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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 10^8cfu 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 poults, 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

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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 nonpathogenic 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 incandescent lighting on a light schedule consisting of 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 (2π) (VW/2) (VL), where VW = 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*., 2007) 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*., 2003). 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; $p \leq 0.05$ 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).

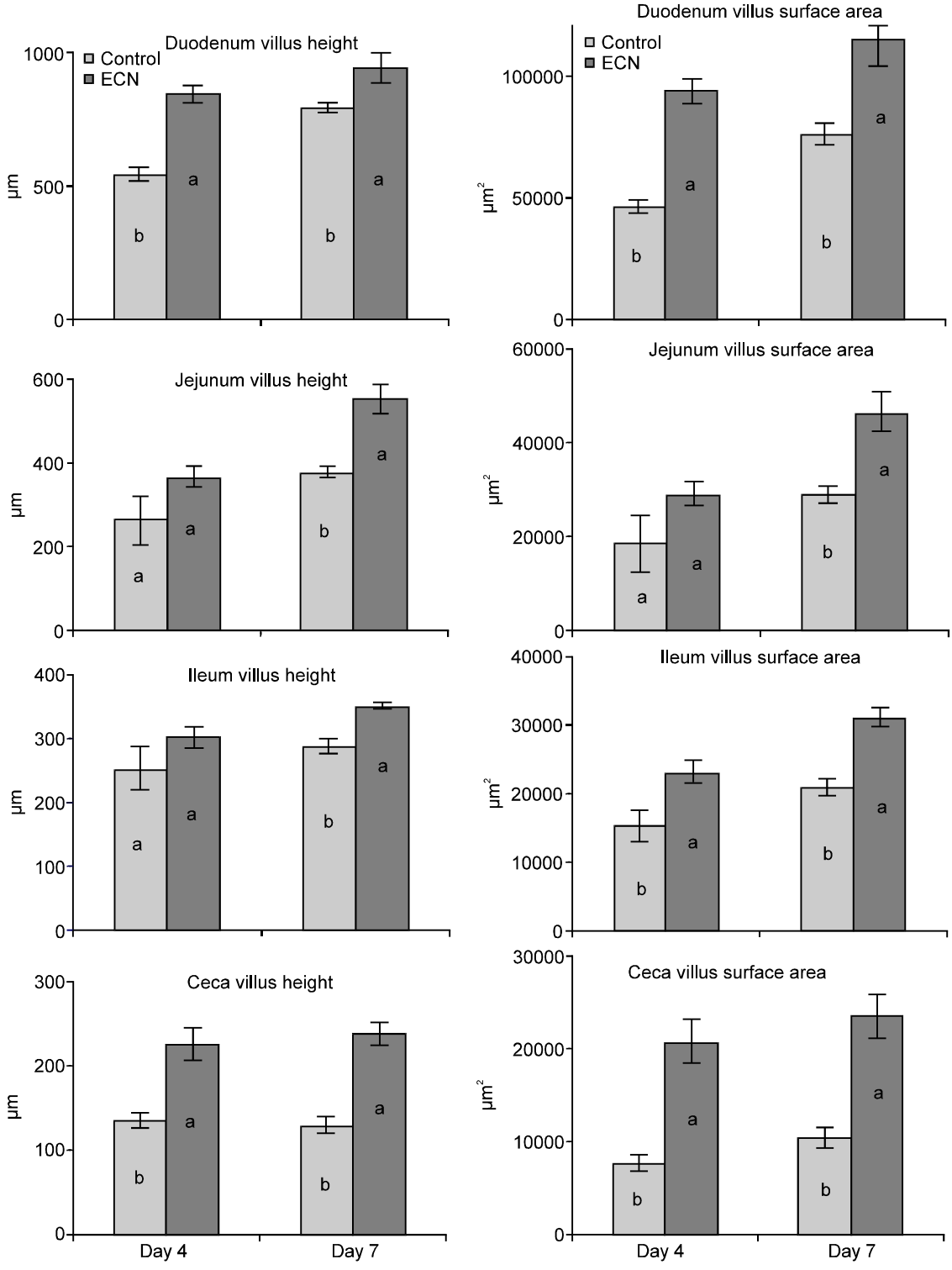

Fig. 1: The effect of feeding EC Nissle (ECN) on villus height and villus surface area in the gut of turkey poults at 4 and 7 days of age. N = 9 poults/treatment/

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 (Caspary, 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 villi height was significantly increased in the duodenum 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 the 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 poults 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 poults 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 jejunum (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.

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

	Day 4	Day 7
Lamina Propria (µm)		
Duodenum		
Control	109.4±05.1^{b}	94.1±05.9^{b}
EC Nissle	137.9±09.7^{a}	138.5±09.7^{a}
Jejunum		
Control	72.5±10.2^{a}	72.4±05.7^{b}
EC Nissle	98.1±10.9^{a}	111.0±04.6^{a}
Ileum		
Control	69.2±07.8^{b}	55.9±02.7^{b}
EC Nissle	102.2±05.9^{a}	102.9±10.4^{a}
Ceca		
Control	56.0±04.2^{b}	62.7±04.7^{b}
EC Nissle	92.2±04.6^{a}	86.4±05.6^{a}
Crypt depth (µm)		
Duodenum		
Control	153.2±05.9^{b}	144.7±05.4^{b}
EC Nissle	205.3±10.4^{a}	173.3±10.7^{a}
Jejunum		
Control	95.3±13.5^{a}	109.3±05.2^{b}
EC Nissle	113.2±04.8^{a}	205.1±12.8^{a}
Ileum		
Control	97.4±07.8^{b}	84.6±03.7^{b}
EC Nissle	138.1±06.3^{a}	152.9±11.3^{a}
Ceca		
Control	73.2±04.7^{b}	67.3±06.6^{b}
EC Nissle	119.9±06.2^{a}	97.8±07.2^{a}

Crypt depth (µm): The intestinal crypts produce goblet cells and enterocytes (Ayabe *et al*., 2000; Geyra *et al*., 2001), 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 villa from damage and acts as a transporter of nutrients from the lumen to the villa (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

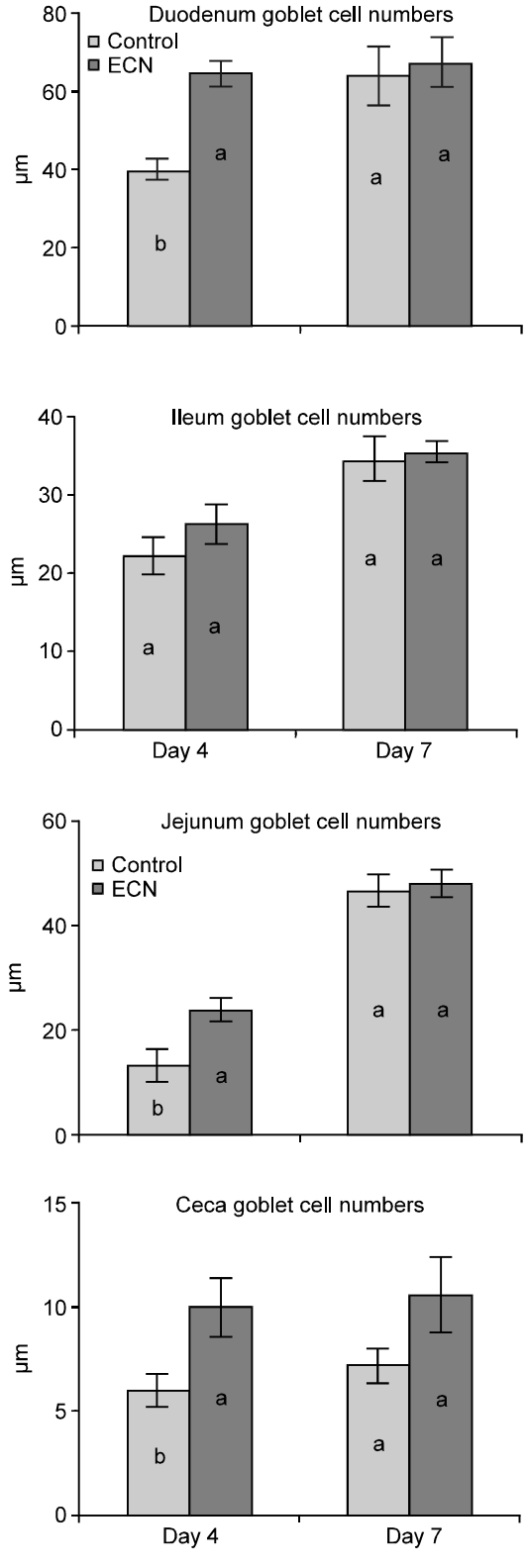

Fig. 2: Effect of the EC Nissle probiotic (ECN) on gastrointestinal neutral goblet cell number. N = 9 poults/treatment/day

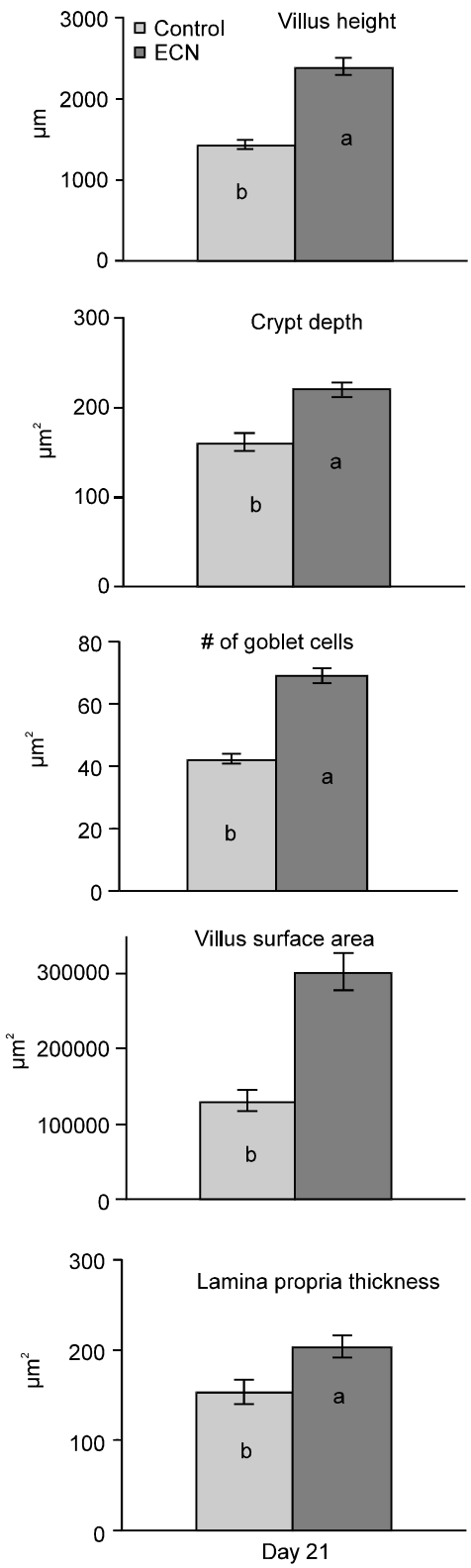

Fig. 3: Effect of the EC Nissle probiotic (ECN) on duodenum at day 21. N = 9 poults/treatment

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

Anonymous, 1999. How do mannanoligosaccharides work? Feed Times, 1: 7-9.

Apata, D.F., 2009. Antibiotic resistance in poultry. Int. J. Poult. Sci., 8: 404-408.

Aptekmann, K.P., S.M. Baraldi Arton, M.A. Stefanini and M.A. Orsi, 2001. Morphometric analysis of the intestine of domestic quails (*Coturnix coturnix japonica*) treated with different levels of dietary calcium. Anat. Histol. Embryol., 30: 277-280.

Awad, W.A., J. Bohm, E. Razzazi-Fazeli, K. Ghareeb and J. Zentek, 2006. Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult. Sci., 85: 974-979.

Awad, W.A., K. Ghareeb, S. Abdel-Raheem and J. Bohm, 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. Poult. Sci., 88: 49-55.

Ayabe, T., D.P. Satchell, C.L. Wilson, W.C. Parks, M.E. Selsted and A.J. Ouelette, 2000. Secretion of microbicidal defensins by intestinal Paneth cells in response to bacteria. Nat. Immunol., 1: 113-118.

Bywater, R.J., 2005. Identification and surveillance of antimicrobial resistance dissemination in animal production. Poult. Sci., 84: 644-648.

Caspary, W.F., 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr., 55: 299S-308S.

Cavazzoni, V., A. Adami and C. Cstrivilli, 1998. Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. Br. Poult. Sci., 39: 526-529.

Chichlowski, M., J. Croom, B.W. McBride, L. Daniel, G. Davis and M.D. Koci, 2007. Direct-fed microbial PrimaLac and salinomycin modulate whole-body and intestinal oxygen consumption and intestinal mucosal cytokine production in the broiler chick. Poult. Sci., 86: 1100-1106.

Cole, C.B., R. Fuller and M.J. Newport, 1987. The effect of diluted yogurt on the gut microbiology and growth of piglets. Food Microbiol., 4: 83-85.

Craven, S.E. and D.D. Williams, 1998. *In vitro* attachment of *Salmonella typhimurium* to chicken cecal mucus: Effect of cations and pretreatment with *Lactobacillus* spp. isolated from the intestinal tracts of chickens. J. Food Prot., 61: 265-271.

Denbow, D.M., 2000. Gastrointestinal anatomy and physiology. In Sturkie's Avian Physiology. 5th Edn., Ed. G.C. Whittow, Academic Press, San Diego, pp: 299-321.

Dibner, J.J. and J.D. Richards, 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci., 84: 634-643.

Fan, Y., J. Croom, V. Christensen, B. Black, A. Bird, L. Daniel, B. McBride and E. Eisen, 1997. Jejunal glucose uptake and oxygen consumption in turkey poults selected for rapid growth. Poult. Sci., 76: 1738-1745.

FAO/WHO, 2002. Working group report on drafting guidelines for the evaluation of probiotics in food. 30 April-1 May, London, UK and Ontario, Canada. FAO, Rome, Italy.

Farnell, M.B., A.M. Donoghue, F.S. De Los Santos, P.J. Blore, B.M. Hargis, G. Tellez and D.J. Donoghue, 2006. Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. Poult. Sci., 85: 1900-1906.

Forstner, J.T., 1978. Intestinal mucins in health and disease. Digestion, 17: 234-263.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365-378.

Geyra, A., Z. Uni and D. Sklan, 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci., 80: 776-782.

Huff, G.R., W.E. Huff, C. Enders, U. Sonnenborn, N.C. Rath, M.B. Farnell, F. Solis De Los Santos and A.M. Donoghue, 2006. 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*. http://www.cabi.org/animalscience/Uploads/File/AnimalScience/additionalFiles/WPSAVerona/10388.pdf accessed July 6, 2012.

Imondi, A.R. and F.H. Bird, 1966. The turnover of intestinal epithelium in the chick. Poult. Sci., 45: 142-147.

Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin, 1997. Probiotics in poultry: Modes of action. World's Poult. Sci. J., 53: 352-368.

Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin, 1998. Growth performance, intestinal microbial populations and serum cholesterol of broiler's diets containing *Lactobacillus* cultures. Poult. Sci., 77: 1259-1265.

Kabir, S.M.L., M.M. Rahman, M.B. Rahman and S.U. Ahmed, 2004. The dynamics of probiotics on growth performance and immune response in broilers. Int. J. Poult. Sci., 3: 361-364.

Koenen, M.E., J. Kramer, R. Van Der Hulst, L. Heres, S.H.M. Jeurissen and W.J.A. Boersma, 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. Br. Poult. Sci., 45: 355-366.

Kruis, W., P. Fric, J. Pokrotnieks, M. Lukas, B. Fixa, M. Kascak, M.A. Kamm, J. Weismuller, C. Beglinger, M. Stolte, C. Wolff and J. Schulze, 2004. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. Gut, 53: 1617-1623.

Macpherson, A.J. and N.L. Harris, 2004. Interactions between commensal intestinal bacteria and the immune system. Nat. Rev. Immunol., 4: 478-485.

Malchow, H.A., 1997. Crohn's disease and *Escherichia coli*. A new approach in therapy to maintain remission of colonic Crohn's disease? J. Clin. Gastroenterol., 25: 653-658.

Matthes, H., T. Krummenerl, M. Giensch, C. Wolff and J. Schulze, 2010. Clinical trial: Probiotic treatment of acute distal ulcerative colitis with rectally administered *Escherichia coli* Nissle 1917 (EcN). BMC Com. Alt. Med., 10: 13.

McManus, J.F.A., 1948. Histological and histochemical uses of periodic acid. Stain Technol., 23: 99-108.

Mohamed, I., W. Schroedl, H.M. Hafez and M. Krueger, 2004. Investigations on the probiotic effects of *Escherichia coli* strain Nissle 1917 after oral infection with *Salmonella enteritidis*. Proc. Soc. Nutr. Physiol., 13: 144.

Mountzouris, K.C., P. Tsistsikos, E. Kalamara, S. Nitsh, G. Schatzmayr and K. Fegeros, 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Pediococcus* strains in promoting broiler performance and modualting cecal microflora composition and metabolic activities. Poult. Sci., 86: 309-317.

Mountzouris, K.C., P. Tsitrsikos, I. Palamidi, A. Arvaniti, M. Mohnl, G. Schatzmayr and K. Fegeros, 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins and cecal microflora composition. Poult. Sci., 89: 58-67.

Nahanshon, S.N., H.S. Nakaue and L.W. Mirosh, 1992. Effects of direct fed microbials on nutrient retention and parameters of laying pullets. Poult. Sci., 71(Suppl. 1):111 (Abstr.).

Nahanshon, S.N., H.S. Nakaue and L.W. Mirosh, 1993. Effects of direct fed microbials on nutrient retention and parameters of Single White Leghorn pullets. Poult. Sci., 72(Suppl. 2):87 (Abstr.).

Pluske, J.R., M.J. Tompson, C.S. Atwood, P.H. Bird, I.H. Williams and P.E. Hartmann, 1996. Maintenance of villus height and crypt depth and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. Br. J. Nutr., 76: 409-422.

Potten, C.S., 1998. Stem cells in the gastrointestinal epithelium: Numbers, characteristics and death. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 353: 821-830.

Rembacken, B.J., A.M. Snelling, P.M. Hawkey, D.M. Chalmers and A.T.R. Axon, 1999. Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: A randomised trial. Lancet, 354: 635-639.

Sakamoto, K., H. Hirose, A. Onizuka, M. Hayashi, N. Futamura, Y. Kawamura and T. Ezaki, 2000. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. J. Surg. Res., 94: 99-106.

Samanya, M. and K. Yamauchi, 2002. Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. *natto*. Comp. Biochem. Physiol., 133: 95-104.

Samli, H.E., N. Senkoylu, F. Koc, M. Kanter and A. Agma, 2007. Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and microbiota. Arch. Anim. Nutr., 61: 42-49.

SAS Institute, 2002. SAS/STAT User's guide: Release 9.03 Edition. SAS Institute Inc., Cary, NC.

Sklan, D., 2001. Development of the digestive tract of poultry. Worlds Poult. Sci. J., 57: 415-428.

Solis de los Santos, F., A.M. Donoghue, M.B. Farnell, G.R. Huff, W.E. Huff and D.J. Donoghue, 2007. Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune™). Poult. Sci., 86: 921-930.

Specian, D. and M. Oliver, 1991. Functional biology of intestinal goblet cells. Am. J. Physiol., 260(Cell Physiol. 29): C183-C193.

Thwaites, R.T. and J.A. Frost, 1999. Drug resistance in campylobacter jejuni, *C. coli* and *C. lari* isolated from humans in North West England and Wales. J. Clin. Pathol., 52: 812-814.

Teo, A.Y. and H.M. Tan, 2007. Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6 (CloSTAT). J. Appl. Poult. Res., 16: 296-303.

Uni, Z., A. Geyra, H. Ben-Hur and D. Sklan, 2000. Small intestinal development in the young chick: Crypt formation and enterocyte proliferation and migration. Br. Poult. Sci., 41: 544-551.

Uni, Z., A. Smirnov and D. Sklan, 2003. Pre- and posthatch development of goblet cells in the broiler small intestine: Effect of delayed access to feed. Poult. Sci., 82: 320-327.

Van den Bogaard, A.E. and E.E. Stobberingh, 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. Int. J. Antimicrob. Agents, 14: 327-335.

Vimal, D.B., M. Khullar, S. Gupta and N.K. Ganguly, 2000. Intestinal mucins: The binding sites for *Salmonella typhimurium*. Mol. Cell. Biochem., 204: 107-117.

Yason, C.V., B.A. Summers and K.A. Schat, 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. Am. J. Vet. Res., 6: 927-938.

Wehkamp, J., J. Harder, K. Wehkamp, B. Wehkamp-Von Meissner, M. Schlee, C. Enders, U. Sonnenborn, S. Nuding, S. Bengmark, K. Fellermann, J.M. Schroder and F.F. Stange, 2004. NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by Escherichia coli Nissle 1917: A novel effect of a probiotic bacterium. Infec. Immun., 72: 5750-5758.

Zulkifli, I., N. Abdullah, N.M. Azrin and Y.W. Ho, 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci., 41: 593-597.