EFFECT OF A DIRECT-FED MICROBIAL ON FEEDLOT PERFMORMANCE, CARCASS CHARACTERISTICS AND PREVALENCE OF *SALMONELLA* IN FED-BEEF HEIFERS

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ABSTRACT

Salmonella is a naturally occurring bacteria that is known to cause upwards of 1.35 million cases of foodborne illnesses annually. Ground beef products may be manufactured from trimmings containing *Salmonella* infected lymph nodes, which has led to pending rulemaking by USDA-Food Safety and Inspection Service to declare *Salmonella* as an adulterant. Direct-fed microbials are a pre-harvest intervention for reduction of *Salmonella*. The objective of this study was to determine the efficacy of a direct-fed microbial upon the prevalence and enumeration of *Salmonella* in feces and lymph nodes. Heifers (n=1,394; 291 ± 9.9 kg) were blocked by day of arrival and randomly allocated to one of two treatments (0 or 2g/animal/d; CON and 10-G, respectively) with ten pens per treatment. Heifers fed 10-G were provided 1 billion CFUs per animal per day of *Lactobacillus acidophilus, Enterococcus faecium, Pediococcus pentosaceus*, *Lactobacillus brevis* and *Lactobacillus plantarum.* Twenty-four animals were randomly selected from each pen for *Salmonella* sampling. Rectoanal 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 170. Mixed models were used to analyze live performance, carcass characteristics and *Salmonella* prevalence and concentration with treatment used as fixed effect, block

used as random effect and pen as experimental unit. Repeated measures was used to analyze *Salmonella* prevalence and concentration across time using the unstructured covariance structure. Dry matter intake ($P = 0.63$), average daily gain ($P = 0.69$), gain:feed ($P = 0.81$) and final body weight ($P = 0.79$) did not differ between treatments. Neither morbidity ($P = 0.90$) nor mortality and railer rates ($P = 0.55$) were different between treatments. Hot carcass weight ($P = 0.14$), dressed carcass yield ($P = 0.53$), 12th rib fat depth ($P = 0.73$), ribeye area ($P = 0.13$), calculated empty body fat ($P = 0.71$) or marbling score ($P = 0.20$) were not different between treatments. Yield grade distributions did not differ between treatments ($P \geq 0.44$), however cattle fed 10-G tended ($P = 0.06$; 15.78 vs 20.34%) to be represented by fewer USDA Select carcasses and more ($P = 0.09$; 73.62 vs 77.97%) USDA Choice carcasses. Livers from cattle fed 10-G tended ($P = 0.10$; 12.26 vs 9.23%) to have a lower frequency of abscesses when compared to CON. Heifers fed 10-G also had fewer ($P = 0.04$; 5.27 vs 8.51%) severe liver abscesses. *Salmonella* prevalence of RAMs did not differ between treatments at initial processing ($P = 0.92$; CON = 11.6%, 10-G = 11.5%) or at harvest ($P = 0.92$; CON = 99.0%, 10-G = 98.6%), however RAMs differed ($P < 0.01$) in *Salmonella* prevalence between the two collection times. Likewise, *Salmonella* log (mpn/g) of RAMs did not differ between treatments at initial processing ($P = 0.63$; CON = 0.28, 10-G = 0.30) or at harvest ($P = 0.63$; CON = 4.40, 10-G = 4.05), while log (mpn/g) of *Salmonella* increased ($P < 0.01$) over the feeding period. Moreover, composited pen level fecal pats were similar for *Salmonella* prevalence ($P = 0.73$; CON = 69.0%, 10-G = 67.0%) between treatments, but prevalence increased ($P < 0.01$) sharply during the initial 52 d then plateaued during the remainder of the finishing period. However, *Salmonella* prevalence

differed ($P < 0.01$) among sampling days. Cattle fed 10-G had a lower frequency of *Salmonella* positive lymph nodes ($P = 0.01$; CON = 15.80%, 10-G = 7.41%) 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). This data indicates that cattle fed 10-G decreased rates of severe liver abscesses without altering live animal performance or carcass characteristics as well as fewer *Salmonella* positive lymph nodes, which in turn can likely improve public health by reducing the number of foodborne illnesses caused by *Salmonella.*

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CHAPTER 1:

INTRODUCTION

Non-typhoidal *Salmonella spp* is a top five pathogen, estimated to cause 1.35 million cases of foodborne illness annually (CDC, 2019). Beef is reported as the eighth leading cause of *Salmonella* illnesses per year (IFSAC, 2020). Behind Norovirus, *Salmonella* causes 11 percent of all foodborne illnesses a year (Scallan et al., 2011). The Interagency Food Safety Analytics Collaboration (IFSAC, 2020) estimates that approximately 38% of foodborne salmonellosis in the United States is attributed to meat and poultry products. *Salmonella* has become a rising concern in ground beef and other comminuted beef products due to the harborage of *Salmonella* within non-mesenteric, peripheral lymph nodes which serve as a potential source of ground beef contamination. Because these peripheral lymph nodes are generally surrounded by a significant amount of adipose tissue, they are typically impervious to in-plant interventions designed to reduce pathogenic bacteria (Brown et al., 2015). Reduction of *Salmonella* in cattle has become a contemporary issue for the industry due to pending rulemaking by Food Safety and Inspection Service (FSIS) that may declare *Salmonella* as an adulterant. *Salmonella* is a naturally occurring bacteria that is known to harbor in beef animals. Direct-fed microbials (DFM) have shown promise as an effective preharvest intervention strategy to mitigate the pathogenic load in beef cattle. When direct-fed

microbials were fed to beef cattle, prevalence of *Salmonella* within lymph nodes has been reported to be reduced when compared to control cattle (Stephens et al., 2007; Vipham et al., 2015; Brown et al., 2019). Direct-fed microbial supplementation may provide an effective preharvest intervention to reduce the burden of *Salmonella* in lymph nodes of cattle and reduce the relative risk of exposing *Salmonella* to humans.

CHAPTER 2:

REVIEW OF THE LITERATURE

2.1 SALMONELLA

Salmonella is a gram-negative, rod-shaped, non-sporeforming bacteria discovered in 1885 by Dr. Daniel Salmon and is naturally occurring in both human and animals. There are more than 2,500 serotypes of *Salmonella* (Hanson et al., 2019) that have been reported thus far, yet only 32 serotypes are known to be virulent to humans (CDC, 2013). *Salmonella* causes 1.35 million cases annually and is the leading cause of foodborne related hospitalizations and deaths with 26,500 hospitalizations and 420 deaths (CDC, 2019). Routes of entry of *Salmonella* can be from contaminated food or water as well as direct contact (human to human or animal to human). Non-typhoidal *Salmonella* is a pathogen that causes foodborne illnesses in humans which can lead to gastroenteritis, bacteremia, and focal infection (Hohmann, 2001). Within those, gastroenteritis is the most common and has an onset of six to seventy-two hours causing diarrhea, abdominal pain, fever and vomiting (Crump et al., 2015). Symptoms vary among individuals due to variance in virulence, immunity and serotype (Jones et al., 2008: Hanson et al., 2019).

The vast majority of *Salmonella* cases originate from agricultural products. *Salmonella enteritidis* is found in the ovaries of hens leading to *Salmonella* infected eggs (Gast and Beard, 1990), whereas *Salmonella* typhimurium is highly associated with

vegetables (Quiroz-Santiago et al., 2009). The most common serotypes detected in ground beef as reported by Bosilevac et al. (2009) were *Montevideo, Anatum* and *Muenster* at 21, 14.8 and 8.5% of samples, respectively. *Salmonella* serotypes present in fecal samples of feedlot cattle differed slightly from those of ground beef with *Anatum* being most commonly found (27.9%) followed by *Montevideo* (12.9%), *Muenster* (11.8%) and *Kentucky* (8.2%) (Fedorka-Cray et al., 1998).

Beef is documented as the eighth leading cause of salmonellosis (IFSAC, 2020) and accounts for 11 percent of all *Salmonella* cases reported (Scallan et al., 2011). When more than two people report an illness from a food pathogen, such as *Salmonella*, it is considered a foodborne disease outbreak. Since 2006, there have been five *Salmonella* outbreaks involving beef across the United States with 504 reported cases, one death, 1,153 hospitalizations and 12,164,611 million pounds of ground beef recalled (CDC, 2021). Utilizing data from FSIS Quaterly sampling reports for 2020 (FSIS, 2021), *Salmonella* prevalence rates were determined from 17,851 raw beef samples with 377 positive samples (8.41%). Fourth quarter report (2020 July – 2020 September) had 156 positives, which was the highest prevalence rate of all quarters. Utilizing the same report, the National Antimicrobial Resistance Monitoring System sampling reports dairy cows to have a prevalence rate of 25.18% as compared to beef cows at 9.68% with steers and heifers at 14.81 and 21.23%, respectively. *Salmonella* is the single most costly bacteria in terms of foodborne illnesses and is an economical burden. The average economic cost of a non-typhoidal *Salmonella* case is $4,312 (Scharff, 2012).

Recent studies have implicated lymph nodes as a source of contamination of ground beef products (Arthur et al., 2008; Koohmaraie et al., 2012; Li et al., 2014) due to trimmings destined for ground beef containing lymph nodes that were not removed during harvest or fabrication procedures. Lymph nodes are impervious to traditional in-plant interventions. Hot water and organic acids are not applicable due to protection provided to the lymph nodes through adipose tissue. Peripheral lymph nodes (PLNs) are more often found in beef trimmings versus mesenteric lymph nodes due to mesenteric lymph nodes being discarded after evisceration; therefore, not posing a food safety risk. Ground beef samples obtained (n=4,136) from commercial processors in different regions across the United States were 4.2% positive for *Salmonella* (Bosilevac et al., 2009). Webb et al. (2017) reported 5.3% of lymph nodes collected to be positive for *Salmonella* and Gragg et al. (2013b) reported lymph nodes to have a prevalence rate of 14.7%.

In 2011, FSIS declared that it was not necessary to sample beef carcasses for *Salmonella* due to extremely low percentage recovered. However, more recently FSIS proposed new performance standards for *Salmonella* in raw ground beef and beef manufacturing trimmings (Docket No. FSIS-2018-0045) to increase *Salmonella* sampling to once per week at plants producing over 50,000 lb of ground beef per day and allowing for only two positive samples out of 48 (FSIS, 2019). Though FSIS is petitioning for a performance standard for *Salmonella* in cattle, Bill Marler, a foodborne illness lawyer, has also filed a petition with USDA Food Safety and Inspection Service requesting that *Salmonella Agona, Anatum, Berta, Blockely, Braenderup, Derby, Dublin, Enteritidis, Hadar, Heidelberg, I 4,[5],12:i:-, Infantis, Javiana, Litchfield, Mbandaka, Mississippi,*

Montevideo, Muenchen, Newport, Oranienburg, Panama, Poona, Reading, Saintpaul, Sandiego, Schwarzengrund, Senftenberg, Stanley, Thompson, Typhi, and Typhimurium be declared adulterants in meat and poultry products (Docket No. FSIS-2020-0007). In 1994, *E. coli* O157:H7 was declared an adulterant by FSIS under the Federal Meat Inspection Act and in 2012 FSIS declared 6 more shiga toxin-producing *E. coli* strains as adulterants. After declaring *E. coli* as an adulterant, the number of *E. coli* cases per year has had an overall decline and Marler believes the same will happen with *Salmonella.*

2.2 PREVELANCE RATES OF *SALMONELLA*

Beef cattle are known reservoirs for *Salmonella,* which has been isolated from hides, feces, and lymph nodes with influence from environmental location, seasonality and cattle type.

2.2.1 Hide

Given hide covers the body of the animal, it is expected that the hide will be contaminated multiple times throughout the animal's life. This could be due to a variety of reasons such as the animal laying down in pens, direct contact with other animals, flies, and transportation.

The prevalence of *Salmonella* found on hides of beef cattle is well established in the literature. Bacon et al. (2002) sampled hides from eight different beef processing plants that slaughtered heifers and steers or cows and bulls. Samples (n=319) were taken after exsanguination but before hide removal in three different anatomical areas (flank, brisket and rump) of the animal. While some processors had zero positive samples for *Salmonella*, other processors had as many as 19 positive samples. Of the 319 samples

taken, 49 were positive for *Salmonella* and had an overall prevalence of 15.4%. An additional study by Barkocy-Gallagher et al. (2002) using different culture methods revealed that out of 50 hide samples per method (Standard, MRU, and MRU-TT), 49, 45 and 49% were positive for *Salmonella*, respectively. Koohmaraie et al. (2012) also used two different methods to analyze *Salmonella* swabs recovered from 100 dairy cow hides at a commercial packing facility. Polymerase chain reaction (PCR) and USDA-FSIS MLG 4.04 procedure were used to analyze all swabs. Samples were 95% positive for *Salmonella* when using PCR, whereas the FSIS procedure detected 96% positive for *Salmonella*. Kunze et al. (2008) sampled four different beef processors and recovered *Salmonella* from 752 of 1,081 hide swabs with an overall presence of 69.6%. From the 22 serotypes identified, *Salmonella enterica* serovars *Anatum*, *Montevideo* and *Cerro* were of greatest prevalence at 25.5, 22.2 and 12.5%, respectively.

Transportation is known to increase stress (Mackenzie et al., 1997) and shedding of *Salmonella* (USDA, 1996) in beef cattle. Trailers not properly cleaned and sanitized between loads can increase chances of animals becoming infected with *Salmonella* during transport. Barham et al. (2002) sampled 10 pens with 20 steers and heifers (n=200) at a large commercial feedlot by taking a ventral midline hide swab from each animal pre-shipment and after arrival at a beef processing facility. There was a significant increase ($P < 0.01$) in *Salmonella* prevalence from pre-shipment swabs to processing swabs. Swabs collected at the feedlot and during processing had an overall prevalence of 6 and 86.9%, respectively. Hide swabs (n=100) were collected pre-shipping and arrival at beef processor from feedlot cattle over four days and adult cattle

(*n*=96) over two days (Beach et al., 2002). There was a significant increase ($P < 0.05$) in *Salmonella* contamination of hides pre-shipping and arrival at the slaughter facility of 18 and 56% positive, respectively. Pre-transit and post-transit swabs were significantly different ($P < 0.05$) in adult cattle. *Salmonella* prevalence on pre-transit hides was 19.8% and increased to 52.2% on post-transit hides. When feedlot and adult cattle hides were compared pre and post transit, they were not significantly different. Dewell et al. (2008) tested multiple factors that could potentially be contaminated with *Salmonella* at the feedyard, transportation and beef processing facility. Out of 40 lots of cattle from 18 different feedyards, hide swabs collected at the feedyard were 6.9% positive for *Salmonella* (54/785) and increased to 19.5% (153/784) positive hide swabs during slaughter. Transportation trailers and lairage pens were 21.9 and 23.3% positive for *Salmonella*, respectively.

Transdermal is known to be a route of entry for *Salmonella* into the body of beef cattle (Edrington et al., 2013a, b). *Salmonella* can enter the host transdermally via biting flies through the hide where it is transported to the peripheral lymph nodes (Edrington et al., 2013b). Olafson et al. (2016) observed than when *Salmonella* Senftenberg was fed to horn flies (*n*=800) using blood meal, which were allowed to feed on the rump or shoulder of the cattle for five days and then replaced with new unfed horn flies once over 11 days, twice over 19 days or not replaced at all. *Salmonella* was recovered from the hide at 88, 100, and 100%, respectively.

2.2.2 Fecal

Salmonella is found in the gastrointestinal tract of cattle (Samuel et al., 1981; Abouzeed et al., 2000) and eventually shed through feces leading to contamination.

Fedorka-Cray et al. (1998) sampled 100 feedyards across 13 major cattle feeding states. Fecal pats (n=25) were collected from two pens from each feedyard; one pen with the shortest days on feed and one pen with the longest days on feed. Thirty-eight percent of feedyards were positive for *Salmonella* with 5.5% (273 of 4,977) positive fecal pats. Cattle that were on feed longer (longest days on feed was 180 days) had a significantly higher prevalence ($P < 0.05$) of *Salmonella* when compared to cattle that had been on feed for a shorter duration (shortest days on feed was 8 days). Several *Salmonella* serotypes were isolated with *Anatum* highest in prevalence (27.9%) followed by *Montevideo* (12.9%), *Muenster* (11.8%), *Kentucky* (8.2%), *and Newington* (4.3%) lowest in prevalence. Fegan et al. (2004) reported low rates of *Salmonella* in fecal grabs of feedlot and grass-fed cattle. Of the 310 fecal grabs, *Salmonella* was only present in 21 grab samples with feedlot and grass-fed cattle having *Salmonella* detected in 14 and 7 fecal grabs, respectively.

Fecal pats (n=50) were collected by rectal palpation from 15 animals at the West Texas A&M University Feedlot and 35 animals from Texas Tech University Burnett Center Feedlot and *Salmonella* was detected through Polymerase Chain Reaction (Stephens et al., 2007). Fecal pats were 50% positive for *Salmonella* and had a lower recovery rate ($P \leq 0.04$) when compared to other locations (back, flank, hock, neck, Perineum, Ventrum, oral) of the animal sampled for *Salmonella* prevalence. An

additional study by Gragg et al. (2013a) sampled (n = 68) cattle in a beef processing facility in Mexico where feces were collected from the recto-colon portion and 94.1% of fecal grabs were positive for *Salmonella.*

2.2.3 Lymph Nodes

The lymphatic system plays an important role in the immune function of an animal because it is used as a filtration system that allows the body to isolate foreign bacteria and viruses and eventually destroy them (Arthur et al., 2008). This could potentially lead to lymph nodes that are loaded with pathogenic bacteria and pose as a risk to ground beef trimmings and the end consumer. Lymph nodes, which are part of the lymphoid system and secondary lymphoid organs (Buettner and Bode, 2012), are found throughout the body of man and animal and serve an important role in the immune system. Immune responses to pathogenic antigens are controlled within the lymph nodes. Lymph nodes identify antigens from lymph fluid by acting as a filter and initiate an immune response to pathogenic antigens or create tolerance against harmless antigens (Buettner and Bode, 2012). Dendritic and T cells play a major role in immune response where naïve T cells navigate to lymph nodes to find antigens presented by dendritic cells (Bousso, 2008). Macrophages (phagocytic cells) destroy antigens through phagocytosis which causes the release of cytokines to alert the adaptive immune system (Willard-Mack, 2006; Gray and Cyster, 2012). Macrophages will catch any bacteria and protect against further invasion. *Salmonella* can survive outside and inside of the host cell due to being a facultative intracellular pathogen. *Salmonella* harbors pathogenicity islands allowing for *Salmonella* virulence to occur (Gerlach and Hensel, 2007). Pathogenicity

islands are large DNA regions that are found in pathogenic bacteria that have virulence properties (Gerlach and Hensel, 2007). *Salmonella* has five *Salmonella* pathogenicity islands (SPI) where SPI 1 is required for bacteria is invade the epithelial cells of the intestine whereas SPI 2, 3 and 4 are necessary for growth and survival of bacteria (Marcus et al., 2000). Instead of being destroyed by macrophages, Salmonella can attack and destroy macrophages while infecting other cells with Salmonella (Holzer, 2020).

Lymph nodes located in the chuck and flank are often encased in fatty tissue and usually are destined for ground beef; in contrast, mesenteric lymph nodes are removed at harvest during evisceration (Arthur et al., 2008). Koohmaraie et al. (2012) sampled (n=100) superficial cervical lymph nodes from dairy cows at a beef processing facility. Trim samples (n=14) and raw ground beef samples (n=60) from sample animals were also collected to determine the prevalence of *Salmonella.* *Salmonella* was determined through mulitplex PCR with positives further being plated by using the USDA FSIS MLG 4.04 procedures and negative samples were further cultured using Rappaport-Vassiliadis broth and tetrathionate broth, PCR assay again and USDA FSIS MLG 4.04 confirmation. Superficial cervical lymph nodes were 18% positive for *Salmonella* through PCR and MLG 4.04. Arthur et al. (2008) sampled subiliac and superficial cervical lymph nodes from fed and cull cattle. Lymph nodes (n=570) had an overall *Salmonella* prevalence of 1.6%; superficial cervical lymph nodes in cull and fed cattle were 1.05 and 0.35% positive, respectively. Subiliac lymph nodes were 3.86% positive for cull cattle and 1.05% positive in fed cattle. Webb et al. (2017) collected subiliac peripheral lymph nodes (n=5,450) from both feedlot and cull cattle from three different

regions (northern, southern and western) of the United States during a span of 11 months from 12 beef processing facilities. Overall, *Salmonella* was recovered from 5.3% of all lymph nodes sampled. Log concentrations of subiliac lymph nodes ranged from 1.6 to 4.9 $\log_{10}$ CFU/PLN with 51 of the 160 had a concentration greater than 3.0 $\log_{10}$.

As mentioned previously, transdermal transmission is a known route of entry for *Salmonella* into peripheral lymph nodes in cattle because of biting flies feeding hourly on the hide (Edrington et al., 2013a, b). Oflason et al. (2016) conducted a study which involved four experiments with different strains of *Salmonella,* number of flies in cages, and different locations of fixed fly cages on the animal. Experiment four evaluated different exposure rates of horn flies and the effects on *Salmonella* prevalence in lymph nodes. Fly cages were adhered to the left and right shoulder or left and right rump for 5, 11, or 19 days. Each fly cage (n=4) contained 200 flies that had been fed bloodmeal that contained *Salmonella Seftenberg.* Cattle affixed with cages on the shoulder had a higher prevalence rate of *Salmonella* positive lymph nodes when compared to the rump. The extending feeding of flies on cattle also had an impact on *Salmonella* prevalence rates with 8% positive after a 5-day exposure period and 50 and 42% positive after 11 and 19-day exposure periods, respectively.

2.2.4 Geographical

Salmonella prevalence can differ by regions across the United States. The southern United States is known to have a higher rate of *Salmonella* when compared to the northern region (Rivera-Betancourt et al., 2004). Hide samples from the southern region were (n=510) were significantly higher ($P < 0.01$) with a prevalence of 91.8%

when compared to the northern region (n=523) cattle who had a prevalence of 50.3%. Holding pens at beef processor that contained southern region cattle had 26.7% more *Salmonella* compared to those that held northern region cattle. Dargatz et al. (2003) sampled fecal pats from 70 feedlots across 12 states and reported that the southern region had a higher *Salmonella* prevalence (7.7%) when compared to northern regions (4.8%). Webb et al. (2017) collected subiliac peripheral lymph nodes (n = 5,450) of cull and fed beef from three regions and 12 beef processing facilities in the United States. The three regions were categorized as followed; Region A (northern region) consisted of Colorado, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Missouri, Nebraska, Ohio, Pennsylvania, Virginia and West Virginia; Region B (southern region) consisted of Arizona, New Mexico, Oklahoma and Texas; Region C (western region) consisted of California, Nevada and Utah. The overall prevalence of *Salmonella* found in subiliac peripheral lymph nodes of cattle sampled was 5.3% with log concentrations ranging from 1.6 to 4.9 $\log_{10}$ CFU/PLN. Region B (southern region) had a prevalence rate of 10.3% as opposed to Region A (northern region) and Region C (western region) where prevalence rates were 1.89 and 2.77%, respectively. An additional study by Haneklaus et al. (2012) collected superficial cervical (n=279) and iliofemoral (n=28) lymph nodes across four different collection times over a 3-month period from July to September from feedlots located in the southern region of the United States. Initial variability amongst feedyards differed considerably for *Salmonella* prevalence; *Salmonella* was not recovered from feedyard A, while feedyard B lymph nodes were 100% positive for superficial cervical location and 80% positive at the iliofemoral location. Second collection results observed

feedyard A still did not have *Salmonella* and feedyard B had a prevalence of 100%. The third collection yielded a prevalence of 0 and 76% for feedyard A and B, respectively. Lymph nodes of the final collection were obtained from Mexican cattle with the hypothesis that cattle origin would alter *Salmonella* prevalence. Cattle of different origin yielded the same results from the above collections with 0% prevalence in feedyard A and 88% prevalence in feedyard B. Though all feedyards collected on were in the southern regions of the United States where it is well-established that *Salmonella* is higher in prevalence, feedyards located in various parts of the southern region plays a role in the varying rates of *Salmonella* found in lymph nodes.

2.2.5 Seasonal

Seasonality is a significant factor in prevalence of *Salmonella* due to *Salmonella* needing warmer conditions to grow; *Salmonella* is seen to increase in warmer months and have a lesser detection in cooler months. *Salmonella* recovered from beef cattle harvested at three processors in the Midwest revealed that fecal samples were significantly higher ($P < 0.05$) in the summer when compared to other seasons (Barkocy-Gallagher et al., 2003). Fecal samples (n=287) in the summer were 9.1% positive for *Salmonella*, while fall, winter and spring were 2.8, 2.5 and 2.1% positive, respectively. Hides from all four seasons had an overall prevalence of 71% and followed the pattern of fecal samples with summer having a prevalence of 97.7% with a decrease during winter to 27.7%. McEvoy et al. (2003) reported that *Salmonella* was most frequently found from August to October and was not found during the months of February, April, May, June, July or November. Dargatz et al. (2003) also reported fecal samples collected from

70 feedlots across the United States were lowest from January to March (2.8%) and October to December (4%) and were highest from July to September (11.4%) and April to June (6.8%) and significant when combined into warm and cool season ($P < 0.01$). In contrast, Brichta-Harhay et al. (2008) reported that seasonality did not effect ($P = 0.67$) prevalence of *Salmonella* found on hides.

Protection is provided to lymph nodes during harvest through carcass fat allowing lymph nodes to avoid any post-harvest interventions that are being implemented to reduce *E. coli* prevalence and end up in ground beef trimmings. Lymph node adulteration can also be affected by seasonality. Gragg et al. (2013b) collected subiliac lymph nodes ($n = 3,327$) from cull and feedlot cattle during three different periods (September-November, February/March and July-September). Lymph nodes from both cattle types and all regions were 14.7% positive for *Salmonella*, while feedlot cattle lymph nodes were significantly higher ($P < 0.05$) in summer/fall than winter/spring with no seasonality affect in cull cattle with a prevalence of 0.65%. Webb et al. (2017) collected subiliac lymph nodes (n=5,450) from cull and feedlot cattle across 11 months to determine the seasonal effect on prevalence in lymph nodes. *Salmonella* prevalence in subiliac lymph nodes during warmer season (June – October) was 8.2% and cooler season (February – May, November – December) was 2.4%. Feedlot cattle lymph nodes had a lower prevalence in the cooler season (2.7%), which increased to 11.6% in the warmer season, whereas cull cattle were low (<2%) during both seasons. An additional study by Nickelson et al. (2019) agrees with the seasonality trend reporting 93 of 200

lymph nodes (46.5%) positive for *Salmonella* in cool season and 115 of 200 (57.5%) lymph nodes positive in warm season.

2.2.6 Cattle Type

Cattle from different production systems and breeds can affect a multitude of factors such as ribeye area (Moore et al., 2012; Boykin et al., 2017), marbling score (Moore et al., 2012; Boykin et al., 2017) and hot carcass weight (Moore et al., 2012; Boykin et al., 2017), as well as pathogenic presence and load on and within an animal.

Cull beef including dairy cows, range cows and bulls are known to have lower *Salmonella* incidence (10.5%) when compared to fed beef (29.5%; Herrick, 2018). Feedlot origin cattle that were harvest ready had a hide Salmonella prevalence of 81.4% with a concentration of 2.32 log10 MPN/cm2. Though they were not different (P = 0.47), dairy and beef cattle with poor productivity both had lower Salmonella prevalence of 71.2 and 59%, respectively when compared to feedlot, harvest ready cattle (Kunze et al., 2008). Webb et al. (2017) reported that cull cattle subiliac lymph nodes (n=1,840) were 1.79% positive for *Salmonella*, whereas fed cattle lymph nodes (n=3,610) were 7.09% positive. In agreeance with Webb et al. (2017), Gragg et al. (2013b) collected subiliac lymph nodes (n=3,327) from fed and cull cattle were significantly higher ($P < 0.01$) in fed cattle when compared to cull cattle with *Salmonella* prevalence of 0.65 and 11.8%, respectively. Contrary to previous studies Arthur et al. (2008) observed that when superficial cervical lymph nodes from the chuck (n=570) and subiliac lymph nodes from the flank (n=570) were collected from fed and cull cattle. Cull cattle had a higher prevalence (2.46%) of *Salmonella* when compared to fed cattle (0.70%). Flank lymph

nodes were 3.86 and 1.05% positive for *Salmonella*, whereas chuck lymph nodes were 1.05 and 0.35% positive for *Salmonella* in cull and fed cattle, respectively.

Brown et al. (2015) reported Holstein steer subiliac lymph nodes (n =467) had a higher prevalence of *Salmonella* (62.1%) when compared to beef cattle (n=462) subiliac lymph nodes at a prevalence rate of 59.7%. Though Holstein steers had an increase in positive lymph nodes, the majority of quantifiable lymph nodes had considerably the same log concentration of *Salmonella* between both beef and Holstein steers from 2.4 to 4.1 log CFU/g and 2.7 and 4.1 log CFU/g, respectively.

Due to flies being a vector in which *Salmonella* reaches the lymph nodes (Edrington et al., 2013b), Brown et al. (2015) hypothesized that Brahman cattle could potentially have lower rates of *Salmonella* prevalence in lymph nodes due to their environmental adaptability of being more resistant to parasites. Lymph nodes collected from Brahman cattle (n=42) and beef cattle (n=31) were 100 and 97% positive for *Salmonella*, respectively and did not differ (P = 0.25).

Gragg et al. (2013a) collected hide, feces and lymph node (mesenteric, mediastinal and subiliac) samples from cattle (n=68) at a beef processing facility in Mexico. *Salmonella* was prevalent in 94.1% and 100% of fecal and hides, respectively. Mesenteric, subiliac, mandibular and mediastinal lymph nodes were 91.2, 76.5, 55.9 and 7.4% positive for *Salmonella*, respectively. Nickelson et al. (2019) compared cattle which came from the same feedlot, but originated from different countries (United States or Mexico). There was no difference (P = 0.48) found in subiliac lymph nodes of cattle originating from Mexico and United States where lymph nodes were 54 and 50%,

respectively. The increase of positive lymph nodes in Mexico compared to cattle originating from Mexico could be indicative of seasonality due to Mexico having higher temperatures year round compared to Mexican cattle fed in the United States were warm and cool season occur.

2.3 INTERVENTIONS FOR *SALMONELLA*

Previous research indicates, *Salmonella* is harbored in peripheral lymph nodes of healthy cattle (Arthur et al., 2008; Koohmaraie et al., 2012; Webb et al., 2017). Due to lymph nodes being encased in fatty tissue and impervious to post-harvest interventions implemented to primarily reduce carcass surface contamination at harvest, do not reduce *Salmonella* prevalence within lymph nodes. Thus, pre-harvest interventions that target *Salmonella* carriage in a beef animal is necessary to mitigate *Salmonella* carriage in lymph nodes. Cattle present a risk to the processor due to potentially introducing pathogenic bacteria to slaughter facility. Additionally, modern post-harvest interventions are largely directed toward mitigating *E. coli*, which is largely controlled by carcass surface measures. Facilities implement Hazard Analysis and Critical Control Points (HACCP) as well as other decontamination interventions such as hide-on wash cabinets (Arthur et al., 2008; Buncic and Sofos, 2012), bunging of the rectum to prevent fecal contamination on carcass (Sheridan, 1998; McEvoy et al., 2000), steam vacuuming and trimming (Kochevar et al., 1997; Sheridan, 1998; Koohmaraie et al., 2005), and pre- and post-evisceration wash (Sheridan, 1998). Though many interventions are implemented during harvest to reduce pathogen load and further contamination of the carcass, lymph nodes are protected during harvest interventions. It is economically impractical to

remove all lymph nodes or implement an intervention at harvest that would reduce *Salmonella* in lymph nodes inadvertenly destined for beef trimmings. In order for *Salmonella* to be reduced in lymph nodes, a pre-harvest intervention is necessary at the feedlot level. Different intervention technologies have been implemented to decrease *Salmonella* pre-harvest.

2.3.1 Feed Additives

Feed additives are used in a feedlot setting typically to benefit not only the animal, but the producer. Ractopamine hydrochloride, commonly known as Optaflexx, is reported to increase live weight, hot carcass weight, and average daily gain (Abney et al., 2007; Scramlin et al., 2010; Boler et al., 2012) in beef cattle and pigs. Ractopamine hydrochloride supplemented to feeder pigs over a four day period reduced *Salmonella* shedding; ractopamine fed pigs shed 0.89% less *Salmonella* compared to control pigs ($P < 0.01$; Edrington et al., 2005). In contrast, cattle fed ractopamine hydrochloride did not differ ($P > 0.10$) in *Salmonella* shedding in fecal samples in two replicates when compared to control (Edrington et al., 2006). When all three replicates were combined, the supplementation of ractopamine hydrochloride tended to increase ($P = 0.08$) overall positive samples when compared to control.

Tylosin phosphate (commonly fed as Tylan®) is a bacteriostartic macrolide antibiotic that is traditionally fed to cattle to reduce severity of liver abscesses in feedlot cattle. Bacterial protein synthesis is inhibited via macrolides due to adhering to the 50S subunit of the ribosome (Pyörälä et al., 2014). Macrolides are typically more effective against gram-positive bacteria as compared to gram-negative bacteria with no

effectiveness against *Salmonella* (Pyörälä et al., 2014). Tylosin supplementation to pigs infected with *Salmonella* did not differ in prevalence ($P = 0.66$) or $\log_{10}$ concentration control ($P = 0.75$) when compared to control; however, tylosin fed pigs shed *Salmonella* a shorter duration compared to control ($P = 0.05$). Steers and heifers (n=5,481) were randomly allocated to four treatments (tylosin, no tylosin, essential oil or Diamond V prototype) (Holzer, 2017). Fifteen subiliac lymph nodes were collected from cattle within each pen (n=40) and cultured for *Salmonella* prevalence. Treatment did not have an effect ($P = 0.84$) on *Salmonella* prevalence with an overall prevalence of 84.6%. Lymph nodes from cattle supplemented with tylosin were 86% positive for *Salmonella* with no tylosin, essential oil and Diamond-V prototype being 83.33, 86.67 and 82% positive, respectively. In contrast, crossbred and Holstein cattle supplemented with tylosin had a prevalence of 13 and 18% in liver abscesses, respectively as compared to control cattle that were 29 and 37% positive, respectively (Amachawadi et al., 2017).

Ascophyllum nodosum, or better known as brown seaweed or Tasco, has been used as a feed additive in ruminants and is reported to reduce *Escherichia coli* O157:H7 (Braden et al., 2004; Bach et al., 2008; Zhou et al., 2018). Steers (n=100) and heifers (n=100) were randomly allocated to 2% Tasco-14 (TRT) supplemented 14 days prior to harvest or control (CON) (Braden et al., 2004). Fecal samples during the pre-feeding period for CON and TRT cattle were 36 and 29% positive for *Salmonella*, respectively. After exsanguination, TRT cattle were 63% positive and CON cattle were 83% positive. Though *Salmonella* prevalence had a significant increase ($P < 0.01$) in both control and

Tasco-14 treated cattle, fecal samples in TRT cattle had lower *Salmonella* prevalence ($P < 0.05$) after the 14 d feeding period when compared to CON.

2.3.2 Vaccines

Vaccines in cattle are typically used for preventive measures against viral respiratory pathogens and clostridial diseases. Previous research indicates, *Salmonella* is harbored in peripheral lymph nodes (Arthur et al., 2008; Koohmaraie et al., 2012; Webb et al., 2017) where postharvest interventions are not efficacious in reducing prevalence of *Salmonella* in lymph nodes inadvertently destined for ground beef. A vaccine for *Salmonella* has been made through purified siderophore receptors and porin proteins which allow vaccinated cattle to produce antibodies to disturb iron acquisition of pathogenic bacteria along with other host defenses (Farrow, 2011). *Salmonella* vaccine administered to dairy cows had a reduced *Salmonella* prevalence when compared to dairy cows that did not receive the vaccine with vaccinated whole-herds feces 8.0% positive for *Salmonella* and 36.8% for herds that did not receive the vaccine (Loneragan et al., 2012). Farrow (2011) also reported a decrease in *Salmonella* prevalence (15.3%) in vaccinated dairy cattle compared to control (27.5%). In contrast, subiliac lymph nodes ($P = 0.52$) and fecal pats ($P = 0.57$) collected from vaccinated and control cattle did not differ (Cernicchiaro et al., 2016). Lymph nodes were 85.7 and 87.4% positive for Salmonella within vaccinated and control cattle, respectively. Though vaccinated cattle had a lower rate of *Salmonella* positive lymph nodes and fecal pats, the vaccine showed no significant differences. Edrington et al. (2020) administered *Salmonella* Newport Bacterial Extract to steers (n=30) and Endovac-Bovi to Holstein steers (n=12) to evaluate efficacy of two

commercially available vaccines on the reduction of *Salmonella* in lymph nodes. Steers were administered *Salmonella* Newport Bacterial Extract with SRP technology on d 0 and d 21. Steers were then inoculated every other day for five days with *Salmonella* intradermally four months after booster vaccination occurred. Cattle from each group where then euthanized on d 6 and d 12 post-inoculation of *Salmonella*. Treatment had no effect (P = 0.89) onperipheral lymph nodes collected on d 6 post-inoculation with vaccinated cattle being 55% positive for *Salmonella* compared to control with an overall prevalence of 52.2%. Twelve days post-inoculation, vaccinated steers had an overall *Salmonella* prevalence of 19.9% whereas control cattle were 62.3% positive for *Salmonella* across all peripheral lymph nodes. Holstein steers were administered Endovac-Bovi on d 0 and 14 and were also inoculated intradermally with *Salmonella* one week after booster vaccination with repeated inoculation every week for three weeks and euthanized seven days following last inoculation. Right popliteal lymph nodes from vaccinated steers were 0% positive for *Salmonella* whereas control right popliteal lymph nodes were 60% positive. There was no significant difference (P = 0.30) between control and vaccinated steers concerning overall prevalence of *Salmonella* in all peripheral lymph nodes. In an additional study by Horton et al. (2020) an autogenous vaccine was administered to feeder calves (n=55) in four treatment groups; control, farm (received at first dose and booster at McGregor Research Center, split (received vaccine at McGregor Research Center and booster upon entry into feedlot, and yard (received first and booster doses at feedlot). Left and right subiliac and superficial cervical lymph nodes (n=508) were obtained from each animal enrolled in the study. All cattle

vaccinated were 0% positive for *Salmonella* in lymph nodes when compared to control cattle lymph nodes (11%).

2.3.3 Direct Fed Microbials

Direct fed microbials (DFM) are defined as feed additives that contain live cultures (Brashears et al., 2005) to improve performance while simultaneously reducing pathogenic load in cattle (Brown et al., 2019). McAllister et al. (2011) proposed that direct fed microbials have antimicrobial effects in the rumen and exhibit competitive exclusion in both the small and large intestine. Studies that have been conducted using a direct fed microbial to reduce pathogenic load have mainly focused on *Escheria coli* O157:H7. Direct fed microbials reduced *Escheria coli* O157:H7 shedding in fecal pats (Brashears et al., 2003; Stephens et al., 2007; Tabe et al., 2008) as well as prevalence on the hide (Brashears et al., 2003; Younts-Dahl et al., 2004).

Direct fed microbial studies involving reduction of *Salmonella* is miniscule and varies by study. Tabe et al. (2008) evaluated the effects of direct fed microbials on fecal shedding of *Salmonella* with no reduction ($P = 0.24$) observed between treated versus control cattle. Though not significantly different, *Salmonella* was recovered from 11.3% of fecal samples compared to control cattle (14%). In an additional study by Stephens et al. (2007), *Lactobacillus acidophilus* (NP 51) and *Propionibacterium freudenreichii* (NP 24) was fed to steers (n=269) at three different concentrations (low, medium and high) to determine the effects on *Salmonella* prevalence in feces and on hides. No differences were found among the three different doses for hides or at low and medium concentrations for feces. The high concentration of NP 51 and NP 24 tended ($P = 0.09$)

to shed less *Salmonella* in feces when compared to control. Brown et al. (2019) also observed no differences in fecal shedding of *Salmonella* when compared to control.

As mentioned previously, peripheral lymph nodes are reservoirs for *Salmonella* with a need for an efficacious pre-harvest intervention to reduce *Salmonella* prevalence in peripheral lymph nodes. Vipham et al. (2015) fed *Lactobacillus acidophilus* (NP 51) and *Propionibacterium freudenreichii* (NP 24) as a direct fed microbial to steers (n = 1794) to determine if NP 51 and NP 24 were efficacious in reducing *Salmonella* prevalence in subiliac lymph nodes. Control cattle lymph nodes were 76.3% positive for *Salmonella* when compared to NP 51 and NP 24 cattle (57.5%) with 176 positives out of 310 lymph nodes in treated cattle and 240 positives out of 317 lymph nodes in control cattle. There was variation across the three slaughter days. *Salmonella* prevalence on first and second slaughter days was significantly reduced ($P < 0.01$) by 50% and 31%, respectively. There was no difference on slaughter day three ($P = 0.18$). This study conducted in a commercial feedlot setting was replicated at the Texas Tech University Research feedlot with a total of 112 steers and pens that consisted of approximately 4 steers (Vipham et al., 2015). Cattle fed NP 51 and NP 24 had a significant reduction of *Salmonella* prevalence in subiliac lymph nodes compared to control cattle and were 82% less likely to shed *Salmonella*. In an additional study by Brown et al. (2019), steers (n = 30) were fed three different blends of direct fed microbials (control, 1:1 ratio of *Lactobacillus acidophilus* and *P. acidilactici;* DFM2 or 1:2 ratio of *Lactobacillus reuteri* and other *Lactobacilus* strains; DFM3) and were then intradermally inoculated with *Salmonella* Montevideo at days 32, 37 and 42. Cattle were then necropsied on day 49 and day 50 where right and

left subiliac, popliteal, superficial cervical and superficial inguinal lymph nodes were collected from all cattle. Though no significant differences ($P > 0.05$) were observed in peripheral lymph nodes, *Salmonella* percent-positive peripheral lymph nodes tended ($P = 0.07$) to be greater in control cattle (80%) when compared to DFM2 and DFM3 that were 63.8% and 68.8% positive, respectively.

The specific mode of action of direct-fed microbials is still unknown and few studies have been addressed the mechanism to which these direct-fed microbials reduce the shedding of pathogenic material. Several theories such as competitive attachment and inhibition leading to an increased immune response and overall better gut health (Krehbiel et al., 2003). Bacteria found in direct-fed microbials could potentially be competing with other bacteria for sites on the intestinal wall (Krehbiel et al., 2003). According to Gilliland and Speck (1977), lactobacilli species have shown inhibitory action against pathogenic bacteria such as *Salmonella*. Direct-fed microbials may also provide an immune response due to promotion of intestinal health through innate and adaptive immune responses against pathogenic bacteria (Krehbiel et al., 2003). These three theories overall promote improved gut health.

2.4 SUMMARY

Salmonella is becoming a rising concern in the meat industry due to pending laws and petitions filed by FSIS and Bill Marler. Due to the impracticality of removing all lymph nodes at harvest and fabrication, it is crucial for mitigation to occur at the finishing phase due to lymph nodes impervious to in-plant interventions that are implemented to

reduce pathogenic load of *E. coli*. Direct-fed microbials may be the most viable option for reduction in *Salmonella* in beef cattle during the finishing phase.

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CHAPTER 3:

EFFECT OF A DIRECT-FED MICROBIAL (10-G ARMOUR) ON FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS AND PREVALENCE OF *SALMONELLA* IN FED BEEF HEIFERS

3.1 INTRODUCTION

An estimated 48 million cases of foodborne illness occur annually in the United States; non-typhoidal *Salmonella* is a top five cause 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) and eggs (Jones et al., 1995; Singh et al., 2010; Jones et al., 2012), but also known to be a risk in produce (Wells and Butterfield, 1997; Quiroz-Santiago et al., 2009; Sant'Ana et al., 2011). However, recent data suggests beef products are also susceptible to *Salmonella* contamination (Rose et al., 2002; Zaidi et al., 2006; Sallam et al., 2014). Lymph nodes in beef cattle are known to harbor *Salmonella* (Samuel et al., 1981; Arthur et al., 2008; Brown et al., 2019); further, the ability to remove all lymph nodes (~115 lymph nodes; Budras et al., 2003) is impractical. As a result, lymph nodes may be incorporated into ground beef trimmings, thus increasing the risk of *Salmonella* contaminated ground beef (Bosilevac et al., 2009; Arthur et al., 2008; Koohmaraie 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 impacts *Salmonella* prevalence; southern regions having a higher prevalence of *Salmonella* than northern regions (Dargatz et al., 2003; Rivera-Betancourt et al., 2004; Haneklaus et al., 2012). Additionally, cattle type impacts *Salmonella* prevalence; feedlot cattle are more frequent carriers than cull cattle (Gragg et al., 2013b; 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, 2018).

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; Holzer, 2017), and direct-fed microbials (Stephens et al., 2007; Vipham et al., 2015; Brown et al., 2019). Direct-fed microbials (DFMs) are commonly used in the industry to improve performance and reduce the pathogenic load in cattle. Shedding of *Salmonella* has been reported to be reduced by DFMs (Stephens et al., 2007; Vipham et al., 2015; Brown et al., 2019), while others report no difference (Tabe et al., 2008). The objective of this study was to determine if direct-fed microbials can decrease the population of *Salmonella* located in fecal matter and lymph nodes.

3.2 MATERIALS AND METHODS

The feeding portion of this experiment was conducted at Wrangler Feedyard (Tulia, TX). All experimental procedures were approved by the Institutional Animal Care and Use Committee at West Texas A&M University (#2020.02.003).

3.2.1 Cattle Processing and Experimental Design

Heifers (n=1,925) were received at Wrangler Feedyard (Tulia, TX) between 28 February 2020 and 14 March 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 coastal Bermuda grass hay. During initial processing, heifers were excluded from the trial if they weighed 68 kg greater than or less than pay weight, deemed unfit due to illness, lameness, or pregnant. 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 Nasalgen IP (Merck Animal Health) for viral respiratory pathogens. Internal and external parasites were controlled for through administration of Synanthic (Boehriner Ingelheim, Duluth, GA) and Dectomax Injectable (Zoetis, Parsippany, NJ). Heifers were identified with visual eartags 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 (291 ± 9.9 kg) were enrolled in this study in a randomized complete block design with time of arrival as blocking factor and pen as 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 grams/animal/day (CON) or 2 grams/animal/day (TRT) of 10-G Armour (Life Products, Inc., Norfolk, NE) to provide 1 billion CFUs per animal per day

of *Lactobacillus acidophilus, Enterococcus faecium, Pediococcus pentosaceus, Lactobacillus brevis* and *Lactobacillus plantarum.* Within each pen, 24 candidate animals were randomly identified for longitudinal Salmonella sampling. Individual BW was collected at initial processing and re-implant; whereas, pen BW were collected on day 0, day 40 and prior to harvest using a platform scale (Model 7531, Mettler-Toledo, Columbus, OH) prior to the morning feeding.

A two percent pencil shrink was applied for day 0 and day 40 body weights, whereas a four percent pencil shrink was applied to final body weight. After randomization but prior to day 0, five heifers assigned to 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 TRT pen due to being pregnant. Upon arrival, all cattle received a starter diet that consisted of RAMP (Cargill Corn Milling, Bovina, TX) and hay for ~3 days. Cattle were then transitioned to a finishing diet in which RAMP was reduced every two to four days at 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 melengesterol acetate (HeifermaX 500, Elanco Animal Health). In addition, racotopamine hydrochloride (Optaflexx, Elanco Animal Health) was fed for the final 35 days 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 Armour was dispensed independently from the Micro Machine (Micro Technologies) into the ration after it was loaded into the delivery truck and mixed for three minutes.

3.2.2 Sample and Data Collection

A longitudinal design was used to investigate Salmonella shedding, with 24 candidate animals selected from each pen. Recto-anal mucosal swab samples (RAMS) were collected during initial processing (-2 d) and at harvest. A sterile foam-tipped applicator swab (FecalSwab®, COPANUSA, Murrieta, CA) was inserted three to five 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 day 0, 52, 120 and 192. Each composite sample consisted of ten individual fecal pats resulting in five composite samples per pen. All bags were labeled with the appropriate pen number and sample day. The left or right subiliac lymph node (n = 429) was collected from each sample 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 (FSNS, San Antonio, TX) for diagnostic analysis.

Carcass data was collected by trained personnel from the West Texas A&M University- Beef Carcass Research Center (Canyon, TX). Eartags were individually recorded and assigned an individual identification by WTAMU personnel. Livers were scored using a modified Elanco Liver Check System (Brown and Lawrence, 2010) where 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 gastrointestinal tract, A+Open = open liver abscesses).

Additionally, other liver abnormalities including telangiectasis, 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 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; 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 was recorded on the harvest floor. Carcass characteristics (marbling, quality grade, 12^{th}-rib subcutaneous fat depth, longissimus muscle area, yield grade) were obtained from USDA camera data.

3.2.3 Salmonella Analysis

Upon arrival at Food Safety Net Services, ten grams of a composite fecal sample were weighed and inserted into a sterile Whirl Pak bag with 90 ml of buffered peptone water. Samples were hand massaged for 30 seconds to create a homogenous sample. Sample liquid of Rectal Swabs was directly transferred to first well of plates.

Lymph node samples were trimmed of excess fat and fascia and submerged into boiling water for three to five seconds to rid the lymph node of any *Salmonella* on the exterior. Lymph nodes were then individually placed into stomacher sample bags and weighed, pulverized by a rubber mallet and enriched with 80 ml of tryptic soy broth

through incubation at 25°C for 2 hours and then 42°C for 12 hours (Brichta-Harhay et al., 2012; 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 ml 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 37ºC for 24 hours. After incubation 96 well pin replicator was used to transfer growth from incubated plates to 1 ml RV broth aliquots. The replicated well pins were covered and incubated at 42ºC for 24 to 48 hours. After incubation, all samples that were indicative of growth changed colors and were streaked on to XLD plates and incubated at 35ºC for 24 hours. After 24 hours, plates that had colony growth were presumed positive for *Salmonella* and underwent serological confirmation with Poly O antisera to confirm *Salmonella*.

3.2.4 Statistical Analysis

The GLIMMIX procedure of SAS (SAS Inst. INC., Cary, NC) was used to analyze all data with least square means determined through use of the LSMEANS option. The model included the fixed effect as treatment and random effect as block with pen as experimental unit. Repeated measures was used to analyze *Salmonella* prevalence and concentration across time using the unstructured covariance structure.

3.3 RESULTS AND DISCUSSION

3.3.1 Live Performance

Initial BW (Table 2) did not differ (P = 0.95) among treatment groups (CON = 290.9 kg, 10-G = 291.0 kg) nor did final body weight (P = 0.79; CON = 543.0 kg, 10-G =

545.3 kg). In a study by Adeyemi et al. (2019), newly weaned steers (n = 40) were supplemented with 19 g of a *Saccharomyces cerevisiae*-based direct-fed microbial for 42 days; steers fed the DFM gained 10 kg more than the control steers after the 42 day receiving period. In contrast Stephens et al. (2007) observed that steers on feed for 139 days and supplemented with 400 mg per animal per day of *S. cerevisiae* and *L. acidophilus* did not have a differing final body weight when compared to control. Heifers supplemented with 10-G did not differ (P = 0.69) in average daily gain (1.38 kg) when compared to control (1.37 kg). In contrast, Kenney et al. (2015) reported a greater average daily gain in steers fed 10-G (1.87 kg) when compared to steers fed Bovamine (1.75 kg); however, average daily gain did not differ between control and 10-G fed cattle. Gain:Feed ratio did not differ (P = 0.81) among treatments (CON = 0.154; 10-G = 0.155). Stephens et al. (2010) also reported no difference in average daily gain and feed efficiency between treated and control cattle. Dry matter intake was similar (P = 0.63) among treatments (CON = 8.89 kg, 10-G = 8.98 kg) which is consistent with the findings from Kenney et al. (2015). Likewise, Cull et al. (2015) reported no difference in dry matter intake between control cattle (8.87 kg) and cattle supplemented with Bovamine (8.85 kg). Morbidity rate for cattle pulled did not differ (P = 0.90) among treatments nor did mortalities and railers (P = 0.55). In contrast, Keyser et al. (2007) supplemented heifers with 1 g of *Saccharomyces cerevisiae* through an oral paste at processing were reported to have a lower percentage (13.78%) of cattle treated for bovine respiratory disease when compared to control (24.0%).

3.3.2 Carcass Performance

Hot carcass weight (Table 4) did not differ (P = 0.14; CON = 370.5; 10-G = 374.2) between treatments. Hot carcass weight did not differ between treatments reported in Kenney et al. (2015). Wilson et al. (2016) fed wet distillers grain or dry-rolled corn with or without Bovamine and reported no difference in hot carcass weight for both Bovamine and Bovamine x Diet. Dressed yield was similar (P = 0.53) for treated and control cattle (64.8 v 64.9%, respectively). Calculated empty body fat (P = 0.71), ribeye area (P = 0.13), marbling (P = 0.20) and 12th-rib fat thickness (P = 0.73) also did not differ among dietary treatments. Wilson et al. (2016) also reported no differences for 12th-rib fat thickness, ribeye area and marbling score between control and Bovamine-fed cattle. However, Brashears et al. (2003) compared *Lactobacillus acidophilus* NPC 747 and *Lactobacillus acidophilus* NPC 750; NPC 750 tended to have less marbling when compared to NPC 747. Ribeye area also tended to be smaller in control cattle when compared to NPC 750 and NPC 747. Percentage Prime and No Roll carcasses were not affected by supplementation of 10-G ($P \geq 0.71$). Heifers supplemented with 10-G tended (P = 0.06) to produce less USDA Select carcasses and tended (P = 0.09) to produce more USDA choice carcasses when compared to control cattle (20.34%). Elam et al. (2003) reported no differences between distribution of Choice and Prime versus Select and Standard in the feeding of various *Lactobacillus* cultures and *Propionibacterium freudenreichii*. Yield grade distributions did not differ between treatment groups ($P \geq 0.66$). Dick et al. (2013) fed various concentrations (0, 1

x 10^5, 1 x 10^6) of Bovamine (NP 51) to calf-fed Holstein steers and reported a 2.6 yield grade for all three concentrations.

3.3.3 Liver and Lung Outcomes

Percentage of edible livers (Table 3) for treated and control cattle were 84.54 and 81.53%, respectively and did not differ ($P = 0.17$) between treatments. Heifers in this study had numerically higher edible liver incidence rates when compared to 76.9% edible livers for cattle in the High Plains reported by Herrick (2018). In contrast, Brown and Lawrence (2010) reported 81.9% edible rate for cattle assessed ($n = 72{,}255$). Conversely, heifers fed 10-G tended ($P = 0.10$) to have a lower frequency of abscesses (9.23%) when compared to control (12.26%). This is consistent with data reported by Brown and Lawrence (2010) where liver abscesses occurred at a 12.2% rate. However, total severe abscess (A+, A+Open, A+Adhesion) incidence rate differed between dietary treatments ($P \leq 0.05$) with control cattle having 8.51% total severely abscessed livers, whereas treated 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., 2016). Livers condemned for reasons other than abscesses (flukes, telangiectasis, cirrhosis, contamination) did not differ ($P \geq 0.28$).

Incidence of lungs (Table 3) without lesions, mycoplasma or consolidation (normal) was 72.62 and 71.93% for 10-G and control cattle, respectively. Lungs scored a one, two, three, five or condemned did not differ ($P \geq 0.17$) between treatments. However, lungs with less than 75 percent adhesion or consolidation (score four) tended

(P = 0.10) to be more frequent in cattle fed 10-G (5.38%) when compared to control (3.38%). Likewise, inflation of lungs tended (P = 0.10) to occur more frequently in control cattle (1.69%) over treated (0.62%).

3.3.4 Salmonella prevalence

Fecal Pats

Salmonella prevalence in fecal pats (Table 5) did not differ (P = 0.73) between dietary treatments (10-G = 67.0%, CON = 69.0%). Brown et al. (2019) reported control and cattle supplemented with either a 1:1 ratio of *Lactobacillus acidophilus* and *Pediococcus acidilactici* or a 1:2 ratio of *Lactobacillus reuteri* and other *Lactobacillus* strains were 90.0, 86.7 and 100.0% positive for *Salmonella.* Likewise, Gragg et al. (2013a) reported *Salmonella* was recovered from 94.1% of cattle sampled. However, a time effect (P < 0.01) was observed across the sampling periods. *Salmonella* was present in 16 percent of all samples on 0 d and increased to 98 percent on 52 d; whereas, prevalence across sample 120 d and 200 d were similar (78.0 and 80.0%, respectively). Log of *Salmonella* followed the same pattern as prevalence rates with an initial log of 0.63 mpn/g followed by a significant increase before plateauing at a log of 3.11 mpn/g on 192 d. A timing effect is most likely due to seasonality. *Salmonella* is known to increase in warmer months and decline in cooler months. Barkocy-Gallagher et al. (2003) reported *Salmonella* prevalence to be highest in summer (9.1%), whereas prevalence in fall, winter and spring were 2.8, 2.5 and 2.1%, respectively. Dargatz et al. (2003) also reported that warm season months were significantly higher for *Salmonella* prevalence when compared to cool season. *Salmonella* log did not differ (P = 0.70), but a time

effect ($P < 0.01$) was observed between treatments. Log concentration increased ($P < 0.01$) sharply from 0 d to 52 d and plateaued for the remainder of the finishing period. The significant increase in log concentration is most likely due to cattle being exposed to *Salmonella* within the first 52 days at the feedyard as well as other stressors including commingling, new environment and new diet.

Rectoanal Mucosal Swabs

Salmonella prevalence of RAMs did not differ ($P = 0.92$) between treated (55.05%) and control cattle (55.30%) across the finishing period. In contrast, Tabe et al. (2008) reported a numerically lower number for *Samonella* prevalence in fecal grabs (12.9%) from control animals as well as steers supplemented with $1x10^9$ CFU of *L. acidophilus* (LA 51) and $1x10^9$ CFU of *P. freudenreichii* (PF 24). However, *Salmonella* prevalence increased ($P < 0.05$) exponentially from initial processing (11.55%) to harvest (98.80%). Increased prevalence can also be attributed to seasonality of *Salmonella* with higher rates detected in warmer seasons and lower rates in cooler seasons (Barkocy-Gallagher et al., 2003; Dargatz et al., 2003). Overall log concentration (mpn/g) between dietary treatments did not differ ($P = 0.63$; 10-G = 2.17, CON = 2.34). Initial log of rectoanal mucosal swabs was 0.29 mpn/g which increased to 4.22 mpn/g on day of harvest. The increase of log can be explained because of the increase in prevalence, which in return would lead to a higher overall log concentration.

Lymph Nodes

Cattle fed 10-G had a lower frequency (P = 0.01; 7.42%) of *Salmonella* positive lymph nodes when compared to control (15.80%). Vipham et al. (2015) supplemented cattle with *Lactobacillus acidophilus* (NP 51) and *Propionibacterium freudenreichii* (NP 24) which resulted in reduction of *Salmonella* in subiliac lymph nodes with treated and control cattle 57.5 and 76.3% positive, respectively. In addition, Brown et al. (2019) fed different blends of direct-fed microbials and reported that control cattle tended to have a greater percent-positive for *Salmonella* in peripheral lymph nodes when compared to treated cattle. *Salmonella* log (mpn/g) of the lymph nodes did not differ (P = 0.34) between dietary treatments (10-G = 0.34, CON = 0.73). Webb et al. (2017) reported log concentrations ranging from 1.6 to 4.9 $\log_{10}$ CFU/PLN from 160 quantifiable subiliac lymph nodes collected. Log of lymph nodes from 10-G cattle was slightly higher (3.88 mpn/g) when compared to control cattle (3.30 mpn/g).

3.4 CONCLUSION

In conclusion, the supplementation of 10-G Armour significantly reduced the frequency of *Salmonella* positive lymph nodes. While reduction of *Salmonella* in the lymph nodes is not necessarily important to the producer, feeding 10-G Armour would be of benefit due to the decrease in USDA Select carcasses and an increase in USDA Choice carcasses. Though log did not differ between treated and control cattle, packers would benefit from this product due to the decrease in *Salmonella* positive lymph nodes resulting in fewer adulterated lymph nodes entering beef trim.

3.5 REFERENCES

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

	STARTER		FINISHER	
Item	**CON**	**10-G**	**CON**	**10-G**
Ingredient, %				
RAMP	100.00	100.00	-	-
10-G, g/hd	-	2.00	-	2.00
Rumensin, g/ton	20.00	20.00	42.00	41.80
Tylosin, g/ton	10.00	10.00	7.20	7.50
Steam flaked corn	-	-	58.98	58.96
Wet distiller's grain	-	-	13.91	13.90
Sweet Bran Plus	-	-	18.29	18.31
Yellow grease fat	-	-	1.44	1.45
Cotton burrs or corn stalks	-	-	7.34	7.33
Micro Ingredients	-	-	0.03	0.05
Vitamin A, IU/lb	-	-	1200.00	200.00
Vitamin D, IU/ lb	-	-	120.00	120.00
MGA, mg/ton	-	-	40.00	39.80
Analyzed Composition, %				
DM, %	65.30	64.20	64.20	63.70
CP, %	21.60	21.40	15.50	15.40
NPN, %	0.90	1.00	1.30	1.30
NDF, %	39.70	39.60	23.70	22.10
CF, %	3.70	3.60	5.10	5.10
Ca, %	1.30	1.40	0.75	0.78
P, %	0.90	0.90	0.49	0.49
Mg, %	0.40	0.40	0.23	0.23
K, %	1.50	1.60	0.83	0.83

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

Item	**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 Railers, %	6.17	6.98	-	0.55

Table 3. Liver and lung outcomes of heifers fed 10-G				
Item	**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	2.69	3.16	-	0.61
Total A+	8.51	5.27	-	0.04
A+	2.20	1.02	-	0.11
A+Open	3.38	2.15	-	0.20
A+Adhesion	1.32	0.80	-	0.29
A+Adhesion/Open	1.23	1.08	-	0.80
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
Lung Score[a], %				
Normal	71.93	72.62	-	0.79
One	5.07	4.59	-	0.69
Two	5.37	4.92	-	0.72
Three	7.21	7.69	-	0.74
Four	3.38	5.38	-	0.10
Five	2.15	1.14	-	0.17
Inflated	1.69	0.62	-	0.10
Condemned	2.91	2.62	-	0.75

[a] N = 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

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
LM Area, cm^2	90.38	91.65	0.8	0.13
Marbling Score[a]	506	516	12.4	0.20
12^{th} rib fat thickness, cm	1.88	1.89	0.05	0.73
Empty body fat[b], %	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
No Roll	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] 300 = Slight, 400 = Small, 500 = Modest, 600 = Moderate

[b] Empty body fat calculated using EBF, % = 17.76107 + (4.68142 × FT) + (0.01945 × HCW) + (0.81855 × QG) − (0.06754 × LMA), where FT = 12th rib fat thickness in cm, HCW = hot carcass weight in kg, QG = quality grade (4 = Select, 5 = Choice−, 6 = Choice, 7 = Choice+, and 8 = Prime), and LMA = longissimus muscle area in cm^2 (Guiroy et al., 2001)

Table 5. *Salmonella* prevalence and log in composite fecal pats collected from cattle fed 10-G										
Item	**TRT**		**DOF**					***P*-VALUE**		
	CON	**10-G**	**0**	**52**	**120**	**191**	**SEM**	**TRT**	**TIME**	**TRTxTIME**
Salmonella prevalence, % positive	69.00	67.00	16.00[a]	98.00[c]	78.00[b]	80.00[b]	-	0.73	<0.01	0.36
Salmonella log, mpn/g	2.73	2.84	0.63[a]	4.53[c]	2.88[b]	3.11[b]	0.44	0.70	<0.01	0.49

Table 6. *Salmonella* prevalence and log from the rectoanal mucosal junction of heifers supplemented with 10-G

Item	TRT		DOF			*P*-VALUE		
	CON	**10-G**	**0**	**192**	**SEM**	**TRT**	**TIME**	**TRTxTIME**
Salmonella prevalence, % positive	55.30	55.05	11.55	98.80	-	0.92	<0.01	0.95
Salmonella log (mpn/g)	2.34	2.17	0.29	4.22	0.46	0.63	<0.01	0.59

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

Item	TRT			*P*-VALUE
	CON	**10-G**	**SEM**	**TRT**
Salmonella, % positive	15.80	7.42	-	0.01
Salmonella log, mpn/g	0.73	0.34	0.28	0.34