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Research Paper

Prevalence of Foodborne Pathogens in Pacific Northwest Beef Feedlot Cattle Fed Two Different Direct-Fed Microbials

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ABSTRACT

In recent years, there has been an increased interest in beef cattle shedding of foodborne pathogens due to the potential to contaminate surrounding food crops; however, the number of studies published on this topic has declined as the majority of research has emphasized on postharvest mitigation efforts. A field study was conducted to determine the prevalence of pathogens and indicator bacteria in beef cattle fed two different direct-fed microbials (DFMs). Fecal samples from a total of 3,708 crossbred yearling cattle randomly assigned to 16 pens and two treatment groups at a commercial cattle feedlot were taken. During the study period, diets were supplemented with two different DFMs i.) *Lactobacillus acidophilus* (NP51) and *Propionibacterium freudenreichii* (NP24) (9 $\log_{10}$CFU/head/day), and ii.) *Lactobacillus salivarius* (L28) (6 $\log_{10}$CFU/head/day). Fecal samples from pen floors were collected on days 0, 21, 42, 63, 103, and analyzed for the presence of *Salmonella* and *E. coli* O157:H7 and concentration of *E. coli* O157:H7, Enterobacteriaceae, and *C. perfringens*. Fecal samples collected from cattle fed L28 had significantly lower concentration of *C. perfringens* ($p < 0.05$) and had a similar prevalence with no significant differences in *E. coli* O157:H7 as those fed NP51/NP24 through the study until day 103. On day 103, the prevalence in cattle fed L28 was 40% with a concentration of 0.95 $\log_{10}$MPN/g while those fed NP51/NP24 were 65% with a concentration of 1.2 $\log_{10}$MPN/g. Cattle supplemented with NP51/NP24 achieved a significant log reduction of EB by 2.4 $\log_{10}$CFU/g over the course of the 103-day supplementation period compared to L28. *Salmonella* prevalence was also measured, but not detected in any samples at significant amounts to draw conclusions. It is evident that *E. coli* O157:H7 and other foodborne pathogens are still prevalent in cattle operations and that preharvest mitigation strategies should be considered to reduce the risk to beef products.

The United States is the world's largest producer and consumer of beef, which makes it an important agricultural commodity (U.S. Department of Agriculture Economic Research Service., 2022). In 2020, U.S. beef consumption was over 26.3 kg per capita, and when included in the diet provides a major source of key nutrients such as, essential amino acids, iron, and high-quality protein (Agriculture Economic Insights., 2021). Beef cattle can host pathogens such as *Escherichia coli* O157:H7 and *Salmonella*, which have resulted in foodborne illness outbreaks (Laufer et al., 2015; Rangel et al., 2005; Tack et al., 2021). Due to reoccurring outbreaks associated with beef products, *E. coli* O157:H7 and six non-O157 Shiga toxin-producing *E. coli* (STEC) serogroups were declared as adulterants in raw ground beef and trimmed by the U.S. Department of Agriculture (USDA) (U.S. Department of Agriculture., 2012). Additionally, the Hazard Analysis and Critical Control Points (HACCP) framework was mandated in facilities that produce meat and poultry along with in-plant validations of interventions (Code of Federal Regulations (CFR), 1996; Food Safety Inspection Services, 2015). Due to the response of the industry, illnesses associated with STECs in ground beef have declined substantially over the past decade. From 2013 to 2019, there has been a 13.6% reduction in beef attribution toward *E. coli* O157:H7 cases in the U.S. (Interagency Food Safety Analytics Collaboration, 2017; Interagency Food Safety Analytics Collaboration, 2021). The implementation of these systems and control measures have greatly contributed to the advancements made in the beef industry to reduce microbial contamination with the use of postharvest interventions.

Salmonella is another common pathogen associated with outbreaks. The Centers for Disease Control and Prevention (CDC) estimates 80,000 annual cases of salmonellosis are associated with the consumption of intact beef cuts and ground beef, in which 53% was estimated

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to be associated with ground beef (U.S. Department of Agriculture Economic Research Service., 2022). Three *Salmonella* outbreaks related to ground beef since 2017 have urged the regulatory agencies to increase the efforts to control contamination, as the total number of cases due to these three outbreaks surpassed the total estimated cases to date (National Center for Emerging and Zoonotic Infections, 2022). As a result of continued outbreaks, in 2019, the FSIS proposed *Salmonella* performance standards for raw ground beef and beef manufacturing trimmings, which involves a 52-week moving sampling window to determine if an establishment is meeting the required pathogen reduction performance standard (U.S. Department of Agriculture – Food Safety and Inspection Service., 2019). These proposed performance standards, which have not been implemented to date, have encouraged novel pre- and postharvest *Salmonella* mitigation strategies within the industry to reduce contamination in the final product.

In addition to the concern about contaminated beef products, there is also a concern about potential *E. coli* O157:H7 contamination of leafy greens from nearby cattle farms. Recent reports from the U.S. Food and Drug Administration (FDA) indicated that the proximity of cattle operations and cattle grazing land could be a risk factor to the recent *E. coli* O157:H7 outbreaks by consumption of raw leafy greens. Between fall 2019 and 2020, four *E. coli* O157:H7 outbreaks linked to leafy greens, produced in the California growing region, resulted in over 220 human illnesses and the strains from these outbreaks were isolated in cattle fecal samples found near the produce fields (U.S. Food and Drug Administration, 2020, 2021). Although the link between these two commodities is not demonstrated through direct empirical data, it is suggested that preharvest interventions that target foodborne pathogens in livestock are expected to improve public health targets by reducing contamination on fresh produce (Benjamin et al., 2015). Preharvest supplements such as direct-fed microbials (DFMs), can reduce multiple pathogens and indicator bacteria present in the animal's GI tract, which can stabilize the gut microflora and improve the animal's health, by reducing undesirable pathogenic bacteria and overall reduce the presence of both foodborne and animal pathogens.

Furthermore, *C. perfringens* is also carried by cattle and is a human foodborne pathogen; however, it is known to be an animal pathogen responsible for the death of ruminants, including cattle, which needs to be addressed in terms of animal health and production economy (Uzal et al., 2015). An important factor to consider with preharvest interventions and research conducted in this area is that many are pathogen-specific, most commonly for *E. coli* O157:H7. For instance, NP51/NP24 has been widely used and studied in feedlot settings but is specific to only targeting *E. coli* O157:H7, not the other STEC serotypes of concern or *Salmonella* and other pathogens. It is important to acknowledge that there is far more than one pathogen from beef cattle that can cause human and animal disease; therefore, using a pathogen-specific approach is not applicable. Cattle have a very complex microbial ecosystem in their gut and when not in a symbiotic relationship with the rumen microbiota, the animal's health and performance are affected (Azad et al., 2018). Therefore, it is important to harmonize the balance and activities of gastrointestinal microbiota to achieve gut homeostasis (Kober et al., 2022; Mansilla et al., 2022). This can be satisfied through the utilization of probiotics in the animal's diet, also referred to as DFMs.

Because of the significant advances in the beef industry in reducing human illnesses of STEC linked to ground beef, there has been a decline in new data published on the presence of pathogens in beef cattle environments. Interest in this area waned as the processing industry took significant steps to control the pathogen, postharvest. However, it is critical to reexamine this reservoir as it could be linked back to outbreaks via cross-contamination of surrounding crops (U.S. Food and Drug Administration, 2019, 2021). Additionally, literature on the use of DFMs, in cattle, for other pathogens and indicator bacteria such as *Salmonella*, *Clostridium perfringens*, and Enterobacteriaceae is limited; therefore, other products targeting a wide variety of pathogens need to be developed for a greater food safety impact. However, novel *Lactobacillus salivarius* L28 (commercially available as Probicon), through various studies, was reported to inhibit *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes,* and *C. perfringens in vitro* and *in vivo* (Ayala et al., 2019; Flach et al., 2022) as well as artificially challenged cattle manure (Castelli, 2018). Our previous work indicates that the supplementation of L28, as a DFM, in the cattle's diet significantly reduced *E. coli* O157:H7 and *Salmonella* prevalence and *C. perfringens* concentration in cattle feces compared to the control group and cattle treated with NP51/NP24, thus providing control of multiple foodborne pathogens in the preharvest stage using a single preharvest intervention (Flach et al., 2022).

The overall aim of this study was to describe the prevalence and concentration of pathogens and indicator microorganisms in a beef cattle feeding operation, located in the Pacific Northwest, from cattle fed NP51/NP24 and L28.

Materials and methods

Cattle feed yards. This study was conducted at a commercial beef cattle feedlot in the Pacific Northwestern (PNW) region of the United States. A total of 3,708 crossbred yearling cattle were randomly assigned to 16 pens, upon arrival. Pens housed approximately 218–264 head, and each pen was randomly assigned to a treatment (n = 8 pens/treatment). Cattle were on the same feeding program for approximately 161 days prior to harvest. All cattle were fed a standard finishing diet with steam-flaked corn, silage, and supplemented with the necessary protein, vitamins, and minerals as well as monensin and tylosin. The study consists of two treatment groups (n = 1,854 head/treatment) whose standard diets were supplemented with i.) Bovamine Defend (CHR-Hansen; Hoersholm, Denmark): *Lactobacillus acidophilus* (NP51) and *Propionibacterium fredenreichii (*NP24) at a target dose of 9 $\log_{10}$CFU/head/day and ii.) Probicon (NexGen Innovation, LLC, Lubbock, TX): *Lactobacillus salivarius* (L28) at a target dose of 6 $\log_{10}$CFU/head/day. DFMs were incorporated into cattle feed rations daily by directly mixing prior to distribution to the pens assigned to each supplement. The cattle did not receive any DFM supplementation prior to arriving at the feedlot.

Fecal sample collection. Feces from feedlot cattle were sampled from the feedlot pens from September to December 2021. The cattle started receiving the supplemented feed rations immediately upon arrival, and they were fed for an average of 161 days prior to harvest. Fecal samples were taken on arrival and after 21, 42, 63 and 103 days of DFM supplementation. Six fresh floor fecal samples from each pen were taken using disposable plastic spoons (n = 96 fecal samples per sampling event), immediately chilled and shipped without indication of treatment group to a third-party laboratory (Food Safety Net Services, San Antonio, TX, USA).

Fecal Sample Analysis. Fecal samples were analyzed by the third-party laboratory using the procedure previously reported by Flach et al. (2022). Briefly, 10 g of subsamples were aseptically transferred to sterile, filtered sampling bags for each separate test and the original sample was retained for further testing if necessary. For the detection of *E. coli* O157:H7, BAX System Real-Time Polymerase Chain Reaction (RT-PRC) Assay for *E. coli* O157:H7 was utilized after primary enrichment in BAX MP Media (Hygiena, Camarillo, CA, USA). Samples tested positive were quantified using 3-tube most probable number (MPN) method, and the positive tubes were further tested using the BAX System for confirmation. For *Salmonella*, BAX System RT-PCR Assay for *Salmonella* was conducted after enrichment in buffered peptone water (BPW); however, no quantitative testing was done.

Culture-based methods were used for enumeration of EB and enumeration of *C. perfringens* by plating tenfold dilutions of the sample

on Petrifilm™ Enterobacteriaceae Count Plates (3M, St. Paul, MN, USA) and Tryptose Sulfite Cycloserine Agar, respectively.

Limits of detection (LOD) were 3 MPN/g for the MPN method for *E. coli* O157:H7 enumeration and 3 $\log_{10}$CFU/g (1000 CFU/g) for the plate counts of EB and *C. perfringens*. For the statistical analysis of plate counts of *C. perfringens*, any value below the LOD was taken as half of the LOD (1.5 $\log_{10}$CFU/g).

Statistical analysis. Data analysis was conducted using the 'geepack' package for R (version 4.1.1) (Liang and Zeger, 1986). Generalized estimating equations were utilized in prior DFM studies (Flach et al., 2022; Peterson et al., 2007) and it is suggested for the analysis of longitudinal dichotomous and continuous data where the repeated measures are expected to have correlation (Ballinger, 2004; Hamza et al., 2018). Continuous dependent variable (concentration) was modeled as Normal distribution and dichotomous dependent variable (prevalence) was included in the GEE models using Binomial distribution with logit link (Hanley et al., 2003). Independent variables (treatment and sampling date) were considered as categorical variables, and each sampled pen was considered as a cluster. The level of statistical significance was $p < 0.05$, and any term with a p value between 0.05 and 0.10 was considered as marginally significant. Any nonsignificant interaction term in the analysis was removed to obtain the most parsimonious model possible.

Results and discussion

Prevalence and concentration of *E. coli* O157:H7. Initial prevalence of *E. coli* O157:H7, prior to any DFM supplementation, was 40% (19/48) and 52% (25/48) for NP51/NP24 and L28, respectively. *Escherichia coli* O157:H7 prevalence for cattle supplemented with L28 on day 21, 52% (25/48), and day 42, 75% (36/48), remained higher than those cattle treated with NP51/NP24, 31% (15/48) and 58% (28/48) for days 21 and 42, respectively. Then, on day 63 the same prevalence, 46% (22/48), for both treatment groups was observed. However, at the end of the study, just before harvest, a lower prevalence was observed in pens treated with L28 compared to NP51/NP24 (Day 103: NP51/NP24: 65% (31/48), L28: 40% (19/48), $p > 0.05$), even though the initial prevalence in the L28 treated pens was higher, as shown in Figure 1. The interaction between L28 treatment and day 103 was significant ($p < 0.05$) as shown in Table 1. Over the course of the study period, the average *E. coli* O157:H7 prevalence was 48% (115/240) and 54% (127/240) for NP51/NP24 and L28, respectively ($p > 0.05$). *E. coli* O157:H7 prevalence was initially higher in pens treated with L28, due to a naturally higher prevalence in the beginning of the study. However, due to the layout of the pens and the sorting of the cattle in the commercial yards, it was not possible to resort the cattle after initial pathogen evaluation and thus the change in pathogen status over time was evaluated as the primary variable of importance.Table 2

The initial (day 0) fecal concentration of *E. coli* O157:H7 was 1.3 $\log_{10}$MPN/g and 1.0 $\log_{10}$MPN/g for NP51/NP24 and L28, respectively. On days 21, 42, and 63, significant increases in total concentration of *E. coli* O157:H7 were observed ($p < 0.05$), when compared to overall loads throughout the study for both treatments as shown in Figure 2. However, on day 103, the final fecal concentration of *E. coli* O157:H7 was 1.2 $\log_{10}$MPN/g and 0.95 $\log_{10}$MPN/g for NP51/NP24 and L28, respectively. From the initial concentration, a 0.10 $\log_{10}$MPN/g and 0.09 $\log_{10}$MPN/g reduction were observed for NP51/NP24 and L28, respectively. Overall, the effects of the DFMs were not significant on the concentration (enumeration) of *E. coli* O157:H7 as similar $\log_{10}$MPN values were observed between both treatment groups when evaluating positive samples (Table 2).

The efficacy of DFM formulations containing *Lactobacillus acidophilus* (NP51) and *Propionibacterium freudenreichii* (NP24) to reduce *E. coli* O157:H7 was previously reported within the range of odds ratios from 0.19 to 1.10 and the reduction in concentration is not widely reported (Sargeant et al., 2007). A quantitative microbial risk assessment in Canada conducted by Smith *et al.* concluded when probiotics were included as a single intervention scenario, the estimated reduction in per serving risk was from 38% to 50% and was further increased by combining probiotics with postharvest interventions and processing stages (Smith et al., 2013). In previous randomized controlled trials, it was proven that some mixtures of NP51 and NP24 significantly reduced the fecal prevalence of *E. coli* O157:H7 (odds ratio point estimates ranging from 0.23 to 0.51) and point estimates from the most trials also favored the use of the DFMs (Sargeant et al., 2007). Results from another independent study conducted in commercial feedlots in Eastern Nebraska and Western Iowa also estimated a significant reduction (OR: 0.42, $p < 0.05$) in cattle fecal samples treated with L28, while the effect of NP51/NP24 was not significant compared to a control group (Flach et al., 2022). The effect of DFMs on the concentration of *E. coli* O157:H7 has not been reported extensively and most probiotic studies come from challenge trials as it is challenging to enumerate this pathogen in naturally contaminated fecal samples.

Prevalence of *Salmonella*. In reference to overall prevalence, fecal samples collected from cattle fed both treatments had a very low *Salmonella* prevalence throughout the study period 0.83% (2/240) and 1.3% (3/240) for NP51/NP24 and L28, respectively, as illustrated in Table 3. The low prevalence did not allow for enough samples to conduct statistical analysis. A study conducted by Tabe et al. (2008) reported no significant reductions in *Salmonella* using the same DFM formulation with the NP51/NP24; however, they noted that the rate of new infections was significantly lower when the cattle's diet was supplemented with the DFM. In another study, Stephens *et al.* reported that fecal *Salmonella* shedding was 48% less likely from cattle treated with high doses of *L. acidophilus* NP51; however, no dose-response relation was identified (Stephens et al., 2007). Lastly, the data from our preliminary work showed significant decreases in *Salmonella* fecal prevalence for both the DFMs; however, the overall prevalence was higher compared to this study, ranging from 2 to 24%, therefore a statistical analysis was possible. Overall, both DFMs, in our previous study, were effective in reducing the prevalence of *Salmonella*, 2% (2/115) and 3% (2/75) for L28 and NP51/NP24, respectively, compared to the control (no DFM supplementation), 24% (26/110), throughout the study period (Flach et al., 2022).

Concentration of *Clostridium perfringens*. The initial (day 0) *C. perfringens* fecal concentrations, prior to any DFM supplementation, were 4.0 $\log_{10}$CFU/g and 3.1 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively. After 21 days of DFM supplementation, fecal concentrations increased by 0.50 $\log_{10}$CFU/g to 4.5 $\log_{10}$CFU/g and decreased by 0.50 $\log_{10}$CFU/g to 2.6 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively ($p < 0.05$). A reduction in *C. perfringens* concentrations was observed on day 42 for both treatment groups as the average fecal concentrations were 1.9 $\log_{10}$CFU/g and 2.1 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively. A further reduction on day 63 was observed and fecal concentrations were 1.5 $\log_{10}$CFU/g for both NP51/NP24 and L28 treatment groups. The final fecal concentration, after 103 days of DFM supplementation, was 1.6 $\log_{10}$CFU/g for both NP51/NP24 and L28 treatment groups.

Overall, a 2.4 $\log_{10}$CFU/g reduction in *C. perfringens* concentration was achieved for cattle supplemented with NP51/NP24 and a 1.5 $\log_{10}$CFU/g reduction was observed for cattle treated with L28 over the course of the 103-day supplementation period. However, it is important to note that average fecal concentrations of *C. perfringens* through the study period were 2.7 $\log_{10}$CFU/g and 2.2 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively. The difference in *C. perfringens* concentration between the two DFMs was statistically significant ($p = 0.04$) with 0.87 $\log_{10}$CFU/g lower concentration for the pens treated with L28. The analysis also shows that there was an increase in *C. perfringens* on day 21 (estimate: 0.48 $\log_{10}$CFU/g) and a decreas-

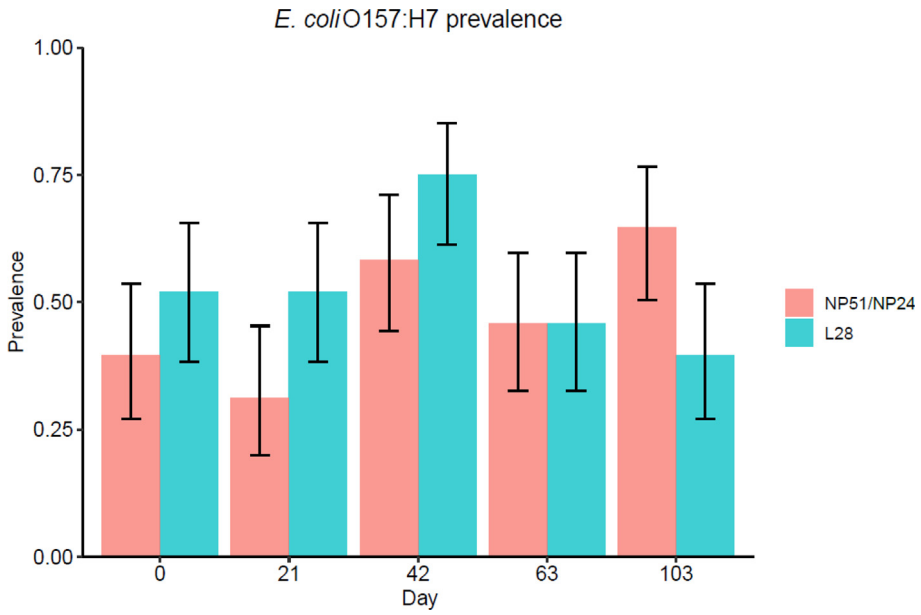

Figure 1. Prevalence (%) and 95% confidence intervals of *E. coli* O157:H7 in bovine fecal samples comparing two different treatment groups throughout 16 different pens. NP51/NP24: (# positive samples – Day 0: 19/48, Day 21: 15/48, Day 42: 28/48, Day 63: 22/48, Day 103: 31/48), L28: (# positive samples – Day 0: 25/48, Day 21: 25/48, Day 42: 36/48, Day 63: 22/48, Day 103: 19/48).

Table 1
GEE model for the prevalence of *E. coli* O157:H7

Term	Estimate (lnOR)	Standard Error	p value
Intercept	0.08	0.32	0.796
NP51/NP24	−0.51	0.53	0.343
Day 21	0.00	0.50	1.000
Day 42	1.02	0.42	0.016
Day 63	−0.25	0.43	0.560
Day 103	0.51	0.40	0.210
Treatment NP51:Day 103	1.53	0.71	0.031

Table 2
E. coli O157:H7 concentration from GEE analysis

Term	Estimate (Mean difference)	Standard Error	p value
Intercept	1.12	0.13	<0.001
NP51/NP24	0.01	0.14	0.950
Day 21	0.43	0.20	0.027
Day 42	1.10	0.20	<0.001
Day 63	1.16	0.25	<0.001
Day 103	−0.06	0.17	0.716

ing trend after 21 days for both DFMs (Fig. 3, Table 4) as also observed in our previous study (Flach et al., 2022). It should also be noted that any values below the LOD were imputed by half of the limit as there were 135 out of 240 samples for NP51/NP24 and 171/240 samples for L28 being below the limit. The mean concentrations for only enumerable samples were 3.9 and 4.2 $\log_{10}$CFU/g for L28 and NP51/NP24, respectively. Although this may cause an overestimation of the effectiveness of both DFMs, considering the amount of data that would have been kept out of the data analysis, it was assumed that the imputation would provide a better estimate of the actual levels in fecal material.Figure 4

In addition to being a human foodborne pathogen, *C. perfringens* also cause animal diseases, and it can be directly or indirectly transmitted from animals to humans or animals to animals (Hamza et al., 2018). The effect of *Lactobacilli*-based DFMs on fecal *C. perfringens* shedding in cattle has not been documented previously; therefore, further research is encouraged. Our previous work indicated that L28 was effective in reducing the concentration of *C. perfringens* in feces and it was more likely to obtain low (≤2 $\log_{10}$CFU/g) counts of *C. perfringens*, compared to the control group, and NP51/NP24 (Flach et al., 2022).

Concentration of Enterobacteriaceae. The initial EB concentration levels, prior to any DFM supplementation, were 8.0 $\log_{10}$CFU/g and 7.2 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively, as illustrated in Table 4. Average fecal concentrations of EB, after 21 days of DFM supplementation, were 6.9 $\log_{10}$CFU/g and 6.5 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively. Then, on day 42, an increase in EB concentrations was observed. Average EB concentrations were 6.9 $\log_{10}$CFU/g and 6.8 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively (Figure 4). On day 63, the average concentration was 6.5 $\log_{10}$CFU/g and 6.2 $\log_{10}$CFU/g NP51/NP24 and L28, respectively. After 103 days of DFM supplementation, the final fecal concentrations of EB were 6.3 $\log_{10}$CFU/g and 6.4 $\log_{10}$CFU/g for NP51/NP24 and L28, respectively. Throughout the study, average counts were 6.6 $\log_{10}$CFU/g for L28 and 6.9 $\log_{10}$CFU/g for NP51/NP2, and the overall effect of the two treatments on EB concentration was significantly different with NP51/NP24 having lower counts than L28 (estimated $\log_{10}$CFU difference = 0.84, $p < 0.001$) (Table 5). The results of this study indicate that both DFMs have the ability to reduce EB fecal concentrations; however, the use of NP51/NP24 will reduce EB levels significantly compared to L28 ($p < 0.05$).

In our previous study, the mean counts of Enterobacteriaceae were above 4 $\log_{10}$CFU/g for all treatment groups throughout the entire sampling period and achieved a minimum of a 1 $\log_{10}$CFU/g reduction, naturally. Additionally, the effect of both treatments was not statistically significant ($p > 0.05$) throughout the experimental period (Flach et al., 2022). In this study, cattle supplemented with NP51/NP24 achieved 1.7 $\log_{10}$CFU/g and cattle supplemented with L28 achieved a 0.8 $\log_{10}$CFU/g reduction from day 0 (no prior DFM supplementation) to day 103. Literature including the effect of DFM supplementation on EB fecal shedding is not previously reported. However, bacteria from the EB family are important indicators of the presence of pathogens as *Salmonella* and Shiga toxin-producing *E. coli* (STEC) are both in the EB family. Additionally, this indicator is a measurement of good hygiene management in commercial abattoirs, and lower concentrations indicate better control of hygiene measures at postharvest

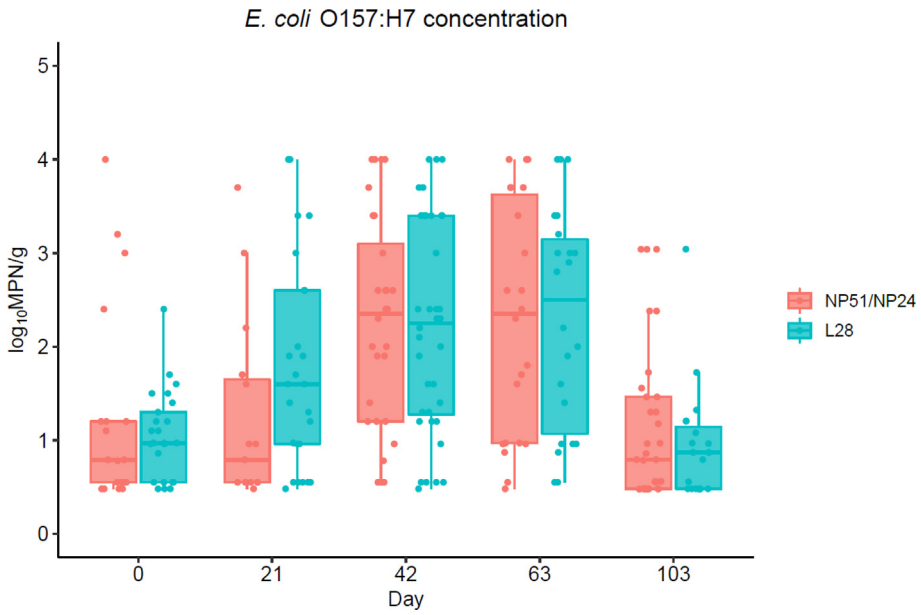

Figure 2. Concentration of *E. coli* O157:H7 (log_{10}MPN/g) in bovine fecal samples comparing two different treatment groups throughout 16 different pens.

Table 3
Prevalence of *Salmonella* (# of positives/# of total) of bovine fecal samples comparing two different treatment groups throughout 16 different pens

Treatment	Day 0	Day 21	Day 42	Day 63	Day 103	Total
NP51/NP24	0/48	1/48	1/48	0/48	0/48	2/240
L28	0/48	0/48	3/48	0/48	0/48	3/240

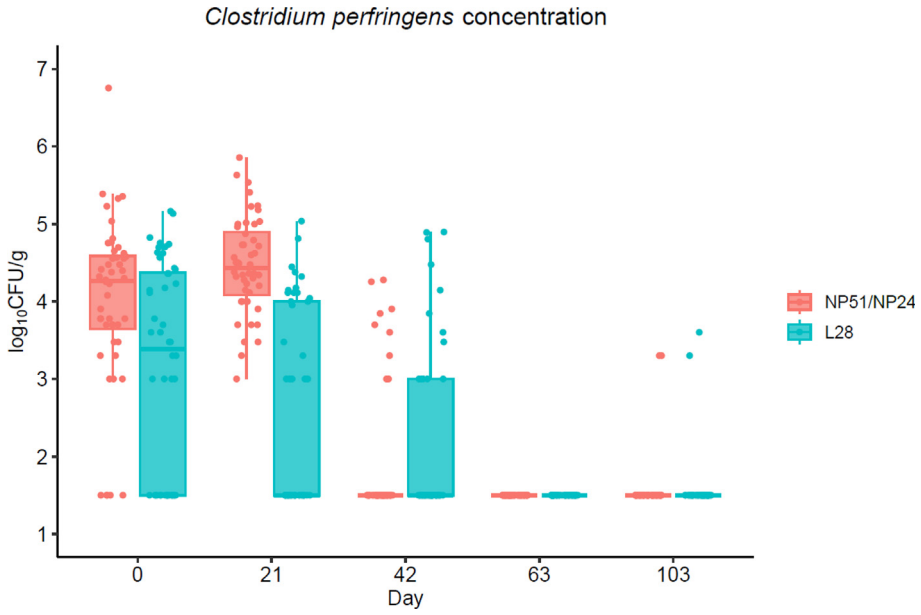

Figure 3. Concentration of *C. perfringens* (log_{10}CFU/g) in bovine fecal samples comparing two different treatment groups throughout 16 different pens.

and reduce the dependence on carcass decontamination (Barco et al., 2015).

Conclusions

It is apparent that foodborne pathogens reside in cattle feed yards; however, most research efforts in recent years have focused on the strides the beef industry has made in reducing pathogens in the final product, postharvest, instead of describing the presence of pathogens in preharvest beef cattle environments. However, the renewed interest in preharvest food safety from both a regulatory perspective and a public health perspective requires a renewed interest in preharvest pathogen presence and control. Our data indicate that *E. coli* O157:H7 prevalence increases over the course of the feeding period initially and then reduces prior to harvest at days 63 and 103 for those cattle

Table 4
Concentration of *C. perfringens* from GEE analysis

Term	Estimate (Mean difference)	Standard Error	p value
Intercept	3.98	0.22	<0.001
NP51/NP24	−0.87	0.43	0.042
Day 21	0.48	0.25	0.049
Day 42	−2.08	0.28	0.000
Day 63	−2.48	0.22	<0.001
Day 103	−2.41	0.23	<0.0016
TreatmentNP51/24:Day 21	−0.99	0.49	0.043
TreatmentNP51/24:Day 42	1.08	0.53	0.040
TreatmentNP51/24:Day 63	0.87	0.43	0.042
TreatmentNP51/24:Day 103	0.88	0.43	0.043

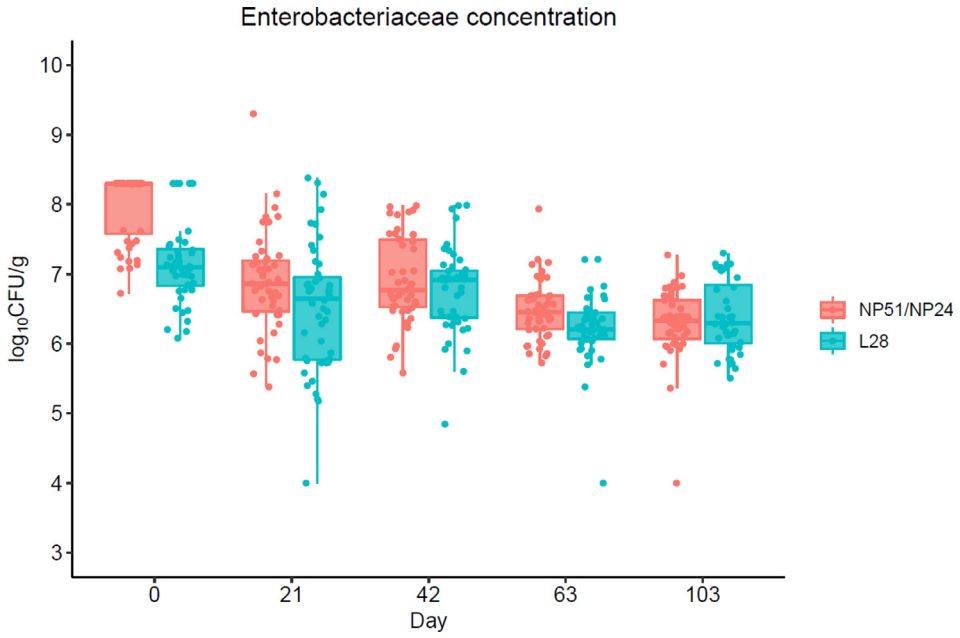

Figure 4. Enterobacteriaceae concentration (log_{10}CFU/g) in bovine fecal samples comparing two different treatment groups throughout 16 different pens.

Table 5
Enterobacteriaceae concentration from GEE analysis

Term	Estimate (Mean difference)	Standard Error	p value
Intercept	7.16	0.11	<0.001
NP51/NP24	0.84	0.14	<0.001
Day 21	−0.64	0.22	0.004
Day 42	−0.40	0.14	0.003
Day 63	−0.95	0.14	<0.001
Day 103	−0.78	0.15	<0.001
TreatmentNP51/24:Day 42	−0.69	0.18	<0.001
TreatmentNP51/24:Day 63	−0.57	0.19	0.003
TreatmentNP51/24:Day 103	−0.92	0.19	<0.001

supplemented with L28. The pathogen was not eliminated. *Salmonella* prevalence was relatively low throughout the study from the beef cattle feeding operation; therefore, no further conclusions were made. Enterobacteriaceae counts in the feces, for both treatment groups, were reduced to similar levels throughout and at the end of the DFM supplementation period. There has been little information on the presence of Clostridia in beef cattle from a food safety perspective. This pathogen is both an animal and human pathogen, and data indicate that the use of L28 could reduce the pathogen and potentially have a positive impact on animal and human health. Further research is encouraged to evaluate different feed yard settings and cattle diets. Geographical data are also important to gain more understanding on the presence of pathogens in beef cattle environments to encourage the use and development of mitigation strategies that could impact public health.

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Data Availability Statement

The data used to support the findings of this study can be made available by the corresponding author upon request.

CRediT authorship contribution statement

Makenzie G. Flach: Software, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Onay B. Dogan:** Soft-

ware, Formal analysis, Data curation, Writing – original draft. **Wanda M. Kreikemeier:** Methodology, Investigation, Resources, Data curation, Writing – review & editing. **Kendra K. Nightingale:** Conceptualization, Writing – review & editing. **Mindy M. Brashears:** Conceptualization, Methodology, Validation, Investigation, Resources, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Drs. Brashears and Nightingale are co-founders and own shares of NexGen Innovations, LLC and their participation is governed by a management plan in place at Texas Tech University to mitigate risks from conflicts of interest. Dr. Brashears receives royalty income from the sales of Bovamine Defend.

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