Comparative effects of probiotic, prebiotic and synbiotic supplements on performance, jejunal morphology, serum lipid profile and antibody response of broiler chicks

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Abstract A 42-d trial was conducted to compare the effects of probiotic, prebiotic and synbiotic supplements on growth performance, morphology of the jejunum, serum lipid profile and antibody response of broiler chickens. A total of 400 one-d-old male broiler chicks were randomly divided into four treatment groups of four replicates (25 birds per replicate) and fed corn-soybean meal diets. The dietary treatments consisted of a basal diet without any feed additive (control diet), a basal diet with added probiotics (diet Pro), a basal diet with added prebiotics (diet Pre), and a basal diet with added synbiotics (diet Syn). The birds fed diet Syn exhibited higher body weight gain (BWG) and better feed conversion ratio (FCR) than those fed the control diet during the entire experimental period (P<0.05). Feeding diets Pro, Pre and Syn significantly increased the villus height and villus height:crypt length ratio in the jejunum of broilers at 42 d of age (P<0.05). The birds fed any of the experimental diets, exhibited lower levels of serum total cholesterol and low-density lipoprotein cholesterol (LDL-C) than those fed the control diet at 28 and 42 days of age (P<0.05). At 41 d of age, the total antibody titer against sheep red blood cell (SRBC) in the birds fed diets Pro, Pre and Syn were higher than in the birds fed the control diet, with the birds fed diet Syn having the highest antibody titer (P<0.05). None of the diets affected feed intake, crypt depth in the jejunum, serum triglyceride level and primary antibody response against SRBC. The results suggested that diets supplemented with probiotics, prebiotics and synbiotics could improve growth performance and health benefits of the broiler chicks by improving intestinal morphology, fat metabolism and immune function.

Keywords: broilers, immunity, intestinal morphology, non-antibiotic supplements, serum lipids

Received: 24 Mar. 2013, *accepted:* 13 Sep. 2013, *published online:* 17 Oct. 2013

Introduction

The rapid development of poultry industry has increased the risk of disease outbreaks and physiological stress in poultry, which has led to production losses and impeded the continual development in this industry (Angel et al., 2005). Under these conditions, antibiotics are commonly the first choice for therapeutic and prophylactic purposes. Sub therapeutic doses of antibiotics have often been added to poultry feeds to promote growth (Erdogan, 1999). The risk of bacteria acquiring resistance to antibiotics and antibiotic residues in meat probably by continuous sub-therapeutic administration of antibiotic growth-promoters in poultry feed led to a ban of antibiotics as growth promoters in European Union in 2006. These restrictions have prompted researchers to explore healthy and safe alternatives to produce safe poultry food (Sorum and Sunde, 2001). Currently, probiotics and prebiotics are the leading candidates as environmentally safe feed additives in the poultry industry (Patterson and Burkhoder, 2003).

Probiotics are live microbial supplements that increase viability, promote growth and general welfare and improve immune and digestive systems of the host when consumed in adequate amounts (Fuller, 1989). Prebiotics are non-digestible substances that are utilized by specific health-promoting bacteria, which have beneficial effects on the health and nutrition of the host (Gibson and Roberfroid, 1995). Recently, synbiotics have been proposed as a new feed additive product for promoting animal industries (Erdoğan et al., 2010). Synbiotics are defined as a combination of prebiotics and

probiotics that reinforce various aspects of health for the host by improving survival and implantation of live microbial dietary supplements in the gastrointestinal tract (Awad et al., 2008).

The data on the impact of non-antibiotic supplements on growth performance of broilers are inconsistent. In some studies, these additives improved the performance of broilers (Jin et al., 1998; Mountzouris et al., 2007; Samli et al., 2007; Awad et al., 2009), whereas others (Erdogan, 1999; Angel et al., 2005; KhodambashiEmami et al., 2012) reported that these additives had no effect on broiler performance. Dietary administrations of probiotics and prebiotics also improved intestinal morphology (Samli et al., 2007; Houshmand et al., 2012; Tsirtsikos et al., 2012), immune function (Huang et al., 2004; Koenen et al., 2004; Janardhana et al., 2009), and lipid metabolism (Mohan et al., 1996; Li et al., 2007; Salma et al., 2007; Velasco et al., 2010). However, little is known regarding the beneficial effects of incorporating synbiotics into the diets of broiler chickens.

Keeping in view the mentioned health benefits of these non-antibiotic additives,a research was designed with the objective to investigate the comparative effects of probiotics, prebiotics and synbiotics on growth performance, morphology of the jejunum, serum lipid profile and antibody response of broiler chicks.

Materials and methods

Dietary supplementations

Non-antibiotic additives are commercial preparations. The probiotic supplement (CYLACTIN® LBC ME10, DSM Nutritional Products Ltd., Birsfelden, Switzerland) was Enterococcus faecium NCIMB 10415 on a carrier of cellulose and sucrose derivative. The prebiotic supplement (Raftilose® P95, ORAFTI, Tienen, Belgium) was based on Fructooligosaccharides (FOS), which are non-digestible oligosaccharides derived from the chicory root rich in inulin. The synbiotic supplement (Biomin IMBO, Biomin GmbH, Herzogenburg, Austria) was a mixture of a probiotic (strain Enterococcus faecium IMB 52), a prebiotic (Fructo-oligosaccharide derived from the chicory root rich in inulin) and immune-stimulating natural materials (derived from sea algal species and the bacterial cell wall preparations).

Husbandry, Diets, and Experimental Designs

Four hundred (400) one-day-old male broiler chicks

Table 1. The feed ingredients and chemical composition of basal diets

Ingredient (%)	0 – 10 d	11 – 28 d	29 – 42 d
Corn	53.19	59.28	64.00
Soybean meal (44% CP)	31.55	27.58	22.96
Gluten meal (60% CP)	7.13	4.49	4.22
Soybean oil	3.00	4.00	4.00
DicalciumPhosphat	2.04	1.80	1.85
Oyster shells	1.41	1.28	1.29
Commen salt	0.36	0.36	0.36
DL-Methionin	0.29	0.25	0.30
L-Lysin,HCL	0.53	0.46	0.52
Vitamin Premix[1]	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25
Calculated composition			
ME, kcal/kg	3010	3150	3200
CP, %	23	20.10	18.50
Ca, %	1.00	0.90	0.90
Non Phytate Phosphorus (NPP), %	0.5	0.45	0.45
Na, %	0.16	0.16	0.16
Lys, %	1.44	1.20	1.00
Met + Cys, %	1.09	0.94	0.80

[1] Supplied per kilogram of diet: 600 IU vitamin A, 800 IU vitamin D, 83 mg vitamin E, 2.2 mg vitamin K3, 2 mg vitamin B6, 8 mg vitamin B12, 10 mg Nicotine amid, 0.3 mg Folic acid, 20 mg D- Biotin and 160 mg Choline Chloride.
[2]Supplied per kilogram of diet: 32 mg Mn, 16 mg Fe, 24 mg Zn, 2 mg Cu, 800 µg I, 200 µg Co and 60 µg Se.

(Ross 308) were purchased from a local hatchery, weighed (43±0.3 g) and randomly allocated into treatments with four replicates of 25 chickens based on a completely randomized design. Birds were fed with regular starter (0-10 d), grower (11-28 d) and finisher (29-42 d) diets (Table 1).

Experimental treatments were as follows: a basal diet without any additives (control diet), basal diet with added probiotic (diet Pro), basal diet with added prebiotic (diet Pre), and basal diet with added synbiotic (diet Syn). The levels of supplements were 1.5 g/kg in starter and 1g/kg in grower and finisher diets. All floor pens measured 1.3 × 2.5 m. Each pen included a hanging nipple waterer and a tube feeder. Feed and water were provided ad libitum throughout the trial. Birds were vaccinated against infectious bronchitis, and Newcastle and Gambaro diseases, but no medication was administered during the experimental. All chickens were kept under uniform temperature and lighting system during the study. All procedures were in accordance with the animal welfare norms.

Body weight gain (BWG) and feed intake (FI) were recorded during each period, and feed conversion ratio (FCR) was calculated as FI: BWG ratio.

Jejunal mucosal morphology

On days 28 and 42, two chickens were randomly selected from each replicate and slaughtered by exsanguinations through a section in the jugular vein. After slaughter, the small intestine was removed, and a 2- cm long segment was dissected from the middle of the jejunum, fixed in 10% buffered formalin for 48 h, dehydrated in increasing concentrations of ethanol ,and placed into paraffin. Sections (5 µm thick) from paraffin-embedded samples were then stained with hematoxylin-eosin for observation with a light microscope. Villus height was measured as the length between the tip of the villus and the villous-crypt axis. Measurements for crypt depth were taken from the valley between individual villi to the baso-lateral membrane.

Serum lipid profile

At 28 and 42 d of age, blood samples were collected from the brachial vein from three birds per replicate. Serum was isolated by centrifugation at 3000 × g for 10 minutes. The serum concentrations of total triglyceride (TG), total cholesterol (CHOL) and high-density lipoprotein cholesterol (HDL-C) in serum samples were analyzed by an automatic biochemical analyzer (Clima, Ral. Co, Spain), following the instructions of the corresponding reagent kit (Pars Azmoon Co., Iran). Concentration of very low-density lipoprotein cholesterol (VLDL-C) was calculated by dividing serum TG by 5. Low-density lipoprotein cholesterol (LDL-C) level was calculated by using the formula: LDL-C = Total cholesterol – HDL cholesterol - VLDL cholesterol (Friedewald et al. 1972).

Antibody response to SRBC

Two birds per replicate were injected (via the brachial vein) by 0.1 ml of a 5% suspension of SRBC in phosphate buffered saline (PBS) at 21 and 35 days of age. Blood samples were collected six days after each injection (at 27 and 41 d). The blood was kept at ambient temperature for at least two hours, and serum separated. Serum samples were stored in a -20°C until analyzed. Antibody titers against SRBC were determined by a hemagglutination method. This assay was conducted in 96-well, U-bottomed micro-titer plates. Briefly, serum samples were placed in a water bath for 30 min at 55° C to neutralize the inhibitory effect of complement regulatory proteins against SRBC. Subsequently, 25 µL serum samples were injected into the wells in the first two columns. The dilution was done from second column with adding PBS solution and continued until the penultimate column in each plate. In the next stage, 25 µL of 1% SRBC suspension in PBS was added to each serum dilution (final volume of 50 µL). Finally, the plates were kept in a wet nylon (to prevent evaporation) for 2 h, allowing the antibody-antigen reaction to occur at room temperature. The hemagglutination titer for each serum sample was reciprocal of the highest serum dilution resultiong in 100% agglutination.

Statistical analysis

Data were analyzed as a completely randomized design using the General Linear Model (GLM) procedure and the means were compared by LSMEANS procedure (SAS (2001). The level of significance was set at $P \leq 0.05$, and a P-value between 0.05 and 0.1 was considered as trend.

Results

Growth Performance

The impact of feed additives on growth performance is shown in Table 2. Significant increase ($P<0.05$) in BWG was observed in chicks fed diet Syn during 0 to 28-day period, and during entire study period (0-42 d). Synbiotics-supplemented diet also improved FCR from 29 to 42 d and from 0 to 42 d of age compared with the control diet; whereas the other two

Table 2. The effects of feed additive supplementations on growth performance in broiler chicks up to the age of 42 days

Item[1]	Dietary treatments[1]				SEM	P-value
	control	probiotic	prebiotic	synbiotic		
BWG						
1–28 d	1322[b]	1375[ab]	1395[ab]	1417[a]	32.4	0.013
28–42 d	1186	1235	1246	1270	40.5	0.154
1–42 d	2508[b]	2610[ab]	2641[ab]	2697[a]	51.32	0.025
FI						
1–28 d	1970	1989	1996	2024	28.7	0.343
28–42 d	2540	2570	2593	2580	34.6	0.619
1–42 d	4510	4559	4589	4604	49.8	0.219
FCR						
1–28 d	1.49	1.45	1.43	1.43	0.026	0.174
28–42 d	2.14[a]	2.08[ab]	2.08[ab]	2.02[b]	0.035	0.023
1–42 d	1.80[a]	1.75[ab]	1.74[ab]	1.71[b]	0.031	0.033

Within each row, mean with common superscript (s) do not differ significantly ($P > 0.5$).
[1]The levels of supplements were 1.5 g/kg in starter and 1g/kg in grower and finisher diets.
[2]BWG, Body weight gain (g); FI, feed intake (g); FCR, feed conversion ratio (FCR)

treatments were intermediate and not statistically different from other groups. There was no significant effect of treatments on feed intake.

Morphological parameters of the jejunum

Morphological data showed that synbiotic- or prebiotic-fed broilers had higher ($P< 0.05$) jejunal villus height at 28 d of age (Table 3). At this age, the villus height:crypt depth ratio tended to be higher in the birds fed diets Pro, Pre and Syn versus the control birds ($P = 0.072$). All experimental diets increased villus height and villus height:crypt depth ratio compared with the control diet at 42 d of age. However, crypt depth in jejunum was not affected by dietary treatments at either stages ($P > 0.05$).

Serum lipid profile

Serum lipid profile is presented in Table 4. At both ages, CHOL and LDL-C levels were significantly lower in the birds fed the diets Pro, Pre and Syn than in the birds fed control diet, with the birds fed diet Syn having the lowest LDL-C levels. At 28 d of age, feeding dietSyn also resulted in significant increases in HDL-C levels compared with the control diet. Although no significant difference was observed in TG and VLDL-C levels during the experiment, the values tended to belower in the birds fed the experimental diets compared to the control diet at 42 d of age ($P = 0.089$).

Overall antibody response against SRBC

The effects of different non-antibiotic supplements on antibody response against SRBC in broiler chicks are presented in Fig. 1. There were no differences in anti-SRBC titer among experimental diets at 21 d of age. At 42 d of age, the antibody titer against SRBC in

Table 3. Effects of feed additives on jejunal histologyin broiler chickens at 28 and 42 d of age

Item	Dietary treatments				SEM	P-value
	control	probiotic	prebiotic	synbiotic		
28 d						
Villus height (µm)	870[b]	945[ab]	953[a]	987[a]	32.17	0.032
Crypt depth (µm)	119	125	121	128	8.95	0.431
Villus height/crypt depth	7.31	7.56	7.88	7.71	0.197	0.072
42 d						
Villus height (µm)	845[b]	921[a]	932[a]	952[a]	19.83	0.009
Crypt depth (µm)	125	128	130	129	9.58	0.557
Villus height/crypt depth	6.76[b]	7.20[a]	7.17[a]	7.38[a]	0.145	0.012

Within each row, mean with common superscript (s) do not differ significantly ($P > 0.5$).

the birds fed diets Pro, Pre and Syn were significantly higher than in the birds fed the control diet; the birdsfed diet Syn had the highest antibody titer ($P< 0.05$).

Discussion

Although, numerical improvement in performance traits was observed in the chicks fed the diet supplemented with probiotics and prebiotics compared with control birds, the data were not significant. The results of this study support the findings of other studies reporting no significant change in BWG and FCR of broilers by dietary probiotics and prebiotics (Erdogan, 1999; Xu et al., 2003; Angel et al., 2005; KhodambashiEmami et al., 2012). In contrast, several reports showed an improvement in growth performance of broiler chickens fed probiotics (Jin et al., 1998; Mountzouris et al., 2007; Samli et al., 2007; Awad et al., 2009) and prebiotics (Zhang et al., 2003; Huang et al., 2005; Li et al; 2007). The contradictory results among the studies can partly be explained by strain of microorganisms and amount of live organisms in the probiotics components, survivability of the microorganisms in the feed, prebiotics ingredients and dietary nutrient levels.

In the present study, BWG and FCR were significantly improved by including synbiotics in the diet. The beneficial effects of synbiotics on broilers performance are in agreement with the finding of Awad et al. (2009). In contrast, although synbiotics supplement improved growth parameters in weekly measurement, no significant response to synbiotics was observed during the whole experimental period (Erdoğan et al., 2010). Presence of prebiotics in synbiotic supplements may boost the growth, colonization, or activity of the probiotics in the gut.

Different feed additives positively influenced the morphological parameters of the jejunum in our study. The increased villus height may be due to the increased colonization of beneficial bacteria that supply nutrients and stimulate enlargement of intestinal villus (Van Leeuwen et al., 2004). Several researchers reported significant increases in the villus height by dietary probiotics and prebiotics (Awad et al., 2006 and 2009; Samli et al., 2007; Houshmand et al., 2012; KhodambashiEmami et al., 2012; Tsirtsikos et al., 2012). The intestinal villi play a crucial role in facilitating nutrient digestion and absorption, as the villi greatly increase small intestine's surface area and are the first tissues in the intestine to make contact with nutrients (Gartner and Hiatt, 2001). The ratio of villus height to crypt depth is a good indicator of intestinal health and digestive tract maintenance (Pluske et al., 1996). In general, the longer villi and higher villus height:crypt depth ratio are associated with increased epithelial cell turnover, which results in improving nutrient absorption (Xu et al., 2003). This may explain, at least in part, the better performance observed in the birds fed with diets supplemented with non-antibiotic supplements.

Single dietary administration of probiotics or prebiotics has been shown to improve serum lipid profile in

Table 4. Effects of feed additives on serum lipid profile[1] (mg/ dL) in broiler chickens at 28 and 42 d of age

Item[1]	Dietary treatments				SEM	P-value
	control	probiotic	prebiotic	synbiotic		
28 d						
TG	105.1	96.8	81.1	95.0	8.1	0.202
CHOL	184.1^{a}	160.9^{b}	150.8^{b}	146.3^{b}	7.8	0.001
HDL-C	70.1^{b}	73.9^{ab}	76.2^{ab}	81.7^{a}	4.3	0.041
VLDL-C	21.0	19.4	16.2	19.0	1.6	0.201
LDL-C	92.9^{a}	67.6^{b}	58.3^{bc}	43.0^{c}	7.3	<0.001
42 d						
TG	85.9	90.3	71.1	71.3	7.7	0.089
CHOL	137.9^{a}	117.2^{b}	117.6^{b}	116.5^{b}	6.5	0.034
HDL-C	65.9	67.7	77.9	78.3	5.9	0.176
VLDL-C	17.2	18.1	14.2	14.3	1.5	0.089
LDL-C	54.8^{a}	31.4^{b}	27.4^{bc}	24.0^{c}	6.1	<0.001

Within each row, mean with common superscript (s) do not differ significantly ($P > 0.5$).
[1]TG=triglyceride, CHOL=total cholesterol, HDL-C=high-density lipoprotein cholesterol, VLDL-C=very low density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol.

broilers, wherein these supplement reduced serum TG, CHOL and/or LDL-C levels and increased HDL-C levels (Mohan et al., 1995, 1996; Kalavathy et al., 2003; Salma et al., 2007; Yalcinkaya et al. 2008; Velasco et al., 2010). There is not sufficient data on the impacts of dietary synbiotics on lipid metabolism in broilers. In this study, both CHOL and LDL-C levels were predominantly lower in the chicks fed the diets containing non-antibiotic additives, especially synbiotics, compared with those fed the control diet. The most important way that probiotics reduce serum cholesterol may be due to the fact that some probiotic bacteria, especially lactic acid-producing bacteria, can interfere with cholesterol absorption in the gut through deconjugating bile salts or by directly assimilating cholesterol (Ooi and Liong, 2010). The most likely mechanism by which prebiotics reduce serum cholesterol would be through binding bile acids, resulting in increased cholesterol removal by hepatic synthesis of new bile acid (Velasco et al., 2010). Serum lipid is very important in evaluating the results related directly to animal health and meat quality (Fletcher, 2002). As a result, meat from broilers fed with diets supplemented with these products is more appropriate for poultry markets due to its beneficial effect on human health. In this experiment, dietary inclusion of several non-antibiotic additives, especially synbiotic, affected the antibody-mediated mediated immune response. Modification of the systemic antibody response to antigens by probiotics has been reported in other experiments with broilers (Huang et al., 2004; Koenen et al., 2004). In addition, the use of oligosaccharides in the broilers' diets are also reported to improve the immune function by increasing IgM and IgG antibody titers in plasma (Janardhana et al., 2009). The synbiotic used in the preset study was a combination of probiotics, prebiotics and other compounds stimulating the immune system. Therefore, it is expected that the synergistic effect of these compounds may have a beneficial effect on the immune system.

Conclusions

Inclusion of synbiotics to broiler diets resulted in improvements in body weight gain and feed conversion ratio. Probiotic and prebiotic supplements also had a slight growth-promoting effect. The supplements used in this study improve jejunal histology and serum lipid profile as well as the antibody-mediated immune function. However, the chicks supplemented with synbiotics showed a greater immune efficiency than birds fed probiotics and prebiotics. In general, the results suggested that these supplements, especially synbiotics, might be a suitable alternative to antibiotic growth-promoters as the efforts to reduce the use of antibiotics growth-promoters in poultry feed increase.

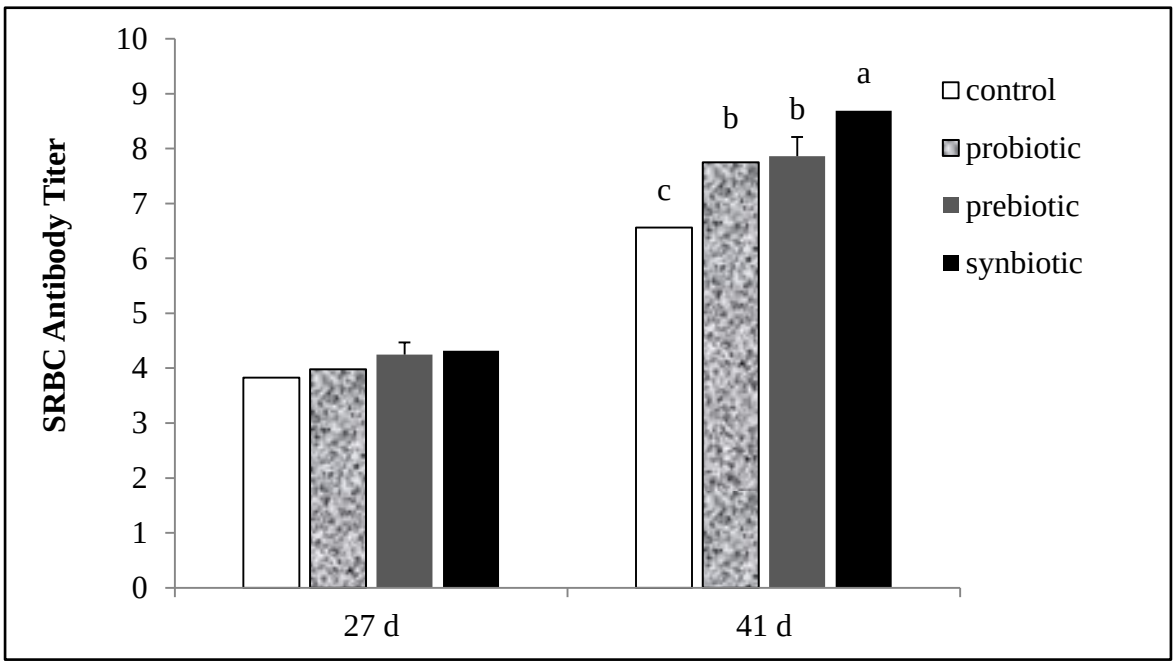

Figure 1. Effect of feed additives on serum anti-SRBC antibody titers of male broiler chickens at 27 and 41 d of age. All data points are mean values from 8 replicates ± SEM ($^{a,b,c}P \leq 0.05$). SRBC = Sheep red blood cell.

Acknowledgments

The authors acknowledge the Razi Vaccine and Serum Research Institute for providing sheep red blood cells.

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Communicating editor: *Mojtaba Zaghari*