Effects of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on serum chemistry, complete blood count, and fecal *Salmonella* spp. count in high-risk cattle during the feedlot receiving and finishing periods[1,2]

Taylor M. Smock,[†] Kendall L. Samuelson,[†] Jim E. Wells,[‡] Kristin E. Hales,[‖] Jerilyn E. Hergenreder,[§] P. Whitney Rounds,[§] and John T. Richeson[†,3]

[†]Department of Agricultural Sciences, West Texas A&M University, Canyon, TX 79016; [‡]U.S. Department of Agriculture, Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, NE 68933; [‖]Department of Animal and Food Science, Texas Tech University, Lubbock, TX 79409; and [§]Animal Nutrition and Health, Kemin Industries, Inc., Des Moines, IA 50317

ABSTRACT: The study objective was to determine the effects of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on serum chemistry, complete blood count, and fecal *Salmonella* spp. count in high-risk beef cattle during a 56-d feedlot receiving period and the subsequent finishing period. Four truckload blocks of crossbred beef bulls (n = 300) and steers [n = 84; total n = 384; average initial body weight (BW) = 220 ± 16.2 kg] were sourced from regional auction markets and assigned randomly to treatments arranged in a 2 × 2 factorial. Blood samples were collected from two bulls nearest to the median BW on arrival in each pen (n = 96) and fecal samples were collected from cattle in block 3 (n = 96). The generalized complete block design consisted of 12 pen replications per treatment with pen as the experimental unit. Treatments were: 1) negative control (CON); 2) 13 g per animal daily of prepared *B. subtilis* PB6 product (CST); 3) 450 ppb dry matter (DM) chromium propionate (CHR); and 4) 13 g per animal daily of prepared *B. subtilis* PB6 product and 450 ppb DM chromium propionate (CST + CHR). Treatments were top dressed in feed bunks daily using 0.45 kg per animal ground corn carrier immediately following feed delivery. Data were analyzed using mixed models with repeated measures. Day affected all serum chemistry variables ($P \leq 0.03$) except total CO_2 (P = 0.34) and all complete blood count variables during receiving ($P \leq 0.02$) except percentage basophils ($P \geq 0.12$). During the overall receiving period, serum calcium was decreased (P = 0.02) by CHR. Cattle fed CHR had greater total leukocyte count (P = 0.04) and neutrophil count (P = 0.02) during the overall receiving period. Fecal *Salmonella* spp. count was markedly reduced in cattle fed CST on day 28 (P = 0.01) and overall (P = 0.07). Overall, these data provide metabolic and hematologic insight into the unique challenges presented by lightweight, high-risk feeder cattle. Notably, CST was found to be effective in mitigating fecal enumeration and presumably replication of *Salmonella* spp. in the gastrointestinal tract.

Key words: *Bacillus subtilis*, cattle, chromium propionate, stress

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[3]Corresponding author: jricheson@wtamu.edu

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INTRODUCTION

Auction-derived feeder cattle are considered high risk because a variety of predisposing factors lend them especially susceptible to digestive disturbances and bovine respiratory disease (BRD) associated morbidity and mortality. Stress and pathogen exposure induced by weaning, marketing, and shipment results in compromised immune function, alteration of and likely negative state of energy and protein metabolism, reduced appetite and growth performance, and compromised digestion and rumen function (Loerch and Fluharty, 1999; Richeson et al., 2019). Cattle feeders employ a variety of pharmacological interventions to address these challenges, such as antimicrobial metaphylaxis and BRD treatments. Although a wealth of research supports antimicrobial efficacy (Ives and Richeson, 2015), there is an increasing demand to reduce antimicrobial use and, therefore, to develop efficacious antimicrobial alternatives. In addition, treatment for BRD is a significant expense to the cattle feeder in the form of drugs, labor, and lost carcass value (Wilson et al., 2017).

An extensive variety of direct-fed microbial (DFM) products with various or unknown efficacy are available for use in feedlot cattle. Bacterial DFM in particular may have beneficial effects in the rumen by reducing acidosis and promotion of desirable microflora and exclusion of pathogenic organisms in the gut (Krehbiel et al., 2003). *Bacillus subtilis* PB6 is a bacterial DFM fed in spore form that germinates in the small intestine among the presence of bile salts and low pH. The organism then produces secondary metabolites and lipopeptide surfactants that target pathogenic organisms, causing cell wall lysis and eventual cell death (Lin et al., 2007).

Supplemental chromium propionate has been found to enhance insulin sensitivity, growth performance, and clinical health of feedlot cattle (Bernhard et al., 2012a, 2012b; Spears et al., 2012). Chromium propionate is the only form of supplemental chromium approved for use in beef cattle in the United States and may be included up to 0.50 mg/kg of diet dry matter (DM) (Spears et al., 2017).

The current study objective was to determine the effect of supplementation with the bacterial DFM *B. subtilis* PB6 and/or chromium propionate on serum chemistry, complete blood count, and fecal *Salmonella* spp. count of high-risk cattle with the hypothesis that *B. subtilis* PB6 and chromium propionate will alter metabolic and immunologic biomarkers and result in less *Salmonella* spp. via improved gastrointestinal tract (GIT) ecology and glucose utilization.

MATERIALS AND METHODS

Live animal procedures were approved by the West Texas A&M University Institutional Animal Care and Use Committee (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 processing, feeding, clinical health management, and carcass data collection procedures were described previously in a companion manuscript (Smock et al., 2020). Briefly, arrival processing included vaccination against viral respiratory, clostridial, and *Mannheimia haemolytica* pathogens, treatment for internal and external parasites using a parenteral anthelmintic, growth implant administration, testing for persistent infection with bovine viral diarrhea virus (PI-BVDV), castration and administration of an oral analgesic when applicable, and antimicrobial metaphylaxis with tilmicosin (Micotil, Elanco Animal Health). Diets were the same for all cattle regardless of experimental treatment and bunks were managed to implement a slick-bunk feeding program. Cattle were administered a terminal growth implant (Revalor XS, Merck Animal Health) on day 84. The average days on feed was 259. Cattle were evaluated daily for health and well-being by trained evaluators and medical treatment followed a preplanned case definition.

Application and Management of Experimental Treatment

Crossbred beef bulls (n = 300) and steers [n = 84; total n = 384; average initial body weight (BW) = 220 ± 16.2 kg] were sourced from regional auction markets in central and south Texas, composited at an order buying facility, and shipped approximately 11 h to WTRF on January 18, 2018 (Block 1), February 8,

2018 (Block 2), May 15, 2018 (Block 3), and June 20, 2018 (Block 4). Cattle were stratified within block by arrival BW, sex, and arrival health status determined via blood leukocyte differential (QScout BLD, Advanced Animal Diagnostics, Morrisville, NC) according to a proprietary algorithm and randomly assigned to treatment. Experimental treatments were: 1) negative control (CON); 2) 13 g per animal daily prepared *B. subtilis* PB6 product (CLOSTAT, Kemin Industries, Des Moines, IA; CST); 3) 450 ppb DM chromium propionate (KemTRACE Chromium, Kemin Industries; CHR); and 4) 13 g per animal daily prepared *B. subtilis* PB6 product and 450 ppb DM chromium propionate (CST + CHR). The feeding rate was established per the recommendation of the manufacturer. The CLOSTAT and KemTRACE Chromium 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, 500-kg-capacity 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 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 (days 0–56) and the finishing period (day 57 through harvest). Experimental treatments were applied throughout both study periods beginning on day 0 until harvest.

Twelve animals were removed from the trial for the following: 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 to the median arrival BW in each pen (n = 96) were selected to obtain 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 evacuated tube (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ) for the analysis of serum chemistry (Preventative Care Profile Plus, VetScan VS2, Abaxis, Union City, CA). Blood samples were allowed to clot, then centrifuged (Allegra 6R Centrifuge, Beckman Coulter, Brea, CA) at 2,791 × g for 20 min at 20 °C. Duplicate serum aliquots were decanted and stored at −20 °C for subsequent quantitative analysis of serum chemistry variables, including albumin, alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, chloride, creatinine, globulin, glucose, potassium, sodium, total carbon dioxide, total bilirubin, and total protein. A second blood sample was collected into an evacuated tube containing 7.2 mg ethylenediaminetetraacetic acid (BD Vacutainer) for the analysis of complete blood count (CBC) variables, including red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, white blood cells, count and percentage of neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and neutrophil:lymphocyte ratio. An automated hemocytometer (ProCyte Dx Hematology Analyzer; IDEXX Laboratories, Westbrook, ME) was used to conduct a CBC analysis. During the finishing period, a single blood sample was collected and analyzed for CBC.

Fecal Enumeration of Salmonella spp.

Fecal samples to determine colony-forming unit (CFU) count of *Salmonella* spp. were collected via rectal grab from cattle in block 3 (n = 96) on days 0, 28, and 196. Population analysis was conducted at the U.S. Meat Animal Research Center (MARC; Clay Center, NE). Fecal enumeration (≥200 CFU/g fecal sample) was determined using direct plating technique and fecal prevalence was determined using enrichment techniques for the designated samples as described previously (Agga et al., 2016).

Statistical Analysis

Data were analyzed as repeated measures using PROC MIXED (SAS v9.4, SAS Inst. Inc., Cary, NC). Experimental treatments were arranged in a 2 × 2 factorial as a generalized complete block design (n = 4 blocks; block = truckload), where factor was CST or CHR and level was "yes" (product was supplemented in the diet) or "no" (product was not supplemented in the diet). There were 12 pen replications per treatment (24 pen replications per main effect) and 8 animals per pen with 2 animals per pen used to quantify blood variables. Pen was the experimental unit for all dependent variables. For serum chemistry and CBC analyses, three- and two-way hierarchical interactions between CST, CHR, and day were evaluated for statistical significance first, then main effects.

Salmonella CFUs were transformed using square root to determine *P*-value and SEM. Fixed effects were analyzed as an interaction of CST × CHR, followed by the main effect of CST and CHR. Slicers were inserted to determine the effect of treatment within an individual day and are presented as *P*-value for TRT. The *P*-value for effect of time was presented as DAY. The interaction of overall treatment and overall day was presented in *P*-value TRT × DAY (Kaps and Lamberson, 2017).

For all analyses, differences between least squares means were determined using the least significant difference. If SEM was different among treatment means for the same dependent variable, the greatest SEM was reported. Results were considered statistically significant when $P \leq 0.05$, and tendencies were discussed when $0.05 > P \leq 0.10$.

RESULTS AND DISCUSSION

Feedlot growth performance, clinical health outcomes, and carcass trait results are presented in a companion manuscript (Smock et al., 2020).

Receiving Period Blood Parameter Analysis

Serum chemistry. Day affected all serum chemistry variables during the receiving period (days 0–56; Table 1; $P \leq 0.03$) except total CO_2 ($P \geq 0.34$). Supplementation with CHR increased serum AST (Table 2; $P = 0.04$), an enzyme associated with highly metabolic tissue, largely cardiac muscle, liver cells, and skeletal muscle cells. Serum AST increases when infection or injury causes cells of these tissues to lyse and AST is liberated into the blood. Increased levels of AST can be an indicator of liver damage, especially when paralleled with increased ALT (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 statistical differences among percentage abnormal livers (Smock et al., 2020); however, in addition to the relatively low sample size, liver score observed at slaughter does not account for microscopic damage or that which was repaired over time. Furthermore, the AST levels reported in the present study fall well within the normal range for bovine (Merck, 2019).

Serum calcium was decreased by CHR (Table 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). The observed serum calcium differences in the present study are small and probably not biologically relevant, especially considering no differences in serum albumin ($P \geq 0.11$), the protein bound to approximately half of serum calcium, were observed.

As a result of limited or no feed and water access and inflammation during marketing and

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

	Day					*P*-value		
Item[b]	Day 0	Day 14	Day 28	Day 56	SEM[c]	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

[a]Serum chemistry analysis conducted using VetScan VS2 Preventative Care Profile Plus, Abaxis, Union City CA.

[b]Items without common superscript differ ($P \leq 0.05$).

[c]Pooled standard error of the least square mean.

transportation, high-risk cattle arrive at the feedlot in a catabolic and chronically stressed state (Richeson et al., 2019). The observed serum chemistry day effects (Table 1) clearly indicate the metabolic disruption high-risk cattle possess at feedlot arrival and how they return to homeostasis later in the receiving period. For example, BUN was greater on day 0 compared to the subsequent interim intervals of the receiving period (Figure 1; linear, quadratic, and cubic, $P < 0.01$). As urea nitrogen is a waste product of protein catabolism, this could indicate that calves were liberating protein stores to compensate for inadequate dietary crude protein during marketing and transportation (Richeson et al., 2015). Furthermore, high-risk cattle experience increased pathogen exposure via

Table 2. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on serum chemistry variables[a] of beef cattle during the feedlot receiving period

	Treatment[b]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[c]	CST	CHR	CST × CHR
Blood urea nitrogen, mg/dL	8.22	8.46	8.79	8.57	0.30	0.97	0.26	0.46
Creatinine, mg/dL	1.19	1.19	1.15	1.13	0.04	0.70	0.17	0.77
Alanine aminotransferase, U/L	19.55	20.25	20.02	26.66	0.76	0.39	0.57	0.97
Alkaline phosphatase, U/L	111.36	107.49	104.53	113.6	7.81	0.74	0.96	0.41
Aspartate aminotransferase, U/L	69.38	70.39	77.51	75.78	3.20	0.91	0.04	0.67
Total bilirubin, mg/dL	0.2854	0.2948	0.2896	0.2823	0.0078	0.89	0.60	0.29
Glucose, mg/dL	94.74	92.76	93.95	99.34	3.06	0.58	0.25	0.23
Calcium, mg/dL	10.27	10.39	10.06	10.19	0.09	0.14	0.02	0.98
Total protein, g/dL	6.96	6.80	6.90	6.90	0.09	0.40	0.82	0.40
Albumin, g/dL	2.45	2.45	2.37	2.37	0.05	1.00	0.11	0.93
Globulin, g/dL	4.50	4.36	4.52	4.54	0.10	0.52	0.30	0.41
Sodium, mmol/L	135.82	135.00	135.91	136.05	0.31	0.27	0.07	0.12
Potassium, mmol/L	5.20	5.30	5.29	5.39	0.07	0.14	0.18	0.96
Chlorine, mmol/L	94.81	95.06	95.53	95.33	0.28	0.93	0.09	0.43
Total CO_2, mmol/L	25.72	26.59	25.57	25.07	0.44	0.70	0.06	0.12

[a]Serum chemistry analysis conducted using VetScan VS2 Preventative Care Profile Plus, Abaxis, Union City, CA.

[b]CON = negative control; CST = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product (CLOSTAT, Kemin Industries, Des Moines, IA); CHR = 450 ppb DM chromium propionate (KemTRACE Chromium, Kemin Industries); CST + CHR = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product + 450 ppb DM chromium propionate.

[c]Standard error of the least square mean.

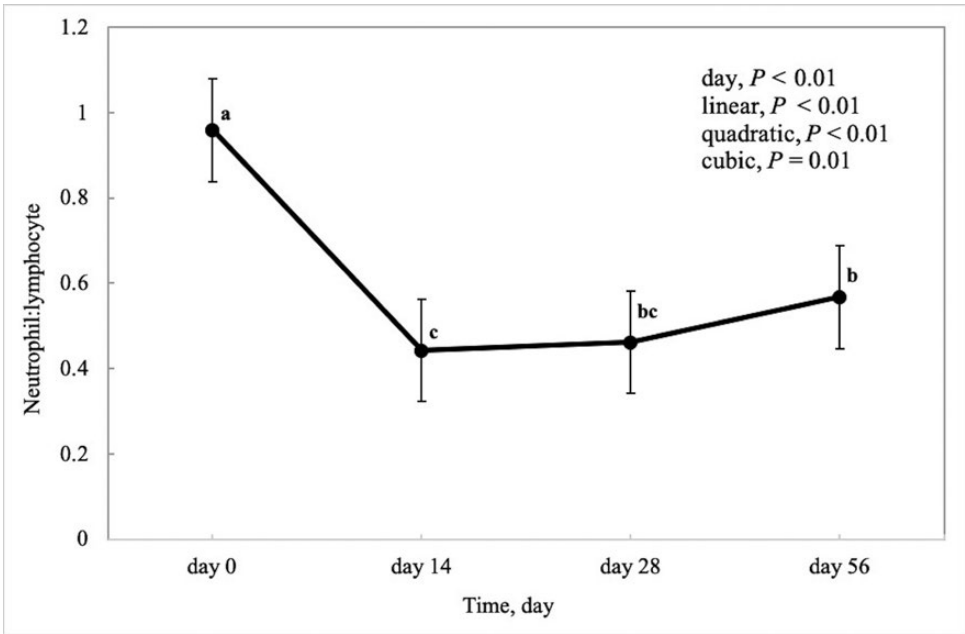

Figure 1. Effect of day on blood urea nitrogen in high-risk cattle. Day means with different superscript letters differ, $P < 0.01$.

commingling during marketing and transportation. A pathogenic challenge will 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). Conversely, total protein was least on day 0, followed by a sustained increase throughout the remainder of the receiving period (linear and quadratic, $P < 0.01$; cubic, $P = 0.02$). This could be indicative of low existing immunoglobulin (antibody) concentration at feedlot arrival, followed by antibody development from vaccination on day 28 or natural infection through the remainder of the receiving period. Furthermore, serum glucose was the least at day 0 than other time points during the receiving period (Figure 2; linear and quadratic, $P < 0.01$). This is intuitive 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 (NASEM, 2016). Collectively, the serum chemistry day effects observed in this study provide compelling evidence for potential use as predictive variables for BRD risk. Cattle were observed to be metabolically imbalanced at feedlot arrival, which resolved over time as cattle consumed feed and water and overcame stress. The temporal changes observed for several of the serum chemistry variables in this study also support their utility as a biomarker for BRD, but further research is needed to better understand metabolic status on arrival and health risk in cattle.

Complete Blood Count

All CBC variables were affected by day during the receiving period (Table 3; $P \leq 0.02$) except percentage basophils ($P \geq 0.12$). Particularly notable is neutrophil:lymphocyte ratio of 0.96 on day 0 (Figure 3; linear and quadratic, $P < 0.01$; cubic, $P = 0.01$), demonstrating the stress and inflammation high-risk cattle harbor at feedlot arrival. Neutrophil:lymphocyte has traditionally been used as a proxy for stress, where a threshold 1.0 ratio would be indicative of a highly stressed bovine (Zahorec, 2001).

Chromium propionate supplementation decreased (Table 4; $P = 0.05$) hemoglobin concentration. 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 are within the normal range for cattle. Supplementation with chromium propionate increased ($P \leq 0.05$) total white blood cell count, neutrophil count and percentage, and neutrophil:lymphocyte ratio. There was a CST × CHR interaction for hematocrit concentration ($P = 0.05$), where CON was greater than CST, CHR, and CST + CHR. Hematocrit is a measure of the

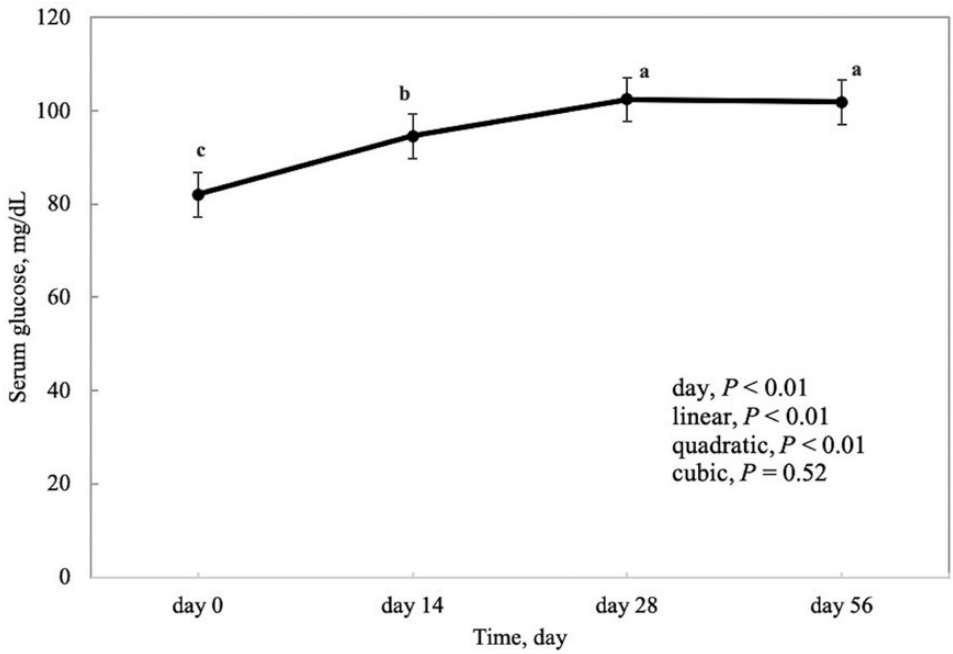

Figure 2. Effect of day on serum glucose concentration in high-risk cattle. Day means with different superscript letters differ, $P < 0.01$.

Table 3. Day effects of complete blood count variables[a] during the feedlot receiving period

	Day					*P*-value		
Item[b]	Day 0	Day 14	Day 28	Day 56	SEM[c]	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]	0.0396	<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
Neutrophil:lymphocyte	0.96[a]	0.44[c]	0.46[bc]	0.57[b]	0.04	<0.01	<0.01	0.01

[a]Complete blood count analysis was conducted using ProCyte Dx Hematology Analyzer, IDEXX Laboratories, Westbrook, ME.

[b]Items without common superscript differ ($P \leq 0.05$).

[c]Pooled standard error of the least square mean.

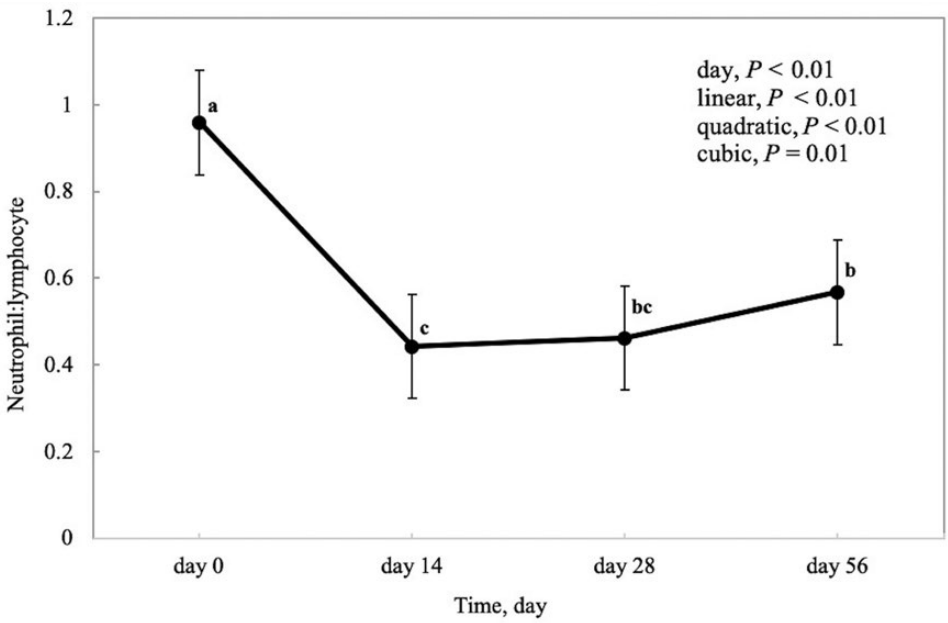

Figure 3. Effect of day on neutrophil:lymphocyte in high-risk cattle. This variable is used as a proxy for stress, where 1.0 indicates high stress. The reference range for bovine is 0.4–0.6. Day means with different superscript letters differ, $P \leq 0.03$.

proportion of total blood volume that is red and white blood cells and is closely linked to dehydration (Pagana and Pagana, 2006). Tomczak et al. (2018) orally drenched high-risk calves with water to rapidly address dehydration at feedlot arrival and observed potential for the practice to improve growth performance during the feedlot receiving period. Mean corpuscular volume was greater ($P < 0.01$) in CON compared to other treatments (CST × CHR; $P < 0.01$). Likewise, mean corpuscular hemoglobin was greater ($P < 0.01$) in CON (CST × CHR; $P = 0.01$). Supplementation with CST resulted in an

Table 4. Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on complete blood count variables[a] of beef cattle during the feedlot receiving period

	Treatment[b]					*P*-value		
Item[c]	CON	CST	CHR	CST + CHR	SEM[d]	CST	CHR	CST × CHR
Red blood cells, M/μL	9.55	9.75	9.44	9.82	0.23	0.14	0.93	0.66
Hemoglobin, g/dL	12.90[a]	12.52[ab]	12.06[b]	12.50[ab]	0.23	0.88	0.05	0.06
Hematocrit, %	39.93[a]	37.94[b]	37.31[b]	38.07[b]	—	0.37	0.07	0.05
Mean corpuscular volume, fL	44.96[a]	40.05[b]	38.35[b]	39.50[b]	1.31	0.03	<0.01	<0.01
Mean corpuscular hemoglobin, pg	14.57[a]	13.37[b]	12.88[b]	12.92[b]	0.38	0.02	<0.01	0.01
Mean corpuscular hemoglobin concentration, g/dL	32.37	33.03	32.38	32.91	0.12	<0.01	0.64	0.57
Platelets, K/μL	431.37	464.89	442.15	475.75	22.40	0.15	0.63	1.00
White blood cells, K/μL	10.93	10.38	11.60	11.23	0.37	0.20	0.04	0.81
Neutrophils, K/μL	3.23	2.90	3.72	3.56	0.21	0.27	0.02	0.69
Lymphocytes, K/μL	5.82	5.60	5.74	5.80	0.18	0.67	0.74	0.44
Monocytes, K/μL	1.76	1.76	1.90	1.76	0.09	0.39	0.40	0.41
Eosinophils, K/μL	0.12	0.18	0.13	0.16	0.03	0.17	0.90	0.71
Basophils, K/μL	0.0027	0.0024	0.0023	0.0014	0.0006	0.33	0.26	0.54
Neutrophils, %	28.11	26.91	30.01	29.71	—	0.53	0.05	0.71
Lymphocytes, %	53.93	54.09	52.17	52.74	—	0.76	0.19	0.86
Monocytes, %	16.86	17.28	16.76	16.11	—	0.84	0.27	0.35
Eosinophils, %	1.12	1.64	1.13	1.41	—	0.16	0.68	0.67
Basophils, %	0.0240	0.0250	0.0219	0.0129	—	0.40	0.17	0.50
Neutrophil:lymphocyte	0.58	0.55	0.67	0.63	0.04	0.39	0.03	0.82

[a]Complete blood count analysis was conducted using ProCyte Dx Hematology Analyzer, IDEXX Laboratories, Westbrook, ME.

[b]CON = control; CST = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product (CLOSTAT, Kemin Industries, Des Moines, IA); CHR = 450 ppb DM chromium propionate (KemTRACE Chromium, Kemin Industries); CST + CHR = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product + 450 ppb DM chromium propionate.

[c]Items without common superscript differ ($P \leq 0.05$).

[d]Pooled standard error of the least square mean.

increase of mean corpuscular hemoglobin concentration ($P < 0.01$). 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 (Table 4). 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 gluconeogenesis but reducing glucose utilization by skeletal muscle and white adipose tissue (Kuo et al., 2015). 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 supports the observed decrease in treatment for BRD in CHR-fed cattle (Smock et al., 2020). Furthermore, CHR did not influence receiving period performance but did decrease performance during the finishing period (Smock et al., 2020). 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 health and stress challenges occur, the satiety effects of glucose and insulin may provide insight to the diminished finishing period performance due to CHR (Smock et al., 2020).

Finishing Period Blood Parameter Analysis

During the finishing period, there was a CST × CHR interaction ($P = 0.04$; *data not shown*) for red blood cell count, where CHR was greatest ($P < 0.01$)

followed by CST + CHR, CON being intermediate, and CST being least. The hemoglobin concentration of CST was less ($P \leq 0.04$) than CON, CHR, and CST + CHR (CST × CHR $P < 0.01$). Likewise, hematocrit of CST was less ($P < 0.01$) compared to CON, CHR, and CST + CHR (CST × CHR, $P = 0.02$). Mean corpuscular volume was decreased by CST supplementation ($P = 0.03$). A CST × CHR × day interaction was observed for mean corpuscular hemoglobin concentration ($P = 0.02$). A CST × CHR interaction occurred for percentage monocytes ($P = 0.03$).

Fecal Salmonella *Prevalence*

Salmonella spp. count was markedly reduced in cattle fed CST and, especially, the CST treatment, both overall (Table 5; $P = 0.07$) and on day 28 (Table 6; $P = 0.01$) when cattle in the CON and CHR treatments were shedding the pathogen. A fecal *Salmonella* spp. count of ≥ 200 CFU/g represents enumerable levels in the fecal sample and indicates that the GIT is colonized and the animal is shedding the pathogen (Berry and Wells, 2016). 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; NASEM, 2016). These results further validate the observed improvements among CST-fed cattle in receiving period performance and overall clinical health (Smock et al., 2020) assuming that the protein and energy that would otherwise be diverted to combating a GIT bacterial infection can instead be used to facilitate tissue growth and/or allow immunological focus and resolution of respiratory infection. Broadway et al. (2020) evaluated the effect of CST in Holstein calves experimentally challenged with *Salmonella typhimurium*. Calves supplemented with CST had reduced concentration of *S. typhimurium* in the jejunum, ileum, and transverse colon 48 h postchallenge and numerically reduced concentration in all GIT tissues 96 h postchallenge. The ability of CST to withhold the colonization of the GIT by *Salmonella* spp. in the present study is a landmark observation among DFM products under natural exposure conditions and provides great potential for future research and use in production as the presence of *Salmonella* spp. is a food safety concern in addition to having

Table 5. Fecal *Salmonella* spp. prevalence in high-risk cattle fed supplemental *Bacillus subtilis* PB6 and/or chromium propionate

	Treatment[a]					*P*-value		
Item	CON	CST	CHR	CST + CHR	SEM[b]	CST	CHR	CST × CHR
Salmonella spp., CFU/g[c]	4,150	4	13,620	220	18.94	0.07	0.33	0.49

[a]CON = negative control; CST = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product (CLOSTAT, Kemin Industries, Des Moines, IA); CHR = 450 ppb DM chromium propionate (KemTRACE Chromium, Kemin Industries); CST + CHR = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product + 450 ppb DM chromium propionate.

[b]Standard error of the least square mean.

[c]*P*-value and SEM reported from data transformed using square root. Treatment means are back-transformed to reflect actual CFU/g.

Table 6. Fecal *Salmonella* spp. prevalence in high-risk cattle fed supplemental *Bacillus subtilis* PB6 and/or chromium propionate

	Treatment[a]					*P*-value[b]		
Item[c]	CON	CST	CHR	CST + CHR	SEM[d]	TRT	DAY	TRT × DAY
Salmonella spp., CFU/g[e]					32.81		0.04	0.68
Day 0	39	1	3	2	32.81	0.99	—	—
Day 28	12,406[ab]	2[b]	40,459[a]	636[b]	32.81	0.01	—	—
Day 196	5	7	397	25	32.81	0.99	—	—

[a]CON = negative control; CST = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product (CLOSTAT, Kemin Industries, Des Moines, IA); CHR = 450 ppb DM chromium propionate (KemTRACE Chromium, Kemin Industries); CST + CHR = 13 g per animal daily DM inclusion of prepared *Bacillus subtilis* PB6 product + 450 ppb DM chromium propionate.

[b]*P*-value for TRT was determined using slicers for experimental treatment within individual day. The *P*-value for DAY represents the effect of overall day. Interaction of TRT × DAY represents the interaction of overall treatment and overall day.

[c]Items without common superscripts differ ($P \leq 0.05$).

[d]Standard error of least square mean.

[e]*P*-value and SEM reported from data transformed using square root. Treatment means are back-transformed to reflect actual CFU/g.

potentially negative impacts on cattle health and performance (Gragg et al., 2013).

While the organism *B. subtilis* PB6 is not itself an antimicrobial, it does produce proteinaceous antimicrobial compounds in the lower gut, bacteriocin (Lin et al., 2007). Bacteriocins are ribosomally synthesized defense mechanisms produced by bacteria, which can kill or inhibit targeted bacteria without harming the host microbe with protection from specific immunity proteins. Multiple pathways exist for a bacteriocin to target specific bacteria. The bacteriocin differentiates into a proteinaceous colicin, an unmodified peptide, or a modified peptide. These compounds act on the target bacteria by entering through a receptor and disrupting the DNA or RNA matrix or by the activity of peptidoglycanase (Yang et al., 2014). As a broad species, *B.* organisms produce a variety of bacteriocins. This DFM product is not functional in the rumen as it is ingested in spore form, passes through the rumen, and germinates in the jejunum and intestinal tract via stimulation of acidic pH and the presence of bile salts (Lin et al., 2007).

CONCLUSIONS

Cattle fed the CST treatment had markedly less fecal *Salmonella* spp. count both overall and on day 28—a landmark observation in beef cattle under natural *Salmonella* spp. challenge, considering increasing concern of *Salmonella* spp. presence in beef at harvest. Day affected almost all serum chemistry and complete blood count variables during the receiving period, but they became relatively homeostatic by the end of the receiving period and throughout the finishing period, demonstrating the metabolic and immunologic disruption high-risk cattle experience at feedlot arrival that resolves over time. Collectively, these data suggest that feeding CST during the feedlot receiving period may be a promising management strategy that improves both performance and health outcomes in high-risk cattle. Furthermore, the CST treatment was effective in mitigating fecal *Salmonella* spp. colonization during both the feedlot receiving and finishing period.

Conflict of interest statement. J. E. Hergenreder and P. W. Rounds are employed by Kemin Industries, Inc. that manufactures the products evaluated in the current study.

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