The Probiotic Escherichia coli Nissle 1917 Enhances Early Gastrointestinal Maturation in Young Turkey Poults


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Abstract: Concerns over the use of antibiotics as growth promoters in poultry production has led to interest in finding alternative growth promoters such as natural compounds and probiotics. Supplementing feed with probiotics has shown to enhance the Gastrointestinal Tract (GIT) development of chickens and turkeys. The human probiotic, E. coli Nissle 1917 (EC Nissle) has been shown to stimulate innate immunity in mammals and to increase body weight in poultry. However, the effect of this probiotic on GIT development has not been studied. The objective of this study was to evaluate the effect of EC Nissle in the maturation of the GIT of young turkey poults. Fifty-four day of hatch turkey poults were housed in battery brooders and fed either a standard diet or the same diet containing of 10⁶cfu EC Nissle /bird/day for 21 days. For GIT morphometric analysis, birds were euthanized on days 4, 7 or 21 and samples collected to evaluate villus height, villus surface area, lamina propria thickness, crypt depth and the number of neutral goblet cells. GIT morphometric analysis was conducted on duodenum, jejunum, ileum and cecum on days 4 and 7 and the duodenum on day 21. Villus height and villus surface of the GIT were higher in the EC Nissle treatments compared to control (p<0.05) on all sampling days with the exception of the jejunum and ileum on day 4. Lamina propria thickness and crypt depth were also increased in the EC Nissle treatment in all sections of the GIT except on day 4 in the jejunum. These data suggest that this human E. coli isolate enhanced the maturation of the GIT in young turkey poults and may have potential as an alternative to growth promoting antibiotics.

Key words: Probiotic, Nissle 1917, turkey poult, gastrointestinal tract, organic, E. coli

INTRODUCTION

The Gastrointestinal Tract (GIT) of poultry species is developmentally very active in the early period following hatch (Uni et al., 2000). The intestinal villi increase in diameter and length significantly during the first 7 to 10 d after hatching (Denbow, 2000; Sklan, 2001). The intestinal crypts form on the day of hatch and become defined in the first 48 to 96 h and continue to grow rapidly during the first seven days (Uni et al., 2000). Traditionally subtherapeutic levels of antibiotics have been used in poultry production as an inexpensive way of increasing growth and preventing disease and are thought to enhance gastrointestinal maturity (Dibner and Richards, 2005). However, the emergence of antibiotic resistance in pathogenic bacteria has led many countries to reconsider the use of antibiotics in animal agriculture (Thwaites and Frost, 1999; Van den Bogaard and Stobberingh, 2000; Bywater, 2005; Apata, 2009). In addition, organic producers need to develop strategies to promote gut health and limit disease/pathogens in birds without relying on restricted compounds. Organic poultry producers have a limited number of safe, effective and approved organic strategies to prevent and treat health problems in their flocks. Therefore, finding natural alternative ways to accelerate gastrointestinal maturation of newly hatched birds may be necessary to help replace antibiotics as growth promoters. Feeding live bacteria such as Lactobacillus and Bifidobacteria has been shown to benefit the development of the GIT and to keep the organism healthy in several species including poultry (Fuller, 1989; FAO/WHO, 2002; Mountzouris et al., 2010). After feeding of probiotics, improvements in growth performance and feed efficiency have been reported in broiler chickens (Cavazzoni et al., 1998; Jin et al., 1998; Zulkifli et al., 2000; Kabir et al., 2004; Mountzouris et al., 2007; Samli et al., 2007). The proposed modes of action of probiotics are not completely understood; however, it is hypothesized that their beneficial effects are created by out competing the pathogens via competitive exclusion...
and antagonism such as producing antimicrobial substances (Fuller, 1989), improving feed intake and digestion (Nahanshon et al., 1992, 1993) and altering bacterial metabolism (Cole et al., 1987; Jin et al., 1997).

The probiotic *E. coli* Nissle 1917 (EC Nissle) isolated in 1917 by Professor Alfred Nissle has been used to prevent and to treat an assortment of human gastrointestinal disorders for several decades (Malchow, 1997; Rembacken et al., 1999; Kruis et al., 2004; Matthes et al., 2010). EC Nissle is a non-pathogenic strain of human origin which has been shown to stimulate innate immunity in mammals (Wehkamp et al., 2004). Additionally, Mohamed et al. (2004) reported that the EC Nissle reduced shedding of *Salmonella* enteritidis in chickens. Very little information exists on how probiotics influence enteric physiology during early development. Thus, the objective of this study was to evaluate the effect of EC Nissle on GIT maturation in turkey poults.

**MATERIALS AND METHODS**

**Housing and experimental design:** All segments of this project complied with the provisions of the Institutional Animal Care and Use Committee at the University of Arkansas, as specified by the Animal and Plant Health Inspection Service, USDA in 9 CFR Part 1(1-91).

Fifty four day of age male Hybrid Converter turkey poults were obtained from a commercial hatchery at day of hatch. All birds were wing-banded and randomly placed into brooder battery pens. Birds were maintained under 23 h light and 1 h dark. Birds were placed into one of two treatments; control birds were fed a standard diet for the duration of the study while the EC Nissle birds received the same diet with the addition of 10^8 cfu EC Nissle/bird/day. Feed and water were provided *ad libitum* for the duration of the study.

**Morphometric analysis of the gut:** For enteric morphometric analysis, birds were euthanized on days 4, 7 or 21 (9 poults/treatment/day) and samples from the small intestines were collected. A 1-cm segment from the duodenum, jejunum, ileum and ceca were removed (at the midpoint) and fixed in 10% buffered formalin for 72 h for day 4 and 7 birds. Only the duodenum samples were prepared for the day 21 sampling. In previous studies evaluating the influence of a yeast extract on early GIT maturation in turkey poults, no difference was detected at day 21 in duodenum samples (Solis de los Santos et al., 2007). Although the focus of the current paper centered on early maturation we added these samples at day 21 to determine if EC Nissle continues to influence GIT development as the birds mature. Segments were subsequently embedded in paraffin and a 2-µm section of each sample was placed on a glass slide and stained with hematoxylin and eosin for examination with a light microscope (Sakamoto et al., 2000). The parameters evaluated were: villus height, villus base, villus surface area, lamina propria thickness, crypt depth and the number of goblet cells. Morphological parameters were measured using the Image Pro Plus v 4.5 software package. Fourteen measurements were taken per GIT section/bird per parameter. Villus height was measured from the top of the villus to the top of the lamina propria. Villus surface area was calculated using the formula (2B) (WV/2) (VL), where WV = villus width and VL = villus length (Sakamoto et al., 2000). The lamina propria thickness was measured from the base of the villus to the top of the muscularis mucosa. Crypt depth was measured from the base upwards to the region of transition between the crypt and villus (Aptekmann et al., 2001).

**Goblet cells quantification:** Neutral goblet cells were detected by staining 5-µm sections with Periodic Acid Schiff (PAS) stain as described previously (McManus, 1948; Solis de los Santos et al., 2005) with minor variations. Following deparaffinization and dehydration, slides were incubated in 0.5% periodic acid for 5 min, washed and incubated with Schiff’s reagent (Sigma Chemical Co., St. Louis, MO) for 20 min and then counter stained with hematoxylin for 5 min. The number of PAS positive cells staining a red color (PAS+) along the villi was counted by light microscopy as described previously (Uni et al., 2005). The number of goblet cells per villus was counted after the staining in 10 well-oriented crypt-villus units of the duodenum, jejunum, ileum and ceca respectively, per bird on days four and seven posthatch.

**Statistical analysis:** Gut morphology, BW data and gut weight data were subjected to ANOVA using SAS (SAS Institute, 2002). Mean separation was accomplished using Duncan’s multiple range tests; p<0.05 was considered significant.

**RESULTS AND DISCUSSION**

**Body weight:** Body weights did not differ between the untreated birds and those treated with the probiotic. On day 4, the mean body weight in the untreated birds and treated birds was similar (84.2 g). On day 7, the mean body weight was 120.6 g in the control birds compared to 125.8 g in the birds receiving the probiotic treatment. At 21 days of age the control birds weighed 604.4 g while the birds treated with EC Nissle weighed 615.2 g (p = 0.055). While the body weights of poults supplemented with the human probiotic EC Nissle were not significantly different from the control group on any of the evaluation days, the GIT architecture was significantly enhanced by supplementation with EC Nissle when evaluated on days 4 and 7 and duodenum on day 21. One possible reason for the lack of difference in body weights is that these birds were a small subset of a larger study which did find that EC Nissle improved weights (Huff et al., 2006).
Fig. 1: The effect of feeding EC Nissle (ECN) on villus height and villus surface area in the gut of turkey poults at 4 and 7 days of age. N = 9 poults/treatment.

Villus height (µm) and villus surface area (µm²): Intestinal villi are structures that protrude from the intestinal lining and are thought to have the utmost impact in the nutrition process of the GIT. Intestinal villi...
increase the wall surface area available for the digestion and absorption of amino acids and sugars (Caspari, 1992). Longer villi increase the absorption area and also stimulate the expression of brush border enzymes and nutrient transport systems (Pluske et al., 1996). The villi are filled with blood vessels where the circulating blood takes away those nutrients.

In this study, the intestinal villus height was significantly increased in the duodenum, jejunum, ileum and ceca on day 4 and in the duodenum, jejunum, ileum and ceca on day 7 by supplementing the diet with the probiotic EC Nissle (Fig. 1). This increase in villus height resulted in a corresponding increase in the villus surface area on both days evaluated in all areas measured, with the exception of the jejunum on day 4 (Fig. 1). This increase in villus height and surface area may explain the heavier gut weight observed on day 4 (data not shown). Chichlowski et al. (2007) reported that feeding a probiotic containing Lactobacilli casei, Lactobacillus acidophilus, Bifidobacterium thermophilum and Enterococcus faecium to broilers increased jejunal villus height when compared to the control birds. Longer villi were found in the ileum of adult male layers along with a slight improvement in feed efficiency after dietary addition of Bacillus subtilis var. natto (Samanya and Yamauchi, 2002) and in broilers after addition of E. faecium (Samli et al., 2007) or Eubacterium sp. (Awad et al., 2006). The increase in villus height and villus surface area may provide poult fed EC Nissle a greater ability to absorb nutrients. This superior ability to absorb nutrients would explain the results seen by Huff et al., (2006) where they reported an increase in growth rate from feeding EC Nissle.

**Lamina propria thickness (µm):** In the present study, the supplementation of the human probiotic EC Nissle significantly increased the lamina propria thickness, crypt depth and the number of goblet cells.

Adding EC Nissle to the diet of young poult resulted in an increase in the thickness of the lamina propria by day 4 in all sections of the GIT measured with the exception of the jejenum (Table 1). By day 7 all areas of the GIT had a thicker lamina propria when supplemented with EC Nissle. Probiotics, in addition to enhancing the intestinal villus and surface area and having a direct impact on nutritional aspects such as feed digestion and absorption, have also demonstrated an immunomodulating effect (Kabir et al., 2004; Koenen et al., 2004; Farnell et al., 2006; Chichlowski et al., 2007; Teo and Tan, 2007). The lamina propria thickness can be used as an indicator of gut health because the lamina propria contains dendritic cells that survey the contents of the lumen and protect against infection by stimulating the adaptive immune response (Macpherson and Harris, 2004). The lamina propria is thought to exert a protective effect due to the quantity and variety of immune cells that it contains and therefore an increase in the thickness may result in improved immunological responses.

**Crypt depth (µm):** The intestinal crypts produce goblet cells and enterocytes (Ayabe et al., 1999; Geyra et al., 2003), which migrate up along the surface of the villus and slough off every 48 to 72 h in the lumen of the intestine (Imondi and Bird, 1966; Potten, 1998); thus, a deeper crypt may suggest higher production of the regenerative cells and therefore, may allow the organism to grow faster (Fan et al., 1997; Yason et al., 1987; Anonymous, 1999; Awad et al., 2009). In this study the addition of EC Nissle resulted in deeper crypt depth on days 4 and 7 in all sections of the GIT, except the jejunum on day 4 (Table 1).

**Goblet cells (count):** The results of the inclusion of EC Nissle on goblet cell numbers are shown in Fig. 2. These results show that on day 4 there was an increased number of goblet cells in the duodenum, jejunum and cecum but not in the ileum. By day 7 there was no difference in the goblet cell numbers. Goblet cells produce mucin, a sticky glycoprotein, that coats and protects the brush border of the villus from damage and acts as a transporter of nutrients from the lumen to the villus (Uni et al., 2003). Additionally, this sticky glycoprotein can trap bacteria and prevent them from entering and invading the organism (Vimal et al., 2000) and allow for competition between pathogenic

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### Table 1: Effect of the probiotic EC Nissle on lamina propria thickness (µm) and crypt depth (µm) of turkey poult at 4 and 7 days of age. N = 9 poult/treatment/day

<table>
<thead>
<tr>
<th>Lamina Propria (µm)</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Ceca</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>109.4±05.1a</td>
<td>137.9±09.7b</td>
<td>98.1±10.9b</td>
<td>56.0±04.2b</td>
</tr>
<tr>
<td><strong>EC Nissle</strong></td>
<td>119.9±06.2a</td>
<td>97.8±07.2b</td>
<td>102.2±05.9a</td>
<td>92.2±04.6b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crypt depth (µm)</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Ceca</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>153.2±05.9b</td>
<td>92.2±04.6b</td>
<td>97.4±07.8b</td>
<td>138.1±06.3a</td>
</tr>
<tr>
<td><strong>EC Nissle</strong></td>
<td>205.3±10.4a</td>
<td>102.2±05.9a</td>
<td>84.6±03.7a</td>
<td>152.3±11.3a</td>
</tr>
</tbody>
</table>

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Fig. 2: Effect of the EC Nissle probiotic (ECN) on gastrointestinal neutral goblet cell number. N = 9 poults/treatment/day

Fig. 3: Effect of the EC Nissle probiotic (ECN) on duodenum at day 21. N = 9 poults/treatment
and beneficial bacteria (Craven and Williams, 1998). In addition, mucus produced by goblet cells may also protect against compounds that could damage the intestinal mucosa (Forstner, 1978; Specian and Oliver, 1991). Furthermore, the mucin lubricates the GIT lining (Forstner, 1978). These benefits of EC Nissle on goblet cells may improve during early GIT development but were not apparent at day 7 in this study.

**Day 21 duodenum development:** Previously Solis de los Santos *et al.* (2007) found that feeding a yeast extract did not enhance 21 day development of the duodenum in turkey poults. To determine if EC Nissle produced similar results the duodenum was evaluated on day 21 (Fig. 3). By adding EC Nissle to the feed, all parameters measured were enhanced, suggesting that EC Nissle can continue to benefit the development of the duodenum for a long period of time.

The results of this study suggest that feeding probiotics may enhance the early maturation of the GIT of poults. In addition, supplementing probiotics may stimulate the development of the immunomodulatory structure of the GIT of turkey poults. The inclusion of EC Nissle in the diet of young poults enhanced the development of the GIT, suggesting that EC Nissle may be useful as a growth promoter. Because EC Nissle is a natural product these results are of particular use to organic poultry producers as it gives them another resource to help in improving production.

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**REFERENCES**


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