Growth performance and immune response of broiler chickens fed diets supplemented with probiotic and (or) prebiotic preparations

H. R. Taheri*, M. Kokabi Moghadam, M. Kakebaveh and T. Harakinezhad

Department of Animal Science, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

* Corresponding author, E-mail address: taherihr@gmail.com

Abstract The objective of this research was to investigate the efficacy of dietary inclusion of probiotics Primalac® and Bactocell® and prebiotic Fermacto® on broiler's performance and immune response, individually or in combination. A total of 540 one-d-old male Ross 308 broiler chicks were allocated into 6 experimental treatments with 6 replicates of 15 birds per replicate from 1 to 42 d of age. The birds received a basal diet (control) or the basal diet supplemented with probiotic Primalac® (PP), probiotic Bactocell® (PB), prebiotic Fermacto® (Pre), probiotic Bactocell® + prebiotic Fermacto® (PBPre) or probiotic Primalac® + prebiotic Fermacto® (PPPre). Performance parameters were measured from 1-42 d of age. A suspension of sheep red blood cells (SRBCs) was injected into the breast of 3 birds from each replicate on d 22, and the antibody titer was measured on d 30. At d 22, blood samples (from 3 birds per replicate) were taken for measuring the white blood cells (WBCs), heterophil (HE) percent, lymphocyte (LY) percent, and the ratio of heterophil:lymphocyte (H/L). No significant differences were found between the control and supplemented groups in average daily gain (ADG), average daily feed intake (ADFI), antibody titer against SRBCs, HE percent, LY percent and H/L. Addition of PBPre or PPPre to the diet improved FCR by 8.5 and 12.7%, respectively, compared with the control group, and PBPre supplementation resulted in an increase in WBCs compared to other treatment groups.

Keywords: broiler, immune response, performance, prebiotic, probiotic, synbiotic

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Introduction

The removal of antibiotics from poultry diets has led farmers to search for new solutions to maintain animal health without affecting performance parameters. A promising strategy may be the use of beneficial microflora in the intestine, which improves the gut immune system; hence increase its protective barrier against enteric bacteria, humoral immune reaction against pathogens and cell immunity response, as well. Such improvments in the immune system are the outcome of the stimulation of immune cells and cytokines production in the gut mucosa (Lillehoj and Trout, 1996; Klasing, 1998; Muir et al., 2000). The high population of beneficial microflora in the gastrointestinal tract may be accomplished by using them as a probiotic product or by the stimulation of the growth of beneficial bacteria already present in the gut by including the specific substrates-prebiotic- in the diet. A prebiotic is a non-digestible feed ingredient that favorably affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the intestine and cecum (Gibson and Roberfroid, 1995).

Several studies have shown that an increase in the digestion and absorption of nutrients is a major mechanism responsible for the enhanced growth performance of broilers in response to probiotic (Mountzouris et al., 2007; Li et al., 2008) and prebiotic (Huang et al., 2005; Biggs et al., 2007) supplements. It has been shown that beneficial bacteria such as *Lactobacillus* spp. produced digestive enzymes which could help to enhance digestion and improve feed conversion in the host animal (Jin et al., 2000). Increased villus height and villus surface area have also been reported as the mechanisms responsible for the enhanced feed efficiency of broilers fed diets supplemented with probiotic or prebiotic preparations (Pluske et al., 1996; Awad et al., 2009).

Although some researchers reported positive effects of probiotics (Taheri et al., 2010a; Ghasemi et al., 2014) or prebiotics (Xu et al., 2003; Chee et al., 2010) on performance, others found no positive responses to probiotics (Angel et al., 2005; Al-Zenki et al., 2009) or prebiotics (Yang et al., 2008; Alzueta et al., 2010). Such inconsistencies may be due to factors such as type and dosage of

the supplements. The efficacy of probiotic preparations may be enhanced by the simultaneous application of the probiotics and prebiotics, which enables the incorporation of probiotic strains into the community of endogenous bacteria, thus stimulating the growth and/or the activities of both the exogenous (probiotic) and endogenous bacteria (Roberfroid, 1998; Suskovic et al., 2001). de Vrese and Schrezenmeir (2008) have defined the mixture of probiotic and prebiotic as synbiotic that exerts synergistic effects in promoting beneficial microorganisms and the health of the digestive tract of the host animal (Gallaher and Khil, 1999). Thus, it may present a considerable biological advantage with respect to growth performance and feed efficiency in poultry production (Awad et al., 2009). Different supplements of probiotic and prebiotic, individually or in combination, have been examined in broiler nutrition, including the *Bifidobacterium lactis*-based probiotic and galactooligosaccharides (Jung et al., 2008), probiotic Bio-Plus 2B® and prebiotic Bio-Mos® (Midilli et al., 2008), *Lactobacillus and Bacillus cereus*-based probiotic and *Astragalus* polysaccharides (Li et al., 2009), *Enterococcus faecium* and inulin (Rodriguez et al., 2012), probiotic Bio K1® and prebiotic Bio-Mos® (Houshmand et al., 2011), probiotic Protexin® and prebiotic SAF-Mannan® (Sohail et al., 2012), *Lactococcus lactis* and raffinose family oligosaccharides (Maiorano et al., 2012) and *Lactobacillus* strains-based probiotic and isomalto-oligosaccharides (Mookiah et al., 2014). Probiotic supplements such as Primalac® (Talebi et al., 2008; Willis and Reid, 2008) or Bactocell® (Al-Zenki et al., 2009; Taheri et al., 2010b) and prebiotics such as Fermacto® (Torres-Rodriguez et al., 2005; Ghasemi et al., 2014) are among the wide variety of additives that have been investigated extensively in broiler nutrition. However, there is no study investigating the combined effect of dietary inclusion of these additives on broiler performance. Therefore, the aim of this research was to determine whether there was a synergistic effect on the broiler performance and immune response when probiotic Primalac® or Bactocell® are used in combination with Fermacto®in the diet.

Materials and methods

A total of 540 one-d-old male Ross 308 broiler chicks were randomly divided into 36 groups. Each treatment consisted of 6 replicates. Each replicate of 15 broilers was assigned to a pen (1.5×1.5 m). Birds were reared in floor pens and in an environmentally controlled house with a 23:1 light:dark cycle. The experimental birds had *ad libitum* access to water and mash diets. They were fed either a basal diet (as a control group) or the basal diet

Table 1. Ingredients and chemical composition of the basal diet (g/kg, unless otherwise indicated)

	1 to 10 d	11 to 24 d	25 to 42 d
Ingredient			
Corn (85 g CP/kg)	553.3	605.7	662.6
Soybean meal (440 g CP/kg)	370.0	320.0	270.0
Soybean oil	30.0	30.0	25.0
Calcium carbonate	11.0	9.7	9.4
Dicalcium phosphate	19.5	17.5	16.5
Common salt	3.0	3.0	2.0
Sodium bicarbonate	1.0	2.5	4.0
DL-Methionine	3.4	3.1	2.6
L-Lysine	2.8	2.6	2.2
L-Threonine	1.0	0.9	0.7
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
Calculated analysis			
ME (MJ/kg)	12.33	12.54	12.72
Crude protein	216.0	198.0	180.0
Methionine+Cystine	10.0	9.3	8.3
Lysine	13.5	12.1	10.6
Calcium	10.0	9.0	8.5
Available phosphorus	4.9	4.5	4.3

[1]The vitamin premix supplied the following per kilogram of complete feed: vitamin A, 9,000 IU (retinyl acetate); cholecalciferol, 2,000 IU; vitamin E, 18 IU (dl-a-tocopheryl acetate); vitamin B_{12}, 0.015 mg; menadione, 2 mg; riboflavin, 6.6 mg; thiamine, 1.8 mg; pantothenic acid, 30 mg; niacin, 10 mg; choline, 500 mg; folic acid, 1 mg; biotin, 0.1 mg; pyridoxine, 3 mg.

[2]The mineral premix supplied the following per kilogram of complete feed: manganese ($MnSO_4 \cdot H_2O$), 80 mg; zinc (ZnO), 80 mg; iron ($FeSO_4 \cdot 7H_2O$), 80 mg; copper ($CuSO_4 \cdot 5H_2O$), 10 mg; selenium (Na_2SeO_3), 0.3 mg; iodine (Iodized NaCl), 0.8 mg; cobalt ($CoCl_2$), 0.25 mg.

supplemented with probiotic Primalac® (PP), probiotic Bactocell® (PB), prebiotic Fermacto® (Pre), probiotic Bactocell® + prebiotic Fermacto® (PBPre) or probiotic Primalac® + prebiotic Fermacto® (PPPre). Probiotic Primalac® (StarLabs Inc., Clarksdale, MO, USA) contained a total of 2×10^8 colony forming unit of *L. acidophilus*, *L. casei*, *E. faecium* and *B. bifidium* per kg supplement. It was administered at a concentration 900, 450 and 225 mg/kg of the diet, respectively, from 1-10, 11-28 and 29-42 d of age. Probiotic Bactocell® (Bactocell PA10, Lallemand SAS, Blagnac Cedex, France) contained a total of 1×10^{10} colony forming unit of *Pediococcus acidilactici* per kg of the supplement. It was administered at a concentration 100 mg/kg of the diet throughout the feeding trial. Prebiotic Fermacto® (PetAg Inc., Hampshire, IL, USA) contained a supplement of *Aspergillus* meal (a dead product of *Aspergillus sp.* with 16% CP, 1% EE, 40% CF and 2% ash). It was administered at a concentration 2000 and 1000 mg/kg of the diet, respectively, from 1-21 and 22-42 d of age. The basal diet was a standard corn-soybean meal-based diet that was formulated to meet Ross 308 broiler nutrient requirements (Ross, 2009) for starter (1-10 d), grower (11-24 d), and finisher (25-42 d) periods (Table 1).

Chicks were weighed at 1 and 42 d of age on the pen basis to determine their average daily gain (ADG). Average daily feed intake (ADFI) per pen was recorded from 1 to 42 d of age and the feed conversion ratio (FCR) was calculated based on ADG and ADFI from 1 to 42 d of age.

On d 22, three birds per replicate (18 birds/treatment) were randomly selected and injected into the breast muscle with one ml of 10% (v/v) suspension of sheep red blood cell (SRBC). Antibody production against SRBC was measured in the serum on d 30. Blood samples were taken from the brachial vein and the serum samples tested in duplicate for antibodies by the hemagglutination inhibition technique (Wegmann and Smithies, 1966). Serum (25 ml) containing antibody was serially diluted into a 96-well plate with physiological saline solution. Red blood cell solution (1% v/v) was added to each well for agglutination. If antibodies during the incubation period were sufficient, hemagglutination would be inhibited completely. The titers were expressed as log2 of the reciprocal of the last serum dilution showing hemagglutation inhibition.

To study the effects of different feed additives on blood leukocyte count, three birds per replicate (18 birds/treatment) were randomly selected and bled at 22 d of age, and blood samples were collected into EDTA anticoagulant-treated tubes to prevent clotting. Leukocytes were counted as described by Lucas and Jamroz (1961). Different leukocyte populations (heterophil (HE), and lymphocyte (LY)) were counted from 200 leukocytes per samples using an optical microscope (Nikon Eclipse 80i, Nikon Corp., Tokyo, Japan). The ratio of heterophil:lymphocyte (H/L) was also calculated.

At 24 and 42 d of age, two birds per replicate (12 birds/treatment) were randomly selected and sacrificed to measure the relative weight (g/g of carcass weight) of the spleen, thymus and bursa of Fabricius.

Data were analyzed as a completely randomized design using the GLM procedure (SAS, 2003). Pen was the experimental unit. HE and LY data presented as percentages were transformed to arcsine square root before statistical analysis, but the non-transformed data are presented in the text. Differences were considered significant at $P < 0.05$ and means were compared using LSD.

Results and discussion

Performance parameters (ADG, ADFI and FCR) are presented in Table 2. Dietary supplementation with the probiotics Primalac® (PP) or Bactocell® (PB) and prebiotic Fermacto® (Pre) had no significant effect on performance traits. Inconsistent results have been reported in

Table 2. Effect of probiotic and (or) prebiotic supplementation on performance[1] of broiler chickens from 1 to 42 d of age

Treatments[2]	ADG, g	ADFI, g	FCR
Control	44.8	84.1	1.88[a]
PP	44.8	84.1	1.88[a]
PB	45.1	83.4	1.85[a]
Pre	45.2	83.6	1.85[a]
PBPre	46.8	80.5	1.72[b]
PPPre	48.1	78.5	1.64[b]
SEM	1.27	2.10	0.044
Significance	NS	NS	***

[ab] Means within columns with a common superscript do not differ ($P < 0.05$).

[1] ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio.

[2]The birds received a corn-soybean meal basal diet that, with the exception of the control, was supplemented with probiotic Primalac® (PP), probiotic Bactocell® (PB), prebiotic Fermacto® (Pre), probiotic Bactocell® + prebiotic Fermacto® (PBPre) and probiotic Primalac® + prebiotic Fermacto® (PPPre).

Table 3. Effect of probiotic and (or) prebiotic supplementation on the antibody titer against sheep red blood cells (SRBCs), white blood cell count (WBC), heterophil percent (HE), lymphocyte percent (LY) and heterophil:lymphocyte (H/L) ratio in broiler chickens on d 22

Treatments[1]	WBC (/*μl*)	HE (%)	LY (%)	H/L	SRBCs titer
Control	25650[b]	27.0	71.0	0.40	4.61
PP	26125[b]	32.8	64.3	0.52	4.94
PB	28020[b]	32.2	66.4	0.49	4.92
Pre	28150[b]	26.3	71.8	0.38	5.65
PBPre	34133[a]	35.7	62.7	0.57	4.85
PPPre	26133[b]	26.0	71.0	0.37	5.00
SEM	1675	3.11	2.96	0.065	0.443
Significance	*	NS	NS	NS	NS

[ab] Means within columns with a common superscript do not differ ($P < 0.05$).

[1]The birds received a corn-soybean meal basal diet that, with the exception of the control, was supplemented with probiotic Primalac® (PP), probiotic Bactocell® (PB), prebiotic Fermacto® (Pre), probiotic Bactocell® + prebiotic Fermacto® (PBPre) and probiotic Primalac® + prebiotic Fermacto® (PPPre).

the literature on the effects of probiotics and prebiotics on broiler's growth performance. Although several researchers reported an improvement in performance due to dietary inclusion of probiotic and prebiotic (Willis and Reid, 2008; Taheri et al., 2010b; Ghasemi et al., 2014), others did not find a positive effect of probiotics Primalac® (Angel et al., 2005) and Bactocell® (Al-Zenki et al., 2009) or prebiotic Fermacto® (Torres-Rodriguez et al., 2005). Such discrepancies are most likely due to differences in the environment in which the experiment was conducted, the degree of stress or microbial challenge, type of diets used, bird characteristics (age, strain, stage of production), type and dosage of probiotic's microbial strains or prebiotic used or a combination thereof.

PBPre and PPPre had no significant effect on ADFI and ADG. Several other studies also showed that even the addition of probiotic and prebiotic combination in feeds had no effect on feed intake or weight gain of broiler chickens (Maiorano et al., 2012; Rudriguez et al., 2012; Sohail et al., 2012). Compared with the control, PBPre and PPPre improved FCR by 8.5 and 12.7%, respectively. In agreement with these results, there are reports of improvements in feed efficiency as a result of simultaneous supplementation of probiotic and prebiotic preparations (Awad et al., 2009; Houshmand et al., 2011). However, there are also studies showing no beneficial effect of probiotic, prebiotic or their combination on feed efficiency (Rudriguez et al., 2012; Sohail et al., 2012). This inconsistency in the effectiveness of probiotic and prebiotic combination may be due to factors such as type and dosage of probiotic and prebiotic preparations used.

The mechanism of beneficial effects of probiotic and prebiotic combination (as a synbiotic) on FCR is unknown. However, there are indications concerning the synergistic effects of synbiotic preparations. Pluske et al. (1996) reported that intestinal villus height was increased after addition of *Bacillus subtilis* in association with prebiotics. Awad et al. (2009) also found increased villus height of ileum in synbiotic supplemented group. 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 (Amat et al., 1996). Mookiah et al. (2014) reported that synbiotic increased total volatile fatty acids (VFA) and non-VFA of intestine compared with probiotic, prebiotic and the control. High VFA and non-VFA concentrations create a lower pH gut environment which inhibits viability and growth of redundant bacteria. This leads to a higher availability of nutrients in the gastrointestinal tract which can improve growth and feed efficiency of broiler chickens by increase in uptake of nutrients (Thanh et al., 2009). The VFA, particularly the short-chain fatty acids (mainly acetic, propionic and butyric acids) also provide energy to the host and are well-known for their health-promoting effects (Corrier et al., 1990). Li et al. (2009) showed that combination of probiotic and *Astragalus* polysaccharide increased the number of *Lactobacilli* and *Bifidobacteria* in the ileum and cecum and decreased *E.coli* in the cecum compared with the probiotic, *Astragalus* polysaccharide and the control.

The results of antibody titer against SRBCs, the count of WBCs, HE percent, LY percent, and H/L are presented in Table 3. No significant differences were found between the control and supplemented groups in the antibody titer against SRBCs, HE percent, LY percent, and H/L. Nevertheless, PBPre resulted in an increase in WBCs compared to other treatments.

Immunological function of gut-associated lymphoid tissue (GALT) is critical for survival of chicks. Beneficial microflora can affect the toll-like receptors (TLRs) of

the gut and, in the literature, it has been shown that probiotics, prebiotics and synbiotics, by supporting the growth of lactic acid bacteria among total microflora, change the balance of gut microflora, hence they can affect the immune system of the host indirectly (McCracken and Gaskins, 1999; de Vrese and Schrezenmeir, 2008).

Although, increased antibody mediated immunity has been reported by the use of probiotic (Midilli et al., 2008), prebiotic (Guo et al., 2004; Janardhana et al., 2009; Khodambashi et al., 2012) and synbiotic (Ghasemi and Taherpour, 2013) in diets, Rahimi et al. (2003), in agreement with our results, showed no increase in the antibody production in broilers and layers fed diets supplemented with such additives.

The higher WBC count of the PBPre group suggested that only the combination of the probiotic Bactocell® and prebiotic Fermacto® could have likely stimulated the proliferation of leukocytes, in this experiment. Chiang et al. (2000) showed that more T helper cells were present due to probiotic supplement. It was further shown that these effects were mediated by cytokines secreted by immune cells, stimulated with probiotic bacteria (Koenen et al., 2004). In agreement with our results, Ghesmi et al. (2014) also found that only the synbiotic Biomin® stimulated the cellular immunity compared to probiotic and prebiotic. This synergistic effect might be related to better attachment and colonisation of beneficial microflora to the intestinal mucosa in PBPre group. Oouwehand et al. (2000) reported that ability to adhere to mucosal surfaces was related to various probiotic health effects, and was a prerequisite for stimulation of the immune system and for antagonistic activity against enteropathogens. Because Oouwehand et al. (2000, 2002) showed that a combination of two or three lactic acid bacteria was more effective than one strain for attachment to the mucosa, therefore the lactic acid bacteria in the PBPre treatment can be closer to the intestinal wall and affect the cytokine production of the gut more than other treatments by releasing short chain fatty acids and other substances.

The H/L appears to be a reliable indicator for stress level in chickens (Maxwell, 1993). The effect of stress in avians is characterized by increased HE and decreased LY due to elevated blood corticosterone level (Khan et al., 2012). In addition to the effect of stress on H/L, it seems that high preparation of beneficial microflora may increase this ratio through the increased stimulation of the gut. Kim et al. (2011) indicated that prebiotic inclusion at high level enhanced H/L. Therefore, lack of significant impact of all feed additives used in this study on H/L could be due to the ideal environmental conditions and the ideal composition and dosage preparation of the supplements during the entire experimental period. The additives used in this study also failed to impart any significant effect on the relative weight of the spleen, thymus and bursa of Fabricius on d 24 and 42 (data not shown).

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References

Al-Zenki, S.F., Al-Nasser, A.Y., Al-Saffar, A.E., Abdullah, F.K., Al-Bahouh, M.E., Al-Haddad, A.S., Alomirah, H., Mashaly, M., 2009. Effects of using a chicken-origin competitive exclusion culture and probiotic cultures on reducing *Salmonella* in broilers. *Journal of Applied Poultry Research* 18, 23-29.

Alzueta, C., Rodriguez, M.L., Ortiz, L.T., Rebole, A., Trevino, J., 2010. Effects of inulin on growth performance, nutrient digestibility and metabolisable energy in broiler chickens. *British Poultry Science* 51, 393-398.

Amat, C., Planas, J.M., Moreto, M., 1996. Kinetics of hexose uptake by the small and large intestine of the chicken. *American Journal of Physiology* 271, 1085-1089.

Angel, R., Dalloul, R.A., Doerr, J., 2005. Performance of broiler chickens fed diets supplemented with a direct-fed microbial. *Poultry Science* 84, 1222-1231.

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

Biggs, P., Parsons, C.M., Fahey, G.C., 2007. The effects of several oligosaccharides on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. *Poultry Science* 86, 2327-2336.

Chee, S.H., Iji, P.A., Choct, M., Mikkelsen, L.L., Kocher, A., 2010. Characterisation and response of intestinal microflora and mucins to manno-oligosaccharide and antibiotic supplementation in broiler chickens. *British Poultry Science* 51, 368-380.

Chiang, B.L., Sheih, Y.H., Wang, L.H., Liao, C.K., Gill, H.S., 2000. Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (*Bifidobacterium lactis* HN019): optimization and definition of cellular immune responses. *European Journal of Clinical Nutrition* 54, 849-855.

Corrier, D.E., Hinton, A., Ziprin, R.L., Beter, R.C., Deloach, J.R., 1990. Effect of dietary lactose on cecal pH, bacteriostatic volatile fatty acids, and *Salmonella* Typhimurium colonization of broiler chicks. *Avian Disease* 34, 617-625.

De Vrese, M., Schrezenmeir, J., 2008. Probiotics, prebiotics, and synbiotics. *Food Biotechnology* 111, 1-66.

Gallaher, D.D., Khil, J., 1999. The effect of synbiotics on colon carcinogenesis in rats. *Journal of Nutrition* 129 (Suppl. 7), 1483S-1487S.

Ghasemi, H.A., Kasani, N., Taherpour, K., 2014. Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. *Livestock Science* 164, 128-134.

Ghasemi, H.A., Taherpour, K. 2013. Comparative effects of probiotic, prebiotic and synbiotic supplements on performance, jejunal morphology, serum lipid profile and antibody response of broiler chicks. *Journal of Livestock Science and Technologies* 2, 20-27.

Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* 125, 1401-1412.

Guo, F.C., Williams, B.A., Kwakkel, R.P., Li, H.S., Li, X.P., Luo, J.Y., Li, W.K., Verstegen, M.W.A., 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science* 83, 175-182.

Houshmand, M., Azhar, K., Zulkifli, I., Bejo, M.H. Meimandipour, A., Kamyab, A., 2012. Effects of non-antibiotic feed additives on performance, tibial dyschondroplasia incidence and tibia characteristics of broilers fed low-calcium diets. *Poultry Science* 91, 393-401.

Huang, R.L., Yin, Y.L., Wu, G.Y., Zhang, Y.G., Li, T.J., Li, L.L., Li, M.X., Tang, Z.R., Zhang, J., Wang, B., He, J.H., Nie, X.Z., 2005. Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. *Poultry Science* 84, 1383-1388.

Janardhana, V., Broadway, M.M., Bruce, M.P., Low-enthal, J.W., Geier, M.S., Hughes, R.J., Bean, A.G.D., 2009. Prebiotics Modulate Immune Responses in the Gut Asso-Ciated Lymphoid Tissue of Chickens. *Journal of Nutrition* 139, 1404-1409.

Jin, L.Z., Ho, Y.W., Abdullah, N., Jalaludin, S., 2000. Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* culture. *Poultry Science* 79, 886-891.

Jung, S.G., Houdge, R., Baurhoo, B., Zhao, X., Lee, B.H., 2008. Effects of galacto-oligosaccharides and a *bifidobacteria lactis*-based probiotic strain on the growth performance and fecal microflora of broiler chickens. *Poultry Science* 87, 1694-1699.

Khan, R.U., Rahman, Z.U., Nikousefat, Z., Javadi, M., Tufarelli, V., Dario, C., Selvaggi, M., Laudadio, V., 2012. Immunomodulating effects of vitamin E in broilers. *World's Poultry Science Journal* 68, 31-40.

Khodambashi Emami, N., Samie, A., Rahmani, H.R., Ruiz-Feria, C.A., 2012. The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamy-cin, on growth performance, digestibility, gut morphology and immune response of male broilers. *Animal Feed Science and Technology* 175, 57-64.

Kim, G.B., Seo, Y.M., Kim, C.H., Paik, I.K., 2011. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science* 90, 75-82.

Klasing, K.C., 1998. Nutritional modulation of resistance to infectious diseases. *Poultry Science* 77, 1119-1125.

Koenen, M.E., Kramer, J., Van der Hulst, R., Heres, L., Jeurissen, S.H.M, Boersma, W.J.A., 2004. Immunomodulation by probiotic *Lactobacilli* in layer- and meat-type chickens. *British Poultry Science* 45, 355-366.

Li, L.L., Hou, Z.P., Li, T.J., Wu, G.Y., Huang, R.L., Tang, Z.R., Yang, C.B., Gong, J., Yu, Hai., Kong, X.F., Pan, E., Ruan, Z., Xhu, W.Y., Deng, Z.Y., Xie, M., Deng, J., Yin, F.G., Yin, Y,L., 2008. Effects of dietary probiotic supplementation on ileal digestibility of nutrients and growth performance in 1- to 42-day-old broilers. *Journal of the Science of Food and Agriculture* 88, 35-42.

Li, S.P., Zhao, X.J., Wang, J.Y., 2009. Synergy of *Astragalus* polysaccharides and probiotics (*Lactobacillus* and *Bacillus cereus*) on immunity and intestinal microbiota in chicks. *Poultry Science* 88, 519-525.

Lillehoj, H.S., Trout, J.M., 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to Eimeria parasites. Clinical Microbiology Reviews 9, 349-360.

Lucas, A.M., Jamroz, C., 1961. Atlas of avian hematology. Agriculture Monograph 25. USDA, Washington, DC.

Maiorano, G., Sobolewska, A., Cianciullo, D., Walasik, K., Elminowska-Wenda, G., Slawinska, A., Tavaniello, S., Zylinska, J., Bardowski, J., Bednarczyk, M., 2012. Influence of *in ovo* prebiotic and synbiotic administration on meat quality of broiler chickens. *Poultry Science* 91, 2963-2969.

Maxwell, M.H., 1993. Avian blood leukocyte responses to stress. *World's Poultry Science Journal* 49, 34-43.

McCracken, V.J. Gaskins, H.R. 1999. Probiotics and the immune system. In: Tannock, G.W., (Ed.), Probiotics, A Critical Review, Hethersett, Horizon Scientific Press, pp. 85-112.

Midilli, M., Alp, M., Kocabach, N., Muglah, O.H., Turan, N., Yilmaz, H., Cakir, S., 2008. Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. *South African Journal of Animal Science* 38, 21-27.

Mookiah, S., Sieo, C.C., Ramasamy, K., Abdullaha, N., Hoa, Y.W., 2014. Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and cae-

cal fermentation concentrations of broiler chickens. *Journal of the Scienec of Food and Agriculture* 94, 341-348.

Mountzouris, K.C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G., Fegeros, K., 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science* 86, 309-317.

Muir, W.I., Bryden, W.L., Husband, A.J., 2000. Immunity, vaccination and the avian intestinal tract. *Developmental and Comparative Immunology* 24, 325-342.

Oouwehand, A.C., Isolauri, E., Kirjavinen, P.V., Tolkko, S. Salminen, S.J. 2000. The mucus binding of *Bifidobacterium lactis* Bb12 is enhanced in the presence of *Lactobacillus* GG and *Lact. Delbrueckii* subsp. *Bulgaricus*. *Letters in Applied Microbiology* 30, 10-13.

Oouwehand, A.C., Suomalainen, T., Tolkko, S., Salminen, S. 2002. In vitro adhesion of propionic acid bacteria to human intestinal mucus. *Le Lait* 82, 123-130.

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

Rahimi, Sh., Khaksefidi, A., Mousavi, T., 2003 Effect of probiotic and antibiotic on immune system. *Journal of Veterinary Research* 58, 159-162

Roberfroid, M.B., 1998. Prebiotics and synbiotics: concepts and nutritional properties. *British Journal of Nutrition* 80 (Suppl. 2), 197-202.

Rodriguez, M.L., Rebole, A., Velasco, S., Ortiz, L.T., Trevino, J., Alzueta, C., 2012. Wheat- and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. *Journal of the Scienec of Food and Agriculture* 92, 184-190.

Ross, 2009. Ross 308 Broiler Nutrition Specifications. Aviagen Ltd. Newbridge, Midlothian, Scotland.

SAS Institute., 2003. SAS User's Guide: Statistics. Version 9.1. SAS Institute Inc. Cary, North Carolina.

Sohail, M.U., Hume, M.E., Byrd, J.A., Nisbet, D.J., Ijaz, A., Sohail, A., Shabbir, M.Z., Rehman, H., 2012. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poultry Science* 91, 2235-2240.

Suskovic, J., Kos, B., Goreta, J., Matosic, S., 2001. Role of lactic acid bacteria and *bifidobacteria* in synbiotic effect. *Food Technology and Biotechnology* 39, 227-235.

Taheri, H.R., Moravej, H., Tabandeh, F., Zaghari, M., Shivazad, M., 2010a. Efficacy of combined or single use of *Lactobacillus crispatus* LT116 and *L. johnsonii* LT171 on broiler performance. *British Poultry Science* 51, 580-585.

Taheri, H.R., Moravej, H., Malakzadegan, A., Tabandeh, F., Zaghari, M., Shivazad, M., Adibmoradi, M., 2010b. Efficacy of *Pediococcus acidlactici*-based probiotic on intestinal Coliforms and villus height, serum cholesterol level and performance of broiler chickens. *African Journal of Biotechnology* 44, 7564-7567.

Talebi, A., Amirzadeh, B., Mokhtari, B., Gahri, H., 2008. Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathology* 37, 509-512.

Thanh, N., Loh, T.C., Foo, H.L., Hair-Bejo, M., Azhar, B., 2009. Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. *British Poultry Science* 50, 298-306.

Torres-Rodriguez, A., Sartor, C., Higgins, S.E., Wolfenden, A.D., Bielke, L.R., Pixley, C.M., Sutton, L., Tellez, G., Hargis, B.M., 2005. Effect of *Aspergillus* meal prebiotic (Fermacto®) on performance of broiler chickens in the starter phase and fed low protein diets. *Journal of Applied Poultry Research* 14, 665-669.

Wegmann, T.G., Smithies, O. 1966. A simple hemagglutination system requiring small amounts of red blood cells and antibodies. *Transfusion* 6, 67-73.

Willis, W.L., Reid, L., 2008. Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and *Campylobacter jejuni* presence. *Poultry Science* 87, 606-611.

Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A., Wang, M.Q., 2003. Effects of dietary fructo-oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science* 82, 1030-1036.

Yang, Y., Iji, P.A., Kocher, A., Thomson, E., Mikkelsen, L.L., Choct, M., 2008. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. *British Poultry Science* 49, 186-194.

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عملکرد رشد و پاسخ ایمنی جوجه‌های گوشتی تغذیه شده با جیره‌های حاوی پروبیوتیک و پری‌بیوتیک

ح.ر. طاهری*، م. کوکبی مقدم، م. کاکه باوه و ط. هرکی‌نژاد

نویسنده مسئول، پست الکترونیک: taherihr@gmail.com

چکیده هدف این تحقیق بررسی اثر استفاده از پروبیوتیک‌های پریمالاک و باکتوسل و پری‌بیوتیک فرمکتو (به طور مجزا و در تلفیق با یکدیگر) در جیره روی عملکرد و پاسخ ایمنی جوجه‌های گوشتی بود. تعداد ۵۴۰ قطعه جوجه گوشتی نر راس ۳۰۸ یک روزه به ۶ تیمار با ۶ تکرار و ۱۵ پرنده در هر تکرار از ۱ تا ۴۲ روزگی اختصاص داده شدند. پرنده‌ها جیره پایه (کنترل) یا جیره پایه به همراه پروبیوتیک پریمالاک، پروبیوتیک باکتوسل، پری‌بیوتیک فرمکتو، پروبیوتیک باکتوسل + پری‌بیوتیک فرمکتو، پروبیوتیک پریمالاک + پری‌بیوتیک فرمکتو را دریافت نمودند. محاسبه فراسنجه‌های عملکرد از ۱ تا ۴۲ روزگی صورت گرفت. محلولی از سوسپانسیون گلبول قرمز گوسفندی به سینه ۳ پرنده هر تکرار در ۲۲ روزگی تزریق شد و تیتر آنتی‌بادی در ۳۰ روزگی مورد اندازه‌گیری قرار گرفت. در ۲۲ روزگی، نمونه‌های خون (از ۳ پرنده به ازاء هر تکرار) گرفته شد تا تعداد کل گلبول‌های سفید خون، درصد هتروفیل، درصد لنفوسیت و نسبت هتروفیل به لنفوسیت اندازه‌گیری شوند. هیچ تفاوت معنی‌داری بین کنترل و گروه‌های دریافت‌کننده افزودنی‌ها برای میانگین افزایش وزن روزانه، میانگین خوراک مصرفی روزانه، تیتر آنتی‌بادی علیه گلبول قرمز گوسفندی، درصد هتروفیل، درصد لنفوسیت و نسبت هتروفیل به لنفوسیت دیده نشد. افزودن پروبیوتیک باکتوسل + پری‌بیوتیک فرمکتو و نیز پروبیوتیک پریمالاک + پری‌بیوتیک فرمکتو باعث بهبود ضریب تبدیل خوراک به ترتیب به میزان ۸/۵ و ۱۲/۷ درصد در مقایسه با کنترل شد و مکمل نمودن پروبیوتیک باکتوسل + پری‌بیوتیک فرمکتو منجر به افزایش تعداد کل گلبول‌های سفید خون نسبت به دیگر گروه‌ها شد.