# Effects of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on clinical health, growth performance, and carcass traits of high-risk cattle during the feedlot receiving and finishing periods[1]

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**ABSTRACT:** The study objective was to determine the effects of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on health, growth performance, and carcass characteristics of 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; body weight (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 12 pen replications per treatment with pen as the experimental unit. Treatments were: 1) placebo control (CON); 2) 13 g per animal daily of *B. subtilis* PB6 (CST); 3) 450 ppb dry matter (DM) chromium propionate (CHR); and 4) 13 g per animal daily of *B. subtilis* PB6 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. During the receiving period, dry matter intake (DMI) increased ($P \leq 0.03$) for CST during each interim period. Overall receiving period daily DMI was 0.35 kg per animal greater for CST ($P = 0.01$). Cattle fed CST had greater ($P \leq 0.06$) BW on days 14, 28, and 56. Likewise, average daily gain (ADG) was improved for CST from day 0 to 14 ($P = 0.04$) and for the overall receiving period (days 0–56; $P = 0.04$). From days 0 to 14, CST tended ($P = 0.08$) to increase gain:feed. During the finishing period, CHR reduced ($P = 0.02$) final BW and ADG (day 56 to final; $P = 0.01$) and ADG was less for CHR over the entire feeding period (day 0 to final; $P = 0.03$). The main effect of both CST ($P = 0.02$) and CHR ($P = 0.03$) decreased the overall treatment rate for bovine respiratory disease (BRD), and CST reduced overall antimicrobial treatment cost by $3.50 per animal compared to CON ($P = 0.03$). Hot carcass weight (HCW) decreased ($P = 0.01$) in cattle fed CHR. The percentage of edible livers tended to increase (CST × CHR; $P = 0.08$) in the CST treatment. Feed intake and growth performance outcomes during the receiving period were improved by CST but not CHR supplementation. However, both CST and CHR supplementation decreased the BRD morbidity rate. During the finishing period, performance and HCW were reduced in cattle supplemented with CHR.

**Key words:** *Bacillus subtilis*, cattle, chromium, health, performance



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## INTRODUCTION

Upon feedlot arrival, high-risk beef cattle have experienced persistent stressors likely over the course of several days, including weaning, transportation, dehydration, commingling coupled with pathogen exposure, castration, and nutrient deficit (Duff and Galyean, 2007). As a result, microbes in both the rumen and gastrointestinal tract are altered, leading to decreased growth performance and increased morbidity and mortality (Krehbiel et al., 2003). Morbidity and mortality events associated with bovine respiratory disease (BRD) result in great economic losses incurred by the producer through labor and treatment expenses and negative impacts on feedlot performance and subsequent carcass merit (Duff and Galyean, 2007). Fulton et al. (2002) reported that cattle treated once for BRD returned $40.64 less than nontreated cohorts, those treated twice returned $58.35 less, and cattle treated three or more times returned $291.93 less. Bovine respiratory disease is multifactorial, influenced by not only viral and bacterial pathogens but also a host of preweaning and postweaning predisposing factors, including marketing stress, preshipment and arrival management, and the plane of nutrition preweaning and postweaning (Duff and Galyean, 2007).

High-risk, lightweight cattle are especially prone to morbidity and mortality events associated with BRD and digestive disturbances at feedlot arrival. Access to feed and water throughout the marketing process is typically 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 (BW) in the first 2 wk following arrival, which further exacerbates immunosuppression due to inadequate nutrient intake (Nagaraja et al., 1998; Richeson et al., 2019). 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 dry matter intake (DMI) being most pronounced within the initial 4 d following arrival (Sowell et al., 1998, 1999). Cattle treated for BRD multiple times during the receiving period will not achieve the same carcass value as nontreated 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).

Nonantimicrobial alternatives to address these health and nutrition challenges in high-risk, newly received feedlot cattle are of considerable interest to the beef production industry, driven in part by consumer preference. Feed additives, such as bacterial direct-fed microbials (DFM) and trace mineral supplements, are designed to address this need. Krehbiel et al. (2003) proposed that bacterial DFM improve feedlot health and performance via alteration of the intestinal microflora with potential effects on ruminal fermentation, competitive exclusion of pathogens for nutrients, influenced gut permeability, and improved immune response (Duff and Galyean, 2007). *Bacillus subtilis* PB6 is a bacterial DFM that passes through the rumen as a spore and initiates activity in the small intestine and lower gut when stimulated by a low pH and the presence of bile salts (Lin et al., 2007). The U.S. Food and Drug Administration (USDA) Center for Veterinary Medicine permitted chromium propionate for use as a source of supplemental chromium in 2009, and it has since been found to enhance insulin sensitivity, growth performance, and health of feedlot cattle (Bernhard et al., 2012a, 2012b; Spears et al., 2012). Chromium propionate may be included up to 0.50 mg/kg of diet dry matter (DM) (Spears et al., 2017).

Therefore, the current study objective was to determine the effect of supplementation with the bacterial DFM *B. subtilis* PB6 and/or chromium propionate on feedlot growth performance, clinical health, and subsequent carcass traits of high-risk cattle with the hypothesis that *B. subtilis* PB6 and chromium propionate will improve feedlot health and performance, and the combination of these will result in additive improvement.

## MATERIALS AND METHODS

All live animal 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 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). Immediately following arrival (day −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 postmetaphylactic 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 (Table 1) 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 day −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 were PI-BVDV negative. Cattle were sorted into their allotted treatment pens and offered a starter diet at 1.5% of the initial BW beginning on day 0.

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

| Item | Starter diet | Finisher diet[b] |
|---|---|---|
| Ingredient, % of DM | | |
| Sweet bran[c] | 56.41 | 43.47 |
| Corn stalks, chopped | 19.05 | 4.30 |
| Steam-flaked corn | 13.90 | 37.34 |
| Brix molasses[d] | 7.30 | 7.31 |
| Corn oil | — | 3.82 |
| Grower supplement[e] | 3.34 | — |
| Finisher supplement[f] | — | 3.76 |
| Nutrient analysis, DM % | | |
| $NE_m$, Mcal/kg | 1.79 | 2.20 |
| $NE_g$, Mcal/kg | 1.15 | 1.52 |
| Crude protein, % | 16.50 | 14.60 |
| Calcium, % | 1.03 | 0.97 |
| Phosphorus, % | 0.64 | 0.56 |

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

[b]Transition from starter diet to finisher diet began on day 57 using a two-ration split system, where, in the total daily feed, call per pen was split as 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.

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

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

[e]Grower supplement formulated to supply 26.47% Ca, 0.07% P, 7.69% Na, 8.83% K, 1.50% Mg, 0.23% S, 1,094 mg/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).

[f]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).

### *Treatment Application*

Experimental treatments were: 1) placebo control (CON); 2) 13 g per animal daily *B. subtilis* PB6 (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 *B. subtilis* PB6 and 450 ppb DM chromium propionate (CST + CHR). Feeding rate was established per the recommendation of the product 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 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 (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.

### *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 (8 animals per pen). Diets were the same for all cattle regardless of treatment (Table 1). Bunks were monitored for residual feed at 1600 and 0700 hours daily and feed calls were managed to implement a slick-bunk feeding program. Cattle were fed once daily beginning at 0730 hours 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) for DM analysis. On day 57, cattle began transition to a finisher diet using a two-ration system such that the 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 28–33 d on feed depending on block and was formulated to supply 250 mg per animal daily.

Interim BW was collected on days 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 on the day of harvest. Average days on feed was 259 (block 1 = 242 d; block 2 = 248 d; block 3 = 282 d; block 4 = 264 d).

Cattle were visually evaluated for health and well-being daily by trained WTRF personnel blinded to experimental treatment. Animals were diagnosed with 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 the 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); and 3) ceftiofur crystalline free acid (Excede, Zoetis). All antimicrobials were administered according to label instructions with consideration of Beef Quality Assurance standards.

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).

### *Harvest and Carcass Data*

All cattle were transported to a commercial abattoir for harvest. Hot carcass weight (HCW), liver scores, and lung scores were collected by trained personnel from the WTAMU Beef Carcass Research Center. The 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 (QG), fat thickness, ribeye area, and percentage kidney, pelvic, and heart fat. Yield grade (YG) was calculated using the USDA regression equation (USDA-ARS, 2017).

### *Statistical Analysis*

Data were analyzed using the MIXED and GLIMMIX procedures of SAS 9.4 (SAS Inst. Inc., Cary, NC) where appropriate. Experimental treatments were arranged in a 2 × 2 factorial as a generalized complete block design ($n$ = 4 blocks; block = truckload) 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. Interactions between CST, CHR, and/or day were evaluated for statistical significance first, then main effects. Differences between least square means were determined using the least significant difference. Results were considered statistically significant when $P \leq 0.05$. Tendencies were identified when $0.05 < P \leq 0.10$.

## RESULTS AND DISCUSSION

### *Clinical Health Outcomes*

The main effects of both CST and CHR reduced treatment rate for BRD (Table 2; $P \leq 0.03$) with the lowest numerical proportion treated being CST + CHR (22.6% less than CON), suggesting a potential additive effect. There were no statistical differences ($P \geq 0.10$) among second and third BRD treatments or respiratory-associated mortality. The lack of statistical difference is likely associated with less frequent occurrence of mortality and secondary morbidity events and small sample size. Days to first treatment were reduced by CHR by 4 d compared to CON ($P = 0.04$) 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 the rectal temperature at second BRD treatment such that CST + CHR had the greatest rectal temperature and CON had the least. There was a tendency ($P = 0.09$) for CHR to have the greatest rectal temperature at third BRD treatment. Antimicrobial treatment cost was reduced by CST ($P = 0.03$) in the amount of $6.30 per animal in CST + CHR and $3.95 per animal in the CST treatment when compared to CON.

Growing evidence supports that unique gut and lung microbial communities are linked in a gut–lung axis, which may include both host–microbe and microbe–microbe interactions, as well as interkingdom crosstalk, which ultimately aids in the maintenance of homeostasis and disease evolution (Enaud et al., 2020). The composition of the gut and lung microbiome are correlated and remain so throughout the life span, indicative of a host-wide microbial network (Grier et al., 2018). For example, Madan et al. (2012) and Liu et al. (2017) reported that fecal transplantation of rats induced changes in the lung microbiome. Likewise, mice challenged with exogenous lipopolysaccharide to the lungs consistently experience disturbance of their gut microbiome (Sze et al., 2012). The gut microbiome is populous and extensive in its impact. Whole bacteria, bacterial fragments, and metabolites from the gut may enter systemic circulation via the mesenteric lymphatic system, which gives the gut microbiome a pathway to modulate the immune response in the lungs (Bingula et al., 2017; McAleer and Kolls, 2018). For these reasons, a probiotic regimen is increasingly encouraged as a novel therapy in human patients with chronic and acute respiratory diseases (Enaud et al., 2020).

Clinical health outcome as a result of providing supplemental chromium in the diet is variable in the

**Table 2.** 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[a] | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | CON | CST | CHR | CST + CHR | SEM[b] | CST | CHR | CST × CHR |
| BRD1[c], % | 43.9 | 34.6 | 35.8 | 21.3 | — | 0.02 | 0.03 | 0.59 |
| BRD2[d], % | 23.4 | 11.9 | 16.3 | 12.4 | — | 0.10 | 0.47 | 0.40 |
| BRD3[e], % | 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 | | | | | | | | |
| First treatment | 13.4 | 14.3 | 9.2 | 11.0 | 2.2 | 0.44 | 0.04 | 0.81 |
| Second treatment | 21.1 | 17.4 | 21.6 | 17.5 | 3.2 | 0.13 | 0.91 | 0.93 |
| Third treatment | 33.2 | 19.9 | 25.2 | 24.4 | 4.5 | 0.07 | 0.63 | 0.13 |
| Rectal temperature, °C | | | | | | | | |
| First treatment | 39.6 | 39.7 | 39.7 | 40.0 | 0.21 | 0.16 | 0.23 | 0.83 |
| Second treatment | 39.6 | 39.3 | 39.4 | 40.0 | 0.31 | 0.68 | 0.28 | 0.09 |
| Third treatment | 39.3 | 40.0 | 40.2 | 40.1 | 0.34 | 0.29 | 0.09 | 0.31 |
| Antimicrobial treatment cost, $/hd[f] | 12.45 | 8.50 | 9.62 | 6.15 | 1.66 | 0.03 | 0.13 | 0.87 |

[a]CON = placebo control; CST = 13 g per animal daily DM inclusion of *Bacillus subtilis* PB6 (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 *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[b]Pooled standard error of least square mean.

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

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

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

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

existing literature. Reduction in BRD morbidity was observed by Moonsie-Shageer and Mowat (1993) and Bernhard et al. (2012b). Conversely, Kegley et al. (1997) reported that supplemental chromium did not effect serum cortisol, leukocyte percentage or concentration, or antibody titer against infectious rhinotracheitis virus. Similarly, Pollard et al. (2002) did not observe supplemental chromium to have long-term effects on serum cortisol.

When stimulated by pathogen-associated molecular patterns, phagocytes (neutrophils and macrophages) engulf pathogens and undergo a series of processes collectively referred to as the respiratory burst to eradicate the 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 nicotinamide adenine dinucleotide phosphate (NADPH). Glucose is then metabolized to regenerate NADPH consumed by the enzyme catalyzation (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 the incidence of 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.

### *Feedlot growth performance*

Although the numerical difference in initial BW between treatments was only 1 kg, a CST × CHR interaction was observed (Table 3; $P = 0.04$); however, this small difference is not biologically relevant. During the receiving period, cattle fed CST had greater BW on days 14, 28, and 56 and were 11 kg heavier than CON at the end of the receiving period ($P \leq 0.06$). Likewise, average daily gain (ADG) was improved in cattle fed CST from day 0 to 14 ($P = 0.04$) and throughout the receiving period

**Table 3.** Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on growth performance, DMI, and feed efficiency of beef steers during the feedlot receiving period

| | Treatment[a] | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | CON | CST | CHR | CST + CHR | SEM[b] | CST | CHR | CST × CHR |
| BW, kg | | | | | | | | |
| Initial[c] | 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 14–28 | 1.12 | 1.20 | 1.16 | 1.12 | 0.11 | 0.84 | 0.86 | 0.62 |
| Days 28–56 | 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 | | | | | | | | |
| Days 0–14 | 3.50 | 3.75 | 3.52 | 3.80 | 0.11 | 0.02 | 0.75 | 0.87 |
| Days 14–28 | 5.13 | 5.55 | 5.13 | 5.50 | 0.17 | 0.02 | 0.86 | 0.90 |
| Days 28–56 | 6.61 | 7.12 | 6.80 | 7.08 | 0.16 | 0.03 | 0.55 | 0.41 |
| Days 0–56 | 5.46 | 5.88 | 5.58 | 5.86 | 0.13 | 0.01 | 0.70 | 0.60 |
| G:F, kg | | | | | | | | |
| Days 0–14 | 0.2241 | 0.2551 | 0.2183 | 0.2681 | 0.0230 | 0.08 | 0.89 | 0.70 |
| Days 14–28 | 0.2082 | 0.2098 | 0.2209 | 0.1833 | 0.0192 | 0.35 | 0.72 | 0.32 |
| Days 28–56 | 0.2253 | 0.2367 | 0.2291 | 0.2250 | 0.0066 | 0.56 | 0.58 | 0.23 |
| Days 0–56 | 0.2223 | 0.2337 | 0.2274 | 0.2245 | 0.0058 | 0.45 | 0.74 | 0.22 |

[a]CON = placebo control; CST = 13 g per animal daily DM inclusion of *Bacillus subtilis* PB6 (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 *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[b]Standard error of least square mean.

[c]Initial = average of day −1 and day 0 BW.

(days 0–56; $P = 0.04$). Cattle fed CST had greater DMI at each interim period ($P \leq 0.03$) and during the overall receiving period by 0.42 kg compared to CON ($P = 0.01$). 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 days 196 and 224 (Table 4; $P \leq 0.09$). In addition, the main effect of CHR reduced ($P = 0.02$) the final BW by 9 kg in cattle fed treatment CHR compared to CON and 11 kg in treatment CST + CHR. Cattle fed CST had lower ADG from day 112 to 140 ($P = 0.05$), and those fed CHR tended ($P = 0.07$) to have lower ADG from day 168 to 196 and day 224 to final. The ADG of cattle fed CHR was lower 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 5; $P = 0.03$) such that the CST and CHR treatments had the greatest DMI, while CST + CHR had the least. From day 224 to final, cattle fed CHR had less DMI. Feed efficiency was reduced 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 reduced by CHR (day 56 to final; $P = 0.09$). A similar study by Wilson et al. (2019) did not observe CST improve BW, ADG, or DMI during either the receiving or finishing periods; however, CST-supplemented cattle tended to have improved feed efficiency over both feeding periods. *Bacillus subtilis* PB6 DFM alone and in combination with other *Bacillus* organism DFM have been reported to improve growth performance and gut integrity of nursery pigs (Cai et al., 2015; Brooks and Kim, 2017; Payling et al., 2017) and have anticlostridial activity against the causative pathogen in necrotic enteritis of poultry (Teo and Tan, 2005). Given the limited existing literature in beef cattle, further research in supplementing *B. subtilis* PB6 is warranted.

Dry matter intake is characteristically low within the first 2 wk of feedlot arrival and is coupled with immunosuppression and negative energy balance induced by traditional beef marketing channels (Nagaraja et al., 1998, Richeson et al., 2019). Sowell et al. (1999) observed that steers diagnosed with BRD spent 30% less time eating than healthy cohorts, especially within the 4-d following feedlot arrival. The observed increase in DMI during the receiving period for CST in the present study may present a nonantimicrobial

**Table 4.** Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on growth performance of beef cattle during the feedlot finishing period

| | Treatment[a] | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | CON | CST | CHR | CST + CHR | SEM[b] | 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[c] | 636 | 644 | 627 | 624 | 5.61 | 0.62 | 0.02 | 0.34 |
| ADG, kg | | | | | | | | |
| Days 56–84 | 1.95 | 1.83 | 1.83 | 1.81 | 0.05 | 0.16 | 0.14 | 0.46 |
| Days 84–112 | 2.05 | 2.12 | 2.10 | 2.14 | 0.05 | 0.24 | 0.56 | 0.76 |
| Days 112–140 | 1.87 | 1.81 | 1.87 | 1.67 | 0.06 | 0.05 | 0.25 | 0.27 |
| Days 140–168 | 1.76 | 1.73 | 1.72 | 1.69 | 0.05 | 0.60 | 0.52 | 0.95 |
| Days 168–196 | 1.52 | 1.47 | 1.42 | 1.39 | 0.05 | 0.50 | 0.07 | 0.76 |
| Days 196–224 | 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[d] to final | 1.61 | 1.63 | 1.57 | 1.56 | 0.02 | 0.73 | 0.03 | 0.40 |

[a]CON = placebo control; CST = 13 g per animal daily DM inclusion of *Bacillus subtilis* PB6 (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 *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[b]Standard error of least square mean.

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

[d]Initial = average of day −1 and day 0 BW.

**Table 5.** Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on DMI and feed efficiency of beef cattle during the feedlot finishing period

| | Treatment[a] | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | CON | CST | CHR | CST + CHR | SEM[b] | CST | CHR | CST × CHR |
| DMI, kg/d | | | | | | | | |
| Days 56–84 | 6.62 | 6.79 | 6.56 | 6.69 | 0.13 | 0.28 | 0.52 | 0.89 |
| Days 84–112 | 8.44 | 8.76 | 8.56 | 8.50 | 0.14 | 0.35 | 0.61 | 0.14 |
| Days 112–140 | 9.21 | 9.48 | 9.48 | 9.07 | 0.15 | 0.68 | 0.64 | 0.03 |
| Days 140–168 | 9.62 | 9.57 | 9.60 | 9.37 | 0.15 | 0.34 | 0.47 | 0.54 |
| Days 168–196 | 9.25 | 9.20 | 9.04 | 8.80 | 0.19 | 0.45 | 0.12 | 0.62 |
| Days 196–224 | 9.24 | 9.32 | 9.14 | 9.02 | 0.17 | 0.91 | 0.27 | 0.56 |
| Day 224 to final[c] | 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 | | | | | | | | |
| Days 56–84 | 0.3067 | 0.2787 | 0.2858 | 0.2759 | 0.0076 | 0.02 | 0.13 | 0.24 |
| Days 84–112 | 0.2462 | 0.2411 | 0.2453 | 0.2516 | 0.0062 | 0.93 | 0.45 | 0.36 |
| Days 112–140 | 0.2070 | 0.1946 | 0.1994 | 0.1883 | 0.0052 | 0.03 | 0.19 | 0.90 |
| Days 140–168 | 0.1829 | 0.1814 | 0.1790 | 0.1799 | 0.0047 | 0.96 | 0.57 | 0.80 |
| Days 168–196 | 0.1649 | 0.1598 | 0.1563 | 0.1587 | 0.0046 | 0.78 | 0.30 | 0.42 |
| Days 196–224 | 0.1601 | 0.1616 | 0.1590 | 0.1628 | 0.0060 | 0.65 | 0.99 | 0.85 |
| Day 224 to final | 0.1451 | 0.1478 | 0.1343 | 0.1465 | 0.0053 | 0.17 | 0.26 | 0.37 |
| Day 56 to final | 0.1978 | 0.1936 | 0.1913 | 0.1921 | 0.0023 | 0.46 | 0.09 | 0.29 |
| Day 0 to final | 0.2013 | 0.1996 | 0.1967 | 0.1971 | 0.0022 | 0.77 | 0.12 | 0.65 |

[a]CON = placebo control; CST = 13 g per animal daily DM inclusion of *Bacillus subtilis* PB6 (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 *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[b]Standard error of least square mean.

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

alternative to mitigate the negative energy balance of high-risk cattle coupled with the potential to reduce BRD morbidity. Wilson et al. (2019) observed no differences in BRD-related morbidity or mortality in cattle supplemented with CST or control, but the calves used in that study did not have a significant natural BRD challenge as the overall BRD morbidity rate was only 10%. In comparison, the overall BRD morbidity rate in the present study was 34%.

Previous literature indicates supplemental chromium to have variable results on feedlot performance. Increases in ADG and feed efficiency were observed in stressed, lightweight feeder calves supplied high-chromium yeast by Chang and Mowat (1992) and Moonsie-Shageer and Mowat (1993). No performance differences were observed in calves under shipping stress fed either high-chromium yeast or a chromium–nicotinic acid complex (Kegley and Spears, 1995); however, a subsequent study by Kegley et al. (1997) observed a tendency for greater ADG over an 80-d feeding period in cattle fed the chromium–nicotinic acid complex. In a dose-titration study using high-chromium yeast, no differences were observed during the receiving period; however, during the subsequent finishing period, steers fed the greatest chromium dose had reductions in final BW, ADG, and gain:feed (G:F) versus steers fed the lower dosage or control (Pollard et al., 2002). Bernhard et al. (2012b) observed an overall increase in ADG and improved feed efficiency during a 56-d receiving period for cattle supplemented chromium propionate. In other instances, researchers have reported no growth performance differences in cattle fed supplemental chromium in the form of an amino acid chelate containing 3.04% chromium (Mathison and Engstrom, 1995), chromium yeast (Danielsson and Pehrson, 1998; Swanson et al., 2000), chromium-L-methionine (Kegley et al., 2000), and chromium propionate (Kneeskern et al., 2016; Van Bibber-Krueger et al., 2016).

### *Carcass Characteristics*

Hot carcass weight was reduced by CHR (Table 6; $P = 0.01$). There tended ($P = 0.10$) to be a CST × CHR interaction for dressing percentage

**Table 6.** Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on carcass traits of beef cattle

| | Treatment[a] | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | CON | CST | CHR | CST + CHR | SEM[b] | CST | CHR | CST × CHR |
| Hot carcass weight, kg | 397 | 401 | 394 | 389 | 3.84 | 0.81 | 0.01 | 0.30 |
| DP | 62.64 | 62.69 | 62.91 | 62.25 | 0.23 | 0.18 | 0.64 | 0.10 |
| Marbling number[c] | 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 |
| Longissimus dorsi area, $cm^2$ | 96.13 | 96.58 | 95.48 | 96.64 | 1.03 | 0.45 | 0.79 | 0.72 |
| Calculated YG[d] | 2.82 | 2.84 | 2.76 | 2.64 | 0.08 | 0.52 | 0.11 | 0.39 |

[a]CON = placebo control; CST = 13 g per animal daily DM inclusion of *Bacillus subtilis* PB6 (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 *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

[b]Standard error of least square mean.

[c]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 the degree of marbling within marbling score.

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

**Table 7.** Effect of *Bacillus subtilis* PB6 and/or chromium propionate supplementation on categorical carcass traits of beef cattle

| | Treatment[a] | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item[b] | CON | CST | CHR | CST + CHR | CST | CHR | CST × CHR |
| QG, % | | | | | | | |
| 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 |
| YG, % | | | | | | | |
| 1[c] | 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[d], % | | | | | | | |
| 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 |

[a]CON = placebo control; CST = 13 g per animal daily DM inclusion of *Bacillus subtilis* PB6 (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 *Bacillus subtilis* PB6 + 450 ppb DM chromium propionate.

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

[c]Due to low frequency of YG 1, means presented are calculated rather than least square means.

[d]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 percentage of lung missing.

(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 7; $P = 0.09$). An interaction of CST × CHR existed such that the percentage of YG 1 ($P < 0.01$) carcasses was greater in cattle fed CST and CHR ($P = 0.05$ and $P = 0.01$, respectively), with CST + CHR being intermediate and CON being the least. Furthermore, a CST × CHR interaction was observed among percentage YG 2 ($P = 0.03$) carcasses where, numerically ($P \geq 0.07$), CON and CST + CHR had a greater percentage. Interaction of CST × CHR ($P = 0.05$) was observed to numerically increase the percentage of YG 4 or 5 carcasses among CST and CHR, with CON being intermediate and CST + CHR being the least ($P \geq 0.05$). The percentage of edible livers tended to increase (CST × CHR; $P = 0.08$) in the CST treatment.

No differences in carcass characteristics in cattle supplemented with CST were observed by Wilson et al. (2019). In cattle fed supplemental chromium, no difference in HCW or DP was observed by Danielsson and Pehrson (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 that steers supplemented with 200 ppb chromium had greater 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 Longissimus muscle (LM) area. In a study with lambs fed chromium yeast (Yan et al., 2008), both chromium supplemented treatments (400 and 800 ppb) deposited less intramuscular fat than control. 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 eight additional days on feed to achieve the same targeted back fat 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; 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.

## 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 and carcass traits were not affected. Supplementation of both CST and CHR reduced the incidence of BRD morbidity. Performance during the finishing period and HCW was reduced by the main effect of CHR. These data suggest that feeding CST during the feedlot receiving period may be a promising alternative to antimicrobials for the improvement of health and performance outcomes in high-risk cattle.

*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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