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# The effect of supplementing CLOSTAT® 500 (*Bacillus subtilis* PB6) to yearling steers in a commercial feedyard on *Salmonella* spp. prevalence

## INTRODUCTION

The active microbial supplement, CLOSTAT® (Kemin Industries, Des Moines, IA) contains a unique, patented strain of *B. subtilis* PB6. Previous research with *B. subtilis* PB6 supplementation to beef steers has demonstrated positive health outcomes, as Smock et al. (2020) reported a decrease in BRD treatment rate and decreased overall disease treatment cost.[1] Broadway et al. (2020) indicated that *B. subtilis* PB6 supplementation improved immune response to a *Salmonella* challenge in Holstein calves and decreased the prevalence of *Salmonella* colonization in small intestinal tissues compared with non-supplemented control calves.[2] In high-producing dairy cows, the rate of cows culled in early lactation was reduced with *B. subtilis* PB6.[3]

## MATERIALS & METHODS

Steers enrolled in the study (*n* = 2,100) were blocked by arrival date and BW such that arrival dates were represented equally within each BW block. Within each group of 2 eligible animals in the chute at processing, one was randomly assigned to each pen (70 steers/pen) in the block. Within blocks, pens were assigned randomly to 1 of 2 dietary treatments, thus, treatments were replicated in 15 pens. Steers were allocated into 30 contiguous, open-air, soil surface pens. Treatments, arranged in a randomized complete block design included: 1) control (CON), diets contained no supplemental bacterial or yeast direct fed microbials and were without *Bacillus subtilis* PB6; 2) CLOSTAT (CLO), diets supplemented with 0.5 g/hd/d *Bacillus subtilis* PB6 (CLOSTAT® 500, Kemin Industries, Des Moines, IA) to provide 6.6 × $10^9$ CFU/g of active ingredient. CLOSTAT was added to the starter and finisher diets through a micro-ingredient machine maintained by Micro Technologies (Amarillo, TX); briefly, CLO was added with water to the Roto-Mix® truck (Roto Mix, Dodge City, KS) via the micro-ingredient machine, and the diet was allowed to mix for at least 3 minutes following the addition of CLO.

Fecal samples were aseptically collected via convenience grab sampling from each pen. Fresh fecal matter was collected from multiple locations throughout a pen and composited. Each pen was analyzed in duplicate, and samples were processed in a similar manner as described by Broadway et al. (2020).[2] Subiliac lymph nodes were obtained from a subset of carcasses within each lot slaughtered between July 26 – September 6, 2021. Samples were processed similarly to that described by Arthur et al. (2008), and quantification and isolation were performed as described previously.[4]

## RESULTS

### *Prevalence of Salmonella*

There was a difference in fecal *Salmonella* prevalence and quantity across sampling days ($P$ < 0.01; Figure 1). However, no differences ($P$ ≥ 0.35) were observed in fecal *Salmonella* prevalence between CON and CLO cattle. Upon arrival, fecal *Salmonella* prevalence was 26.7% and 20% for CON and CLO respectively; however, by d 45 the percentage of prevalence positive pens had risen to 86 and 93% for CON and CLO, respectively. There was a tendency ($P$ = 0.07) for overall mean fecal *Salmonella* counts to be decreased in CLO (1.59 log CFU/g) compared with CON (2.04 log CFU/g; Figure 2). Similarly, there was a day effect for fecal *Salmonella* concentration ($P$ <0.01) by which *Salmonella* concentrations were very few upon arrival but spiked to over 3.5 log CFU/g by d 45. Each subsequent collection from d 45 to d 180 yielded a slight decrease in overall fecal *Salmonella* concentrations. Fecal *Salmonella* concentrations were numerically reduced in CLO steers on all days with the exception of d 45 ($P$ = 0.59; 3.62 vs 3.88 log CFU/g for CON and CLO, respectively). The largest difference between treatments in fecal *Salmonella* concentrations occurred on d 135 ($P$ = 0.01; 2.50 vs. 1.23 log CFU/g for CON and CLO, respectively).

Subiliac lymph nodes were collected at harvest. There was no difference ($P$ = 0.62) between CON (0.22 log CFU/g) and CLO (0.19 log CFU/g) when evaluating *Salmonella* concentrations within the lymph nodes. Steers were harvested on 4 separate days, and there was a difference in lymph node *Salmonella* concentrations across harvest days (Figure 3). *Salmonella* prevalence did not differ by treatment in subiliac lymph nodes ($P$ = 0.46; Figure 4); however, there was a 46% decrease in the overall mean lymph node *Salmonella* prevalence (28.66% vs. 15.48% for CON and CLO, respectively). There was no difference ($P$ = 0.16) for lymph node *Salmonella* prevalence between harvest dates. The percentage of *Salmonella* positive lymph nodes was numerically reduced in CLO compared to CON on all days except for harvest day 3.

## CONCLUSIONS

In a large-pen, commercial feedyard setting, supplementing CLO to feedlot steers resulted in a lower fecal prevalence of *Salmonella* compared to un-supplemented CON steers. *Bacillus subtilis* PB6 works through multifaceted modes to decrease enteric pathogen load and improve intestinal integrity. These results demonstrate that *Bacillus subtilis* PB6 is an effective active microbial for improving the overall health of feedlot cattle.

**Table 1.** Ingredient and nutrient composition of starting and finishing diets.

| | Dietary Treatment[1] | |
|---|---|---|
| Ingredient, % of DM | CON | CLO |
| *Starting diet* | | |
| RAMP[2] | 100 | 99.98 |
| Micro-ingredients | -- | 0.02 |
| CLOSTAT® 500[3], g/T | -- | 0.50 |
| *Composition, % of DM[5]* | | |
| Crude protein, %[5] | 21.5 | 21.3 |
| Calcium, %[5] | 1.53 | 1.66 |
| Phosphorus, %[5] | 0.82 | 0.39 |
| *Finishing diet* | | |
| Steam flaked corn | 53.67 | 53.63 |
| Wet distiller's grains | 19.15 | 19.16 |
| Sweet Bran® Plus[4] | 18.34 | 18.37 |
| Ground corn stalks | 7.45 | 7.44 |
| Fat | 1.36 | 1.37 |
| Micro-ingredients[4] | 0.03 | 0.03 |
| CLOSTAT® 500[3] | -- | 0.50 |
| *Composition, % of DM[5]* | | |
| $NE_m$, Mcal/kg[6] | 2.17 | 2.17 |
| $NE_g$, Mcal/kg[6] | 1.45 | 1.45 |
| Crude protein, %[5] | 14.7 | 14.8 |
| Calcium, %[5] | 0.79 | 0.82 |
| Phosphorus, %[5] | 0.51 | 5.51 |

[1]CON = control; CLO = CLOSTAT steers fed control diet supplemented with 0.5 g/hg/d *Bacillus subtilis* PB6 (CLOSTAT® 500, Kemin Industries, Des Moines, IA)

[2]RAMP = commercially manufactured complete starter feed (Cargill Corn Milling, Dalhart, TX), including minerals, vitamins, 20 g/T monensin (Rumensin® 90, Elanco Animal Health, Greenfield, IN) and 10 g/T tylsoin (Tylan®, Elanco Animal Health).

[3]CLOSTAT 500, Kemin Industries, delivered through micro-ingredient machine, containing 6.6 × $10^9$ CFU *Bacillus subtilis* PB6.

[4]Micro-ingredients for finishing diet included minerals, vitamins, 42 g/T monensin (Rumensin® 90, Elanco Animal Health), 7.5 g/T tylosin (Tylan®, Elanco Animal Health), and Ractopamine-hydrochloride, 27.3 g/T the final 31 days on feed (Optaflexx®, Elanco Animal Health).

[5]Analysis by Servi-Tech Laboratories, Amarillo, TX.

[6]Formulated values based on NASEM (2016).

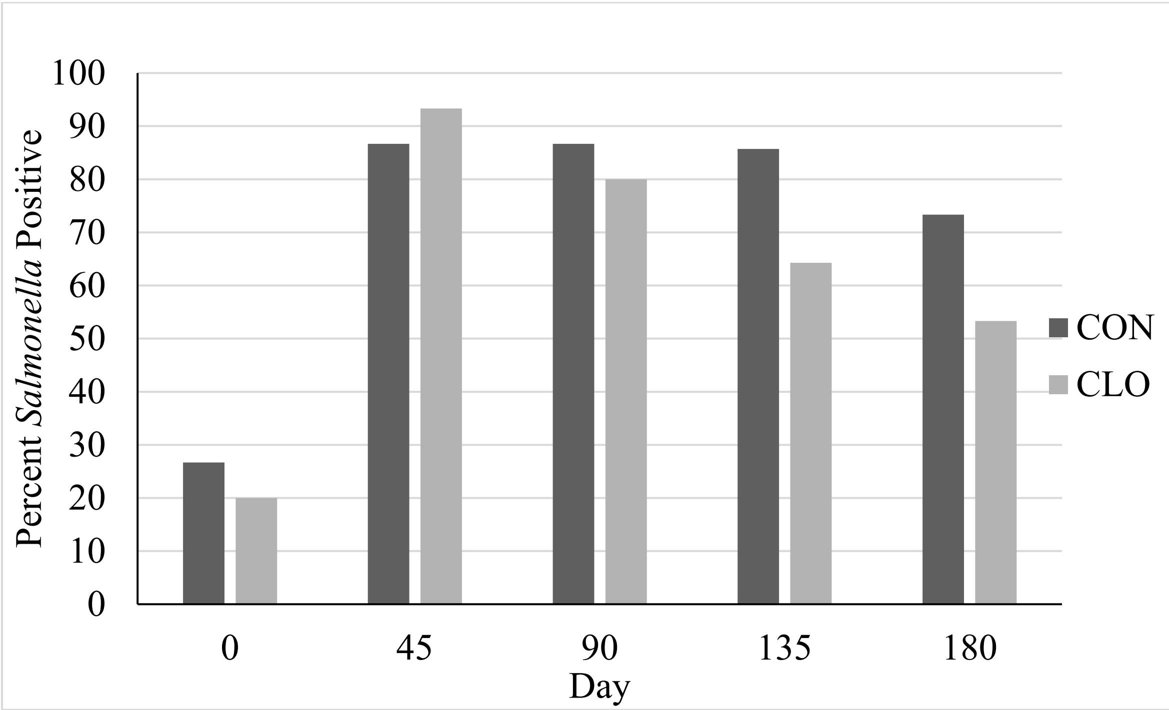


**Figure 1.** Fecal *Salmonella* prevalence of feedlot cattle sampled throughout the feeding period supplemented with 0.5 g/hd per day of *Bacillus subtilis* PB6, CLOSTAT ® 500, Kemin Industries, Des Moines, IA, (CLO) or not (CON). Treatment: $P = 0.35$, Day: $P < 0.01$, Treatment*Day: $P = 0.76$; SEM = 5.4.

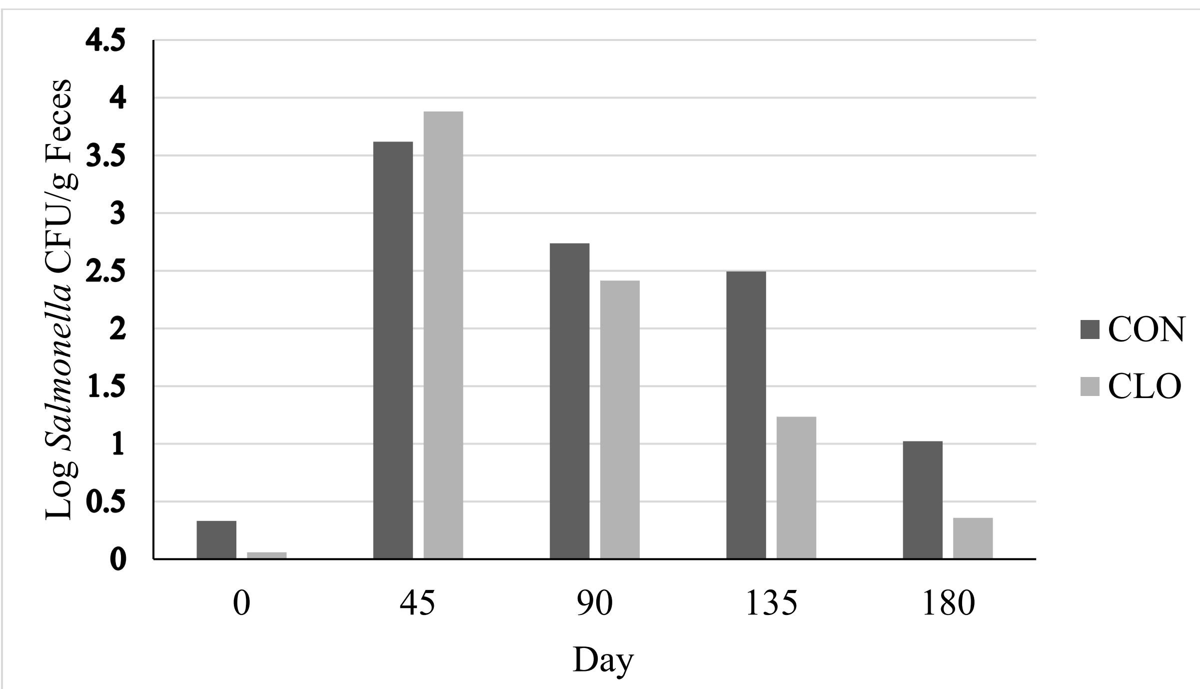


**Figure 2.** Fecal *Salmonella* concentrations from feedlot cattle sampled throughout the feeding period supplemented with 0.5 g/hd per day *Bacillus subtilis* PB6, CLOSTAT ® 500, Kemin Industries, Des Moines, IA, (CLO) or not (CON). Treatment: $P = 0.07$, Day: $P < 0.01$, Treatment*Day: $P = 0.3$; SEM = 0.3.

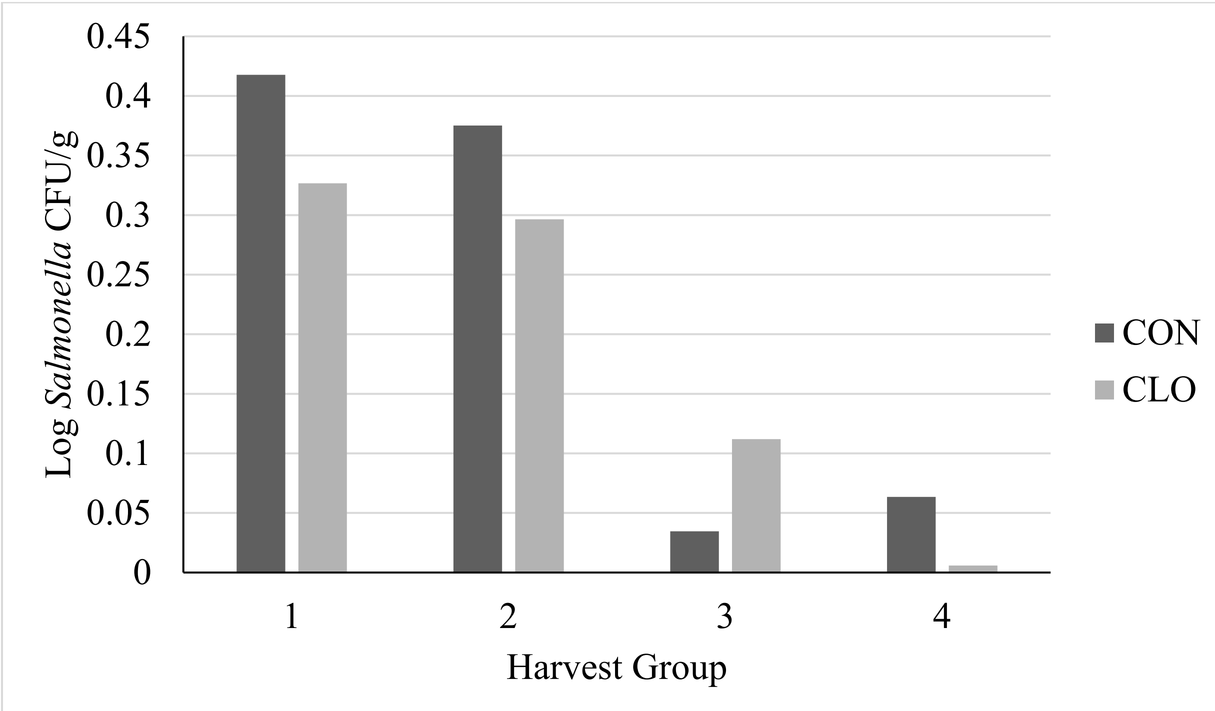


**Figure 3.** Lymph node *Salmonella* concentrations from feedlot cattle sampled across four harvest dates that were supplemented with 0.5 g/hd per day *Bacillus subtilis* PB6, CLOSTAT ® 500, Kemin Industries, Des Moines, IA, (CLO) or not (CON). Treatment: $P = 0.62$, Day: $P < 0.01$, Treatment*Day: $P = 0.87$.

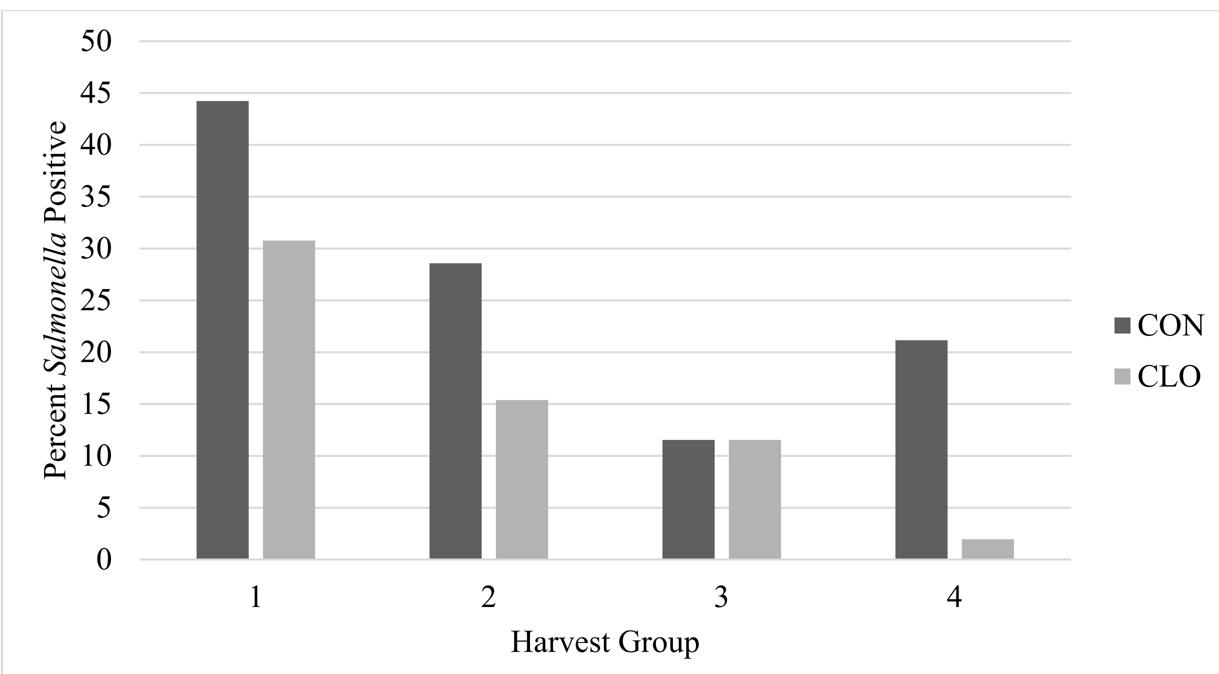


**Figure 4.** Lymph node *Salmonella* prevalence from feedlot cattle sampled across four harvest dates that were supplemented with 0.5 g/hd per day *Bacillus subtilis* PB6*,* CLOSTAT ® 500, Kemin Industries, Des Moines, IA, (CLO) or not (CON). Treatment: $P = 0.45$, Day: $P = 0.16$, Treatment*Day: $P = 0.93$, SEM=18.68.

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