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Effect of Indigenous Probiotics Lactic Acid Bacteria on the Small Intestinal Histology Structure and the Expression of Mucins in the Ileum of Broiler Chickens

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Abstract: Intestinal mucous containing mucins play an essential role as mucosal barrier to prevent invasion in the intestinal tissue of broilers. The aim of the study was to investigate the effect of supplementation of indigenous probiotics lactic acid bacteria on small intestinal histology structure and expression of mucins in the ileum of broiler chicken raised for 35 days. A total of 60 day old chick Lohmann strain broilers were randomly divided into four treatment groups, namely T0, T1, T2 and T3. The T0 group was raised with unsupplemented probiotics, while T1, T2 and T3 were orally supplemented multistrain probiotics at concentration 10^7, 10^8, 10^9 CFU/ml/bird/day, respectively. The results showed that supplementation of indigenous lactic acid bacteria probiotics significantly ($P<0.05$) increased villus height, villus width of duodenum, jejunum and ileum, as well as increased expression of *mucin* mRNA in the ileum compared to the control one in broilers. This results suggest that probiotics may stimulate proliferation of intestinal epithelium and regulate mucosal barrier formed by mucin in the intestine of broiler chickens.

Key words: Broilers, probiotics, small intestinal histology structure, mucins

INTRODUCTION

The mucosal tissue of the oviduct fulfills defense functions that are essential for maintaining its health. Mucosal epithelial cells form a contagious lining that acts as a barrier against the moist exterior environment. The surface of the epithelial cell lining is covered by a mucus layer which protects the underlying epithelium from pathogenic microorganisms (Corfield *et al*., 2000; Perez-Vilar, 2007; Linden *et al*., 2008). Mucins are composed of glycoproteins and secreted by mucosal epithelium (Gendler and Spicer, 1995; Linden *et al*., 2008). The presence of this mucous layer prevents bacterial translocations, because gut pathogens must pass through this mucous layer before adherence to and invade the epithelial cells. Studies showed various interactions between intestinal mucin and intestinal microflora (Gork *et al*., 1999). *Lactobacillus* strains adhered to chicken intestinal mucin (Gusils *et al*., 2003), in addition Johnson *et al*. (2001) showed that the presence of mucin in the growth medium initiates mucin binding properties in several strain of *Lactobacillus*. Other studies indicated that mucin was a site for bacterial adhesion (Vimal *et al*., 2000), with subsequent competition between pathogenic and beneficial bacteria (Pascual *et al*., 1999).

Supplementation of indigenous lactic acid bacteria probiotics affected significantly the productive performance and the short chain fatty acids production such as propionate and butyrate in the ileum and caecum of broiler chicken (Sri-Harimurti *et al*., 2013). The short chain fatty acids, as metabolite products of bacterial fermentation, have ability to stimulate the proliferation of epithelial cells of the intestine (Gunal *et al*., 2006). Thus, the goal of this study was to determine the effect of supplementation of indigenous probiotics lactic acid bacteria on small intestinal histology structure and expression of mucins in the ileum of broilers.

MATERIALS AND METHODS

Experimental birds: The probiotics consisted of three indigenous lactic acid bacteria strains *Lactobacillus murinus* (Ar3), *Streptococcus thermophilus* (Kp2) and *Pediococcus acidilactici* (Kd 6). A total of 60 day old chick Lohmann strain broilers were randomly divided into four treatment groups: T0, T1, T2 and T3. The T0 group was raised with unsupplemented probiotics, while T1, T2 and T3 were orally supplemented multistrain probiotics at concentration 10^7, 10^8, 10^9 CFU/ml/bird/day, respectively. All of treatment groups were replicated three times, with five chickens each. The broiler diet was formulated to meet the National Research Council recommendation, without antibiotic and coccidiostat. Feed and drinking water were provided *ad libitum*. To study the intestinal histology structure (histological process followed haematoxylin-eosin stained) and mucins in the ileum of birds, three birds from each treatment group were randomly sacrificed on 5th week of age.

Quantitative reverse-transcription PCR analysis for expression of mucins: Quantitative reverse-transcription PCR analysis was performed as described previously

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(Ariyadi *et al*., 2012). Briefly, total RNA was extracted from the mucosal tissues of ileum using Sepasol RNA I Super (Nacalai Tesque Inc., Kyoto, Japan). The extracted total RNA samples were dissolved in TE buffer (10 mM Tris, pH 8.0, with 1 mM EDTA). They were treated with 1 U of RQ1 RNase-free DNase (Promega Co., Madison, WI) on a PTC-100 programmable thermal controller (MJ Research Inc., Waltham, MA), programmed at 37°C for 45 min and 65°C for 10 min. The concentration of RNA in each sample was measured using Gene Quant Pro (Amersham Pharmacia Biotech, Cambridge, UK).

RNA samples were reverse-transcribed using ReverTra Ace (Toyobo Co. Ltd., Osaka, Japan) according to the manufacturer's instructions. The reaction mixture (10 µL) consisted of 1 µg of the total RNA, 1 x RT buffer, 1 mM dNTP mixture, 20 U of RNase inhibitor, 0.5 µg oligo(dT)20 primer and 50 U ReverTra Ace. Reverse transcription was performed at 42°C for 30 min, followed by heat inactivation for 5 min at 99°C using the PTC-100 Programmable Thermal Controller (MJ Research Inc.). PCR was performed using Takara Taq (Takara Bio Inc., Shiga, Japan) according to the manufacturer`s protocol. Primers used in this study are shown in Table 1. The PCR mixture (25 µL) contained 0.5 µL cDNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 1.25 U Takara Taq and 0.5 µM each primer. Mucin was amplified in a PTC-100 Programmable Thermal Controller (MJ Research Inc.) under the following conditions: 94°C for 30 sec, then 34 cycles at 95°C for 30 sec to denature, 58°C for 60 sec to anneal, 72°C for 60 sec for extension. The PCR products were separated by electrophoresis on a 2% (w/v) agarose gel containing 0.4% (w/v) ethidium bromide.

Table 1: Primer Sequences for Mucin and RPS-17

Target genes	Sequences 5' - 3'	Accession Number
Mucin	F: TCT TCC GCT ACC CTG GGC TCT GTAA R: CTC ATG CAG TTC TAG CAA GAT ACT	GI_45125071
RPS-17	F: AAG CTG CAG GAG GAG GAG AGG R: GGT TGG ACA GGC TGC CGA AGT	NM_204217

Statistical Analysis: Fold changes in the mucin expressions were expressed as the mean ± SEM. The data were analyzed by one way ANOVA of Completely Randomized Design (CRD) followed by Duncan New Multiple Range Test (DMRT). Differences were considered significant at P<0.05.

RESULTS

After 28 days of probiotics supplementation to broilers the villi height, villi width, crypth depth of duodenum, jejunum and ileum of them were improved significantly (P<0.05) compared to the control groups (unsupplementation probiotics) as presented in the Table 2.

Table 2: Effect of probiotics supplementation on intestinal morphology in 35-day old broiler

Parameter	T0	T1	T2	T3
Duodenum				
Villus height (mm)	497.23^{a}	697.20^{b}	713.87^{b}	688.87^{b}
Villus width (mm)	73.33^{a}	104.47^{b}	122.20^{b}	111.13^{b}
Crypt depth (mm)	90.53^{a}	141.70^{b}	125.03^{b}	134.46^{b}
Jejunum				
Villus height (mm)	558.33^{a}	811.32^{b}	791.66^{b}	775.56^{b}
Villus width (mm)	75.53^{a}	136.10^{c}	119.43^{b}	122.23^{b}
Crypt depth (mm)	92.80^{a}	113.90^{b}	120.57^{b}	114.43
Ileum				
Villus height (mm)	516.66^{a}	738.90^{b}	747.23^{b}	722.23^{b}
Villus width (mm)	69.97^{a}	132.20^{b}	113.90^{b}	121.67^{b}
Crypt depth (mm)	76.10	108.33	114.43	123.86

^{abcd} Means values with different superscripts differ significantly (P<0.05)

Figure 1 shows effect of supplementation of indigenous probiotics lactic acid bacteria on the relative expression of *mucin* mRNA of ileum in the broilers. Expression of *mucin* mRNA in the ileum were higher in T1, T2 and T3 compared to the T0 (control) group.

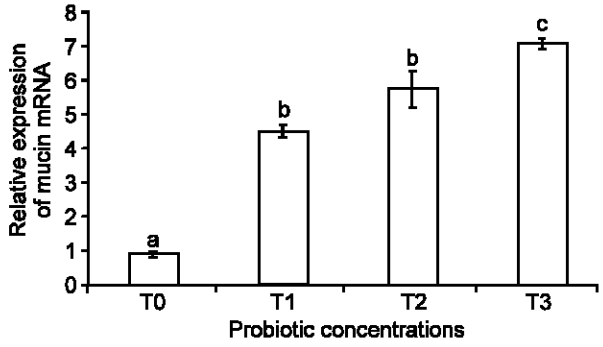

Figure 1: Effect of supplementation of indigenous probiotics lactic acid bacteria on the relative expression of *mucin* mRNA in the ileum of broilers. Expression of *mucin* mRNA in the ileum were higher in T1, T2 and T3 compared to the T0 group. Values are the mean ± SEM of fold change. Values with different letters (a-c) are significantly different among T0, T1, T2 and T3. (P<0.05). T0 = un supplemented probiotics, T1, T2, T3 = probiotics at concentrations of 10^7, 10^8, 10^9 CFU, respectively.

DISCUSSION

We here identify effect of indigenous probiotics lactic acid bacteria on the intestinal histology structure and the expression of mucins in the ileum of broiler chickens. Significant findings were: (1) the villi height, villi width, crypth depth of duodenum, jejunum and ileum were improved significantly (P<0.05) compared to the control groups (unsupplementation probiotics), (2) expression of *mucin* mRNA in the ileum were higher in T1, T2 and T3 compared to the T0 (control) group.

It was supported by Gunal *et al.* (2006) and Sri-Harimurti *et al.* (2013) that oral supplementation of mixture *Lactobacillus murinus* (Ar3), *Streptococcus thermophilus* (Kd2) and *Pediococcus acidilactici* (Kp6) as probiotics, increased the production of short chain fatty acids propionate and butyrate in ileum and caecum of broiler chicken. The short chain fatty acids, as the metabolite products of bacterial fermentation, have an ability to stimulate the proliferation of intestinal epithelium.

Effect of supplementation of indigenous probiotics lactic acid bacteria on the relative expression of *mucin* mRNA in the ileum are shown in Fig. 1. Expression of *mucin* mRNA in the ileum were higher in T1, T2 and T3 compared to the T0 group (unsupplemented birds). The relative expression levels of *mucin* in the ileum were significantly increased by approximately 4, 6 and 7-fold in T1, T2 and T3, respectively. It was supported by Smirnov *et al.* (2005) that mucin mRNA and the level of mucin glycoprotein were increased by probiotics in the jejunum of chicks.

Conclusions: The indigenous lactic acid bacteria probiotics increased villus height, villus width of duodenum, jejunum and ileum, as well as increased expression of *mucin* mRNA in the ileum compared to the control one in broilers. This results suggest that probiotics may stimulate proliferation of intestinal epithelium and regulate mucosal barrier formed by mucin in the intestine of broiler chickens.

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