Beef Day 2021

Evaluation of Bacillus subtilis PB6 probiotic (CLOSTAT®500) on feedlot phase growth performance, efficiency of dietary net energy utilization, and fecal and subiliac lymph node Salmonella prevalence in spring placement yearling beef steers fed in southeastern South Dakota

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Objective

The objective of this research was to determine the influence of *Bacillus subtilis* PB6 administration had on growth performance, carcass traits, and *Salmonella* prevalence in yearling steers placed on feed in March in southeastern South Dakota that were not subjected to marketing or environmental stressors during the finishing phase.

Study Description

Yearling crossbred beef steers (N = 238; initial shrunk BW = 886 ± 68.8 lbs) were used in a 140-d finishing study at the Southeast Research Farm (SERF) in Beresford, SD. Steers were allotted to one of 24 pens (N = 9 to 10 steers/pen) and assigned to one of two dietary treatments (12 pens/treatment): no probiotic (CON) or 0.5 g·steer-1·d-1 of a *Bacillus subtilis* PB6 probiotic (CLOSTAT®500, Kemin Industries, Des Moines, IA; CLO). Data were analyzed as a randomized complete block design and pen served as the experimental unit; an α of 0.05 determined significance.

Take Home Points

Live-basis final BW and ADG tended (P ≤ 0.06) to be greater for CON. Upon harvest, no *Salmonella* was recovered in any subiliac lymph nodes. Carcass traits were not altered (*P* ≥ 0.08) by dietary treatment. *Salmonella* was not observed in the subiliac lymph nodes of any steers upon harvest. If Salmonella is determined to be an adulterant in raw beef products, then cattle feeders might be able to exploit regional differences in Salmonella prevalence and regional based assessment of identified feed additives that have proven efficacy to mitigate Salmonella prevalence in beef cattle should be conducted.

Introduction

Food safety is an issue of concern for producers, consumers, and processors of livestock products. Food-borne pathogens such as *Salmonella* can result in human disease. Recently, there have been efforts to include specific *Salmonella* serotypes as adulterants in raw beef products (Gremillion, 2018). Furthermore, as of summer 2020, United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) has received a citizen petition asking to declare 31 *Salmonella* serotypes as adulterants of meat and poultry products (FSIS Salmonella Petition 1.19.20). *Salmonella* can infect the gastrointestinal tract of beef animals at a variety of time points in the life of the beef animal (Gragg et al., 2013; Broadway et al., 2020). In the United States, there are regional differences in *Salmonella* prevalence in fed cattle populations (Green et al., 2010; Gragg et al., 2013). Steers fed and harvested in the Northern Plains region of the United States (e.g., South Dakota) have been shown to have little to no *Salmonella* positive lymph nodes in upon harvest (Gragg et al., 2013). *Salmonella* can proliferate in the gastrointestinal tract and subsequently takes residence in subiliac lymph nodes where it can then contaminate beef trim (Gragg et al., 2013; Gremillion, 2018). Many cattle may harbor and shed *Salmonella* but remain asymptomatic; however, there is still the risk of reduced feed intake and growth performance in these cattle infected with *Salmonella*. Currently, many antimicrobial alternatives are being investigated to determine their pre-harvest efficacy to reduce foodborne pathogens (Broadway et al., 2014). One of the primary goals of the feedlot industry is to increase animal growth performance and gain efficiency during all stages of the feedlot production phase. Production enhancement technologies (e.g., steroidal implants with anabolic activity and beta-adrenergic agonist) are routinely employed in North American feedlots to increase production efficiencies (Johnson et al., 2013; Smith and Johnson, 2020). Additionally, feed grade and injectable antimicrobials are used in North American beef production to prevent and treat illness in cattle. The safety of these production enhancement technologies, feed grade and injectable antimicrobials have been confirmed through many thorough evaluations; however, there is still widespread concern surrounding the safety of these products amongst consumers (Sánchez-Mendoza et al., 2014). Thus, there has been considerable attention focused on non-pharmaceutical antibiotic alternatives to the use of these compounds in food production (Sánchez-Mendoza et al., 2014). *Bacillus subtilis* PB6 (CLOSTAT®500, Kemin Industries, Des Moines, IA) is a patented spore-forming bacterium that has been shown to impact clostridia and *Salmonella* in livestock species (Broadway et al., 2020; Smock et al., 2020b). Reducing subclinical illness in livestock that is associated with *Clostridia* and *Salmonella* challenges can in turn improve immunological responses to more severe diseases associated with the respiratory tract in cattle. This ultimately could reduce the need for therapeutic and sub-therapeutic administration of antimicrobials during the feedlot production phase, and in turn enhance growth performance and growth efficiency (Broadway et al., 2020). The objective of this research was to determine the influence of *Bacillus subtilis* PB6 administration in yearling feedlot steers on growth performance, efficiency of dietary net energy (NE) utilization, carcass trait responses, and *Salmonella* prevalence.

Experimental Procedures

Animal care and handling procedures used in this study were approved by the South Dakota State University Animal Care and Use Committee (Approval Number: 2003-019E). The study was conducted at the Southeast Research Farm (SERF) Feedlot located near Beresford, SD (43.0805° N, 96.7737 °W).

Dietary Treatments

This study used 12 replicate pens of 9 to 10 steers/pen assigned to one of two dietary treatments. Dietary treatments included:

1) No probiotic (CON).
2) Fed 0.50 g·steer^{-1}·d^{-1} of a *Bacillus subtilis* PB6 probiotic (CLOSTAT®500, Kemin Industries, Des Moines, IA; CLO).

Animal, initial processing, and study initiation

A total of 238 crossbred beef steers (initial BW 886 ± 68.8 lbs) were used in this study. Steers were sourced from a grow yard in northwest Iowa and transported 99 miles to the SERF on March 17, 2020. All steers were processed on March 20, 2020. At the time of initial processing, individual body weight (BW) was collected, and a unique identification tag was applied to each steer. Steers were also vaccinated against respiratory pathogens: infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) types 1 and 2, parainfluenza-3 virus (PI_3), and bovine respiratory syncytial virus (BRSV) (Bovi-Shield Gold® 5, Zoetis, Parsippany, NJ) and clostridial species (ULTRABAC® 7/Somubac®, Zoetis), administered pour-on moxidectin (Cydectin®, Bayer Healthcare LLC, Pittsburgh, PA) and administered a steroidal implant (200 mg trenbolone acetate and 28 mg estradiol benzoate; SYNOVEX® PLUS, Zoetis). On study d 28, all steers were re-vaccinated for clostridial species (ULTRABAC® 7/Somubac®, Zoetis). The study was initiated on March 23, 2020 (6 d following arrival to the SERF). Steers were housed in open-lot, soil-surfaced pens, with 20.0 ft of bunk space, a 19.7 ft concrete bunk apron, and 650 or 585 ft^2 of pen space per steer (9 or 10 steers/pen).

Weather measurement and THI estimation

Climatic variables (ambient temperature, relative humidity, and wind speed) were obtained every 5 min from a weather station (Mesonet at SDState) located at the SERF throughout the experimental period. The Temperature-humidity index (THI) was calculated using the following formula: THI = 0.81 × ambient temperature, °C + [relative humidity × (ambient temperature, °C - 14.40)] + 46.40 (Hahn, 1999).

Diet and intake management

Steers were fed once daily in the morning. Bunks were managed to be slick to traces of feed (less than 1 lb) at 0700 h. Steers were stepped up to their final diet over a 14-d period with two step-up diets fed. Feed intake and diet formulations were summarized at weekly intervals. Steers were fed common diets only differing in regards to the addition of the *Bacillus subtilis* PB6 probiotic (Table 1). Individual ingredient samples (except for the dietary treatment pellet and liquid supplement) were collected weekly and dry matter (DM) calculated after drying in a forced-air oven at 140°F until no further weight change to determine DM intake (DMI). Proximate analysis of each ingredient (except for pelleted treatment supplement and liquid supplement) was conducted weekly according to: DM [method no. 935.29; (AOAC, 2012)], N [method no. 968.06; (AOAC, 2016); Rapid Max N Exceed; Elementar; Mt. Laurel, NJ], and ash [method no. 942.05; (AOAC, 2012)]. Modified distillers grains samples were analyzed for ether extract content using an Ankom Fat Extractor (XT10; Ankom Technology, Macedon, NY) and tabular values for the remainder of the ingredients were used (NASEM, 2016). Percentages of ADF and NDF were assumed to be 3 and 9% for corn, respectively. Analysis of ADF and NDF composition for all other ingredients was conducted as described by (Goering and VanSoest, 1970).

Weekly DM and assayed nutrient composition values were used to tabulate actual DM ingredient inclusions and assayed nutrient composition of the diets fed along with tabular ingredient energy values presented in Table 1 according to (NASEM, 2016).

Cattle management and growth performance parameters

Steer BW was recorded at the time of study initiation and on d 28, 56, 84, 112, and 140 for the calculation of average daily gain (ADG) and feed conversion efficiency (G:F). Body weights were measured before the morning feeding and a 4% pencil shrink was applied to initial BW and final BW (BW from d 140) for the calculation of cumulative steer growth performance. Carcass-adjusted final BW was calculated from hot carcass weight (HCW)/0.625 for the calculation of carcass-adjusted growth performance.

Carcass-adjusted growth performance was used to calculate performance-based dietary NE to determine efficiency of dietary net energy utilization. The performance-based dietary NE was calculated from daily energy gain (EG; Mcal/d): $EG = ADG^{1.097} \times 0.0557W^{0.75}$, where W is the mean equivalent shrunk BW [kg; (NRC, 1996)] from median feeding shrunk BW, and final BW at 28% estimated empty body fatness (AFBW) calculated as: [median feeding shrunk BW × (478/AFBW), kg; (NRC, 1996)]. Maintenance energy (EM) was calculated by the

equation: EM = 0.077 × $BW^{0.75}$. Dry matter intake is related to energy requirements and dietary NEm (Mcal/kg) according to the following equation: DMI = EG/(0.877NEm − 0.41), and can be resolved for estimation of dietary NEm by means of the quadratic formula $x = \frac{-b \pm \sqrt{b^2-4ac}}{2c}$, where a = −0.41EM, b = 0.877EM + 0.41DMI + EG, and c = −0.877DMI (Zinn and Shen, 1998). Dietary NEg was derived from NEm using the following equation: NEg= 0.877NEm − 0.41 (Zinn, 1987).

Management of pulls and removals

All steers that were pulled from their home pen for health evaluation were then monitored in individual hospital pens prior to being returned to their home pens. When a steer was moved to a hospital pen, the appropriate amount of feed from the home pen was removed and transferred to the hospital pen. If the steer in the hospital returned to their home pen, this feed remained credited to the home pen. If the steer did not return to their home pen, all feed that was delivered to the hospital pen was deducted from the feed intake record for that particular pen back to the date the steer was hospitalized. Four steers died during the course of the experiment for reasons determined to be health anomalies not related to dietary treatment. Two steers from CON died of heart failure, and two steers from the CLO died due to pneumonia associated with Bovine Respiratory Disease Complex.

Study termination and carcass data collection

The study was terminated on August 10, 2020 when steers were visually appraised to have 0.50 in of rib fat (RF). Cattle were shipped the same day as study termination and harvested the following day at Tyson Fresh Meats in Dakota City, NE. Individual steer identity was tracked through the harvest facility by trained personnel from South Dakota State University. Hot carcass weight (HCW) and liver abscess scores were recorded during the harvest procedure. Liver scores were classified according to the Elanco Liver Scoring System: Normal (no abscesses), A- (1 or 2 small abscesses or abscess scars), A (2 to 4 well organized abscesses less than 2.54 cm diameter), or A+ (1 or more large active abscesses greater than 2.54 cm diameter with inflammation of surrounding tissue). Video image data were obtained from the abattoir for ribeye area (REA), RF, kidney-pelvic-heart fat (KPH), and USDA marbling scores. Dressing percentage was calculated as: (HCW/final BW shrunk 4%) × 100. Estimated empty body fat (EBF) percentage and AFBW were calculated from observed carcass traits (Guiroy et al., 2002). Yield grade was calculated according to the USDA regression equation (USDA, 1997). Estimated proportion of closely trimmed boneless retail cuts from carcass round, loin, rib, and chuck (Retail Yield; RY) was also calculated from carcass traits (Murphey et al., 1960).

Salmonella prevalence determination

Fecal grab samples were aseptically collected via rectal palpation during the weighing procedure, from the same steers throughout the course of the study, at study initiation and on d 28, 56, 112, and 140 (6, 34, 62, 118, and 146 d following arrival to the SERF) according to (Broadway et al., 2020). Briefly, samples were obtained from the 5 steers closest to the initial pen mean average from each of the 24 pens (12 pens/treatment). Samples were aseptically transferred to sealable bags and shipped overnight to USDA-ARS in Lubbock, TX in shipping coolers maintaining 32 to 38°F. Upon arrival, samples were weighed, and an equal portion of feces from each steer were pooled by pen and homogenized for determination of *Salmonella* prevalence using selective enrichment and culture medias. Subiliac lymph nodes were collected from every other carcass during the harvest procedure. Samples were de-nuded and subjected to similar procedures as outlined above for determination of *Salmonella*. Using the same selective enrichment and culture medias used for fecal determination.

Statistical analysis

Growth performance data were analyzed as a randomized complete block design using the MIXED procedure of SAS® 9.4 (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The model included fixed effects of block (location) and dietary treatment. No random effects were included in the model. Least squares means

were generated using the LSMEANS statement of SAS. Treatment means were compared using the F-test statistic. An α of 0.05 or less determined significance and tendencies were declared from 0.051 to 0.10.

Results and Discussion

Weather measurements

Ambient weather conditions during the course of the study are presented in Table 2. Average THI during the course of the 140-d study was 61.6. The THI was above 75 for 21-d of the 140-d study. The average total precipitation at the SERF for the past 67 y from March to August is 17.9 in. The precipitation during the course of this experiment was below historical records. Two heat events occurred during period 4 of the present study (d 85 to 112) in which the average THI was greater than 75 for 10-d of the 28-d period.

Animal growth performance

Animal growth performance responses for the 140-d study are located in Table 3. There was no difference detected for initial on test BW (P = 0.37; 886 vs. 884 ± 2.0 lbs) for CON and CLO steers, respectively. Final BW (live-basis) from the 140-d experiment tended to be decreased for CLO steers compared to CON steers (P = 0.09; 1424 vs. 1407 ± 7.5 lbs). Dietary treatment had no influence on live-basis ADG or feed efficiency. Dry matter intake was not different (P = 0.63) between treatments. Carcass-adjusted final BW, ADG, and G:F were not impacted by dietary treatment ($P \geq 0.29$). This is similar to what has been reported by Smock et al. (2020a) who noted no improvements in cumulative growth performance responses in steers when *Bacillus subtilis* PB6 was fed to high-stressed feeder steers. Alternatively, Smock et al. (2020a) noted an improvement in ADG and DMI during the initial 56-d feedlot receiving phase when *Bacillus subtilis* PB6 was supplemented to high-stressed feeder steers. Finally, observed dietary NE and the ratio of observed to expected dietary NE were not altered by dietary treatment ($P \geq 0.46$). It has been reported previously that *Bacillus subtilis* supplementation increased ADG of broiler chicks (Sen et al., 2012). Others have reported that *Bacillus subtilis* PB6 supplementation increased DMI when weaned Holstein steers were experimentally infected with *Salmonella* (Broadway et al., 2020). Improvements in feed conversion efficiency have been reported by others in broiler chicks and feedlot steers when *Bacillus subtilis* was fed compared to non-supplemented controls (Sen et al., 2012; Zhang et al., 2013; Kemin, 2018). No appreciable differences for animal growth performance responses in the present study is likely due to the steers being under minimal amounts of environmental stress during the course of the study.

Carcass characteristics

Carcass trait responses are located in Table 4. Previous data in regards to *Bacillus subtilis* supplementation to feedlot finishing cattle is limited. There were no differences ($P \geq 0.15$) among treatments for any carcass traits measured in the present experiment. Moreira et al. (2016) indicated that Nellore bulls supplemented with 10 $g \cdot bull^{-1} \cdot d^{-1}$ of calcium butyrate (ButiPEARL®, Kemin Industries) and 10 $g \cdot bull^{-1} \cdot d^{-1}$ of *Bacillus subtilis* (CLOSTAT®, Kemin Industries) had greater intramuscular fat accumulation compared to cattle not supplemented with *Bacillus subtilis*, but indicated no differences in any other carcass parameters. In transit-stressed steers from the Southeastern U.S. transported and fed in Oklahoma, the supplementation of *Bacillus subtilis* PB6 (CLOSTAT®, Kemin Industries) had no influence on HCW, dressing percentage, RF, REA, USDA Marbling score, or calculated yield grade (Kemin, 2018). Smock et al. (2020a) detected no differences for HCW, dressing percentage, USDA marbling scores, RF, REA, or calculated yield grade when *Bacillus subtilis* PB6 was fed to finishing steers. Additionally, there were no differences ($P \geq 0.16$) among treatments for the distribution of USDA Yield or Quality grades in the present study. Finally, there were no treatment effects ($P \geq 0.54$) for prevalence of abscessed livers in this experiment. These findings are similar to Smock et al. (2020a), who indicated that the distribution of USDA Yield and Quality grade, or condemned livers were not influenced by the supplementation of *Bacillus subtilis* PB6 to finishing beef steers.

Salmonella prevalence

There was no *Salmonella* recovered from any fecal samples collected on study d 1, 28, or 56 (6, 34, or 62 d following arrival to the SERF). On study d 112 (118-d following arrival to the SERF) there was numerically

greater (*P* = 0.17; 8.3 vs. 25.0%) fecal prevalence of *Salmonella* in CON steers compared to CLO steers. Study d 112 was during a heat event that occurred in the Northern Plains and Midwest region. On d 140 of the present study there was no difference between treatments (*P* =0.34; 0.0 vs. 8.3%) in fecal *Salmonella* prevalence for CON and CLO steers, respectively. Smock et al. (2020b) noted an appreciable decrease in fecal *Salmonella* prevalence in high-stressed feeder steers supplemented with *Bacillus subtilis* PB6 on d 28 of the feedlot receiving period. However, no differences among treatments for fecal *Salmonella* incidence was noted on d 196 of the feeding period (Smock et al., 2020b). The lack of detectable *Salmonella* in these steers could be due to the fact that the steers used in the present experiment were not transitioned through a cattle auction facility, and due to a relatively short transit distance from the grow yard to the feedlot research facility. Thus, the steers experienced minimal transit stress, no marketing stress, and minimal environmental stressors during the present study which could have reduced *Salmonella* exposure and/or shedding (Gragg et al., 2013). Additionally, steers from the Northern Plains region of the United States (e.g., South Dakota) have been shown to have no *Salmonella* positive lymph nodes in finished cattle upon harvest (Gragg et al., 2013), suggesting regional differences in *Salmonella* prevalence in fed cattle populations (Green et al., 2010; Gragg et al., 2013). Regional differences in fecal and subiliac lymph node *Salmonella* prevalence in beef cattle should be investigated further. Regional differences in *Salmonella* prevalence should be exploited by cattle feeders and this might provide opportunities to increase cattle feeding numbers in specific regions of the United States such as the Northern Great Plains.

Implications

These data indicate that *Bacillus subtilis* PB6 had no influence on feedlot phase growth performance, efficiency of dietary NE utilization, or carcass traits. Also, fecal *Salmonella* prevalence was rarely (only on d 112 and d 140) observed in yearling steers placed on feed in March in southeastern South Dakota, and no *Salmonella* was detected in any subiliac lymph nodes upon harvest. If *Salmonella* is determined to be an adulterant in raw beef products, then cattle feeders might be able to exploit regional differences in *Salmonella* prevalence and regional based assessment of identified feed additives that have proven efficacy to mitigate *Salmonella* prevalence in beef cattle should be conducted.

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Tables

Table 1. Actual diet formulation fed and nutrient composition from weekly ingredient analyses.[1]

Item	Days fed						
	1 to 7	8 to 14	15 to 21	22 to 56	57 to 74	75 to 116	117 to 140
Dry-rolled corn, %	39.00	48.75	64.23	64.94	64.20	68.87	69.33
Modified distillers grains plus solubles, %	20.01	20.82	14.19	16.57	18.26	17.42	17.27
Alfalfa-grass hay blend, %	29.49	17.54	5.03	-	-	-	-
Millet hay, %	-	-	-	2.92	3.05	7.90	-
Corn silage, %	4.74	5.69	10.27	9.30	8.56	-	-
Grass hay, %	-	-	-	-	-	-	7.58
Liquid supplement[2], %	3.90	4.11	4.03	4.02	3.96	3.88	3.88
Pelleted treatment supplement[3], %	2.86	3.09	2.25	2.25	1.97	1.93	1.94
Diet dry matter, %	68.46	66.28	65.48	64.62	65.62	76.51	76.46
Crude protein, %	13.73	13.33	11.29	12.17	12.11	12.83	12.51
Neutral detergent fiber, %	34.92	28.83	19.95	18.79	18.15	18.77	17.96
Acid detergent fiber, %	19.79	15.62	10.24	9.88	9.22	9.53	9.46
Ash, %	7.18	6.43	4.90	5.27	5.55	5.58	5.36
Ether extract, %	3.31	3.51	3.60	4.47	4.79	4.42	4.28
Net energy for maintenance, Mcal/cwt	84.8	89.4	93.0	93.9	94.3	93.9	94.8
Net energy for gain, Mcal/cwt	54.0	58.5	63.0	63.5	64.0	63.5	64.0

[1] All values except diet dry matter on a dry matter basis.
[2] The liquid supplement provide micronutrients to exceed requirements and: 11,078.69 ppb chromium propionate and 730 g/ton of monensin sodium.
[3] Pelleted treatment supplement consisted of exclusively soybean hulls for control (CON) steers and soybean hulls plus CLOSTAT® 500 (CLO; Kemin Industries, Des Moines, IA) at 2,080 g/Mg sufficient to provide 0.50 $g \cdot steer^{-1} \cdot d^{-1}$ for CLO steers.

Table 2. Ambient temperature (T_a), mean relative humidity (RH), and Temperature-humidity index (THI) throughout the course of the experiment.

Period[1]	Mean Ta, (°F)	Mean RH, (%)	Mean THI2	Days with THI >75	Wind speed, MPH	Total precipitation, in
Pre-trial (6 d)	32.9	83.9	35.4	0	9.8	0.8
1	40.3	73.4	42.7	0	8.9	2.2
2	53.2	64.5	54.1	0	7.8	1.3
3	69.6	67.6	67.2	4	9.3	1.8
4	74.5	77.4	72.5	10	6.7	3.5
5	72.5	80.9	71.2	7	5.7	2.1
Average[3]	62.1	72.7	61.6	21	7.7	11.7

[1] Each period represents 28 days. Period 1: March 23 to April 20; Period 2: April 21 to May 18; Period 3: May 19 to June 15; Period 4: June 16 to July 13; Period 5: July 14 to August 10.
[2] THI = 0.81 × ambient temperature, °C + [relative humidity × (ambient temperature, °C - 14.40)] + 46.40.
[3]Average of the 140-d study, except for days with THI >75 and precipitation, which is total days with THI >75 and total precipitation during the course of the 140-d study.

Table 3. Cumulative growth performance responses.

Item	Treatment[1]		SEM	P-value
	CON	CLO		
Pens, n	12	12	-	-
Steers, n	119	119	-	-
DOF	140	140	-	-
Initial body weight (BW)[2], lbs	886	884	2.0	0.37
Live-basis				
Final BW[2], lbs	1424	1407	7.5	0.09
Average daily gain (ADG), lbs	3.86	3.73	0.051	0.13
Dry matter intake (DMI),lbs	25.15	25.04	0.154	0.63
ADG/DMI (G:F)	0.153	0.149	0.0018	0.14
DMI/ADG (F:G)	6.54	6.71	-	-
Carcass-adjusted basis				
Final BW[3],lbs	1448	1440	6.6	0.29
ADG, lbs	4.01	3.97	0.044	0.39
DMI, lbs	25.15	25.04	0.154	0.63
G:F	0.160	0.158	0.0018	0.58
F:G	6.25	6.33	-	-
Observed dietary NE, Mcal/cwt				
Maintenance	92.53	92.08	0.635	0.46
Gain	62.59	62.14	0.544	0.46
Observed/expected dietary NE[4]				
Maintenance	0.99	0.98	0.007	0.46
Gain	1.00	0.99	0.009	0.46

[1] Fed no probiotic (CON) or fed g·steer^{-1}·d^{-1} of *Bacillus subtilis* PB6 (CLOSTAT® 500, Kemin Industries, Des Moines, IA; CLO).
[2] A 4% pencil shrink was applied to account for gastrointestinal tract fill.
[3] Calculated as: HCW/0.625.
[4] Observed dietary NE/tabular trial NE: where tabular trial NEm was 92.99 Mcal/cwt and NEg was 62.60 Mcal/cwt.

Table 4. Carcass trait responses.

Item	Treatment[1]		SEM	P-value
	CON	CLO		
Pens, n	12	12	-	-
Steers, n	119	119	-	-
HCW, lbs	906	899	4.2	0.29
Dressing percent[2], %	63.56	64.01	0.245	0.23
Rib fat, in	0.54	0.52	0.009	0.15
Ribeye area, in^2	13.50	13.45	0.120	0.80
Marbling[3]	442	438	9.8	0.77
KPH, %	1.71	1.71	0.013	0.97
Calculated YG[4]	3.31	3.28	0.047	0.38
Retail yield[5], %	49.92	50.04	0.103	0.41
Estimated EBF[6], %	30.71	30.39	0.173	0.22
Final BW at 28% EBF[6], lbs	1323	1325	8.4	0.88
USDA Yield Grade (YG) distribution				
YG 1, %	0.0	0.0	-	-
YG 2, %	27.4	23.0	4.44	0.50
YG 3, %	53.8	64.4	4.93	0.16
YG 4, %	18.8	12.6	3.66	0.26
YG 5, %	0.0	0.0	-	-
USDA Quality Grade distribution				
Select, %	41.9	36.0	5.09	0.43
Low Choice, %	32.3	44.9	6.04	0.17
Average Choice, %	17.0	15.5	3.34	0.75
High Choice, %	7.1	3.6	3.33	0.47
Prime, %	1.7	0.0	0.79	0.17
Liver abscess scores[7]				
Normal, %	67.4	65.1	5.57	0.77
A$^-$, %	12.9	15.3	3.81	0.66
A, %	9.4	6.9	3.11	0.57
A$^+$, %	10.3	12.7	2.77	0.54

[1] Fed no probiotic (CON) or fed g·steer^{-1}·d^{-1} of *Bacillus subtilis* PB6 (CLOSTAT® 500, Kemin Industries, Des Moines, IA; CLO).
[2] Calculated as: HCW/(final BW pencil shrunk 4%).
[3] 400 = small^{00} (USDA Low Choice).
[4] Calculated according to the USDA regression equation (USDA, 1997).
[5] As a percentage of HCW according to Murphey et al. (1960).
[6] Calculated according the equations described by Guiroy et al. (2002).
[7] According to the Elanco Liver Scoring System: Normal (no abscesses), A- (1 or 2 small abscesses or abscess scars), A (2 to 4 well organized abscesses less than 1.0 in diameter), or A+ (1 or more large active abscesses greater than 1.0 in diameter with inflammation of surrounding tissue).