Reduced Burden of *Salmonella enterica* in Bovine Subiliac Lymph Nodes Associated with Administration of a Direct-fed Microbial

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**Abstract**

Non-typhoidal *Salmonella* is a leading cause of death due to bacterial contamination of food. In the United States, *Salmonella enterica* causes an estimated 1.3 million cases of gastroenteritis annually (5). Despite effective food safety interventions within abattoirs, *Salmonella enterica* remains a common contaminant of raw ground beef. Research has recently implicated peripheral lymph nodes (PLNs) as a potential route by which *Salmonella enterica* contaminates ground beef (3,4). Direct fed microbials (DFM) provide promise as a pre-harvest intervention for reducing the carriage of *Salmonella enterica* and *Escherichia coli* O157:H7 in feedlot cattle (2), and may be an effective control for *Salmonella enterica* harbored in bovine lymph nodes.

This study examined the efficacy of using *Lactobacillus animalis* (formerly designated *Lactobacillus acidophilus*; NP51) and *Propionibacterium freudenreichii* (NP24), at 10⁹ cfu/head/day, as a DFM in feedlot cattle diets to control *Salmonella enterica* in subiliac lymph nodes (SLNs) of feedlot cattle (2). Optimal dry-matter intake (DMI) and concentration of *Salmonella enterica* were measured on days 1, 3, and 5 of the study using Enterobacteriaceae (EB) petrifilm for enumeration. RV was streaked onto Xylose Desoxycholate (XLD) and Brilliant Green Fuso (BGF) as a confirmatory step.

**Methods**

Lymph nodes were trimmed. Weights were recorded and the outside of nodes were sterilized by immersing in boiling water for 3-5 seconds. Lymph nodes were homogenized with a mallet. Pulverized nodes were homogenized with 80 ml of tryptic soy broth (TSB) in a mallet. Pulverized using rubber mallet. RV was streaked onto XLD and Brilliant Green Fuso agar. Homogenate was plated using Enterobacteriaceae (EB) petrifilm for enumeration. Immunomagnetic separation (IMS) was conducted and transferred into 3 ml of Rappaport Vassiliadis (RV) to detect *Salmonella enterica*.

**Results**

- *Salmonella enterica* was 82% less likely (p=0.008) to be recovered from subiliac lymph nodes of treatment cattle in research feedlot study.
- DFM supplementation varied across slaughter days in the commercial feedlot study. On the first and second slaughter day, prevalence was reduced by 50% (p=0.0072) and 31% (p=0.009), respectively. No significant difference was observed on slaughter day three (p=0.176).
- A significant reduction in concentration of *Salmonella enterica* in subiliac lymph nodes (p<0.0001) on a cfu/g and cfu/node basis was also observed in cattle administered NP51 and NP24 in the first study.

**Conclusions**

The results indicate that NP51 and NP24 supplementation may aid in reducing the prevalence and concentration of *Salmonella enterica* in SLNs and, therefore, serve as an effective control measure to reduce *Salmonella enterica* in ground beef products.

**References**

Figure 1. Model adjusted prevalence of Salmonella in subiliac lymph nodes in the research feedlot for control cattle and cattle fed 10^9 CFU/head/day of Lactobacillus animalis (NP51) and Propionibacterium freudenreichii (NP24). The total number of positives from the research feedlot study was 14/47 from the control group and 3/52 from the treatment group. Bars represent upper and lower model adjusted 95% confidence levels. Columns with different letters are significantly different (p<0.10).

Figure 2. Model adjusted prevalence of Salmonella in subiliac lymph nodes in commercial feedlot for control cattle and cattle fed 10^9 CFU/head/day of Lactobacillus animalis (NP51) and Propionibacterium freudenreichii (NP24) for each slaughter day. The total number of positives from slaughter day one was 82/157, with total number of 58/86 positives in the control group and 24/71 positives in the treatment group. The total number of positives from slaughter day two was 137/208, with a total number of 78/99 positives in the control group and 59/109 positives in the treatment group. The total number of positives from slaughter day three was 197/262, with 104/132 positives in the control group and 93/130 positives from the treatment group. Bars represent upper and lower model adjusted 95% confidence levels. Columns with different letters are significantly different (p<0.10).

Figure 3. Concentration of Salmonella per gram and per node for control and supplemented cattle for the commercial feedlot study. The total number of quantifiable SLNs from the control group was 191 while the total number of quantifiable SLNs from the treatment group was 150. Bars represent upper and lower 95% confidence levels. Columns with different letters are significantly different (p<0.10).
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**Background on Cattle**

- Steers for the research study were predominantly of British breeding whereas steers of the second study were crossbred cattle with varying amounts of *Bos indicus* influence.
- Commercial steers were located at a feedlot in the Panhandle of Texas, while the research feedlot steers were housed at the Texas Tech University Burnett Center. Arrival weights were recorded and standard health treatments and feeding routines were followed as specified by protocols at the commercial feedlot and the research feedlot.

**Pen Assignments**

- For the commercial feedlot study, approximately 1800 steers were allocated to 12 blocks. Within each block animals were randomly allocated to the treatment or control cohort with approximately 75 animals/pen. The cattle were slaughtered in 3 groups and therefore housed at the feedlot for 129, 142, and 151 days, respectively.
- In the research feedlot study a total of 112 steers were blocked by weight and randomly allocated within block into either the treatment or control cohort with approximately 4 steers per pen. The cattle were housed at the research feedlot for 117 days.

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**Diets**

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<th>Ingredient</th>
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<td>Corn Grain, Steam Flaked</td>
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<td>Corn distillers grains, dry</td>
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<td>Triticale silage</td>
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<td>Corn Silage</td>
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<td>Commodity liquid</td>
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<tr>
<td>Supplement</td>
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</table>

**Table 1.** Ingredient composition of the commercial feedlot diet, on a dry matter basis.

- *Sweet Bran*, Cargill Corn Milling, Dalhart, TX.
- Triticale silage fed for the first 15 d; corn silage was fed for the remainder of the study. A 50:30:12:8 blend of condensed corn distiller’s solubles: glycerin: cane molasses: water was fed for the first 10 d and a 70:30 blend of condensed corn distiller’s solubles: glycerin was fed for the remainder of the study. A dry meal supplement manufactured by Cargill Animal Nutrition, Guymon, OK.

<table>
<thead>
<tr>
<th>Ingredient</th>
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<td>Corn Grain, Steam Flaked</td>
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<td>Urea</td>
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<td>TTU-2.0 Supplement</td>
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<tr>
<td><em>Sweet Bran</em></td>
<td>24.98</td>
</tr>
</tbody>
</table>

**Table 2.** Ingredient composition of the research feedlot diet, on a dry matter basis.

- *Sweet Bran*, Cargill Corn Milling, Dalhart, TX.
- TTU-2.0 supplement included 29.9mg/kg of monensin (Rumensin, Elanco Animal Health, Greenfield, IN 46140 USA) 10.0mg/kg of tylosin (Tylan® Premix, Elanco Animal Health, Greenfield, IN 46140 USA), Ractopamine hydrochloride (Optaflexx®, Elanco Animal Health, Greenfield, IN 46140 USA) at a level of 200mg/head/d fed for the last 30 d.

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**Lymph Node Processing**

- As described previously (3,4), visible fat and fascia were trimmed from subiliac lymph nodes and the weight of each trimmed node was recorded. Nodes were then immersed in boiling water for 3-5 seconds, placed into plastic bags, and pulverized using a rubber mallet. Samples were subsequently homogenized by adding 80 ml of trypticase soy broth (TSB) to each bag and stomached at 230 rpm for 2 minutes. For qualitative culture purposes, homogenates were incubated at room temperature for 2 hours and then at 42°C for 12 hours.
- Immunomagnetic separation (IMS) was conducted using anti-*Salmonella* paramagnetic beads and an automated bead retriever according to the manufacturer’s instructions. One hundred microliters of the bead-bacteria suspension were added to 3 ml of Rappaport-Vassiliadis (RV) broth and incubated at 42°C for 18-20 hours. Enrichments were streaked onto brilliant green sulfa (BGS) and xylose desoxycholate (XLD) agar plates. Characteristic colonies of *Salmonella* on at least one type of agar were considered presumptive positives. Commercial agglutination kits were used for further identification of morphologically typical colonies.
- Quantitative culture methods were conducted as described by Gragg et al., (2013) in that 1 ml of the TSB/SLN homogenate was removed prior to initial incubation, plated in duplicate onto *Enterobacteriaceae* count plates and incubated for 22-26 hours at 37°C. Colonies were counted according to manufacturer’s instructions and recorded. Bacterial growth on EB count plates was transferred to XLD agar and incubated for 16 hours at 37°C. Morphologically typical colonies on XLD plates were counted and comparisons were made with EB count plate counts.
Basic Lymph Node Structure

Lymph nodes are specific tissues that can be found daisy-chained throughout the mammalian body. These tissues have a very specific structure and are the site in which intact pathogens or pathogen components can be trafficked to; via infected dendritic cells or the lymph (1). The lymph flows to lymph nodes is a single direction, and enters the lymph node through the afferent lymphatic vessel and exits through the efferent lymphatic vessel (1,3). The inside of a lymph node contains a specific architecture. The cortex makes up the majority of the tissue and is made up of the paracortex (T-cell area) and the lymphoid follicle (1,2). The lymphoid follicle (outer cortex) is mostly made up of B-cells and contains germinal centers during an infection (1). Germinal centers are created when activated B-cells begin to proliferate and form a dense structure in the lymph node follicle (1). Lymph nodes are vascularized from the bottom. High endothelial venules are adapted branches of the vascular system of a lymph node and are associated with the paracortex (1,3). These venules allow lymphocytes to enter the lymph nodes from the blood. Lymphocytes that enter through high endothelial venules enter the second smaller compartment of the lymph node; the medullary region (3). The medullary region contain a large number of macrophage which function as antigen presenting cells, or as a means of removing large particles and molecules from the incoming lymph; via the capsular sinus. The third, less recognized, portion in a lymph node is the conduit system (3). This system behaves as a messenger system, or a “freeway” type system, and transports molecules—such as chemokines and cytokines—as well as immune cells from lymph node draining regions, to the high endothelial venule, and the circulatory system (3).

References
