

# Reduction of inoculated *Salmonella* cocktail in ground turkey and turkey breasts using *Lactobacillus*-based intervention

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**ABSTRACT** Lactic acid bacteria (LAB) have been shown to be inhibitory toward various pathogenic bacteria during refrigerated storage of ground beef samples and in subprimals. In this study, the effectiveness of a combination of 4 strains of *Lactobacillus* at reducing *Salmonella* in turkey products was evaluated to improve the final safety of the product. Turkey breasts (hot and chilled) were inoculated with a 3-strain *Salmonella* cocktail (Typhimurium ATCC 14028, Enteritidis PT 13 NVSL 96-18535, and Heidelberg 3347-1 Sheldon) and then treated with  $1 \times 10^6$  cfu/cm<sup>2</sup> of a *Lactobacillus*-based intervention comprising NP51, NP35, NP3, and NP7 (Lactiguard, Nutrition Physiology Corp., Kansas City, MO). The turkey breasts were stored at either 5°C to simulate refrigerated storage and sampled on d 0, 1, 2, and 3 or at 37°C to simulate hot

carcass applications and sampled at h 0, 2, and 4. Additionally, ground turkey was inoculated with a 3-strain *Salmonella* cocktail, treated with  $1 \times 10^6$  cfu/g of the LAB intervention, and stored at 5°C with sampling on d 0, 1, 2, and 3. The reduction of *Salmonella* in the turkey breasts stored at 5°C treated with *Lactobacillus* was 2 log ( $P < 0.05$ ) compared with the control turkey breasts after d 2. *Salmonella* in the turkey breasts held at 37°C was reduced by 1.5 log ( $P < 0.05$ ) by h 2 and 2 log ( $P < 0.05$ ) by h 4. The *Salmonella* in the ground turkey held at 5°C using the LAB exhibited a 2 log ( $P < 0.05$ ) reduction compared with the control after d 1. These results show that the addition of a *Lactobacillus*-based treatment in turkey products significantly reduces *Salmonella* during storage.

**Key words:** *Salmonella*, lactic acid bacteria, turkey

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## INTRODUCTION

*Salmonella* is one of the leading food-borne pathogens in the United States with infections leading to gastroenteritis including diarrhea, abdominal cramps, fever, nausea, and vomiting (CDC, 2008). *Salmonella* is highly prevalent in poultry products and has been isolated from raw turkey products (Harrison et al., 2001).

The prevalence of *Salmonella* in the food supply has led to several studies using interventions to reduce the total numbers of the pathogen in different products. The use of lactic acid bacteria (LAB) has been successful in reducing the growth of various pathogens in ground beef and in live cattle. Lactic acid bacteria has been used in previous studies because of its ability to form an antagonistic environment through the production of organic acids, hydrogen peroxide, and bacteriocins (Brashears et al., 2003). Given the ability of LAB to inhibit other bacterial growth, several studies have been conducted to better understand the range by

which LAB can successfully inhibit other bacteria and the various areas of application in the food supply.

In a previous study in which *Listeria monocytogenes* growth was inhibited in cold cuts and frankfurters, it was determined that the addition of LAB reduced the pathogen by 3 log cycles (Amezquita and Brashears, 2000). That study also determined that the addition of LAB did not affect the sensory attributes of the product. In beef carcasses and ground beef, it was determined that the production of hydrogen peroxide by LAB inhibited both *Escherichia coli* O157:H7 and *Salmonella* (Senné and Gilliland, 2003; Smith et al., 2005). In this study it was determined that an inoculation of LAB at  $10^8$  cfu/g of meat provided a greater amount of overall reduction in the growth of *E. coli* O157:H7 and *Salmonella*. In *E. coli* O157: H7 there was a 3 log reduction and no detectable *Salmonella* at the end of 5 d of storage (Smith et al., 2005). These studies determined that LAB were proficient in reducing the total numbers of different bacteria and higher concentrations of LAB provided greater reductions. The objective of this study was to determine whether adding LAB to selected turkey products inhibited the growth of *Salmonella* under warm and refrigeration conditions.

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## MATERIALS AND METHODS

### **Preparation and Inoculation of Turkey Products**

The ground turkey and turkey breasts were obtained from a local grocery store for each of the 3 studies (refrigerated turkey breasts, turkey breasts stored at increased temperatures, and refrigerated ground turkey). Prior to the start of the study the turkey products were tested for the presence of *Salmonella* by the processor and the products were determined to be negative for *Salmonella*. The turkey was then tested using the Bax (Dupont Qualicon, Wilmington, DE) method to verify the absence of *Salmonella* upon receipt.

A 1,000-g sample of each of the turkey products was used for each replication; there were 3 replications total for each of the 3 projects. To inoculate the ground turkey,  $1 \times 10^4$  cfu/g of *Salmonella* cocktail (Typhimurium ATCC 14028, Enteritidis PT 13 NVSL 96-18535, and Heidelberg 3347-1 Sheldon) was added to the ground turkey in an industrial mixer and homogenized for 2 min. After inoculation the ground turkey was separated into two 500-g portions. One portion was separated further into five 100-g portions. These portions were placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI) and stored at 5°C and served as the control.

The remaining 500-g portion was then treated with an appropriate amount of the LAB to yield  $1 \times 10^6$  cfu/g [strain NP 51, *Lactobacillus acidophilus*, cattle; strain NP 35, *Lactobacillus crispatus*, cattle; strain NP 3, *Pediococcus acidilactici*, hot dogs (commercial); strain NP 7, *Lactobacillus lactis* ssp. *lactis*, alfalfa sprouts]. The LAB cocktail was added to the turkey and then homogenized in the product with an industrial mixer. The treated 500 g of turkey was then separated into five 100-g portions, placed into Whirl-Pak bags, and stored at 5°C.

Turkey breasts for both the hot and cold studies were inoculated by cutting the breasts into ten 100-g cubes and immersing the cubes in a solution containing  $1 \times 10^6$  cfu/mL of a cocktail mixture of *Salmonella*. Cubes were immersed in the solution for 30 min to allow for attachment and then dried in the hood for 30 min and added to Whirl-Pak bags. Five of the cubes were treated with a  $1 \times 10^6$  LAB mixture by spraying with a solution containing the LAB suspended in buffered peptone water. All pieces were packaged in individual Whirl-Pak bags. Two separate studies were conducted and replicated 3 times. In one study, the pieces were stored at 5°C and in the other they were stored at 37°C.

### **Sampling of Turkey Products**

The control and treated ground turkey and turkey cubes stored at 5°C were sampled on d 0, 1, 2, and 3. Ten grams of turkey was taken from each ground sample and placed into a stomacher bag. Then, 90 mL

of buffered peptone water was added to each bag and stomached at 230 rpm for 2 min. A total of 90 mL of buffered peptone was added to each Whirl-Pak bag and agitated for 2 min to suspend any surviving bacteria. The turkey cubes stored at 37°C were sampled at 0, 2, and 4 h as described for the refrigerated cubes.

### **Enumeration of Microorganisms**

Dilutions were made from the stomacher bags and plated onto xylose lysine deoxycholate agar (Fisher Scientific, Pittsburgh, PA). The *Salmonella* were enumerated by spiral plating 100 µL onto respective plates and incubating at 36°C for 24 h, after which the total plate count was determined using a plate counter.

### **Statistical Analysis**

The data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC) and the means were separated using Duncan's multiple range test with a significance level of  $P < 0.05$ . No interactions were found between replications and treatment so the data were pooled by treatment.

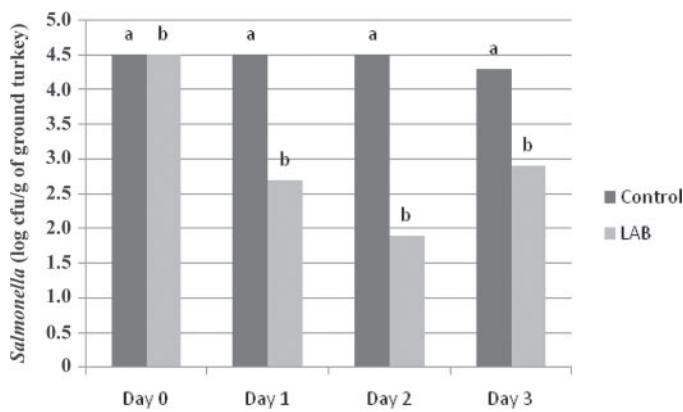
## **RESULTS AND DISCUSSION**

Salmonellosis is one of the leading food-borne illnesses in the United States with approximately 40,000 cases occurring each year (CDC, 2008). *Salmonella* is known to be ubiquitous, but its primary reservoir is the intestines of animals (Antunes et al., 2003). Poultry products are the leading source of animal-originating *Salmonella* that is transmitted to people (Bryan and Doyle, 1995).

Contamination of turkey carcasses from *Salmonella* occurs as a result of direct contact with the pathogen via contact with the intestines, feathers, or feet and also through cross-contamination (Uyttendaele et al., 1998). Controlling the contraction of *Salmonella* through turkey products has proven to be difficult, thus increasing the need for new methods of control.

Lactic acid bacteria have been used in several studies to help reduce and control pathogens such as *E. coli* 0157:H7, *Salmonella*, and *Listeria monocytogenes*. This study was conducted to determine whether LAB could help reduce the amount of *Salmonella* present in turkey products. The effectiveness of the LAB treatment was determined after 4 d at 5°C in ground and whole turnkey and 4 h at 37°C on whole muscle.

In the ground turkey samples a reduction in the total numbers of *Salmonella* could be detected after d 1 of storage (Figure 1) at 5°C. This reduction was maintained after d 2 and 3 of storage with no additional reductions observed after d 1, indicating that although the LAB were inhibitory to the pathogen, little residual inhibition was observed. On the other hand, the *Salmo-*

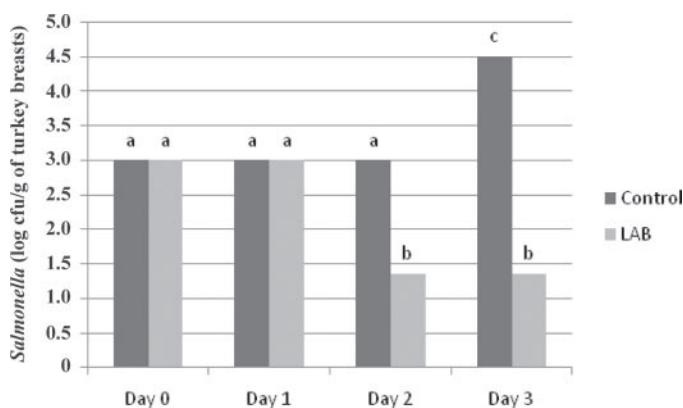


**Figure 1.** The reduction of *Salmonella* in ground turkey stored at 5°C. <sup>a,b</sup>Means with different letters differ,  $P < 0.05$ . Pooled SEM: Day 0 = 0.22, Day 1 = 0.33, Day 2 = 0.35, Day 3 = 0.52. LAB = lactic acid bacteria.

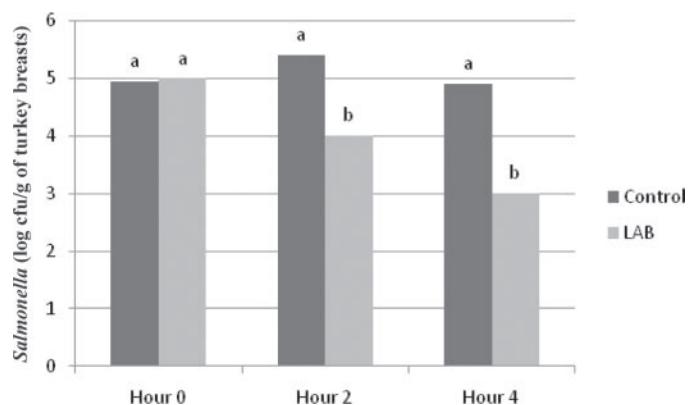
*Salmonella* did not increase after d 1, so the inhibition was maintained at cold storage temperatures.

In the turkey breasts, a reduction in the total numbers of *Salmonella* was observed after d 2 of storage (Figure 2) at 5°C. The reduction was maintained during d 3 of storage with no additional reductions observed after d 2, indicating that although the LAB were inhibitory to the pathogen, little residual inhibition was observed. The *Salmonella* did not increase after d 2, indicating that the inhibition was maintained at cold storage temperatures.

The increase of temperature in this study determined that by 2 h of incubation a reduction in the total number of *Salmonella* was observed (Figure 3) at 37°C. The reduction in total numbers increased from 1.5 log at 2 h to 2 log after a storage period of 4 h. This indicated that the LAB were inhibitory to the pathogen and that during storage at increased temperatures the rate of reduction was increased over time. This data are consistent with results obtained from a previous study that combined 4 strains of LAB to reduce the presence of *E. coli* 0157: H7 and *Salmonella* in ground beef (Smith et



**Figure 2.** The reduction of *Salmonella* in turkey breasts stored at 5°C. <sup>a-c</sup>Means with different letters differ,  $P < 0.05$ . Pooled SEM: Day 0 = 0.24, Day 1 = 0.26, Day 2 = 0.33, Day 3 = 0.28. LAB = lactic acid bacteria.



**Figure 3.** The reduction of *Salmonella* in turkey breasts stored at 37°C. <sup>a,b</sup>Means with different letters differ,  $P < 0.05$ . Pooled SEM: Hour 0 = 0.50, Hour 2 = 0.37, Hour 4 = 0.46. LAB = lactic acid bacteria.

al., 2005). This study determined that the combination of the LAB strains provided a 3 log reduction in *E. coli* 0157: H7 after 5 d and a 3 log reduction in *Salmonella* after 3 d (Smith et al., 2005).

In conclusion, this study determined that the addition of LAB reduced the amount of *Salmonella* present in the turkey products. The results of this study confirm the results of previous studies in which LAB were used as a means of pathogen reduction. Therefore, conclusive evidence exists that LAB has the ability to control and reduce the amount of *Salmonella* present in turkey products.

Lactic acid bacteria were successful in reducing the growth of *Salmonella* in turkey products. The higher concentration of LAB was significant in producing an overall greater reduction in the total numbers of the pathogen. Although LAB had an effect in cold storage temperatures, it was determined that 37°C provided a faster rate of reduction in total numbers compared with 5°C. The increased temperature produced a 2 log reduction at the end of 4 h, whereas the cold storage temperature achieved this same reduction after d 1 for the ground turkey and d 2 for the turkey breasts. This showed that the storage temperature was significant in determining the effectiveness of LAB. The data obtained through this study indicate that LAB are capable of significantly reducing the total numbers of *Salmonella* in turkey products and that the reduction at higher temperatures would mean that LAB could be applied to hot carcasses.

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