

Evaluation of Spray Application of a *Lactobacillus*-Based Probiotic on *Salmonella enteritidis* Colonization in Broiler Chickens

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Abstract: Spray application offers low-cost and efficient application of biologic and reduced concerns regarding diverse water quality and medicator/proportioner function. The objective of the present study was to evaluate the spray application of a *Lactobacillus*-based probiotic on *Salmonella enteritidis* (SE) colonization in broiler chickens. Day-of-hatch chicks were challenged with *Salmonella enteritidis* (SE) by oral gavage alone, challenged with SE and treated by coarse spray application of a commercially-available Lactic-acid bacterial probiotic (FM-Probiotic™), or challenged with SE and treated with B11 continuously in the Drinking Water (DW). Five days post-challenge, cecal tonsils were collected for presence or absence of SE. In Exp. 1, probiotic treatment by either spray or DW application significantly ($p < 0.05$) reduced SE recovery (55% and 50% respectively; controls 85%) when chicks were held for 8h prior to challenge and placement. Similarly, when probiotic spray treatment or water treatment and challenge occurred simultaneously, with placement 8h after treatment, a marked and significant reduction of SE recovery was noted after 5d (10% and 40% respectively, controls 55%). In Exp. 2, when probiotic spray treatment and challenge occurred simultaneously, with placement 8h after treatment, a significant reduction of SE recovery was again noted in both the spray and DW application (80% controls, 15% spray, 15% DW). Taken together, these results suggest that spray application of this probiotic, when performed in the manner described above, can be effective for protection of chicks against *Salmonella* infection.

Key words: Probiotic, *Salmonella enteritidis*, broiler chicks, spray application

Introduction

Poultry producers are challenged to improve production while using fewer antibiotics due to increased restriction on antimicrobial usage. Researchers worldwide are working on alternatives due to the ban of a wide range of drugs for animal production. Probiotics consisting of live or dead organisms and spores (Patterson and Burkholder, 2003), non-traditional chemicals (Moore *et al.*, 2006), bacteriophages (Higgins *et al.*, 2005), organic acids (Jarquin *et al.*, 2007; Wolfenden *et al.*, 2007) and others have emerged in the last decades as some of the tools that could be potentially useful in the near future for pathogen control and poultry performance improvement. An effective and defined *Lactobacillus*-based probiotic has been developed and is commercially available (Tellez *et al.*, 2006). Experimental and commercial studies conducted have shown that these selected probiotic organisms are able to reduce idiopathic diarrhea in commercial turkey brooding houses (Higgins *et al.*, 2005) and also to significantly reduce *Salmonella* colonization in turkeys (Vicente *et al.*, 2007a) and broilers (Higgins *et al.*, 2007; Vicente *et al.*, 2007b). Application to large numbers of chicks under commercial conditions must be efficient, should be

administered as early in life as possible (Schneitz *et al.*, 1992) and should minimize uncontrolled variables such as water quality and proportioner/medicator function and consistency. These issues can be addressed and minimized if the probiotic was administered at the hatchery by spray application. Automated spray application offers several advantages over drinking water or individual administration by gavage. The objective of the present study was to evaluate the spray application of a *Lactobacillus*-based probiotic on *Salmonella enteritidis* colonization in broiler chickens.

Materials and Methods

Salmonella amplification: A primary poultry isolate of *Salmonella enteritidis* (SE), phage type 13A, was originally obtained from the National Veterinary Services Laboratory (Ames, Iowa). This isolate was selected for resistance to nalidixic acid (NA)¹. For these experiments, *Salmonella* was grown in tryptic soy broth (TSB)² for approximately 8 h. The cells were washed three times with 0.9% sterile saline by centrifugation (3,000×g) and the approximate concentration of the stock solution was determined spectrophotometrically. The stock solution was serially diluted and confirmed by colony counts of

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Table 1: Treatments for experiments 1 and 2

Treatment Regimes	Treatment Groups	Treatments	SE Challenge
Treat-Challenge-Place Immediately	Control	Water	10 ⁴ cfu/chick
	Probiotic DW ¹	10 ⁶ cfu/ml	
	Probiotic Spray	10 ⁷ cfu/ml	
Treat-Hold 8h-Challenge-Place	Control	Water	10 ⁴ cfu/chick
	Probiotic DW	10 ⁶ cfu/ml	
	Probiotic Spray	10 ⁷ cfu/ml	
Treat-Challenge-Hold 8h-Place	Control	Water	10 ⁴ cfu/chick
	Probiotic DW	10 ⁶ cfu/ml	
	Probiotic Spray	10 ⁷ cfu/ml	

¹DW = drinking water

three replicate samples (0.1 mL/replicate) that were spread plated on xylose lactose differential agar (XLD)³ plates containing 25 µg/mL novobiocin (NO)⁴ and 20 µg/mL NA. The colony-forming units of *Salmonella* determined by spread plating were reported as the concentration of *Salmonella* (in cfu/mL) for *in vitro* experiments and total colony-forming units for *in vivo* challenge experiments.

Probiotic culture: Eleven lactic acid bacterial isolates, of poultry gastrointestinal origin, were previously selected and described (Higgins *et al.*, 2005). This commercial product (FM-B11™)⁵ was diluted in reconstituted powdered skim milk according to manufacturer's directions to an expected concentration of 4×10⁶ cfu/mL for oral gavage of chicks or 10⁷ cfu/ml for spray treatment for these studies. Actual cfu administered per chick from each experiment were determined retrospectively from spread plating on Mann Rogosa sharp agar⁶.

Experimental design: For experiments 1 and 2, 900 day-of-hatch chicks, 100 chicks per box, were obtained from a local hatchery. Chicks were held in a dark room until time of treatment. For these experiments, 3 different treatment regimes were administered with 3 different groups within each treatment regime for a total of 9 groups per experiment (Table 1). To encourage preening activity after spray application (Caldwell *et al.*, 2001a,b), green food coloring was added to either the probiotic or the control groups prior to administration. Briefly, chicks were sprayed using a hand-held garden sprayer⁷, adjusted to a coarse spray, with either water (DW) containing green dye⁸ (0.5 mg/ml) (untreated controls) or probiotic containing green dye (25 ml/100 chicks). Increased photointensity has also been shown to increase preening activity (Caldwell *et al.*, 2001c). Therefore, chicks were then placed under a halogen light for 2.5 minutes to stimulate preening (~95 Lux). Chicks were then held according to treatment regimes and then challenged with approximately 10⁴ cfu/chick of SE. Chicks were then placed in brooder batteries (n = 40). For each of the experiments the chicks were housed in brooder batteries with food and water *ad libitum*. At 5 days post-challenge, all chicks, were humanely killed by

CO₂ inhalation and cecal tonsils from 20 chicks per group were aseptically harvested. *Salmonella* recovery procedures have been previously described by our laboratory and were followed with some modifications (Tellez *et al.*, 1993). Briefly, cecal tonsils were enriched in 20 mL of tetrathionate broth⁹ overnight at 37°C. Following enrichment, each sample was streaked for isolation on XLD plates containing 25 µg/mL NO and 20 µg/mL NA. The plates were incubated at 37°C for 24 h and examined for the presence or absence of the antibiotic resistant SE.

Statistical analysis: The incidence of *Salmonella* recovery within experiments was compared using the chi-square test of independence (Zar, 1984) testing all possible combinations to determine significant (p<0.05) differences between control and treated groups.

Results and Discussion

Effective probiotics have been shown to accelerate development of normal microflora in chicks and poults, providing increased resistance to infection by some enteric bacterial pathogens (Higgins *et al.*, 2007; Vicente *et al.*, 2007a; Vicente *et al.*, 2007b). Spray application offers low-cost and efficient application of biologics and reduced concerns regarding diverse water quality and medicator/proportioner function. Results from both experiments 1 and 2 are summarize in Table 2. In Experiment 1, near-simultaneous administration of probiotic with spray and immediate placement of chicks did not significantly reduce SE recovery. However, probiotic treatment, by either spray or DW application significantly (p<0.05) reduced SE recovery to 55% for spray application and 50% when administered in the drinking water compared to an 85% recovery from negative controls when chicks were held for 8h prior to challenge and placement. Similarly, when probiotic spray treatment and challenge occurred simultaneously, with placement 8h after treatment, a marked and significant reduction of SE recovery was noted after 5d (55% recovery in the negative controls compared to 10% in the spray treated or 44% in the DW treated). In Exp. 2, when probiotic spray treatment and challenge occurred simultaneously, with placement 8h after treatment, a

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Table 2: *S. enteritidis* recovery from cecal tonsils of broiler chicks 5 days post-challenge

Treatment Regimes	Treatment Groups	Cecal Tonsils	
		Exp 1	Exp 2
Treat-Challenge-Place Immediately	Control	95% (19/20)	95% (19/20)
	Probiotic DW ¹	75% (15/20)	25% (5/20)**
	Probiotic Spray	90% (18/20)	80% (16/20)
Treat-Hold 8h-Challenge-Place	Control	85% (17/20)	70% (14/20)
	Probiotic DW	50% (10/20)*	70% (14/20)
	Probiotic Spray	55% (11/20)*	80% (16/20)
Treat-Challenge-Hold 8h-Place	Control	55% (11/20)	80% (16/20)
	Probiotic DW	44% (7/20)*	15% (3/20)*
	Probiotic Spray	10% (2/20)**	15% (3/20)*

*Indicates significant (p<0.05) differences was observed between control and treated within a single experiment and treatment regime in each column, **Significantly (p<0.05) different than all groups within a single experiment and treatment regime in each column

significant reduction of SE recovery was noted in both the spray and DW application (80% controls, 15% spray, 15% DW). These results suggest that spray application of a *Lactobacillus*-based probiotic is as effective at reducing *Salmonella* in chicks as the drinking water application of this probiotic when the chicks are held 8 hours in chick boxes prior to challenge and placement. This model simulates an on farm challenge with *Salmonella*. In experiment 2, a reduction in *Salmonella* occurred when the chicks were sprayed with the probiotic and challenge and then held for 8 hours prior to placement, simulating a challenge occurring at the hatchery. Taken together, these results suggest that spray application of this probiotic, when performed in this manner, can be effective for protection of chicks against *Salmonella* infection. Spray application of probiotics at the hatchery can lessen the variables that can occur with drinking water administration on the poultry farm. Furthermore, hatchery administration could prove to be a more effective way to administer probiotics because the chicks will be receiving the beneficial bacteria at the earliest possible time, short of *in ovo* administration.

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