Effect of a direct-fed microbial (10-G Armor) on feedlot performance, carcass characteristics, and prevalence of Salmonella in fed-beef heifers

Lauren M. Mayer,† Kevin Martens,‡ Alyssa B. Word,‖ Ben P. Holland,‖ Loni L. Lucherk,†
Ty E. Lawrence,†‡ and Travis C. Tennant,†

†Beef Carcass Research Center, West Texas A&M University, Canyon, TX 79016, USA
‡Life Products, Inc., Norfolk, NE 68701, USA
‖Cactus Research, Amarillo, TX 79101, USA

ABSTRACT

Crossbred beef heifers (N = 1,394; initial shrink body weight [BW] 291 ± 9.9 kg) were used to investigate the efficacy of 10-G Armor (Life Products, Inc., Norfolk, NE; 10-G) upon feedlot performance, carcass characteristics, and fecal and subiliac lymph nodes Salmonella prevalence. Heifers were blocked by day of arrival and allocated to 1 of 20 pens (10 pens/treatment) and assigned one of two treatments (10 pens/treatment): no direct-fed microbial (CON) or 2 g/heifer/d of Lactobacillus acidophilus, Enterococcus faecium, Pediococcus pentosaceus, Lactobacillus brevis and Lactobacillus plantarum, respectively (Life Products, Inc., Norfolk, NE; 10-G). Twenty-four animals were selected from each pen for Salmonella sampling. Recto-anal mucosal swab samples (RAMS) were obtained at initial processing and harvest; subiliac lymph nodes were collected at harvest. In addition, pen surface fecal pats were collected and composited by pen (10 pats per composite, 5 composites per pen) on days 0, 52, 120, and 192. Data were analyzed as a generalized complete block design, and pen served as the experimental unit. No differences were observed in live growth performance metrics (P > 0.55). Yield grade distributions did not differ between treatments (P > 0.62); however, cattle fed 10-G tended (P = 0.06; 14.6% vs. 18.9%) to have fewer USDA Select carcasses and more (P = 0.09; 73.6% vs. 78.0%) USDA Choice carcasses. Cattle fed 10-G tended (P = 0.10; 9.2% vs. 12.3%) to have fewer liver abscesses and had fewer (P = 0.04; 5.3% vs. 8.5%) severe liver abscesses. Salmonella prevalence of RAMS did not differ between treatments at initial processing (P = 0.97; CON = 11.6%, 10-G = 11.5%) or at harvest (P = 0.91; CON = 99.0%, 10-G = 98.6%); however, RAMS differed (P < 0.01) in Salmonella prevalence between the two collection times. Cattle fed 10-G had a lower frequency of Salmonella positive lymph nodes (P = 0.01; CON = 15.8%, 10-G = 7.4%) than CON. However, Salmonella log (mpn/g) of lymph nodes did not differ between treatments at harvest (P = 0.34; CON = 0.73, 10-G = 0.34). These data indicate that cattle fed 10-G have decreased rates of severe liver abscesses without altering live animal performance or carcass characteristics. Supplementation of 10-G significantly reduced the prevalence rate of Salmonella recovered from the subiliac lymph nodes. The factors responsible for the observed difference in the effects of 10-G on Salmonella warrant further investigation.

Key words: carcass, direct-fed microbial, heifers, performance, prevalence, Salmonella

INTRODUCTION

An estimated 48 million cases of foodborne illness occur annually in the United States; non-typoidal Salmonella is the leading cause of bacterial foodborne illnesses with 1.35 million cases, 26,500 hospitalizations, and 420 deaths (CDC, 2019). Salmonella is a naturally occurring bacterial pathogen historically associated with poultry (Whyte et al., 2002; Parveen et al., 2007; Foley et al., 2008) eggs (Jones et al., 1995, 2012; Singh et al., 2010) and produce (Wells and Butterfield, 1997; Quiroz-Santiago et al., 2009; Sant’Ana et al., 2011). Studies have shown that beef products are also susceptible to Salmonella contamination (Rose et al., 2002; Zaidi et al., 2008; Sallam et al., 2014). Lymph nodes in beef cattle are known to harbor Salmonella (Samuel et al., 1980; Arthur et al., 2008; Brown et al., 2020); furthermore, the ability to remove all lymph nodes from a beef carcass is impractical. As a result, lymph nodes may be incorporated into ground beef trimmings, thus increasing the risk of Salmonella-contaminated ground beef (Arthur et al., 2008; Bosilevac et al., 2009; Kooohmaraei et al., 2012). Salmonella prevalence differs seasonally; frequency peaks during the summer through early fall and troughs during the winter (Barkocy-Gallagher et al, 2003; Dargatz et al., 2003; McEvoy et al., 2003). Feedyard location also affects Salmonella prevalence; southern regions have a higher prevalence of Salmonella than northern regions (Dargatz et al., 2003; Rivera-Betancourt et al., 2004; Haneklaus et al., 2012). Additionally, cattle type affects Salmonella prevalence; feedlot cattle are more frequent carriers than cull cows and bulls (Gragg et al., 2013a; Webb et al., 2017). Fed Holstein steers had a higher prevalence of Salmonella than beef-type steers, whereas cull dairy cows had a higher prevalence than range cows (Herrick, 2022).

Effective intervention technologies implemented to decrease Salmonella include vaccines (Edrington et al., 2013; Cernicchiaro et al., 2016) and various feed additives, including...
seaweed extract (Braden et al., 2004), tylosin (Amachawadi et al., 2017), and direct-fed microbials (DFMs; Stephens et al., 2007; Vipham et al., 2015; Brown et al., 2020). Direct-fed microbials are commonly used in the industry to improve performance and may reduce the pathogenic load in cattle. Shedding of Salmonella has been reported to be reduced by DFM (Stephens et al., 2007; Vipham et al., 2015; Brown et al., 2020), while others reported no difference (Tabe et al., 2008). Bacterial DFM have been reported to reduce pathogenic microorganisms in cattle via modifying the balance of intestinal microorganisms, competitive attachment to the intestinal mucosa, influencing gut permeability, formation of antimicrobial proteins or bacteriocins, and modulating immune function (Krehbiel et al., 2003). Yet, the effects of a mixture of Lactobacillus acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum on growth performance, carcass merit, and Salmonella prevalence of fed-beef heifers have not been reported, even though there is evidence that these microbes may have beneficial properties in this regard when fed individually (Luebbe et al., 2013). The objectives of this study were to evaluate the inclusion of L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum in fed-beef heifers on growth performance, carcass characteristics, and Salmonella prevalence.

MATERIALS AND METHODS

The feeding portion of this experiment was conducted at a commercial feedyard in the Texas Panhandle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at West Texas A&M University (#2020.02.003).

Cattle Processing and Experimental Design

Crossbred beef heifers (N = 1,925) were received at a commercial feedyard in the Texas Panhandle between February 28, 2020, and March 14, 2020, from Texas, Alabama, and Tennessee. Prior to initial processing, cattle were penned together by source and were provided ad libitum access to water and prairie grass hay. During initial processing, heifers were excluded from the trial if their initial body weight (BW) deviated more than 68 kg from the average pay weight and were deemed unfit due to illness, lameness, or pregnancy. Heifers were initially implanted with Revalor-IH (Merck Animal Health, Summit, NJ) and at re-implant (77-79 DOF) were administered Titanium 3 (Elanco Animal Health, Indianapolis, IN) and NaSalgen IP (Merck Animal Health) for viral respiratory pathogens. Internal and external parasites were controlled through the administration of Synanthic (Boehringer Ingelheim, Duluth, GA) and Dectomax Injectable (Zoetis, Parsippany, NJ). Heifers were identified with visual ear tags that contained the last three digits of the lot associated with the pen as well as an individual number specific to the animal.

In total, 1,400 heifers with an initial BW of 291 ± 9.9 kg were enrolled in this study in a generalized complete block design with time of arrival as blocking factor and pen as the experimental unit. Each arrival block (N = 5) contained four pens with two replications of each dietary treatment. Pens (N = 20) each housed 70 heifers. Animals were randomly assigned to pens within blocks using a computer-generated schedule. Heifers were randomly allocated to one of two treatments: 0 g/animal/d (CON) or 2 g/animal/d (10-G) of 10-G Armor (Life Products, Inc., Norfolk, NE) to provide 1 billion colony forming units per animal per day of L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum. Within each pen, 24 candidate animals were randomly identified for longitudinal Salmonella sampling. Individual BW was collected at initial processing and re-implant; pen BW was collected on day 0 and prior to harvest using a platform scale (Model 7531, Mettler-Toledo, Columbus, OH) prior to the morning feeding.

A 2% pencil shrink was applied to the initial BW, whereas a 4% pencil shrink was applied to the final BW due to differences in proportional BW. After randomization but prior to day 0, five heifers assigned to the CON treatment died. The cause of death was liver failure (1), peritonitis (2), and bovine respiratory disease (2). Also prior to day 0, one heifer was removed from a 10-G pen due to being pregnant. Upon arrival, all cattle received a starter diet (Table 1) that consisted of

Table 1. Ingredient formulation and analyzed composition of starter and finishing diets for 1×××

| Item                  | Dietary treatment | CON   | 10-G  |
|-----------------------|-------------------|-------|-------|
|                       |                   |       |       |
| Ingredient, %         |                   |       |       |
| RAMP                  |                   | 100.00| 100.00|
| Steam-flaked corn     | —                 | 58.98 | 58.96 |
| Wet distillers grain  | —                 | 13.91 | 13.90 |
| Sweet bran plus       | —                 | 18.29 | 18.31 |
| Yellow grease fat     | —                 | 1.44  | 1.45  |
| Cotton burrs or corn  | —                 | 7.34  | 7.33  |
| Stalks                | 0.03              | 0.03  | 0.05  |
| Nutrient composition, |                   |       |       |
| Diet DM, %            |                   | 65.30 | 64.20 |
| Crude protein, %      |                   | 21.60 | 21.40 |
| Nonprotein nitrogen   |                   | 0.90  | 1.00  |
| compounds, %          |                   | 1.30  | 1.30  |
| Neutral detergent     | 39.70             | 39.60 | 23.70 |
| fiber, %              |                   | 22.10 |
| Crude fiber, %        | 3.70              | 3.60  | 5.10  |
| Ca, %                 | 1.30              | 1.40  | 0.75  |
| P, %                  | 0.90              | 0.90  | 0.49  |
| Mg, %                 | 0.40              | 0.40  | 0.23  |
| K, %                  | 1.50              | 1.60  | 0.83  |

aTreatments included no DFM contained in the diet (CON) and a diet containing L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum fed at 2 g/heifer/d providing 1 × 10^9 CFU (10-G) (Life Products, Inc., Norfolk, NE).
bAll values except DM on a DM basis.
cAnalysis and calculation performed by Servi-Tech Laboratories, Amarillo, TX.
dComplete starter feed (Cargill Inc., Blair, NE).
eWet corn gluten feed (Cargill Inc., Blair, NE).
fGrower supplement formulated to supply 22.04 mg/kg Rumensin (Elanco, Indianapolis, IN) and 11.02 mg/kg Tylan (Elanco, Indianapolis, IN).
gFinisher supplement formulated to supply 46.30 mg/kg Rumensin (Elanco, Indianapolis, IN) and 11.02 mg/kg Tylan (Elanco, Indianapolis, IN), 544.31 IU/kg Vit A, 54.4 IU/kg Vit D, and 44.09 melengestrol acetate.

MATERIALS AND METHODS

The feeding portion of this experiment was conducted at a commercial feedyard in the Texas Panhandle between February 28, 2020, and March 14, 2020, from Texas, Alabama, and Tennessee. Prior to initial processing, cattle were penned together by source and were provided ad libitum access to water and prairie grass hay. During initial processing, heifers were excluded from the trial if their initial body weight (BW) deviated more than 68 kg from the average pay weight and were deemed unfit due to illness, lameness, or pregnancy. Heifers were initially implanted with Revalor-IH (Merck Animal Health, Summit, NJ) and at re-implant (77-79 DOF) received Revalor-200 (Merck Animal Health). Heifers were administered Titanium 3 (Elanco Animal Health, Indianapolis, IN) and Nasalgren IP (Merck Animal Health) for viral respiratory pathogens. Internal and external parasites were controlled through the administration of Synanthic (Boehringer Ingelheim, Duluth, GA) and Dectomax Injectable (Zoetis, Parsippany, NJ). Heifers were identified with visual ear tags that contained the last three digits of the lot associated with the pen as well as an individual number specific to the animal.

In total, 1,400 heifers with an initial BW of 291 ± 9.9 kg were enrolled in this study in a generalized complete block design with time of arrival as blocking factor and pen as the experimental unit. Each arrival block (N = 5) contained four pens with two replications of each dietary treatment. Pens (N = 20) each housed 70 heifers. Animals were randomly assigned to pens within blocks using a computer-generated schedule. Heifers were randomly allocated to one of two treatments: 0 g/animal/d (CON) or 2 g/animal/d (10-G) of 10-G Armor (Life Products, Inc., Norfolk, NE) to provide 1 billion colony forming units per animal per day of L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum. Within each pen, 24 candidate animals were randomly identified for longitudinal Salmonella sampling. Individual BW was collected at initial processing and re-implant; pen BW was collected on day 0 and prior to harvest using a platform scale (Model 7531, Mettler-Toledo, Columbus, OH) prior to the morning feeding.

A 2% pencil shrink was applied to the initial BW, whereas a 4% pencil shrink was applied to the final BW due to differences in proportional BW. After randomization but prior to day 0, five heifers assigned to the CON treatment died. The cause of death was liver failure (1), peritonitis (2), and bovine respiratory disease (2). Also prior to day 0, one heifer was removed from a 10-G pen due to being pregnant. Upon arrival, all cattle received a starter diet (Table 1) that consisted of

Table 1. Ingredient formulation and analyzed composition of starter and finishing diets for 1×××
of RAMP (Cargill Corn Milling, Bovina, TX), and hay was top-dressed for the first 3 d. Cattle were then transitioned to a finishing diet in which RAMP was reduced every 2 to 4 d at a 10% to 15% rate. Both starter and finishing diets included monensin (Rumensin, Elanco Animal Health) and tylosin (Tylan, Elanco Animal Health). Finishing diets also included melengestrol acetate (HeiferMax 500, Elanco Animal Health). In addition, ractopamine hydrochloride (Optaflexx, Elanco Animal Health) was fed for the final 35 d prior to slaughter. Inclusion of micro-nutrients occurred via a Micro Machine (Micro Technologies, Amarillo, TX) and were added directly to each feed batch. 10-G Armor was dispensed independently from a Micro Machine (Micro Technologies) into the ration after it was loaded into the delivery truck (Roto-Mix, Dodge City, KS) and mixed for 3 min.

Sample and Data Collection
A longitudinal design was used to investigate Salmonella shedding, with 24 candidate animals randomly selected from each pen. Recto-anal mucosal swab samples (RAMS) were collected during initial processing (day −2) and at harvest. A sterile foam-tipped applicator swab (FecalSwab, COPANUSA, Murrieta, CA) was inserted 3 to 5 cm into the recto-anal canal junction of each designated heifer. The swab was then placed into a sterile sample bag (WhirlPak, Nasco, Modesto, CA) that was labeled with a sample number that was correlated back to the heifer ID and sealed.

Composite fecal pat samples were collected from each pen on days 0, 52, 120, and 192. Each composite sample represented 10 individual fecal pats; five composite samples were collected per pen. Sample bags were labeled with the appropriate pen number and sample day.

Heifers were fed an average of 192 d (range of 183 to 204 d) prior to being transported 92 km to a commercial beef processor (USDA Establishment #245E) for harvest. Carcass data were collected by trained personnel from the West Texas A&M University—Beef Carcass Research Center (Canyon, TX). Ear tags were individually recorded and assigned an individual identification by West Texas A&M University personnel. Livers were scored using a modified Elanco Liver Check System (Brown and Lawrence, 2010) in which abscesses were evaluated based on severity (edible = no abscesses, A− = 1 or 2 small abscesses, A = 2 to 4 small active abscesses, A+ = 1 or more large active abscesses, A + Adhesion = liver adhered to the gastrointestinal tract, and A + Open = open liver abscesses). Additionally, other liver abnormalities including telangiectasia, cirrhosis, flukes, and contamination were recorded.

Individual lungs were evaluated to determine the presence and severity of lung lesions, interlobular adhesions, and plural adhesions, and missing lobes were recorded. Lung scores were N = normal; 1 = presence of mycoplasma-like lesion > 15%; 2 = plural adhesions, a portion of the lung missing, or a combination of these affecting <25% of lung tissue; 3 = plural adhesions, a portion of lung missing, or a combination of these affecting >25% to <50% of lung tissue; 4 = plural adhesions, a portion of lung missing, or a combination of these affecting >50% to <75% of lung tissue; and 5 = plural adhesions, a portion of lung missing, or a combination of these affecting >75% of lung tissue. Lungs that were contaminated, inflated, or skipped received a C, I, or S score, respectively. Hot carcass weight (HCW) was recorded on the harvest floor. The left or right subiliac lymph node (n = 429) was collected from each sampled animal at harvest. Lymph nodes were excised, kept intact, and encased in fat, placed in a bag with a label corresponding to the sample animal. All samples were placed on wet ice and shipped to Food Safety Net Services (San Antonio, TX) for diagnostic analysis. Carcass characteristics (marbling, quality grade, 12th-rib subcutaneous fat depth, longissimus muscle area [LMA], and yield grade) were obtained from USDA camera data.

Salmonella Analysis
Upon arrival at Food Safety Net Services, 10 g of a composite fecal sample was weighed and inserted into a sterile WhirlPak bag with 90 mL of buffered peptone water. Samples were hand massaged for 30 s to create a homogenous sample. Sample liquid of RAMS was directly transferred to the first well of plates. Lymph node samples were trimmed of excess fat and fascia and submerged into boiling water for 3 to 5 s to rid the lymph node of any Salmonella on the exterior surface. Lymph nodes were then individually placed into stomacher sample bags and weighed, manually pulverized using a rubber mallet, and enriched with 80 mL of tryptic soy broth through incubation at 25 °C for 2 h and then 42 °C for 12 h (Brichta-Harhay et al., 2008; Gragg et al., 2013b). Each sample was replicated three times and serially diluted eight times onto a 96-well plate with four samples per well pin. One milliliter of aliquot was put into each well pin with a serial dilution of 10−8 through 10−1. The well pin plate was then covered and incubated at 37 °C for 24 h. After incubation, a 96-well pin replicator was used to transfer growth from incubated plates to 1 mL Rappaport-Vassiliadis broth aliquots. The replicated well pins were covered and incubated at 42 °C for 24 to 48 h. After incubation, all samples that were indicative of growth changed colors and were streaked onto xylose lysine deoxycholate agar plates and incubated at 35 °C for 24 h. After 24 h, plates that had colony growth were presumed positive for Salmonella and underwent serological confirmation with Poly O antisera to confirm Salmonella.

Statistical Analysis
The GLIMMIX procedure of SAS (version 9.4, SAS Inst. INC., Cary, NC) was used to model the fixed effect of dietary treatment utilizing block as a random effect and pen as the experimental unit. Means were generated using the LSMEANS option and separated using the PDIF option. Repeated measures were used to analyze Salmonella prevalence and concentration across days on feed using the unstructured covariance structure. Nominal data were analyzed as a series of binomial distributions; treatment proportions and standard errors were calculated using the ILINK option. Differences were considered significant at α ≤ 0.05, and trends were noted at 0.05 < α ≤ 0.10.

RESULTS AND DISCUSSION
Growth performance data are presented in Table 2. Supplementation with 10-G did not affect growth performance as average daily gain (ADG), dry matter intake (DMI), gain:feed (G:F), and final BW did not differ (P ≥ 0.63) between treatments. Likewise, no differences (P ≥ 0.55) were observed for morbidity or mortalities and removals. Other studies in which 10-G was supplemented to cattle reported similar results for ADG, DMI, F:G, and final BW (Neuhold et al., 2012; Luebbe et al., 2013; Kenney et al., 2015). Live
Liver and Lung Outcomes

The percentage of edible livers (Table 3) for 10-G and CON cattle were 84.54% and 81.53%, respectively, and did not differ ($P = 0.17$) between treatments. Conversely, heifers fed 10-G tended ($P = 0.10$) to have a lower frequency of abscesses (9.23%) when compared with CON (12.26%). The rates of edible or abscessed livers observed in the current study are similar to those reported by Brown and Lawrence (2010) and Herrick (2022). The total severe abscess (A+, A + Adhesion, A + Adhesion/Open) incidence rate differed between dietary treatments ($P = 0.04$) with CON cattle having 8.51% severely abscessed livers, whereas 10-G cattle had 5.27% severely abscessed livers. Severely abscessed incidence rate for CON was numerically higher than the 6.0% incidence rate reported by the National Beef Quality Audit (Eastwood et al., 2017). Livers condemned for reasons other than abscesses (flukes, telangiectasis, contamination) did not differ ($P \geq 0.28$).

The incidence of normal lungs (Table 3) was 72.62% and 71.93% for 10-G and CON cattle, respectively. Lungs scored 1, 2, 3, 5, or condemned did not differ ($P \geq 0.17$) between treatments. However, lungs with 50% to 75% adhesion or consolidation (score four) tended ($P = 0.10$) to be more frequent in cattle fed 10-G (5.38%) when compared with CON (3.38%). Likewise, lungs that did not deflate at harvest tended ($P = 0.10$) to occur more frequently in CON cattle (1.69%) over 10-G (0.62%).

Carcass Performance

Hot carcass weight (Table 4) did not differ ($P = 0.14$; CON = 370.5; 10-G = 374.2) between treatments. Several studies have reported no difference in HCW for cattle fed 10-G (Neuhold et al., 2012; Luebbe et al., 2013; Kenney et al., 2015). The dressed yield was similar ($P = 0.53$) for 10-G and CON cattle (64.8% vs. 64.9%, respectively). Calculated empty body fat ($P = 0.71$), LMA ($P = 0.13$), marbling ($P = 0.20$), and 12th-rib fat thickness ($P = 0.73$) also did not differ between dietary treatments. Wilson et al. (2016) also reported no differences in 12th-rib fat thickness, LMA, and marbling score between control and DFM-fed cattle.

Percentage USDA Prime and Ungraded carcasses were not affected by supplementation of 10-G ($P \geq 0.71$). Heifers supplemented with 10-G tended ($P = 0.06$) to be represented

Table 2. Live growth performance of heifers fed 10-G

| Item                        | TRT7 | CON  | 10-G | SEM  | P-value |
|-----------------------------|------|------|------|------|---------|
| $n$ pens                    |      | 10   | 10   | —    | —       |
| Initial BW, kg              |      | 290.9| 291.0| 9.9  | 0.95    |
| Final BW, kg                |      | 543.0| 545.3| 8.9  | 0.79    |
| ADG, kg                     |      | 1.37 | 1.38 | 0.02 | 0.69    |
| DMI, kg                     |      | 8.89 | 8.98 | 0.27 | 0.63    |
| G:F                         |      | 0.154| 0.155| 0.005| 0.81    |
| Morbidity, %                |      | 9.57 | 9.37 | —    | 0.90    |
| Mortalities and removals, % |      | 6.17 | 6.98 | —    | 0.55    |

7Treatments included no DFM contained in the diet (CON) and a diet containing L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum fed at 2 g/heifer/d providing 1 x 109 CFU (10-G) (Life Products, Inc., Norfolk, NE).

Table 3. Liver and lung outcomes of heifers fed 10-G

| Item                        | TRT8 | CON  | 10-G | SEM  | P-value |
|-----------------------------|------|------|------|------|---------|
| Liver score,%               |      |      |      |      |         |
| Edible                      |      | 81.53| 84.54| —    | 0.17    |
| Abscessed                   |      | 12.26| 9.23 | —    | 0.10    |
| A                           |      | 0.92 | 1.17 | —    | 0.65    |
| A + Adhesion                |      | 2.69 | 3.16 | —    | 0.61    |
| Total A+                    |      | 8.51 | 5.27 | —    | 0.04    |
| A + Open                    |      | 2.20 | 1.02 | —    | 0.11    |
| A + Adhesion/Open           |      | 3.38 | 2.15 | —    | 0.20    |
| Total Other                 |      | 5.99 | 5.85 | —    | 0.91    |
| Flukes                      |      | 2.58 | 2.39 | —    | 0.81    |
| Telangiectasis              |      | 0.22 | 0.56 | —    | 0.28    |
| Contamination               |      | 2.06 | 2.08 | —    | 0.98    |

8Treatments included no DFM contained in the diet (CON) and a diet containing L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum fed at 2 g/heifer/d providing 1 x 109 CFU (10-G) (Life Products, Inc., Norfolk, NE).

9N = normal; 1 = presence of mycoplasma-like lesion greater than 25%; 2 = plural adhesions, a portion of lung missing, or a combination of these affecting <25% of lung tissue; 3 = plural adhesions, a portion of lung missing, or a combination of these affecting 25% to 50% of lung tissue; 4 = plural adhesions, a portion of lung missing, or a combination of these affecting >50% to 75% of lung tissue; 5 = plural adhesions, a portion of lung missing, or a combination of these affecting >75% of lung tissue; C = contaminated; I = inflated.
Effects of 10-G Armor in fed-beef heifers

Effects of 10-G Armor in fed-beef heifers

Table 4. Carcass performance of heifers supplemented with 10-G

| Item                  | CON   | 10-G  | SEM  | P-value |
|-----------------------|-------|-------|------|---------|
| HCW, kg               | 370.5 | 374.2 | 4.9  | 0.14    |
| Dressed yield, %      | 64.90 | 64.80 | 0.001| 0.53    |
| LMA, cm³              | 90.38 | 91.65 | 0.8  | 0.13    |
| Marbling score,a      | 506   | 516   | 12.4 | 0.20    |
| 12th-rib fat thickness, cm | 1.88 | 1.89 | 0.05 | 0.73    |
| Empty body fat,a, %   | 32.00 | 32.11 | 0.35 | 0.71    |
| Quality grade, %      |       |       |      |         |
| Prime                 | 5.70  | 5.73  |      | 0.98    |
| Choice                | 73.62 | 77.97 |      | 0.09    |
| Select                | 18.94 | 14.64 |      | 0.06    |
| Ungraded              | 0.62  | 0.27  |      | 0.71    |
| Yield grade,%         |       |       |      |         |
| YG1                   | 6.37  | 7.01  |      | 0.72    |
| YG2                   | 29.38 | 28.80 |      | 0.85    |
| YG3                   | 37.40 | 36.24 |      | 0.62    |
| YG4                   | 23.18 | 23.94 |      | 0.82    |
| YG5                   | 3.67  | 4.01  |      | 0.78    |

a Treatments included no DFM contained in the diet (CON) and a diet containing L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum fed at 2 g/heifer/d providing 1×10⁹ CFU (10-G) (Life Products, Inc., Norfolk, NE).

b Day 0 = Slight, 400 = Small, 500 = Modest, and 600 = Moderate.

c Empty body fat calculated using EBF, %

Table 5. Salmonella prevalence and log of composited fecal pats collected from cattle fed 10-G

| Item                  | CON   | 10-G  | SEM  | P-value |
|-----------------------|-------|-------|------|---------|
| Salmonella prevalence,%|       |       |      |         |
| Day 0                 | 11.5  | 11.6  |      | 0.97    |
| Day 192               | 99.0  | 98.6  |      | 0.91    |
| Salmonella log (MPN/g)|       |       |      |         |
| Day 0                 | 0.28  | 0.30  | 0.48 | 0.97    |
| Day 192               | 4.40  | 4.05  | 0.48 | 0.47    |

a Treatments included no DFM contained in the diet (CON) and a diet containing L. acidophilus, E. faecium, P. pentosaceus, L. brevis, and L. plantarum fed at 2 g/heifer/d providing 1×10⁹ CFU (10-G) (Life Products, Inc., Norfolk, NE).
80.0%, respectively). Salmonella concentration did not differ $(P \geq 0.28)$ between treatments but followed the same pattern as prevalence rates with an initial log of $0.63 \text{ mpn/g}$ followed by a significant increase before plateauing at a log of $3.11 \text{ mpn/g}$ at 192 d. The significant increase in log concentration is most likely due to environmental seasonality. Salmonella is known to increase in warmer months and decline in cooler months. Barkocy-Gallagher et al. (2003) reported Salmonella prevalence to be more than 3.5-fold greater in summer compared with prevalence in fall, winter, and spring. Dargatz et al. (2003) also reported that warm-season months were significantly higher for Salmonella prevalence when compared with cool season.

**Rectoanal mucosal swabs.** Salmonella prevalence of RAMS (Table 6) was similar at day 0 $(P = 0.97; 11.6\%)$ and also did not differ $(P = 0.91)$ between 10-G $(99.0\%)$ and CON cattle $(98.6\%)$ at the end of the finishing period. Tabe et al. (2008) reported a numerically lower percentage for overall Salmonella prevalence in fecal grabs $(12.7\%)$ from both non-supplemented or DFM-supplemented cattle. Conversely, Salmonella shedding increased throughout the feeding period leading to an increasing trend in Salmonella prevalence from the initial sampling.

Salmonella prevalence increased $(P < 0.01)$ dramatically from initial processing in March $(11.55\%)$ to harvest $(98.80\%)$ in September. The increase in prevalence observed can be largely attributed to the seasonal nature of when samples were collected. Other researchers have described a seasonal effect with prevalence rates peaking in warmer months and troughing in cooler months (Barkocy-Gallagher et al., 2003; Gragg et al., 2013a; Webb et al., 2017). The increase in the prevalence of Salmonella would suggest that environment is an important component for Salmonella harborage in peripheral lymph nodes, especially in regions where it is consistently warm.

Overall log concentration (mpn/g) between dietary treatments did not differ at day 0 $(P = 0.97; 10-G = 0.30, \text{CON} = 0.28)$ or at harvest $(P = 0.47; \text{CON} = 4.4, 10-G = 4.1)$ but increased $(P < 0.01)$ more than 14-fold during the 192-d study.

**Lymph nodes.** Salmonella prevalence and concentration data are presented in Table 7. Cattle fed 10-G had a lower frequency $(P = 0.01; 7.42\%)$ of Salmonella positive lymph nodes when compared with CON $(15.80\%)$. Vipham et al. (2015) supplemented cattle with *L. acidophilus* and *P. freudenreichii*, which resulted in an $18.8\%$ reduction of Salmonella in subiliac lymph nodes. In addition, Brown et al. (2020) fed different blends of bacterial DFMs and reported that control cattle tended to have a numerically greater percent-positive of Salmonella in peripheral lymph nodes when compared with treated cattle, suggestive of a potential treatment effect. Concomitantly, Salmonella concentration (mpn/g) of the lymph nodes did not differ $(P = 0.34)$ between dietary treatments $(10-G = 0.34, \text{CON} = 0.73)$ of all samples. Additionally, DFMs may elicit stimulation or alteration of the immune system as DFM treatments had improved immune function via suppressed or downregulated innate immunity and differences in pathogen prevalence. Webb et al. (2017) reported log concentrations ranging from 1.6 to 4.9 log$_{10}$ CFU/g PLN from 160 quantifiable subiliac lymph nodes collected.

### Table 7. Salmonella prevalence and log of subiliac lymph nodes collected from heifers fed 10-G

| Item                                      | TRT$^a$ | CON  | 10-G | SEM | $P$-value |
|-------------------------------------------|---------|------|------|-----|-----------|
| Salmonella, % positive                    |         | 15.80| 7.42 | 0.00| 0.01      |
| Salmonella log, MPN/g (mean of all samples) |         | 0.73 | 0.34 | 0.28| 0.34      |
| Salmonella log, MPN/g (mean of positive samples) |     | 3.30 | 3.75 | 0.94| 0.65      |
| Salmonella, minimum log MPN/g             |         | 0.00 | 0.00 | 0.00|           |
| Salmonella, maximum log MPN/g             |         | 6.54 | 5.64 | 0.00|           |

$^a$Treatments included no DFM contained in the diet (CON) and a diet containing *L. acidophilus*, *E. faecium*, *P. pentosaceus*, *L. brevis*, and *L. plantarum* fed at 2 g/heifer/d providing 1 × 10$^7$ CFU (10-G) (Life Products, Inc., Norfolk, NE).

#### CONCLUSION

These data indicate that supplementation of 10-G had no influence on feedlot performance or carcass traits. Conversely, feeding 10-G may directly benefit the producer via improved carcass grading outcomes and reduced frequency of severe liver abscesses. Supplementation of 10-G did significantly reduce the frequency of Salmonella positive lymph nodes, which may translate into improved public health outcomes by reducing the number of foodborne illnesses caused by Salmonella.

#### Acknowledgments

Funding for this project was provided by Life Products, Inc., Norfolk, NE. We wish to acknowledge the efforts of the Beef Carcass Research Center personnel for data collection.

#### Conflict of interest statement

No potential conflict of interest is reported by L.M.M., A.B.W., B.P.H., L.L.L., T.E.L., or T.C.T. Life Products Inc. provided funding for this research; K.M. is employed by Life Products Inc., the manufacturer of the product evaluated in the current study.

#### LITERATURE CITED

Amachawadi, R. G., T. J. Purvis, B. V. Lubbers, J. W. Homm, C. L. Maxwell, and T. G. Nagaraja. 2017. Bacterial flora of liver abscesses in crossbred beef cattle and Holstein steers fed finishing diets with or without tylosin. *J. Anim. Sci.* 95:3425–3434. doi:10.2527/jas2016.1198

Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, M. N. Guerini, N. Kachayanan, J. E. Wells, S. D. Shackelford, T. L. Wheeler, and M. Koochmarie. 2008. Prevalence and characterization of *Salmonella*...
in bovine lymph nodes potentially destined for use in ground beef.

J. Food Prot. 71:1685–1688.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing Escherichia coli, including O157:H7 and non-O157:H7 serotypes, and Salmonella in commercial beef processing plants. J. Food Prot. 66:1978–1986.

Bosilevac, J. M., M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2009. Prevalence and characterization of salmonella in commercial ground beef in the United States. Appl. Environ. Microbiol. 75:1892–1900. doi:10.1128/AEM.02530-08

Braden, K. W., J. R. Blanton, Jr., V. G. Allen, K. R. Pond, and M. F. Miller. 2004. Ascophyllum nodosum supplementation: a preharvest intervention for reducing Escherichia coli O157:H7 and Salmonella spp. in feedlot steers. J. Food Prot. 67:1824–1828.

Brichta-Harhay, D. M., M. N. Guerini, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. Salmonella and Escherichia coli O157:H7 contamination on hides and carcasses of cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. Appl. Environ. Microbiol. 74:6289–6297. doi:10.1128/AEM.00700-08

Bosilevac, J. M., N. Kalchayanand, R. Wang, J. W. Schmidt, J. C. Brooks, M. M. Brashears, and S. M. Younts-Dahl. 2003. Effects of live inoculation of Escherichia coli O157:H7 fecal shedding. J. Anim. Sci. 81:2686–2698. doi:10.2527/2003.81112686x

Foley, S. L., A. M. Lynne, and R. Nayak. 2008. Salmonella challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. J. Anim. Sci. 86:E149–E162. doi:10.2527/jas.2007-0464

Gragg, S. E., G. H. Loneragan, M. M. Brashears, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, R. Wang, J. W. Schmidt, J. C. Brooks, S. D. Shackelford, et al. 2013b. Cross-sectional study examining Salmonella enterica carriage in subiliac lymph nodes of cattle and feedlot cattle at harvest. Foodborne Pathog. Dis. 10:368–374. doi:10.1089/fpd.2012.1275

Gragg, S. E., G. H. Loneragan, K. K. Nightingale, D. M. Brichta-Harhay, H. Ruiz, J. R. Elder, L. G. Garcia, M. E. Miller, A. Echeverry, R. G. Ramírez Porras, et al. 2013a. Substantial within-animal diversity of Salmonella isolates from lymph nodes, feces, and hides of cattle at slaughter. Appl. Environ. Microbiol. 79:4744–4750. doi:10.1128/AEM.01020-13

Guiryo, P. J., D. G. Fox, L. O. Tedeschi, M. J. Baker, and M. D. Cravey. 2001. Predicting individual feed requirements of cattle fed in groups. J. Anim. Sci. 79(8):1993–1995. doi:10.2527/2001.7991983x

Hanekelaus, A. N., K. B. Harris, D. B. Griffin, T. S. Edrington, L. M. Lucia, and J. W. Savell. 2012. Salmonella prevalence in bovine lymph nodes differs among feedyards. J. Food Prot. 75:1131–1133.

Herrick, R. T., C. L. Rogers, T. J. McEvers, R. G. Amachawadi, T. G. Nagaraja, C. L. Maxwell, J. B. Reinbold, T. E. Lawrence. 2022. Exploratory observational quantification of liver abscess incidence, specific to region and cattle type, and their associations to viscera value and bacterial flora. Appl. Anim. Sci. 38(2):170–182. doi:10.11252/aas.2021-02228.

Huck, G. L., K. K. Kreikemeier, and G. A. Ducharme. 2000. Effects of feeding two microbial additives in sequence on growth performance and carcass characteristics of finishing beef steers. Kansas Agricultural Experiment Station Research Reports. 01(1). doi:10.4148/2378-5977.1788

Jones, D. R., K. E. Anderson, and J. Y. Guard. 2012. Prevalence of coliforms, Salmonella, Listeria, and Campylobacter associated with eggs and the environment of conventional cage and free-range egg production. Poult. Sci. 91:1195–1202. doi:10.3382/ps.2011-01795

Jones, F. T., D. V. Rives, and J. B. Carey. 1995. Salmonella contamina in commercial eggs and an egg production facility. Poult. Sci. 74:753–757. doi:10.3382/ps.7430753

Kenney, N. M., E. S. Vangskerken, D. L. Harmon, and K. R. McLeod. 2015. Direct-fed microbials containing lactate-producing bacteria influence ruminal fermentation but not lactate utilization in steers fed a high-concentrate diet. J. Anim. Sci. 93:2336–2348. doi:10.2527/2014-0870

Koohmaraie, M., J. A. Scanga, M. J. De La Zerda, B. Koohmaraie, L. Topay, V. Besklehnaya, T. Mai, G. Greeson, and M. Samadpour. 2012. Tracking the sources of Salmonella in ground beef produced from nonfed cattle. J. Food Prot. 75:1464–1468. doi:10.4315/0362-028X.JFP-11-540

Krehbiel, C. R., S. R. Rust, G. Zhang, and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: performance response and mode of action. J. Anim. Sci. 81(14_suppl_2):120–132. doi:10.2527/2003.8114_suppl_2E120x

Luebbe, M. K., K. H. Jenkins, S. A. Furman, and K. K. Kreikemeier. 2013. Effects of feeding microbial feed additives on growth performance and carcass traits of steers fed steam-flaked corn-based diets with wet distillers grains plus solubles. Nebraska Beef Cattle Reports. Report No.: 730. https://digitalcommons.unl.edu/animalscinbc730. Accessed May 5, 2021.

McEvoy, J. M., A. M. Doherty, J. J. Sheridan, I. S. Blair, and D. A. McDowell. 2003. The prevalence of Salmonella spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. J. Appl. Microbiol. 94:693–700.
