

Research Note

Effect of lactic acid bacteria probiotic culture for the treatment of *Salmonella enterica* serovar Heidelberg in neonatal broiler chickens and turkey poults

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ABSTRACT In the present study, a series of experiments was conducted to evaluate the ability of a commercial probiotic culture (FloraMax, IVS-Wynco LLC, Springdale, AR) to reduce *Salmonella enterica* serovar Heidelberg (SH) in chicks and turkey poults. In experiments 1 and 2, chicks were randomly assigned to treatment groups and then challenged via oral gavage with SH. Chicks were treated 1 h following SH challenge with the probiotic culture via oral gavage. At 24 and 72 h posttreatment, cecal tonsils and ceca were collected for recovery and enumeration of enteric *Salmonella* Heidelberg, respectively. In experiment 3, day-of-hatch turkeys were randomly assigned to treatment groups and then challenged via oral gavage with SH. Poults were treated 1 h following challenge with the probiotic via oral gavage. At 24 and 72 h post probiotic treatment, cecal tonsils and ceca were collected for recovery

and enumeration of enteric SH, respectively. The probiotic culture significantly reduced the incidence of SH in cecal tonsils at both time points in chicks in both experiments ($P < 0.05$). These data demonstrate that administration of probiotic 1 h post SH challenge significantly reduced the incidence of SH recovery from cecal tonsils of chicks compared with controls 24 and 72 h following treatment. Similarly, probiotic treatment resulted in significant reductions in the concentrations of SH within the ceca in both experiments. Although similar significant results were observed at both 24 and 72 h in experiment 3, it was clear that poults were more susceptible to SH colonization than chicks. Overall, a *Lactobacillus*-based probiotic significantly reduced *Salmonella enterica* serovar Heidelberg in chicks and turkey poults.

Key words: *Lactobacillus*, poultry, probiotic, *Salmonella* Heidelberg

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INTRODUCTION

Despite advances in the treatment of infectious diseases, pathogenic microorganisms, including *Salmonella*, are an important threat to both human and animal health worldwide (Kim et al., 2007). Among them, *Salmonella enterica* serovar Heidelberg (SH) ranks among the top 3 serovars isolated from persons infected with *Salmonella* in North America, higher than in other regions of the world (Bucher et al., 2007; Zaidi et al., 2008; Elgroud et al., 2009; Wales et al., 2009; Dutil et al., 2010; Vaz et al., 2010). Sources of human SH infection include consumption of undercooked poultry or eggs and egg-containing products (Currie et al., 2005;

Chittick et al., 2006; Zhao et al., 2008; Borsoi et al., 2009). Recent restrictions on the use of antimicrobials as growth promoters in animal production have pressured the poultry industry to look for alternatives that can continue to provide performance benefits. Probiotics, although not a new concept, have only recently begun to receive an increasing level of scientific interest. January 2006 was the date for complete ban of antibiotics in animal feed within Europe (Anadón et al., 2006). A viable alternative to antibiotics is therefore an important venture (Foley and Lynne, 2008; Dutil et al., 2010; Vaz et al., 2010). For this reason, the development of new probiotic products that could be licensed for animal use is receiving considerable interest (Patterson and Burkholder, 2003; Hong et al., 2005). Currently, no universal class of probiotic bacterium exists, although the most common types available are lactic acid bacteria (LAB). These bacteria are found normally in the gastrointestinal tract of humans and animals, and the vague no-

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tion exists that the use of indigenous or commensal microorganisms somehow restores the natural microflora of the gut. Research conducted in our laboratory has elucidated an effective in vitro screening technique for identification of candidate probiotic organisms (Bielke et al., 2003). Further screening allowed the identification of 11 LAB of the genus or related to *Lactobacillus* in the product FM-B11 (FloraMax, IVS-Wynco LLC, Springdale, AR) that were more efficacious in the treatment of *Salmonella*-infected chickens and poult. This probiotic culture has been shown in both laboratory and field studies to increase performance and accelerate development of normal microflora in chicks and turkeys, providing increased resistance to infection by enteric bacterial pathogens (Farnell et al., 2006; Higgins et al., 2007, 2008, 2010; Torres-Rodriguez et al., 2007; Vicente et al., 2008). In the present study, we evaluated the effect of this LAB probiotic culture for the treatment of a *Salmonella enterica* serovar Heidelberg infection in neonatal broiler chickens and turkey poult.

MATERIALS AND METHODS

Salmonella Amplification

A primary poultry isolate of SH, resistant to novobiocin (NO; Sigma, St. Louis, MO) and nalidixic acid (NA; Sigma), was used for these experiments. Briefly, SH was incubated at 37°C for 24 h and passed every 8 h. Cells were then washed 3 times in sterile saline by centrifugation at $1,864 \times g$. Concentrations of SH were retrospectively determined by spread plating on brilliant green agar (BGA; Becton Dickinson, Sparks, MD) plates containing NO (25 µg/mL) and NA (20 µg/mL). Actual colony-forming units administered per chick were determined from spread plating on BGA agar.

Probiotic Culture

Eleven lactic acid bacterial isolates of poultry gastrointestinal origin were described previously (Higgins et al., 2007, 2010). This commercial product (FloraMax)

was diluted in reconstituted powdered skim milk to an expected concentration of 4×10^6 cfu/mL for oral gavage to chicks in these studies. Actual colony-forming units administered per chick were determined from spread plating on de Man, Rogosa, and Sharpe agar (Becton Dickinson).

Experimental Design (Experiments 1 and 2)

Experiments 1 and 2 were conducted as follows. Day-of-hatch male broiler chicks were obtained from a local hatchery. Chicks used in all experiments were cared for using procedures approved by the University of Arkansas Institutional Animal Care and Use Committee. Heated brooder batteries were used for housing and chicks were allowed ad libitum access to unmedicated broiler starter ration, formulated to meet or exceed NRC (1994) recommended levels of critical nutrients, and water for the duration of the experiment. Chicks were randomly assigned to treatment groups and then challenged via oral gavage (0.25 mL) with approximately 10^5 cfu/chick of SH in experiment 1 or 10^6 cfu/chick of SH in experiment 2 and placed into pens ($n = 40$ /pen). Chicks were treated 1 h following *Salmonella* challenge with approximately 10^6 cfu/chick of FloraMax culture via oral gavage (0.25 mL), and PBS as vehicle was administered to control groups. At 24 and 72 h following treatment, broilers were humanely killed by CO₂ inhalation and cecal tonsils were collected aseptically and enriched in 10 mL of tetrathionate broth (Becton Dickinson) overnight at 37°C. Following enrichment, each sample was streaked for isolation on BGA plates containing 25 µg/mL of NO and 20 µg/mL of NA. The plates were incubated at 37°C for 24 h and examined for the presence or absence of antibiotic-resistant SH. Ceca were homogenized within sterile sample bags (Nasco, Fort Atkinson, WI) using a rubber mallet. Sterile saline (3 mL) was added to each sample bag and hand stomached with the cecal contents. Dilutions were spread plated on BGA plates containing 25 µg/mL of NO and 20 µg/mL of NA. The plates were incubated at 37°C for 24 h and total colony-forming units of SH per cecal pair were determined.

Table 1. In vivo evaluation of FloraMax-B11 against *Salmonella* Heidelberg (SH) at 24 and 72 h in broiler chickens from experiment 1¹

Item	24 h		72 h	
	Cecal tonsils ²	SH ³ (log ₁₀ cfu/g of ceca content)	Cecal tonsils ²	SH ³ (log ₁₀ cfu/g of ceca content)
Control SH	18/20 (90)	3.02 ± 0.53 ^a	7/20 (35)	1.08 ± 0.57 ^a
FloraMax-B11	1/20 (5.2)*	0.27 ± 0.27 ^b	0/20 (0)*	0 ± 0 ^b

^{a,b}Different superscripts within columns indicate significant differences ($P < 0.05$).

¹Chickens were orally gavaged with 10^5 cfu/chicken of SH at hatch. One hour later treated chickens were gavaged with FloraMax-B11 (IVS-Wynco LLC, Springdale, AR). Control chickens were gavaged with PBS.

²Data expressed as positive/total chickens (%).

³Data expressed as mean ± SE.

* $P < 0.001$.

Table 2. In vivo evaluation of FloraMax-B11 against *Salmonella* Heidelberg (SH) at 24 and 72 h in broiler chickens from experiment 2¹

Item	24 h		72 h	
	Cecal tonsils ²	SH ³ (log ₁₀ cfu/g of ceca content)	Cecal tonsils ²	SH ³ (log ₁₀ cfu/g of ceca content)
Control SH	16/20 (80)	3.44 ± 0.47 ^a	14/20 (70)	2.96 ± 0.76 ^a
FloraMax-B11	0/20 (0)*	0 ± 0 ^b	0/20 (0)*	0 ± 0 ^b

^{a,b}Different superscripts within columns indicate significant differences ($P < 0.05$).

¹Chickens were orally gavaged with 10⁶ cfu/chicken of SH at hatch. One hour later treated chickens were gavaged with FloraMax-B11 (IVS-Wynco LLC, Springdale, AR). Control chickens were gavaged with PBS.

²Data expressed as positive/total chickens (%).

³Data expressed as mean ± SE.

* $P < 0.001$.

Experimental Design (Experiment 3)

Day-of-hatch turkey poults were obtained from a local hatchery and were housed and cared for as described for experiments 1 and 2. Poults were randomly assigned to treatment groups and then challenged via oral gavage (0.25 mL) with approximately 10⁶ cfu/poult of SH and placed into pens ($n = 40/\text{pen}$). Poults were treated 1 h following challenge with approximately 10⁶ cfu/chick of FloraMax culture via oral gavage (0.25 mL), and PBS as vehicle was administered to control groups. At 24 and 72 h following challenge, poults were humanely killed and cecal tonsils and ceca were collected and processed as above.

Statistical Analysis

The incidence of SH recovery within experiments was compared, testing all possibilities, using the chi-squared test of independence (Zar, 1984) to determine significant ($P < 0.05$) differences between groups within experiments. Ceca colony-forming unit data were converted to log₁₀ colony-forming unit numbers and then compared using the GLM procedure of SAS (SAS Institute, 2002) with significance reported at $P < 0.05$.

RESULTS AND DISCUSSION

Salmonella enterica serovar Heidelberg has been recognized as one of the most common serovars associated with foodborne infections in several countries around

the world (Zaidi et al., 2008; Borsoi et al., 2009; El-groudi et al., 2009; Wales et al., 2009; Dutil et al., 2010). It is also frequently isolated from nonhuman sources and has increasingly shown resistance to various antimicrobial agents (Patchanee et al., 2008; Zhao et al., 2008; Borsoi et al., 2009; Dutil et al., 2010; Vaz et al., 2010).

In the present study, the probiotic treatment significantly reduced the incidence of SH in cecal tonsils at both 24 and 72 h in broiler chickens in both experiments (Tables 1 and 2). These data demonstrate that administration of probiotic 1 h post SH challenge significantly reduced the incidence of *Salmonella* recovery from cecal tonsils of broiler chicks compared with untreated controls 24 and 72 h following treatment. Similarly, probiotic treatment resulted in significant reductions in the concentrations of SH within the ceca in both experiments (Tables 1 and 2). Although similar significant results were observed at both 24 and 72 h in experiment 3, it was clear that poults were more susceptible to SH colonization than chickens (Table 3). Despite considerable published data regarding the efficacy of probiotics in reducing intestinal colonization by enteric pathogens, the mechanisms of action of probiotics are not fully understood. Several mechanisms have been proposed for probiotic functions, among which modulation of the immune system has recently received attention (Shahani and Ayebo, 1980; Jijon et al., 2004; Flore et al., 2010). Probiotic bacteria can exert immunomodulatory activities through their interactions with the host immune system. These interactions may lead to enhancement of

Table 3. In vivo evaluation of FloraMax-B11 against *Salmonella* Heidelberg (SH) at 24 and 72 h in poults¹

Item	24 h		72 h	
	Cecal tonsils ²	SH ³ (log ₁₀ cfu/g of ceca content)	Cecal tonsils ²	SH ³ (log ₁₀ cfu/g of ceca content)
Control SH	20/20 (100)	7.04 ± 0.19 ^a	20/20 (100)	6.05 ± 0.28 ^a
FloraMax-B11	13/20 (65)*	4.36 ± 0.74 ^b	9/20 (45)*	2.15 ± 0.75 ^b

^{a,b}Different superscripts within columns indicate significant differences ($P < 0.05$).

¹Poults were orally gavaged with 10⁶ cfu/chicken of SH at hatch. One hour later treated poults were gavaged with FloraMax-B11 (IVS-Wynco LLC, Springdale, AR). Control poults were gavaged with PBS.

²Data expressed as positive/total poults (%).

³Data expressed as mean ± SE.

* $P < 0.001$.

natural and antigen-specific antibodies (Davies et al., 2009; Amit-Romach et al., 2010; Cai et al., 2010), activation or suppression of T cells (Górska et al., 2009; Sjögren et al., 2009; Starovoítova et al., 2009; Foligné et al., 2010), and changes in cytokine expression profiles (Shida et al., 2009; Nayak, 2010; Tsai et al., 2010). Moreover, probiotics are able to induce the expression of antimicrobial peptides by host cells (Li et al., 2009; Shida et al., 2009; Dong et al., 2010; Mohamadzadeh, 2010). Collectively, the above-mentioned mechanisms contribute to the immunomodulatory activities of probiotics. Our laboratory is currently studying the source of antimicrobial peptides in the ileum and cecal tonsils of *Salmonella*-infected chicks as well as the mechanisms of action of probiotics in downregulating antimicrobial peptide genes in infected chickens. Overall, more research must be conducted to elucidate the conditions necessary for probiotic bacteria to elicit a beneficial immune response from the host that prevents or treats enteric infections. In the present study, LAB treatment significantly reduced recovery of SH in neonatal broilers and poults. Further research will be conducted using this probiotic to determine the exact mechanism of pathogen reduction

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