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Effect of Direct-Fed Microbial Supplementation on Pathogenic *Escherichia coli* Fecal Shedding, Live Performance, and Carcass Characteristics in Feedlot Steers

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Abstract

Three experiments were conducted to evaluate direct-fed microbial (**DFM**) supplementation on live performance, carcass characteristics, and fecal shedding of *E. coli* in feedlot steers. In Exp. 1, 400 steers (BW = 348 kg) were assigned to treatments: **CON** = lactose carrier only, **BOV** = *P. freudenreichii* (NP24) + *L. acidophilus* (NP51), **BOVD** = *P. freudenreichii* (NP24) + *L. acidophilus* (NP51), and **COMB** = BOV fed for the first 101 d on feed, followed by BOVD for the final 28 d prior to harvest. In Exp. 2 (n = 1800; BW = 354 kg) and Exp. 3 (n = 112; BW = 397 kg), steers were utilized in a randomized complete block design and assigned to DFM treatments using low dose and high dose, respectively. Fecal samples were collected prior to harvest and analyzed for *E. coli* serogroups. In Exp. 1, DFM reduced ($P < 0.01$) the concentration of *E. coli* O157. Prevalence of O157 was reduced by BOVD supplementation in Exp. 2 and 3 ($P < 0.01$ and $P = 0.08$, respectively), and concentration of *E. coli* O157 in positive samples was reduced in both experiments where enumeration was performed ($P \leq 0.02$). Weighted mean differences across the three experiments were equal to a 33% reduction in the prevalence of *E. coli* O157:H7 in BOVD treated cattle. A significant reduction in prevalence of O26, O45, O103, and O121 was observed in Exp. 2 ($P \leq 0.03$). These results indicate that high levels of *L. acidophilus* (NP51) may represent an effective pre-harvest food safety intervention to reduce fecal shedding of several *E. coli* serogroups.

Keywords

Beef Cattle, Direct-Fed Microbial, *Escherichia coli* O157, *Lactobacillus acidophilus*, Pre-Harvest Intervention

1. Introduction

The United States Center for Disease Control (**CDC**) estimates that 48 million Americans are affected by foodborne illness each year, and *E. coli* O157:H7 alone causes more than 70,000 illnesses and 61 deaths [1]. This and other Shiga toxin-producing *E. coli* (**STEC**) have been linked to numerous incidents [2]. Specifically, the STEC O-serogroups known as the "big-six" (O26, O45, O111, O121, O103, and O145) are responsible for over 250,000 illnesses and 3500 hospitalizations each year in the U.S. [3].

Cattle are a natural reservoir of STECs, and while illness associated non-O157 STECs are rare, *E. coli* O157:H7 has been associated with numerous outbreaks [4]. Because STECs are natural flora of the gastrointestinal tract of cattle, they can create substantial challenges upon harvest. Moreover, pathogen-contaminated hides appear to be a primary source of contamination. During the de-hiding process, fecal material can be transferred from the hide to the carcass and, despite many post-harvest interventions, high microbial loads on hides can increase this risk [5]. A 2011 study [6] reported a 24.3% prevalence of STECs in U.S. ground beef, which indicates that improvements to current interventions are still needed. The USDA has suggested that, in addition to current HACCP plans, harvest facilities should source and receive cattle from producers who implement pre-harvest interventions to reduce shedding of these pathogens [7].

Direct Fed Microbials (DFM) are products containing live microorganisms to be used in animal feed due to their potential health benefit [8] [9]. Various DFM formulations have been proposed to enhance ruminal microbiota and improving immune response, control shedding of pathogenic Enterobacteriaceae, improve feed efficiency, animal performance and carcass characteristics in beef cattle [10] [11] [12] [13]. Due to the increased concern in the use of chemical interventions or antimicrobial treatments to reduce foodborne pathogen shedding by beef cattle, alternatives such as DFM in animal diets are gaining popularity.

Therefore, the objectives of this study were to: 1) evaluate various feeding strategies of commercially available direct-fed microbial (**DFM**) on pathogen shedding, live performance, and carcass characteristics in feedlot steers (Exp. 1), and 2) further evaluate a specific DFM as an intervention to reduce shedding of harmful *E. coli* serotypes, and evaluate its effects on performance and carcass characteristics in feedlot steers (Exp. 2 and 3).

2. Materials and Methods

Three independently conducted feeding trials were carried out in order to eva-

luate the effects of commercially available direct-fed microbials (**DFM**; Bovamine® and Bovamine Defend®, Nutrition Physiology Company, Overland Park, KS) on the prevalence and concentration of *E. coli* O157:H7 and non-O157 O-groups, as well as live performance and carcass characteristics in feedlot steers. Experiment 1 formed the basis for the treatment selection and experimental design for the subsequently conducted experiments (Exp. 2 and Exp. 3). All procedures involving the use of live animals were conducted within the guidelines of, and approved by, the Federation of Animal Science Societies guide for the use of farm animals in research [14], and the Texas Tech University Animal Care and Use Committee (#12033-04). Experiments were conducted at three different locations, including Johnson Research Facility in Parma, ID (Exp. 1), Cactus Feeders in Cactus, TX (Exp. 2), and Texas Tech University Burnett Center in Lubbock, TX (Exp. 3).

2.1. Experiment 1

2.1.1. Animals

British × Continental crossbred steers (n = 400; BW = 348 ± 30 kg) were sourced from 3 locations in California and shipped to a research feedyard (Johnson Research Facility) located in Parma, ID. On arrival, all animals were processed and individually weighed. Initial processing included the following procedures: application of a unique identification tag, application of a hot-iron brand, vaccinations against viral (Bovi-Shield Gold 5; Zoetis, Florham Park, NJ) and clostridial (Cavalry 9; Merck Animal Health, Florham Park, NJ) diseases, treatment for internal and external parasites (Cydectin Injectable; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), and administration of a terminal implant (Revalor-XS; Merck Animal Health, Florham Park, NJ).

On d − 1, steers were randomized to provide an even distribution of body weights across all study pens (n = 40). Within each origin, 120 steers were divided into 3 weight groups of 40 steers each. Each weight group within origin was then randomly allocated to 1 of 40 study pens using a random number generator. At the conclusion of this randomization, 8 steers remained from 2 sources, and 24 remained from the third source. Utilizing the previous methodology, these final 40 steers were then randomly allocated to the 40 study pens. Following randomization, steers were sorted into their 40 respective home pens (10 head/pen; 7.6 × 21.3 m with 7.6 m of linear bunk space). Treatments were introduced on d 0, and rations were fed to provide *ad libitum* access to feed once daily in the morning for the duration of the experiment.

2.1.2. Treatments and Experimental Design

Four treatments were used in a completely randomized design. Treatments consisted of the following: 1) **CON** = 1 g/(head·d) of an inert lactose carrier, 2) **BOV** = 1.0 g/(head·d) of a commercially available DFM (Bovamine®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* (NP24 strain) at a concentration of 1.00×10^9

CFU/g and *Lactobacillus acidophilus* (NP51 strain) at a concentration of 1.00×10^7 CFU/g, 3) **BOVD** = 1 g/(head·d) of a commercially available DFM (Bovamine Defend®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* at a concentration of 1.00×10^9 CFU/g, and 4) **COMB** = BOV fed for the first 101 d on feed, followed by BOVD which was fed for the final 28 d on feed.

2.1.3. Treatment Application and Routine Management

Diets were formulated to meet or exceed National Research Council requirements for growing-finishing beef cattle and were prepared on site [15]. The same basal diets (Table 1) were fed to all steers for the entire experiment, and all pens were fed once daily (0800 h) in the following sequence: 1) CON, 2) BOV, and 3) BOVD in order to reduce cross contamination. Feed bunks were evaluated prior to feeding in order to estimate orts and adjust feed calls to ensure *ad libitum* access to feed. The feed bunk management approach was to achieve ≤1.0 kg of dry orts in the bunk each day. Previous intake pattern and residual feed from the previous day were used to determine the amount to be fed. Damaged or excessive amounts of feed remaining in the bunks was removed, weighed, and discarded.

Treatment packages were color-coded and stored in a freezer until use. Color-coded packages were removed from the freezer daily immediately prior to supplement preparation. A total of 22.68 kg of each respective supplement was prepared daily for each of the four treatments. Supplements were prepared in a laboratory setting using commercial grade Kitchen-Aid® and cement mixers. Separate color-coded mixers, bowls, whisks, and buckets were used to prepare each of the supplements. In addition, the mixers were spatially separated in the laboratory to prevent aerosol contamination of the different treatment preparations. Treatments were prepared in the following order: 1) a premix was first made by adding 1 g/(head·d) of the respective product (CON, BOV, or BOVD) to 2.27 kg of ground corn and mixing for 3 min in a Kitchen-Aid® mixer, 2) this premix was then added to 20.41 kg of ground corn and mixed for another 5 min in a cement mixer, 3) dispensed into a corresponding receptacle which was delivered to the feed mill, and finally 4) the supplement was added to the respective total mixed ration and mixed for an additional 5 min before delivery to the appropriate treatment pens. Addition of the supplement, total daily feed delivery (as-fed), and time of feed delivery were all recorded daily.

2.1.4. Performance and Carcass Evaluation

Individual BW measurements were collected before feed delivery on the morning (0600 h) of d − 1, 0, 101, and 129. Before collection of BW measurements, feed bunks were cleaned of residual feed. Orts were weighed and sampled for DM content, and the DMI of each pen was adjusted to reflect the total DM delivered to each pen after subtracting the quantity of dry orts for each interim period. Adjusted final BW were calculated as HCW divided by a standard dressing

Table 1. Ingredient and analyzed chemical composition (DM basis) of experimental diets.

	Trial[1]		
Diet Composition	Exp. 1	Exp. 2	Exp. 3
Ingredient, % DM basis			
Alfalfa hay, mid bloom	4.12	--	10.05
Cabbage	33.30	--	--
Corn grain, rolled	13.92	--	--
Corn grain, steam flaked	--	62.10	58.20
Corn gluten feed, wet[2]	--	12.00	24.98
Corn silage	--	8.90	--
Corn syrup	3.54	--	--
Distillers grain, dry	17.06	8.10	--
Wheat	23.26	--	--
Commodity Liquid[3]	--	4.20	--
Limestone	--	--	1.35
Supplement[4]	4.80	3.30	2.00
Tallow	--	1.40	3.00
Urea	--	--	0.42
Nutrient Composition[5]			
DM, %	72.30	66.50	--
CP, %	14.50	14.40	--
EE, %	--	5.70	--
NEg, Mcal/kg	1.24	--	--
NEm, Mcal/kg	1.96	--	--
ADF, %	8.60	--	--
NDF, %	16.90	15.40	--

[1]Exp. 1: Conducted in Parma, ID (n = 400); Exp. 2: Conducted in Cactus, TX (n = 1,800); Exp. 3: Conducted in New Deal, TX (n = 112). [2]Sweet Bran®, Cargill Corn Milling, Dalhart, TX. [3]A 70:30 blend of condensed corn distiller's solubles:glycerin. [4]Exp. 1: Liquid supplement provided 25.63 mg/kg monensin and 7.05 mg/kg tylosin (DM basis, Elanco Animal Health, Greenfield, IN); Exp. 2: Dry meal supplement (Cargill Animal Nutrition, Guymon, OK) provided 37 mg/kg monensin and 10 mg/kg tylosin (Elanco Animal Health, Greenfield, IN), vitamin A (2600 IU/kg), and 8.33 mg/kg zilpaterol hydrochloride (Merck Animal Health, Florham Park, NJ) for 20 d followed by a 3 d withdrawal; Exp. 3: Dry meal supplement (Texas Tech University Burnett Center Feed Mill, New Deal, TX) provided 37 mg/kg monensin, 10 mg/kg tylosin, and 200 mg/(hd·d) ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) for the final 30 d on feed. [5]Proximate analysis of the diet for Exp. 3 is not provided due to mechanical failure leading to spoilage of diet samples prior to analysis.

percentage (63.0%) for all steers. Steers were harvested at a commercial abattoir (Washington Beef, Agri Beef Co., Toppenish, WA), and carcass data were collected via a computerized carcass evaluation system and were provided electronically to researchers. To confirm animal identification from the computerized system, harvest sequence, electronic identification, and ear tag numbers were

collected upon harvest by Johnson Research personnel.

2.1.5. Fecal Sample Collection

Fecal grab samples were collected at two times during this experiment, prior to dosage change (d 101), and immediately prior to shipping (d 129). Palpation sleeves were used to collect the sample directly from the rectum of each animal, and sleeves were replaced between animals. Feces were placed individually into pre-labeled specimen cups and transferred immediately to a cooler with ice packs for shipment to Texas Tech University International Center for Food Industry Excellence (**ICFIE**) Food Microbiology Laboratory located in Lubbock, TX for subsequent microbial analysis.

2.1.6. *E. coli* O157:H7 Detection and Isolation

Microbial analyses were conducted at the ICFIE at Texas Tech University and samples were processed upon arrival. Enrichment was performed by adding 10 g of feces to 90 ml of modified Tryptone Soy Broth (**mTSB**; Oxoid Ltda., Hampshire, England) containing bile salts and novobiocin (Novobiocin Sodium Salt, Sigma-Aldrich, St Louis, MO). Samples were then incubated at 42˚C for 16 h. Following incubation, recovery of *E. coli* O157:H7 was conducted using automated immunomagnetic separation (**IMS**). Samples were loaded into five-well tube strips with phosphate buffered saline solution (PBS Tween, pH = 7.4 with 0.05% Tween, Sigma-Aldrich, St. Louis, MO) and Dynabeads anti-*E. coli* O157 (Invitrogen, Dynal AS, Oslo, Norway). For the IMS cell recovery, a Bead Retriever (Invitrogen Corporation, Carlsbad, CA) was used following the standardized protocol from the supplier (Invitrogen, Dynal AS, Oslo, Norway). Fifty-µl of the cell-bead complex obtained after IMS was spread-plated on *E. coli* O157 medium (CHROMagar NT; Paris, France), supplemented with novobiocin (5.0 mg/L) and potassium tellurite (2.5 mg/L), and incubated at 37˚C for 18 h. Presumptive *E. coli* O157:H7 colonies were confirmed by conducting a latex agglutination test Dry Spot *E. coli* O157 (Oxoid Ltd., Basingstoke, Hants, UK).

2.1.7. *E. coli* O157:H7 Enumeration

Based on the concentration of colonies recovered after IMS, Most Probable Number technique (**MPN**) or direct plate (**DP**) was utilized for enumeration. For the MPN procedure, 10-fold dilutions were prepared by adding 11g of fecal sample into 99 ml of buffered peptone water (**BPW**; EMD Chemicals, Darmstadt, Germany). To create a 3 × 5 MPN scheme, three 1-ml aliquots of each dilution were transferred to MPN tubes containing Gram Negative broth (**GN**; Difco, Sparks, MD), and incubated at 37˚C for 6 h. Following incubation, enrichments were plated on sorbitol MacConkey agar containing the antibiotic cefixime and potassium tellurite (CT-SMAC, Difco, Sparks, MD), and further incubated at 37˚C for 24 h. For DP, 1 g of feces from each *E. coli* O157 positive sample was enriched in 9 ml mTSB and incubated at 42˚C for 16 h; serial dilutions were prepared in BPW, spread-plated on *E. coli* O157 medium supplemented with novobiocin (5.0 mg/L) and potassium tellurite (2.5 mg/L), and incubated at 37˚C

for 18 h. For both MPN and DP techniques, presumptive *E. coli* O157:H7 colonies were confirmed via latex agglutination tests Dry Spot *E. coli* O157.

2.1.8. Non-O157 Detection

Fecal samples were enriched in 9 ml of GN broth and incubated at 37˚C for 6 h. Shiga toxin-producing *E. coli* serogroups were detected using a scorpion polymerase chain reaction (**PCR**) analysis utilizing a commercially available system which tested for the presence of genes encoding for the *E. coli* O-serogroups O26, O45, O103, O111, O121 and O145 (BAX Q7, DuPont Qualicon, Wilmington, DE). Procedures were carried out according to the supplier's standard protocol. A first screening was conducted to detect the presence of the virulence factors *stx* and *eae*, and a second PCR analysis was performed to confirm the O-serogroups present in the fecal samples.

2.1.9. Statistical Analysis

All live performance and carcass data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Pen was considered the experimental unit, and treatment was included as a fixed effect. For microbial analysis, binomial response models were created for the detection of *E. coli* O157, O26, O45, O103, O111, O121 and O145 genes within each block (0 = negative, 1 = positive). *E. coli* O157:H7 enumeration data were $\log_{10}$-transformed and analyzed using a mixed linear model. Concentration analysis was only performed on positive samples. In instances where a sample was positive but could not be enumerated (*i.e.* above the limit of detection but below the limit of quantification), a fixed value of 1 was included for CFU/g concentration. This value was calculated using half of the limit of quantification for EB count plates. To obtain log CFU/g, the above value was $\log_{10}$-transformed and yielded a value of 0. For analysis of fecal shedding, single-degree-of-freedom preplanned contrasts were used to compare 1) inclusion of a DFM (CON vs BOV + BOVD + COMB) and 2) if linear effects of increased inclusion rate existed (CON, BOV, and BOVD used in this analysis). Results are reported as least squares means and were separated using the PDIFF option of SAS. For all analyses, an alpha level ≤ 0.05 was considered significant, and values between 0.05 and 0.10 were considered a tendency.

2.2. Experiment 2

2.2.1. Animals

British × Continental crossbred steers (n = 1800; BW = 354 kg) were sourced from Oklahoma and Texas and received at a commercial feedyard (Cactus Feeders, Cactus, TX) from April 17 to April 21 of 2012 (days on feed ranged from 129 to 151 d). Upon arrival, animals were processed and weighed. Initial processing included the following procedures: application of a unique identification tag, vaccination against viral (Bovi-Shield Gold; Zoetis, Florham Park, NJ) diseases, treatment for internal and external parasites (Dectomax Pour-on; Zoetis, Florham Park, NJ), administration of a broad spectrum antibiotic (Draxxin; Zoetis,

Florham Park, NJ), and administration of a terminal implant (Revalor-XS; Merck Animal Health, Florham Park, NJ).

A prepared randomization schedule was used to assign steers to pens within blocks. One block of 2 pens was filled at a time, and each receiving group was randomized separately. The candidate pool for each block was group-weighed immediately prior to processing, and the mean BW was calculated. The coefficient of variation of initial individual BW was assumed to be 10% and only steers within ±2 standard deviations [calculated as 2 × (mean BW/10)] of the mean BW were included in the study. The randomization schedule was constructed so that within succeeding groups of 2 steers each, one was randomly assigned to each of the two treatments. At the completion of the physical randomization, 1800 steers (n = 24 pens; 12 pens/treatment; 75 steers/pen) were included in the study.

2.2.2. Treatments and Experimental Design

Two treatments were used in a randomized complete block design. Treatments consisted of the following: 1) **CON** = 50 mg/(head·d) of an inert lactose carrier, and 2) **BOVD** = 50 mg/(head·d) of a commercially available DFM (Bovamine Defend®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* and *Lactobacillus acidophilus* at a concentration of 1.0×10^{11} CFU/g. Although included at a different rate than in Exp. 1 [1 g/(head·d)], a greater concentration of CFU in the supplied product meant that equal doses of the DFM were fed in both Exp. 1 and Exp. 2.

2.2.3. Treatment Application and Routine Management

Cattle were fed three times daily (0600, 0900 and 1230 h) during transition to the finishing diet. All steers began on a complete-feed starter ration (RAMP®; Cargill Corn Milling, Dalhart, TX) with substitution of the finishing diet at increasing rates (10% to 15% addition every 2 to 4 d); all cattle were transitioned to the final diet by d 20 of the experiment. Cattle were fed the final diet (**Table 1**) twice daily (0600 and 1230 h) for the remainder of the study. The objective was for all feed issued to be consumed daily. When it was necessary to discard feed, the amount was recorded and subtracted from the total amount issued.

Treatment packages were stored in a freezer and a new bag was opened daily. Treatments were added to the basal starting and finishing diets using equipment supplied, calibrated and maintained by Nutrition Physiology Company, LLC (Overland Park, KS). The treatments were added to the respective rations as follows: 1) supplement [fed at a rate of 50 mg/(head·d)] was dispensed into approximately 25 to 30 L of water and mixed thoroughly for 3 min., 2) sprayed directly onto the total mixed ration in the delivery truck, and 3) mixed for another 3 min. (1800 rpm). Separate feed trucks were used to feed each treatment in order to eliminate potential for contamination of unsupplemented feed.

Zilpaterol HCL (Zilmax, Merck Animal Health) was included in the diet (8.33 mg/kg DM) for 20 days followed by a 3 d withdrawal immediately prior to harv-

est. Throughout the experiment, diet samples were obtained daily directly from the feed bunks during morning feed delivery (0600). A portion of each sample was dried at 100˚C for 24 h in a forced air oven in order to monitor diet DM content and the remainder of the sample was frozen for analysis. Upon conclusion of the study, daily diet samples were composited by week, and weekly composites were composited within month before being submitted to a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX) for proximate analysis (**Table 1**) using AOAC procedures [16].

2.2.4. Performance and Carcass Evaluation

Initial and final BW were determined by group-weighing pens of steers on a platform scale. Total pen weight was divided by the number of steers within the pen to establish mean BW. All BW were obtained prior to the morning feeding and the final BW was multiplied by 0.96 to account for gastrointestinal fill. Trained personnel from the Cattlemen's Carcass Data Service (West Texas A&M University, Canyon, TX) recorded individual animal identification numbers in the sequence of harvest and affixed the harvest sequence number to each carcass. Plant carcass identification numbers and hot carcass weights were recorded by carcass sequence number. Carcasses were graded after approximately 36 h of chill. Quality grade (USDA Grader), yield grade (from camera data), and carcass weight listed by plant carcass identification number were obtained from the packing plant. Dressing percent for each pen was calculated as the mean HCW/mean shrunk final BW × 100.

2.2.5. Fecal Sample Collection

On the morning prior to shipping for harvest, 25 fecal pats were taken from each pen (n = 600 fecal pats). Cattle were shipped in 3 groups spanning a 22-d period (August 27th, September 10th, and September 17th, 2012). During the first harvest group, six pens were sampled for a total of 150 fecal pat samples, for the second group, eight pens were sampled (n = 200 samples), and prior to shipment of the final harvest group, ten pens were sampled (n = 250 fecal samples). Sample collection personnel were trained to prevent cross-contamination and proper fecal pat sampling. Fecal pats were collected by taking four spoon scoops from top to bottom and four spoon scoops from left to right on the fecal patty. All fecal pats were collected using aseptic technique and stored in separate, pre-labeled, sterile fecal cups (VWR International, LLC, Sugarland, TX). Samples were stored in coolers that contained ice packs and were subsequently transported to the Texas Tech University ICFIE Food Microbiology Laboratory located in Lubbock, TX for microbial analysis. All sample collection personnel were blinded to treatment.

2.2.6. Detection, Isolation, and Enumeration of *E. coli* O157:H7 and Non-O157 Serogroups

Detection, isolation, and enumeration of *E. coli* O157:H7 and non-O157 serogroups were conducted according to the same procedures outlined in Exp. 1. Of

the 600 enrichment samples collected, 56% (166 treatment samples and 167 control samples) were selected to determine the presence of the 6 additional O-serogroup genes. A scorpion polymerase chain reaction (PCR) analysis was conducted utilizing a commercially available system to test for the presence of genes encoding for the *E. coli* O-serogroups O26, O45, O103, O111, O121 and O145 (BAX Q7, DuPont Qualicon, Wilmington, DE).

2.2.7. Statistical Analysis

All live performance and carcass data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Dressing percent, quality grade, and yield grade data were rank transformed within block prior to analysis. Pen was considered the experimental unit, treatment was included as a fixed effect, and receiving group was used as the random blocking effect. For microbial analysis, binomial response models were created for the detection of *E. coli* O157, O26, O45, O103, O111, O121 and O145 genes within each block (0 = negative, 1 = positive). Least squares means were generated utilizing the PROC GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Random residual and random block were accounted for with the exception of serogroups O111 and O145. Due to the prevalence levels being low, only random residual was accounted for during the statistical analysis for these two serogroups. With the exception of serogroups O111 and O145 a relative risk (RR) was generated and confidence intervals around the relative risk were calculated using a coefficient of 1.96. *E. coli* O157:H7 enumeration data were log10-transformed and analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Concentration analysis was only performed on positive samples. In instances where a sample was positive but could not be enumerated (*i.e.* above the limit of detection but below the limit of quantification), a fixed value of 1 was included for CFU/g concentration. This value was calculated using half of the limit of quantification for EB count plates. To obtain log CFU/g, the above value was log10-transformed and yielded values of 0. Results are reported as least squares means. For all analyses, an alpha level ≤ 0.05 was considered significant, and values between 0.05 and 0.10 were considered a tendency.

2.3. Experiment 3

2.3.1. Animals, Treatments, and Experimental Design

British × Continental crossbred steers (n = 112; BW = 397 ± 30 kg) were sourced from a commercial feedlot and received at the Texas Tech University Burnett Center (Lubbock, TX). On arrival, animals were processed and individually weighed (Silencer chute; Moly Manufacturing, Lorraine, KS; mounted on Avery Weigh-Tronix load cells, Fairmount, MN; readability ± 0.45 kg). Initial processing included the following procedures: application of a unique identification tag, vaccination against viral (Vista 5; Merck Animal Health, Florham Park, NJ) and clostridial (Vision 7 + SPUR; Merck Animal Health, Florham Park, NJ) diseases, treatment for internal and external parasites (Vetrimec Pour-on; Dur-

vet, Inc., Blue Springs, MO), and administration of an implant (Ralgro; Merck Animal Health, Florham Park, NJ). Cattle were re-implanted on d 28 with a terminal implant (Revalor-S; Merck Animal Health, Florham Park, NJ).

Based on d 0 BW, steers were allocated to weight blocks (n = 7). Within each weight block, steers were randomly allocated to pen (n = 4), and each of two treatments was randomly assigned to 2 pens within each block. Following blocking and assignment to pen, steers were sorted into 28 concrete, partially slotted-floor pens (4 steers/pen; 2.9 × 5.5 m with 2.4 m of linear bunk space). The respective treatments were introduced to the diet on d 0, and were fed to provide *ad libitum* access to feed once daily in the morning for the duration of the experiment.

Two treatments were used in a randomized complete block design, and consisted of the following: 1) **CON** = 1 g/(head·d) of an inert lactose carrier, and 2) **BOV** = 1.0 g/(head·d) of a commercially available DFM (Bovamine®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* (NP24 strain) at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* (NP51 strain) at a concentration of 1.00×10^7 CFU/g.

2.3.2. Treatment Application and Routine Management

Steers began the study (d 0) on a 65% concentrate receiving ration and were stepped up over the following 22 d to a 90% concentrate finishing ration (Table 1). Diets were formulated to meet or exceed NRC (1996) requirements. Feed bunks were evaluated at approximately 0730 h daily to estimate orts and adjust feed calls to ensure *ad libitum* access to feed. The feed bunk management approach was to achieve ≤0.45 kg of dry orts in the bunk each day. Diets were mixed in a paddle type mixer, transferred by drag chain conveyor to a tractor pulled mixer (Rotomix 540-14 wagon mixer; Rotomix, Dodge City, KS), and delivered once daily beginning at 0900 h. Ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN) was fed at 200 mg/(head·d) for the final 30 d on feed.

Treatment packages with respective product were received at Texas Tech University prior to the start of the study, were placed in a freezer (−4˚C) for storage and remained frozen until use. Each package contained 56 g of product, providing sufficient product for 1 d of use at the assigned feeding rate of 1 g/(head·d). Treatments were prepared for addition to respective rations as follows: 1) one package was removed from the freezer immediately prior to use, 2) the entire contents of the package (56 g) were placed into an assigned glass bottle with a screw cap, 3) 2.5 L of distilled water was added to the glass bottle, the bottle was capped and then inverted several times, 4) the contents of the bottle were immediately transferred to an assigned spray bottle, 5) contents of the bottle were applied directly and uniformly to the total mixed ration while the ration was being continually mixed in the feed delivery wagon, 6) after product was applied, the ration was allowed to mix for an additional 3 min. before delivery to

respective pens. Unsupplemented control diets were fed first, followed by BOVD, with a flush batch following feeding in order to reduce cross contamination.

Ration samples were collected weekly, with one aliquot being dried in a forced air oven (24 h at 100˚C) to determine dry matter. An average DM was calculated for the feeding period, and actual DMI consumption was calculated at the end of the study by dividing total kg of feed consumed per pen by total head days of a pen. Another aliquot was stored for later analysis for chemical composition. Due to mechanical failure of the storage cooler, samples were rendered unusable due to spoilage, and therefore, no chemical analysis exists for Exp. 3.

2.3.3. Performance and Carcass Evaluation

Individual BW measurements were taken prior to morning feed delivery (0600 h) on d 0, 28, and 117. Before collection of BW measurements, feed bunks were cleaned of residual feed. Orts were weighed and sampled for DM content, and the DMI of each pen was adjusted to reflect the total DM delivered to each pen after subtracting the quantity of dry orts for each interim period. Adjusted final BW were calculated as HCW divided by the mean dressing percentage (64.15%) of all steers. On d 117, steers were weighed and shipped to a commercial abattoir (Cargill Meat Solutions, Plainview, TX). Carcass measurements were collected by trained personnel (West Texas A&M University) and included individual HCW, LM area, and marbling score. During the harvest process extra carcass trim, fat, and hide pulls of soft tissue ≥ 6.8 kg, were noted. Yield grade was calculated using the USDA regression equation [17].

2.3.4. Fecal Sample Collection

The day cattle were shipped to the harvest facility (d 117), each animal was placed into a working chute and fecal grabs were collected directly from the rectum of each animal (n = 112 fecal grabs). Sample collection personnel were trained to prevent cross-contamination and proper fecal grab sampling. Fecal grabs were collected using large, arm length, plastic palpation sleeves and placed in separate, pre-labeled, sterile fecal cups (VWR International, LLC, Sugarland, TX). Samples were stored in coolers that contained ice packs and were subsequently transported to the Texas Tech University ICFIE Food Microbiology Laboratory located in Lubbock, TX for microbial analysis. All sample collection personnel were blinded to treatment. Detection and isolation of *E. coli* O157:H7 and non-O157 serotypes was conducted according to the same procedures outlined in Exp. 1. Due to insufficient sample size, enumeration of samples was not carried out for this experiment.

2.3.5. Statistical Analysis

All live performance and carcass data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Pen was considered the experimental unit, treatment was included as a fixed effect, and bodyweight block was used as the random blocking effect. For microbi-

al analysis, binomial response models were created for the detection of *E. coli* O157, O26, O45, O103, O111, O121 and O145 genes within each block (0 = negative, 1 = positive). Least squares means were generated utilizing the PROC GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Random residual and random block were accounted for in each serogroup analyzed with the exception of serogroups O26 and O45. Due to the prevalence levels being low, only random residual was accounted for during the statistical analysis for these two serogroups. A relative risk (RR) was generated and confidence intervals around the relative risk were calculated using a coefficient of 1.96. Due to the low number of quantitated observations in this study, formal statistical analysis was not performed for enumeration of *E. coli* O157 as previously described in Exp. 1 and 2. For all analyses, an alpha level ≤ 0.05 was considered significant, and values between 0.05 and 0.10 were considered a tendency.

3. Results

3.1. Experiment 1

No significant differences or tendencies were observed for any feedlot performance parameters or carcass characteristics measured in this study (Table 2 and Table 3, respectively). Prevalence of *E. coli* O157:H7 (Table 4) was unaffected by treatment, however, concentration of this serotype in fecal samples was significantly reduced ($P < 0.01$) with DFM supplementation. A linear reduction ($P < 0.01$) in concentration was observed with increasing CFU of NP51 through the addition of BOV [1.01 CFU/(head·d)] and BOVD [2.00 CFU/(head·d)]. Both BOVD and COMB treatments had significantly lower ($P \leq 0.05$) *E. coli* O157:H7 concentrations in fecal samples than unsupplemented controls, while BOV was intermediate. Overall, *E. coli* O157:H7 enumeration of positive samples resulted in a reduction of 2.13 $\log_{10}$ cycles ($P < 0.01$) when cattle were supplemented with BOVD versus CON.

Prevalence of non-O157 O-serogroups in fecal samples was also analyzed (Table 4). Direct-fed microbial supplementation reduced the prevalence ($P = 0.02$) of serogroup O45, and tended to reduce ($P = 0.09$) prevalence of O26. A linear reduction in the prevalence of both O26 and O45 ($P < 0.01$ and $P = 0.03$, respectively) was achieved by increasing NP51 supplementation in BOV and BOVD cattle. Furthermore, although treatment and linear contrasts were significant for these serotypes, pairwise comparisons revealed that only BOVD supplemented cattle had significantly lower prevalence than CON, with reductions of 41% and 34% for O26 and O45 serogroups, respectively. Although numerical reductions in the prevalence of all other evaluated serogroups were observed when cattle were supplemented with BOVD, no other significant differences were detected.

3.2. Experiment 2

No significant differences were observed for any of the feedlot performance

Table 2. Effect of direct-fed microbials on adjusted beef cattle performance.

	Treatment[1]				
Item	CON	BOV	BOVD	COMB	SEM
			Experiment 1		
Pens	10	10	10	10	
Days on Feed	129	129	129	129	
Initial BW, kg	345	346	344	344	0.9
Final BW, kg[2]	597	602	599	594	3.1
DMI, kg	11.66	11.61	11.52	11.61	0.10
ADG, kg	1.97	2.00	1.99	1.95	0.02
G:F	0.170	0.172	0.173	0.167	0.002
			Experiment 2		
Pens	12	--	12	--	
Days on Feed	142	--	142	--	
Initial BW, kg	354	--	355	--	1.0
Final BW, kg[3]	610	--	613	--	2.0
DMI, kg	9.88	--	9.97	--	0.05
ADG, kg	1.80	--	1.82	--	0.01
G:F	0.182	--	0.183	--	0.002
			Experiment 3		
Pens	14	--	14	--	
Days on Feed	117	--	117	--	
Initial BW, kg	398	--	397	--	1.0
Final BW, kg[4]	582	--	587	--	6.3
DMI, kg	9.84	--	9.84	--	0.20
ADG, kg	1.57	--	1.63	--	0.05
G:F	0.166	--	0.160	--	0.005

[1]CON = lactose carrier only; BOV = 1.0 g/(head·d) of a commercially available DFM (Bovamine®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* (NP24 strain) at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* (NP51 strain) at a concentration of 1.00×10^7 CFU/g; BOVD = 1 g/(head·d) of a commercially available DFM (Bovamine Defend®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* at a concentration of 1.00×10^9 CFU/g; COMB = BOV fed for the first 101 d on feed, followed by BOVD which was fed for the final 28 d on feed. [2]Calculated as HCW divided by 0.63. [3]Carcass-adjusted final live weight = ((total final pen scale weight, kg/final pen head) × 0.96) × (pen dressing %/trial mean dressing %). [4]Calculated as HCW divided by 0.6415.

Table 3. Effect of direct-fed microbial on carcass characteristics of feedlot steers.

	Treatment[1]				
Item	CON	BOV	BOVD	COMB	SEM
			Experiment 1		
HCW, kg	376	379	377	375	2.0
Dressing percent	61.6	61.9	61.7	61.5	0.14

Continued

LM area, cm^2	88.74	89.97	89.68	87.53	1.06
Yield grade	2.91	2.95	2.86	2.98	0.07
Marbling score[2]	422	420	417	425	7.5
		Experiment 2			
HCW, kg	398	--	400	--	1.3
Dressing percent	65.2	--	65.3	--	0.08
Quality Grade distribution[3], %					
Prime + Choice	41.20	--	45.00	--	2.23
Select	54.60	--	51.60	--	2.09
No Roll and lower	4.30	--	3.40	--	0.60
Yield Grade distribution[3], %					
Yield Grade 1	21.70	--	17.30	--	2.25
Yield Grade 2	44.90	--	46.60	--	1.15
Yield Grade 3	29.30	--	29.70	--	1.31
Yield Grades 4 + 5	4.10	--	6.40	--	0.92
		Experiment 3			
HCW, kg	373	--	377	--	4.1
Dressing percent	64.0	--	64.4	--	0.42
LM area, cm^2	91.60	--	92.20	--	1.74
Yield Grade	2.76	--	2.77	--	0.16
Marbling Score[2]	436	--	439	--	14.1

[1]CON = lactose carrier only; BOV = 1.0 g/(head·d) of a commercially available DFM (Bovamine®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* (NP24 strain) at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* (NP51 strain) at a concentration of 1.00×10^7 CFU/g; BOVD = 1 g/(head·d) of a commercially available DFM (Bovamine Defend®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* at a concentration of 1.00×10^9 CFU/g; COMB = BOV fed for the first 101 d on feed, followed by BOVD which was fed for the final 28 d on feed. [2]Marbling score: 400 = small[00]; 500 = modest[00]; [3]Distributions = carcass kg of each quality or yield grade expressed as a percentage of total carcass kg. Statistical analysis was on treatment numerical rank within blocks.

Table 4. Effect of direct-fed microbial supplementation on fecal shedding of *E. coli* O157:H7 and non-O157 serotypes in feedlot cattle (Exp. 1).

	Treatment[1]					*P*-value[2]	
Item	CON	BOV	BOVD	COMB	SEM	TRT	Linear
E. coli O157:H7							
Prevalence, %	10.0	9.2	13.3	8.2		0.73	0.50
Concentration, Log CFU/g	2.26^a	1.04^{ab}	0.13^b	0.49^b	0.46	<0.01	<0.01
Non-O157 Prevalence, %							
O26	61.7^a	54.3^a	36.3^b	51.3^{ab}		0.09	<0.01
O45	67.9^a	72.8^a	45.0^b	72.5^a		0.02	0.03

Continued

O103	55.6	58.0	40.0	48.8	0.25	0.11
O111	6.2	3.7	1.3	2.5	0.57	0.24
O121	3.7	2.5	1.3	1.3	0.67	0.34
O145	8.6	9.9	7.5	10.0	0.94	0.79

[a,b]Row means that do not have a common superscript differ ($P \leq 0.05$). [1]CON = lactose carrier only; BOV = 1.0 g/(head·d) of a commercially available DFM (Bovamine®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* (NP24 strain) at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* (NP51 strain) at a concentration of 1.00×10^7 CFU/g; BOVD = 1 g/(head·d) of a commercially available DFM (Bovamine Defend®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* at a concentration of 1.00×10^9 CFU/g; COMB = BOV fed for the first 101 d on feed, followed by BOVD which was fed for the final 28 d on feed. [2]Preplanned contrasts were used to evaluate TRT = CON vs. BOV + BOVD + COMB and Linear = CON, BOV, and BOVD.

parameters or carcass characteristics measured in this study (Table 2 and Table 3, respectively). Supplementation with BOVD tended to increase ($P = 0.08$) the percentage of USDA Prime and Choice carcasses when compared to unsupplemented controls. Percentage of USDA Yield Grade (**YG**) 1 carcasses tended to be reduced ($P = 0.08$) by supplementation with BOVD, while percentage of YG 2 carcasses tended to be greater ($P = 0.08$) in BOVD supplemented cattle. Overall, carcass characteristics between the two treatments were similar.

The prevalence of *E. coli* O157:H7 in fecal samples was reduced by 45% ($P < 0.01$) in pens of cattle that were supplemented with BOVD (Table 5). Furthermore, *E. coli* O157 enumeration of positive samples indicated that there was a significant reduction of 1.23 $\log_{10}$ cycles/g ($P = 0.02$) when cattle were supplemented with BOVD versus unsupplemented CON. Genes encoding for the *E. coli* serogroups O26, O45, O103, O111, O121 and O145 were screened in 56% of the fecal samples collected. From CON pens, 6.8% (n = 17) of fecal samples tested positive for O26 compared to 3.2% (n = 8) of fecal samples collected from BOVD pens (Table 5). This was equal to a significant ($P = 0.02$) 53% reduction in the prevalence of this serogroup, similar to the 41% reduction observed in Exp. 1. Additionally, fecal samples from BOVD supplemented cattle had a significant reduction in the prevalence of O45, O103, and O121 ($P = 0.01$, 0.03, and 0.02, respectively) compared to CON. Serogroups O111 and O145 were not significantly different between treatments ($P = 0.97$), but prevalence in CON samples was near zero (0.6% for both).

3.3. Experiment 3

No significant differences or tendencies were observed for any feedlot performance parameters or carcass characteristics measured in this study (Table 2 and Table 3, respectively). Although not significant, BOVD supplementation tended to reduce ($P = 0.08$) fecal shedding of *E. coli* O157:H7. The 4-fold reduction in prevalence of this serotype was greater than, but similar to, the reduction reported in Exp. 2 (45%). Concentration of *E. coli* O157:H7 in fecal samples was

Table 5. Effect of direct-fed microbial supplementation on fecal shedding of *E. coli* O157:H7 and non-O157 serotypes in feedlot cattle (Exp. 2 and 3).

	Treatment[1]				CI for RR	
Item	CON	BOVD	RR[2]	*P*-value	Lower	Upper
			Experiment 2			
E. coli O157:H7						
Prevalence, %	25.1	13.9	0.55	<0.01	0.41	0.74
Concentration, Log CFU/g	1.88	0.65	--	0.02	--	--
Non-O157 Prevalence, %						
O26	6.8	3.2	0.47	0.02	0.24	0.90
O45	31.6	18.6	0.59	<0.01	0.43	0.80
O103	29.8	19.5	0.65	0.03	0.45	0.95
O111	0.6	0.0	--	0.97	--	--
O121	11.1	5.8	0.53	0.02	0.31	0.90
O145	0.6	0.0	--	0.97	--	--
			Experiment 3			
E. coli O157:H7						
Prevalence, %	14.8	3.7	0.25	0.08	0.05	1.14
Concentration[3]	--	--	--	--	--	--
Non-O157 Prevalence, %						
O26	74.6	52.7	0.71	0.02	0.53	0.95
O45	76.4	58.2	0.76	0.05	0.58	1.00
O103	25.5	18.2	0.71	0.36	0.35	1.48
O111	38.2	31.0	0.81	0.43	0.50	1.30
O121	20.0	34.6	1.73	0.10	0.90	3.30
O145	5.5	3.6	0.67	0.65	0.19	2.32

[1]CON = lactose carrier only; BOVD = 1 g/(head·d) of a commercially available DFM (Bovamine Defend®; Nutrition Physiology Company, LLC, Overland Park, KS) comprised of a combination of *Propionibacterium freudenreichii* at a concentration of 1.00×10^9 CFU/g and *Lactobacillus acidophilus* at a concentration of 1.00×10^9 CFU/g; [2]RR = risk ratio; [3]Sample size was too small for enumeration in this experiment.

not evaluated for this study, as sample size was too small for enumeration. Direct fed microbial supplementation reduced fecal shedding of *E. coli* serogroups O26 and O45 ($P = 0.02$ and 0.05, respectively). Similar to previously reported results, prevalence of serotype O26 was reduced by 29%. Although numeric reductions in prevalence were observed in serogroups O103, O111 and O145 for BOVD treated cattle, no significant differences were observed ($P \geq 0.36$).

4. Discussion

According to the most recent Feedlot Nutritionist Survey [18], which represents approximately half of the U.S. fed cattle population, 56.9% of clients represented

by respondents utilized direct-fed microbials or probiotics in their finishing diets. While the potential benefits have been described, previous studies assessing the impact of DFM on feedlot performance and carcass characteristics have yielded conflicting results. Vasconcelos *et al.* (2008) evaluated increasing doses of live cultures of *L. acidophilus* (**LA**) combined with a single dose of *P. freudenreichii* (**PF**) [19]. Two of the three treatments were equal to the BOV and BOVD treatments utilized in the current studies, and the third contained an intermediate level of LA. Vasconcelos *et al.* (2008) concluded that supplementation with various concentrations of DFM in feedlot steers for 140 d prior to harvest had no effect on final BW or DMI [19]. Numerous other studies have also reported similar results that support the current data, where LA supplementation did not alter DMI [20] [21] [22]. Rust *et al.* (2000), Galyean *et al.* (2000), and Elam *et al.* (2003) all evaluated the same bacterial strains at similar or identical levels as reported in the current study. Although DMI was unaffected by DFM supplementation [20] [21] [22]. Galyean *et al.* (2000) noted that final BW and carcass-adjusted final BW were greater for the average of all DFM treatments vs. the control diet [21]. Similarly, Rust *et al.* (2000) also reported that DFM supplementation yielded greater final BW than controls [20]. These trials contradict the results reported by Elam *et al.* (2003) [22], which agreed with the current study and others [19] [23], and concluded that supplementation of a DFM did not affect final BW.

In the current study, no differences among treatments were observed for ADG ($P \geq 0.30$). Two of the more recent studies [19] [22] support these results and also concluded that DFM treatment had no effect on ADG. In a commercial study conducted by Cull *et al.* (2015), which represented more than 15,000 cattle, a combination of LA and PF was fed at 1.01×10^9 CFU/(head·d) and no differences were observed in DMI, final BW, or ADG [24]. Alternatively, a review by Krehbiel *et al.* (2003) included data from six experiments, which summarized the effects of varied concentrations and strains of LA and PF and concluded that feeding bacterial DFM to feedlot cattle resulted in a 2.5% to 5% increase in ADG with an inconsistent effect in DMI [25]. Ware *et al.* (1988) had previously summarized data from eight trials and also reported that supplementation with LA increased ADG in steers by approximately 4% [25], and Galyean *et al.* (2000) reported that DFM-supplemented steers tended to have improved ADG when compared to unsupplemented controls [21].

With inconsistent effects on DMI and ADG, feed efficiency has also shown varied results. Similar to previously discussed measures of performance, feed efficiency was also unaffected by DFM treatment in any of the current study. Kiesling *et al.* (1982) reported that DFM supplementation did not affect G:F during a 28-d receiving or 209-d finishing period [23]. Elam *et al.* (2003) also supported the current results, where treatments similar to those evaluated in the current study produced no differences in feed efficiency [22]. Conversely, in 2008, Vasconcelos *et al.* concluded that G:F was improved when all DFM treatments were pooled versus unsupplemented controls [19]. It was further reported

that the two treatments which were analogous to the current BOV and BOVD treatments were most favorable for improving feed efficiency. Rust *et al.* (2000) also reported improved G:F in LA and PF supplemented cattle compared to controls [20]. Finally, a more recent study by Cull *et al.* (2015) reported a significant improvement in G:F of 2.5% with DFM supplementation [20], which agreed with the previously cited review by Krehbiel *et al.* (2003), where it was concluded that DFM supplementation results in an average improvement of 2% in feed efficiency [25].

No significant differences were observed for any carcass characteristics in the current study, however, BOVD tended to increase the percent of Choice + Prime carcasses when compared to control. Huck *et al.* (2000) reported a similar increase in Prime + Choice carcasses in heifers receiving *Propionibacterium* DFM versus controls, which agrees with the tendency that we observed [26]. Other studies have also failed to identify differences in carcass characteristics outside of conflicting results on HCW that are likely a function of the varied responses on ADG and final BW. Galyean *et al.* (2000) and Peterson *et al.* (2007) both reported significant increases in HCW for cattle supplemented with DFM [21] [27]. Similarly, both a 2011 meta-analysis [28] and summarized results of multiple studies by Krehbiel *et al.* (2003) demonstrated similar improvements [25]. The differences reported in these studies coincided with a comparable improvement in final BW. Still, many investigations [20] [22] [23] have not identified significant improvements in carcass weights. Besides HCW responses reported in some studies, other carcass characteristics rarely differ. Improvements in these traits due to DFM supplementation would be difficult to explain beyond changes in HCW as a function of ADG in treated animals. Although metabolic manipulation of VFA profiles as a result of DFM supplementation could lead to differences in fat synthesis and distribution [22], the mechanisms to explain these changes are not well understood. Overall, performance and carcass characteristics in DFM supplemented cattle remain inconsistent.

A number of studies have evaluated *L. acidophilus* supplementation as a pre-harvest intervention to reduce shedding of *E. coli* O157 in the cattle production system. Peterson *et al.* (2007) collected fecal samples from 448 steers every three weeks during the course of a two-year period [27]. Half of the cattle were fed NP51 at a rate of 10^9 CFU/(head·d), and half of the cattle were unsupplemented controls. During the first year, 21% of fecal samples collected from control steers tested positive for *E. coli* O157:H7, while 13% were positive in NP51 supplemented cattle. In the second year, fecal samples from control animals tested positive for *E. coli* O157:H7 28% of the time and 21% were positive in treated cattle. Overall, this resulted in a 35% reduction in *E. coli* O157:H7 prevalence in fecal samples from DFM treated cattle [27]. In the current study, control cattle shed *E. coli* O157 in 23.2% of fecal samples (weighted mean), while BOVD supplemented cattle had a prevalence of 13.8% (weighted mean). This was equal to a 40% reduction in the prevalence of *E. coli* O157:H7 in BOVD samples, which agrees with Peterson *et al.* (2007) [27]. Younts-Dahl *et al.* (2004) evaluated

the effect of four DFM treatments [**CON**: no DFM; **HNP51**: *L. acidophilus* (NP51) fed at 10^9 CFU/(head·d); **HNP51 + 45**: HNP51 + *L. acidophilus* (NP45) fed at 10^6 CFU/(head·d); **LNP51 + 45**: NP51 fed at 10^6 CFU/(head·d); all DFM treatments also included *P. freudenreichii* fed at 10^9 CFU/(head·d)] on fecal shedding of *E. coli* O157:H7 [29]. Prior to harvest, cattle receiving HNP51 (analogous to BOVD in Exp. 1, 2, and 3) were 57% less likely to shed detectable levels of *E. coli* O157 in their feces than controls. This same treatment reduced *E. coli* O157 prevalence in both fecal and hide samples upon harvest, and it was concluded that the HNP51 treatment may be an efficacious pre-harvest intervention to reduce fecal shedding of this pathogen [29]. A subsequent study by Younts-Dahl *et al.* (2005) evaluated a dose titration of the NP51 strain of LA [**CON**, **LNP51**, **MNP51**, and **HNP51** fed at rates of 0, 10^7, 10^8, and 10^9 CFU/(head·d); all DFM treatments also included *P. freudenreichii* fed at 10^9 CFU/(head·d)] [11]. *Lactobacillus acidophilus* supplementation significantly reduced *E. coli* O157 prevalence throughout the feeding period, and the response was a linear decrease in prevalence with increasing NP51 dose. Similar to previously cited reports [27] [29], Younts-Dahl *et al.* (2005) saw a 71.5% reduction in *E. coli* O157 prevalence when cattle were fed the highest dose of NP51, analogous to BOVD in the current study [11]. Finally, a study conducted in 2007 [30] indicated that cattle fed a high level of NP51 were 51% less likely to shed *E. coli* O157 in feces than control cattle, and of those testing positive, a significant reduction in the concentration of *E. coli* O157 (MPN/g feces) was observed.

In Experiments 1 and 2, enumeration results indicated a 94% and 65% $\log_{10}$ reduction of *E. coli* O157 ($P \leq 0.02$) when cattle were supplemented with BOVD compared to controls. To our knowledge, only one other published dataset evaluating the impact of *L. acidophilus* NP51 on the concentration of *E. coli* O157 has been reported [30]. Stephens *et al.* (2007) reported a 2.3 $\log_{10}$ reduction in *E. coli* O157 when cattle were supplemented with a high dose of NP51, supporting the current results from Exp. 1 and 2 [30]. The data from these three experiments support the concept that *L. acidophilus* NP51 at fed at 10^9 CFU/(head·d) is a valuable pre-harvest intervention to not only reduce the prevalence *E. coli* O157, but also to reduce the concentration of this pathogen in the feces of feedlot cattle. Although the scientific evidence supports *L. acidophilus* NP51 supplementation as a successful pre-harvest intervention to reduce *E. coli* O157 shedding in feedlot cattle, the efficacy of this DFM to reduce the prevalence of other serogroups (including O26, O45, O103, O111, O121 and O145) have not previously been reported. The data presented in the current study suggest that feeding BOVD may be an effective pre-harvest food safety intervention to control some non-O157 O-serogroups. Specifically, serogroups O26 and O45 were significantly reduced in all three experiments. Furthermore, serogroups O103 and O121 were significantly reduced in a large pen commercial setting (Exp. 2), and although they were not significantly reduced in Exp. 1, reductions in prevalence of 28% (O103) and 65% (O121) were observed. Results from Exp. 3 did not show significant reductions in *E. coli* serotype O157:H7 ($P = 0.08$), however, se-

rogroups O157, O26, O45, O103, O111, and O145 were reduced by 75, 29, 24, 29, 19, and 35%, respectively. The fact that O157, O103, O111, and O145 did not suggest significant reductions could be a function of the reduced sample size (n = 56 animals/treatment), or the production system. Whereas Exp. 1 and 2 were conducted in dirt lot pens, Exp. 3 was conducted in a facility where animals were housed in 28 concrete, partially slotted-floor pens (4 head/pen). Prior to the experiment, we hypothesized that lower shedding incidence may be observed based on smaller pen-size (less contamination of low fecal shedding animals by a high shedding animal) or based on sanitation (fecal matter was collected in drains below the slotted-floors so direct fecal contact was reduced).

Based on the collective results reported in the current study, we conclude that the supplementation of a commercially available DFM [BOVD; providing a combination of *P. freudenreichii* and *L. acidophilus* at a rate of 1.00×10^9 CFU/g and 1.00×10^7 CFU/g/(head·d), respectively], to reduce fecal shedding of *E. coli* O157:H7 and other *E. coli* serogroups is an effective pre-harvest food safety intervention. Though its effects on *E. coli* O157 have been thoroughly reported, further evaluation of *L. acidophilus* NP51 as a means to reduce other *E. coli* serogroups is warranted due to the potential implications for food safety in the beef industry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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