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Effects of Dietary Inclusion of Probiotic or Prebiotic on Growth Performance, Organ Weight, Blood Parameters and Antibody Titers Against Influenza and Newcastle in Broiler Chickens

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Abstract: An experiment was performed to evaluate the effects of dietary supplementation of probiotic (Biosof®) and prebiotic (Active-Mos®) on broiler performance, organ weights, blood parameters and antibody titers against Influenza and Newcastle. Three hundred twelve 7-d-old male broiler chicks were randomly assigned to one of 3 dietary treatments for 6 weeks. The dietary treatments were: (1) control diet (without additive), (2) control diet plus probiotic (0.1% of Biosof®/ton of feed), (3) control diet plus prebiotic (0.1% of Active-MOS/ton of feed). Overall body weight and feed conversion ratio were significantly ($p<0.05$) improved by dietary inclusion of the probiotic and the prebiotic compared with the control diet. The relative weights of duodenum, jejunum and ileum were greater ($p<0.05$) for probiotic-fed birds than the control group, however, duodenum relative weight in the prebiotic group was also significantly greater than control group. The serum concentration of cholesterol was lower in the probiotic fed group than prebiotic and control groups. The serum concentrations of total protein, albumin, globulin and albumin/globulin ratio were not affected by dietary treatments. The number of heterophils, monocytes, lymphocyte, eosinophils were not affected by dietary treatments. However, the heterophils to lymphocyte ratio was lower in the probiotic group than prebiotic and control groups. Serum antibody titers against Newcastle was higher in probiotic treatments compared with prebiotic and control groups, but no significant changes were observed in the antibody titers against Influenza. The results of this study suggest that the probiotic and prebiotic can be used as an alternative to antibiotic growth promoters in broiler diets and can improve the immune response to some vaccines.

Key words: Broiler, probiotic, prebiotic, performance, immune response

INTRODUCTION

Antibiotics are generally used in the poultry industry to prevent poultry pathogens and diseases as growth promoters. However, using of antibiotic in the diet caused the development drug-resistant bacteria (Edens, 2003), drug residues in the bird's body (Pelicano *et al*., 2004) and imbalance of normal microflora (Barton, 2000). As a result, there is an increasing interest in finding a suitable substitute to antibiotics in poultry production. The use of probiotics and prebiotics can be a suitable alternative and successful approach to antibiotic application in the poultry industry.

Probiotics, based on Fuller (1989) definition, "are live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance". Probiotic efficacy depends upon several factors, such as microbial species composition (e.g., single or multi strain) and viability, application procedure, dosing level, frequency of application, age, type of diet, sanitation and environmental stressors factors. However, beneficial effects of probiotic on broilers including: performance (Mountzouris *et al*., 2007; Kralik *et al*., 2004), pathogen inhibition (Mountzouris *et al*., 2009), modification of intestinal microflora (Teo and Tan, 2007; Mountzouris *et al*., 2009), nutrient digestibility (Apata, 2008) and immunomodulation and gut mucosal immunity (Farnell *et al*., 2006; Teo and Tan, 2007) have been reported. These positive effects by application of probiotics could be related to increasing population of beneficial microflora and removal of pathogenic bacteria by means of competitive exclusion and antagonism (Fuller, 1989); adapting bacterial metabolism (Jin *et al*., 1997); improving feed intake, digestion and absorption (Nahanshon *et al*., 1993) and stimulating the immune system (Havenaar and Spanhaak, 1994). The enhancement of the immune system may be in relation to increase production of antibodies particularly IgG and IgA classes and also, increase local antibodies at mucosal surface such as gut wall (usually IgA) (Koenen *et al*., 2004).

According to Gibson and Roberfroid (1995), a prebiotic is a non-digestible food ingredient that can be utilized by intestinal microflora, which beneficially affects the host. The beneficial effects of prebiotics on performance, feed

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conversion ratio (Iji *et al*., 2001), reducing microbial pathogens (Jung *et al*., 2008) and improving immune system have been reported (Savage *et al*., 1996). In addition, dietary prebiotics are stable in feed, resistance to the conditions available in crop and stomach and improve developing beneficial bacteria in the intestine (Gibson and Roberfroid, 1995; Jung *et al*., 2008). The present study was performed to evaluate the effects of dietary probiotic and prebiotic on broiler performance, organ weight, blood parameter and antibody titers against Influenza and Newcastle.

MATERIALS AND METHODS

Housing and management: Three hundred twelve 1-d-old male broiler chicks (Ross-308) were purchased from a commercial hatchery. All of the chicks were fed with the same diet until 7 d of age. Thereafter, the birds were randomly divided into 3 treatments (104 birds/group) and housed in pens measuring 1.50 x 1.50 m concrete floor covered with new wood shavings. Each treatment had 8 replicates (13 birds/pen). Birds had free access to water and feed during the experiment. The lighting program and temperature were according to Ross guidelines throw-out all the experiment.

Dietary treatments: The dietary treatments were: (1) basal diet (control), (2) basal diet plus 0.1% of the probiotic Biosof®/ton of feed and (3) basal diet plus 0.1% of the prebiotic product Active-MOS/ton of feed. All of the chicks were fed with a diet without any additive until 7 d of age. After that, the chicks were fed with the starter diets from d 7 to 21 and grower diets from d 22 to 42 (Table 1). Active-MOS® (Biorigin, Lençóis Paulista, SP) prebiotic contained manan-oligosaccharide which is extracted from yeast cell wall. The probiotic Biosaf® SC 47 (SC) was composed of a minimum of $5x10^{10}$ colony forming units (cfu) gr *Saccharomyces cerevisiae* (strain NCYC sc 47). This product was obtained from the company of Society Industrielle LESAFFRE (Maisons Alfort Cedex, France).

Growth performance traits and organ weights: Feed intake (FI) and body weight (BW) were measured and feed conversion ratio (FCR) was calculated at the end of each period (days 21 and 42 of age). Mortality was recorded daily. At the end of the experiment, 16 birds per treatment were randomly selected and blood samples were taken from the wing vein using a syringe. Then, blood samples were centrifuged at 3,000×g for 10 min at 4°C to obtain serum for analysis of hematology. After blood sampling, the birds were killed by severing the jugular vein. The proventriculus, gizzard, heart, liver, spleen, bursa of Fabricius, small intestine (duodenum, jejunum and ileum) and ceca were excised and weighed.

Table 1: Composition of experimental diets in starter (7-21 d) and grower (22-42 d) period

Item	Starter	Grower
Ingredient (% of diet)		
Corn	60.55	65.8
Soybean meal	30.32	26.8
Fish meal	3	1.5
Vegetable oil	2.35	1.86
Dicalcium phosphate	1.4	1.55
Calcium carbonate	1.11	1.1
Mineral and vitamin premix[1]	0.6	0.6
Salt	0.3	0.3
DL-Met	0.2	0.3
L-Lys, HCL	0.17	0.19
Composition, calculated		
ME (kcal/kg)	3000	3010
CP (%)	21.1	19.1
Met (%)	0.5	0.42
Met+Cys (%)	0.95	0.86
Lys (%)	1.22	1.1
Calcium (%)	0.9	0.85
Available phosphorus (%)	0.45	0.42

[1]Mineral and vitamin premix provided the following:

Mn, 89 mg	Zn, 88 mg
Fe, 34 mg	Cu, 63 mg
Se, 0.3 mg	I, 1.8 mg
vitamin A, 6,238 IU	vitamin D 3, 2,275 IU
vitamin E, 20 IU	vitamin B 12, 0.013 mg
vitamin K, 2.9 mg	niacin, 75 mg
folic acid, 0.86 mg	biotin, 0.1 mg
riboflavin, 5.5 mg/kg	of the starter diet
Mn, 71 mg	Zn, 71 mg
Fe, 27 mg	Cu, 50 mg
Se, 0.24 mg	I, 1.4 mg
vitamin A, 4,990 IU	vitamin D 3, 1,820 IU
vitamin E, 16 IU	vitamin B 12, 0.011 mg
vitamin K, 2.3 mg	niacin, 60 mg
folic acid, 0.69 mg	biotin, 0.08 mg
riboflavin, 4.4 mg/kg	of the grower diet

Analyses of blood parameters and humoral immunity: The heterophils, lymphocytes, monocytes, eosinophils of blood samples were enumerated using Hemavet Multi species Hematology Systems (Drew Scientific Inc., Oxford, CT). Cholesterol level was also tested using the enzymatic colorimetric method by means of a Cholesterol Liquicolor kit (GmbH, Wiesbaden, Germany). Albumin, Globulin and total protein levels were determined by appropriate commercial diagnostic kits for avian species (BioSystems, S.A. Barcelona, Spain and GmbH, Wiesbaden, Germany). In order to evaluate the effects of probiotic and prebiotic on antibody titers against Influenza and Newcastle, a live (ocular) and inactivated (subcutaneous) Influenza (Razi® co. Tehran, Iran, strain H_9N_2) and Newcastle (Razi® co. Tehran, Iran) vaccine were injected to the chicks at 21 and 35 d of age. Seven d after each injection, 4 birds of each pen were randomly selected and blood samples were taken from the wing vein using a syringe. Antibody titers against Influenza and Newcastle in serum were measured by hemagglutination inhibition test (Alexander and Chettle, 1977).

Statistical analysis: All data were analyzed by the GLM procedure of SAS 9.1 (SAS Institute Inc., 2003). Duncan's multiple rage test was used to compare the means. All statements of significance were based on $p<0.05$.

RESULTS

Growth performance: The effects of probiotic and prebiotic on performance of broilers has been shown in Table 2. During the starter phase (7 to 21 d), broilers in probiotic group had higher ($p<0.05$) BW and FI compared with control group. However, in the grower period (22 to 42 d) BW and FI were higher in the prebiotic group than the control and birds fed probiotic-containing diet. There was a significant difference in FCR during the starter phase as well as in whole experiment among the treatment groups. During starter phase, probiotic treatment had better FCR values compared with prebiotic and control groups. None of the treatment groups had a significant effect on FCR during the grower phase. For the whole period of experiment, BW was higher in prebiotic treatment than in control (probiotic and prebiotic were the same) group. Overall, total FI for the whole period of the experiment was higher in prebiotic and probiotic treatment compared with control group and it was higher for birds supplemented with prebiotic than probiotic. For the whole period of experiment, FCR values were better for prebiotic fed group.

Relative organ weights: The relative weights of the proventriculus, gizzard liver, heart, ceca, spleen and bursa of Fabricius were not affected by the dietary treatments. However, relative weight of duodenum and jejunum were significantly higher in the probiotic and prebiotic-supplemented group compared with the control group, but only the relative weight of the ileum was higher in the probiotic group when compared with prebiotic and control groups (data not shown).

Blood chemistry: The effects of dietary treatments on blood parameter are presented in Table 3. Concentration of total protein, albumin and globulin was not affected by any of dietary treatments, however, serum cholesterol concentration was decreased in the probiotic supplemented group compared with the prebiotic supplemented and control group.

Immune response and antibody titers: The effects of dietary treatments on the relative percent of white blood cells and antibody titers against Influenza and Newcastle are presented in Table 4. The heterophil: lymphocyte ratio was significantly ($p<0.05$) decreased in the probiotic supplemented group compared with either the control group or prebiotic supplemented group. However, the relative percent of heterophil, monocyte, lymphocyte and eosinophil were not affected by dietary supplementations. Serum antibody titers against Newcastle was significantly ($p<0.05$) increased in the probiotic supplemented group compared to the prebiotic

Table 2: Effect of probiotic and prebiotic on growth performance for the 2 growth phases (starter and grower) and the whole experiment[1]

	Dietary treatment				
Item	Control	Probiotic	Prebiotic	SEM	p-value
Starter phase (7 to 21 d)					
BW (g)	494.6^{b}	561.33^{a}	528.3^{ab}	17.51	0.03
Feed intake (g)	834^{b}	893^{a}	876.32^{ab}	15.82	0.001
FCR[2]	1.76^{a}	1.59^{b}	1.66^{ab}	0.045	0.04
Grower phase (22 to 42 d)					
BW (g)	1559^{b}	1683^{ab}	1716.33^{a}	34.46	0.04
Feed intake (g)	3190.66^{b}	3298.33^{ab}	3368.66^{a}	51.45	0.003
FCR	2.05	1.959	1.96	0.031	NS
Whole period (7 to 42 d)					
BW (g)	2053.33^{b}	2164.33^{ab}	2244.63^{a}	41.39	0.03
Feed intake (g)	4024.66^{c}	4154.33^{b}	4244.98^{a}	55.52	0.002
FCR	1.96^{a}	1.92^{ab}	1.89^{b}	0.019	0.01

^{a,b}Within the same row, means with different superscripts are significantly different ($p<0.05$)

[1]Data represent means from 8 replicates (i.e., pens) per treatment

[2]FCR: Feed conversion ratio (feed intake: BW gain)

Table 3: Effects of dietary treatments on blood chemistry

	Dietary treatment				
Item	Control	Probiotic	Prebiotic	SEM	p-value
Cholesterol (mg/dL)	136.6^{a}	94.7^{b}	121^{a}	7.32	0.001
Total protein (g/dL)	3.50	3.56	3.53	0.16	0.125
Albumin (g/dL)	1.63	1.88	1.77	0.12	0.246
Globulin (g/dL)	1.87	1.92	1.79	0.12	0.326
Albumin/globulin	0.87	0.98	0.99	0.09	0.185

^{a,b}Within the same row, means with different superscripts are significantly different ($p<0.05$)

Table 4: Effects of dietary treatments on leucocytes and antibody titers against Influenza and Newcastle in the blood

	Dietary treatment			
Item	Control	Probiotic	Prebiotic	SEM
Heterophils[1](%)	49.33	37.33	46.00	7.49
Monocytes[1] (%)	1.50	1.00	1.50	0.34
Lymphocytes[1] (%)	48.00	58.75	52.33	7.8
Eosinophils[1] (%)	2.33	3.66	2.25	1.45
H:L[2]	1.03[a]	0.63[b]	0.88[ab]	0.06
Influenza				
28 d	0.83	2.50	4.25	1.17
42 d	4.00	5.50	6.33	0.93
Newcastle				
28 d	4.50[b]	6.67[a]	6.00[ab]	0.70
42 d	5.25[b]	7.00[a]	6.67[ab]	0.50

[a,b]Within the same row, means with different superscripts are significantly different ($p<0.05$)
[1]Expressed as a percent of total white blood cell [2]H:L: heterophil:lymphocyte ratio

supplemented group and the control at days 28 and 42. Serum antibody against Influenza did not show any difference among the dietary treatments.

DISCUSSION

The beneficial effects of probiotic and prebiotic on broiler performance in the present study are in agreement with previous reports (Kralik *et al*., 2004; Sims *et al*., 2004; Mountzouris *et al*., 2007; Kim *et al*., 2011). Improving performance of broiler chickens fed probiotic is thought to be because of maintaining of beneficial microbial population (Teo and Tan, 2007) improving feed intake and nutrient digestibility (Apata, 2008; Nahanshon *et al*., 1992), altering bacterial metabolism (Jin *et al*., 1997) and reducing of cell turnover of the intestinal epithelium (Awad *et al*., 2009).

Dietary probiotic administration increased the relative weights of duodenum jejunum and ileum when compared with control group. Dietary prebiotic only increased the relative weight in the duodenum when compared with control group. These findings are in agreement with the results of Awad *et al*. (2009) and Watkins and Kratzer (1984). Similar results have also shown that the improvement in the relative weight of small intestine, by dietary probiotic or prebiotic supplementation is correlated to morphometric histological changes, improved surface of absorption and decrease in pathogenic bacteria (Awad *et al*., 2009, Tellez *et al*., 2010).

Supplementing broiler diet with probiotic and prebiotic did not have any effect on total protein, albumin, globulin and albumin to globulin ratio. These findings are in line with the results of Dimcho *et al*. (2005), Alkhalf *et al*. (2010) and Sadeghi *et al*. (2008). The results showed that the probiotic have a cholesterol decreasing effect on broiler chickens. These results are in agreement with those reported by Mohan *et al*. (1996) and Jin *et al*. (1998). The decrease in cholesterol level could be related to de-conjugating of bile salts by means of bacteria of probiotic, as a result they are absorbed less from the intestine and are excreted more in the feces (Klaver and Van der Meer, 1993). Plasma immunoglobulins were not affected, but the heterophil:lymphocyte ratio was affected by probiotic. Regarding to the effects of prebiotic on the parameters of leucocytes, Kim *et al*. (2010), reported that the heterophil:lymphocyte ratio increased by adding prebiotic in the diet. Serum antibody titer against Newcastle was affected by the use of probiotic in the diet. This result is in agreement with results reported by Khaksefidi and Ghoorchi (2006). They reported an increase of antibody production against Newcastle in 50 mg/kg probiotic supplementation at 10 days of post immunization compared to control group. Cross *et al*. (2002) and Dalloul *et al*. (2003), stated that some probiotics may stimulate a protective immune response and improve resistance to microbial pathogens.

Conclusion: The results of the current study suggest that the dietary supplementation with probiotic in the starter period and prebiotic in the grower period increased BW. Also, dietary addition of probiotic and prebiotic in the starter period improved FCR. For the entire period of the experiment, probiotic showed a growth-promoting effect, but lower than prebiotic. Prebiotic supplementation did not have any significant effect on the blood parameters, antibody titer against Influenza and Newcastle and parameters of leukocyte. However, the relative weight of the duodenum was increased by adding prebiotic in the diet. Serum antibody titer against Newcastle was increased by supplementation of probiotic in the diet. Therefore, these products might be suitable substitutes for antibiotic growth promoters as interests to eliminate antibiotic growth promoters in animal diets are increasing.

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