AMI Final Report

Executive Summary Sheet

Project Title: Mitigation of Salmonella in Lymph Nodes Using a Pre-Harvest Intervention

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Objectives:

1. Determine if supplementing cattle diets with a 10^9/head/day dose of *Lactobacillus acidophilus* NP51 will reduce *Salmonella* in lymph nodes at slaughter.
2. Determine if lymph node contamination increases the risk of carcass contamination and/or trim from the carcass.

Conclusions: The results indicated a 25% reduction ($p=0.005$) in *Salmonella* prevalence in the subiliac lymph nodes of cattle fed a 10^9/head/day dose of NP51. A significant reduction ($p<0.05$) in *Salmonella* concentration in the subiliac lymph nodes was also observed when compared to the control cattle. Our team has concluded that this direct-fed microbial treatment with the high dose of NP51 is a viable intervention to reduce the carriage of *Salmonella* in the lymph nodes of cattle. This could potentially aid in reducing ground beef contamination from lymph nodes harboring *Salmonella*. If so, a decrease in the prevalence of *Salmonella* in lymph nodes could help to protect public health by helping to decrease the illnesses caused by ground beef.

Deliverables: The use of well characterized direct-fed microbial interventions such as *Lactobacillus acidophilus* NP51 can have a positive impact on public health by reducing *Salmonella* in the beef supply. The product is currently available from Nutrition Physiology Company, LLC as "Bovamine Defend". This pre-harvest intervention is an additional hurdle when combined with current pre- and post-harvest interventions. In most situations, the efficacy of post-harvest interventions may be greater if the pathogen load coming into the processing plant is less due to effective pre-harvest interventions as described in this research. However, current in-plant interventions such as hot water washes, chemical treatments, etc do not impact the *Salmonella* harbored and protected inside the lymph nodes.

Technical Abstract:

A cattle feeding study was conducted at a commercial feed yard located in the Texas panhandle. There were approximately 1,800 head of cattle randomly allocated into two treatments. The treatments consisted of a control treatment and a direct-fed microbial treatment containing 10^9/head/day *Lactobacillus acidophilus* NP51. The cattle were housed in pens with approximately 75 head/pen and 12 pens/treatment. Subiliac and mandibular lymph nodes were obtained from freshly harvested carcasses of a targeted 25 animals/pen (n= 600 at each site) at the slaughter facility. The lymph nodes were transported on ice to the laboratory at Texas Tech for analysis. The lymph nodes were trimmed, weighed, and surface sterilized by dipping in boiling water for 3-5 seconds. Sterilized nodes were analyzed for the presence of internalized *Salmonella* with qualitative and quantitative analyses. The adipose tissue surrounding the lymph node was reserved from 10 lymph nodes/pen for *Salmonella* detection. The results indicated a 25% reduction (p=0.005) in *Salmonella* prevalence in the subiliac lymph nodes of cattle fed 10^9/head/day NP51. Quantitatively the NP51 cattle had significantly less ($p<0.05$) *Salmonella* in subiliac lymph nodes (3.1 vs 4.2 $\log_{10}$ cfu/lymph node) and per gram of lymph nodes (1.9 vs. 2.9 $\log_{10}$ cfu/g). *Salmonella* was only recovered from two mandibular node samples both of which were from the control group. Additionally, adipose tissue trimmed from the nodes had a very low prevalence of *Salmonella* with only 4 positive adipose samples out of a total of 240 samples. Out of the 4 positive adipose samples, 3 were associated with a *Salmonella* positive lymph node. This indicates that the presence of *Salmonella* in the lymph nodes could potentially impact the prevalence in the surrounding tissue. More research is needed to determine the significance of these observations because most of the fat surrounding positive lymph nodes was negative for the presence of *Salmonella*. Our research team has concluded that this pre-harvest direct-fed microbial intervention is effective at decreasing the prevalence and concentration of *Salmonella* in the subliliac lymph nodes of feedlot cattle. This intervention can be used to reduce the contamination of beef products and protect public health from *Salmonella* contamination.

Goals/Objectives

The overall goal of this project was to reduce the prevalence and concentration of *Salmonella* in the subiliac and mandibular lymph nodes of cattle. The following objectives were established to achieve this goal:

1. Determine if supplementing cattle diets with a 10^9/head/day dose of *Lactobacillus acidophilus* NP51 will reduce *Salmonella* in lymph nodes at slaughter
2. Determine if lymph node contamination increases the risk of carcass contamination and/or trim from the carcass

Materials and Methods

Cattle and Treatment Assignment

Approximately 1,800 cattle were housed in a commercial feed yard in the Texas Panhandle. Upon arrival cattle were randomly assigned to pens. A lot tag and Ralgro implant was administered to each animal. Standard health procedures were followed according to the feed yard practices. Cattle were randomized to either the control group receiving the standard ration or the NP51 group receiving

the standard ration plus 10^9/head/day NP51. There were 12 pens/treatment with approximately 75 head/pen.

Cattle Feeding and Rations

Cattle were fed according to the standard feeding procedures of the feed yard with Rumensin and Tylan included in all rations. The cattle in the control group were fed with the main yard truck to prevent contamination with the treatment. The feed for the cattle fed the standard ration with added 10^9/head/day NP51 was mixed and delivered with a separate truck.

Cattle Shipment

The shipping dates were balanced across each replication. The date was estimated for each pen based on pay weight. The cattle were supplemented with Zilmax for 20 days plus a 3 day withdrawal prior to shipment. All cattle in this study were shipped to a commercial slaughter facility in the Texas Panhandle.

Sample Collection

Subiliac and mandibular lymph nodes were obtained from 25 animals/pen (n= 600) from the carcasses after slaughter in the harvest facility. The lymph nodes and surrounding fat tissue were collected at the processing facility from freshly harvested carcasses. All samples were placed on ice for transport to the laboratory and then refrigerated to prevent sample degradation. For 10 subiliac lymph nodes/pen, 10-20 g of adipose tissue was reserved to test for the presence of *Salmonella*. The lymph nodes and adipose samples were processed within 24 hours of collection.

Lymph Node and Adipose Microbial Testing

Lymph nodes were trimmed of fat and fascia and weighed. Weights were recorded and the outside of the node was surface sterilized by immersing the trimmed lymph node into boiling water for 3-5 seconds. Sterilized nodes were placed into sterile whirl pak bags and pulverized with a rubber mallet. Adipose tissue was reserved from 10 of the 25 lymph nodes/pen. Eighty (80) ml of tryptic soy broth (TSB) was added to each lymph node and adipose sample and stomached at 230 rpm for 2 minutes. Once stomached all samples were incubated at room temperature for 2 hours and then incubated at 42°C for 12 hours. After incubation samples underwent immunomagnetic separation (IMS) with anti-*Salmonella* Dynabeads and an automatic bead retriever following manufacturer's guidelines. The resulting 100 µl bead-bacteria complex was added to 3 ml of Rappaport Vassiliadis (RV) broth and incubated at 42°C for 18-20 hours. The RV enrichments were streaked to Brilliant Green Sulfa (BGS) and Xylose Desoxycholate (XLD) agar plates. Plates with characteristic colonies of *Salmonella* on either or both types of agar were considered positive. A commercial agglutination kit was used to further characterize positive colonies.

Lymph node enumeration

Enterobacteriaceae (EB) petrifilm was used to enumerate all lymph node samples. Prior to incubation, 1 ml of the TSB/lymph node homogenate was plated in duplicate onto EB petrifilm and incubated for 24 hours at 37°C. The petrifilms were counted according to manufacturer's instructions and recorded. The EB petrifilm growth was transferred to XLD agar and incubated for 16 hours at 37°C. All XLD plates displaying characteristic *Salmonella* growth were counted and compared with petrifilm

counts in order to achieve an accurate enumeration. The counts obtained were used to calculate the concentration of *Salmonella* on a CFU/g and CFU/lymph node basis.

Statistical Analysis

Binomial response models were created for the detection (yes/no) of the pathogens in the lymph node samples. Response variables were generated for each treatment by block observation. Concentration data were $\log_{10}$-transformed for analysis. Generalized and general linear mixed models were used to analyze the data. Block was treated as a random variable in all models. Alpha was set at 0.10.

Results

The prevalence of *Salmonella* in the subiliac lymph nodes for the control cattle and the cattle fed 10^9/head/day NP51 is illustrated in Figure 1. The cattle supplemented with NP51 had 25% less *Salmonella* detected which was statistically significant at $p = 0.005$. Quantitatively the NP51 cattle had significantly less ($p<0.05$) *Salmonella* in subiliac lymph nodes (3.1 vs 4.2 $\log_{10}$ cfu/lymph node) and per gram of lymph nodes (1.9 vs. 2.9 $\log_{10}$ cfu/g). Thus, control samples were more likely to have higher concentrations of *Salmonella* than nodes collected from animals supplemented with NP51. These results are included in Figure 2. Figure 3 illustrates the shift in concentration of the *Salmonella* in the lymph nodes as influenced by supplementation with NP51. Only 2 mandibular lymph nodes tested positive and both samples were from the control group. There were only 4 samples that tested positive for *Salmonella* in the adipose tissue collected from around selected lymph nodes.

Conclusions

These results indicate that 10^9/head/day supplementation of NP51 is a successful pre-harvest intervention to reduce the prevalence of *Salmonella* in the lymph nodes of cattle. The concentration of *Salmonella* was also reduced in the cattle supplemented with NP51 on a CFU/g and CFU/lymph node basis. The use of this intervention can reduce the pathogen loads coming into a processing/harvest facility. This could potentially improve the efficacy of interventions in the plant which could help to prevent outbreaks, product recalls, and most importantly protect public health. While only 2 mandibular lymph nodes tested positive for *Salmonella* in this study, in other studies we have isolated *Salmonella* from this testing site. Also, while the fat surrounding an intact lymph node was not positive for *Salmonella,* if during processing, the lymph node is cut, there is a potential for the *Salmonella* from the node to cross-contaminate the meat surfaces.

Recommendations for Future Research

Future research in this area may benefit from investigating a combination approach of this direct-fed microbial and a vaccine targeted to reduce *Salmonella*. These treatments may have a synergistic effect when combined and result in even greater reductions. Additionally, examination of the other lymph node sites and reduction of *Salmonella* would be valuable. While three of the 4 fat samples that tested positive were also associated with a positive lymph node, these could have been positive by chance alone or could have become contaminated during processing and not necessarily from the lymph node itself. We plan to serotype the *Salmonella* from the lymph nodes and the fat

samples. If the serotypes match we will follow up with PFGE to determine if they are the same isolates. Finally, the impact of supplementation of the cattle with the 10^9/head/day dose of NP51 and the ultimate impact on the prevalence of *Salmonella* in ground beef would be of great value to the industry.

Presentations and Publications

Abstracts for poster presentations of this research will be submitted to the International Association for Food Protection and the Beef Industry Food Safety Council. This research will also be submitted for publication in peer-reviewed journals.

FIGURES:

FIGURE 1. *Salmonella prevalence in lymph nodes collected from cattle fed 10^9/head/day NP51 at a commercial feedlot*

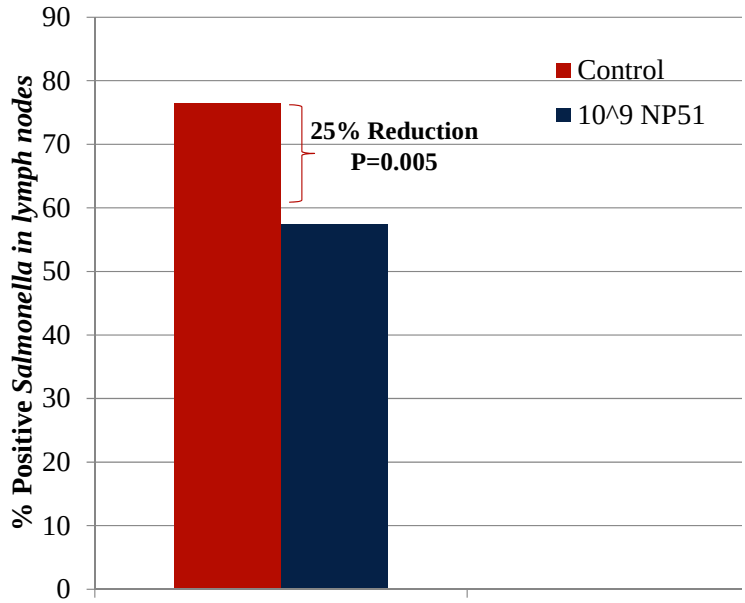


FIGURE 2. *Concentration of Salmonella in lymph nodes of cattle fed 10^9/head/day NP51 at a commercial feedlot*

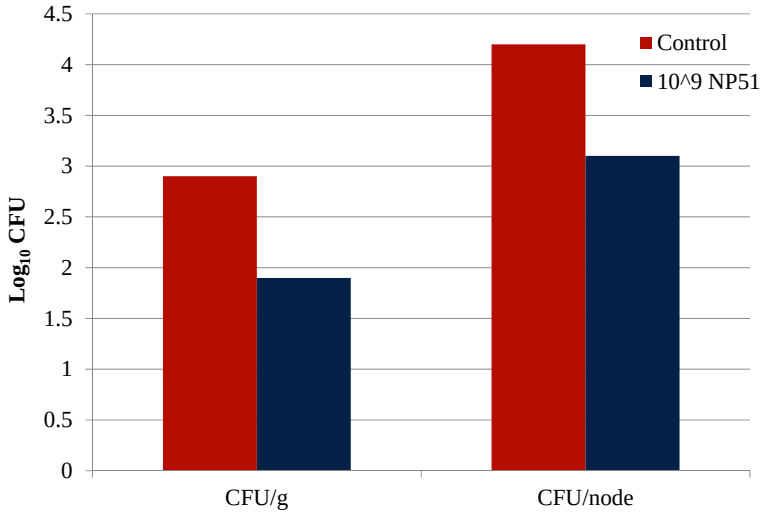
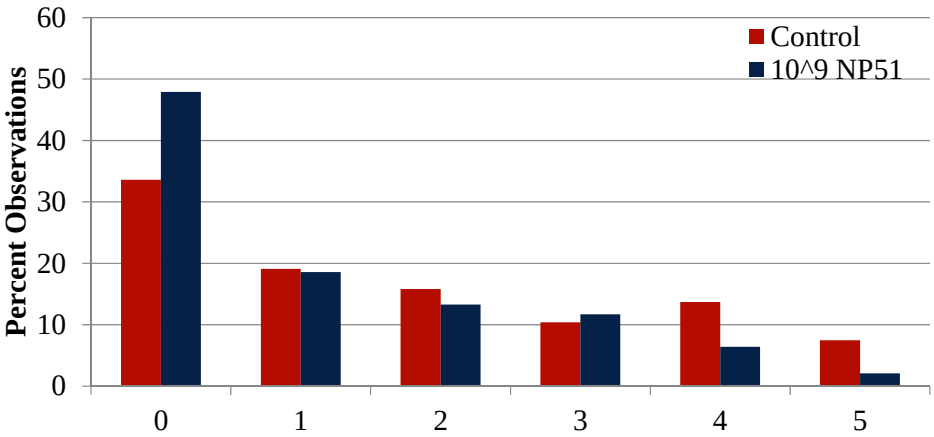


FIGURE 3. *Shift in concentration of Salmonella in cattle lymph nodes collected from cattle fed 10^9 NP51/head/day at a commercial feedlot*



0: positive but below limit of concentration & le 1 log10 cfu/g
1: > 1 log10 cfu/g & le 2 log10 cfu/g
2: > 2 log10 cfu/g & le 3 log10 cfu/g
3: > 3 log10 cfu/g & le 4 log10 cfu/g
4: > 4 log10 cfu/g & le 5 log10 cfu/g
5: > 5 log10 cfu/g