

# Effect of Probiotic Culture Candidates on *Salmonella* Prevalence in Commercial Turkey Houses

J. Vicente, S. Higgins, L. Bielke, G. Tellez, D. Donoghue, A. Donoghue, and B. Hargis<sup>1</sup>

Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville 72701

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**Primary Audience:** Veterinarians, Turkey Producers, Production Managers, Researchers

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## SUMMARY

The ability of 2 probiotic cultures (P1 and P2) to reduce environmental *Salmonella* in commercial turkey flocks 2 wk prior to processing with or without the use of a commercial organic acid (OA) was evaluated. *Salmonella*-positive flocks were identified 3 to 4 wk before processing by using standard assembled drag swabs. Two weeks after treatment (prior to live haul), drag swabs were used again for *Salmonella* recovery. In the first trial, 6 *Salmonella*-positive houses were selected to evaluate 4 treatments: P1 ( $1.0 \times 10^8$  cfu/mL), OA + P1 ( $1.0 \times 10^8$  cfu/mL), OA + P1 ( $1.0 \times 10^6$  cfu/mL), and OA + P2 ( $1.0 \times 10^6$  cfu/mL). Two weeks after treatment, reductions ( $P < 0.05$ ) of *Salmonella* recovery (90, 100, 100, and 86%, respectively) were observed in all treatments. In the second trial, 22 *Salmonella*-positive houses were selected to evaluate 6 treatments: control, OA, P1, P2, OA + P1, and OA + P2. Two weeks after treatment, the recovery of *Salmonella* was significantly reduced ( $P < 0.05$ ) in houses in which P1 and P2 cultures were administered in combination with the OA product. Our results suggest that the administration of selected probiotic candidate bacteria in combination with OA may reduce environmental *Salmonella* in turkey houses prior to live haul, and that this practice could help to reduce the risk of *Salmonella* cross-contamination in the processing plant.

**Key words:** probiotic, organic acid, *Salmonella*, turkey house

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## DESCRIPTION OF PROBLEM

*Salmonella* is a major concern to the worldwide poultry industry because it has been involved in human infections. Although outbreaks of this pathogen have been reported for decades, within the past 25 yr the disease has increased its incidence in many countries. *Salmonella* Enteritidis has become the predominant strain [1], and it continued to lead the list of foodborne pathogens in 2002 [2]. In fact, about 2 million

*Salmonella* cases are found in livestock in the United States each year and are estimated to cost more than \$1.4 billion [3].

Because of emerging pressure by the public health community and consumer groups to reduce the use of antibiotics in animal production, alternative methods for the control of pathogens are necessary. Competitive exclusion (CE), first described by Nurmi and Rantala in 1973 [4], has proven to be effective in the reduction of

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<sup>1</sup>Corresponding author: bhargis@uark.edu

*Salmonella* infection and other pathogens in either experimental or commercial trials with chickens [5, 6] and turkeys [7]. Extensive research has demonstrated the utility of administering normal gastrointestinal flora (aerobic and anaerobic bacteria) from healthy adult birds to day-of-hatch chickens to reduce intestinal colonization of *Salmonella* [8, 9, 10] by participating actively in intestinal epithelium development, nutrition, and immune response [11]. Competitive exclusion cultures fit within the category of probiotics, which are live microbial supplements that have a beneficial effect on health [12]. Because we do not have a clear understanding of the mechanisms by which our cultures provide health benefits, we have described them as probiotic candidates.

The objective of this study was to evaluate the effect of 2 probiotic cultures, alone or in combination with a commercial organic acid (OA) product, on the prevalence of *Salmonella* in commercial turkey houses previously identified by environmental sampling with drag swabs. In the present study, we used 7 enterobacteriaceae species described by Bielke et al. [7] as nonpathogenic and capable of reducing *Salmonella* colonization under experimental conditions. Additionally, 2 lactic acid bacteria were chosen because of their ability to inhibit the growth of foodborne pathogens with in vitro studies [13]. These isolates were either combined (culture P1) or the lactic acid bacteria were used alone (P2).

## MATERIALS AND METHODS

### *Evaluation of Salmonella Prevalence in Turkey Houses*

*Salmonella*-positive flocks were identified 3 to 4 wk prior to processing by standard drag swabs of each house, with subsequent enrichment and selective plating [14, 15]. Eight swabs per house were dragged twice through the house and collected with clean gloves to avoid cross-contamination [16]. Samples were transported on ice to the Poultry Health Laboratory at the University of Arkansas for enrichment and culture [15]. Only houses with 6 out of 8 swabs positive for *Salmonella* were included in these experiments.

### *Experimental Design*

In experiment 1, 6 *Salmonella*-positive houses were selected to evaluate 4 treatments, which are described in Table 1. In the second experiment, 22 *Salmonella*-positive houses were selected to evaluate 6 treatments, which are also described in Table 1. Organic acid treatments were administered in the appropriate houses for 8 h [17]. After cleaning the water lines by flushing with tap water, the cultures were administered in the vaccine medicator for 3 consecutive days, with skim milk added as a stabilizer [18]. Fourteen days after treatment, 8 drag swab samples per house were taken for *Salmonella* isolation [15, 16]. Statistical comparisons were made between times and within treatments by using the chi-squared test of independence [19].

## RESULTS AND DISCUSSION

In the preliminary experiment, a significant ( $P < 0.05$ ) reduction [19] of environmental *Salmonella* was observed within each treatment 2 wk posttreatment. Houses receiving a high dose of P1 culture alone experienced a 90% reduction in *Salmonella*-positive drag swabs (Figure 1). In addition, no *Salmonella* was recovered in houses pretreated with OA for 8 h before the administration of either a high or low dose of P1 culture. In houses that received the P2 culture after 8 h of OA consumption, a significant decrease in *Salmonella*-positive samples was also observed (Figure 1). The results of this first trial suggested that administration of OA before the application of probiotic cultures could have an additive effect on the reduction of *Salmonella* under commercial conditions.

To further evaluate the potential advantage of pretreatment with OA prior to probiotic administration, a second experiment was designed. In the second study, nontreated control houses were included. Nontreated control houses and houses in which OA, P1, or P2 were administered alone did not result in statistically reduced *Salmonella* (26.7, 48.6, and 35% reduction; Figure 2). However, a significant decrease in *Salmonella* prevalence was observed when OA was used for 8 h before the administration of P1 and P2 (87.5 and 75% reductions, respectively) 2 wk after treatment (Figure 2). The results of this second experiment suggest that the effect

**Table 1.** Description of treatments in experiments 1 and 2

Experiment	Treatment group <sup>1</sup>	Probiotic, cfu/mL of drinking water	Houses/treatment group, n
Experiment 1	P1 <sup>2</sup>	$1 \times 10^8$	2
	OA + P1	$1 \times 10^8$	1
	OA + P1	$1 \times 10^6$	1
	OA + P2 <sup>3</sup>	$1 \times 10^6$	2
Experiment 2	Control	0	8
	OA	0	2
	P1	$1 \times 10^6$	5
	P2	$1 \times 10^6$	4
	OA + P1	$1 \times 10^6$	2
	OA + P2	$1 \times 10^6$	1

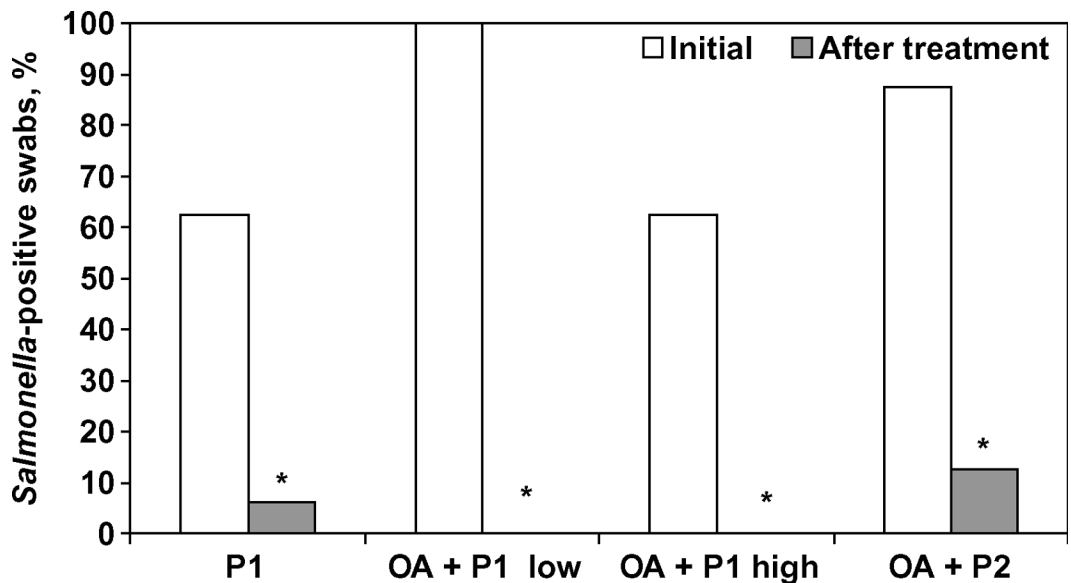
<sup>1</sup>Organic acid (OA) was administered according to the manufacturer's directions in the vaccine medicator for 8 h prior to probiotic administration. Water lines were flushed before administration of probiotic cultures. Probiotic cultures were also administered through the vaccine medicator for 3 consecutive days. Skim milk was used as a stabilizer.

<sup>2</sup>P1 consisted of 5 strains of *Escherichia coli*, *Kluyvera ascorbata*, *Klebsiella travesanii*, *Lactobacillus casei*, and *Lactobacillus cellobiosus*.

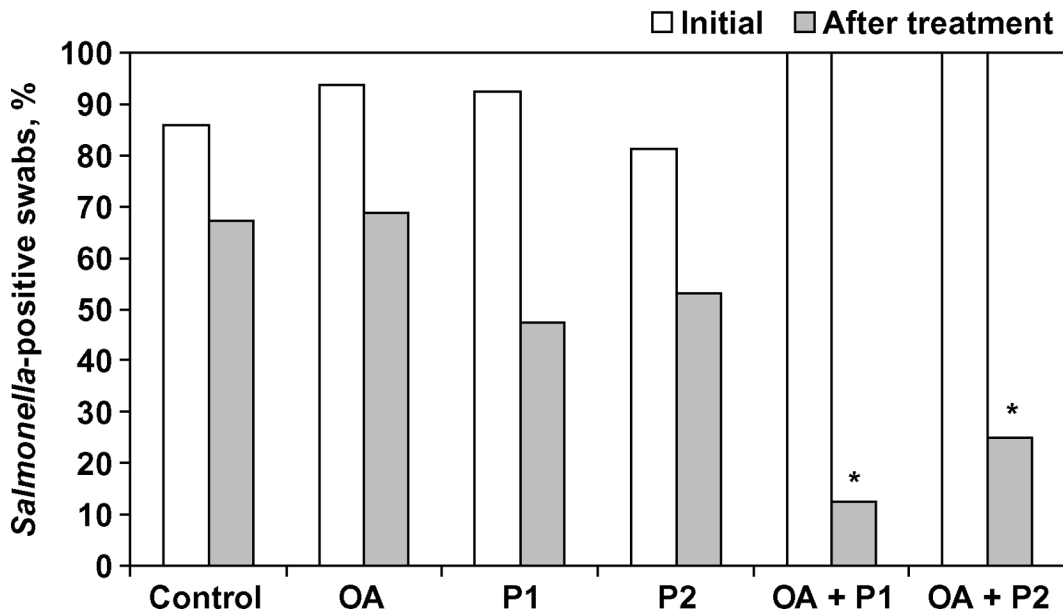
<sup>3</sup>P2 consisted of *L. casei* and *L. cellobiosus*.

of probiotic cultures on *Salmonella* reduction in commercial turkey houses was improved by using OA. It appears that an additive effect was observed, because the sum of the reduction in *Salmonella* between OA (26.7%) and probiotic (P1 = 48.6% and P2 = 35%) cultures alone is close to the overall reduction seen from coadministration of OA and probiotic cultures.

The beneficial effect of probiotic cultures is well documented [5, 6, 7, 8, 9, 10, 11], although the specific mechanisms by which they protect against colonization and invasion of pathogenic bacteria to the host remain unknown. Some of the mechanisms that have been hypothesized are the competition for nutrients and receptor sites in the gastrointestinal tract, as well as stimula-



**Figure 1.** Administration of probiotic cultures (P1 and P2) in the drinking water alone or after administration of an organic acid (OA; experiment 1). \* = significant differences ( $P > 0.05$ ) within a treatment within sampling times.



**Figure 2.** Administration of probiotic cultures (P1 and P2) or organic acid (OA) in the drinking water alone or in combination (experiment 2). \* = significant differences ( $P > 0.05$ ) within a treatment within sampling times.

tion of the immune system [20, 21, 22]. Recent reports showed that intestinal microflora stimulate the production of fucosylated glycoconjugates and  $\alpha$ -1,2-fucosyltransferase messenger RNA in the small-intestinal epithelium that are required for continuous enterocytic replacement [23]. Additional studies have revealed that intestinal microflora can influence host programs of cellular metabolism and differentiation [24, 25], resulting in an improvement of the epithelial barrier [26, 27]. More research is needed to definitively elucidate the mechanisms by which probiotics eliminate pathogens.

Along with the beneficial effect of the probiotic culture on *Salmonella* excretion used in our study, the OA treatment had an apparent additive effect. Organic acids have the ability to disrupt bacterial cell membranes [28] because of the high concentration of undissociated ions that diffuse across the membrane, altering bacterial enzyme reactions and nutrient transport [29, 30]. Lactic acid added to the drinking water during feed withdrawal in broilers has been reported to reduce the number of *Salmonella* Typhimurium and *Campylobacter*-positive crop samples and contaminated carcasses prechill [31]. Additionally, tannic acid has been shown to inhibit the

growth of intestinal bacteria (*Bacteroides fragilis*, *Clostridium clostridioforme*, *Clostridium perfringens*, *Clostridium paraputrificum*, *Escherichia coli*, *Enterobacter cloacae*, *S. Typhimurium* TA98 and *S. Typhimurium* YG1041), but had no effect on *Bifidobacterium infantis* and *Lactobacillus acidophilus* populations [32], possibly because of their strong iron-binding capacity [31]. These data suggest that the administration of probiotic cultures immediately following OA treatment in these experiments exerted a detrimental effect on *Salmonella* viability.

The reduction of environmental *Salmonella* prior to slaughter in poultry is necessary to reduce cross-contamination in the processing plant and, along with this, to reduce the risk of foodborne outbreaks, especially *Salmonella* and *Campylobacter* infections. A survey conducted by the Food Safety and Inspection Service [33] reported that even though a low incidence of birds that enter the processing plant were *Salmonella* positive (3 to 4%), the percentage was increased when processed birds left the plant (35%). From the literature [5, 6, 7, 8, 9, 10] and the results obtained in this study, the use of these probiotic cultures may serve as an alternative for controlling pathogens on poultry farms.

## CONCLUSIONS AND APPLICATIONS

1. In experiment 1, the administration of culture P1 alone or following OA significantly reduced recoverable *Salmonella*. In addition, administration of P2 following OA significantly reduced recoverable *Salmonella*.
2. In experiment 2, the administration of OA followed by probiotic cultures significantly reduced recoverable *Salmonella*.
3. These data show an apparent additive effect of OA and probiotics when administered to turkeys preslaughter.

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14. Drag swabs were assembled as previously described by Caldwell et al. [16], with some modification. Briefly, 2 sets of sterile gauze pads (10.2 × 10.2 cm) were tied both at the end (2 m) and at 30 cm above (1.70 m) the end of the string. The assembled swabs were placed in a sterile whirl plastic bag and heated at 60°C overnight in a dry oven. Before use, swabs were wet with skim milk.
15. Samples were enriched with tetrathionate broth (210420, Becton Dickinson, and Co., Sparks, MD; ~60 mL), and incubated for 24 h at 37°C. They were then streaked for isolation on novobiocin-containing (20 µg/mL) brilliant green agar (BGA, 228530, Becton Dickinson and Co.) plates and incubated for an additional 24 h at 37°C. Plates were examined for the presence or absence of typical lactose-negative colonies of *Salmonella*. Suspected colonies were serologically evaluated with O antigen (229511, Becton, Dickinson, and Co.).
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17. A 950-mL quantity of OA was added to 7.8 L of tap water in the medicator bucket. The OA reached the turkeys at the end of the water line at a final dilution of 1:128 (vol/vol).
18. The 7 enterobacteriaceae (5 strains of *E. coli*, *Kluyvera ascorbata*, and *Klebsiella travesanii*) species and the 2 lactic acid bacteria (*L. casei* and *L. cellobiosus*) were individually grown on tryptic soy broth (TSB, 211822, Becton Dickinson, and Co.) and de Mann, Rogosa, Sharpe (MRS, 288130, Becton Dickinson, and Co.) broth, respectively, during overnight incubation at 37°C. Bacterial cultures were concentrated by centrifugation at 1,864 × g for 10 min and resuspended in fresh media at a final concentration of 1.0 × 10<sup>9</sup> cfu/mL. Enterobacteriaceae bacteria species were combined and kept in 1 bottle separate from the 2 lactic acid bacteria. All bacteria were maintained at 4°C until their use.
19. Significant differences between the isolation frequency of environmental *Salmonella* before and after treatment were determined by using the chi-squared test for independence [Zar, J. 1984. *Biostatistical Analysis*. 2nd ed. Prentice-Hall, Engelwood Cliffs, NJ] and the significance level is reported as  $P < 0.001$  and  $P < 0.05$ .
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