Oral treatment with the probiotic *Escherichia coli* Nissle 1917 improves body weight and modulates the stress response of poultry in respiratory challenges with avian pathogenic *E. coli*\(^1\)

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Systemic infection with *Escherichia coli* (colibacillosis) is an important problem in poultry production and requires antibiotics for both treatment and prevention. A commercially available human probiotic, *E. coli* Nissle 1917 (EcN), which has been shown to stimulate innate immunity in mammals, was tested for its ability to prevent the effects of respiratory *E. coli* challenge in both chickens and turkeys. In Trial 1, broiler chicks were housed in battery brooders and were treated with an oral gavage of \(10^8\) cfu EcN at day of age, followed by the addition of \(10^8\)cfu EcN/bird/day in feed for 3 weeks. At 1 week of age birds were challenged by airsac inoculation of 250 cfu of a non-motile serotype 02 strain of avian pathogenic *E. coli* (APEC). In Trial 2 turkey poultts housed in battery brooders were treated with \(10^8\) cfu EcN/bird/day in feed for 3 weeks. Birds were challenged by either airsac inoculation with 150 cfu APEC at 1 week of age, followed by transport stress at 3 weeks of age, or were challenged by coarse spray of \(3\times10^8\) cfu APEC at 1 week of age followed by cold stress during weeks 1 and 2. A sample of birds from Trial 2 was bled for leukocyte differential counts. Birds in both trials were necropsied at 3 weeks of age. Pen means were analyzed by ANOVA using the general linear models procedure of SAS software. In Trial 1 percent mortality was decreased from 52% to 37% by EcN (\(P=0.1\)) and week 3 body weight (BW) gain (\(P=0.04\)) and total BW (\(P=0.1\)) were increased by EcN. In Trial 2, there was no effect of EcN on percent mortality, both stress challenges decreased BW and gain, cold stressed birds had lower BW than transport stressed birds, overall BW was increased by EcN (\(P=0.02\)) and EcN protected the BW of transport stressed birds (\(P=0.007\)). In Trial 2 both stress challenges decreased the percentage of lymphocytes in peripheral blood and increased the heterophil/lymphocyte (H/L) ratio, which is an accepted indicator of stress in birds. EvN prevented the decrease in lymphocyte percentage in cold stressed birds (\(P =0.01\)) and the increase in H/L ratio in both cold stressed (\(P =0.02\)) and transport stressed birds (\(P =0.03\)). These data suggest that this human *E. coli* isolate may modulate the stress response of birds and may have potential for development as an alternative to growth promoting antibiotics.

**Keywords:** Probiotic; Nissle 1917; poultry; *Escherichia coli*; antibiotic alternative

\(^1\)Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.
Introduction

The consumption of beneficial bacterial cultures to improve health and decrease pathogen contamination of poultry has had a long history and has been reviewed recently (Schneitz, 2005; Nava et al., 2005). It has been suggested that the immunomodulation provided by such probiotics may provide the poultry industry with a reliable alternative to antibiotics for reduction of the stress response and improvement of the safety of poultry products (Edens, 2003).

_E. coli_ Nissle 1917 (DSM 6601)(EcN) is the active compound of a microbial drug for use in humans (Mutaflor^TM_), commercially available in Central Europe for nearly 90 years. This _E. coli_ strain of human origin has been shown to be non-pathogenic and to stimulate innate immunity. The mechanisms detected so far include the upregulation of beta defensins (Wehkamp et al., 2004) and interference with bacterial invasion of intestinal epithelial cells (Altenhoefer et al., 2004), augmentation of the host defense of mice against both bacterial and fungal challenges (Hockertz, 1997), and clinical benefit in treatment of ulcerative colitis and Crohn’s disease (Malchow, 1997; Rembacken et al., 1999;Kruis et al., 2004). _E. coli_ Nissle 1917 has also been administered to both full-term and premature infants to prevent colonization of the intestine with pathogens and to stimulate the early development of immune defenses (Lodinova-Zadnikova and Sonnenborn, 1997; Cukrowska et al., 2002). In addition, EcN has been shown recently to prevent neonatal diarrhea in calves (von Buenau et al., 2005) and to reduce the shedding of _Salmonella enteritidis_ in chickens (Mohamed et al., 2004).

Airsacculitis, a respiratory disease which most frequently involves _E. coli_ infection, is the most important condemnation problem for all commercial poultry and _E. coli_ systemic infection (colibacillosis) is the most frequently reported poultry disease (Barnes et al., 2003). While colibacillosis has a complex etiology, host susceptibility is thought to be more important than bacterial virulence and stress has been shown to be associated with infection (Barnes et al., 2003). The objective of the following studies was to determine whether feed supplementation with the EcN strain would have efficacy in preventing the sequelae of stress-induced infection with an avian pathogenic _E. coli_.

Materials and methods

**Trial 1.** Day-old male Cobb broiler chicks were obtained from a commercial hatchery and were wing-banded and placed in battery brooder pens containing plastic-coated paper liners. Birds were provided _ad libitum_ access to water and to a corn and soybean broiler ration meeting or exceeding the NRC recommended allowances (National Research Council, 1994), and were kept under incandescent lighting on a light schedule consisting of 23 h day and 1 h night. The experimental design was a 2 x 2 factorial with 2 treatments and 2 challenges, each consisting of 3 replicate pens of 10 birds/pen. At one day of age, half of the birds were treated with an oral gavage of $10^8$ cfu of EcN, followed by the addition of $10^8$ cfu EcN/bird/day added to feed daily for 3 weeks. At 1 week of age half of the birds were challenged by airsac inoculation of 250 cfu of a non-motile serotype 02 strain of avian pathogenic _E. coli_ (APEC) originally isolated from a chicken with septicemia. Birds and feed were weighed weekly. All birds were necropsied at 3 weeks of age.

**Trial 2.** Day-old male Hybrid Converter turkey poultts were obtained from a commercial hatchery and were wing-banded and placed in battery brooder pens containing plastic-coated paper liners. Birds were provided _ad libitum_ access to water and a corn and soybean turkey ration meeting or exceeding the NRC recommended allowances (National Research Council, 1994), and were kept under incandescent lighting on a light schedule consisting of 23 h day and 1 h night. The experimental design was a 2 x 3 factorial with 2 treatments and 3 challenges, each consisting of 3 pens of 10 birds/pen. Half of the birds were provided with $10^5$ cfu EcN/bird/day added to feed daily for 3 weeks.

Birds in the cold stress challenge were brooded in a separate room. At 1 week of age the temperature of this room was lowered from 26.7° C to 15.5° C. Challenged birds were removed from their battery pens and placed into plastic baskets where they were inoculated with approximately 1 ml of a coarse spray of a $3 \times 10^8$ cfu culture of APEC sprayed directly on the eyes and nares. Birds
remained in the baskets for 2 hours and were then returned to their pens and the temperature was returned to 26.7°C. For the next 7 days cold stressed birds were given a 6 hour period of cold stress at an average of 15.5°C each day while control birds were maintained at 26.7°C. On day 14 birds were again challenged by coarse spray of approximately 3 ml of a 3 x 10^8 cfu/ml culture of APEC. During the next week the cold stress temperature was maintained at 12.6°C for 24 hours while the temperature in the control room was lowered to 24°C to maintain an ambient temperature.

Birds in the transport stress challenge were inoculated with 150 cfu APEC directly into the airsac at 1 week of age as previously described (Huff et al., 1998). These birds were also subjected to transport stress which occurred 12 d after the bacterial challenge and included a total of 12 hours of holding time in the transport vehicle. Birds were placed in transport coops by treatment group and were driven for 3 hours by truck and then were held in the truck for a further 8 hours. At the end of the transport stress birds were returned to their pens and were provided with feed and water.

The following morning 3 birds/pen were bled by cardiac puncture into EDTA tubes. All treated birds and untreated control birds from both stress treatments were bled at the same time, which was 1 day after transport and 13 days after challenge with E. coli. Total leukocyte counts (WBC) and the numbers and percentage of heterophils, lymphocytes, monocytes, eosinophils, and basophils were determined using a Cell-Dyn 3500 blood analysis system (Abbott Diagnostic Systems, Abbott, Il) which employs both electronic impedance and laser light scattering and has been standardized for analysis of turkey blood. Heterophil/lymphocyte ratios (H/L), an indicator of stress in birds (Gross and Siegel, 1983), were calculated by dividing the number of heterophils in 1 mL of peripheral blood by the number of lymphocytes. The next day all birds were weighed and necropsied. Pen means were analyzed by ANOVA using the general linear models procedure of SAS software. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Arkansas.

**Results and discussion**

In Trial 1 percent mortality of broiler chickens was decreased from 52% to 37% by EcN supplementation (P=0.1) (Figure 1). Week 3 body weight gain was increased by EcN (P = 0.04) (Figure 2) and total body weight at week 3 tended to be higher in EcN supplemented birds (P = 0.1) (Figure 3).

In Trial 2, mortality was not increased by either stress challenge and there was no effect of EcN on percent mortality (data not shown). Both stress challenges decreased body weight and gain (P = 0.0001) and cold stressed birds had lower BW than transport stressed birds; overall body weight was increased by EcN (P=0.02) (Figure 4), and EcN protected the body weight of transport stressed birds (P=0.007). In Trial 2 both stress challenges decreased the percentage of lymphocytes in peripheral blood and increased the heterophil/lymphocyte (H/L) ratio, which is an accepted indicator of stress in birds. EcN prevented the decrease in lymphocyte percentage in cold stressed birds (P =0.01) (Figure 5) and the increase in H/L ratio in both cold stressed (P =0.02) and transport stressed birds (P =0.03) (Figure 6).

![Figure 1](image_url). Trial 1. Percent mortality of broiler chickens supplemented with EcN tended to be lower than non-supplemented birds, P = 0.1. There was no mortality in non-challenged birds. Values are means ± SEM of 3 pens of 10 birds/ group.
Figure 2. Trial 1. Week 3 body weight gain was significantly higher in broiler chickens supplemented with EcN, \( P = 0.04 \). Values are means ± SEM of 6 pens of 10 birds/group. Means with no common superscript differ significantly (\( P \leq 0.05 \)).

Figure 3. Trial 1. Total body weight gain tended to be higher in broiler chickens supplemented with EcN, \( P = 0.1 \). Values are means ± SEM of 3 pens of 10 birds/group.

Figure 4. Trial 2. Week 3 body weight of turkey poults was significantly decreased by both stress treatments (0.0001). EcN prevented the decrease in body weight due to transport stress (\( P = 0.007 \)). The main effect mean body weight was increased from 550 g to 574 g by EcN (\( P = 0.05 \)). Values are means ± SEM of 3 pens of 10 birds/group. Means with no common superscript differ significantly (\( P \leq 0.05 \)).

Figure 5. Trial 2. The percentage of lymphocytes in peripheral blood of turkey poults was decreased by APEC + transport stress (\( P = 0.03 \)) and APEC + cold stress (\( P = 0.003 \)). EcN prevented the decrease in lymphocyte percentage of APEC + cold stress birds. Values are means ± SEM of 3 pens of 10 birds/group. Means with no common superscript differ significantly (\( P \leq 0.05 \)).
These data suggest that this *E. coli* probiotic can modulate the stress response of birds and may have potential for development as an alternative to growth promoting antibiotics. Opportunistic infections of poultry with pathogens such as APEC are prevented by the use of both prophylactic and therapeutic antibiotics. The stressors that are inherent in growing poultry quickly and inexpensively can impair the immune response and lead to disease and loss of production value. The recent ban of growth-promoting antibiotics in the European Union and the general trend toward limiting antibiotic usage in the rest of the world will require the development of alternatives that are as effective and consistent as antibiotics for poultry production to continue at its present pace.

Probiotics appear to have potential for replacing or supplementing antibiotics in poultry production, however most commercial probiotics are prepared from undefined mixed cultures of bacteria obtained from cecal contents of adult chickens (Schneitz, 2005). Maintaining the ecology of such mixed cultures can be a problem over time due to the inherent ability of bacteria to change and compete and may result in a high level of variability in the product. The ability of a single, safe, and well characterized bacterial strain such as EcN to modulate the stress response and improve production values in poultry is an important advantage in the development of a probiotic product that can help the industry in their search for antibiotic replacements.

References


