Effects of *Bacillus subtilis* PB6 (CLOSTAT 500) Incorporation Into a Commercial Mineral Supplement on Growth Performance and Health of Beef Stocker Calves Grazing in the Kansas Flint Hills

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Abstract

The objective of this experiment was to evaluate the effects of incorporating *Bacillus subtilis* PB6 (CLOSTAT; Kemin Industries, Inc., Des Moines, IA) into a commercial mineral supplement on growth performance and health of crossbred beef steers grazing in the Kansas Flint Hills. Eighteen pastures were randomly assigned to receive one of two free choice mineral supplements (n = 9): a commercial mineral supplement (CON) or a commercial mineral supplement formulated to provide 0.5 g/head/day CLOSTAT 500 (CLO). A total of 495 steers (initial body weight [BW]= 477 ± 5.7 lb) were purchased in Texas and transported to the Kansas State Beef Stocker Unit. On day -1, steers were individually weighed, treated for internal and external parasites, and randomly assigned to pasture. The following day (day 0), steers were reweighed individually, given a growth-promoting implant, and walked to their respective pastures. Steers were grazed for 90 days at a targeted stocking density of 250 lb of live weight per acre. At the completion of the grazing period, steers were gathered and individually weighed. Prior to grazing, a supplement feeder was placed in each pasture, and mineral was delivered twice weekly to provide 4 oz/head/day. Initial BW, final BW, and average daily gain did not differ ($P \geq 0.29$) between steers supplemented with CON or CLO. Similarly, mineral consumption and the proportion of steers treated for bovine keratoconjunctivitis did not differ ($P \geq 0.16$) between treatments. Overall, incorporating CLOSTAT into a commercial mineral supplement did not improve growth performance of beef cattle grazing in the Kansas Flint Hills.

Introduction

Each year beef cattle from around the United States are sent to Kansas to graze the native warm-season pastures of the Flint Hills. Cattle are traditionally grazed at a high stocking density from May to August (i.e., intensive-early stocking) and are often provided with a mineral supplement to improve weight gains. CLOSTAT 500 (Kemin Industries, Inc., Des Moines, IA) is a direct-fed microbial that contains a proprietary strain of *Bacillus subtilis*, PB6. Previous research evaluating CLOSTAT supplemen-

[1] Kemin Industries, Inc., Des Moines, IA

tation to feedlot cattle has demonstrated improvements in health and growth performance (Ryan et al., 2023; Smock et al., 2020; Word et al., 2022); however, supplementing CLOSTAT to grazing cattle has not been evaluated. The objective of this experiment was to determine if incorporation of CLOSTAT 500 into a commercial mineral supplement would improve growth performance and health of stocker cattle grazing in the Kansas Flint Hills.

Experimental Procedures

This experiment was conducted between May and August 2024 at the Kansas State Beef Stocker Unit. The Beef Stocker Unit includes approximately 1,100 acres of native tallgrass prairie and is divided into 18 pastures that range from 40 to 70 acres. Prior to the start of the experiment, a spring-season prescribed fire was applied to 11 pastures on April 20 and seven pastures on April 24. Pastures were randomly assigned to receive one of two mineral supplements (n = 9): a free choice commercial mineral supplement (CON; Table 1) or a free choice commercial mineral supplement that contained CLOSTAT 500 (CLO). Mineral supplements were delivered twice weekly (i.e., Monday and Friday) to provide 4 oz/head/day. For CLO, CLOSTAT 500 was included in the mineral supplement at 9 lb/ton as-fed (AF) to provide 0.5 g of CLOSTAT/head/day.

A total of 495 crossbred beef steers (initial body weight [BW]= 477 ± 5.7 lb) were purchased in Texas and transported to the Kansas State Beef Stocker Unit between April 29 and May 8, 2024. Upon arrival, steers received a visual identification ear tag and were fed a growing diet at 2.0% of body weight (BW; dry matter basis) until May 12, 2024 (day -1). On day -1, steers were weighed individually using a hydraulic squeeze chute (SILENCER, Moly Manufacturing, LLC, Lorraine, KS), treated for internal (SAFE-GUARD; Intervet International B.V., Madison, NJ) and external (Clean-Up II; Elanco Animal Health, Greenfield, IN) parasites, and were randomly assigned to pasture (20 to 34 head). The following day (day 0), steers were reweighed individually, given a pasture ear tag, and administered a growth-promoting implant (Revalor-G, Intervet International B.V., Madison, NJ). Following processing, steers were sorted and then walked to their respective pastures. Steers were grazed for 90 days at a targeted stocking density of 250 lb of live weight per acre. At the completion of the grazing period, steers were gathered and reweighed individually.

Prior to the start of the experiment, a supplement feeder (Bull Master; F&B Mann Products, Waterville, KS) was placed in each pasture. Feeders were placed near the water tank with their flaps up to allow steers to acclimate. If the mineral was consumed within 3 days of delivery, feeder flaps were lowered. Similarly, if the mineral was consumed within 3 days of delivery with the flaps lowered, feeders were moved approximately 1,000 to 1,500 ft away from the water tank. An individual salt block was placed in each pasture at the start of the experiment. Salt blocks were removed from all pastures on day 26.

Growth performance data were analyzed using the MIXED procedure of SAS (SAS 9.4, SAS Inst. Inc., Cary, NC). The model included treatment as a fixed effect. Mineral consumption data were analyzed using the MIXED procedure of SAS. The model included fixed effects of treatment, week, and treatment × week. No treatment × week interactions were observed ($P = 0.98$); therefore, main effects of treatment are discussed. Health data were analyzed as a binomial proportion of the pasture using the GLIMMIX procedure of SAS. The model included treatment as a fixed effect.

Results and Discussion

A total of four steers were removed from the experiment due to lameness. Three steers were removed from CLO and one steer was removed from CON. In addition, one steer from CON died. All performance data from lame and dead animals were removed prior to analysis.

At the completion of the 90-day grazing period, final BW and average daily gain did not differ ($P \geq 0.57$; Table 2) between mineral treatments. Average daily gains were 2.03 and 1.99 lb/day for steers supplemented with CON and CLO, respectively. Previous research demonstrated improvements in cattle health and growth performance when CLOSTAT was supplemented to feedlot cattle (Ryan et al., 2023; Smock et al., 2020; Word et al., 2022). In this experiment, no steers were treated for bovine respiratory disease (BRD), which may have contributed to the lack of a treatment response. The incidence of bovine keratoconjunctivitis (i.e., pinkeye) was relatively high in our experiment. Despite this, the proportion of steers treated for pinkeye prior to grazing, treated once during the grazing season, or treated twice during the grazing season did not differ ($P \geq 0.16$; Table 3) between CON or CLO.

Overall mineral consumption was similar ($P = 0.54$; Table 2; Figure 1) between treatments and averaged 3.38 and 3.33 oz/head/day for steers supplemented with CON and CLO, respectively. Mineral consumption during the first two weeks averaged 1.71 and 1.29 oz/head/day, respectively. Low mineral consumption during this period may have been influenced by precipitation. The Beef Stocker Unit received approximately 4.5 in of rainfall during the first two weeks of the experiment (Figure 2). Mineral feeder flaps were placed down during periods of rainfall, which likely increased the amount of time steers took to acclimate to the feeders. Regardless, mineral consumption increased (week: $P < 0.01$; Figure1) from week 2 to 5, where it remained relatively constant throughout the remainder of the grazing season.

Implications

Feeding a commercial mineral supplement that contained *Bacillus subtilis* PB6 (CLOSTAT 500) did not improve growth performance or health of crossbred beef steers grazing in the Kansas Flint Hills.

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Table 1. Composition of mineral treatments

	Treatment	
Ingredient, % AF	**Control**	**CLOSTAT**
Calcium carbonate	31.03	30.58
Salt	25.13	25.13
Distillers grains	20.54	20.54
Molasses, 5% fat	17.99	17.99
Dicalcium phosphate	2.00	2.00
Beef MFG TM II	1.25	1.25
Mineral oil	0.75	0.75
Selenium 0.4% premix	0.55	0.55
Iron oxide	0.50	0.50
Vitamin A 650,000 IU	0.01	0.01
Beef MFG vitamin premix	0.20	0.20
Pur licorice/anise flavor	0.03	0.03
Magnesium oxide bulk	0.03	0.03
CLOSTAT 500[1]	---	0.45

[1]*Bacillus subtilis* PB6 (CLOSTAT 500; Kemin Industries, Inc., Des Moines, IA).

Table 2. Effects of *Bacillus subtilis* PB6 incorporation into a free choice commercial mineral supplement on growth performance of beef stocker calves grazing in the Kansas Flint Hills

	Treatment[1]			
Item	Control	CLOSTAT	SEM[2]	*P*-value[3]
Number of pastures	9	9		
Number of head[4]	246	244		
Initial body weight, lb	476.0	479.0	1.88	0.29
Final body weight, lb	658.8	657.8	5.30	0.90
Average daily gain, lb/day	2.03	1.99	0.053	0.57
Mineral consumption, oz/head/day	3.38	3.33	0.050	0.54

[1] Commercial mineral supplement (Control) or commercial mineral supplement + 0.5 g/head/day *Bacillus subtilis* PB6 (CLOSTAT 500, Kemin Industries, Inc., Des Moines, IA) provided at 4 oz/head/day.
[2] Largest standard error of the mean.
[3] Treatment main effect.
[4] Four steers were removed from the study due to lameness; CLOSTAT: three; Control: one. One steer from Control died. All data presented with dead and lame steers removed.

Table 3. Effects of *Bacillus subtilis* PB6 incorporation into a commercial mineral supplement on bovine keratoconjunctivitis (pinkeye) prevalence in beef stocker calves grazing in the Kansas Flint Hills

	Treatment[1]			
Item, % treated	Control	CLOSTAT	SEM[2]	*P*-value[3]
Prior to grazing	2.4	3.7	1.21	0.44
Treated once	26.8	22.5	2.83	0.29
Treated twice	1.2	3.3	1.14	0.16

[1] Commercial mineral supplement (Control) or commercial mineral supplement + 0.5 g/head/day *Bacillus subtilis* PB6 (CLOSTAT 500, Kemin Industries, Inc., Des Moines, IA) provided at 4 oz/head/day.
[2] Largest standard error of the mean.
[3] Treatment main effect.
[4] Four steers were removed from the study due to lameness; CLOSTAT: three; Control: one. One steer from Control died. All data are presented with dead and lame steers removed.

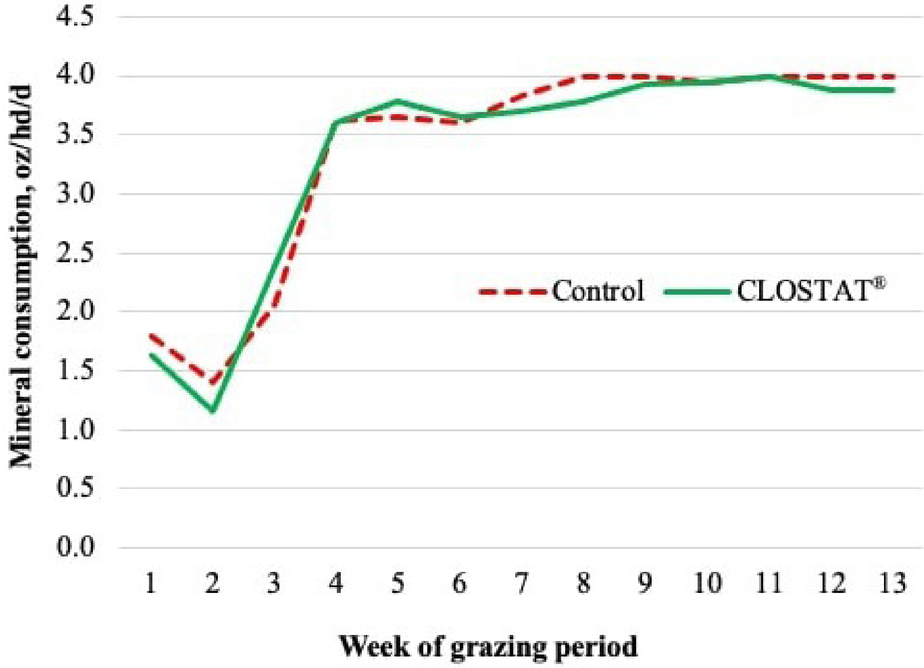

Figure 1. Free choice mineral consumption of a commercial mineral supplement (Control) or a commercial mineral supplement + 0.5 g/hd/d *Bacillus subtilis* PB6 (CLOSTAT® 500) for beef stocker calves grazing in the Kansas Flint Hills. Treatment × week: $P = 0.9730$; Treatment: $P = 0.54$; Week: $P < 0.01$.

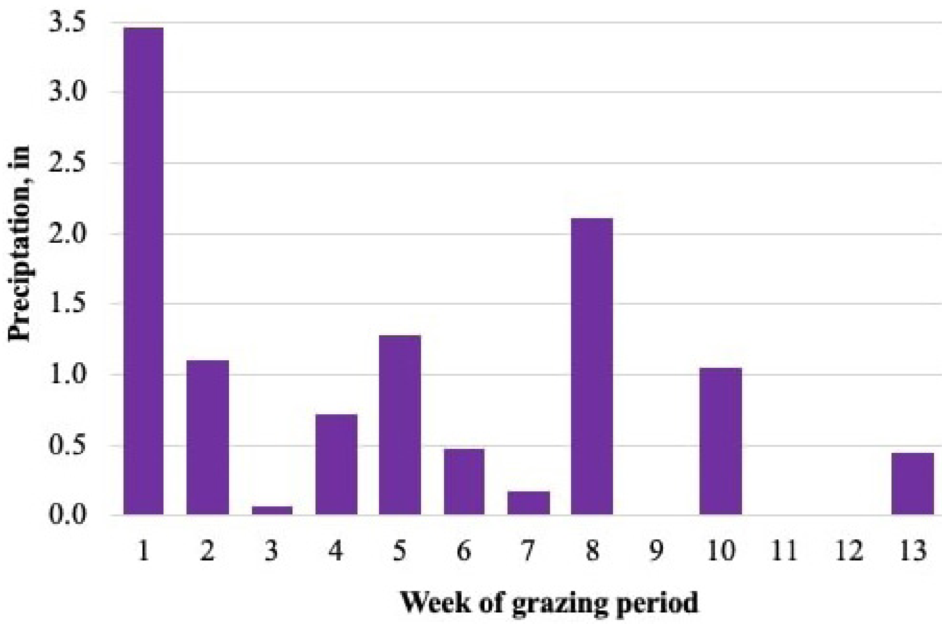

Figure 2. Precipitation received near the Kansas State Beef Stocker Unit between May 13, 2024 and August 11, 2024. Data was obtained from the Kansas Mesonet database using the Rocky Ford weather station, located approximately 3 miles east of the Kansas State Beef Stocker Unit.