EFFECT OF A SELECTED *LACTOBACILLUS* SPP-BASED PROBIOTIC ON *SALMONELLA ENTERITIDIS*-INFECTED BROILER CHICKS

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**Key Words:** chick, probiotic, *Salmonella, Lactobacillus*

**Abreviation keys:** GRAS = General recognized as safe; SE = *Salmonella enteritidis*; NA = Nalidixic acid; NO = Novobiocin; LIQ = Liquid probiotic culture, LYO = Lyohilized probiotic culture; CE = Competitive exclusion.

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SUMMARY

The effect of a *Lactobacillus*-based probiotic (FM-B11™) on *Salmonella* recovery was evaluated in liquid (Exp. 1) and lyophilized (Exp. 2) forms in two separate experiments with two trials each. For each trial, eighty broiler chicks were randomly allocated into two treatments: control and probiotic culture. All chicks were challenged with *S. enteritidis* (SE) (~10⁴ cfu) upon arrival at our laboratory. In both experiments, probiotic culture was administered in the drinking water for three consecutive days at a final concentration of approximately 10⁶ cfu/mL, beginning one hour after SE challenge. Cecal tonsils were aseptically removed at 24 and 72 h post-challenge, followed by enrichment and plating on XLD agar for the presence or absence of *Salmonella*-typical colonies. In Exp. 1, a significant reduction (P<0.05) of SE positive samples was observed in both trials at 24 and 72 h post-challenge. Additionally, in Exp. 2, the lyophilized probiotic decreased (P<0.05) SE recovery at both 24 and 72 h post-challenge compared to the control group in trial 1. In trial 2, SE evaluation was performed only at 72 h after challenge and fewer (P<0.001) treated samples were SE positive. Results showed that application of either liquid or lyophilized probiotic culture for three consecutive days in the drinking water may help to reduce SE recovery from young birds, although further research is needed to elucidate the mechanism of this response.

RESUMEN. Efecto terapéutico de un probiótico en pollos de engorda infectados con *S. enteritidis*.

Dos experimentos con dos repeticiones cada uno fueron conducidos para evaluar el efecto de un probiótico a base de *Lactobacillus* (FM-B11™) en sus presentaciones
liquida (Exp. 1) o liofilizada (Exp. 2) sobre la colonización de S. enteritidis (SE) en pollos de engorda de un día de edad. Ochenta pollos en cada repetición fueron obtenidos de una incubadora comercial y asignados aleatoriamente en dos tratamientos: control y probiótico. El probiótico fue administrado en el agua de bebida a una concentración final de ~$10^6$ ufc/mL por tres días iniciando una hora después de desafió con SE. Al arribo, los pollos fueron desafíados vía oral con SE (~$10^4$ ufc). Venticuatro y 72 h después del desafío, 20 pollos por grupo fueron sacrificados y muestras de tonsilas cecales fueron obtenidas asépticamente para evaluar la colonización por SE. En el experimento 1, una disminución significativa ($P<0.05$) fue observada en el grupo tratado con el probiótico en ambos tiempos de evaluación. Una disminución significativa a la colonización por SE fue observada entre los pollos del grupo tratado a las 24 y 72 h posdesafío en ambas repeticiones ($P<0.05$). Similares resultados fueron obtenidos en la repetición 1. En la repetición 2, muestras para SE fueron tomadas únicamente a las 72 h postdesfío. El número de muestras positivas a SE fue reducido significativamente ($P<0.001$) en el grupo tratado. Los resultados de este reporte sugieren que el probiótico tanto líquido como liofilizado ayuda a reducir SE en aves previamente infectadas aunque el mecanismo específico de acción del probiótico requiere ser estudiado.

**INTRODUCTION**

Despite advances in the treatment of infectious diseases, pathogenic microorganisms, including *Salmonella*, are an important threat to health worldwide (20). The FoodNet surveillance program has estimated about 1.4 million cases of Salmonellosis occur annually in the United States, resulting in ~16,000 hospitalization
and > 500 deaths (12). Poultry products have been implicated as the most common vehicle of Salmonellosis transmission. Poultry often become infected by consuming contaminated feed, by cross-contamination in brooding houses or, during slaughter and processing (9).

Recent restrictions on the use of some antimicrobials as growth promoters in animal production have pressured the poultry industry to look for alternatives for pathogen control. Since Nurmi and Rantala (15) proposed that competitive exclusion could be used as a method to prevent *Salmonella* infection, numerous researchers have reported the ability of these cultures (2,13,14) and probiotic organisms (3,11,18) to reduce colonization of opportunistic microorganisms in the gastrointestinal tract by competition for receptor sites, stimulation of the immune system, and production of some active antimicrobial substances (17).

Probiotic organisms are live microbial feed supplements that exert beneficial effects on the host by improving the microbiologic balance of the intestine (7). Lactic acid bacteria are considered to be optimal probiotic bacterial candidates because they are generally recognized as safe (GRAS). Unpublished data from our laboratory has shown that probiotic organisms contained in the culture used in these experiments inhibit the growth of food-borne pathogens under *in vitro* conditions. The aim of this study was to evaluate the therapeutic effect of a commercial probiotic (FM-B11™, IVESCO, LLC.) in both liquid and lyophilized form when provided in the drinking water for three consecutive days on *Salmonella enteritidis* (SE) colonization in day-of-hatch broiler chicks.
MATERIALS AND METHODS

Salmonella enteritidis

A primary poultry isolate of *Salmonella enteritidis* PT13A (SE) was obtained from the USDA National Veterinary Services Laboratory. This isolate was resistant to novobiocin (NO) (25 µg/mL) and was selected for resistance to naladixic acid (NA) (20 µg/mL) in our laboratory. For these studies, SE was grown in tryptic soy broth (TSB) at 37°C for eight hours, and passed to fresh TSB for three incubation periods. Cells were washed 3 times in sterile saline by centrifugation at 1,864 x g, and the concentration was estimated with a spectrophotometer, using a previously generated standard curve, to approximately 10^8 cfu/mL in sterile saline. The culture was then diluted to inoculated concentrations as described below. Concentrations of SE and ST were retrospectively determined by spread plating on XLD agar containing NO (25 µg/mL) and NA (20 µg/mL), followed by enumeration for each experiment. Actual determined colony-forming units for each experiment are reported.

Probiotic administration

Eleven lactic acid bacterial isolates were previously selected and have been previously described (Higgins et al., 2005). This mixture, FM-B11™ (IVESCO, LLC), now commercially available in both liquid and powdered (lyophilized) forms, was used for these experiments. For experiment 1, the liquid probiotic culture (LIQ) containing 10^9 cfu/mL was diluted 10 fold in MRS broth. Thirty-five mL was then added to 3, 425 mL of fresh drinking water and given to the chicks approximately 1 h after SE challenge. For experiment 2, the lyophilized probiotic culture (LYO) was obtained which contained ~10^{11} cfu viable organisms. A one-thousand-fold dilution (1:1000) was made (final
concentration $10^8$ cfu/mL) in MRS broth, and 35mL was added to 3,425 mL of drinking water. Thirty-five mL of skim milk was added to the drinking water in both experiments as a stabilizer. Enumeration of viable organisms of the probiotic cultures was performed on MRS agar plates, and the final concentrations were $\sim 10^6$ cfu/mL (Tables 1 and 2).

**Salmonella Recovery**

Briefly, cecal tonsils were aseptically removed and incubated for 24 h in tetrathionate broth at 37ºC. After incubation, a sample of broth was streaked for isolation on XLD agar plates containing NO/NA antibiotics as described above, and incubated for an additional period of 24 h at 37ºC. Following incubation, agar plates were evaluated for the presence or absence of typical antibiotic resistant *Salmonella* colonies.

**Experiment 1**

This experiment was replicated in two separate trials. Day-of-hatch broiler chicks were obtained from a commercial hatchery and placed in brooder batteries located in an isolation room in the Poultry Health Laboratory at the University of Arkansas. Prior to the start of the experiment, 5 chicks and feed were cultured for *Salmonella* using a previously described procedure (1). Chicks were provided with unmedicated chicken starter feed *ad libitum* and fresh water daily, with or without probiotic treatment according to the experimental design. In trial 1, 80 broiler chicks were randomly assigned to either the control or probiotic treatment (LIQ). Chicks were individually gavaged with $6.0 \times 10^3$ cfu of SE prior to placement in brooder batteries. Probiotic was provided for three consecutive days beginning 1 h after *Salmonella* challenge. For trial 2, eighty chicks were treated as described in trial 1 and challenged with $7.5 \times 10^3$
cfu/bird SE. In both trials, twenty chicks per group were humanely killed at 24 or 72 h post-challenge according to the National Poultry Improvement Plan (NPIP) guidelines (19).

**Experiment 2**

Two trials were performed at different times using the same model as experiment 1. For this experiment, probiotic treatment (LYO) was added to drinking water 1 h after *Salmonella* challenge and continued for three days. In trial 1, the SE challenge was $1.0 \times 10^4$ cfu per chick and in trial 2, the challenge was $5.0 \times 10^3$ cfu per chick. Trial 2 used only 25 chicks in each group instead of 40, as described in previous trials.

**Statistical analysis**

Chi-square analysis was performed in each experiment to determine significant differences ($P<0.05$) between groups in SE recovery rate (21).

**RESULTS**

**Experiment 1**

In experiment 1, a significant reduction ($P<0.01$) in *Salmonella* recovery was observed among chicks that received LIQ (1/20; 5%) compared to the control group (14/20; 70%) after 24h of treatment (Table 1). At 72 h after challenge, significantly fewer samples ($P<0.05$) were positive for SE in the LIQ group (5/20; 25%) in comparison with the control group (13/20; 65%). Likewise, in trial 2, a reduction ($P<0.01$) of SE recovery was observed among LIQ-treated chicks compared with untreated chicks at both 24 and 72 h.

**Experiment 2**
In the first trial of experiment 2 (Table 2), the number of SE-positive samples was significantly lower (P<0.05) among chicks treated with LYO than among the control group at 24 and 72 h post-infection. In trial 2, *Salmonella* evaluation was performed only at 72 h postchallenge, and the colonization of SE was reduced (P<0.001) among LYO treated broiler chicks in comparison to the control group at this timepoint.

**DISCUSSION**

Many reports support the benefits of administering normal microflora of healthy adult poultry to young chicks to prevent infections (2,14,18). Organisms present in the *Lactobacillus*-based probiotic (FM-B11™) include live, poultry-origin, lactic acid bacteria: *Lactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus paracasei*, *Lactobacillus salivarius*, and *Pediococcus parvulus* (based upon 16s RNA sequencing). Data obtained in this report suggest that the combination of these probiotic strains may be used as a tool to significantly reduce SE colonization in broiler chicks from day-of-hatch. Furthermore, the supplementation of this culture for three consecutive days continued to maintain reduced levels of SE recovery from cecal tonsils.

Administration of a probiotic culture may modify the ecology of the gastrointestinal tract during the first days of a chick’s life, a time that is considered an open window for the establishment of pathogens such as *Salmonella*. Neonates are born with an almost a sterile gastrointestinal tract, but microorganisms present in the environment after birth rapidly begin colonize (4). Although lactic acid bacteria are normal inhabitants of the gastrointestinal tract, their presence follows a succession with *L. delbrueckii* as the major species at day 3, *L. acidophilus* and *Weissella sp* dominate
at day 7, and *L. crispatus* predominates from days 14 to 49. Lastly, *L. salivarius* appeared at day 49 (10). In addition, Guan *et al.*, (5) observed that *L. acidophilus* and *L. salivarius*, appeared in developmental succession while other species such as *L. reuteri* and *L. johnsonii* were consistently detected.

Reduction of pathogens has been observed by others following administration of probiotic cultures. Pascual and coworkers (16) observed that oral administration of *L. salivarius* strain probiotic organism in day-old leghorn chicks caused no *Salmonella* to be recovered after 21 days. The same results were observed when the probiotic strain was also administered through the feed and drinking water apart from oral gavage. In other studies, application of lactose and a probiotic culture (10⁹ cfu/gram) during the growout period not only improved performance in broiler chicks, but also reduced coliforms in the cecum at 10 days of age (8). Oral gavaged of a lyophilized CE product in broiler chicks reduced coliforms in the small intestine, large intestine and cecum at days 7 and 14 (6). However, to our knowledge, this is the first study to evaluate both liquid and lyophilized forms of the same culture for reduction of a pathogen.

In conclusion, the administration of either a liquid or lyophilized *Lactobacillus*-based probiotic (FM-B11™) in the drinking water may help to reduce the incidence of *Salmonella* recovery in broiler chicks. Further research is necessary to elicit the specific mechanisms of the probiotic culture’s protection benefits.
REFERENCES


Table 1. Therapeutic effect of *Lactobacillus*-based probiotic (FM-B11<sup>TM</sup>) in liquid form (LPC) administered in drinking water for three consecutive days on *Salmonella enteritidis* recovery in broiler chicks.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatments</th>
<th>Probiotic concentration in the drinking water (cfu/ml)</th>
<th>No. <em>S. enteritidis</em>-positive samples/ total (%)&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>4.0x10&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>6.0x10&lt;sup&gt;6&lt;/sup&gt;</td>
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</tbody>
</table>

* Values in the same column are significantly different from the control value (P<0.01)
**Values in same column are significantly different from the control value (P<0.05)

<sup>1</sup> 6.0 x 10<sup>3</sup> cfu/bird *Salmonella enteritidis*

<sup>2</sup> 7.5 x 10<sup>3</sup> cfu/bird *Salmonella enteritidis*
Table 2. Therapeutic effect of a *Lactobacillus*-based probiotic (FM-B11™) in lyophilized form (LYP) in the drinking water for three consecutive days *Salmonella enteritidis* recovery in broiler chicks.

<table>
<thead>
<tr>
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<th>Probiotic concentration in drinking water (cfu/ml)</th>
<th>No. <em>S. enteritidis</em>-positive samples / total (%)&lt;a&gt; &lt;sup&gt;a&lt;/sup&gt;</th>
<th>24h</th>
<th>72h</th>
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<td>LYP</td>
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<td>5.0x10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2.0x10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>10 / 20 (50) *</td>
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<tr>
<td></td>
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<td>2.0x10&lt;sup&gt;6&lt;/sup&gt;</td>
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* Values in the same column significantly different from the control value (P<0.001)
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<sup>1</sup> 1.0 x 10<sup>4</sup> cfu/bird *Salmonella enteritidis*
<sup>2</sup> 5.0x10<sup>3</sup> cfu/bird *Salmonella enteritidis*
<sup>3</sup> NE: Not evaluated