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Influence of BOVAMINE DEFEND Plus on growth performance, carcass characteristics, estimated dry matter digestibility, rumen fermentation characteristics, and immune function in finishing beef steers

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ABSTRACT

One hundred and eighty crossbred beef steers (406.0 ± 2.2 kg) were used to determine the impact of a novel direct-fed microbial (DFM) on growth performance, carcass characteristics, rumen fermentation characteristics, and immune response in finishing beef cattle. Steers were blocked by body weight (BW) and randomly assigned, within block, to 1 of 2 treatments (3 replicates/treatment: 30 steers/replicate). Treatments included: (1) no DFM (control) and (2) DFM supplementation at 50 mg · animal^{-1} · d^{-1} (BOVAMINE DEFEND Plus). All steers were fed a high-concentrate finishing diet and individual feed intake was recorded daily via the GrowSafe system. BWs were collected every 28 d. On day 55, 10 steers per pen were injected with ovalbumin (OVA). Jugular blood samples were collected from each steer on days 0, 7, 14, and 21 post injection. On day 112, the same steers were injected again with OVA and intramuscularly with a pig red blood cell solution. Jugular blood samples were collected from each steer on days 0, 7, 14, and 21 post injection. On day 124, rumen fluid was collected from 3 steers per treatment and used to estimate in vitro rumen fermentation characteristics. Equal numbers of steers per treatment were transported to a commercial abattoir on days 145, 167, and 185 of the experiment, harvested, and carcass data were collected. Initial BW was similar across treatments. On days 28 and 55, steers receiving DFM had heavier BW ($P < 0.01$) compared to controls. The average daily gain was greater in DFM-supplemented steers from days 0 to 28 ($P < 0.01$) and days 0 to 55 ($P < 0.01$) of the experiment compared to controls. Overall dry matter intake (DMI) was greater ($P < 0.04$) and overall feed efficiency was similar in DFM-supplemented steers compared to controls. Dressing percentage ($P < 0.02$) was greater in steers receiving DFM compared to controls. Antibody titers to injected antigens were similar across treatments. However, red blood cell superoxide dismutase activity was greater ($P < 0.05$) in DFM-supplemented steers compared to controls. In vitro molar proportions of isobutyric and butyric acid were greater ($P < 0.01$) and dry matter (DM) digestibility tended ($P < 0.07$) to be greater in rumen fluid obtained from steers supplemented with DFM. These data suggest that BOVAMINE DEFEND Plus supplementation improves growth performance during the initial period of the finishing phase, increases overall DMI and dressing percentage, and may impact antioxidant status in beef cattle.

LAY SUMMARY

Direct-fed microbials (DFMs) are live microorganisms, from naturally occurring sources, that can be added to feedlot cattle diets to help improve gut health and overall feedlot performance. Bacterial DFM products have the potential to positively benefit the balance of intestinal microorganisms ultimately improving digestion and gut health. BOVAMINE DEFEND Plus is a DFM that contains four different strains of bacteria that aim to improve ruminal fermentation and overall gut health. We tested the effect of BOVAMINE DEFEND Plus on the performance of feedlot steers during the finishing period. Steers receiving BOVAMINE DEFEND Plus had increased body weight gains early during the feeding period, greater overall dry matter intakes, and improved dressing percentages at harvest.

Key words: cattle, digestibility, in vitro, fermentation

Abbreviations: ADG, average daily gain; BW, body weight; CP, crude protein; DFM, direct-fed microbial; DM, dry matter; DMD, dry matter disappearance; DMI, dry matter intake; IgG, immunoglobulin G; IgM, immunoglobulin M; IVDMD, in vitro dry matter disappearance; LMA, longissimus muscle area; OVA, ovalbumin; PRBC, pig red blood cell; SCFA, short-chain fatty acids; TiO2, titanium dioxide; TMR, total mixed ration; USDA, United States Department of Agriculture

INTRODUCTION

Direct-fed microbials (DFMs) have been used to aid livestock production systems for over 20 years (LeJeune and Wetzel, 2007), and are defined, by The office of Regulatory Affairs of the Food and Drug Administration, as products fed to livestock that contain live microorganisms from naturally

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occurring sources (Brashears et al., 2005). Bacterial DFM products have the potential to positively benefit the balance of intestinal microorganisms while preventing pathogen adherence, modulating immune function, and influencing the permeability of gut tissues (Krehbiel et al., 2003). Furthermore, published data would suggest that cattle supplemented with DFM have improved feed conversion efficiency and average daily gains (ADGs; Krehbiel et al., 2003; Cull et al., 2015).

BOVAMINE DEFEND Plus, a DFM, is a combination of live bacterial cultures with the aim of improving normal functions of the gastrointestinal (GI) tract such as digestion, absorption, immune function, and barrier function. Research conducted by Silva et al. (2022) and Preedy et al. (2023) has demonstrated positive benefits of DFM supplementation on several production variables of finishing feedlot cattle. BOVAMINE DEFEND Plus (*Lactobacillus animalis* 506, *Propionibacterium freudenreichii* 507, *Bacillus licheniformis* 809, and *Bacillus subtilis* 597) supplementation has been reported to inhibit *Clostridium perfringens* types A and C growth in vitro and reduced the proportion of newborn beef calves with abnormal diarrhea after being challenged with *C. perfringens* type A (Guimaraes et al., 2023). We hypothesized that BOVAMINE DEFEND Plus would increase growth performance while improving carcass characteristics and immune function in finishing beef steers. Therefore, the objective of the current experiment was to investigate the impact of BOVAMINE DEFEND Plus on growth performance, carcass characteristics, estimated dry matter (DM) digestibility, and immune parameters in finishing beef cattle.

MATERIALS AND METHODS

Prior to the initiation of this experiment all animal care, handling, and procedures were approved by the Colorado State University Animal Care and Use Committee (approval no. 2453).

Cattle Processing

Two hundred and twenty-nine crossbred beef steers (BW = 415.9 ± 2.3 kg) were transported to the Colorado State University Agriculture, Research, Development, and Education Center, in Fort Collins, CO. Upon arrival, steers were handled, processed, and allotted to treatments, according to our standard animal processing procedures (Caldera et al., 2017; Budde et al., 2019). Briefly, steers were individually weighed, identified with a unique ear tag, vaccinated with Presponse (*Pasteurella multocida* Bacterial Extract-*Mannheimia haemolytica* Toxoid, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and Pyramid 2 plus Type II BVD (Infectious Bovine Rhinotracheitis Virus and Bovine Viral Diarrhea [Types I and II], Boehringer Ingelheim Vetmedica, Inc.) respiratory vaccines, injected with Promectin (Ivermectin, Vedco, Inc.), drenched with Synanthic (Oxfendazole, Boehringer Ingelheim Vetmedica, Inc.) for parasite control, and implanted with Revalor—XS (200 mg trenbolone acetate and 40 mg estradiol, Merck Animal Health, DeSoto, KS). Following initial weighing, steers were housed together in groups of approximately 40 animals per pen with ad libitum access to long-stem grass hay and water overnight.

After initial weighing, steers were ranked by body weight (BW), and individuals that were beyond ±2 SD from the mean BW were eliminated from further consideration for the experiment as described by Caldera et al. (2017). Briefly, the remaining steers were assigned a random number from 1 to 1,000 using the random number function in Excel 2007 (Microsoft Corporation, Redmond, WA). Steers with the lowest random numbers were eliminated from the experiment reducing the number of remaining steers to 180. The 180 eligible steers were ranked by weight and divided into 3 weight block replicates, each one consisting of 60 steers. Within each weight block replicate, steers were ranked by weight and randomly assigned to one of two pens. This randomization schedule resulted in 3 weight block replicates, each containing 2 pens with 30 steers per pen with similar BW distribution for a total of 6 pens. Replicates within a weight block were randomly assigned to treatments. Treatments consisted of (1) no DFM (control) and (2) DFM supplementation at 50 mg · animal^{-1} · d^{-1} (BOVAMINE DEFEND Plus). The following day, steers were weighed prior to feeding and visual ear tags identifying each animal were applied. Steers were then sorted into their respective treatment pens and the experiment was initiated. The initial BW used for the experiment was the average of the two full BW obtained on days –1 and 0. Each pen (15 × 43 m) housing 30 animals was equipped with four GrowSafe units (in order to determine individual animal feed intake for the duration of the experiment), an automatic waterer, a concrete bunk pad, and a metal roof (15 × 3 m) covering the GrowSafe units and approximately 7% of the entire pen.

Pens were checked daily to ensure that cattle were in the appropriate pens, had ad libitum access to water, and that all GrowSafe units had enough feed to supply all cattle in the pen with 24 h of feed. Furthermore, all cattle were monitored for health and locomotion daily. Steers exhibiting symptoms of respiratory disease were removed from the pen and rectal body temperatures were recorded. Steers with body temperatures greater than 39.4 °C were considered to have clinical disease. All clinically ill steers were treated according to the appropriate treatment protocol and immediately returned to their original pen.

Diets

All steers were fed a steam-flaked corn-based high-energy finishing diet. Steers were adjusted to the finishing diet using a series of step-up diets where the roughage portion of the diet was replaced with corn during each step-up diet. Diet changes during the step-up program were simultaneous (every 5 to 7 d) for both treatments and cattle reached the finishing diet by approximately day 24 of the experiment. The finishing diet (Table 1) was formulated to meet or exceed NASEM (2016) requirements for growing and finishing beef cattle. Nutrient target values were 13.1% crude protein (CP) with 3.5% CP equivalent from nonprotein nitrogen, 0.7% calcium, 0.36% phosphorus, 0.6% potassium, 0.25% magnesium, 0.15 mg Co/kg DM; 0.3 mg Se/kg DM, 30 g/metric ton of monensin (Rumensin, Elanco Animal Health, Greenfield, IN), and 10 g/metric ton of tylosin on a DM basis. Vitamins A and E were included in the diets at 2,200 and 9.4 IU/kg of DM, respectively, and were administered in the liquid supplement, and macro and micro minerals were added as inorganic sources to meet the targeted values of the total mixed ration (TMR). DFM supplement was added to the diet fresh daily from preweighed packages. The DFM supplement (BOVAMINE DEFEND Plus; Chr. Hansen A/S, Hørsholm, Denmark) contained a combination of *Lactobacillus animalis* 506,

Table 1. Dry matter ingredient composition of the basal finishing diet[a]

Ingredient	Percent
Steam-flaked corn	60.3
Distillers grains	14.3
Corn silage	11.0
Liquid supplement[b]	6.8
Wheat straw	4.8
Limestone	1.6
Rumensin/Tylan supplement[c]	0.9
White Salt	0.3
Chemical composition	
DM, % as fed	71.3
CP, %	13.0
Acid detergent fiber, %	9.9
Neutral detergent fiber, %	17.7
Ether extract, %	4.2
NEg, Mcal/kg	1.54
NEm, Mcal/kg	2.2
Calcium, %	0.68
Phosphorus, %	0.32
Magnesium, %	0.22
Potassium, %	0.64
Sulfur, %	0.21
Copper, mg/kg	15.2
Selenium, mg/kg	0.24
Manganese, mg/kg	36.7
Zinc, mg/kg	54.3
Cobalt, mg/kg	0.16
Iron, mg/kg	81.2

[a]Optaflexx was included in the diet at a rate of 400 mg · animal^{-1} · d^{-1}.
[b]Liquid supplement provided in a molasses base included: 3.72% NPN (urea), 0.61% Ca ($CaCO_3$), 0.26% salt (NaCl), 0.05% K (KCl), 2,343 IU/kg vitamin A, 9.4 IU/kg vitamin E.
[c]Formulated to provide 30.0 g of Rumensin/metric ton and 10.0 g of Tylan/metric ton.

Propionibacterium freudenreichii 507, *Bacillus licheniformis* 809, and *Bacillus subtilis* 597. A beta agonist (ractopamine hydrochloride; Optaflexx; 400 mg · animal^{-1} · d^{-1}) was fed to all cattle for the last 29 d on feed. Diets were delivered once daily in the morning (0800 h) in amounts to allow all steers ad libitum access to feed over a 24-h period.

Weighing, Sampling, and Carcass Data Collection

Steers were individually weighed on days −1, 0, approximately every 28 d, and on 2 consecutive days at the end of the experiment. At the time of slaughter, control and DFM-supplemented steers from the same BW block (n = 3 BW blocks) were transported to a commercial abattoir and slaughtered. Weight blocks were harvested after receiving the finishing diet for 145, 167, or 185 d. Following harvest, hot carcass weight was determined, and liver abscesses were scored, as described by Elanco (2019). Carcasses were allowed to chill for approximately 24 to 36 h before additional carcass data were obtained. Carcass data were collected by trained professionals. Carcass data collected included dressing percentage, longissimus muscle area (LMA), subcutaneous adipose tissue thickness, adjusted subcutaneous adipose tissue thickness (USDA, 1989), kidney, pelvic, and heart fat, marbling score, quality grade, and yield grade (calculated).

Titanium dioxide (TiO_2) was added to the diet on day 98 of the experiment as described by others (Titgemeyer, et al., 2001; Ebert et al., 2016). Fecal samples were collected directly from the rectum and collections were conducted from the same 10 randomly selected animals per pen, once daily on days 112, 113, 114, 121, 122, and 123. For every 24-h period, the time of collection was advanced by 2 h to minimize the effects of diurnal variation (Titgemeyer et al., 2001; Ebert et al., 2016). The fecal samples with TiO_2 were used to estimate DM digestibility. TiO_2 was determined as described by Myers et al. (2004).

Immune Parameters

On days 0, 55, and 112, all cattle were bled via jugular venipuncture to assess immune parameters. Blood was collected into three separate 7-mL vacutainer tubes. Two nonheparinized vacutainer tubes for serum collection, and the other vacutainer tube was a heparinized trace-mineral-free vacutainer tube for red blood cells and plasma (Becton Dickinson Co., Franklin Lakes, NJ). Total serum immunoglobulin G (IgG) concentrations were determined using a radial immunodiffusion assay kit (Kent Laboratories, Bellinghan, WA). Superoxide dismutase enzyme activity and interferon-gamma concentrations were determined using a SOD 525 Assay Kit (Biotech 21010; Oxis Health Products, Inc., Portland, OR) and ELISA assay (Biosource KBC1231, Biosource International, Inc., Camarillo, CA), respectively.

On day 55 of the experiment, 10 steers from each pen (n = 60 steers total) were randomly selected and injected with ovalbumin (OVA). Briefly, as described by Dorton (2005), 2 mL of a solution containing 160 mg of OVA (Sigma A5503), 60 mL Freund's Incomplete Adjuvant (FIA; Sigma F-5506), and 60 mL of sterile phosphate-buffered saline were injected subcutaneously and 1 mL was injected intradermally to give a total injection of 4,000 µg of OVA/animal. Blood samples were collected via jugular venipuncture in nonheparinized vacutainer tubes (Becton Dickenson Co.) prior to injection and 7, 14, and 21 d post injection.

On day 112, the same subset of cattle was then injected with OVA (as described previously) and with 5 mL of a 25% purified pig red blood cell (PRBC; i.m. in the neck muscle) solution as described by Kuhlman et al. (1988). Blood samples were collected from these animals immediately prior to OVA and PRBC injection on day 112, and again on days 119, 126, and 133. All serum samples were analyzed for antibody titers specific to OVA using an ELISA procedure described by Engvall and Perlmann (1972). Antibody titers specific for PRBC were measured using a microtiter hemagglutination assay to determine total immunoglobulin, IgG, and immunoglobulin M (IgM) concentrations specific for PRBC (Ferket and Qureshi, 1992).

In Vitro Fermentation

Rumen fluid was collected, on day 124, using a stomach tube as described by Engle and Spears (2000), from three control and three DFM-supplemented steers from the same weight block replicate and used as inoculum for in vitro analysis of diet DM digestibility. A composite rumen fluid sample was made from the three steers per treatment. The rumen fluid composite for each treatment was mixed with McDougall's solution and incubated for 0, 6, 12, and 24 h (in quadruplicate)

for in vitro analysis. The TMR for each treatment was used as the fermentation substrate for the rumen fluid obtained from steers on the same treatments. A modified McDougall's buffer solution (39.20 g $NaHCO_3$, 14.80 g Na_2HPO_4, 2.28 g KCl, 1.88 g NaCl, and 0.48 $MgSO_4 \cdot 7H_2O$ per 2 L H_2O) was mixed with rumen fluid at a 1:1 ratio, simulating saliva production during rumination (Tilley and Terry, 1963). Short-chain fatty acids (SCFA), and in vitro dry matter disappearance (IVDMD), were determined as described by Levenson et al. (2022). Briefly, to simulate rumen motility, vaccine bottles were gently swirled every 4 h. Samples were removed at each time point and centrifuged at 1,000 × *g* for 30 min (Beckman Model TJ-6; Beckman Coulter, Indianapolis, IN). A 2.0-mL aliquot of the supernatant was extracted from the in vitro vessel post centrifugation, acidified with *meta*-phosphoric acid, and frozen at −80 °C until analyzed for SCFA concentrations. The remaining supernatant was aspirated, and the indigestible residue dried in a forced air-drying oven at 60 °C for 120 h to determine IVDMD.

After thawing at room temperature, the samples designated for SCFA analysis were centrifuged at 28,000 × *g* at 5 °C for 15 min and the supernatant was removed and placed into a 1.5-mL gas chromatography vials and analyzed for SCFA (Levenson et al., 2022). The SCFA concentrations were determined via gas chromatography (Agilent 6890N, Santa Clara, CA, USA) fitted with a fused silica capillary column (30 m × 0.25 µm × 0.25 µm) and a flame ionization detector as described by Gifford et al. (2021).

Dry matter disappearance (DMD) was determined for all samples by weighing the 50-mL conical tubes prior to dispensing the vaccine bottle rumen contents into the tube and after drying in the forced air-drying oven at 60 °C for 120 h. The IVDMD was calculated as follows: IVDMD, % = (initial substrate DM mass − [undigested DM mass − microbial DM residue mass])/(initial substrate DM mass) × 100.

Statistics

Feedlot performance, immune parameters, carcass characteristics, in vivo DM apparent digestibility, and in vitro fermentation data were analyzed on an individual animal or digestion vessel basis for a randomized complete block design using PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA). Treatment and where appropriate, time and the interactions of treatment × time were included in the model as a fixed classification effect, and weight block was included in the model as a random effect. Covariates of initial BW were used in the analysis of all performance and carcass response variables. Outlier tests were performed on all data. Data points exceeding three SDs above or below the mean were removed from the data set prior to analysis. A type 3 ANOVA table was constructed using the Kenward–Roger method of computing denominator degrees of freedom. Backward elimination with Akaike's Information Criterion (AIC) criteria was used to remove nonsignificant ($P \geq 0.05$) covariates from the model. The main effect of treatment, time, and the treatment × time interactions (where appropriate) were determined significant at $P < 0.05$. For the appropriate response variables, if the treatment × time interaction was not significant, overall treatment means were reported. Treatment means were separated ($P \leq 0.05$) using the PDIFF option of the LSMEANS statement of SAS (SAS Inst. Inc.). Categorical data were evaluated using PROC GLIMMIX of SAS assuming a binomial distribution. The Link = Logit option was included in the model, and the LSMEANS and SEM were calculated from the output statement. Significance was determined at $P \leq 0.05$ for all response variables.

Results

A total of five steers were removed from the experiment due to lameness associated with severe foot rot. Two steers were removed within the first 28 d of the experiment and euthanized (Control; $n = 90$, Treatment; $n = 88$). Three additional steers were removed from the experiment approximately 28 d prior to slaughter. These three animals were slaughtered but their carcass data were not included in the statistical analysis (Control; $n = 90$, Treatment; $n = 85$). All five steers were from the DFM treatment.

The impacts of the DFM on the growth performance of feedlot steers are presented in Table 2. Initial BW was similar across treatments ($P = 0.88$), but on days 28 and 55 of the experiment, steers receiving DFM had heavier BW ($P < 0.01$) compared to controls. ADG was greater in DFM-supplemented steers from days 0 to 28 ($P < 0.01$) and days 0 to 55 ($P < 0.01$) of the experiment compared to controls. Overall, dry matter intake (DMI) was greater ($P < 0.04$) in DFM-supplemented steers when compared to controls. However, final BW, overall ADG, and feed efficiency were similar across treatments ($P \geq 0.1$).

The influence of DFM on carcass data is presented in Table 3. Dressing percentage ($P < 0.02$) and United States Department of Agriculture (USDA) yield grade ($P < 0.05$) were greater, and 12th rib subcutaneous fat depth tended ($P < 0.10$) to be greater in DFM-supplemented steers when compared to controls. Hot carcass weights, LMA, marbling score, USDA quality grade, calculated yield grade, and percent liver abscesses were similar across treatments ($P \geq 0.1$).

There was no treatment-by-time interactions for any of the in vitro rumen fermentation characteristics measured, therefore only the main effects are reported in Table 4. Isobutyric and butyric acid concentrations (mM) were greater ($P < 0.01$) and DM disappearance tended ($P < 0.07$) to be greater for digestion vessels containing inoculum obtained from DFM-supplemented steers compared to controls. Acetic and propionic acid concentrations (mM) and total SCFA concentrations (mM) were similar across treatments ($P \geq 0.1$). Furthermore, in vivo estimates of DM digestibility (using TiO_2 as an indigestible marker) were similar across treatments (78.3% and 83.8% ± 2.9 for Control and DFM treatments, respectively; data not shown).

The influence of DFM supplementation on immune parameters measured in this experiment is presented in Table 5. There were no treatment-by-time interactions for any of the immune parameters measured; therefore, only treatment main effects are presented. Total serum IgG concentrations, IgG and IgM antibodies specific to OVA, and total immunoglobulins specific for PRBC were similar across treatments ($P \geq 0.1$). However, treatment was a significant source of variation for plasma interferon-gamma concentrations and superoxide dismutase activity. Interferon-gamma concentrations ($P < 0.04$) and superoxide dismutase activity ($P < 0.02$) were greater in DFM-supplemented steers compared to control steers.

Table 2. Influence of BOVAMINE DEFEND Plus on growth performance of feedlot steers

Item	Treatment[a]		SEM	*P*-value
	Control	DFM		
Initial, *n*	90	90	—	—
Body weight, kg				
Initial	405.7	406.2	2.2	0.88
Day 28	457.9	468.4	4.9	0.01
Day 55	521.1	528.8	5.7	0.01
Day 84	578.7	582.0	5.4	0.34
Day 112	626.6	634.0	6.8	0.10
Day 144	668.6	672.9	8.6	0.44
Day 168	685.1	692.2	9.4	0.36
Day 185	700.7	705.0	12.5	0.50
Average daily gain, kg · animal^{-1} · d^{-1} [b]				
Days 0 to 28	1.86	2.22	0.10	0.01
Days 0 to 55	2.10	2.23	0.07	0.01
Days 0 to 84	2.05	2.10	0.06	0.33
Days 0 to 112	1.97	2.04	0.06	0.11
Days 0 to 144	1.82	1.85	0.06	0.44
Days 0 to 168	1.73	1.78	0.07	0.33
Days 0 to 185	1.79	1.83	0.04	0.52
Overall dry matter intake, kg · animal^{-1} · d^{-1}	10.37	10.74	0.12	0.04
Overall feed efficiency (gain:feed)	0.173	0.170	0.003	0.41

[a]CON, control diet; DFM, direct-fed microbial treatment BOVAMINE DEFEND Plus fed at 50 mg · animal^{-1} · d^{-1}.
[b]Weight blocks were slaughtered on days 145, 167, and 185.

Table 3. Influence of BOVAMINE DEFEND Plus on carcass characteristics of feedlot steers

Item	Treatment[a]		SEM	*P*-value
	CON	DFM		
Hot carcass weight, kg	418.4	424.0	2.97	0.18
Dressing percentage[b]	62.2	63.0	0.22	0.02
12th rib subcutaneous fat depth, cm	1.36	1.45	0.04	0.10
Longissimus muscle area, cm^2	91.6	92.7	0.84	0.37
Marbling score[c]	629.0	627.5	11.77	0.92
USDA yield grade	2.79	2.95	0.06	0.05
USDA quality grade	5.80	5.79	0.12	0.95
Calculated yield grade	3.19	3.33	0.06	0.11
Liver abscess, % (*n*/total)	42.2 (38/90)	53.4 (47/88)	5.41	0.44

[a]CON: control diet, DFM: Direct-Fed Microbial Treatment BOVAMINE DEFEND Plus fed at 50 mg · animal^{-1} · d^{-1}.
[b]Final live body weight pencil-shrunk by 4% prior to dressing percentage calculation.
[c]Slightly abundant = 800, Moderate = 700, Modest = 600, Small = 500, Slight = 400.

Discussion

The DFM (BOVAMINE DEFEND Plus) used in this study contained a combination of *Lactobacillus animalis* 506, *Propionibacterium freudenreichii* 507, *Bacillus licheniformis* 809, and *Bacillus subtilis* 597. *Lactobacillus animalis* used in the current experiment is a lactic acid-producing bacteria that has barrier function capabilities within the rumen environment, and *P. freudenreichii*, a Gram-positive, lactic acid-utilizing bacteria that has been reported to increase propionate production in the rumen (Brashears et al., 2005; McAllister et al., 2011). *Bacillus licheniformis* is a potent competitor of potentially harmful bacteria within the rumen, while *B. subtilis* is a growth inhibitor of potentially harmful bacteria within the GI tract of cattle (Mingmongkolchai and Panbangred, 2018). Initial research has demonstrated that the aforementioned combination tested improved the health of newborn male beef calves inoculated with *C. perfringens* type A (Guimaraes et al., 2023). Based on our data, BOVAMINE DEFEND Plus improves growth performance during initial periods of the finishing phase, increases overall DMI, may impact antioxidant status, and increases dressing percentage.

Numerous experiments have been conducted investigating the impacts of individual or combinations of DFM bacteria on cattle performance, pathogenic challenge growth, and animal health. The results of these experiments have varied. In a pooled summary of eight feedlot experiments, Ware et al. (1988) reported that steers receiving *L. acidophilus* (1×10^8 cfu · steer^{-1} · d^{-1}) had improved ADG and feed efficiency compared to nonsupplemented controls. The inclusion of *L. animalis* as a DFM in the diet of finishing feedlot steers has also been reported to improve weight gains, gain-to-feed ratio, and hot carcass weights (Hanford et al., 2011; Cull et al., 2012; Cull et al., 2015). Supplementing *P. freudenreichii* at 1×10^9 cfu · animal^{-1} · d^{-1} dose decreased shedding of *E. coli* in feces of mixed breed beef steers fed a high-concentrate finishing diet (Elam et al., 2003) and reduced total CH_4 production as a result of increasing propionate production (Meale et al., 2014). Reid and Burton (2002) reported that DFM containing *P. freudenreichii* can prevent *E. coli* O157 infections, and therefore shedding, by inhibiting penetration of *E. coli* O157 into the intestinal mucosal layer. *Propionibacterium* strains have also been reported to increase ruminal propionate production therefore reducing enteric methane emissions from forage-fed cattle (Jeyanathan et al. 2014). It is difficult to determine the specific mode of action that DFM has on animal performance due to the variety of bacteria included in different DFM products. However, it appears that the bacteria mentioned previously can improve rumen fermentation characteristics and reduce GI tract growth of pathogens which may help explain the improvement in animal performance.

Previous studies have investigated the impact of supplementing a combination of *L. animalis* and *P. freudenreichii* on cattle performance and disease resistance. Supplementing *L. animalis* and *P. freudenreichii*, at a combined dose of 4×10^9 cfu · animal^{-1} · d^{-1}, to lactating (120 d in milk) dairy cows, improved milk yield, protein yield, and energy-corrected milk by 7.6%, 6.9%, and 6.0%, respectively, compared to nonsupplemented cows (Boyd et al., 2011). Furthermore, DM digestibility was increased by 3% in DFM-supplemented dairy cows compared to controls. In feedlot cattle consuming a high-concentrate diet, Galyean et al. (1995) investigated the inclusion of 1×10^6 cfu · animal^{-1} · d^{-1} or 1×10^9 cfu · animal^{-1} · d^{-1} *L. animalis* with 1×10^9 cfu · animal^{-1} · d^{-1} *P. freudenreichii*. The researchers reported heavier final BWs and greater ADGs when cattle were supplemented with *L. animalis* and *P. freudenreichii*, irrespective of the dose of *L. animalis*.

However, other researchers have reported no impact of single bacterial strains of *L. animalis* or *P. freudenreichii* or combinations of *L. animalis* and *P. freudenreichii*, on feedlot cattle growth performance and carcass characteristics (Vasconcelos et al., 2008; Luebbe et al., 2013; Thompson et al., 2020; Cull et al., 2022). The reason for the variable impacts of DFM supplementation on beef cattle growth and carcass characteristics is unknown. There are many factors that can impact an animal's response to DFM supplementation such as: (1) species and strain supplemented, (2) dose and duration of DFM supplementation, (3) stage of growth, and (4) environmental stressors.

Bacillus licheniformis and *B. subtilis* are both spore-forming bacteria that are stable in the digestive tract of mammals. They can function as competitors of pathogens and improve nutrient digestion (Mingmongkolchai and Panbangred, 2018; Su et al., 2020). The majority of research investigating the addition of *B. licheniformis* and *B. subtilis* to livestock diets has been conducted in nonruminant species with little published

Table 4. Influence of ruminal inoculum from control and BOVAMINE DEFEND Plus supplemented steers on in vitro fermentation characteristics

Item	Treatment[a]		SEM	*P*-value
	CON	DFM		
Dry matter digestion, %	50.49	58.13	2.89	0.07
Acetic acid, mM	39.65	39.39	1.32	0.89
Propionic acid, mM	25.38	25.09	0.76	0.79
Isobutyric acid, mM	0.83	1.12	0.07	0.01
Butyric acid, mM	9.83	11.61	0.46	0.01
Total SCFA[b], mM	75.01	77.90	2.97	0.55

[a]CON, control diet; DFM, direct-fed microbial treatment BOVAMINE DEFEND Plus fed at 50 mg · animal^{-1} · d^{-1}.
[b]Short chain fatty acids.

Table 5. Influence of BOVAMINE DEFEND Plus on blood immune parameters in feedlot steers

Item	Treatment[a]		SEM	*P*-value
	Control	DFM		Treatment
Total serum immunoglobulin G, mg/mL	2565.9	2586.0	28.9	0.63
Ovalbumin IgG Titers, $\log_{10}$	0.011	0.010	0.001	0.64
Ovalbumin IgM Titers, $\log_{10}$	0.053	0.055	0.002	0.20
Total PRBC titers, $\log_2$	1.321	1.363	0.094	0.76
Superoxide dismutase, U/mg hemoglobin	0.344	0.372	0.020	0.02
Interferon gamma, $\log_{10}$	0.133	0.148	0.005	0.04

[a]CON, control diet; DFM, direct-fed microbial treatment using BOVAMINE DEFEND Plus fed at 50 mg · animal^{-1} · d^{-1}.

research in cattle. Other authors have reported that the inclusion of *B. licheniformis* and *B. subtilis*, at a combined dose of 3.2×10^9 cfu · g^{-1}, improved in vitro DM, fiber, and starch digestibility of different forage-based substrates, dairy TMR, and high-starch feedstuffs (Pan et al., 2022; Cappellozza et al., 2023). Oyebade et al. (2023) reported that supplementing multiparous dairy cows (41 ± 7 d in milk) with a mixture of *L. animalis* and *P. freudenreichii* at 3×10^9 cfu · d^{-1} or *L. animalis*, *P. freudenreichii*, *B. subtilis*, and *B. licheniformis* at 11.8×10^9 cfu · d^{-1} improved dietary crude fat digestibility and certain immune parameter measurements compared to nonsupplemented controls. Other researchers have reported improvements in milk production, milk components, and fermentation characteristics in dairy cows supplemented with *B. subtilis* (Sun et al., 2013) and improved health in newly received feedlot cattle beef cattle (Colombo et al., 2021). These data indicate that certain bacterial DFM alone or in combination can impact cattle performance and health and are in agreement with the improvement in cattle performance and greater red blood cell superoxide dismutase activity observed in the DFM-supplemented cattle compared to controls in the current experiment.

Conclusion

To our knowledge, the current experiment is the first to examine the impact of the DFM BOVAMINE DEFEND Plus containing *L. animalis*, *P. freudenreichii*, *B. licheniformis*, and *B. subtilis* on feedlot cattle performance, carcass characteristics, immune function, and rumen fermentation characteristics. These data suggest that BOVAMINE DEFEND Plus supplementation improves growth performance during initial periods of the finishing phase, increases overall DMI and dressing percentage, and may impact antioxidant status in beef cattle. Future research examining the impact of DFM on feed intake and disease resistance is warranted.

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Conflict of Interest Statement

The authors from Colorado State University and the Universidade Federal Rural da Amazonia-UFRA have no conflict of interest with this study. The funders, Octavio Guimaraes, Bruno I. Cappellozza, and Jennifer S. Schutz, assisted with the design of the experiment but had no role in the collection, analyses, or interpretation of data.

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