

Article

A Novel Direct-Fed Microbial Impacts Growth Performance and Supports Overall Health of Feedlot Cattle

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Simple Summary: Scientific research and industry support for DFMs have been growing steadily, with studies consistently demonstrating their ability to improve nutrient digestibility and overall performance in beef and dairy cattle. By combining different bacterial strains, DFMs can enhance immune response, increase feed efficiency, and improve carcass characteristics in beef cattle. *Lactobacillus* and *Bacillus* strains have emerged as popular choices for their beneficial effects on ruminal fermentation, stability, and enzymatic activity. These bacteria improve cattle's nutrient digestion and overall performance. A novel DFM mixture called BOVAMINE DEFEND[®] Plus, containing a combination of *L. animalis* 506, *P. freudenreichii* 507, *B. licheniformis* 809, and *B. subtilis* 597, has shown promising results in improving beef cattle health and performance and a reduction in liver abscesses. BOVAMINE DEFEND[®] Plus shows multifaceted benefits that suggest the potential to revolutionize livestock agriculture by promoting economic viability and animal health within a probiotic. Continued research efforts will shed more light on the broader implications of this novel DFM mixture, shaping the future of sustainable and efficient beef production practices.



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Abstract: Non-hormone-treated beef steers ($n = 1625$; 371 ± 8.4 kg) were used to determine the impact of a direct-fed microbial (DFM) on growth performance, carcass characteristics, health parameters, and economic outcomes in finishing beef cattle. Steers were blocked based on initial BW, sorted into three optimal marketing groups for each day of enrollment, randomly assigned to one of two treatments (12 replicates per treatment), and fed for an average of 133 days before harvest. Treatments included the following: (1) control (CON) and (2) 50 mg/head per day of a DFM (BDP) containing *Lactobacillus animalis* 506, *Propionibacterium freudenreichii* 507, *Bacillus licheniformis* 809, and *Bacillus subtilis* 597 (BOVAMINE DEFEND[®] Plus). Steers were weighed in drafts by pen on a certified scale at closeout, and both pens within a block were harvested on the same day. Carcass characteristics and liver scores were collected upon slaughter. Data were analyzed as part of a completely randomized block design with the pen as the experimental unit. There was no difference in dry matter intake and final body weight between treatments. The BDP steers exhibited a lower number of total outs (deaths and removed) throughout the experiment ($p < 0.01$). Average daily gain (ADG) tended to be greater ($p < 0.06$), and feed efficiency ($p < 0.01$) was greater, for steers receiving BDP. Although there were no statistical differences in hot carcass weight and quality grade between treatments, there was a lower incidence of liver abscess scores ($p < 0.01$) in the BDP steers. These data suggest that BOVAMINE DEFEND[®] Plus supplementation improves growth performance during the finishing phase, reduces overall mortality, and improves feed to gain efficiency.

Keywords: *Bacillus* spp.; beef cattle; direct-fed microbial; *Lactobacillus animalis*; liver abscess; *Propionibacterium freudenreichii*

1. Introduction

Consumer apprehension related to antibiotic use within production agriculture has inspired ruminant nutritionists to find alternative feed-additive strategies to support production animals' overall health and performance. Over the past twenty years, probiotics have greatly expanded within livestock production. In 1989, the FDA strictly defined the use of probiotics in livestock production as "direct fed microbials" (DFMs), which were naturally occurring microorganisms that altered ruminal fermentation and intestinal function [1,2].

The ruminant scientific community and industry leaders have started to show increasing support for DFMs. These additives support normal gut functions, and consistent results [3–8] have demonstrated health support and improved nutrient digestibility and performance for beef and dairy cattle.

A range of DFMs have been developed to enhance immune response, increase feed efficiency, and improve carcass characteristics in beef cattle [9–11]. Most DFMs on the market are a combination of multiple bacteria strains; such combination is used to elicit the most significant response via numerous modes of action. The most common bacterial strains in cattle diets are lactate-producing and lactate-utilizing bacteria. This combination is used due to their synergistic effects on ruminal fermentation [12]. Lactate-producing bacteria, such as *Lactobacillus animalis*, have shown resilience within acidic conditions, allowing the ruminal microbiota to adjust to the environment. One benefit of lactate production in the rumen is stimulating lactate-utilizing bacteria, such as *Propionibacterium freudenreichii* [1,10,13]. By utilizing lactate, *Propionibacterium* spp. produces propionate, the primary glucose precursor in ruminants [14]; therefore, the combination of both strains allows the conversion of lactate into propionate, which favors the rumen's energetic efficiency while maintaining a more stable microbial ruminal environment [15]. Moreover, numerous studies have demonstrated growth performance improvements and immunological benefits when this combination of bacteria was fed to beef cattle [3,10,16–19].

In addition to *Lactobacillus animalis* and *Propionibacterium freudenreichii*, spore-forming bacteria such as Bacilli have become a popular DFM choice. Bacilli have various beneficial characteristics, such as germination and proliferation in the rumen, providing long-lasting benefits within the entire gastrointestinal tract [20]. Species such as *Bacillus subtilis* have demonstrated enhanced growth of proteolytic bacteria because of greater nitrogen digestibility and increasing ammonia concentrations [21–23]. More specifically, *Bacillus subtilis* is reportedly capable of maintaining beneficial bacterial populations throughout the GI tract [24]; therefore, its use can promote beneficial, durable changes to the microbiota within the intestine [25], recovery from diarrhea [26], and improvements in average daily gain and feed efficiency [27–29].

Other *Bacillus* species, such as *Bacillus licheniformis*, have been found to produce specific enzymes, such as amylase and cellulase [30], leading to improved starch [23] and fiber digestibility [22]. Hence, both Bacilli species have demonstrated improvements in the digestibility of dry matter and neutral detergent fiber in various forages [6,31] while simultaneously improving starch digestion. [31].

Considering the individual benefits found within these unique bacteria for the gastrointestinal tracts of ruminants, a novel DFM containing *L. animalis* 506, *P. freudenreichii* 507, *B. licheniformis* 809, and *B. subtilis* 597 (BOVAMINE DEFEND[®] Plus) has been developed with favorable in vitro and in vivo effects on the health and performance of beef cattle [7,8]. We hypothesize that feeding a multispecies bacterial DFM would improve growth performance and carcass weights. Therefore, our objective was to evaluate the effects of feeding a multispecies bacterial DFM on the growth performance, carcass characteristics, and health variables of finishing-feedlot beef cattle.

2. Materials and Methods

2.1. Study Population

The study began in August 2022 at a commercial cattle feedlot (Hy-Plains Feedyard, LLC) near Montezuma, KS, USA. Before the study's initiation, the Veterinary Research and Consulting Services, LLC, and Institutional Animal Care and Use Committee (IACUC number 1014) approved all animal procedures.

A total of 1625 black- and black–white-faced steers (average body weight [BW] = 371 ± 8.4 kg) were sourced from grazing stocker operations in New Mexico and California and enrolled in the study from August to September 2022. Steers were age- and source-verified and part of a non-hormone-treated cattle (NHTC) branded-beef program. Upon arrival at the feedlot, steers were unloaded and housed in pens by origin and provided ad libitum access to prairie hay and water. Before initial processing, a rest period of 4 to 6 days was allowed. During the rest period, steers had access to the starter ration (without DFM) and ad libitum access to prairie hay and water.

2.2. Arrival Processing

Before processing, all steers were evaluated by a licensed veterinarian to identify any chronic animals to be removed from the study. All steers were processed following the same protocol. Serially numbered ear tags, color-coded for each pen, were matched to the low-frequency electronic identification tags. Modified live virus vaccine (Pyramid[®] 3, Boehringer Ingelheim, Duluth, GA, USA) containing Infectious Bovine Rhinotracheitis Virus, Bovine Viral Diarrhea Virus (types 1 and 2), and Bovine Respiratory Syncytial Virus was administered subcutaneously (SC). Ivermectin (1.0 mL/50 kg of body weight; Ivermax[®] 1%, Aspen Veterinary Resources, Liberty, MO, USA) was administered SC. Oxfendazole (1.0 mL/50 kg of body weight; Synanthic[®], Boehringer Ingelheim Animal Health USA, Inc., Duluth, GA, USA) was administered orally.

All products were administered in accordance with Beef Quality Assurance guidelines. After processing, all steers were weighed by pen in drafts on a certified platform scale to determine pen enrollment weight.

2.2.1. Treatment Allocation

At arrival processing, steers were sorted into three optimal marketing groups using a chute-side technological program to optimize sorting decisions (PenPoint, Elanco Animal Health, Greenfield, IN, USA) over each day of enrollment. Within each marketing group, steers were assigned to 1 of 2 treatment groups, resulting in 6 pens enrolled per day and a total of 24 pens across the study:

- (1) Negative control—(CON; no DFM feeding)
- (2) Direct-fed microbial—feeding of 50 mg/head per day of a DFM containing *L. animalis* 506, *P. freudenreichii* 507, *B. licheniformis* 809, and *B. subtilis* 597 (BDP; BOVAMINE DEFEND[®] Plus; Chr Hansen, Milwaukee, WI, USA).

According to the marketing group allocation outcome, steers were assigned treatment groups according to the order in which they entered the chute. Treatment groups were randomly selected from sorting, with the initial treatment group drawn being assigned to the first group within a marketing cluster and the subsequent treatment group allocated to the second group in the same marketing cluster. This process was repeated thrice daily, and a new sequence was randomly chosen at the start of each block.

2.2.2. Feed, Housing, and Water

Upon arrival, steers were offered long-stemmed prairie hay with starter ration delivered on top (1% of BW, DM basis, Table 1). Steers were stepped up onto the finisher ration over 21 days and were fed twice daily using a slick bunk feeding program. Feed was delivered twice daily in commercial Roto-mix feed delivery trucks at 0800 h and 1300 h, and bunks were evaluated each morning at 0630 h for feed refusals. Diet samples were collected

from bunks weekly and frozen at $-20\text{ }^{\circ}\text{C}$. Samples were composited at the conclusion of the experiment and submitted to a commercial laboratory (Servitech Laboratories, Dodge City, KS, USA) for analysis of DM, CP, NDF, ADF, Ca, P, and S (Table 1). Both pens within each block were transitioned to the next ration on the same day, and prairie hay was available (free choice) to each pen during each ration transition. Monensin (RumensinTM, Elanco Animal Health, Greenfield, IN, USA) was fed for improved feed efficiency, and tylosin (TylanTM, Elanco Animal Health, Greenfield, IN, USA) was fed to reduce liver abscesses. No additional feed-grade antibiotics were fed throughout the trial.

Table 1. Macro ingredient composition (percent on dry matter basis) and calculated nutrients for each ration fed throughout the study.

Ingredient	Ration		
	Starter	Intermediate	Finish [†]
Steam flaked corn	32.0	53.4	76.4
Wet distillers grain	8.5	8.6	8.5
Ground alfalfa hay	47.6	25.3	9.4
Corn steep	8.2	7.1	0.0
Fat	0.0	1.4	1.4
Liquid finisher [§]	3.7	4.2	4.3
Calculated nutrients			
Dry matter, %	70.69	69.83	70.84
NEm, Mcal/kg	1.72	2.00	2.17
NEg, Mcal/kg	1.10	1.35	1.51
Crude protein, %	17.25	15.19	13.83
Non-protein nitrogen, %	2.52	2.47	2.55
Crude fat, %	3.13	4.81	5.47
Crude fiber, %	18.02	10.97	6.51
Calcium, %	1.20	1.00	0.78
Phosphorus, %	0.36	0.41	0.37
Potassium, %	1.46	1.04	0.61

[§] Supplement to provide macro (calcium, phosphorus, potassium, sodium, magnesium, and sulfur) and micro trace minerals (zinc, iron, copper, selenium, cobalt, and iodine). [†] Formulated to provide 60 to 90 mg/steer/day tylosin (90% DM basis) and 26 g/ton monensin.

The two pens within each block were housed in adjacent pens, and all pens were within the same area of the feedlot. Water was provided ad libitum through an automatic float-activated system shared between pens. An average of 68 steers (range: 55 to 85) were enrolled per pen, with an average enrollment weight of 371 kg (range: 324 to 418 kg) across all blocks. Pen area per steer averaged 26.5 m² (range: 20 to 32 m²), and bunk space averaged 32 cm (range: 24 to 38 cm) per steer enrolled. Pen metrics were similar (within one steer) for each experimental block.

2.2.3. Interim Weight

Both pens within a block had an interim pen weight collected on the same day at an average of 82 days on feed (DOF; range: 72 to 88 DOF). Steers were weighed in drafts by pen for interim body weight on certified platform scales (WH. Scale Co., Topeka, KS, USA).

2.2.4. Harvest

Steers were harvested based upon visual estimation of adequate finish, feed intake, and cattle supply availability for the NHTC branded-beef program. The average DOF at the time of harvest was 133 (range 106 to 151 days). Steers were fed the morning of the shipment and shipped in late afternoon, and both pens within a block were shipped on the same day. Steers were weighed in drafts on a certified platform scale by pen prior to being loaded on commercial trucks with a 5% shrink applied to final live weight, gain, and feed conversion calculations. Cattle arrived at a commercial packing plant in Arkansas City, KS,

USA (375 km) to begin harvest the following morning (fasting period of approximately 8 h). All steers were harvested from 5 December 2022 to 2 February 2023 and shipped on 5 dates (2–8 blocks shipped per date).

2.2.5. Carcass Outcomes and Liver Scoring

At the harvest facility, trained personnel from West Texas A&M University—Beef Carcass Research Center cross-referenced individual animal identification tags with plant carcass identification. Carcass quality grade was provided by a manual USDA grader at the plant, and yield grade was generated on an individual carcass basis based on the processor's visual camera grading system. Carcasses that did not have a camera score recorded ($n = 34$; CON = 20 and BDP = 14) were assigned a yield grade of 3 if the quality grade was USDA Choice or better or assigned a yield grade of 2 if the quality was USDA Select or USDA Standard. Personnel from Beef Carcass Research Center, blinded to the treatment group, scored livers based on the Elanco Liver Scoring System (Elanco Animal Health, Greenfield, IN, USA). Edible livers without any abnormalities were classified as normal while livers with 1 or 2 small abscesses or inactive scars were classified as A–; livers with 1 to 2 large abscesses or multiple small abscesses were classified as A; and the A+ score was used to describe multiple large abscesses present. Ruptured abscesses and those with tissue adhesions were categorized as A+ liver scores. The presence of liver flukes, telangiectasis, congestive heart failure, and cirrhosis were also recorded and categorized as “other” under liver abnormalities. No samples were collected for culture or histopathology evaluation.

2.2.6. Economic Analysis

Cost of gain was calculated on a deads-in and deads-out basis. Briefly, deads-in used initial body weight and head days from mortalities and removals were included in the calculation whereas deads-out used the initial body weight and head days from mortalities and removals were excluded from the calculation. Cost of gain was determined by dividing the total costs for the pen (feed costs, medicine costs, processing costs, and yardage) by the total kilograms of weight gained during the feeding period. Commercial prices were included in the cost of BDP feeding. All other feed ingredients used during the study were included in feedlot closeout expenses. Sale weight had a 5% shrink applied to all final weights, and head days were used to determine performance outcomes.

2.2.7. Statistical Analysis

Data were evaluated using a commercial software program (R Studio Team 2023, Version 4.2, Boston, MA, USA). Pen served as the experimental unit for all outcomes comparing treatment groups. Continuous outcomes (body weight, average daily gain [ADG], feed to gain [F:G], dry matter intake, cost of gain, carcass weight, and dressing percent) were evaluated as a randomized complete-block design with linear mixed models. Binomial outcomes (BRD first and second treatment, BRD mortality, digestive mortality, other mortality, overall mortality, removals, and total outs (deads + removals)) were evaluated using generalized mixed models. Health and performance outcomes were assessed at time of interim weight and closeout. Health data were also evaluated from interim weight to closeout. All models included a fixed effect of treatment group and random effect of block. Differences having $p \leq 0.05$ were considered statistically significant, with tendencies described as $0.05 < p \leq 0.10$. Descriptive cumulative mortality and total outs were evaluated by treatment group and DOF. Multinomial cumulative link mixed models were used to evaluate distribution outcomes by treatment group for quality grade, yield grade, and liver scores. Multinomial models included fixed effects of treatment group and random effects of block and repeated measures of the lot. Pairwise comparisons were performed within each category across treatment groups if the overall distribution was significantly ($p \leq 0.05$) different.

3. Results

Initial BWs were not different ($p = 0.92$) among the treatments (370 ± 8.4 kg) at the beginning of the experiment (Table 2). Steers in the BDP group tended to have a heavier body weight ($p = 0.06$; 530.0 vs. 533.6 kg for CON vs. BDP, respectively), greater ADG (both deads-in and deads-out; $p = 0.06$), and lower F:G conversion deads-in ($p = 0.06$; 5.39 vs. 5.19; CON vs. BDP, respectively) when compared to the CON group. Steers fed BDP had significantly ($p = 0.05$; 5.26 vs. 5.09; NC vs. BDP, respectively) improved F:G deads-out through the interim weight period compared to those fed CON. No differences due to treatment in morbidity or mortality were identified at the time of interim weight measurement.

Table 2. Growth, performance, and health parameters (\pm SEM) at interim weight check (average: 82 days on feed) by treatment group in steers offered a steam-flaked-corn finishing diet with or without BOVAMINE DEFEND® Plus. Models included fixed effect of treatment group and random effect for block.

Variable	CON ¹		BDP ²		<i>p</i> -Value
No. Calves (Pens)	811 (12)		814 (12)		-
Enrollment weight, kg	370.5	\pm 8.42	370.6	\pm 8.42	0.92
Interim body weight, kg	530.3	\pm 9.56	533.6	\pm 9.56	0.06
ADG, kg [*]	1.90	\pm 0.04	1.95	\pm 0.04	0.06
ADG, kg [†]	1.96	\pm 0.03	2.00	\pm 0.03	0.06
F:G [*]	5.39	\pm 0.12	5.19	\pm 0.12	0.06
F:G [†]	5.26	\pm 0.10	5.09	\pm 0.10	0.05
Average dry matter intake, kg	10.22	\pm 0.20	10.14	\pm 0.20	0.30
BRD first treatment, %	2.75	\pm 0.62	2.86	\pm 0.64	0.89
BRD second treatment, %	0.37	\pm 0.21	0.12	\pm 0.12	0.34
Overall mortality, %	0.58	\pm 0.33	0.48	\pm 0.28	0.76

^{*} Dead and removed steers included in analysis. [†] Dead and removed steers excluded in analysis. ¹ Control. ² BOVAMINE DEFEND® Plus, Chr Hansen, Milwaukee, WI, USA.

There was a tendency for fewer total deads ($p = 0.07$; 0.72 vs. 0.10%) and a significant reduction in the number of total outs ($p = 0.01$; 1.61 vs. 0.11%) in the BDP group compared to the CON group from the time of interim weight checking to closeout (Table 3). A total of nine steers were removed from the study and not marketed with the cohort (BDP = 1; CON = 8). Causes for removals included BRD (CON = 4), not qualifying for the NHTC program (BDP = 1; CON = 1), being non-performing (CON = 2), and being musculoskeletal (CON = 1). Steers in the BDP group had fewer removals ($p < 0.05$) and total outs (deads and removals; $p < 0.01$) compared to the CON group (Table 4). The cumulative mortality and total outs are shown in Figures 1 and 2.

Table 3. Health outcomes (\pm SEM) from interim weight to closeout in steers offered a steam-flaked-corn finishing diet with or without BOVAMINE DEFEND® Plus. Models included fixed effect of treatment group and random effect for block.

Variable	CON ¹		BDP ²		<i>p</i> -Value
BRD first treatment, %	0.11	\pm 0.15	0.21	\pm 0.27	0.42
BRD second treatment, %	0.12	\pm 0.12	0.12	\pm 0.12	0.99
Overall mortality, %	0.72	\pm 0.41	0.10	\pm 0.11	0.07
Total outs (deads + removals [§]), %	1.61	\pm 0.56	0.11	\pm 0.12	0.01

[§] Not marketed with cohort due to BRD, not qualifying for NHTC, being non-performing, or being musculoskeletal. ¹ Negative control. ² BOVAMINE DEFEND® Plus, Chr Hansen, Milwaukee, WI, USA.

Table 4. Health parameters (\pm SEM) at closeout (average 133 days on feed) by treatment group in steers offered a steam-flaked-corn finishing diet with or without BOVAMINE DEFEND® Plus. Models included fixed effect of treatment group and random effect for block.

Variable	CON ¹		BDP ²		<i>p</i> -Value
BRD first treatment, %	2.84	\pm 0.69	3.17	\pm 0.75	0.68
BRD second treatment, %	0.49	\pm 0.25	0.25	\pm 0.17	0.42
Overall mortality, %	1.18	\pm 0.52	0.54	\pm 0.29	0.11
BRD mortality, %	0.37	\pm 0.21	0.12	\pm 0.12	0.34
Digestive mortality, %	0.46	\pm 0.45	0.12	\pm 0.15	0.21
Other mortality, %	0.47	\pm 0.33	0.31	\pm 0.23	0.52
Removals, § %	0.99	\pm 0.35	0.12	\pm 0.12	0.05
Total outs (deads + removals), %	2.23	\pm 0.69	0.74	\pm 0.32	0.01

§ Not marketed with cohort due to BRD, not qualifying for NHTC, being non-performing, or being musculoskeletal.
¹ Negative control. ² BOVAMINE DEFEND® Plus, Chr Hansen, Milwaukee, WI, USA.

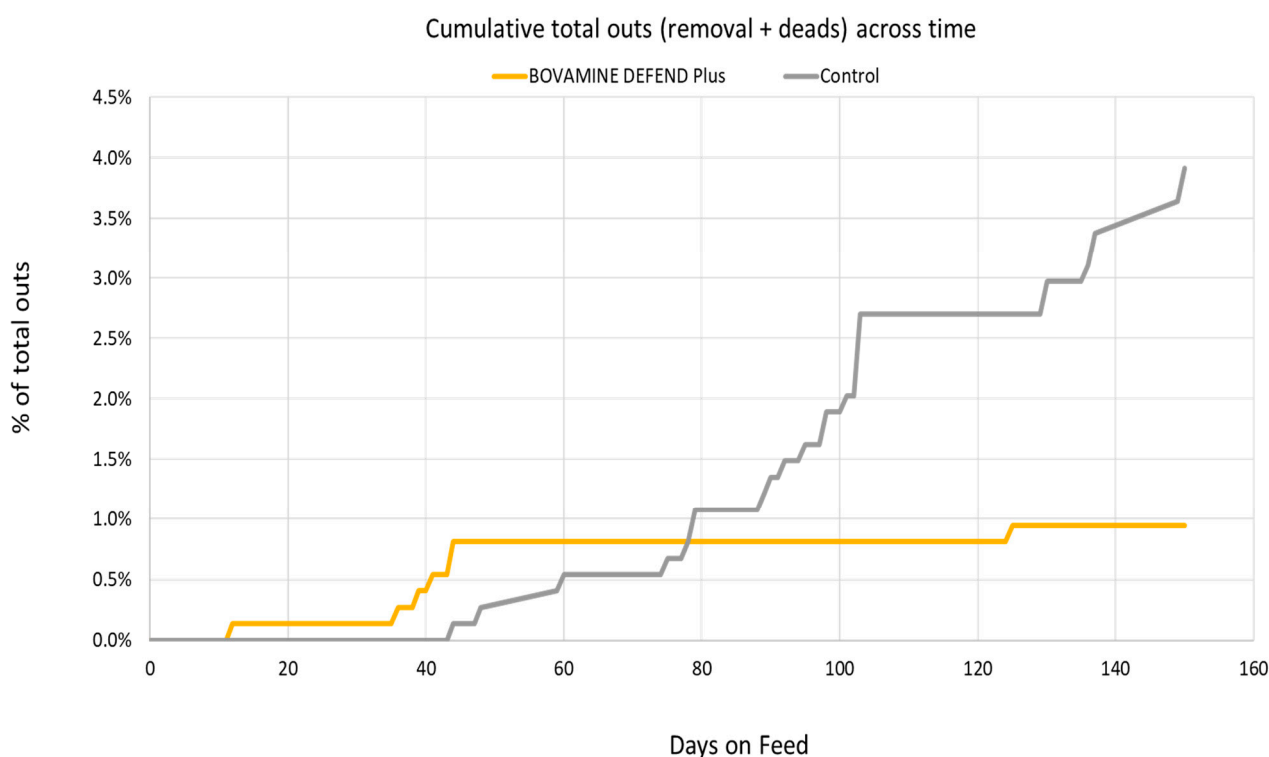


Figure 1. Cumulative total outs (deads + removals) by days on feed and treatment group (control, gray line; BOVAMINE DEFEND® Plus, Chr Hansen, Milwaukee, WI, USA, gold line).

Performance outcomes at closeout (Table 5) showed that the F:G deads-in basis was significantly ($p = 0.05$; 7.08 vs. 6.71) improved for the BDP group compared to the CON group. The average daily gain on the deads-in basis ($p = 0.06$), F:G deads-out basis, and cost of gain for both deads-in ($p = 0.06$; 159.83 vs. 151.87), and deads-out ($p = 0.08$; 155.02 vs. 150.20) tended to be improved for the BDP group compared to the CON group.

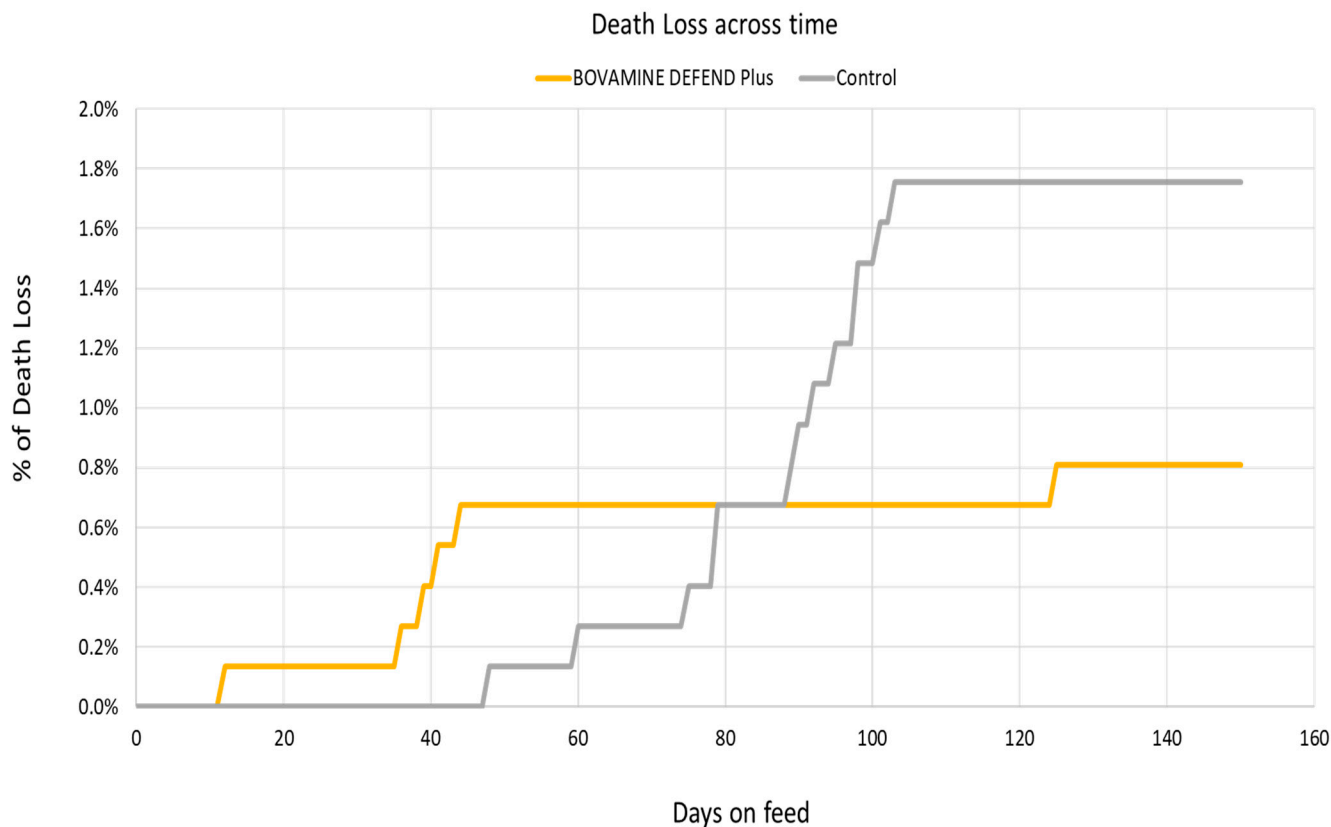


Figure 2. Descriptive cumulative death loss by days on feed and treatment group (control, gray line; BOVAMINE DEFEND® Plus, Chr Hansen, Milwaukee, WI, USA, gold line).

Table 5. Growth performance (±SEM) at closeout (average 133 days on feed) by treatment group in steers offered a steam-flaked-corn finishing diet with or without BOVAMINE DEFEND® Plus. Models included fixed effect of treatment group and random effect for block.

Variable	CON ¹		BDP ²		p-Value
Final body weight, kg [¶]	576.2	± 6.30	577.8	± 6.30	0.53
ADG, kg [*]	1.47	± 0.04	1.54	± 0.04	0.06
ADG, kg [†]	1.55	± 0.03	1.56	± 0.03	0.51
F:G [*]	7.08	± 0.14	6.71	± 0.14	0.05
F:G [†]	6.86	± 0.12	6.63	± 0.12	0.06
Average dry matter intake, kg	10.39	± 0.18	10.33	± 0.18	0.42
Cost of gain [§] , \$/45.5 kg [*]	159.83	± 3.23	151.87	± 3.23	0.06
Cost of gain [§] , \$/45.5 kg [†]	155.02	± 2.66	150.20	± 2.65	0.08

[¶] Adjusted for 5% shrink. ^{*} Dead and removed steers included in analysis. [†] Dead and removed steers excluded in analysis. [§] Calculated by dividing total costs for the pen (feed costs, medicine costs, processing costs, DFM cost, and yardage) by the total kilograms of weight gained. ¹ Negative control. ² BOVAMINE DEFEND® Plus, Chr Hansen, Milwaukee, WI, USA.

There were no significant differences ($p > 0.36$) in carcass characteristics (Table 6), indicating that the improved growth rate and feed efficiency outcomes did not impact carcass traits. The distribution of liver abscess scores was not different between the treatment groups ($p = 0.52$), but total liver abscesses were reduced in the BDP group compared to the CON group ($p = 0.01$).

Table 6. Carcass characteristics (\pm SEM) of steers offered a steam-flaked-corn finishing diet with or without BOVAMINE DEFEND[®] Plus. Models included fixed effect of treatment group and random effect for block.

Variable	CON ¹		BDP ²		<i>p</i> -Value
Hot carcass weight, kg	358.90	\pm 3.39	359.80	\pm 3.39	0.53
Dressing percent [§] , %	62.30	\pm 0.20	62.23	\pm 0.20	0.54
Ribeye area, in ²	12.51	\pm 0.16	12.48	\pm 0.16	0.54
Backfat, in	0.54	\pm 0.02	0.55	\pm 0.02	0.81
Marbling score	464.94	\pm 12.31	465.90	\pm 12.31	0.84
Quality grade distribution					0.61
Prime, %	1.48	\pm 0.48	1.64	\pm 0.53	
Upper 2/3 choice, %	34.24	\pm 6.13	36.47	\pm 6.27	
Lower 1/3 choice, %	52.38	\pm 3.75	51.03	\pm 4.12	
Select, %	11.60	\pm 2.95	10.59	\pm 2.74	
Standard, %	0.30	\pm 0.14	0.27	\pm 0.13	
Yield grade	3.28	\pm 0.09	3.31	\pm 0.09	0.36
Yield grade distribution					0.38
1, %	1.55	\pm 0.46	1.41	\pm 0.42	
2, %	28.16	\pm 4.80	26.37	\pm 4.63	
3, %	59.44	\pm 3.00	60.43	\pm 2.68	
4, %	10.44	\pm 2.34	11.34	\pm 2.52	
5, %	0.41	\pm 0.17	0.45	\pm 0.18	
Liver abnormalities					0.52
Normal, %	83.32	\pm 2.21	84.50	\pm 2.11	
A-, %	8.72	\pm 1.18	8.16	\pm 1.13	
A, %	1.26	\pm 0.32	1.17	\pm 0.30	
A+, %	2.32	\pm 0.47	2.15	\pm 0.44	
Other [†] , %	4.35	\pm 0.78	4.01	\pm 0.73	
Total liver abscesses, %	14.81	\pm 1.26	10.41	\pm 1.07	0.01

[§] Dressing percent calculated as HCW divided by the unshrunk final BW. [†] Composed of contamination, flukes, telangiectasis, heart failure, and cirrhosis. ¹ Negative control. ² BOVAMINE DEFEND[®] Plus, Chr Hansen, Milwaukee, WI, USA.

4. Discussion

A 6% greater overall ADG for steers offered BDP corroborates with the findings reported by Galyean et al. [32] and Swinney-Floyd et al. [33], which supplemented *Lactobacillus acidophilus* (1×10^8 CFU/animal-daily) and *Propionibacterium* strain P63 (1×10^9 CFU/animal-daily) to cattle fed a 90% concentrate diet, wherein researchers found a 2–3% increase in feed efficiency. Dry matter intake was not affected by the supplementation of the current DFM, which corroborates results found in several experiments using similar DFM combinations [9,16–18,32,34]. The increased ADG and similar DMI levels resulted in an improved F:G of approximately 6% for animals offered BDP. Previous studies evaluating the supplementation of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* showed 2% to 7.3% improvements in F:G [16,19,32]. When cattle were offered a 90% concentrate diet (65% steam-flaked corn-based diet) with a DFM containing *Lactobacillus* (*L. acidophilus*; 1×10^9 CFU/animal-daily), a tendency for a 5% improvement in F:G was observed for cattle offered the DFM [9]. Lawrence et al. [35] evaluated the effects of *Lactobacillus animalis* (1×10^9 CFU/animal-daily) and *Propionibacterium freudenreichii* (2×10^9 CFU/animal-daily) on dairy cattle performance. They reported high rates of mastitis in the herd, which may have masked any DFM effect. Although the literature results are not directly comparable due to differences in bacterial species, strains, concentrations, and diet types, it seems reasonable to assume that supplementing current bacterial DFM mixtures to cattle does not seem to cause adverse effects. In contrast, potential improvements in cattle growth performance have been repeatedly reported. Krehbiel et al. [10] suggested that using a combination of lactate-producing bacteria and lactate-utilizing bacteria improved the ADG (2.5% to 5%). At the same time, other performance variables, such as DMI and F:G, are less

consistent across DFM studies. This highlights the importance of designing experiments to measure variables other than growth performance, in which potential mechanisms of action, such as nutrient digestion and ruminal morphology, can be elucidated.

Similarly, the utilization of Bacilli as a DFM for cattle has been justified by its spore-forming and stability features [36], as well as its enzyme production [37]. Recently, *in vitro* DM and NDF digestibility have improved after incubating *B. licheniformis* and *B. subtilis* with different forage-based substrates [31,38]. The various types and amounts of fibrolytic enzymes produced by the different Bacilli strains may explain these improvements [37,39]. Moreover, *in vitro*, starch degradation has been enhanced in high-starch concentrates incubated with the same *Bacillus* spp. [31]. This indicates that the broad range of enzymes produced may lead to enhanced nutrient utilization and a rumen fermentation profile necessary to improve beef cattle performance [5,40]. Therefore, the different modes of action that Bacilli present may complement the efficacy of *L. animalis* and *P. freudenreichii* in enhancing the performance and health of feedlot cattle, as reported herein.

Hot carcass weight was not affected by the treatments. Galyean et al. [32] offered *L. acidophilus* and *P. freudenreichii* at various concentrations, and HCW increased on average by 2.2%. However, Vasconcelos et al. [18] used DFM treatments like those reported by Galyean et al. [32] and did not observe differences in HCW or other carcass characteristics. Due to cattle availability, the current study population comprised NHTC, and these were harvested earlier than was optimal based on carcass weights and yield grades. The dressing percentage, longissimus muscle area, liver scores, and USDA quality grades were not affected by treatment, like in the data reported by Galyean et al. [32], Brashears et al. [9], and Vasconcelos et al. [18].

The modulation of rumen pH using a DFM may be a potential reason for the decrease in total liver abscess as a reduction in the number of total outs through closeout for the current study. The time when the ruminal pH of feedlot cattle is below 5.6 (subacute rumen acidosis) is associated with increased liver abscesses [41,42]. Liver abscesses are primarily believed to be secondary to rumen acidosis, which allows bacteria to access the portal vein [43]; damage to the epithelial lining of the gastrointestinal tract can be a causative agent. In addition to pH, another causative agent of liver abscesses, *Salmonella enterica*, is found throughout the gastrointestinal system [44,45]. Rumen pH was not monitored in the current study, and tylosin was administered to both treatment groups to control liver abscesses. Additional research is needed to determine the repeatability of feeding *Lactobacillus animalis*, *Propionibacterium freudenreichii*, *Bacillus licheniformis*, and *Bacillus subtilis* for mitigating liver abscesses.

Direct-fed microbials have been shown to improve cattle health outcomes [46,47]. Studies with DFMs have focused on binding undesirable Gram-negative pathogens, such as *Escherichia coli* O157:H7, from the intestinal tract [9]. Feeding DFMs may improve an animal's immune response, but the response magnitude depends on the species and bacterial strain [47,48]. A yeast product with the primary mechanism of action occurring in the small intestine reduced the BRD first treatment by 28.4% and severe A+ liver abscesses [49]. The current study did not identify a difference in morbidity, but the cattle had a low incidence of BRD morbidity. The total-outs separation primarily occurred later in the feeding period. The cattle removed later in the feeding period had most of the feeding costs incurred throughout the feeding period. The authors hypothesize that the reduction in the number of total outs may be attributed to improved gastrointestinal health from the DFM fed in this study. Additional research is warranted to evaluate the health findings further.

Limitations of the current study include that the study cattle were NHTC and harvested earlier than the industry standard; however, the study cattle yield was still greater than 10% yield (grades 4 and 5). An interim weight was collected to mimic when a re-implant would occur for traditional feedlot cattle. The authors anticipate a similar magnitude of response in cattle administered an implant and fed for a more extended period. Still, additional research is needed to support or refute the hypothesis.

5. Conclusions

In summary, the novel mixture of *Lactobacillus animalis* 506, *Propionibacterium freudenreichii* 507, *Bacillus licheniformis* 809, and *Bacillus subtilis* 597 (BOVAMINE DEFEND® Plus) improved the finishing-feedlot steer growth performance, cost of gain, and health parameters. Additional research efforts are warranted to understand the long-term health and performance effects of the presented novel DFM mixture for beef animals.

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