid detergent fiber (ADF), and DE. Because the main effects of both protease and DFM were not observed to influence growth performance, the authors concluded the interaction of protease and DFM to be additive rather than synergistic. There is little comparative literature related to DFM fed in combination with protease in grower/finisher pigs; however, Narasimha et al. (2013) reported a combination of DFM, carbohydrase, phytase, and prebiotic to synergistically improve feed conversion ratio in broilers.

Prior studies have indicated that yeast facilitate a production response by stimulating cellulolytic and lactate-utilizing bacteria activity in the rumen, subsequently improving fiber digestion and flow of microbial crude protein to the abomasum (Newbold et al., 1995). Therefore, the objective of a two-trial study conducted by Beauchemin et al. (2003) was to determine the effect of yeast supplementation alone or in combination with *Enterococcus faecium*, a lactate-producing bacterium, on performance and acidosis risk in feedlot cattle consuming a high-concentrate diet. Eight cannulated steers were used in each experiment, both of which were conducted as a metabolism study designed as a Latin square. Steers in Group 1 were naïve in their exposure to grain

diets, whereas steers in Group 2 had previously been fed a high-grain barley-based diet for four months. To minimize carryover effect between trial periods, rumens of steers in Group 1 were manually emptied and the contents were then placed in the rumen of the steer in Group 2 such that the steer beginning the new feeding period received the ruminal contents from the steer which previously received the same treatment. In Exp. 1, steers received one of two dietary treatments: 1) control diet supplemented with calcium carbonate carrier only (CON), or 2) control diet supplemented with *E. faecium* EF212 (EF). In Exp. 2, steers received one of two dietary treatments: 1) control diet homologous to that of Exp. 1 (CON), or 2) control diet supplemented with both *E. faecium* EF212 and yeast (EF + Yeast). In both experiments, DFM were blended with calcium carbonate such that steers received 6 x 10^9 cfu/d of bacteria and 6 x 10^9 cfu/d of yeast. No treatment interactions for BW were observed; however, steers fed EF were heavier than those fed CON. Ruminal pH was numerically lower in steers that received EF during Exp. 1 when compared to steers receiving EF + Yeast during Exp. 2. The mean ruminal pH values found in Beauchemin et al. (2003) were in agreement with Ghorbani et al. (2002); however, were lower than that demonstrated by Beauchemin et al. (2001). Despite these findings, treatments in both Exp. 1 and Exp. 2 were unsuccessful in decreasing acidosis risk based upon the proportion of time which ruminal pH was below 5.8 and/or 5.5. However, as time progressed across each experiment, acidosis risk decreased. In Exp. 1, ruminal pH remained below 5.8 for 74% of the time, and below 5.5 for 55% of the time. In Exp. 2, ruminal pH was below 5.8 for 57% of the time and below 5.5 for 39% of the time. Regardless, treatment did not affect the proportion of time spent or area below pH 5.8 or 5.5. In fact, the incidence of subclinical ruminal acidosis was more severe in

comparison to similar studies conducted in cattle receiving high-grain diets which fed lactic acid-utilizing bacteria in combination with yeast. This was further confirmed by the lack of treatment effect on blood cation levels, which decreases during acute acidosis, and amylase activity, which would increase during such an event (Owens et al., 1998). In a study conducted by Williams et al. (1991) in dairy cattle, supplementing the diet with yeast mitigated the decline in ruminal pH following a meal. Also in dairy cattle, Nocek et al. (2000) reported greater daily low pH and reduced area below pH 5.5 for cows that were supplemented with *Enterococus* and *Lactobacillus*. Huffman et al. (1992) demonstrated a reduced proportion of time in which ruminal pH was below 6.0 in steers supplemented with *Lactobacillus acidophilus*. For these reasons, Beauchemin et. al (2013) concluded that a crossover experimental design was not the ideal method for evaluating DFM effects on pH, even with ruminal wash.

In addition to growth performance, DFM have been evaluated for their ability to influence animal health, blood profile, and gut integrity. Cai et al. (2015) evaluated such effects of a three-strain *Bacillus* DFM on 128 piglets fed in two stages of growth, beginning at 24 d of age (d 0) and subsequently on d 14 (Phase I) and 42 (Phase II). Pigs fed DFM had greater ADG, lower blood urea N (BUN) concentration, and lower fecal ammonia emission compared to their control counterparts across both feeding phases. In addition, intestinal histomorphology analysis revealed longer duodenal and jejunal villi in pigs fed DFM during Phase II. These results are not in agreement with previous studies by Walsh et al. (2007) and Choi et al. (2011), both of which found that supplementation with multi-microbe DFM did not affect duodenal villus height in nursery pigs. Pigs fed DFM exhibited greater ATTD of N during Phase II. Because BUN is a product of protein

metabolism, the lower BUN concentration in DFM-fed piglets likely contributed to the observed increase in ATTD of N (Cai et al., 2015). These results agree with a study by Ranganathan et al. (2010) conducted in humans, which reported lower BUN concentration in patients administered a dietary probiotic compared to control patients.

Recent studies demonstrate microbiome and GIT interactions with the brain (Yano et al., 2016) and immunity (Brandsma et al., 2015) are able to alleviate the effects of inflammation and stress-induced behavior in humans and rodents. These functions can be improved with the use of DFM, as they regulate these pathways via immunomodulation and metabolic homeostasis. Lee et al. (2010, 2015) reported that supplementation with *B. subtilis* resulted in improved performance in broilers exposed to *Eimeria* spp. and *Clostridium perfringens*. Therefore, Wang et al. (2018) examined the effects of dietary *B. subtilis* on performance, behavior, and immunity of broiler chickens under heat stress (HS) conditions. Two hundred and forty broiler chicks were assigned to one of four dietary treatments: 1) TN (thermoneutral conditions)–RD (regular diet); 2) TN-PD (RD + 250 ppm Sporulin); 3) HS-RD; and 4) HS-PD. Final BW, ADG, average feed intake (AFI) and feed conversion ratio (FCR) were improved in birds fed PD compared to their RD counterparts regardless of temperature. No temperature × diet interactions were observed. Further, no temperature × diet interactions were observed in hepatic concentrations of IL-6, IL-10, or HSP70 and mRNA expression. However, regardless of diet, HS birds had greater concentrations of IL-6, IL-10, and HSP70 accompanied by greater mRNA expression. Heterophil to lymphocyte (H/L) ratio and cecal concentrations of IgA and IgY and were increased in birds exposed to HS among both dietary treatment groups. No temperature × diet interaction on H/L ratio was

observed. Therefore, under the conditions of this study, supplementation with *B. subtilis* improved broiler performance regardless of ambient temperature and alleviated the bird's inflammatory response.

Dick et al. (2013) evaluated the effects of long-term DFM supplementation on intestinal morphology, performance, and carcass characteristics of Holstein bull calves from birth through slaughter. In Exp. 1, 1-d-old Holstein bull calves were fed a commercial milk replacer containing no DFM (control) or 5×10^8 cfu *L. acidophilus* and *Propionibacterium freudenreichii*. All calves were also provided an 80% concentrate starter diet. At milk weaning (day 50), a subset of calves were sacrificed for collection of GIT tissues. The remaining calves were weaned from milk and continued to be grain-fed for an additional 14 d, during which time the appropriate DFM treatment was top-dressed on the diet. Calves were harvested on day 64 for collection of GIT tissues. No differences in BW, ADG, DMI, or G:F were observed at both the weaning interval or during the 2 week post-weaning interval. At both the weaning and post-weaning intervals, treatment did not affect rumen papillae height; however, papillae were wider in calves supplemented with DFM during the post-weaning interval. This result suggests that calves fed DFM had greater ruminal surface area with which to absorb nutrients than their non-supplemented cohorts. The authors suggest this boost in papillae growth in DFM-supplemented calves could have been aided by increased VFA production, as butyrate and some propionate is used by rumen epithelium as an energy source. Morphology of the small intestine was also investigated. At the weaning interval, calves supplemented with DFM had greater total height, villus height, and crypt depth in the ileum compared to the control group. No differences in morphology of the small intestine

were observed during the post-weaning interval, which the authors suggest is due to milk bypassing the rumen and lack of ruminal development during the milk-feeding stage and therefore the DFM exert their effects in the small intestine. In experiment 2 of the same study, 1 to 3-d old Holstein calves were transported approximately 2 h from a commercial dairy to the research facility. Upon arrival, calves were placed in hutches and provided commercial milk replacer and free-choice access to a starter diet. After weaning, calves were moved to feedlot pens for the remainder of the study period. Treatments were 0 (control), 1×10^5 *L. acidophilus* strain NP 51 + 1×10^9 *P. freudenreichii* strain NP24 (Diet 1), or 1×10^6 *L. acidophilus* strain NP 51 + 1×10^9 *P. freudenreichii* strain NP24 (Diet 2). There were 11 pens per treatment, containing 6 to 8 steers per pen. During the preweaning phase, DFM treatment was supplied via the milk replacer. During the feeding period, DFM treatment was blended with ground corn and top dressed on the diet. Overall feedlot performance, including both the growing and finishing phases, was not affected by DFM supplementation. Although not statistically significant, steers fed Diet 1 had a final BW 22 kg greater than control and 27 kg greater than steers fed Diet 2. In addition, steers fed Diet 1 had an overall ADG numerically 0.5 kg greater than control steers, and 0.6 kg greater than steers fed Diet 2. Again, although not statistically significant, overall DMI was 0.19 kg greater in steers fed Diet 1 compared to control steers, and 0.24 kg greater than steers fed diet 2. No statistical differences were observed in carcass characteristics; however, again numerically, HCW of steers fed Diet 1 was 12 kg greater than steers fed control and 14 kg greater than steers fed Diet 2.

Chromium

Chemical Properties & mode of action

Chromium is a trace element that acts in parallel to insulin function (Pechova and Pavlata, 2007). This function potentiates the action of insulin by decreasing the half-life of glucose and increasing the disappearance rate. Therefore, it is involved in insulin-dependent processes, such as metabolism of carbohydrates, protein, and lipids (Kegley and Spears, 1995; Danielsson and Pehrson, 1998; Odgaard and Greaves, 2001). It is proposed that chromium plays a role in glucose tolerance factor with nicotinic acid, a member of the B-vitamin complex, and amino acids glycine, cysteine, and glutamic acid, and it is further proposed that this synchrony of organic complexes increases chromium absorption (Chang and Mowat, 1992; Lukaski, 1999). Research focus has shifted less on chromium's role in glucose tolerance factor activity and turned more to the functions of chromodulin, a low-molecular weight oligopeptide comprised of aspartate, cysteine, glutamate, and glycine bonded with four Cr^{+3} ions. Chromodulin is present in mammals and has been isolated and purified from pig kidney, cattle kidney, and colostrum (Pechova and Pavlata, 2007).

Clodfelder et al. (2001) reported that chromodulin would bind to activated insulin receptors, therefore stimulating kinase activity and subsequently improved glucose absorption. The proposed mechanism of action by Vincent (2000) of kinase activity by chromodulin in response to insulin begins when the receptor is converted to the active form by binding to insulin. Chromium, in the form of Cr-transferrin, moves from the blood into insulin-dependent cells, forming apochromodulin. Holochromodulin binds to the insulin receptor and further activates receptor kinase activity, and therefore

apochromodulin is excluded from binding. Holochromodulin is released from the binding site when insulin concentration drops (Vincent, 2000). Both Shepherd and Kahn (1999) and Cefalu et al. (2002) support the hypothesis that chromium enhances insulin-sensitive glucose uptake through chromium binding with low molecular weight peptides, which enhances insulin action following binding to the receptor. As a result, there is an increase in translocation of glucose transporters, primarily GLUT4, to the cell membrane.

Chromium occurs in a variety of oxidative states, as elemental chromium does not occur naturally and is biologically inert (Pechova and Pavlata, 2007). The most notable isotopes are Cr^{+3} and Cr^{+6}, the former being most predominant in biological systems and the latter having many industrial uses (Lukaski, 1999). Hexavalent chromium compounds have been known to be toxic, mutagenic, and carcinogenic in humans as well as potentially carcinogenic to animals, therefore leading to amendment in mining practices. Trivalent chromium is much more ubiquitous and is the ion considered to be nutritionally essential (Anger et al., 2005). Plant stores of chromium are largely concentrated in the roots. Therefore, only small amounts of chromium are present in the foliage of plants, and less than 1% of plant-sourced chromium would be available for absorption upon consumption. Therefore, dietary chromium should be sourced from a biologically suitable form (Anger et al., 2005) such as chromium propionate, a bioavailable source of chromium which is currently the only source of chromium approved to be supplemented to cattle in the United States (Bernhard et al., 2012a). Chromium is not currently recognized as a mineral requirement for beef cattle until further research elucidates a necessity for setting a dietary chromium requirement (NRC, 2016).

Chromium is absorbed into the blood, circulates attached to the β-globulin plasma fraction and is then bound to transferrin for transport to tissues. Chromium is absorbed readily into bones and also accumulates in the liver, kidneys, spleen, lungs, and large intestine. Virtually no chromium accumulates in muscle and/or adipose tissues. Although a small amount of chromium is excreted in epidermal tissues, perspiration, and bile, the majority of excretion occurs in urine. Excretion of chromium may increase 10 to 300 times the normal rate under conditions of stress or consumption of a diet high in carbohydrates (Pechova and Pavlata, 2007). Studies by Borel et al. (1984) and Anderson et al. (1988) demonstrated increased urinary excretion of chromium due to various stressors in humans and mice. Research by Schrauzer et al. (1986) demonstrated in mice that supplemental chromium protected against loss of several other trace elements due to stress (Chang and Mowat, 1992). A study in mice by Schrauzer et al. (1986) suggested that supplemental chromium may aid in prevention of stress-induced loss of copper, zinc, magnesium, and iron.

A meta-analysis of the relationship between stress, carbohydrate intake, and urinary chromium excretion in humans was conducted by Anderson (1997). The basal state of no stress measured 0.16 ± 0.02 μg/d urinary chromium. In response, an acute stress factor measured 0.30 ± 0.07 μg/d, chronic stress factor 0.09 ± 0.01 μg/d, and a diet rich in carbohydrates measured 0.28 ± 0.01 μg/d urinary excretion of chromium. These results suggest that an acute stress event and/or a diet high in carbohydrates is capable of increasing the rate of urinary chromium excretion beyond that of the basal rate. Therefore, cattle consuming a feedlot diet and especially high-risk, stressed calves may be at a greater propensity to be in a state of increased urinary excretion of chromium.

These implications are to be expected, as stress increases urination (Moberg and Mench, 2000), and both excess and absorbed chromium are primarily excreted in the urine (Lukaski, 1999).

Chromium compounds in feed ingredients

Chromium concentration in cattle diets was thought to be adequate before foundational research in the 1990's demonstrating supplemental chromium to modulate performance, health, and milk production (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Yang et al., 1996; Hayirli et al., 2001). The United States Food and Drug Administration Center for Veterinary Medicine approved chromium propionate as a source of supplemental chromium in 2009, and it has since been found to enhance insulin sensitivity, performance, and health of feedlot cattle (Bernhard et al., 2012a; Bernhard et al., 2012b; Spears et al., 2012). Chromium propionate may be included up to 0.50 mg/kg of diet DM. To clarify chromium concentration in feed ingredients commonly fed to cattle, Spears et al. (2017) analyzed a variety forages, concentrates, by-product feedstuffs, and feed additives sourced from locations across the US. Greater chromium concentration was observed in forages rather than in concentrates, as was the expectation of the authors. Corn samples that had been processed by either cracking or steam flaking had chromium concentrations nearly twice as high compared to whole corn samples, likely because processed grain samples were ground before chromium analysis, leading to a greater likelihood of chromium contamination. Among the harvested forages analyzed, alfalfa hay or haylage had the highest chromium concentration. Beet pulp and corn steep liquid had the greatest chromium concentrations compared to other by-product feedstuffs

analyzed. Regardless of differences observed among classes of commodities, the general outcome confirms that chromium concentration in most common ruminant feedstuffs and ingredients are low. The authors recognize the difficulty of accurately quantifying naturally occurring chromium in feed ingredients, due to the potential of chromium contamination throughout the processes of harvesting, processing, contact with metal and chrome plating, and sample grinding for laboratory analysis. Regardless, chromium absorbed from soil to plant roots is poorly translocated to other parts of the plant. Furthermore, the bioavailability of naturally occurring chromium is low. The solubility of chromium in soil may be improved by acid conditions introduced during fermentation of silage or haylage.

Development of chromium propionate and further research

Foundational research by Schwarz and Mertz (1959) demonstrated the essentiality of dietary chromium in rats, and by Jeejebhoy et al. (1977) in humans. It was not until the late 1990's that animal scientists began extensively researching chromium in livestock. Given the lack of widespread clinical symptoms attributed to chromium deficiency, concentration of chromium is considered to be adequate in livestock diets. Regardless, animal scientists have researched the effects of supplemental chromium on a variety of production outcomes (Lukaski, 1999). The results of trials conducted to examine the effects of supplemental chromium in feeder calves have been relatively inconsistent.

In a 28-d receiving trial, Chang and Mowat (1992) reported an overall increase in DMI among lightweight, stressed feeder calves that received long-acting oxytetracycline (LAOTC) upon initial processing regardless of supplementation or no supplementation

with chromium. Intake was characteristically low, especially during the first week following arrival; however, DMI for all treatments increased to a similar amount by week 4. Calves that received chromium and no LAOTC had increased ADG and feed efficiency, while LAOTC alone increased ADG, DMI, and feed efficiency while also tending to reduce morbidity. During a subsequent 70-day growing period, supplemental chromium had no effect on ADG, DMI, or feed efficiency. During the growing period, serum glucose in steers supplemented with chromium tended to be lower than their non-supplemented counterparts. There were no effects on insulin, cholesterol, or free fatty acid concentrations.

Steers influenced by shipping stress were fed supplemental chromium in the form of high-chromium yeast in a 30-day feedlot receiving study by Moonsie-Shageer and Mowat (1992). Dietary treatments were 0, 0.2, 0.5, or 1 ppm supplemental chromium. Steers fed 0.2 ppm and 1 ppm of chromium had increased ADG. Dry matter intake was greater in steers fed 0.2 or 0.5 ppm chromium. No differences in feed efficiency were observed. Morbidity was reduced by chromium supplementation from days 2 to 30, with the 0.2 ppm chromium dose being most effective. As dose of chromium increased, serum cortisol decreased at day 28; however, no effects were observed on days 7, 14, or 21. At day 7, supplemented steers had increased serum calcium. Serum albumin increased linearly across treatments on days 7 and 21. Hematocrit was increased by chromium supplementation at any dose on days 14 and 21. All steers supplemented with chromium had a greater primary antibody response to human erythrocytes on day 14, and all animals had anamnestic response to HRBC on day 21. No differences in IgM or IgG_2

were observed; however, there was a tendency for chromium-supplemented steers to have greater IgG_1 on day 14.

In Exp. 1 of Mathison and Engstrom (1995), growing steers were fed supplemental chromium under additional simulated stress induced by withholding feed and water for 12 h following feedlot arrival and commingling with new cattle 12 and 72 after arrival. Treatments were 1) control diet, 2) supplemental chromium, 3) control diet with additional stress, or 4) supplemental chromium with additional stress. No interaction between additional stress and supplemental chromium was observed over the course of the 28-day feeding period. However, the additional stress model did cause calves to lose 7 kg more than their non-stressed cohorts, reduced DMI by 19%, and increased incidence of morbidity.

No performance differences or morbidity events were observed in calves under shipping stress fed organic or inorganic chromium in a study by Kegley and Spears (1995). In a later study, Kegley et al. (1997) evaluated the performance of calves supplemented a chromium-nicotinic acid complex under the influence of shipping stress. A tendency for chromium calves to increase ADG over the 80 d period was observed; however, there were no differences in DMI or G:F. Among calves fed chromium, the final BW of calves under shipping stress was numerically lower than calves not shipped. Supplemental chromium did not affect serum cortisol concentration, plasma glucose, total leukocyte count, or percentage of neutrophils or lymphocytes. There were no observed treatment effects on antibody titer against infectious bovine rhinotracheitis virus (IBRV).

In a study using growing bulls fed a grain-based diet, Danielsson and Pherson (1998) evaluated the effects of supplemental chromium in the form of fodder yeast on

performance and carcass quality. Bulls were provided *ad libitum* access to hay and pelleted concentrate with experimental treatments being 0 or 400 mg of fodder yeast, which provided 0.2 mg chromium/kg of concentrate. No differences in final BW, ADG, HCW, or DP were observed. No differences in quality based upon the EUROP-classification system were observed. The authors concluded that in growing bulls under no apparent stress, the baseline diet which contained 0.72 mg chromium/kg appeared to be adequate in satisfying chromium requirements and that supplemental chromium did not pay dividends in the form of added performance or carcass merit.

Kegley et al. (2000) evaluated the effects of supplemental chromium in the form of chromium-L-methionine fed to growing beef steers on glucose metabolism using a glucose tolerance test (GTT) and insulin sensitivity test (IST). Three dietary treatments consisted of the basal diet (control), basal diet + 400 or 800 μg chromium-L-methionine/kg of DM. During the 21- to 23-day feeding period, no effects on ADG, DMI, or G:F were observed. A time × dietary treatment interaction was observed for plasma glucose concentration following glucose infusion. Calves fed 400 μg chromium had lower plasma glucose compared to control calves and calves fed 800 μg chromium 5 and 10 min after the glucose infusion. After 15 min, no further differences in plasma glucose concentrations were observed. There were no treatment effects on glucose area under the curve (AUC), glucose clearance rate, and glucose half-life following the GTT. A linear affect for serum insulin concentration was observed, such that calves fed an increasing dose of chromium had greater insulin response after glucose infusion compared to control. Calves fed supplemental chromium-L-methionine had greater insulin AUC between 0 and 60 min following the GTT. The results of this study demonstrated

chromium-L-methionine to be a bioavailable source of chromium because it altered glucose and insulin metabolism of growing beef steers.

A study by Swanson et al. (2000) was conducted to determine the efficacy of feeding supplemental chromium yeast to growing beef steers limit-fed a diet consisting of 90% corn silage and 10% soybean meal-based supplement. Experimental treatments were 0, 100, 200, or 400 μg chromium via high-chromium yeast/kg diet DM. Steers were administered a GTT and insulin challenge test (ICT) via jugular catheter at weeks 3 and 6 of the study. No differences in BW, ADG, or feed efficiency were observed. Overall analysis of the GTT indicated that plasma glucose concentration 15 min after dosing was greatest in steers supplemented with 200 μg chromium compared to other treatment groups. Glucose half-life tended to increase with increasing supplemental chromium dose. No difference in glucose AUC was observed. Overall analysis of the ICT found no difference in basal glucose concentration and glucose concentration 30 min after dosing. Given this data, the authors concluded supplemental chromium had minimal effects on glucose kinetics and did not influence growth or feed efficiency.

In the development of chromium propionate by Odgaard and Greaves (2001), the objective was to provide a feed additive that would maintain performance when metabolizable energy of the diet is low or decreased, expedite improved performance when metabolizable energy is increased, and improve subsequent meat quality. Developers stated it is preferred that the product be administered to the animal as a feed additive; however, it can alternatively be supplied as an injectable, an implant, in water, or as a tablet. When used as a feed additive, the acceptable range to be included in cattle diets is up to 500 parts per billion (ppb). The author-defined optimal range for pigs was

100 ppb to 200 ppb. This is in contrast to the study concurrent with this literature review, in which chromium propionate was supplied at a rate of 450 ppb/animal/d. The study by Odgaard and Greaves (2001) was conducted to include 144 barrows in a grower-finisher program in which pigs were fed one of four experimental treatment diets: 1) corn-soybean meal basal diet (B), 2) B+200 ppb chromium propionate, 3) B+200 kcal ME/kg, or 4) B+200 kcal ME/kg+200 ppb chromium propionate. During the grower phase, G:F tended to decrease in pigs fed the high-ME diet. Average daily gain was increased in pigs fed diet 2, containing low ME and chromium propionate, but decreased in pigs that were fed diet 4, high ME and chromium propionate. During the early finishing phase, ADG tended to decrease when chromium propionate was fed in both low- and high-ME diets. No differences in carcass traits were attributed to dietary chromium propionate supplementation. However, a tendency was observed for pigs fed the diet with low ME with chromium propionate supplement, to have increased ham-fat when compared to pigs fed both high ME diets.

Pollard et al. (2002) conducted a 196-d feedlot study to evaluate the effect of supplemental organic chromium on growth, feed efficiency, and carcass characteristics of steers. Chromium was supplied as a premix to treatment diets at 0 (control), 200, or 400 ppb. During the first 56 days of the study, no differences were observed for ADG, DMI, or G:F. During days 57-196, steers fed 400 ppb chromium had lower ADG and G:F, although no observed differences in DMI. Overall, steers fed 400 ppb chromium had lighter final BW, ADG, and G:F than steers fed 200 ppb chromium or control. Steers fed 200 ppb and control did not differ. Steers fed 200 ppb chromium had increased HCW when compared to those fed 400 ppb. Dressing percent, marbling score, and YG was

lower in steers fed 400 ppb chromium compared to both those fed 200 ppb chromium and control. Chromium supplementation at either dose tended to increase LM area. Serum cortisol levels were not affected by treatment until day 196, when steers fed 200 ppb chromium were higher than control and those fed 400 ppb, indicating that steers experienced similar levels of stress throughout the feeding period and that supplemental chromium did not improve the immune response or performance under the conditions of this study.

Sumner et al. (2007) evaluated the response to a GTT in nonpregnant growing Holstein heifers supplemented with chromium propionate. The study was designed as a replicated 4 × 4 Latin Square and experimental treatments were 0, 5, 10, or 15 mg/day supplemental chromium propionate. On day 14 of each experimental period, heifers were fitted with a jugular catheter and issued a GTT. Basal glucose tended to be increased by 10 and 15 mg/day chromium doses. Glucose clearance rate during the GTT was increased by all doses of chromium when compared to control. Across the three treatments fed supplemental chromium, the reduction in AUC was 36%. The authors noted this faster clearance rate was biologically significant, given that other studies in cattle generally report a 10 to 20% change (Bunting et al., 2000; Hayirli et al., 2001). No differences in insulin clearance were observed. The authors suggest the rapid glucose clearance coupled with no differences in insulin clearance are indicative of greater insulin sensitivity at the tissue level.

Yan et al. (2008) provided supplemental chromium in the form of chromium yeast to 9-week-old weaned lambs. A 2 × 3 factorial arrangement was used, which included 3 levels of chromium supplementation (0 ppb; control, 400 ppb, or 800 ppb) and two levels

of protein (low, 157-171 g/animal/d; or high, 189-209 g/animal/d). No treatment differences among final BW were observed, however, lambs supplemented with chromium and those fed a high protein diet had numerically higher final BW than control and low protein fed lambs. Lambs fed the high protein diet and 400 ppb chromium supplement had higher ADG. Both treatments supplemented with chromium deposited less intramuscular fat than non-supplemented lambs, with those fed 400 ppb being the lowest. No treatment differences for DP and pelvic fat percentage were observed. Plasma glucose was not affected by treatment. A chromium × crude protein interaction was observed for NEFA, where NEFA was increased in both the 400 ppb chromium supplemented lambs as well as those fed a high protein diet. Lambs supplemented with 400 ppb chromium had lower plasma triglycerides and decreased lipoprotein lipase activity in subcutaneous adipose tissue (aLPL). Given the lower insulin concentration and insulin:glucose ratio in lambs supplemented with 400 ppb chromium, the authors conclude there was an improvement in insulin activity. The increase in NEFA suggests enhanced lipid catabolism via chromium supplementation, which is supported by the reduction in backfat. The authors also suggest decreasing plasma triglyceride could improve hepatic transport ability and promote triglyceride catabolism due to increased activity of plasma lipoprotein lipase (LPL). In accordance with previous literature, the conditions of this study indicate changeable trends in LPL activity in adipose tissue and skeletal muscle. The observed decrease in aLPL:lipoprotein lipase activity in longissimus muscle (mLPL) activity may suggest chromium plays a role in energy redistribution.

In a 53 d feedlot receiving trial, Bernhard et al. (2012a) evaluated the effect of supplemental chromium propionate on glucose and lipid metabolism of steers using a

GTT and IST via infusion with jugular catheter. Experimental treatments were formulated to provide 0 (control) or 0.2 mg/kg DM chromium propionate to the total diet. During the GTT, NEFA concentration was lower in chromium supplemented steers both pre- and post-infusion. No differences were observed in glucose concentration or clearance rate. Post-infusion insulin concentration tended to be greater in steers supplemented with chromium, which caused an increase in insulin:glucose ratio (I:G) from 0 to 150 following infusion. During the IST, post-infusion glucose concentration was greater in chromium supplemented steers and glucose clearance rate was faster from 30 to 45 min post-infusion when compared to control. No treatment effects were observed for insulin concentration or I:G. Chromium supplemented steers had lower NEFA concentration both pre- and post-infusion. The authors indicated the collective data suggests chromium supplementation can alter lipid metabolism, and that chromium supplementation may reduce reliance on lipid metabolism for energy.

Bernhard et al. (2012b) provided supplemental chromium in increasing dosages paralleled with a lipopolysaccharide challenge to lightweight, high-risk bulls and steers at feedlot arrival. From day 0 to 14, no differences in BW, ADG, DMI, or G:F were detected; however, from day 0 to 28, as chromium dosage increased, ADG increased linearly with a tendency to improve DMI. Throughout the entire 56-d receiving period, ADG and G:F increased linearly, although no difference in DMI was detected linearly or orthogonally. Body weight on day 56 tended to increase linearly with increasing chromium level in the diet. As chromium dosage increased, percentage of steers treated for BRD at least once tended to decrease linearly. When compared to CON, steers

supplemented with 0.3 mg/kg of chromium reduced the number of steers treated for BRD at least once.

Spears et al. (2012) evaluated supplemental chromium propionate in growing heifers. Experimental treatments were 0, 3, 6, or 9 mg supplemental chromium/day blended with ground corn and 0.4 mg/day melengesterol acetate (MGA) top dressed daily on the basal diet. A GTT was administered for 3 days, beginning on day 44 of the trial. Chromium supplementation did not affect final BW or DMI. Following the GTT, a treatment × time interaction for serum insulin was observed, with heifers supplemented with 6 mg/chromium/day having the lowest concentration. Control heifers had higher serum insulin concentration compared to heifers supplemented with chromium 5, 10, 15, and 30 min following glucose administration. No difference among treatments was observed by 45 min post-glucose administration. In heifers supplemented with chromium, glucose AUC was lower from 0 to 45 min following glucose infusion. The authors suggest these findings indicate that reduced insulin secretion did not compromise glucose uptake.

In a study of preconditioned steers during the feedlot finishing phase, Kneeskern et al. (2016) reported no differences in ADG, DMI, feed efficiency, final BW, or HCW in steers provided supplemental chromium propionate compared to non-supplemented control steers. Supplemented steers tended to require 8 additional days on feed to reach the same targeted back fat (BF) as control steers. Supplemented steers had greater LM area and DP; however, no differences were observed among YG. A tendency was observed for supplemented steers to have lower marbling scores and less percent intramuscular fat (IMF); however, no difference in QG was observed among treatments.

A treatment × DOF interaction occurred for glucose area under the curve (gAUC), clearance rate from 5 to 20 min (*k*), and half-life from 5 to 20 min ($T_{1/2}$). No treatment effects were observed on measured insulin variables, however, steers had increased insulin area under the curve (iAUC), increased peak and baseline insulin concentration, and increased insulin:glucose ratio at 98 DOF regardless of treatment compared to 21 DOF. This result suggests all steers became insulin resistant with increasing DOF.

Van Bibber-Krueger et al. (2016) evaluated the effects of chromium supplementation in combination with *S. cerevisiae* on the performance and carcass merit of steers during the feedlot finishing phase. No treatment effect across both heavy and light study groups were observed for interim or final BW. Steers fed the combination of yeast and chromium had lower ADG from day 85 to 105 compared to their non-supplemented counterparts, however there was no effect during other interim periods or for overall ADG. No treatment × weight group or treatment effects were observed for HCW, DP, incidence of liver abscess, LM area, or fat thickness. Chromium supplemented steers tended to have a decreased marbling score compared to control steers.

Conclusions from the Literature

Results of bacterial DFM use in the commercial fed-beef system have been variable. Improvements in feedlot performance attributed to DFM are documented, although these results do not always pay dividends in subsequent carcass characteristics. Likewise, health outcomes are inconsistent for bacterial DFM, but some studies found improvement.

Supplemental chromium has inconsistent influence on cattle performance; however, it has consistently shown to alter glucose and insulin metabolism and modulate immune response.

MATERIALS & METHODS

All procedures were approved by the West Texas A&M University Institutional Animal Care and Use Committee prior to study initiation (IACUC #02-12-17). The experiment was conducted from January 2018 to March 2019 at the West Texas A&M University Research Feedlot (WTRF), located 11.7 km east of Canyon, TX.

Arrival Procedures

A total of 384 crossbred beef bulls (n=300) and steers (n=84; BW = 220 ± 16.2 kg) were sourced from regional auction markets and shipped approximately 11 hours to the WTRF on 18Jan2018 (Block 1), 8Feb2018 (Block 2), 15May2018 (Block 3), and 20Jun2018 (Block 4). Immediately following arrival (d -1), cattle were individually weighed (All Hydraulic Squeeze Chute, Daniels Manufacturing Co., Ainsworth, NE), ear tagged with a unique identification number (Allflex, Fort Worth, TX), vaccinated against clostridial pathogens with tetanus toxoid (Covexin 8, Merck Animal Health, Madison, NJ), administered *Mannheimia haemolytica* bacterin (Nuplura, Elanco Animal Health, Greenfield, IN), treated for internal and external parasites with a parenteral anthelmintic (Noromectin, Norbrook Labs, Overland Park, KS), administered a growth implant containing 200 mg progesterone + 20 mg estradiol benzoate + 29 mg tylosin tartrate (Component E-S, Elanco Animal Health), tested for persistent infection with bovine viral diarrhea virus (PI-BVDV) via ear cartilage sample submitted to a commercial laboratory

(Cattle Stats, Oklahoma City, OK), and treated metaphylactically with 1.5 mL/45.5 kg BW of tilmicosin (Micotil, Elanco Animal Health) with a 3-d post-metaphylactic interval. Indication of arrival health status was determined via jugular venipuncture blood sample analyzed for blood leukocyte differential (QScout BLD, Advanced Animal Diagnostics, Morrisville, NC) and was recorded as normal or abnormal according to a proprietary algorithm. Following initial processing on day -1, cattle were penned together overnight and provided *ad libitum* access to water, starter diet at 0.5% of arrival BW, and coastal Bermudagrass hay at 1% of arrival BW. On day 0, cattle were stratified by arrival BW, sex, and arrival health status. Body weight was recorded and averaged with the d -1 BW to determine initial BW. Bulls were castrated by banding (Callicrate Pro Bander, No-Bull Enterprises LLC, St. Francis, KS) and orally administered 1 mg/kg BW meloxicam (Unichem Pharmaceuticals, Hasbrouck Heights, NJ). All cattle in the study were PI-BVDV negative. Cattle were sorted into their allotted treatment pens and offered starter diet at 1.5% of initial BW beginning on day 0.

Treatment Application

Experimental treatments were: 1) placebo control (CON); 2) 13 g/animal/d *Bacillus subtilis* PB6 (CLOSTAT, Kemin Industries, Des Moines, IA; CST); 3) 450 ppb DM chromium propionate (KemTRACE Chromium, Kemin Industries; CHR); and 4) 13 g/animal/d *Bacillus subtilis* PB6 and 450 ppb DM chromium propionate (CST + CHR). Dosage was determined at the discretion of the product manufacturer. The CLOSTAT and KemTRACE products were mixed into a ground corn base weekly using a ribbon mixer (Davis Precision Horizontal Batch Mixer, H. C. Davis Sons Mfg. Co., Bonner

Springs, KS) and stored in covered 20 bu commodity bins. Experimental treatments were top dressed daily immediately following feed delivery. Cattle fed CON received an equivalent amount of ground corn only. Morbidity investigators and feeding technicians were blinded to experimental treatment by assignment of color codes to treatment pens and ear tags. The two study periods were the receiving period (day 0 through 56) and the finishing period (d 57 through harvest). Experimental treatments were applied throughout both study periods beginning on day 0.

Housing and Management

Cattle were housed in soil-surfaced outdoor pens without shade providing 20.8 m^2 of pen space and 76 cm of linear bunk space per animal. Diets were the same for all cattle regardless of treatment (Table 1). Bunks were monitored for residual feed at 1600 and 0700 h and feed calls were managed to implement a slick-bunk feeding program. Cattle were fed once daily beginning at 0730 h by a trailer-type rotary mixer (274-12B Forage Express Feed Mixer, Roto-Mix, Dodge City, KS). Orts were collected before each BW collection and factored into DMI calculations. Diet samples were collected daily, of which 100 g was dried at 60 °C for 48 h in a forced air oven (Mechanical Convection Oven 645, Precision Scientific, Winchester, VA). On d 57, cattle began transition to a finisher diet using a two-ration system such that total daily ration per pen was divided as 75% starter diet and 25% finisher diet for 3 d, 50% starter diet and 50% finisher diet for 7 d, 25% starter diet and 75% finisher diet for 3 d, followed by 100% finisher diet for the remainder of the study. Ractopamine hydrochloride (Actogain, Zoetis) was fed during the

final 28 to 33 days on feed depending on block and was formulated to supply 250 mg/animal/d.

Interim BW was collected on day 14, 28, and 56 (receiving period), and every 28 d thereafter until harvest (finishing period). On day 28, cattle were vaccinated against viral respiratory pathogens (Titanium 5, Elanco Animal Health). Cattle were administered a terminal implant containing 200 mg trenbolone acetate + 40 mg estradiol (Revalor-XS, Merck Animal Health) on day 84. Final BW was determined using the average BW recorded the day before and the day of harvest.

Cattle were visually evaluated for health and well-being daily by trained WTRF personnel blinded to treatment assignment. Animals were diagnosed with bovine respiratory disease (BRD) according to a clinical illness score (CIS) assigned by the evaluator. Clinical illness score was based on a 1 to 4 severity scale, where 1 was healthy and 4 was moribund. When CIS was 2 or greater, cattle were removed from their home pen for determination of rectal temperature. If rectal temperature was 39.7° C or greater, cattle were treated with antimicrobial therapy up to three times. Antimicrobial treatments were: 1) florfenicol (Nuflor, Merck Animal Health); 2) enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, KS); 3) ceftiofur crystalline free acid (Excede, Zoetis). All antimicrobials were administered according to label instructions with consideration of Beef Quality Assurance standards.

Fecal samples to determine count of S*almonella* spp. were collected using cattle in Block 3 (n=96) on day 0 (week 0), 28 (week 4), and 196 (week 28). Collection and analysis were conducted by USDA Meat Animal Research Center (MARC; Clay Center, NE; Patton et. al (2009)).

Over the course of the study, 12 animals were removed from the trial: poor performance due to chronic BRD (n=8), poor performance with suspected *Mycoplasma bovis* infection (n=1), difficulty standing with suspected peritonitis/enteritis (n=1), castration error (n=1), and rectal prolapse (n=1).

Blood Sampling

Two bulls nearest the median arrival BW in each pen (n=96) were selected as representative blood samples for the entirety of the study. Blood samples were collected at each BW collection beginning on day 0. During the receiving period, blood was collected via jugular venipuncture into a plain vacuum tube (BD Vacutainer, Franklin Lakes, NJ) for analysis of serum chemistry (Preventative Care Profile Plus, VetScan VS2, Abaxis, Union City, CA). Samples were allowed to clot, then centrifuged (Allegra 6R Centrifuge, Beckman Coulter, Brea, CA) at 2791 x *g* for 20 min at 20° C. Duplicate aliquots were decanted and stored at -20° C for subsequent quantitative analysis of serum chemistry, including albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), blood urea nitrogen (BUN), calcium (Ca), chloride (Cl-), creatinine (CRE), globulin (GLOB), glucose (GLU), potassium (K+), sodium (Na+), total carbon dioxide (tCO_2), total bilirubin (TBIL), and total protein (TP). A second blood sample was collected into a tube containing 7.2 mg EDTA (BD Vacutainer) for analysis of complete blood count (CBC) using an automated hemocytometer (ProCyte Dx Hematology Analyzer; IDEXX Laboratories, Westbrook, ME). During the finishing period, a single blood sample was collected and analyzed for CBC.

Harvest & Carcass Data

All cattle were transported 51.5 km to a commercial abattoir (USDA Est. No. M245E). Hot carcass weight, liver scores, and lung scores were collected by trained personnel from the WTAMU Beef Carcass Research Center. Determination of liver score followed the Eli Lilly Liver Check System (Elanco Animal Health) as described by Brown and Lawrence (2010). After carcasses were chilled for 24 h, individual measurements were collected, including marbling score, color score, quality grade, fat thickness, ribeye area, and percentage kidney, pelvic, and heart fat. Yield grade was calculated using the USDA regression equation (USDA, 2017).

Statistical Analysis

Data were analyzed using the MIXED and GLIMMIX procedures of SAS (SAS Inst. Inc., Cary, NC) where appropriate. Experimental treatments were assigned in a 2 × 2 factorial as a generalized complete block design (n=4 blocks) with 12 pen replications per treatment (24 pen replications per main effect) and 8 animals per pen. Pen served as the experimental unit for all dependent variables. Repeated measures analysis was used for blood variables and fecal *Salmonella* counts. *Salmonella* colony forming units were transformed using square root and slicers were inserted to determine the effect of treatment within week. Interactions between CST, CHR and/or day were evaluated for statistical significance first, then main effects. Differences between least squares means were determined using least significant difference. Results were considered statistically significant when $P \leq 0.05$. Tendencies were declared when $0.05 > P \leq 0.10$.

Table 1. Ingredient and nutrient composition of experimental diets[1]

Item	Starter diet	Finisher diet[2]
Ingredient, % of DM		
Sweet Bran[3]	56.41	43.47
Corn stalks, chopped	19.05	4.3
Corn grain, steam flaked	13.9	37.34
Brix molasses[4]	7.3	7.31
Corn oil	-	3.82
Grower supplement[5]	3.34	-
Finisher supplement[6]	-	3.76
Nutrient Analysis, DM %		
NE_m, Mcal/kg	1.79	2.20
NE_g, Mcal/kg	1.15	1.52
CP, %	16.5	14.6
Ca, %	1.03	0.97
P, %	0.64	0.56

[1]Analysis and calculation performed by Servi-Tech Laboratories, Amarillo, TX.

[2]Transition from starter diet to finisher diet began on d 57 using a two-ration split system, where in the total daily feed call per pen was split 75% starter/25% finisher for 3 d, 50% starter/50% finisher for 7 d, and 25% starter/75% finisher for 3 d. The finisher diet was fed for the remainder of the study.

[3]Wet corn gluten feed (Cargill Inc., Blair, NE).

[4]Cane molasses with condensed whey solubles (Westway Feed Products, Tomball, TX).

[5]Grower supplement formulated to supply 26.47% Ca, 0.07% P, 7.69% Na, , 8.83% K, 1.50% Mg, 0.23% S, 1,094mg/kg Mn, 2,052 mg/kg Zn, 237.2 mg/kg Fe, 756.8 mg/kg Cu, 5.47 mg/kg Se, 2.32 mg/kg Co, 12.03 mg/kg I, 27.32 IU/kg Vit A, 124.05 IU/kg Vit E, and 603.64 mg/kg monensin sodium on a DM basis (Hi-Pro Feeds, Friona, TX).

[6]Finisher supplement formulated to supply 26.36% Ca, 0.07% P, 7.72% Na, 2.83% K, 1.50% Mg, 0.22% S, 1,096 mg/kg Mn, 2,054 mg/kg Zn, 236.3 mg/kg Fe, 555.0 mg/kg Cu, 5.48 mg/kg Se, 2.32 mg/kg Co, 12.03 mg/kg I, 27.33 IU/kg Vit A, 124.06 IU/kg Vit E, 996.16 mg/kg monensin sodium, and 271.42 mg/kg tylosin phosphate on a DM basis (Hi-Pro Feeds, Friona, TX).

RESULTS AND DISCUSSION

Feedlot performance

Although the numerical difference in initial BW between treatments was only 1 kg, a CST × CHR interaction was observed (Table 2; $P = 0.04$); however, this difference is not biologically relevant. During the receiving period, cattle fed CST had greater BW on day 14, 28, and 56 ($P \leq 0.06$). Likewise, ADG was improved in cattle fed CST from day 0 to 14 ($P = 0.04$) and throughout the receiving period (day 0 to 56; $P = 0.04$). Cattle fed CST had greater DMI at each interim period ($P \leq 0.03$) and during the overall receiving period ($P = 0.01$). Feed efficiency tended ($P = 0.08$) to be improved by CST from day 0 to 14 but did not differ through the remainder of the receiving period.

During the finishing period, cattle fed CHR tended to have lighter BW on day 196 and 224 (Table 3; $P \leq 0.09$). In addition, the main effect of CHR reduced ($P = 0.02$) final BW numerically by 9 kg in cattle fed treatment CHR compared to CON, and 11 kg in treatment CST + CHR. Cattle fed CST had less ADG from day 112 to 140 ($P = 0.05$), and those fed CHR tended ($P = 0.07$) to have less ADG from day 168 to 196 and day 224 to final. The ADG of cattle fed CHR was less during both the overall finishing period (day 56 to final; $P = 0.01$) and throughout the overall feeding period (day 0 to final; $P = 0.03$). A CST × CHR interaction was observed for DMI from day 112 to 140 (Table 4; $P = 0.03$) such that CST and CHR had the greatest DMI, while CST + CHR was the least. From day 224 to final, cattle fed CHR had less DMI. Feed efficiency was improved from

day 56 to 84 and day 112 to 140 by CST ($P \leq 0.03$). There was a tendency for finishing period feed efficiency to be improved by CHR (day 56 to final; $P = 0.09$).

A similar study by Wilson et al. (2019) did not report CST to improve BW, ADG, or DMI during both the receiving and finishing periods; however, CST-supplemented cattle tended to have improved feed efficiency during both feeding periods. Previous literature indicates supplemental chromium to have variable results in feedlot performance. Increases in ADG and feed efficiency were observed in stressed, lightweight feeder calves by Chang and Mowat (1992) and Moonsie-Shageer and Mowat (1992). No performance differences were observed in calves under shipping stress (Kegley and Spears, 1995); however, a concurrent study by Kegley et al. (1997) observed a tendency for greater ADG over an 80-d feeding period. In a dose titration study conducted by Pollard et al. (2002), no differences were observed during the receiving period; however, during the subsequent finishing period, steers fed the greatest dosage had reductions in final BW, ADG, and G:F vs. steers fed the lower dosage or control. Bernhard et al. (2012b) observed an overall increase in ADG and improved feed efficiency during a 56-d receiving period for CHR supplemented cattle. In other instances, researchers have reported no performance differences in cattle fed supplemental chromium (Mathison and Engstrom, 1995; Danielsson and Pherson, 1998; Kegley et al., 2000; Swanson et al., 2000; Kneeskern et al., 2016, Van Bibber-Krueger et al., 2016).

It has been reported that a constant supply of glucose for a longer time induces prolonged satiety and lower feeding motivation in pigs (De Leeuw et al., 2005; Sapkota et al., 2016). In ruminants, insulin is itself considered a putative satiety hormone. In

addition, insulin can indirectly cause satiety by increasing the rate of hepatic oxidation of energy. When energy is available, i.e. during meals, hepatic oxidation increases, which increases the energy status of hepatocytes and decreases the discharge rate of hepatic vagal afferents, causing satiety (Allen et al., 2009). It has been frequently reported that supplemental chromium modulates glucose and insulin metabolism of cattle such that insulin concentration is increased and therefore so is the disappearance rate of glucose from blood into tissue (Kegley et al., 2000; Swanson et al., 2000; Sumner et al., 2007; Yan et al., 2008; Bernhard et al., 2012a; Spears et al., 2012; Kneeskern et al., 2016), which may provide insight to the instances of lower DMI for chromium-fed cattle and therefore decreased finishing period performance in the present study. In the study associated with the patent for chromium propionate, Odgaard and Greaves (2001) describe the optimal range of inclusion for pigs being 100 to 200 ppb. Although the legal concentration in cattle is up to 500 ppb, the statement by Odgaard and Greaves (2001) is in contrast with the current study, where a dose of 450 ppb was fed per the recommendation of the manufacturer. Further research may be justified to elucidate the effect of supplemental chromium propionate at a lower dose in beef cattle.

Clinical health outcomes

The main effects of both CST and CHR reduced treatment rate for bovine respiratory disease (BRD; Table 5; $P \leq 0.03$) with the lowest numerical proportion treated being treatment CST + CHR, suggesting a potential synergistic affect. There were no statistical differences ($P \geq 0.10$) among second and third BRD treatments or respiratory-associated mortality. Days to first treatment was reduced by CHR ($P = 0.04$) and days to

third treatment tended to be reduced by CST ($P = 0.07$). There tended ($P = 0.09$) to be a CST × CHR interaction for rectal temperature at second BRD treatment, such that CST + CHR had the highest temperature, and CON had the lowest. There was a tendency ($P = 0.09$) for CHR to have the highest rectal temperature at third BRD treatment. Likewise, Broadway et al. (2017) observed that calves supplemented with *B. subtilis* PB6 were less likely to sustain a febrile response following a challenge with *Salmonella typhimurium*. Antimicrobial treatment cost was reduced by CST ($P = 0.03$) in the amount of $6.30/animal in CST + CHR and $3.95/animal in the CST treatment when compared to CON.

Feed intake is characteristically low within the first two weeks of feedlot arrival and is coupled with immunosuppression and negative energy balance induced by traditional beef marketing channels (Nagaraja et al., 1998). Sowell et al. (1999) observed that steers diagnosed with BRD spent 30% less time eating than healthy cohorts, especially within 4 days following feedlot arrival. The observed increase in DMI during the receiving period for CST in the present study may present a non-antimicrobial alternative to mitigate the negative energy balance of high-risk cattle by increasing DMI coupled with potential to reduce BRD morbidity. However, Wilson et al. (2019) observed no differences in BRD related morbidity or mortality in cattle supplemented with CST or control, but these calves did not have a significant natural BRD challenge as the average BRD morbidity rate across all cattle was 10%.

Clinical health outcome as a result of providing supplemental chromium in the diet is also variable in the existing literature. Reduction in BRD morbidity was observed by Moonsie-Shageer and Mowat (1992) and Bernhard et al. (2012b). Conversely, Kegley

et al. (1997) reported supplemental chromium to have no effect on serum cortisol, leukocyte percentage and concentration, or antibody titer to IBRV. Similarly, Pollard et al. (2002) did not observe supplemental chromium to have long-term effects on serum cortisol.

When stimulated by an immune insult, phagocytes (neutrophils and macrophages) undergo a series of processes collectively referred to as the respiratory burst to eradicate engulfed pathogen. Following a more than 50-fold increase in oxygen uptake by the cells, superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are formed via enzyme activation that catalyzes the reduction of oxygen to O_2^- at the cost of NADPH. Glucose is metabolized through the hexose monophosphate shunt to regenerate NADPH consumed by the enzyme catalyzation as well as glutathione-depended H_2O_2-detoxifying system in the cytoplasm of the phagocyte itself (Babior, 1984). It is well documented that supplemental chromium modulates glucose and insulin metabolism of cattle (Kegley et al., 2000; Swanson et al., 2000; Sumner et al., 2007; Yan et al., 2008; Bernhard et al., 2012a; Spears et al., 2012; Kneeskern et al., 2016). Therefore, as chromium aids in glucose uptake, glucose may be more readily available to regenerate the respiratory burst process, and potentially improve the immune response of the animal. In the present study, CHR was effective in reducing incidence of first BRD treatment. In addition, CHR reduced days to first treatment. Potentially, chromium's indirect role in the respiratory burst process may have caused cattle supplemented with CHR to exhibit clinical signs of BRD earlier if inflammatory products were enhanced by CHR.

Receiving period blood parameter analysis

Day affected all serum chemistry variables during the receiving period (day 0 to day 56; Table 6; $P \leq 0.03$) except total CO_2. Supplementation with chromium propionate resulted in increased serum aspartate aminotransferase (AST; Fig. 1; $P = 0.04$), an enzyme associated with highly metabolic tissue; largely cardiac muscle, liver cells, and skeletal muscle cells. Serum levels increase when disease or injury causes cells of these tissues to lyse and AST is released into the blood. High levels can be an indicator of liver damage, especially when paralleled with high levels of alanine aminotransferase (Pagana and Pagana, 2006). In the present study, using the observed AST levels as a proxy for liver damage was not supported by liver outcome as there were no observed statistical differences among percentage abnormal livers (Table 10; $P \geq 0.15$), however, liver score does not account for microscopic damage or that which repaired over time.

Serum calcium was decreased by supplementation with chromium propionate (Fig. 2; $P = 0.02$). Serum calcium is used in many metabolic enzymatic pathways and is a critical contributor to muscle contraction, cardiac function, neural transmission, and blood clotting. When blood levels decrease, parathyroid hormone is released and stimulates calcium to be released from bone and teeth reservoirs (Pagana and Pagana, 2006). However, no differences in serum albumin ($P \leq 1.00$), the protein bound to approximately half of serum calcium were observed.

As a result of limited to no feed and water access and inflammation during marketing and transportation, high-risk cattle arrive to the feedlot in a catabolic and chronically stressed state (Richeson et al., 2019). The observed serum chemistry day effects (Table 6) clearly indicate the metabolic disruption high-risk cattle harbor at

feedlot arrival, and how they return to homeostasis later in the receiving period. For example, BUN was significantly greater at day 0 compared to the subsequent interim intervals of the receiving period. As urea nitrogen is a waste product of protein catabolism (Richeson, 2017), this could indicate calves were liberating protein stores to compensate for inadequate dietary CP during marketing and transportation. Furthermore, high-risk cattle experience pathogen exposure via commingling during marketing and transportation. This pathogen challenge may initiate an acute phase response, an early and nonspecific defense mechanism of the immune system that results in the production of proinflammatory cytokines, therefore promoting skeletal muscle catabolism to supply amino acids to immune tissues (Peterson, 2004; NRC, 2016). Conversely, TP was least on day 0, followed by a sustained increase throughout the remainder of the receiving period. This could be indicative of low existing immunoglobulin concentration at feedlot arrival, followed by antibody development from vaccination or natural infection through the remainder of the period. Further, serum glucose was the least at day 0 than other time points during the receiving period. This is intuitive when considering the lack of access to feed during transport and therefore less rumination activity. Subsequently, less propionate would be made available for transport to the liver to complete gluconeogenesis and be dispatched to target tissues as an energy source (NRC, 2016).

All CBC variables were affected by day during the receiving period (Table 7, $P \leq 0.02$) except percentage basophils. Particularly notable is neutrophil:lymphocyte ratio of 0.96 on day 0, clearly demonstrating the stress and inflammation high-risk cattle experience at feedlot arrival. Neutrophil:lymphocyte ratio has traditionally been used as a

proxy for stress level, where a threshold 1.0 ratio would be indicative of a highly stressed calf (Zahorec, 2001).

Chromium propionate supplementation decreased hemoglobin concentration (Fig. 3; $P = 0.05$). Hemoglobin transports oxygen and carbon dioxide throughout the body; therefore, the oxygen carrying capacity of the blood is determined by hemoglobin concentration. Although decreased hemoglobin indicates anemia (Pagana and Pagana, 2006), both values presented in Figure 3 are within the normal range for cattle. Supplementation with chromium propionate increased total white blood cell count, neutrophil count and percentage, and neutrophil:lymphocyte ratio (Figures 8-11; $P \leq 0.05$). There was a CST × CHR interaction for hematocrit concentration (Figure 4; $P = 0.05$), where CON was greater than CST, CHR, and CST + CHR ($P \leq 0.03$). Hematocrit is a measure of the proportion of total blood volume that is red and white blood cells and is closely linked to dehydration (Pagana and Pagana, 2006). Mean corpuscular volume was greater ($P < 0.01$) in CON compared to other treatments (Figure 5; CST × CHR $P < 0.01$). Likewise, mean corpuscular hemoglobin was greater ($P < 0.01$) in CON (Figure 6; CST × CHR $P = 0.01$). Supplementation with CST resulted in an increase of mean corpuscular hemoglobin concentration (Figure 7; $P < 0.01$). Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration collectively provide information about the size, weight, and hemoglobin concentration, respectively of individual red blood cells (Pagana and Pagana, 2006).

Glucocorticoids are known to increase total white blood cell count (Davies and Lefkowitz, 1980) as was observed in CHR-fed cattle during the receiving period of the present study (Fig. 8). Glucocorticoids are also known to extensively influence

mammalian glucose homeostasis. This is likely a defense mechanism, as the overall function is to reserve plasma glucose for maximal brain function during a stress event by promoting hepatic gluconeogenesis, but reducing glucose utilization by skeletal muscle and white adipose tissue (Kuo et al., 2015). As previously discussed, marketing, transportation, and feedlot arrival of high-risk cattle is a stressful event (Richeson et al., 2019), and the stress-induced production of glucocorticoid and subsequent increase in total white blood cell count may validate the observed decrease in treatment for BRD in CHR-fed cattle. Furthermore, CHR was not found to have an effect on receiving period performance but did decrease performance during the finishing period. As previously discussed, glucose and insulin are potent satiety signals (De Leeuw et al., 2005; Allen et al., 2009) and supplemental chromium is known to modulate glucose and insulin metabolism (Kegley et al., 2000; Swanson et al., 2000; Sumner et al., 2007; Yan et al., 2008; Bernhard et al., 2012a; Spears et al., 2012; Kneeskern et al., 2016). Therefore, the detrimental effects of glucocorticoid on glucose absorption may have been mitigated by CHR during the receiving period, allowing cattle more energy to combat the initial immune challenge with no apparent decrease in performance. However, as cattle sustained homeostasis and entered the finishing period where fewer stress events occur, the satiety effects of glucose and insulin may provide insight to the diminished finishing period performance due to CHR.

Finishing period blood parameter analysis

During the finishing period, day effects were observed for CBC variables of hemoglobin, hematocrit, mean corpuscular volume, platelet count, neutrophil,

lymphocyte, and monocyte count and percentage, and ratio of neutrophils to lymphocytes (Table 8; $P \leq 0.04$). There was a CST × CHR interaction for red blood cell count, where CHR was greatest ($P < 0.01$) followed by CST + CHR, CON being intermediate, and CST being least (Figure 12; $P = 0.04$). The hemoglobin concentration of CST was less ($P \leq 0.04$) than CON, CHR, and CST + CHR (Figure 13; CST × CHR $P < 0.01$). Likewise, hematocrit of CST was less ($P < 0.01$) compared to CON, CHR, and CST + CHR (Figure 14; CST × CHR $P = 0.02$). Mean corpuscular volume was decreased by CST supplementation (Figure 15; $P = 0.03$). A CST × CHR × day interaction was observed for mean corpuscular hemoglobin concentration (Figure 16; $P = 0.02$). A CST × CHR interaction occurred for percent monocytes (Figure 17; $P = 0.03$).

Carcass characteristics

Hot carcass weight was reduced by CHR (Table 9; $P = 0.01$). There tended ($P = 0.10$) to be a CST × CHR interaction for dressing percent (DP), although no statistical differences were observed between treatments. A tendency was observed for CHR to reduce the incidence of both USDA Prime and Select carcasses (Table 10; $P = 0.09$). An interaction of CST × CHR existed such that percentage of YG1 ($P < 0.01$) carcasses was greater in cattle fed CST and CHR, with CST + CHR being intermediate and CON being the least. Further, a CST × CHR interaction was observed among percent YG2 ($P = 0.03$) carcasses where numerically, CON and CST + CHR had a greater percentage. Interaction of CST × CHR ($P = 0.05$) was observed to increase percentage of YG 4 or 5 carcasses among CST and CHR, with CON being intermediate and CST + CHR being the least.

Percentage of edible livers tended to increase (CST × CHR; $P = 0.08$) numerically in the CST treatment.

No differences in carcass merit in cattle supplemented with CST were observed by Wilson et al. (2019). In cattle fed supplemental chromium, no difference in HCW or DP were observed by Danielsson and Pherson (1998). Similarly, Odgaard and Greaves (2001) observed no differences in carcass traits of pigs fed chromium propionate. In a chromium dose titration study, Pollard et al. (2002) reported steers supplemented with 0.2 ppm chromium had increased HCW compared to those fed 400 ppb chromium. In addition, DP, marbling score, and YG was lower in steers fed 400 ppb chromium compared to those fed 200 ppb chromium and control. However, chromium supplementation at either dose tended to increase LM area. In a study with lambs fed chromium yeast (Yan et al., 2008), both treatments supplemented (400 ppb or 800 ppb) deposited less intramuscular fat than control lambs, with those fed 400 ppb chromium being the lowest. Kneeskern et al. (2016) fed supplemental chromium propionate to preconditioned steers during the feedlot finishing phase and reported no differences in HCW. However, supplemented steers tended to require 8 additional days on feed to achieve the same targeted back fat (BF) as control steers. No differences were observed for YG; however, chromium supplemented steers had greater LM area and DP. Chromium steers tended to have lower marbling score and less intramuscular fat (IMF); however, no differences in QG were observed among treatments. Van Bibber-Krueger et al. (2016) fed a combination of chromium and the DFM *S. cerevisiae* to steers during the feedlot finishing phase. No treatment effects were observed for HCW, DP, incidence of

liver abscess, LM area, or fat thickness. Supplemented steers tended to have decreased marbling score compared to control steers.

Fecal Salmonella prevalence

Salmonella spp. count was markedly reduced in cattle fed CST, and especially the CST treatment, both overall (Table 11; $P = 0.07$) and on day 28 (Table 12; $P = 0.01$) when cattle among other treatments appeared to be shedding the pathogen. A fecal *Salmonella* spp. count of ≥ 200 CFU/g indicates the GIT is colonized and the animal is shedding the pathogen (Wells, 2019). Although *Salmonella* spp. are relatively ubiquitous in certain environments, cattle are not considered a major reservoir and are therefore susceptible to salmonellosis (Fedorka-Cray et al., 1998; McGuirk and Peek, 2003; NRC, 2016). These results further validate the observed improvements among CST-fed cattle in receiving period performance and overall clinical health results assuming the protein and energy that would otherwise be diverted to combating a bacterial infection can instead be used to facilitate tissue growth.

While the organism *B. subtilis* PB6 is not itself an antimicrobial, it does produce proteinaceous antimicrobial compounds in the lower gut called bacteriocin (Lin et al., 2007). Bacteriocins are defense mechanisms produced by bacteria which can kill or inhibit targeted bacteria without harming the host microbe with protection from specific immunity proteins (Yang et al., 2014). PB6 is not functional in the rumen as it is ingested as a spore, passes through the rumen, and becomes active in the lower digestive tract via stimulation of an acidic pH and the presence of bile salts. As a broad species, *Bacillus* organisms produce a variety of bacteriocins such as subtilin of *B. subtilis,* lichenin of *B.*

licheniformis, and tochicin of *B. thuringiensis.* The PB6 strain, otherwise known as *Bacillus megaterium,* primarily produces megacin (Lin et al., 2007). Megacin is a potent antimicrobial compound and has been reported to be lethal to target microorganisms at a concentration of 10 μmg/ml or 136 molecules of megacin per treated cell *in vitro* (Holland, 1961) and has a broad spectrum of activity (Von Tersch and Carlton, 1983).

CONCLUSIONS

The results of this study indicate health and performance benefits for CST during the receiving period; however, gain improvement was not maintained during the finishing period or in carcass merit. Conversely, CHR did not affect performance during the receiving period; however, steers provided CHR had decreased performance during the finishing period. Clinical health outcomes were improved by reducing incidence of first BRD treatment by both CST and CHR; however, there were no statistical differences in respiratory associated mortality. Cattle fed the CST treatment had markedly less fecal *Salmonella* spp. count both overall and on day 28. Hot carcass weight was reduced by the main effect of CHR. There was a tendency for CST to have the most frequent occurrence of edible livers. No statistical differences were observed among lung consolidation, fibrin, or status. Therefore, these data suggest feeding CST during the feedlot receiving period is a promising management strategy that improves both performance and health outcomes in high-risk cattle.

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Table 2. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on growth performance, dry matter intake, and feed efficiency of beef steers during the feedlot receiving period

	Treatment[1]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[2]	CST	CHR	CST × CHR
BW, kg								
Initial[3]	219	220	220	220	0.39	0.13	0.86	0.04
Day 14	230	234	230	233	1.43	0.04	0.99	0.73
Day 28	246	250	247	249	1.79	0.06	0.87	0.48
Day 56	287	298	291	293	2.67	0.02	0.95	0.16
ADG, kg								
Initial to Day 14	0.78	0.95	0.77	1.01	0.10	0.04	0.85	0.69
Days 14 to 28	1.07	1.16	1.14	1.01	0.11	0.84	0.73	0.32
Days 28 to 56	1.47	1.68	1.57	1.59	0.06	0.06	0.95	0.12
Initial to Day 56	1.21	1.37	1.27	1.31	0.05	0.04	0.95	0.26
DMI, kg/d								
Day 0 to 14	3.50	3.75	3.52	3.80	0.11	0.02	0.75	0.87
Day 14 to 28	5.13	5.55	5.13	5.50	0.17	0.02	0.86	0.90
Day 28 to 56	6.61	7.12	6.80	7.08	0.16	0.03	0.55	0.41
Day 0 to 56	5.46	5.88	5.58	5.86	0.13	0.01	0.70	0.60
G:F, kg								
Day 0 to 14	0.0998	0.1146	0.0973	0.1200	0.0103	0.08	0.89	0.70
Day 14 to 28	0.0945	0.0951	0.1002	0.0832	0.0009	0.35	0.72	0.32
Day 28 to 56	0.1020	0.1074	0.1039	0.1021	0.0030	0.56	0.58	0.23
Day 0 to 56	0.1005	0.1059	0.1030	0.1017	0.0027	0.45	0.74	0.22

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[2]Standard error of the mean.

[3]Initial = average of day -1 and day 0 BW.

Table 3. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on growth performance of beef cattle during the feedlot finishing period

	Treatment[1]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[2]	CST	CHR	CST × CHR
BW, kg								
Day 84	342	349	342	344	3.08	0.19	0.38	0.45
Day 112	394	404	396	397	3.56	0.11	0.47	0.24
Day 140	453	461	454	450	3.80	0.55	0.22	0.17
Day 168	502	509	502	498	4.26	0.73	0.19	0.21
Day 196	545	550	542	537	4.66	0.91	0.08	0.29
Day 224	586	593	583	578	5.17	0.92	0.09	0.28
Final[3]	635	643	626	624	5.61	0.62	0.02	0.34
ADG, kg								
Day 56 to 84	1.95	1.83	1.83	1.81	0.05	0.16	0.14	0.46
Day 84 to 112	2.05	2.12	2.10	2.14	0.05	0.24	0.56	0.76
Day 112 to 140	1.87	1.81	1.87	1.67	0.06	0.05	0.25	0.27
Day 140 to 168	1.76	1.73	1.72	1.69	0.05	0.60	0.52	0.95
Day 168 to 196	1.52	1.47	1.42	1.39	0.05	0.50	0.07	0.76
Day 196 to 224	1.50	1.51	1.47	1.47	0.06	0.82	0.57	0.91
Day 224 to Final	1.32	1.36	1.19	1.28	0.05	0.24	0.07	0.70
Day 56 to Final	1.72	1.71	1.66	1.63	0.02	0.50	0.01	0.73
Initial[4] to Final	1.61	1.63	1.57	1.56	0.02	0.73	0.03	0.40

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[2]Standard error of the mean.

[3]Final = average BW of day prior to and day of harvest.

[4]Initial = average of day -1 and day 0 BW.

Table 4. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on dry matter intake and feed efficiency of beef cattle during the feedlot finishing period

	Treatment[1]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[2]	CST	CHR	CST × CHR
DMI, kg/d								
Day 56 to 84	6.62	6.79	6.56	6.69	0.13	0.28	0.52	0.89
Day 84 to 112	8.44	8.76	8.56	8.50	0.14	0.35	0.61	0.14
Day 112 to 140	9.21	9.48	9.48	9.07	0.15	0.68	0.64	0.03
Day 140 to 168	9.62	9.57	9.60	9.37	0.15	0.34	0.47	0.54
Day 168 to 196	9.25	9.20	9.04	8.80	0.19	0.45	0.12	0.62
Day 196 to 224	9.24	9.32	9.14	9.02	0.17	0.91	0.27	0.56
Day 224 to Final[3]	9.17	9.29	8.94	8.78	0.15	0.91	0.02	0.35
Day 56 to Final	8.71	8.85	8.68	8.53	0.12	0.97	0.15	0.25
Day 0 to Final	8.01	8.20	8.00	7.94	0.11	0.53	0.25	0.25
G:F, kg								
Day 56 to 84	0.1391	0.1264	0.1296	0.1251	0.0034	0.02	0.13	0.24
Day 84 to 112	0.1117	0.1094	0.1113	0.1141	0.0028	0.93	0.45	0.36
Day 112 to 140	0.0939	0.0883	0.0904	0.0854	0.0024	0.03	0.19	0.90
Day 140 to 168	0.0830	0.0823	0.0812	0.0816	0.0021	0.96	0.57	0.80
Day 168 to 196	0.0750	0.0725	0.0709	0.0720	0.0021	0.78	0.30	0.42
Day 196 to 224	0.0726	0.0733	0.0721	0.0738	0.0027	0.65	0.99	0.85
Day 224 to Final	0.0658	0.0670	0.0609	0.0665	0.0024	0.17	0.26	0.37
Day 56 to Final	0.0897	0.0878	0.0867	0.0871	0.0010	0.46	0.09	0.29
Day 0 to Final	0.0913	0.0905	0.0892	0.0894	0.0010	0.77	0.12	0.65

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[2]Standard error of the mean.

[3]Final = average BW of day prior to and day of harvest.

Table 5. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on clinical health outcomes of beef cattle during the feedlot receiving and finishing periods

	Treatment[1]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[5]	CST	CHR	CST × CHR
BRD1[2], %	43.9	34.6	35.8	21.3	-	0.02	0.03	0.59
BRD2[3], %	23.4	11.9	16.3	12.4	-	0.10	0.47	0.40
BRD3[4], %	12.7	5.4	8.8	6.7	-	0.19	0.71	0.46
Respiratory mortality, %	4.2	1.1	3.3	2.2	-	0.34	0.95	0.65
Days to								
1st treatment	13.4	14.3	9.2	11.0	2.2	0.44	0.04	0.81
2nd treatment	21.1	17.4	21.6	17.5	3.2	0.13	0.91	0.93
3rd treatment	33.2	19.9	25.2	24.4	4.5	0.07	0.63	0.13
Rectal temperature, °C								
1st treatment	39.6	39.7	39.7	40.0	0.21	0.16	0.23	0.83
2nd treatment	39.6	39.3	39.4	40.0	0.31	0.68	0.28	0.09
3rd treatment	39.3	40.0	40.2	40.1	0.34	0.29	0.09	0.31
Antimicrobial treatment cost, $/hd[6]	12.45	8.50	9.62	6.15	1.66	0.03	0.13	0.87

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[2]Percentage of cattle treated for BRD at least once.

[3]Percentage of cattle treated for BRD at least twice.

[4]Percentage of cattle treated for BRD three times.

[5]Pooled standard error of the mean.

[6]Antimicrobial treatment cost assumes the following: $0.59/mL for florfenicol (Nuflor, Merck Animal Health), $0.47/mL for enrofloxacin (Baytril, Bayer Animal Health), $2.00/mL for ceftiofur crystalline free acid (Excede, Zoetis).

Table 6. Day effects of serum chemistry variables[1] during the feedlot receiving period

	Day					*P*-value		
Item	Day 0	Day 14	Day 28	Day 56	SEM[2]	Linear	Quadratic	Cubic
Blood urea nitrogen, mg/dL	10.14[a]	7.34[d]	8.04[c]	8.52[b]	0.23	<0.01	<0.01	<0.01
Creatinine, mg/dL	1.49[a]	1.09[b]	1.06[bc]	1.02[c]	0.03	<0.01	<0.01	<0.01
Alanine aminotransferase, U/L	23.41[a]	17.42[c]	18.31[c]	21.34[b]	0.57	0.02	<0.01	0.03
Alkaline phosphatase, U/L	69.09[d]	86.34[c]	123.10[b]	158.45[a]	5.10	<0.01	0.02	0.22
Aspartate aminotransferase, U/L	94.63[a]	61.00[c]	61.59[c]	75.83[b]	2.38	<0.01	<0.01	0.03
Total bilirubin, mg/dL	0.39[a]	0.26[b]	0.25[b]	0.25[b]	0.01	<0.01	<0.01	0.01
Glucose, mg/dL	81.99[c]	94.57[b]	102.39[a]	101.84[a]	1.88	<0.01	<0.01	0.52
Calcium, mg/dL	9.42[d]	10.24[c]	10.43[b]	10.81[a]	0.06	<0.01	<0.01	<0.01
Total protein, g/dL	6.27[c]	6.76[b]	7.24[a]	7.31[a]	0.06	<0.01	<0.01	0.02
Albumin, g/dL	2.50[a]	2.29[b]	2.36[b]	2.50[a]	0.03	0.42	<0.01	0.02
Globulin, g/dL	3.77[c]	4.47[b]	4.88[a]	4.81[a]	0.06	<0.01	<0.01	0.41
Sodium, mmol/L	134.77[c]	135.85[b]	135.54[b]	136.61[a]	0.25	<0.01	0.98	0.01
Potassium, mmol/L	5.14[c]	5.29[b]	5.23[bc]	5.52[a]	0.05	<0.01	0.08	<0.01
Chlorine, mmol/L	96.10[a]	95.46[a]	94.47[b]	94.72[b]	0.22	<0.01	0.03	0.07
Total CO_2, mmol/L	25.44	25.90	25.78	25.81	0.30	0.34	0.37	0.50

[1]Serum chemistry analysis conducted using VetScan VS2 Preventative Care Profile Plus, Abaxis, Union City CA.
[2]Pooled standard error of the mean.

Table 7. Day effects of complete blood count variables[1] during the feedlot receiving period

	Day					*P*-value		
Item	Day 0	Day 14	Day 28	Day 56	SEM[2]	Linear	Quadratic	Cubic
Red blood cells, M/μL	9.55^{bc}	9.33^{c}	9.66^{b}	10.02^{a}	0.18	<0.01	<0.01	0.26
Hemoglobin, g/dL	12.20^{bc}	11.85^{c}	12.53^{b}	13.41^{a}	0.18	<0.01	<0.01	0.17
Hematocrit, %	36.92^{c}	36.87^{c}	38.71^{b}	40.75^{a}	-	<0.01	0.02	0.39
Mean corpuscular volume, fL	39.62^{c}	40.52^{b}	41.08^{ab}	41.64^{a}	1.07	<0.01	0.53	0.77
Mean corpuscular hemoglobin, pg	13.24^{c}	13.19^{c}	13.46^{b}	13.85^{a}	0.31	<0.01	<0.01	0.54
Mean corpuscular hemoglobin concentration, g/dL	33.11^{a}	32.21^{b}	32.43^{b}	32.93^{a}	0.12	0.56	<0.01	0.12
Platelets, K/μL	401.40^{b}	527.38^{a}	448.51^{b}	436.87^{b}	21.70	0.77	<0.01	<0.01
White blood cells, K/μL	11.93^{a}	9.44^{c}	10.55^{b}	12.23^{a}	0.31	0.12	<0.01	0.02
Neutrophils, K/μL	4.95^{a}	2.28^{c}	2.58^{c}	3.61^{b}	0.21	<0.01	<0.01	0.02
Lymphocytes, K/μL	5.33^{c}	5.30^{c}	5.86^{b}	6.47^{a}	0.17	<0.01	0.05	0.46
Monocytes, K/μL	1.56^{b}	1.71^{b}	1.96^{a}	1.95^{a}	0.07	<0.01	0.17	0.18
Eosinophils, K/μL	0.0856^{b}	0.1518^{ab}	0.1460^{ab}	0.2045^{a}	0.03	<0.01	0.89	0.28
Basophils, K/μL	0.0028	0.0026	0.0017	0.0018	0.0006	0.16	0.80	0.54
Neutrophils, %	40.66^{a}	23.14^{b}	22.53^{b}	28.40^{c}	-	<0.01	<0.01	0.05
Lymphocytes, %	45.32^{b}	56.83^{a}	56.94^{a}	53.83^{a}	-	<0.01	<0.01	<0.01
Monocytes, %	13.28^{c}	18.45^{a}	19.12^{a}	16.16^{b}	-	<0.01	<0.01	0.68
Eosinophils, %	0.73^{b}	1.56^{a}	1.40^{a}	1.60^{a}	-	0.02	0.18	0.20
Basophils, %	0.0278	0.0247	0.0167	0.0167	-	0.12	0.79	0.63
Neutrophils:Lymphocytes	0.96^{a}	0.44^{c}	0.46^{bc}	0.57^{b}	0.04	<0.01	<0.01	0.01

[1]Complete blood count analysis was conducted using ProCyte Dx Hematology Analyzer, IDEXX Laboratories, Westbrook, ME.
[2]Pooled standard error of the mean.

Table 8. Day effects of complete blood count variables[1] during the feedlot finishing period

	Day								*P*-value		
Item	Day 84	Day 112	Day 140	Day 168	Day 196	Day 224	Final	SEM[2]	Linear	Quadratic	Cubic
Red blood cells, M/μL	10.32^{a}	10.02^{ab}	10.24^{a}	10.15^{a}	10.10^{ab}	9.83^{bc}	9.55^{c}	0.45	0.78	0.26	0.49
Hemoglobin, g/dL	13.77^{c}	13.80^{c}	14.41^{b}	14.60^{ab}	14.92^{a}	14.97^{a}	14.81^{ab}	0.20	<0.01	0.38	0.45
Hematocrit, %	41.16^{c}	42.59^{c}	44.20^{b}	44.78^{ab}	45.80^{a}	45.96^{a}	45.36^{ab}	-	<0.01	0.58	0.53
Mean corpuscular volume, fl	41.22^{e}	44.40^{d}	44.58^{cd}	45.64^{bc}	46.95^{ab}	48.43^{a}	47.18^{a}	0.78	<0.01	0.47	0.90
Mean corpuscular hemoglobin, pg	13.85	14.16	14.60	14.95	15.36	15.86	18.88	1.38	0.49	0.99	0.98
Mean corpuscular hemoglobin concentration, g/dL	33.57	32.50	32.65	32.64	32.61	32.67	31.60	0.32	0.79	0.75	0.90
Platelets, K/μL	388.88^{a}	394.11^{a}	348.32^{b}	319.93^{b}	313.03^{b}	269.47^{c}	297.00^{bc}	17.50	<0.01	0.20	0.95
White blood cells, K/μL	12.23^{a}	12.33^{a}	11.98^{ab}	11.32^{b}	10.57^{c}	10.71^{bc}	10.72^{bc}	0.34	0.59	0.92	0.98
Neutrophils, K/μL	3.25	3.29	2.94	2.67	2.79	2.92	2.96	0.22	0.04	0.20	0.69
Lymphocytes, K/μL	6.77^{a}	6.99^{a}	7.33^{a}	7.08^{ab}	6.40^{ac}	6.40^{ac}	6.57^{ab}	0.23	0.01	0.003	0.85
Monocytes, K/μL	1.85^{a}	1.64^{b}	1.36^{c}	1.21^{d}	1.03^{e}	1.05^{e}	0.99^{e}	0.07	<0.01	0.42	0.55
Eosinophils, K/μL	0.28	0.33	0.27	0.28	0.28	0.27	0.36	0.04	0.39	0.33	0.54
Basophils, K/μL	0.0041	0.0019	0.0030	0.0029	0.0024	0.0018	0.0040	0.0007	0.60	0.17	0.71
Neutrophils, %	26.21^{ab}	26.53^{ab}	23.87^{b}	23.73^{b}	26.23^{ab}	27.09^{a}	26.88^{a}	-	0.98	0.03	0.93
Lymphocytes, %	56.48^{b}	57.75^{b}	65.45^{a}	63.03^{a}	61.24^{a}	60.34^{ab}	60.79^{a}	-	0.04	<0.01	0.73
Monocytes, %	15.02^{a}	13.28^{ab}	11.46^{bc}	10.75^{cd}	9.82^{d}	10.02^{cd}	9.16^{d}	-	<0.01	0.26	0.41
Eosinophils, %	2.25	2.71	2.18	2.45	2.67	2.53	3.12	-	0.91	0.16	0.47
Basophils, %	0.0396	0.0204	0.0260	0.0281	0.0240	0.0184	0.0405	-	0.62	0.38	0.91
Neutrophils:Lymphocytes	0.49^{a}	0.48^{a}	0.41^{ab}	0.40^{b}	0.45^{ab}	0.47^{ab}	0.46^{ab}	0.03	0.37	0.03	0.87

[1]Complete blood count analysis was conducted using ProCyte Dx Hematology Analyzer, IDEXX Laboratories, Westbrook, ME.
[2]Pooled standard error of the mean.

Table 9. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on carcass traits of beef cattle

	Treatment[1]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[2]	CST	CHR	CST × CHR
Hot carcass weight, kg	399	402	393	388	3.84	0.81	0.01	0.30
Dressing percent	62.64	62.71	62.91	62.23	0.23	0.18	0.64	0.10
Marbling number[3]	41.97	43.12	43.38	43.17	0.75	0.53	0.34	0.37
Fat thickness, cm	1.40	1.40	1.35	1.32	0.05	0.92	0.21	0.83
Ribeye area, cm^2	96.13	96.58	95.48	96.64	1.03	0.45	0.79	0.72
Calculated yield grade[4]	2.82	2.84	2.76	2.64	0.08	0.52	0.11	0.39

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[2]Standard error of the mean.

[3]Leading digit in marbling number indicates marbling score; 2=trace, 3=slight, 4=small, 5=modest, 6=moderate, 7=slightly abundant, 8=moderately abundant, 9=abundant. Following digits indicate degree of marbling within marbling score.

[4]Calculated using the USDA regression equation, 2017.

Table 10. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on categorical carcass traits of beef cattle

	Treatment[1]				*P*-value		
Item	CON	CST	CHR	CST + CHR	CST	CHR	CST × CHR
Quality grade, %							
Prime	0.18	0.89	0.16	0.16	0.12	0.09	0.11
Premium Choice	7.73	15.11	12.20	11.07	0.32	0.82	0.18
Choice	43.02	39.43	51.48	46.56	0.40	0.13	0.90
Select	48.98	42.79	33.68	40.93	0.88	0.09	0.19
Standard	0.05	0.68	1.42	0.68	0.54	0.28	0.28
Yield grade, %							
1	1.14[b]	7.69[a]	10.23[a]	4.55[ab]	0.18	0.05	<0.01
2	36.48	25.36	27.21	38.01	0.95	0.72	0.03
3	47.51	44.96	40.97	46.43	0.77	0.62	0.43
4 or 5	13.12[ab]	17.00[a]	16.87[a]	7.76[b]	0.33	0.32	0.05
Liver score, %							
Edible	81.58	89.58	88.19	82.16	0.78	0.87	0.08
Abnormal	16.41	9.53	10.94	14.58	0.65	0.98	0.15
Lung consolidation score[2], %							
1	15.18	12.03	14.24	12.96	0.53	0.99	0.79
2	4.43	7.50	6.58	6.59	0.53	0.75	0.53
3	2.84	1.73	2.66	0.86	0.25	0.59	0.65
Lung fibrin score, %							
Minor	9.99	6.99	14.29	13.36	0.49	0.11	0.65
Extensive	11.00	9.34	11.72	9.46	0.53	0.90	0.93
Lung status, %							
Normal	42.32	48.42	34.93	38.84	0.38	0.14	0.87
Abnormal	44.75	40.17	50.49	46.47	0.39	0.23	0.95
Condemned	10.00	9.74	13.44	13.59	0.98	0.33	0.95

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.
[2]Lung consolidation score parameters are as follows: 1 = 0-15% consolidation of tissue or mycoplasma-like lesion; 2 = 15-50% consolidation of tissue or mycoplasma-like lesion; 3 = >50% consolidation of tissue or percent of lung missing.

Table 11. Fecal *Salmonella* spp. prevalence in high-risk cattle fed supplemental *Bacillus subtilis* PB6 and/or chromium propionate

	Treatment[1]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[2]	CST	CHR	CST × CHR
Salmonella spp., CFU/g[3]	4,150.05	3.98	13,620.00	220.91	18.94	0.07	0.33	0.49

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.
[2]Standard error of the mean.
[3]*P*-value and SEM reported from data transformed using square root. Treatment means are back-transformed to reflect actual CFU/g.

Table 12. Fecal *Salmonella* spp. prevalence in high-risk cattle fed supplemental *Bacillus subtilis* PB6 and/or chromium propionate

	Treatment[1]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[2]	TRT	DAY	TRT × DAY
Salmonella spp., CFU/g[3]								
Day 0	39.10	1.45	3.81	1.51	32.81	0.99	0.04	0.68
Day 28	12,406.00[ab]	2.88[b]	40,459.00[a]	636.17[b]	32.81	0.01	-	-
Day 196	4.56	7.61	397.53	25.05	32.81	0.99	-	-

[1]CON = placebo control; CST = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6; CHR = 450 ppb DM chromium propionate; CST + CHR = 13 g/animal/d DM inclusion of *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.
[2]Standard error of the mean.
[3]*P*-value and SEM reported from data transformed using square root. Treatment means are back-transformed to reflect actual CFU/g.

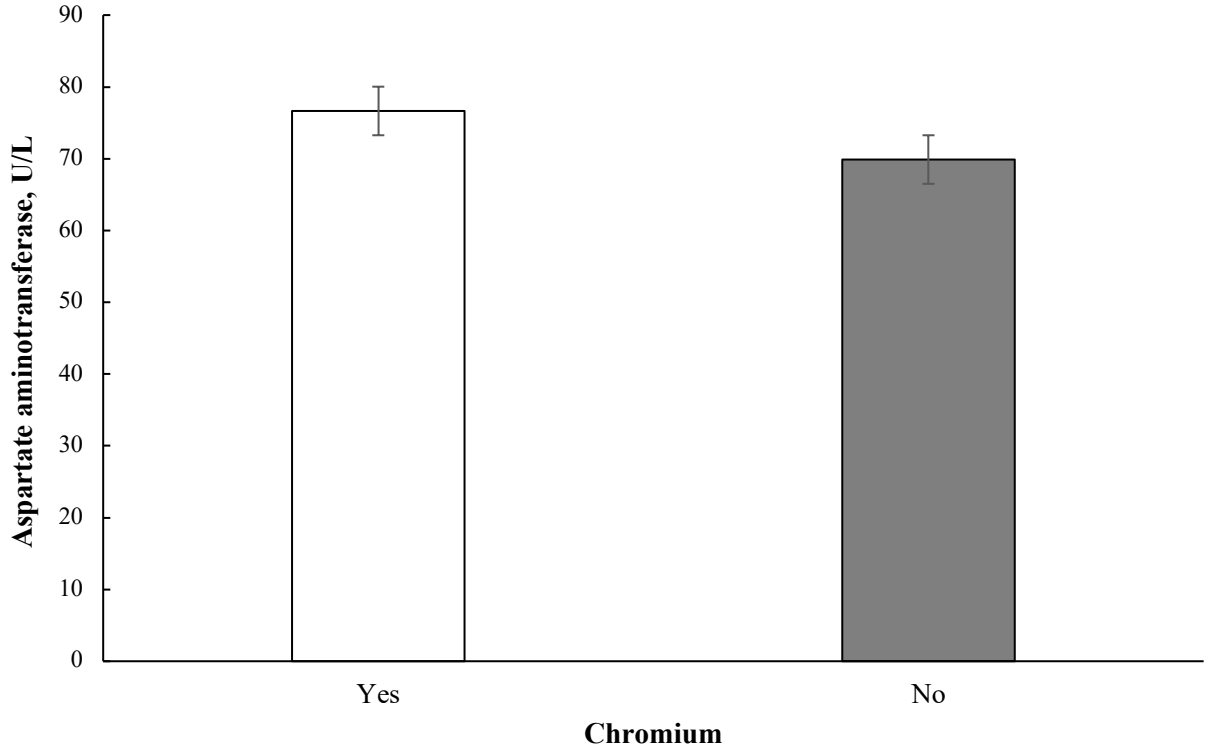

Figure 1. Effect of chromium propionate supplementation on serum aspartate aminotransferase of high-risk cattle during the feedlot receiving period ($P = 0.04$).

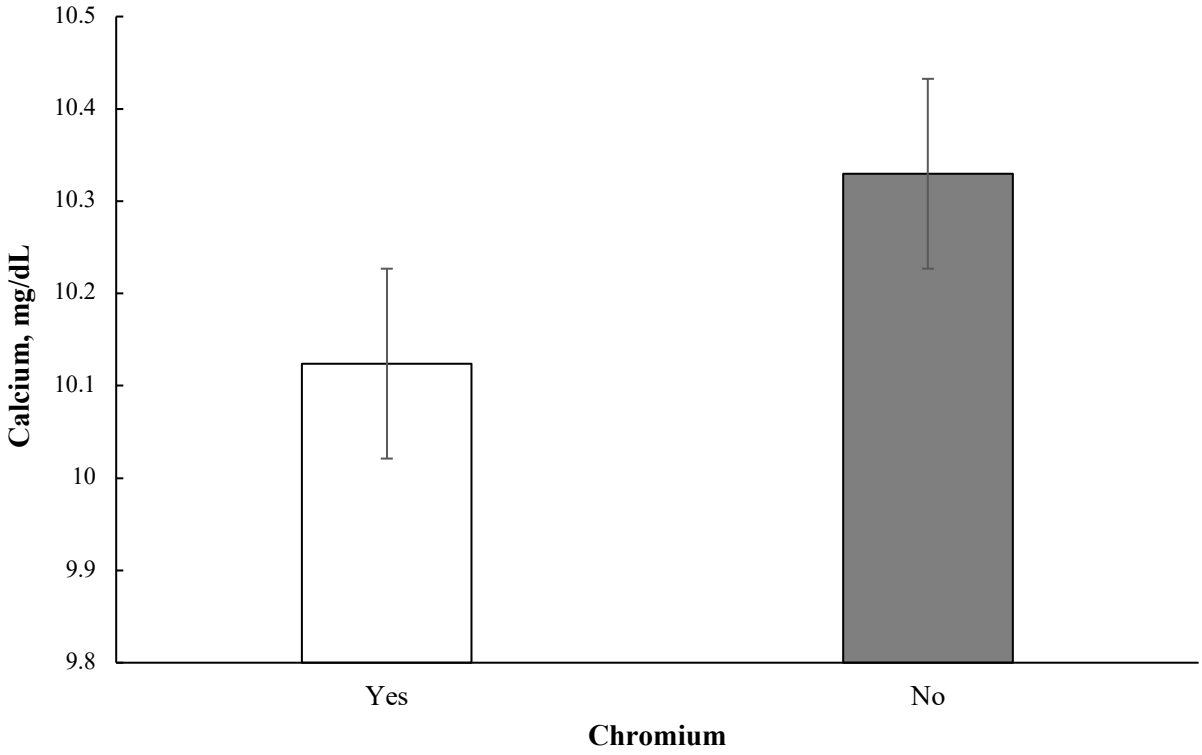

Figure 2. Effect of chromium propionate supplementation on serum calcium of high-risk cattle during the feedlot receiving period ($P = 0.02$).

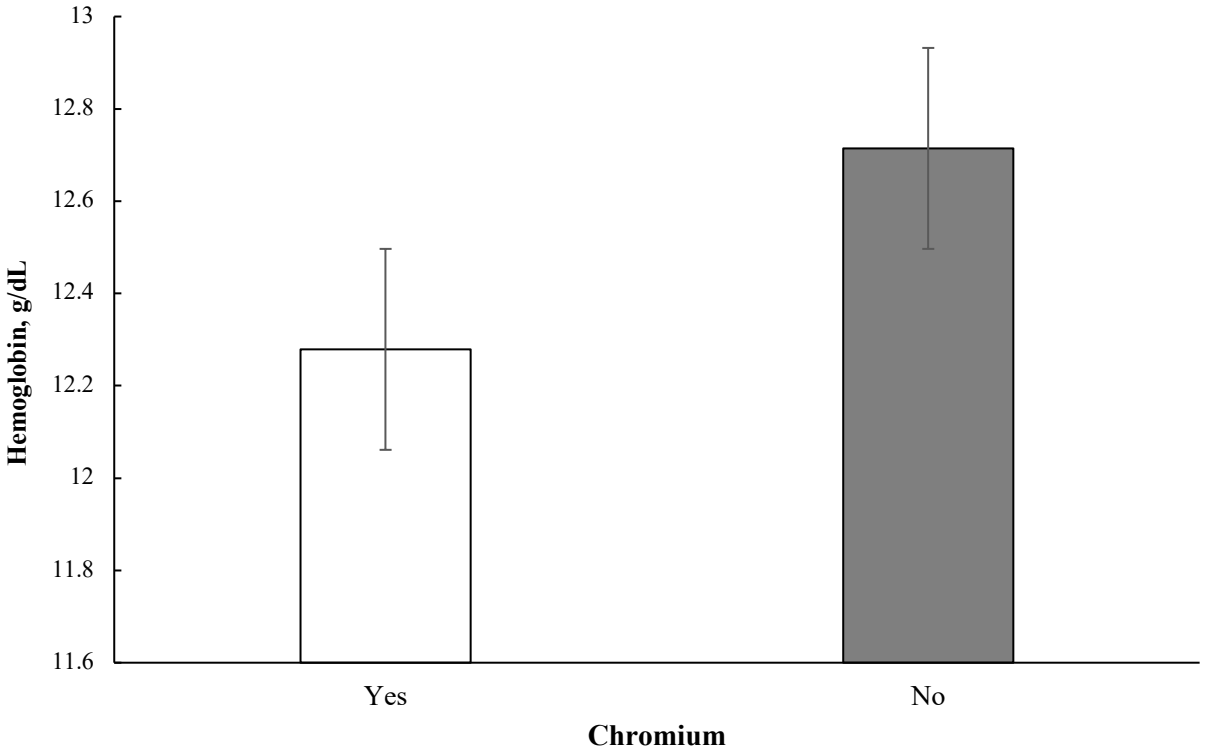

Figure 3. Effect of chromium propionate supplementation on hemoglobin concentration of high-risk cattle during the feedlot receiving period ($P = 0.05$).

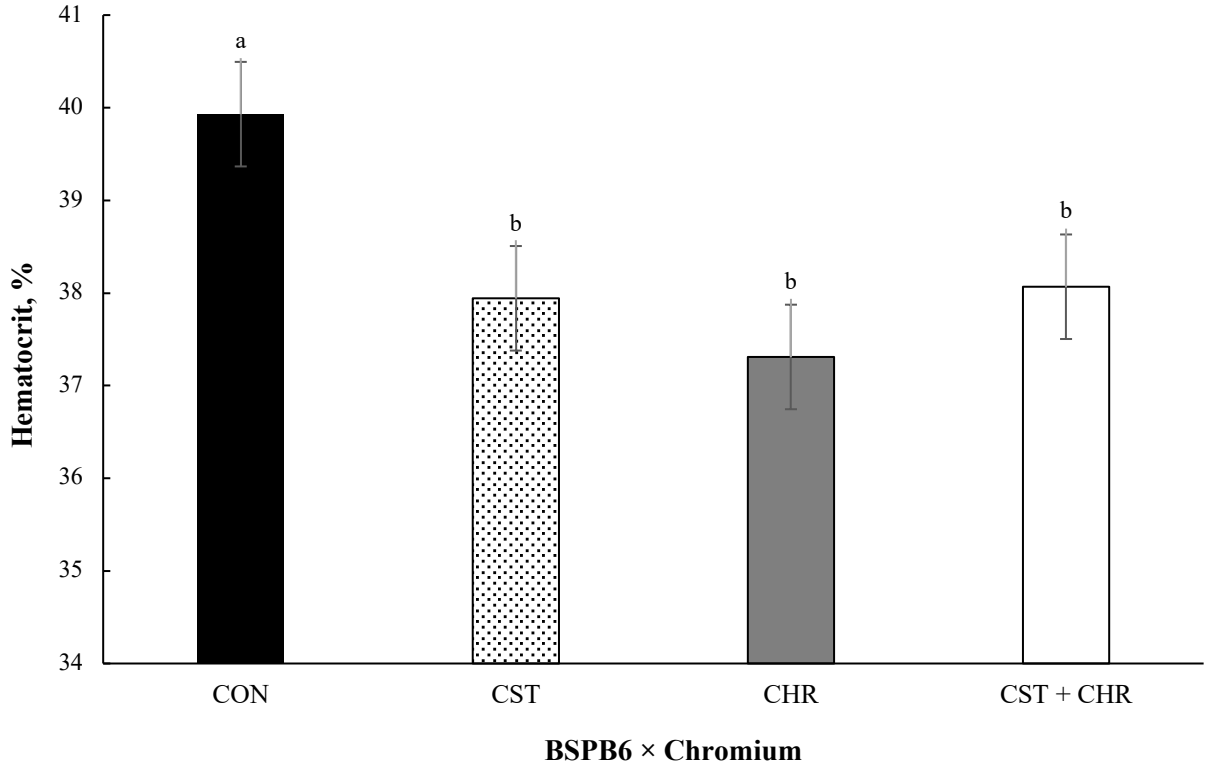

Figure 4. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on hematocrit concentration of high-risk cattle during the feedlot receiving period ($P = 0.05$). CST, CHR, and CST + CHR differ from CON, ($P \leq 0.03$).

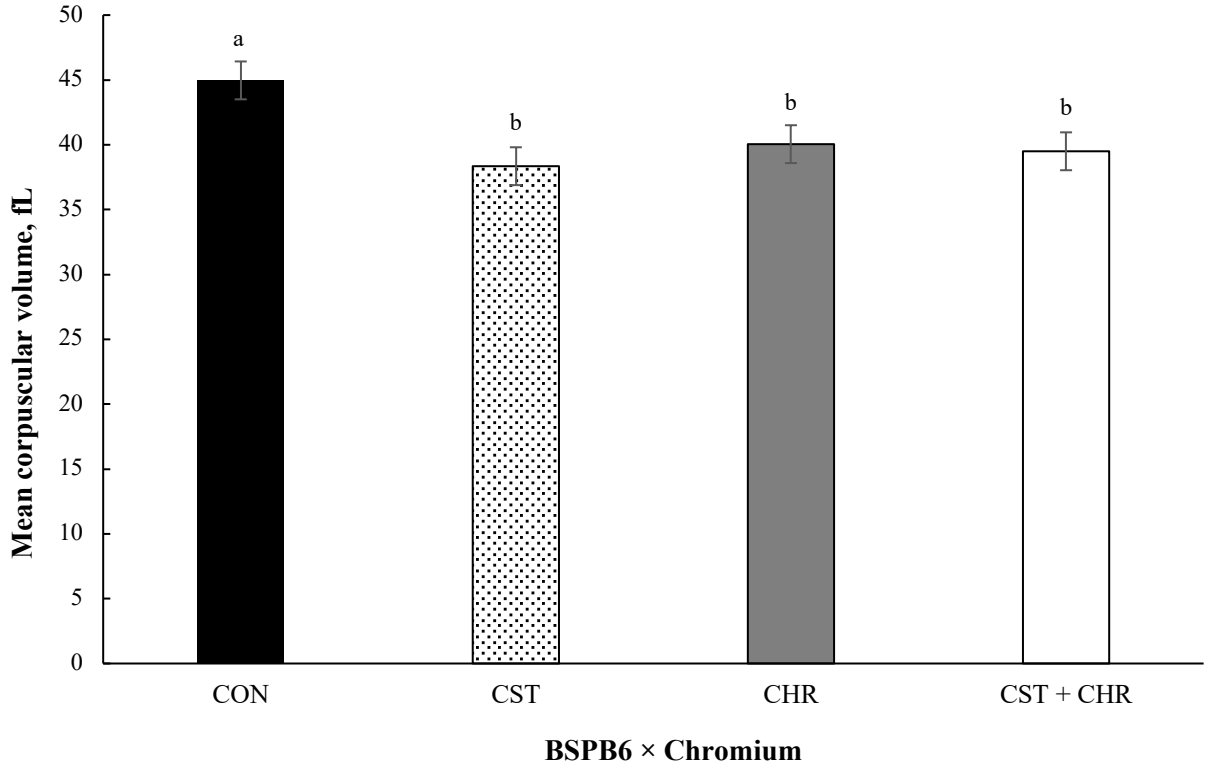

Figure 5. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on mean corpuscular volume of high-risk cattle during the feedlot receiving period ($P < 0.01$). CST, CHR, and CST + CHR differ from CON, ($P < 0.01$).

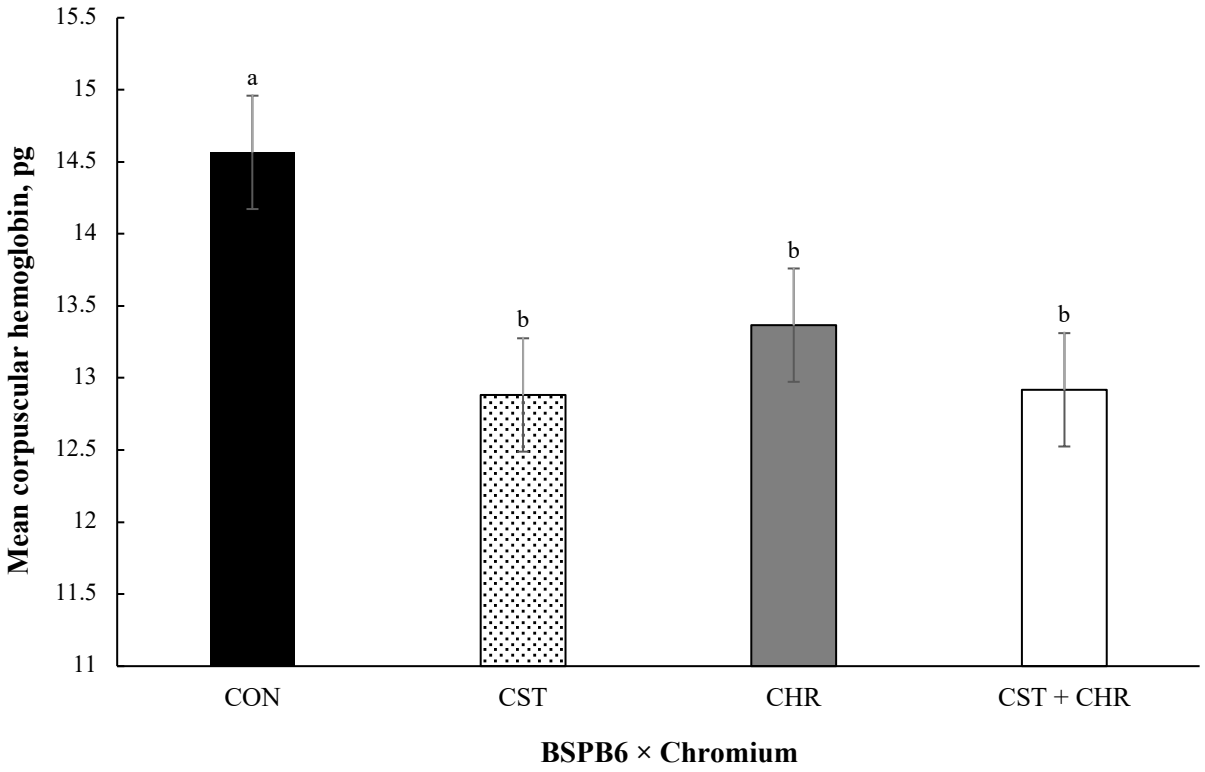

Figure 6. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on mean corpuscular hemoglobin of high-risk cattle during the feedlot receiving period ($P = 0.01$). CST, CHR, and CST + CHR differ from CON, ($P < 0.01$).

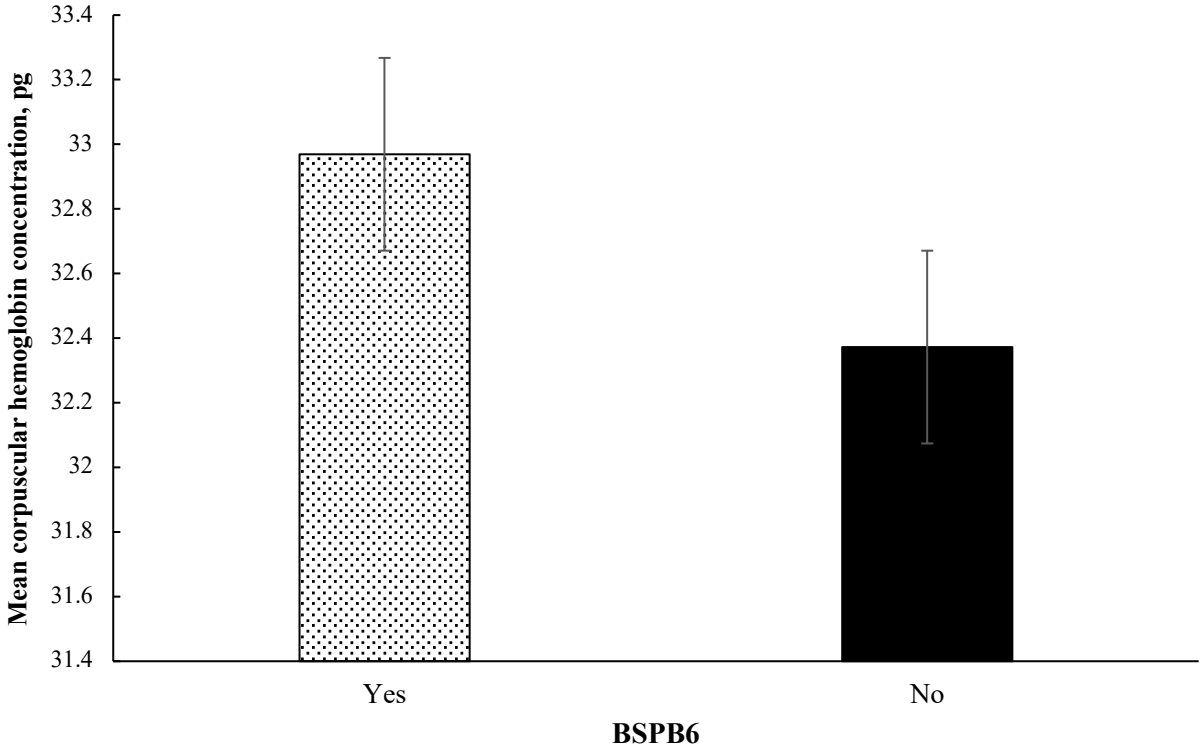

Figure 7. Effect of *Bacillus subtilis* PB6 supplementation on mean corpuscular hemoglobin concentration of high-risk cattle during the feedlot receiving period ($P < 0.01$).

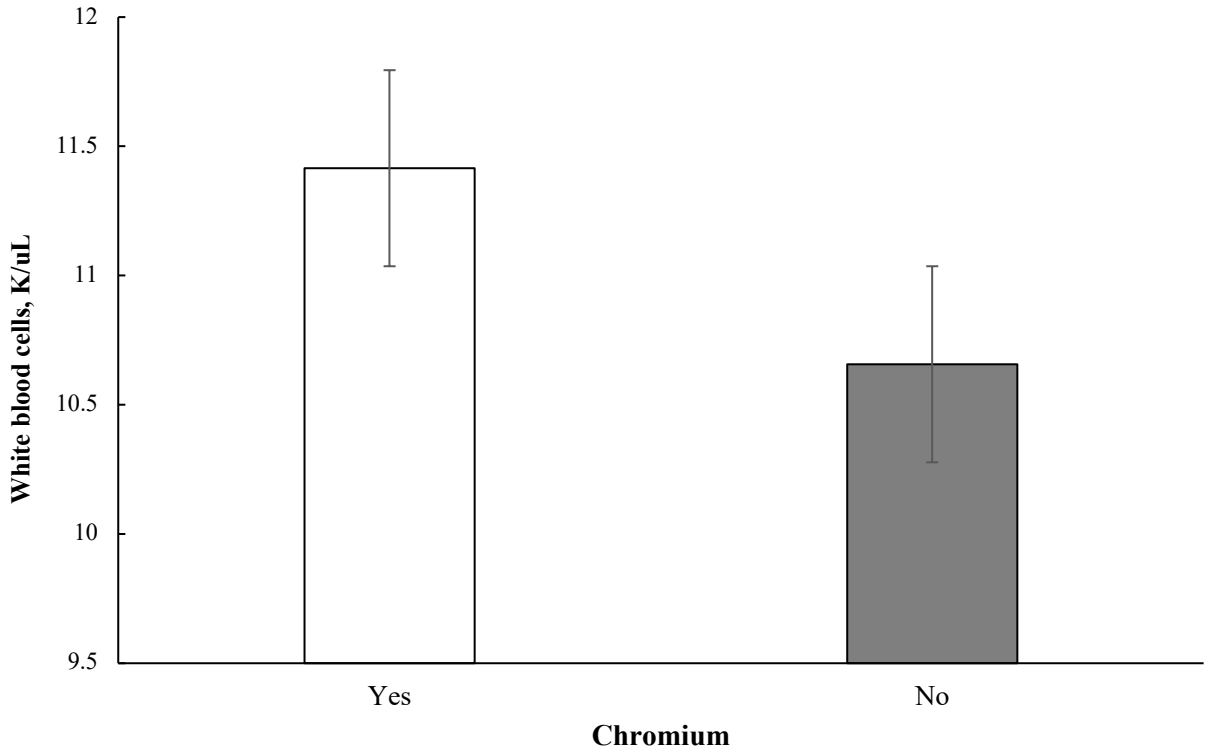

Figure 8. Effect of chromium propionate supplementation on white blood cell count of high-risk cattle during the feedlot receiving period ($P = 0.04$).

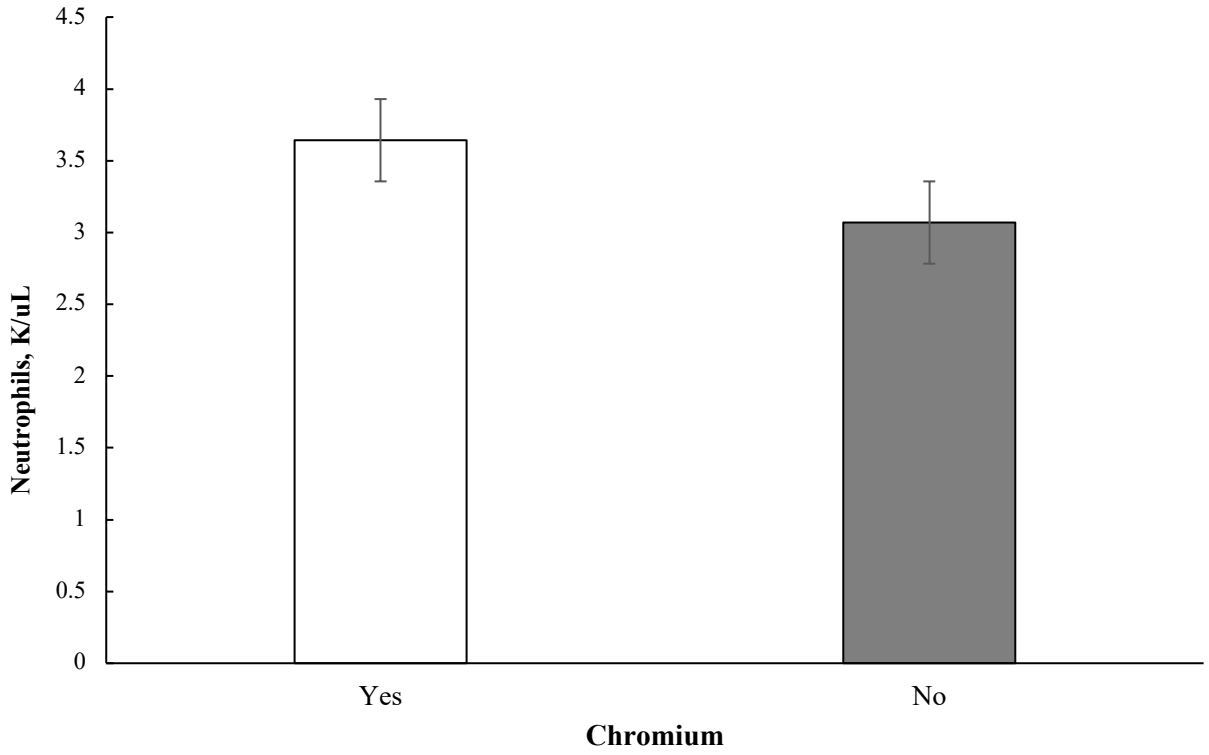

Figure 9. Effect of chromium propionate supplementation on neutrophil count of high-risk cattle during the feedlot receiving period ($P = 0.02$).

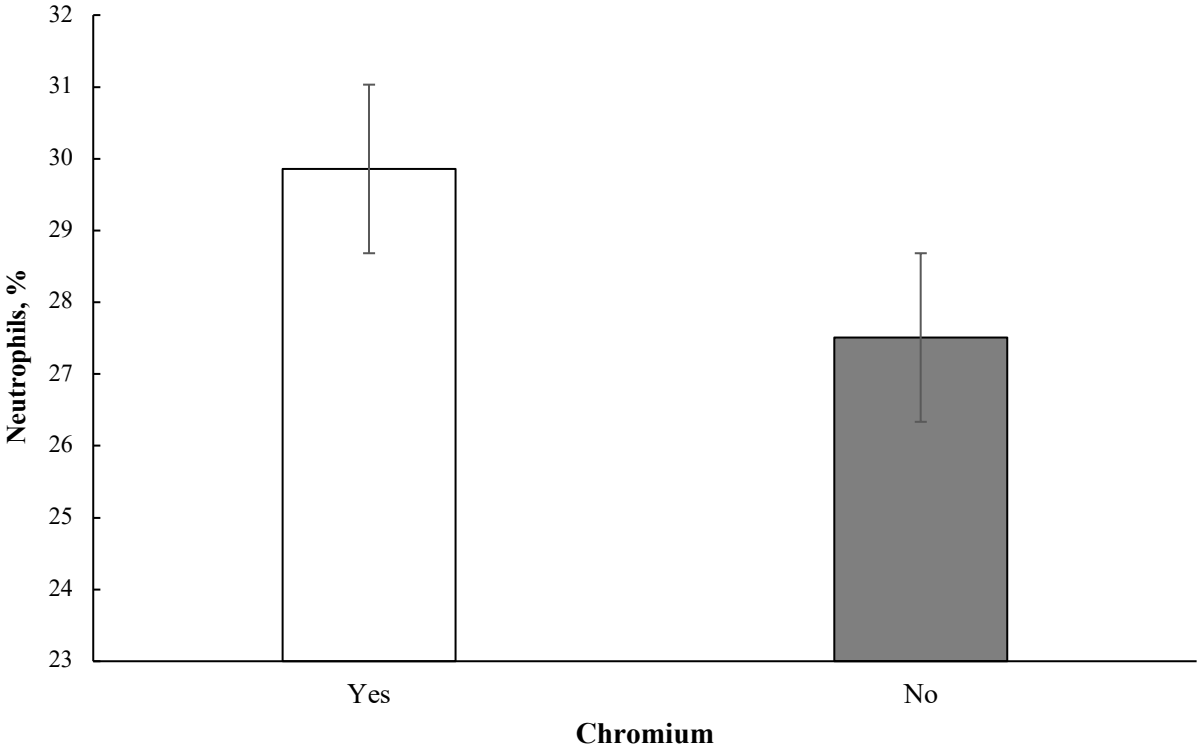

Figure 10. Effect of chromium propionate supplementation on percent neutrophil concentration of high-risk cattle during the feedlot receiving period ($P = 0.05$).

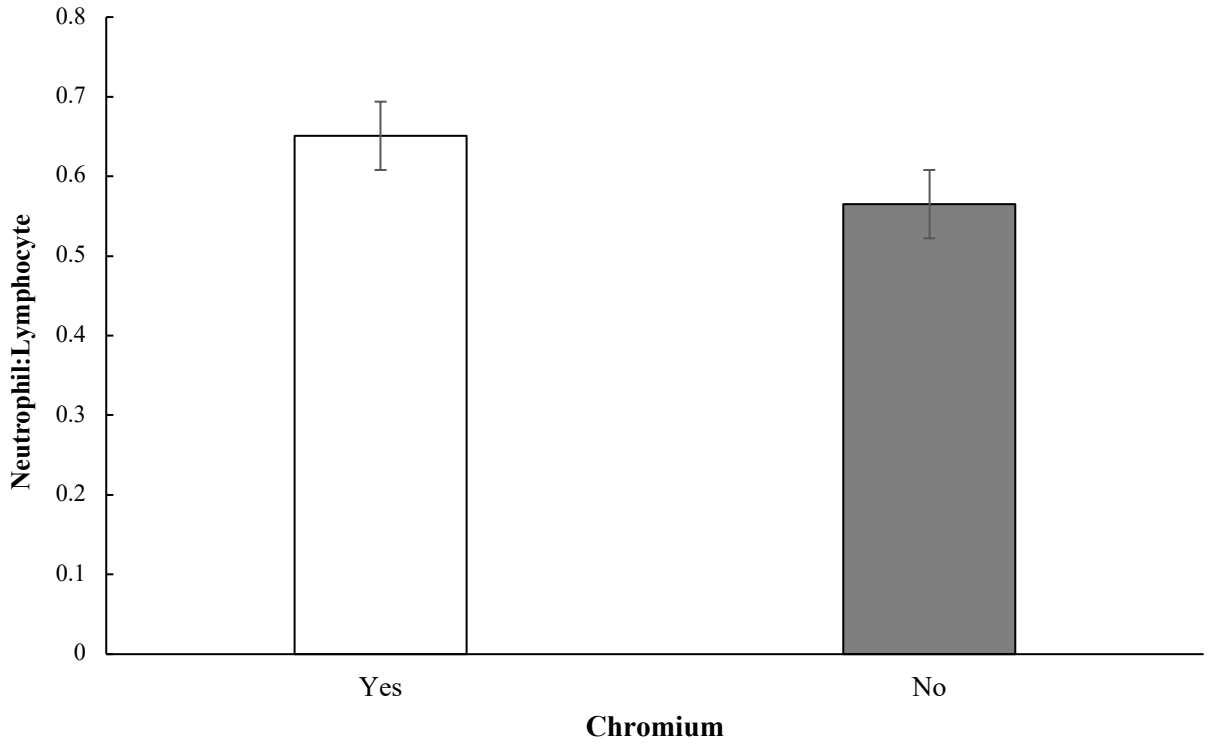

Figure 11. Effect of chromium propionate supplementation on neutrophil:lymphocyte ratio of high-risk cattle during the feedlot receiving period ($P = 0.03$).

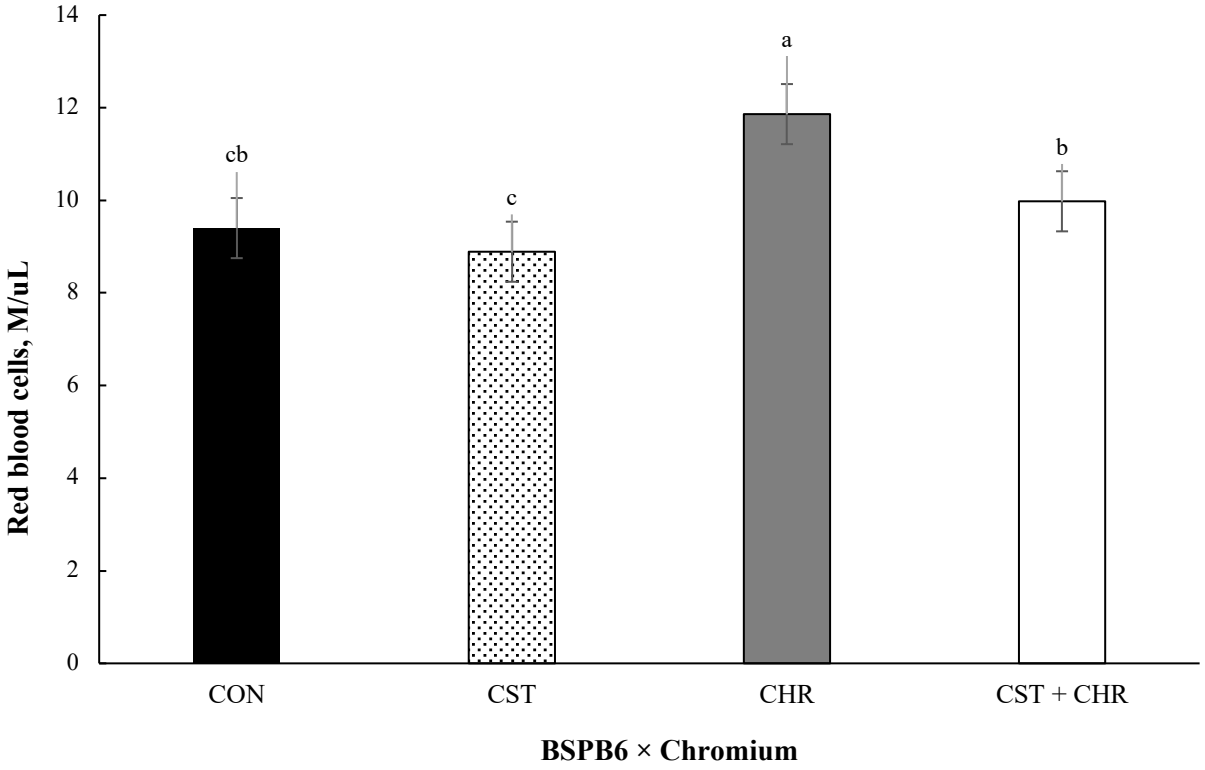

Figure 12. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on red blood cell count of high-risk cattle during the feedlot finishing period ($P = 0.04$). CHR differs from CON, CST, and CST + CHR, ($P < 0.01$).

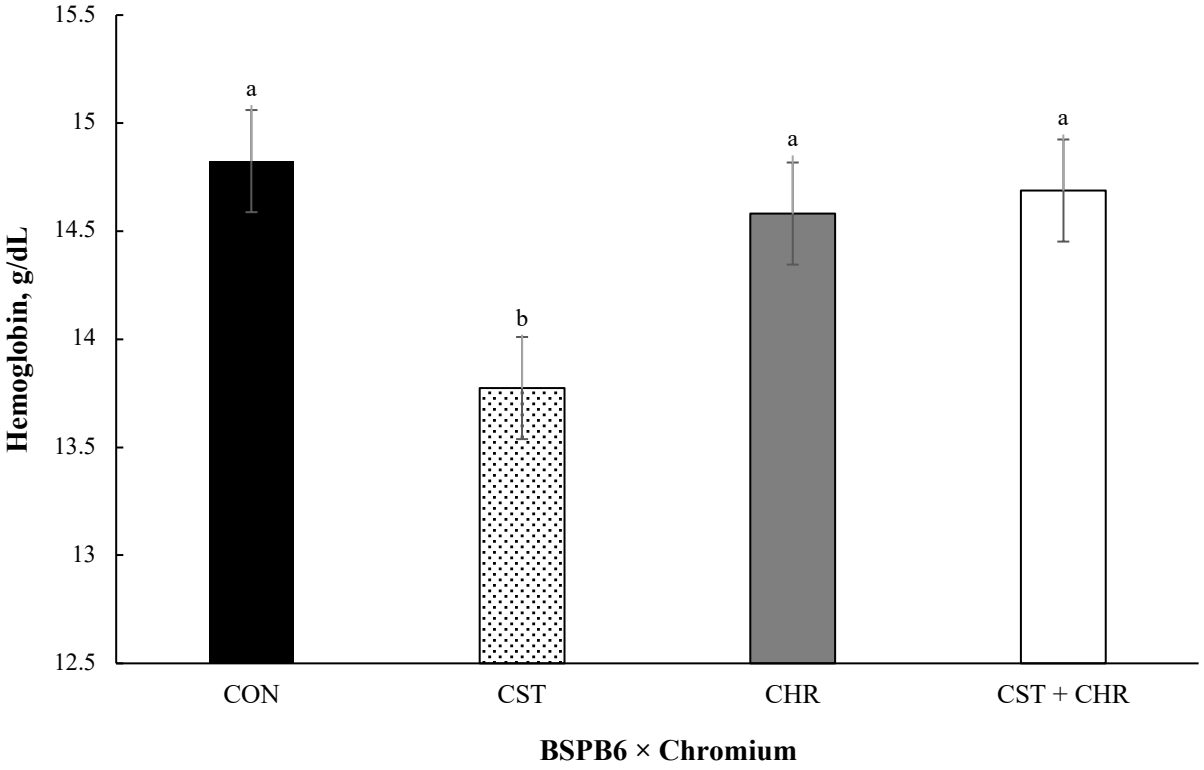

Figure 13. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on hemoglobin concentration of high-risk cattle during the feedlot finishing period ($P < 0.01$). CST differs from CON, CHR, and CST + CHR, ($P \leq 0.04$).

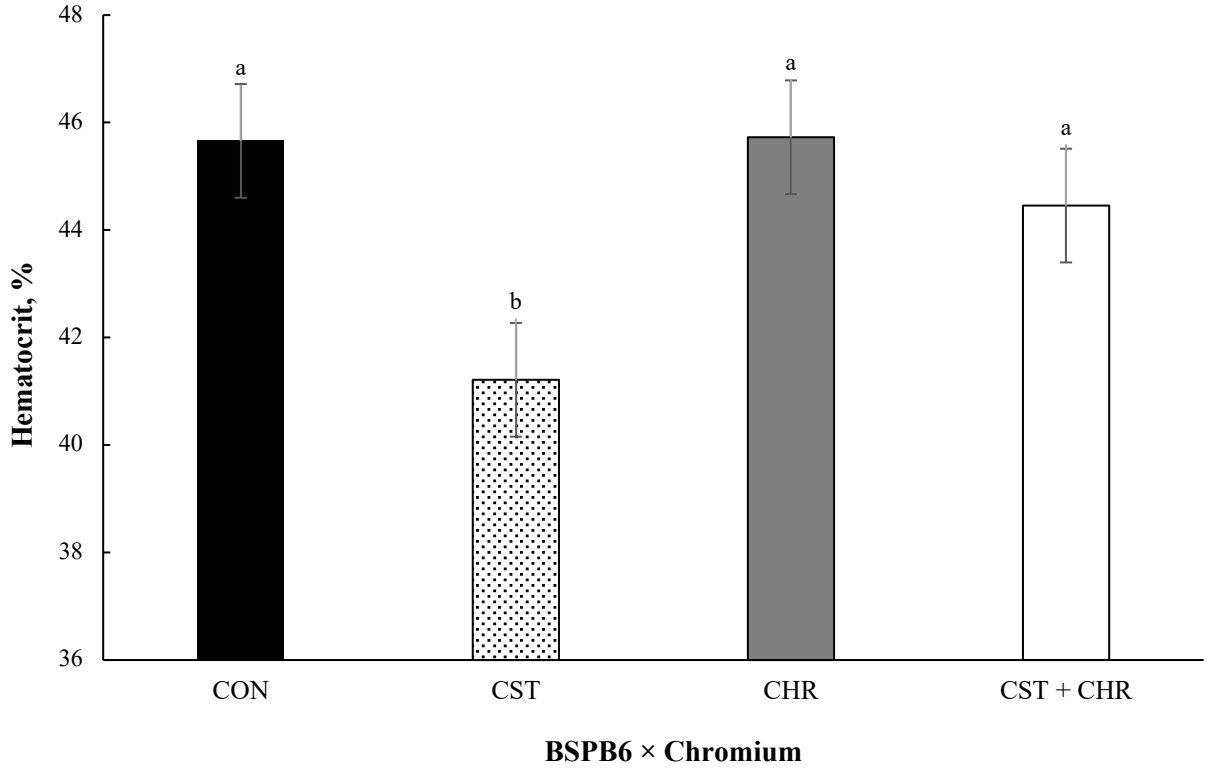

Figure 14. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on hematocrit of high-risk cattle during the feedlot finishing period ($P = 0.02$). CST differs from CON, CHR, and CST + CHR ($P < 0.01$).

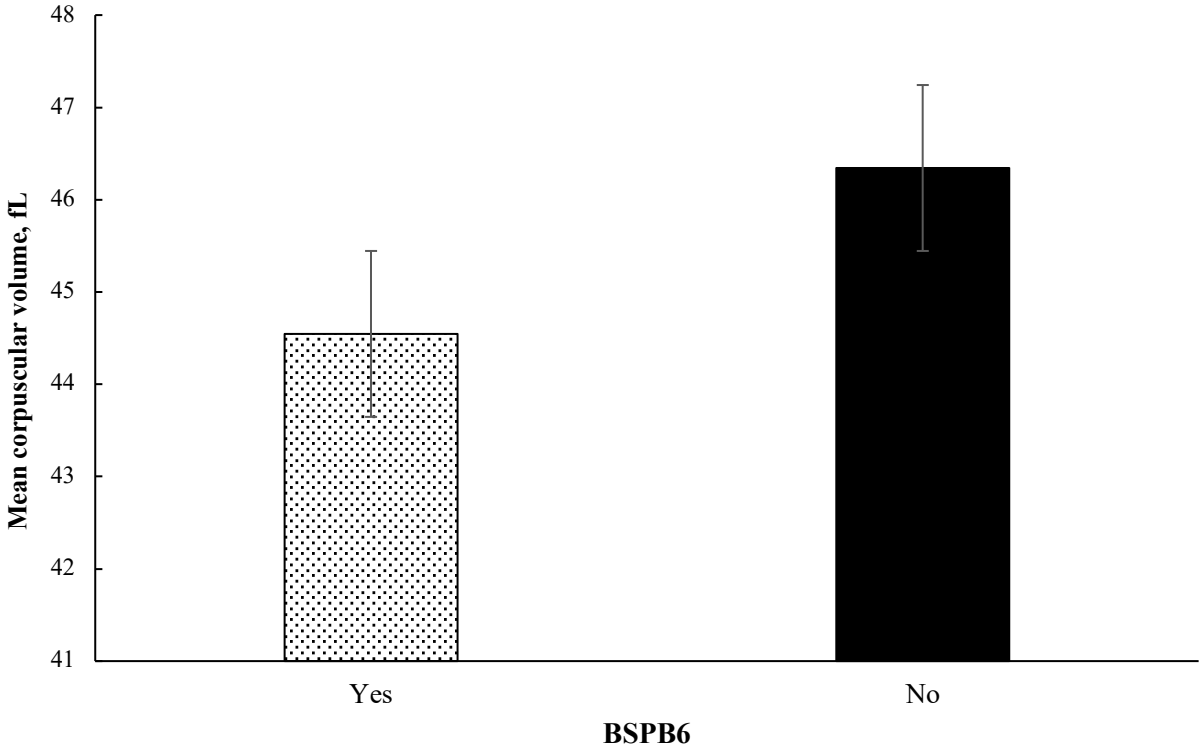

Figure 15. Effect of *Bacillus subtilis* PB6 supplementation on mean corpuscular volume of high-risk cattle during the feedlot finishing period ($P = 0.03$).

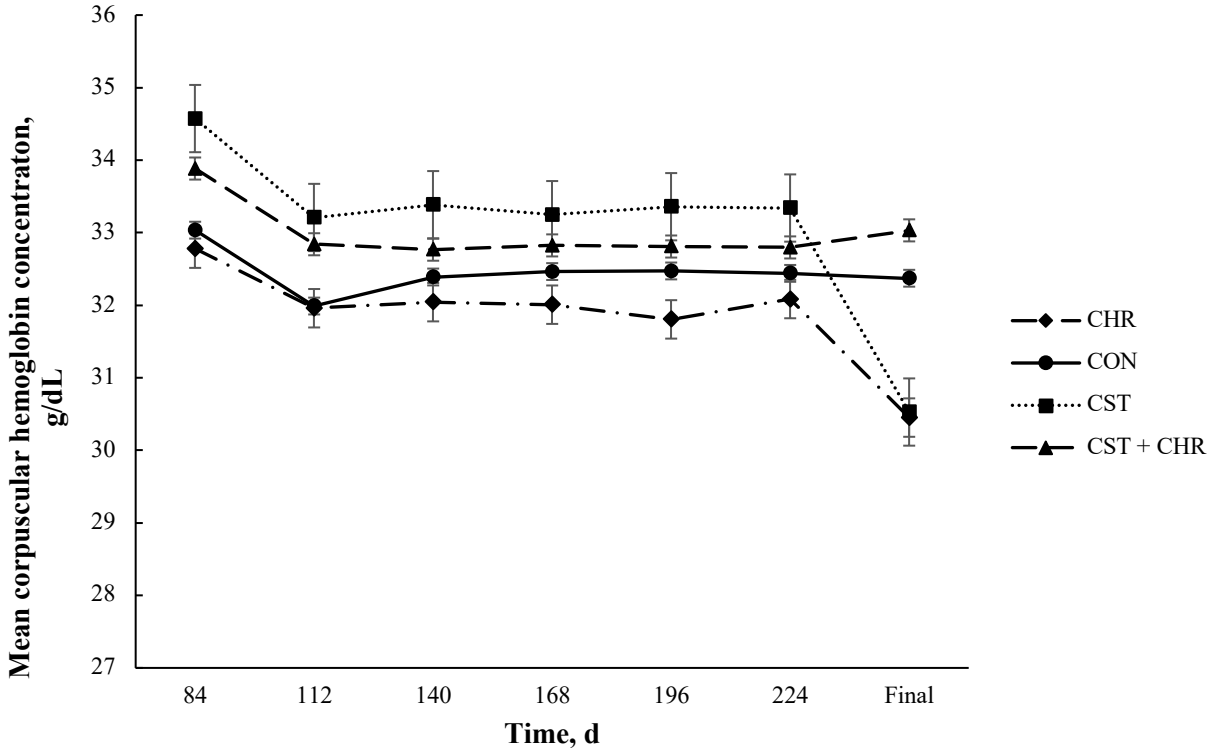

Figure 16. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on mean corpuscular hemoglobin concentration of high-risk cattle during the feedlot finishing period (P = 0.02).

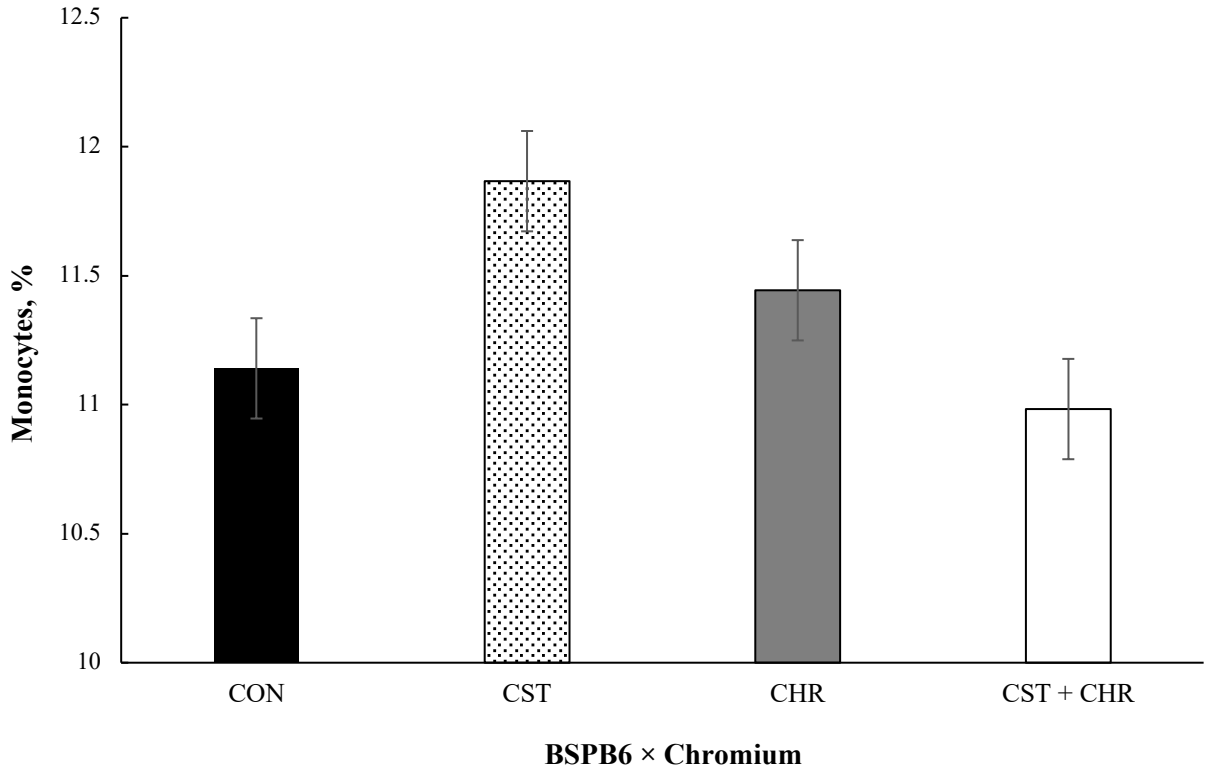

Figure 17. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on monocyte percentage of high-risk cattle during the feedlot finishing period ($P = 0.03$).

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