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Efficacy of Antibiotic, Probiotic, Prebiotic and Synbiotic on Growth Performance, Organ Weights, Intestinal Histomorphology and Immune Response in Broiler Chickens

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ABSTRACT

A feeding trial was conducted to investigate the effects of dietary supplementations of antibiotic, probiotic, prebiotic and synbiotic on broiler performance, histomorphologic measurements of small intestine and immune response. A total number of 432, day-old broiler chicks (Ross308) were obtained and randomly assigned to 1 of 9 dietary treatments for 6 weeks. The dietary treatments were: 1) basal diet ; 2,3) basal diet plus (400, 600) g of phosphomycin product/ton of starter and grower feeds, respectively; 4,5) basal diet plus (150,200) g of probiotic product/ton of the starter feed and (100,150) g/ton of the grower feed, respectively; 6,7) basal diet plus (500, 1000) g of a prebiotic product /ton of starter and grower feeds, respectively, 8 and9) basal diet plus (1000,1250) g of synbiotic product /ton of the starter feed and (500,750) g/ton of the grower feed, respectively. Birds supplemented with the synbiotic had a greater ($P < 0.01$) feed intake and body weight gain compared with those of others treatments. Feed conversion rate was lower in birds supplemented with all additives than in control birds ($P < 0.01$). The carcass weight was significantly increased in feed additives compared with that of control treatment group ($P < 0.05$). The villus height was significantly increased in feed additives compared with that of control group ($P < 0.01$). Synbiotic treated animals showed increase ($p<0.05$) in antibody titers against NDV compared to those of the control groups at 28, 35 and 42 days of age. The result of the present study revealed that these products had promising effects as alternatives for antibiotics in parallel to demand for elimination of growth-promotant antibiotics.

Key words: broiler, feed additives, performance, histomorphology, immune response.

INTRODUCTION

Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics in broiler industry. Therefore, it is highly essential to improve feed efficiency in poultry to produce meat economically and, food safety is also more seriously considered than before. On the other hand, economy of food production is also a factor that cannot be ignored. A huge amount of antibiotics have been used to control diseases and improve performances in livestock. The mechanisms for the observed improvement in productive parameters (body weight gain and feed conversion) have not been completely elucidated. However, it is suspected that an overall reduction in bacterial load within the intestine is responsible for increased availability of nutrients to the animal. Theoretically, a decrease in pathogenic bacteria and their metabolites could contribute to reduce subclinical lesions on the intestinal mucosa. Since the healing process involves the use of resources to repair the damaged cells, less epithelial damage can be indeed an efficient way to save energy. However, the use of dietary antibiotics has resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1999), the presence of antibiotic residues in poultry meat and eggs that may have deleterious effects on human consumers, imbalance of normal microflora (Andremont, 2000), and the ban on subtherapeutic antibiotic usage in many countries. There is increasing interest in finding alternatives to antibiotics for poultry production.

Because of the general problem of increased resistance of bacteria and the decreasing acceptance of the consumers for Antibacterial Growth Promoters (AGPs), different substances, referred as Natural Growth Promoters (NGPs), have been identified as effective and safe alternatives to AGPs. At present, there is a large number of NGPs available in the market, including probiotics, prebiotics and synbiotics.

Substitution of conventional and prohibited AGPs with probiotics has received much attention in the recent years. One of the major reasons for increased interest in the use of probiotics is because they are natural alternatives to antibiotics for growth promotion in poultry. Recently, it was shown that addition of probiotic containing Enterococcus faecium microorganisms to broiler diets has increased the jejunal villus height (Chichowski *et al.*, 2007) and ileal villus height (Samli *et al.*, 2007). Probiotics act through six different means (Menten, 2002): (a) adherence to the binding sites of the intestinal epithelium (competition with pathogenic bacteria); (b) direct antagonism through the production of bactericidal substances; (c) stimulus to the immune system; (d) facilitating the digestion and absorption of nutrients; (e) suppression of ammonia production, which might be toxic to intestinal cells; and (f) neutralization of enterotoxins. The health promoting effect of probiotic in the gastrointestinal tract has been mainly associated with their capacity to stimulate the immune response and to inhibit the growth of pathogenic bacteria (Barnes *et al.*, 1972).

Prebiotics are substances that act as microbial modulators and are defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve host health” (Gibson and Roberfroid, 1995). This definition was revised in 2004 and prebiotics are now defined as “selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health” (Gibson *et al.*, 2004). Intake of prebiotics can either significantly modulate the colonic microbiota by increasing the number of specific beneficial bacteria such as lactobacilli and bifidobacteria (Rycroft *et al.*, 2001) or reducing undesired intestinal colonization of pathogenic bacteria by mimicking their attachment sites on the intestinal mucosa (Iji and Tivey, 1998).

Moreover, increased intestinal villi height was reported after addition of Bacillus subtilis in association with prebiotics (Pluske *et al.*, 1996). Several studies have shown that administration of prebiotics can improve weight gain, feed intake and feed convention rate in broiler (Rodrigues *et al.*, 2005). However, some reports indicated that prebiotic supplementation did not affect body weight gain, feed intake or feed convention (Stanczuk *et al.*, 2005).

Synbiotics is defined as a mixture of probiotics and prebiotics that beneficially affects the host by activating the metabolism of one or a limited number of health promoting bacteria and/or by stimulating their growth selectively, improving the host's welfare (Gibson and Roberfroid, 1995). Synbiotic products contain viable bacterial cultures that establish easily in the gut while the prebiotic present in the synbiotic serve as a source of nutrient for the probiotics in addition to dietary sources. Recent research and development of synbiotic products have been increasingly focused on functional benefits including resistance to gastrointestinal bacterial infection, antibacterial activity, and improved immune status in broiler chicks. In addition, Mohnl *et al.*, (2007) found that the synbiotic had a comparable potential to improve broiler performance as avilamycin (an antibiotic growth promoter). It seems that synergistic effects of prebiotics and probiotics can be useful in stimulating beneficial bacteria and improving the health of the gut. To the best of the author's knowledge there is scarce information available to date on synbiotics and the possible mechanisms in broiler chickens. Little information is available regarding the effect of adding synbiotic product to broiler diets on the immune status of broiler chickens. The aim of the present study was to assess the effects of antibiotic, probiotic, prebiotic and synbiotic on the performance, intestinal histomorphology and immune response of broiler chickens.

MATERIALS AND METHODS

Birds and Housing

A total number of 432, day-old broiler chicks (Ross308) were obtained from a commercial hatchery. A completely randomized experimental design was used and chicks were divided into nine treatment groups, with four replicates per treatment, each group with the equal numbers of male and female included and 12 chicks per replicate. Chicks were raised in floor pens with *ad libitum* access to feed and water and controlled ventilation. Temperature was maintained at 32 °C for the first 4 days and then gradually reduced. According to normal management practices a temperature of 22 °C was achieved at day 28. The lightning regimen was 23 hours of light and 1 hour of dark.

The dietary treatments

The dietary treatments were: 1) basal diet (control) ; 2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively, 4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively, 6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively, 8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750) g/ton of the grower feed, respectively. All diets were formulated to provide 3000 kcal of ME/kg and to meet the amino acid ratios and all other nutrients as suggested by the NRC, 1994 for broilers from 0 to 6 week of age (Table 1).

Growth Performance Traits

All birds were weighed individually after their arrival from the hatchery to the experimental farm (initial weight) and chicks of a uniform body weight (BW) were placed in individual pens and average initial body weight was 48 g. Weekly weight gain for each dietary treatment was calculated. Feed consumption was recorded weekly and in the course of the whole experiment for each treatment, and subsequently the feed conversion rates were calculated.

Organ Weights and Carcass Weights

At the end of experiment, after weighing, 8 birds per treatment were randomly selected and euthanized by cervical dislocation. The gizzard, heart, liver, pancreas, proventriculus, spleen, bursa of Fabricius, small intestine, (duodenum, jejunum, and ileum) and cecum were excised and weighed. The gastrointestinal tract was weighed after removal of the content. Afterward, the birds were scalded, defeathered, and carcasses were eviscerated. The head, neck, and feet were removed, and the carcass subsequently was ready to cook (RTC). The RTC carcass weight was then determined.

Histomorphological Samples

The tissue samples for histology were taken from the ileum. 10 cm proximal to the ileocecal junction (from Meckel's diverticulum to the ileocecal colonic junction) was referred as the ileum.

Light Microscopy

The samples were fixed in 4% buffered formalin for 48 h. The processing consisted of serial dehydration, clearing, and impregnation with wax. Semithin sections, 5 μm thick (3 cross-sections from each sample), were cut by a microtome and were mounted on slides. A routine staining procedure was carried out using hematoxylin and eosin. The slides were examined under an Olympus AX70 microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera (Sony DXC-930P, Sony Corporation, Tokyo, Japan). The images were analyzed using stereological image software, Cast Image System (Version 2.3.1.3, Visiopharm Albertslund, Hørsholm, Denmark).

Histomorphological Measurements

The intact well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section for each sample. The criterion for villus selection was based on the presence of intact lamina propria. Villus height was measured from the tip of the villus to the villus-crypt junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi.

Vaccination and serology

At 9th day chicks were vaccinated with Hitchner B1 NDV (Newcastle Disease Virus) vaccine via eye I/O route and bivalent killed vaccine (Newpasol 102, Inactivated W/O Emulsion ND + AI (H9N2) Vaccine, Pasouk Biological Co) by I/M route. Blood samples were collected every week from the wing veins of broiler chickens and their sera were separated and inactivated at 56° C for 30 min and kept at −20° C until analysis of NDV antibody level. Serum Antibody titer was measured using hemagglutination-inhibition test as described by Alexander *et al.*,1983 (Alexander *et al.*, 1983) on d7, 14, 21, 28, 35, and 42.

Table 1. Composition of experimental diets[1]

Ingredients %	Starter (0-21 d)	Grower (22-42)
Corn grain	52.89	64.09
Soybean meal	38.87	30.23
Soybean oil	4.04	2
Oyster shell	1.63	1.69
Ca phosphate	1.52	1.09
Salt	0.38	0.28
Mineral premix[2]	0.25	0.25
Vitamin premix[3]	0.25	0.25
DL-methionine	0.17	0.03
Nutrient composition		
ME, kcal/kg	3000	3000
Crude protein %	21.6	18.75
Lys%	1.3	0.9375
Met %	0.4874	0.3659
Ca %	0.937	0.843
P (Total) %	0.42	0.33
Antibiotic[4]		
Probiotic[5]		
Prebiotic[6]		
Synbiotic[7]		

[1]Calculated from NRC (1994).
[2]provides per kilogram of diet: Cu (CuSO4-5 H2O), 4.0 mg; I (potassium iodate), 1.0 mg; Fe (ferrous sulfate-7 H2O), 60 mg; Mn (manganese sulfate-H2O), 60 mg; Se (sodium selenite), 0.1mg; Zn (zinc sulfate-7H2O), 44 mg; and Ca (calcium carbonate), 723 mg.
[3]For experiment, provides per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D3, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 2.4 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; pyridoxine (pyridoxine_HCl), 6.4 mg;riboflavin, 15 mg; and thiamin (thiamin_HCl), 15.2 mg.
[4]phosphomycin -Bedson co S.A.,La Lonja,Argentina.
[5]Probiotic- each kilogram contained: *Lactobacillus plantarum*, $1.89 \cdot 10^{10}$ cfu; *Lactobacillus delbrueckii* subsp. *bulgaricus*, $3.09 \cdot 10^{10}$ cfu; *Lactobacillus acidophilus*, $3.09 \cdot 10^{10}$ cfu; *Lactobacillus rhamnosus*, $3.09 \cdot 10^{10}$ cfu; *Bifidobacterium* 10^{10} cfu; *Aspergillus oryza*, $7.98 \cdot 10^{9}$ cfu; *Candida pintolopesii*, $7.98 \cdot 10^{9}$ cfu. Protexin Compounder, Novaritis Inc., Istanbul, Turkey.
[6]Prebiotic- Techno Mos (25% mannan-oligosaccharides, Alltech, Nicholasville, KY).
[7]Synbiotic- each kilogram contained 5×10^{8} cfu/kg- Biomin IMBO, Etouk Farda Feed Additives Co,Tehran,Iran.

Statistical analysis

When the chicks reached 42 d of age, the feeding trial was terminated. Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software. The treatment means with significant differences were compared using Duncan's new multiple range tests. All statements of differences were based on significance level set at $P \leq 0.05$.

RESULT

Mortality was low (<1%) and not treatment associated.

Feed Intake

The effects of treatments on feed intake (FI) are presented in Table 2. Birds supplemented with the synbiotic had a greater ($P < 0.05$) FI compared with that of control and other treatments. Moreover, prebiotic supplemented birds had a greater ($P < 0.05$) FI than that of probiotic and

phosphomycin supplemented birds. No significant differences on feed intake were observed between the probiotic and phosphomycin-treated birds in the entire experimental period ($P > 0.05$).

Table 2. Effect of feed supplementations on feed intake of broiler chickens (g)

Dietary treatment[1](n=12)[3]	FI (0-3)week	FI (3-6)week	FI (0-6)week
T1	1057.79 ± 74.36^{b}	3222.80 ± 91.82^{c}	4280.64 ± 136.12^{c}
T2	1131.03 ± 21.63^{ab}	3263.49 ± 18.80^{bc}	4394.52 ± 16.23^{ab}
T3	1101.85 ± 29.55^{ab}	3223.54 ± 18.28^{c}	4325.40 ± 26.29^{bc}
T4	1062.13 ± 12.93^{ab}	3263.85 ± 46.73^{bc}	4325.98 ± 43.72^{bc}
T5	1108.63 ± 55.59^{ab}	3257.83 ± 66.39^{bc}	4341.45 ± 62.53^{bc}
T6	1085.00 ± 39.40^{ab}	3233.23 ± 65.73^{ab}	4447.23 ± 99.66^{ab}
T7	1136.63 ± 21.82^{a}	3343.48 ± 152.67^{abc}	4477.85 ± 154.62^{ab}
T8	1125.93 ± 28.18^{ab}	3338.50 ± 73.14^{abc}	4440.70 ± 107.17^{ab}
T9	1111.40 ± 77.09^{ab}	3397.08 ± 55.95^{a}	4508.52 ± 88.68^{a}
P-value	*[2]	*	*

1 The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
2 a-d Means within a column with differing superscripts are significantly different at $P < 0.05$.
3 n=the number of birds/pen

Body Weight Gain

The initial body weight (BW) of chicks did not differ significantly ($P > 0.05$) between the dietary treatments (48 g). Responses to dietary treatments were significant ($P < 0.01$) for BWG (Body weight gain) in starter and grower periods (Table 3).

Table 3. Effect of feed supplementations on body weight gain of broiler chickens (g)

Dietary treatment[1](n=12)[3]	Initial BW	BWG (0-3)week	BWG (3-6)week	BWG (0-6)week
T1	48	619.77 ± 14.55^{c}	1570.29 ± 2760^{e}	2190.06 ± 18.29^{f}
T2	48	686.08 ± 42.94^{ab}	1666.84 ± 49.00^{d}	2353.00 ± 32.12^{e}
T3	48	709.49 ± 54.02^{ab}	1712.95 ± 6593^{cd}	2224.45 ± 97.00^{de}
T4	48	703.50 ± 7.33^{ab}	1718.63 ± 37.38^{cd}	2422.13 ± 33.62^{de}
T5	48	703.50 ± 71.1^{ab}	1734.75 ± 32.11^{cd}	2438.25 ± 31.19^{de}
T6	48	715.25 ± 11.09^{ab}	1755.50 ± 97.58^{bc}	2470.75 ± 101.10^{dc}
T7	48	713.50 ± 9.47^{ab}	1813.75 ± 32.85^{ab}	2527.25 ± 40.26^{bc}
T8	48	753.74 ± 33.25^{b}	1850.77 ± 18.28^{a}	2593.25 ± 54.29^{ab}
T9	48	755.12 ± 19.35^{b}	1874.11 ± 32.53^{a}	2629.21 ± 41.57^{a}
P-value	ns[2]	**[2]	**	**

The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-f Means within a column with differing superscripts are significantly different at $P < 0.01$.
3 n=The number of birds/pen

At the end of the experiment (d 42), birds supplemented with the synbiotic had a greater ($P < 0.01$) BWG compared with that of control and other treatments. Moreover, prebiotic supplemented birds had a greater ($P < 0.01$) BWG than that of probiotic and phosphomycin

supplemented birds. However, birds supplemented with the probiotic had a greater BWG than phosphomycin supplemented birds but difference was not significant (P > 0.05).

Feed Conversion Rate

Feed conversion rate (FCR) was lower for birds supplemented with synbiotic, prebiotic, probiotic and phosphomysin than that of control birds (P < 0.01). In addition, no significant differences on FCR were found among treatments (Table 4).

Table 4. Effect of feed supplementations on feed conversion rate of broiler chickens

Dietary treatment[1] (n=12)[3]	FCR(0-3)week	FCR(3-6)week	FCR(0-6)week
T1	1.70 ± 0.08^{a}	2.05 ± 0.06^{a}	1.95 ± 0.06^{a}
T2	1.65 ± 0.09^{ab}	1.96 ± 0.06^{ab}	1.86 ± 0.02^{b}
T3	1.53 ± 0.16^{bc}	1.88 ± 0.08^{bcd}	1.78 ± 0.06^{c}
T4	1.50 ± 0.02^{c}	1.90 ± 0.02^{bcd}	1.79 ± 0.01^{c}
T5	1.54 ± 0.02^{bc}	1.89 ± 0.03^{bcd}	1.79 ± 0.04^{c}
T6	1.53 ± 0.13^{bc}	1.92 ± 0.12^{bc}	1.77 ± 0.06^{c}
T7	1.59 ± 0.06^{abc}	1.82 ± 0.10^{cd}	1.73 ± 0.07^{c}
T8	1.46 ± 0.08^{c}	1.80 ± 0.03^{d}	1.71 ± 0.05^{c}
T9	1.46 ± 0.07^{c}	1.81 ± 0.05^{cd}	1.71 ± 0.04^{c}
P-value	**[2]	**	**

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2] a-d Means within a column with differing superscripts are significantly different at $P < 0.01$.

3 n=The number of birds/pen

Carcass Weight and Live Weight

The means of carcass weight and live weight are shown in Table 5. The carcass weight was significantly higher in synbiotic treated group compared with control and phosphomycin treated groups (P < 0.05), and it was significantly increased for prebiotic and probiotic compared with that of control treatment (P < 0.05). No significant differences on carcass weight were found between synbiotic, prebiotic and probiotic treatments with each other (P > 0.05). Birds supplemented with the synbiotic had a greater (P < 0.01) live weight compared with that of control and other treatments. Moreover, prebiotic supplemented birds had a greater (P < 0.01) live weight than probiotic and phosphomycin supplemented birds. However, birds supplemented with the probiotic had a greater live weight than that of phosphomycin supplemented birds but the difference was not significant (P > 0.05). Both probiotic and phosphomycin increased live weight (P < 0.01) compared with that of the control group.

Weights of Organs

The means of the absolute weights of organs for dietary treatments are presented in Table 6a and b. The weight of liver, pancreas and small intestine were decreased (P < 0.05) for the synbiotic-supplemented group compared with that of the control group and other dietary supplemented groups. Moreover, the synbiotic- supplemented group showed a decrease (P < 0.01) in heart weight compared with that of either the control group or other treatments groups. The weight of small intestine was significantly greater (P < 0.05) in the probiotic-supplemented group than that in the control group and other treatment groups.

Table 5. Effects of dietary treatments on live weight and carcass weight (g) of broiler chickens (42 day)

Dietary treatment[1](n=12)[4]	Carcass weight	Live weight
T1	1303.60 ± 118.27^{d}	2243.09 ± 18.34^{f}
T2	1387.18 ± 48.00^{cd}	2405.98 ± 32.14^{e}
T3	1403.25 ± 102.10^{bcd}	2475.33 ± 97.02^{de}
T4	1408.68 ± 43.18^{abcd}	2475.13 ± 33.62^{de}
T5	1429.70 ± 27.75^{abc}	2491.25 ± 31.19^{de}
T6	1425.50 ± 63.33^{abc}	2523.75 ± 101.10^{dc}
T7	1453.63 ± 38.20^{abc}	2580.25 ± 40.26^{bc}
T8	1515.53 ± 108.37^{ab}	2646.23 ± 54.32^{ab}
T9	1529.50 ± 70.47^{a}	2682.20 ± 41.56^{a}
P-value	*2	**3

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-d Means within a column with differing superscripts are significantly different at $P < 0.05$.
[3] a-f Means within a column with differing superscripts are significantly different at $P < 0.01$.
4 n=The number of birds/pen

The weight of heart was increased (P < 0.01) in the prebiotic-supplemented group compared with that of the control group and other treatment groups. In addition, the absolute weights of gizzard, proventriculus, spleen, cecum and bursa did not show any significant differences among the dietary treatments.

Table 6a. Effect of dietary treatments on absolute organ weights of broiler chickens (g)

Dietary treatment[1] (n=12)[5]	Heart	Liver	Cecum	Small intestine	Proventriculus
T1	46.20 ± 5.04^{abc}	39.68 ± 3.48^{ab}	11.33 ± 3.02	74.38 ± 5.40^{ab}	7.43 ± 0.59
T2	48.40 ± 4.58^{ab}	39.73 ± 2.90^{ab}	10.53 ± 0.72	64.35 ± 11.08^{abc}	6.90 ± 0.65
T3	40.80 ± 7.59^{bc}	37.18 ± 0.59^{b}	11.10 ± 2.10	62.73 ± 7.78^{bc}	6.45 ± 0.24
T4	49.63 ± 5.45^{a}	40.43 ± 3.11^{ab}	11.25 ± 1.01	77.53 ± 1.36^{a}	7.10 ± 1.28
T5	52.00 ± 5.05^{a}	43.68 ± 1.14^{a}	12.28 ± 1.86	77.25 ± 3.99^{a}	7.40 ± 0.35
T6	47.00 ± 4.34^{abc}	43.93 ± 3.12^{a}	10.88 ± 3.30	71.85 ± 10.83^{ab}	8.28 ± 0.61
T7	54.43 ± 3.81^{a}	40.30 ± 5.06^{ab}	11.38 ± 0.98	68.48 ± 6.67^{abc}	7.00 ± 0.37
T8	45.70 ± 4.10^{abc}	37.38 ± 2.35^{b}	11.50 ± 2.15	62.48 ± 10.35^{bc}	6.35 ± 1.31
T9	39.30 ± 7.54^{c}	36.98 ± 3.23^{b}	11.55 ± 1.39	55.88 ± 12.52^{bc}	6.83 ± 0.97
P-value	[2]**	[3]*	NS[4]	*	NS

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-c Means within a column with differing superscripts are significantly different at $P < 0.01$.
[3] a-c Means within a column with differing superscripts are significantly different at $P < 0.05$.
[4] $P \geq 0.05$.
5 n=The number of birds/pen

The means of weight of organs relative to the BW are shown in Table7a and b. The weight of heart, liver, small intestine, pancreas relative to the BW tended to be lower (P < 0.01) for synbiotic-fed birds than those of control group and other product-fed birds. The relative weight of heart, liver and small intestine were significantly greater (P < 0.01) for probiotic compared with

synbiotic-fed birds. In addition, the relative weights of proventriculus, cecum, spleen, and bursa remained unaffected by dietary supplementations.

Table 6b. Effect of dietary treatments on absolute organ weights of broiler chickens (g)

Dietary treatment[1] (n=12)[4]	Gizzard	Pancreas	Bursa	Spleen
T1	42.00 ± 7.16	5.35 ± 0.17[a]	3.68 ± 0.42	2.50 ± 0.58
T2	40.38 ± 7.28	4.00 ± 0.67[b]	4.00 ± 1.23	2.35 ± 0.72
T3	38.48 ± 3.13	4.35 ± 1.26[ab]	3.28 ± 1.33	2.15 ± 0.53
T4	39.65 ± 6.63	4.78 ± 0.66[ab]	3.23 ± 0.26	2.48 ± 0.35
T5	42.55 ± 5.91	4.70 ± 0.54[ab]	3.30 ± 0.27	2.23 ± 0.32
T6	37.58 ± 6.03	4.48 ± 0.33[ab]	3.28 ± 0.13	2.35 ± 0.35
T7	36.33 ± 3.17	5.05 ± 0.64[ab]	3.18 ± 0.48	2.33 ± 0.58
T8	37.58 ± 6.35	4.00 ± 0.47[b]	3.28 ± 0.38	2.65 ± 0.13
T9	36.18 ± 5.67	4.23 ± 0.86[ab]	0.93 ± 1.44	2.23 ± 0.39
P-value	NS[2]	*[3]	NS	NS

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2] $P \geq 0.05$.

[3] a-b Means within a column with differing superscripts are significantly different at $P < 0.05$.

4 n=The number of birds/pen

Histomorphological Measurements

Ileum

The means of ileal villus height, crypt depth, and villus height: Crypt depth ratios for dietary treatments are shown in Table 8. The villus height was significantly increased for synbiotic compared with that of control and other treatment groups ($P < 0.01$), and it was significantly increased for prebiotic compared with that of control, probiotic and phposphomycin- treatments ($P < 0.01$). The villus height were significantly increased ($P < 0.01$) for both probiotic and phosphomycin compared with that of control but no significant differences were found between these treatments with each other. Moreover, synbiotic supplementation increased the villus height: crypt depth ratio compared with that of control and other treatment groups ($P < 0.01$). The villus height: crypt depth ratio was significantly increased ($P < 0.01$) for prebiotic compared with that of control, probiotic and phposphomycin- treatments. In addition, the crypt depth remained unaffected by dietary supplementations ($P > 0.05$).

Immunological Measurements

Antibody titers

The effect of treatments on antibody production against NDV in broilers from 7 d to 42d are presented in Table 9. On the day 7th, no differences among antibody titers of experimental groups were observed. Animals of synbiotic treatment showed increase ($p<0.05$) in antibody titers against NDV as compared to those of the control at 28, 35 and 42 days of age. Other supplementation of the diet showed increase in antibody titers against NDV compared to those of the control but were not significant ($P > 0.05$). No significant differences on antibody titers were

found among the prebiotic, probiotic and phosphomycin-treated birds in the entire experimental period ($P > 0.05$).

Table 7a. Effect of dietary treatment on organ weights relative to BW of broiler chickens (g/100g)

Dietary treatment[1] (n=12)[4]	Heart	Liver	Cecum	Small intestine	Proventriculus
T1	2.05 ± 0.21^{a}	1.76 ± 0.16^{a}	0.50 ± 0.13	3.31 ± 0.26^{a}	0.33 ± 0.03
T2	2.01 ± 0.21^{a}	1.65 ± 012^{abc}	0.43 ± 0.03	2.67 ± 0.47^{bc}	0.28 ± 0.03
T3	1.65 ± 0.36^{bc}	1.50 ± 0.08^{dc}	0.44 ± 0.09	2.53 ± 0.25^{dc}	0.28 ± 0.05
T4	2.00 ± 0.24^{a}	1.63 ± 0.15^{abc}	0.45 ± 0.04	3.13 ± 0.08^{ab}	0.28 ± 0.05
T5	2.09 ± 0.22^{a}	1.75 ± 0.05^{ab}	0.49 ± 0.08	3.10 ± 0.18^{ab}	0.29 ± 0.02
T6	1.86 ± 0.06^{ab}	1.74 ± 0.12^{ab}	0.43 ± 0.15	2.85 ± 0.51^{abc}	0.29 ± 0.04
T7	2.11 ± 0.17^{a}	1.55 ± 0.23^{bcd}	0.44 ± 0.04	2.65 ± 0.29^{bc}	0.27 ± 0.02
T8	1.63 ± 0.17^{bc}	1.41 ± 0.08^{d}	0.43 ± 0.08	2.35 ± 0.35^{dc}	0.41 ± 0.33
T9	1.46 ± 0.26^{c}	1.37 ± 0.13^{d}	0.42 ± 0.05	2.07 ± 0.44^{d}	0.25 ± 0.04
P-value	[2]**	**	NS[3]	**	NS

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2] a-d Means within a column with differing superscripts are significantly different at $P < 0.01$.

[3] $P \geq 0.05$

4 n=The number of birds/pen

Table 7b. Effect of dietary treatment on organ weights relative to BW of broiler chickens (g/100g)

Dietary treatment[1] (n=12)[5]	Gizzard	Pancreas	Bursa	Spleen
T1	1.87 ± 0.32^{a}	0.24 ± 0.01^{a}	0.16 ± 0.02	0.11 ± 0.03
T2	1.67 ± 0.31^{ab}	0.16 ± 0.03^{b}	0.16 ± 0.05	0.10 ± 0.03
T3	1.55 ± 0.14^{ab}	0.17 ± 1.05^{b}	0.13 ± 0.05	0.08 ± 0.03
T4	1.28 ± 0.79^{ab}	0.19 ± 0.03^{b}	0.13 ± 0.01	0.10 ± 0.01
T5	1.71 ± 0.25^{ab}	0.18 ± 0.02^{b}	0.13 ± 0.01	0.09 ± 0.01
T6	1.49 ± 0.27^{ab}	0.17 ± 0.02^{b}	0.13 ± 0.01	0.09 ± 0.01
T7	1.08 ± 0.63^{a}	0.19 ± 0.03^{b}	0.12 ± 0.02	0.09 ± 0.03
T8	1.02 ± 0.57^{a}	0.15 ± 0.02^{b}	0.12 ± 0.01	0.10 ± 0.01
T9	1.34 ± 0.19^{ab}	0.16 ± 0.03^{b}	0.14 ± 0.05	0.09 ± 0.02
P-value	*[2]	**[3]	ns[4]	ns

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2] a-b Means within a column with differing superscripts are significantly different at $P < 0.05$.

[3] a-b Means within a column with differing superscripts are significantly different at $P < 0.01$.

[4] $P \geq 0.05$

5 n=The number of birds/pen

Table 8. Effect of feed additive supplementations on histomorphological parameters of the ileum in broilers chickens

Dietary treatment[1] (n=12)[4]	Villus height (µm)	Crypt depth (µm)	Villus height:crypt depth
T1	523.00 ± 27.17^{d}	131.00 ± 4.32	3.99 ± 0.1^{e}
T2	563.00 ± 10.80^{c}	136.00 ± 8.12	4.15 ± 0.28^{e}
T3	564.25 ± 22.91^{c}	137.00 ± 6.27	3.48 ± 18.74^{e}
T4	581.00 ± 17.68^{c}	134.25 ± 8.54	4.34 ± 0.26^{de}
T5	578.75 ± 31.94^{c}	133.00 ± 7.12	4.36 ± 0.29^{de}
T6	676.25 ± 11.84^{b}	141. 50 ± 4.93	4.79 ± 0.23^{bc}
T7	656.75 ± 10.90^{b}	139.25 ± 5.56	4.72 ± 0.24^{dc}
T8	724.00 ± 8.87^{a}	134.75 ± 8.10	5.39 ± 0.29^{a}
T9	714.75 ± 4.35^{a}	139.50 ± 11.12	5.15 ± 0.41^{ab}
P-value	[2]**	NS[3]	**

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2] a-e Means within a column with differing superscripts are significantly different at $P < 0.01$.

[3] P ≥0.05

4 n=The number of birds/pen

Table 9. Effect of feed additive supplementations on NDV antibody titers in broiler chicenks from 7 to 42 days of age

Dietary treatment[1] (n=12)[5]	Antibody titers					
	7th day	14th day	21st day	28th day	35th day	42nd day
T1	6.15 ± 0.45	5.03 ± 0.55^{ab}	4.68 ± 0.49	5.13 ± 0.43^{c}	4.40 ± 0.20^{c}	4.48 ± 4.40^{c}
T2	6.03 ± 0.36	4.58 ± 0.05^{b}	4.93 ± 0.49	5.33 ± 0.13^{c}	4.95 ± 1.27^{bc}	4.93 ± 4.95^{c}
T3	5.73 ± 0.17	5.05 ± 0.52^{ab}	4.52 ± 0.04	5.35 ± 0.70^{c}	5.15 ± 0.90^{abc}	5.30 ± 5.15^{bc}
T4	5.75 ± 0.06	5.00 ± 0.58^{ab}	4.92 ± 0.45	5.30 ± 0.70^{c}	5.35 ± 0.70^{abc}	5.00 ± 5.35^{c}
T5	5.90 ± 0.80	5.25 ± 0.50^{ab}	4.83 ± 0.39	5.95 ± 0.53^{abc}	5.20 ± 0.83^{abc}	4.53 ± 5.20^{c}
T6	5.85 ± 0.44	4.78 ± 0.49^{ab}	4.93 ± 0.49	5.73 ± 0.55^{bc}	5.98 ± 0.50^{ab}	5.03 ± 5.98^{c}
T7	5.73 ± 0.15	5.15 ± 0.44^{ab}	4.88 ± 0.43	5.98 ± 0.61^{abc}	5.60 ± 0.91^{abc}	5.10 ± 5.60^{bc}
T8	5.73 ± 0.83	5.53 ± 0.78^{a}	5.10 ± 0.40	6.28 ± 0.83^{ab}	6.28 ± 0.05^{a}	5.89 ± 6.28^{ab}
T9	5.88 ± 8.96	5.25 ± 0.50^{ab}	5.13 ± 0.43	6.80 ± 0.54^{a}	6.18 ± 0.47^{a}	6.29 ± 6.18^{a}
P-value	ns[2]	*[3]	NS	**[4]	*	**

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2]P ≥0.05

[3] a-c Means within a column with differing superscripts are significantly different at $P < 0.05$.

[4] a-c Means within a column with differing superscripts are significantly different at $P < 0.01$.

5 n=The number of birds/pen

DISCUSSION AND CONCLUSION

In the recent decades, deficiencies in feed formulation and management practices have been masked by the routine use of antibiotic growth promoters (AGP). However, the ban of AGP in

Europe has driven the implementation of alternative strategies in order to maintain health and performance status and optimizing digestion in poultry production. Several feed additives have been used to manipulate microbial communities in the digestive tract. However, their efficacy has not always been proven and their modes of action require further research. The present study focused on the role and the efficacy of the antibiotic, probiotic, prebiotic and synbiotic products as potential modulators of gut health, immune responses and growth performance in poultry production.

Data of this research showed that probiotic can be good alternative for antibiotic because it had positive influence on growth performance, organ weights, intestinal histomorphology and immune response in broiler chickens compared with those of the control group (but in some parameters it is not significant). Improvement in growth performance and feed efficiency of broiler chickens fed probiotics (Falaki *et al.*, 2011; Naseri *et al.*, 2012; Houshmand *et al.*, 2012) is thought to be induced by the total effects of probiotic action including the maintenance of beneficial microbial population (llerFu, 1989), improving feed intake and digestion (Nahanshon *et al.*, 1993), and altering bacterial metabolism (Jin *et al.*, 1997). The mechanism that explains the action of probiotics is focused on gastro intestinal tract, because, most of these products are not absorbed and are not efficient as growth promoters in germ-free animals (Coates *et al.*, 1963). Therefore, it may be speculated that there is a strong interaction between probiotics and the intestinal micro flora. Hence, this improvement in performance due to the action of probiotics on the micro flora can be interpreted in two ways: the first is related to the reduction in the utilization of nutrients by micro organisms and the second is the decrease of microbial metabolites that interfere with host growth (Anderson *et al.*, 1999). In addition, maintaining the integrity of the intestinal mucosa results in high energy requirements, and the decrease of pathogens and intestinal metabolites can also decrease intestinal cell turnover, resulting in more energy available for production. Finally, the reduction of opportunistic pathogens and subclinical infections can also be associated with the use of probiotics (Dibner and Richards, 2005). In the present study, the beneficial effects of probiotic product on broiler performance parameters, histomorphological parameters and immune responses are in agreement with previous studies (Midilli *et al.*, 2008; Awad *et al.*, 2009; Ashayerizadeh *et al.*, 2011).

Serum antibody titers against Newcastle disease virus based on hemagglutination-inhibition test (HI) in broiler chickens fed commercial diet supplemented with probiotic (group 4and5) was higher than those of chickens in group 1. The positive effect of feeding diet containing probiotic on the immune response indicates the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The direct effect might be related to stimulate the lymphatic tissue (Kabir *et al.*, 2004), whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract. Shoeib *et al.* (1997) reported that the bursa of probiotic-treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Christensen *et al.*, (2002) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Commensally, bacteria presented in intestinal microbiota are in close contact with cells of the immune system. It has recently been demonstrated that resident dendritic cells (DC) in the intestinal lamina propria have the capacity to directly sample the gut lumen by projecting their dendrites through the tight junctions of epithelial cells (Rescigno *et al.*, 2001). The recognition of commensal bacteria or their structural components by Toll like receptors (TLR) presented on surfaces of DC could lead to the activation and maturation of these cells (Rakoff-Nahoum *et al.*, 2004). Differential activation of DC by commensal bacteria promotes the establishment of T-helper 1 (Th1), Th2, and Th3 responses and the secretion of cytokines, such as interleukin 4 (IL-

4), IL-10, and transforming growth factor β, that are important for antibody production and isotype switching (Christensen *et al.*, 2002; Di Giacinto *et al.*, 2005).

In this study we found that, broilers fed prebiotic were more efficient than probiotic and antibiotic on broiler performance parameters, immune responses and histomorphological parameters, however, in some parameters this differences was not significant. Prebiotics can serve as substrate for beneficial bacteria mainly located in the hind gut. We think they can enhance the digestibility and performance parameters by creating the favorable conditions for beneficial bacteria. However, they are exclusively fermented by beneficial bacteria such as Lactobacillus, Bifidobacteria and Bacteroides, thereby having the potential to modulate the composition of microbial communities in the gut (Chen *et al.*, 2005). According to our data, prebiotic supplementation of the diet showed increase in antibody titers against NDV compared to that of the control but were not significant ($P > 0.05$). Much of the nature of mechanism accountable for immunomodulation associated with the prebiotic remains to be delineated. One hypothesis is that defense cells in the gut-associated lymphoid tissue (GALT) detect the presence of microbes by recognizing molecules unique to microorganisms that are not associated with host cells. We think it is also possible that prebiotic may enhance the secretion of plasma serum IgG and intestinal mucosa IgA, increasing the number of lymphocytes and/or leukocytes in the GALT and in peripheral blood (Kaufhold *et al.*, 2000). In the intestine, secretory IgA binds to pathogenic organisms and provides protection by preventing their attachment to mucosal cells (Abbas *et al.*, 2000). It is the most prominent antibody present at mucosal surfaces, and provides passive immunoprotection against invading pathogens in the gastrointestinal tract. Gao *et al.* (2008) reported that birds fed prebiotic -supplemented diets had greater sIgA content in the duodenum so with increasing concentration of dietary prebiotic, IgA content increased linearly. We speculated that it may stimulate the humoral immune system to produce more antibodies therefore increased antibodies cover the surface of intestinal mucosa and can protect villi from damage (Toloei *et al.*, 2010; Ghahri *et al.*, 2010). However, in a commercial poultry setting, the impact of nutrition on the immune competence that underpins the production traits is difficult to quantify. As such, laboratory research is essential for a better understanding of the immunomodulatory properties of feed additives, particularly in light of the need for alternatives to in-feed antibiotics.

Conclusions obtained by this study represent that synbiotics had a positive effect on growth performance, immune responses and histomorphological parameters, that is in agreement with previous studies (Awad *et al.*, 2009; Naseri *et al.*, 2012) It might be combination of probiotics and prebiotics, also referred as synbiotics, improve the survival rate of probiotics during their passage through the digestive tract, thus contributing to enhancement of the probiotic effects. A synbiotic relationship between a prebiotic substance and a probiotic organism suggests synergism. There are many discussions about synbiotic but their mechanism of action is not completely known and there are a few articles about the effectiveness of this product in the world. Our data indicated that synbiotic supplementation of the diet increased antibody titers against NDV compared to that of the control on 28, 35 and 42 days ($P < 0.05$). These results are in agreement with that of Haghighi *et al.* (Haghighi *et al.*, 2006) who found that probiotics enhance the systemic antibody response to some antigens in chickens and Talebi *et al.* (2008) who found that administration of a multi-strain probiotic improve the antibody responses to ND. It is possible that, binding of structural components of commencal bacteria to Toll-like receptors expressed on the surface of macrophage and dendritic cells in the lamina propria may lead to their activation and differentiation. Upon its activation, they promote the activation and

differentiation of different subsets of other immune system cells, leading to the production of cytokines such as IL4, IL10 and transforming growth factor ß, that are important for antibody production and isotype switching (Di Giacinto *et al.*, 2005; Mohamadzadeh *et al.*, 2005).

In the present study, supplementation of broilers with probiotic, prebiotic and synbiotic increased the villus height and villus height: crypt depth ratio in ileum significantly ($P < 0.01$), suggesting an increased epithelial cell turnover due to feeding of direct-fed microbials. The histomorphological changes in the intestine of broiler chickens reported in the present study provide useful information regarding the potential for using probiotic, prebiotic and synbiotic in broiler feed. Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). The villus crypt is considered as the villus factory and deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demands for tissue. The intestinal epithelial cells originating in the crypt migrate along the villus surface upward to the villus tip and are extruded into the intestinal lumen within 48 to 96 h (Potten, 1998). We believe shortening of the villi and deeper crypts may lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, and lower performance (Xu *et al.*, 2003). In contrast, increase in the villus height and villus height: crypt depth ratios are directly correlated with increased epithelial cell turnover and longer villi are associated with activated cell mitosis (Dunham *et al.*, 1993). Longer villi were found in the ileum of chicks and turkeys treated with Lactobacillus reuteri (Dunham *et al.*, 1993) and in the ileum of adult male layers with slight improvement in feed efficiency after dietary addition of Bacillus subtilis var. natto (Samanya and Yamauchi, 2002). Feeding of probiotics has been shown to induce gut epithelial cell proliferation in rats (Ichikawa *et al.*, 1999). In addition, longer villi were induced by dietary amylase (Ritz *et al.*, 1995). The concentrations of amylase in broiler intestine were increased after supplementation of diet with either a single strain of Lactobacillus acidophilus or a mixture of Lactobacillus strains (Jin *et al.*, 2000). However, amylase concentrations were not estimated in the present study, and further experiments are needed to verify this effect. It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes, and nutrient transport systems (Pluske *et al.*, 1996). It is understood that greater villus height is an indicator that the function of intestinal villi is activated (Shamoto and Yamauchi, 2000). This fact suggests that the villus function is activated after feeding of dietary probiotic, prebiotic and synbiotic.

In conclusion, the present study indicated that the synbiotic had the best effect on performance, immune responses, histomorphological parameters in comparison with probiotic and prebiotic products. Also prebiotic had better effect than probiotic and, both of them had better effect than phosphomycin. Therefore, these products might be promising alternatives for antibiotic growth promoters as pressure to eliminate antibiotic growth promoters in animal feed increases. The synbiotic offers a good alternative to improve poultry production.

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