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Effect of Inulin on Some Hematological, Immunological Parameters and Broiler Chickens Performance

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Abstract: The objective of this experiment was to study the influence of inulin on blood parameters, humoral immune response and performance of broiler chickens. After sexing, one hundred and sixty eight, day old male Ross 308 broiler chicks were randomly allotted to 3 treatments with 4 replicates of 14 chicks each. The experimental diets including 0, 0.5 and 1% of inulin were fed for 42 days. The experimental diets were formulated based upon corn-soybean meal. Results showed that livability and Body Weight Gain (BWG) were numerically improved for broilers fed diets supplemented with inulin but differences did not vary significantly from one another and from the control. The live body weight on d 42 significantly increased when the diet supplemented with 1% inulin. Feed Intake (FI) was not affected in birds fed diets with inulin supplement compared to control bird. Feed Conversion Ratio (FCR) significantly improved when diet supplemented with 1% inulin. Indices of main immune organs except Bursa of fabricius at 42 days old were not affected in birds fed diets with inulin supplement in comparison with control group. The results of this experiment showed that inulin could affect the immune response. Total anti-SRBC and IgM titers of broiler chickens significantly increased at 35 days of age when diets supplemented with inulin. There was no significant differences in Hemoglobin concentration (Hb) and Red Blood Cells (RBCs) but the differences in White Blood Cells (WBCs), heterophils, lymphocytes and heterophils to lymphocytes ratios were significant ($p<0.05$) at 42 days of age. In conclusion, dietary inulin supplementation significantly improved the growth performance and may enhance immune response in broiler chickens.

Key words: Broiler chickens, inulin, blood parameters, humoral immune response, performance

INTRODUCTION

Antibiotics have been widely used in animal production for many years. Although, some antibiotics are used therapeutically to improve the health and well-being of animals a large portion was employed for prophylactic purposes and to improve growth rate and feed conversion ratio. The long-term presence of antibiotics in the microbial population has made antibiotic-resistant strains by gene mutation more efficient in survival compared to normal microbes (Murray and Moellering, 1978). However, due to the emergence of microbes resistant to antibiotics which are used to treat human and animal infections, antibiotics are being taken out of poultry and pig diets around the world, beginning in Sweden in the year 1986 (Dibner and Richards, 2005).

On the other hand, selection for increased Body Weight (BW) has been shown to be genetically associated with a reduction in immune competence and disease resistance in chickens (Qureshi and Havenstein, 1994; Cheema *et al*., 2003). Therefore, increasing immune function is of crucial importance. Diet is known to modulate immune functions in multiple ways and to affect host resistance to infections. The immune system highly depends on an adequate supply of nutrients to function properly. Besides the essential nutrients, non-essential food constituents such as non-digestible carbohydrates may also have an impact on the immune system (Seifert and Watzl, 2007). Prebiotics cause potential possibilities of their practical using in non-specific immunoprophylaxis lots of animal diseases. The ban placed on using antibiotic growth promoters raised an

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interest in prebiotics as alternative solution in cattle, swine and poultry farms. They also could be used in various infection prophylaxis and treatment by virtue of their efficacious mobilization of animal immune system and in consequence protect them from being afflicted with various contagions. Additives containing prebiotics could be used to correct often stated in animals defective immunity and immunological deficiency (Krol, 2011).

A prebiotic was defined by Gibson and Roberfroid (1995) as a non-digestible food ingredient that beneficially affects the host by selectively stimulating growth, activity or both of one or a limited number of bacterial species already resident in the colon. To exhibit such effects a prebiotic must neither be hydrolysed nor absorbed in the upper part of the gastrointestinal tract and must be selective for one or a limited number of potentially beneficial bacteria residing in the colon (Collins and Gibson, 1999). Prebiotics offer several advantages over probiotics in that they increase populations of bacteria already present can affect multiple species of beneficial bacteria at the same time (Yang *et al*., 2009; Buddington, 2009) are cheaper and easier to include in the diet and more likely to reach the lower gastrointestinal tract (Dhama *et al*., 2008).

Inulin and Fructo Oligo Saccharides (FOS) are the most studied oligosaccharides for their prebiotic properties. Inulin is a set of fructans with monomers linked by β (2-1) bonds (Roberfroid *et al*., 1998; Pool-Zobel *et al*., 2002) mainly extracted from the chicory root (*Cichorium intybus*), contains molecules with a Degree of Polymerization (DP) of 3-60 mean being 10 (Crittenden, 1999). Therefore, inulin contains oligosaccharide components and polysaccharides. Because of the β (2→1) glycosidic bond, it is resistant to host-derived digestive enzymes and is believed to enhance the growth of health-promoting bacteria and to suppress the growth of potential pathogenic bacteria (Zentek *et al*., 2003). Microflora use multiple means to exclude pathogens from the gut. The first of these is competitive exclusion for nutrients and for space. A mature microbiota that occupies all niches is effective in preventing pathogens from colonizing the gut (Dunkley *et al*., 2009). Bifidobacterium and Lactobacillus were shown to successively inhibit adherence of *E. coli* (Howarth, 2010; Roselli *et al*., 2006) and culture supernatants from lactic acid producing bacteria inhibited the growth and the attachment of *Helicobacter pylori* (Howarth, 2010). The microbiota also produce substances which have direct antibacterial effects such as organic acids, acidolin, acidophilin, reuterin, lysozyme, lactoferrin, hydrogen peroxide, lactoperoxidase and bacteriocins like lactocin and lactocidin (Dibner and Richards, 2005; Dhama *et al*., 2008). By promoting the growth of lactic acid producing bacteria, prebiotics have an indirect, beneficial effect on the immune system of the host. These bacterial populations produce immune stimulating substances that react with the immune system at different levels, including the production of cytokines, mononuclear cells and macrophage phagocytosis as well as the induction of synthesis of large amounts of Ig, particularly IgA (Yasui and Ohwaki, 1991; Macfarlance and Cummings, 1999). Available information that concerning the effect of inilin on broiler performance shows conflicting results. Rebole *et al*. (2010), Verdonk and van Leeuwen (2004) showed inulin to increase BWG. Wu *et al*. (1999) found FOS to increase BW and improve FCR and Catala-Gregori *et al*. (2008) found FOS to increase BWG in broilers. However, other studies did not find inulin or FOS to increase growth performance (Geier *et al*., 2009; Janardhana *et al*., 2009; Rehman *et al*., 2008; Biggs *et al*., 2007). Information on the effect of inulin on the immune system, haematological and serum biochemical parameters of broiler chicks is still very scanty in literature. These facts require more carefully examine at deferent condition that can give confidential results.

The present study was therefore carried out to assess the effect of inulin on blood parameters, immune response and performance of broiler chickens.

MATERIALS AND METHODS

Birds and housing: A total of 168 male, day old Ross 308 broiler chicks were obtained from a commercial hatchery with an average body weight of 41.1 g and randomly allocated to 3 treatments of 4 replicate pens of 14 birds each. Each pen was 1.28 m^2. This research was conducted from 25 May to 6 July 2011 and was carried out in a private farm that belongs to Movahhed Gostaran Jovain Company (MGJC) in Iran. All chicks had *ad libitum* access to feed and water and controlled ventilation throughout the experiment. During the 1st 3 days, chicks were provided with 24 h of light after which 18L:6D was supplied until the end of the trial. Temperature was maintained at 32°C for the 1st 3 days and then gradually was reduced according to the normal management practices until a temperature of 21°C was achieved at day 24. To protect birds against viral diseases specially, Newcastle Disease (ND) and Infectious Bursal Disease (IBD)/Gumboro disease all birds were vaccinated as per scheduled followed by MGJC.

Diets: Three experimental diets including 0, 0.5 and 1% of inulin (Orafti® GR, BENEO-Orafti B 3300, Tienen, Belgium) were used. Diets were isocaloric and isonitrogenous based on Ross Broiler Nutrition Specification (2007) recommendation and were offered in mash form. The composition of the diets is shown in Table 1. The

Table1: Composition and nutrient concentration of diets

	Starter (inulin %)			Grower (inulin %)			Finisher (inulin %)		
Ingredients	0	0.5	1	0	0.5	1	0	0.5	1
Corn grain	50.24	49.12	48.00	46.38	45.36	44.87	50.89	50.24	48.08
Soybean meal	38.82	39.05	39.27	35.90	36.17	36.21	30.34	30.25	30.28
Wheat grain	1.00	1.00	1.00	6.73	6.58	6.47	7.97	8.12	9.31
Soybean oil	5.16	5.56	5.96	7.00	7.40	7.45	7.00	7.09	7.54
Limestone	1.44	1.44	1.44	1.11	1.11	1.11	1.06	1.06	1.05
Dicalcium	1.77	1.77	1.77	1.63	1.63	1.63	1.53	1.53	1.53
Phosphate									
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.33	0.33	0.33	0.18	0.18	0.19	0.15	0.15	0.15
L-Lysine HCl	0.34	0.33	0.33	0.17	0.17	0.17	0.16	0.16	0.16
Inulin	0.00	0.50	1.00	0.00	0.50	1.00	0.00	0.50	1.00
Calculated analysis									
ME (kcal kg^{-1})	3025.00	3025.00	3025.00	3150.00	3150.00	3150.00	3200.00	3200.00	3200.00
Protein (%)	22.00	22.00	22.00	21.00	21.00	21.00	19.00	19.00	19.00
Ca (%)	1.05	1.05	1.05	0.90	0.90	0.90	0.85	0.85	0.85
Available P (%)	0.50	0.50	0.50	0.45	0.45	0.45	0.42	0.42	0.42
Na (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Lysine (%)	1.43	1.43	1.43	1.24	1.24	1.24	1.09	1.09	1.09
Met + Cys (%)	1.02	1.02	1.02	0.85	0.85	0.85	0.77	0.77	0.77

[1]Each kg of diet contained: Vitamin A, 9000 IU; Vitamin D_3, 2000 IU; Vitamin E, 18 IU; Vitamin K_3 , 2 mg; Vitamin B_1, 1.8 mg; Vitamin B_2, 6.6 mg; Vitamin B_3, 10 mg; Vitamin B_5 30 mg; Vitamin B_6 3 mg; Vitamin B_9 1 mg; Vitamin B_{12} 0.015 mg; Vitamin H_2 0.1 mg; Choline choloride, 500 mg. [2]Each kg of diet contained: Mn, 100 mg; Fe, 50 mg; Zn, 100 mg; Cu, 10 mg; Mg, 3.5 mg; Se, 0.2 mg

Table 2: The chemical compounds and characteristics of inulin

Compound	Inulin content	Glucose/Fructose/Sucrose	Average DP (Degree of Polymerization)	Sweetness	Technical properties
Orafti® GR	92%	48%	≥10	10%	Granulated inulin

chemical compounds of inulin and it's characteristics according to the manufacturer data sheet is shown in Table 2.

Performance measurements: Records for live body weight and feed consumption were obtained at the end of each period. Weight gain and feed conversion ratio were calculated. Mortality was weighed and recorded as it occurred and was added to the total pen live body weight for the calculation of feed conversion ratio at each period. Feed conversion ratios were calculated by dividing total feed intake by weight gain of birds. The European Production Efficiency Factor (EPEF) was calculated according to the following equation:

$$\text{EPEF} = \frac{\text{Livability (\%)} \times \text{Liveweight (kg)}}{\text{Age (day)} \times \text{FCR}} \times 100$$

Hematological and serum analysis: At the 24 and 42 days of ages, following 6h of fasting, 8 birds with average body weight from each treatment (2 birds per replicate) were selected and weighed. Blood samples were collected into labeled sterile tubes with Ethyl Diamin Tetra Acetic (EDTA) and without EDTA from the bronchial vein during slaughter. A part of blood samples that were collected on EDTA were used for the hematological study. RBCs and WBCs counts were performed using improved hemocytometer according to Dacie and Lewis (1991). Hb was estimated according to Dacie and Lewis (1991). To obtain the heterophils, lymphocytes and heterophils to lymphocytes ratio, blood samples were smeared on glass slide. The smears were stained using May-Grunwald and Giemsa stains, approximately 2-4 h after methylalcohol fixation, one hundred leukocytes including granular(heterophils, eosinophils, and basophils) and non granular (lymphocytes and monocytes) were counted and the heterophil to lymphocyte ratio were calculated. Another part of blood samples without EDTA, transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for determination of total proteins and albumin. The total proteins and albumin concentrations in the plasma were determined by an automatic analyzer (Operator Manual BT 3500 Plus, Biotecnica Instruments S.P.A. Via Licenza, Rome, Italy). Serum globulins were calculated as the difference between total protein and albumin.

Humoral immune response to SRBC: Non pathogenic antigens of Sheep Red Blood Cells (SRBC) were used to monitor the immune response of broiler chickens. The anti-SRBC titers for total antibodies were measured by a hemagglutination test. For providing SRBC, first of all, blood of a male sheep was collected into labeled sterile tubes containing EDTA and then were centrifuged for 15 min at 2000 rpm the supernatant throw away and SRBC were washed three times in Phosphate Buffered Solution (PBS) and were diluted in PBS to 15% (vol./vol.). Four chicks in each treatment were intramusculary injected with 1 mL of a 15% suspension of SRBC at 21st day of age. Antiserums were collected 7 and 14 days after the immune challenge in 28 and 35 days of age. The serum from each sample was collected, heat inactivated at 56°C for 30 min and then analyzed for total, Mercaptoethanol-Resistant (MER) and Mercaptoethanol-Sensitive (MES) antibody titers. MER and MES titers are presumably IgG and IgM, respectively. Antibody titers were determined by the method of Cheema *et al.* (2003). Briefly, 50 μL of serum was added in an equal amount of PBS in the first column of a 96 well v-shaped bottom plate and the solution was incubated for 30 min at 37°C. A serial dilution was then made (1:2) and 50 μL of 2% SRBC suspension was added to each well. Total antibody titers were then read after 30 min of incubation at 37°C. The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titer for agglutination. For IgM response, 50 μL of 0.01 M mercaptoethanol in PBS was used instead of PBS alone followed by the aforementioned procedure.

Statistical analysis: Data were analyzed in a completely randomized design by one way Analysis of Variance (ANOVA) in non-orthogonal designs by using MSTATC Software package. Means were compared using Duncan's multiple-range test. A difference with a probability of $p \leq 0.05$ was considered significant.

Table 3: Body weight gain, feed intake and feed conversion ratio of broiler chickens

	Dietary inulin (%)			
Parameters	0	0.5	1	SEM
Body weight gain (g/chick)				
1-10 days	225.9	227.4	221.1	3.798
11-24 days	632.4	615.9	625.1	27.30
25-42 days	1247.0	1265.0	1347	31.76
1-42 days	2105.0^b	2107.0^b	2194^a	22.02
Feed intake (g/chick)				
1-10 days	231.8	231.6	227.0	6.938
11-24 days	1064.0	1037.0	1031	20.80
25-42 days	2468.0	2438.0	2405	43.37
1-42 days	3764.0	3707.0	3664	52.51
Feed conversion ratio (g/g)				
1-10 days	1.014	1.020	1.027	0.047
11-24 days	1.685	1.695	1.658	0.059
25-42 days	1.984^a	1.931^{ab}	1.788^b	0.012
1-42 days	1.789^a	1.759^{ab}	1.671^b	0.027
Livability (%)	96.43	98.22	100	1.574
EPEF	270.4^b	280.0^b	313.1^a	7.252

Means in the same row with uncommon letter are significantly different at $p \leq 0.05$. SEM = Standard Error Mean

Table 4: Effect of various levels of inulin on relative main immune organ weights of broiler chicks at days 24 and 42 of age

	Dietary inulin (%)			
Parameters	0	0.5	1	SEM
Thymus				
24 days old	0.410	0.477	0.395	0.045
42 days old	0.325	0.438	0.350	0.039
Spleen				
24 days old	0.093	0.110	0.120	0.016
42 days old	0.095	0.115	0.115	0.016
Bursa of fabricius				
24 days old	0.248	0.310	0.308	0.032
42 days old	0.078^b	0.098^a	0.110^a	0.005

Means in the same row with uncommon letter are significantly different at $p \leq 0.05$. SEM = Standard Error Mean. Organ weight; percentage of live body weight

RESULTS

Body weight gain, feed intake, feed conversion ratio, the European Production Efficiency Factor (EPEF), livability percent, initial body weight and final body weight in broiler-fed diets with different levels of inulin are shown in Table 3. The chicken BWG were not affected by inulin treatment diets during the starter (1-10 days of age), grower (11-24 days of age) and finisher (25-42 days of age). Birds fed diet supplemented with 1% inulin had significantly higher live body weight compared to those fed diet with 0 or 0.5% inulin at 42 days of age (Table 3). The dietary inulin treatments had no effect on feed intake throughout the whole phase of experiment. During the starter and grower phase were not observed significant effect on feed conversion ratio but at finisher and total phase of trial the chickens using ration supplemented with 1% inulin had lower ($p<0.05$) feed conversion ratio than birds fed other diets. The EPEF was significantly increased by the increase in inulin level. Increasing the inulin level did not significant affect the livability. Relative organs weights of broiler chicks fed different levels of inulin are shown in Table 4. Inulin had no significant effect on relative weight of thymus and spleen at the end of the grower and finisher period and bursa of Fabricius relative weight at grower period. Relative weight of bursa of Fabricius significantly increased when diet was supplemented with inulin measured at the end of the finisher period.

Effect of inulin supplementation on total anti-SRBC, IgG and IgM titers of broiler chickens at 28 and 35 days of age are shown in Table 5. There was not a significant effect of inulin on total anti-SRBC, IgG and IgM at 28 day of age (7 days after injection). Total anti-SRBC and IgM antibody titers were significantly altered when diets were supplemented with inulin at 35 day of age (14 days after

Table 5: Effect of inulin supplementation on total anti-SRBC, IgG and IgM titers of broiler chickens at 28 and 35 days of age

	Dietary inulin (%)			
Parameters	0	0.5	1	SEM
Total anti-SRBC (mg d L^{-1})				
28 days old	366.50	368.25	368.25	2.163
35 days old	446.00[c]	689.00[a]	592.30[b]	28.520
IgG (mg d L^{-1})				
28 days old	308.00	309.25	307.00	22.530
35 days old	323.00	435.30	352.30	34.390
IgM (mg d L^{-1})				
28 days old	58.50	59.00	61.25	0.909
35 days old	123.00[b]	253.75[a]	237.50[a]	9.724

Means in the same row with uncommon letter are significantly different at $p \leq 0.05$. SEM = Standard Error Mean

Table 6: Haematological and serum biochemical parameters of 24 and 42 days old broiler chicks fed deferent levels of inulin

	Dietary inulin (%)			
Parameters	0	0.5	1	SEM
Hb (g dL^{-1})				
24 days old	13.03	11.93	13.03	0.407
42 days old	12.53	11.23	11.98	0.413
RBC (106 dL^{-1})				
24 days old	3.100	2.825	3.100	0.087
42 days old	2.975	2.750	2.725	0.187
WBC (104 dL^{-1})				
24 days old	2.125[b]	2.875[a]	2.700[a]	0.057
42 days old	2.500[c]	3.550[a]	3.125[b]	0.100
Heterophils (%)				
24 days old	27.00	27.25	24.75	1.577
42 days old	21.75[c]	37.25[a]	31.75[b]	1.250
Lymphocytes (%)				
24 days old	60.75	59.50	62.00	1.420
42 days old	65.25[a]	51.50[b]	55.75[b]	1.339
H/L ratio				
24 days old	0 .447	0.462	0.401	0.035
42 days old	0.335[c]	0.726[a]	0.574[b]	0.035
Total proteins (g dL^{-1})				
24 days old	5.225[b]	6.400[a]	6.00[ab]	0.243
42 days old	5.575	5.700	5.400	0.252
Albumin (g dL^{-1})				
24 days old	2.375[b]	2.675[a]	2.400[b]	0.039
42 days old	3.175	3.425	2.975	0.257
Globulins (g dL^{-1})				
24 days old	2.775[b]	3.725[a]	3.600[a]	0.243
42 days old	2.400	2.275	2.425	0.256

Means in the same row with uncommon letter are significantly different at $p \leq 0.05$. SEM = Standard Error Mean; H = Heterophils, L = Lymphocytes

injection). The total antibody titer was significantly higher in birds fed 0.5% inulin diet compared to those fed other diets.

The effect of various levels of inulin on haematological and serum biochemical parameters of 24 and 42 days old broiler chicks is shown in Table 6. Inulin supplementation did not have any significant effect on Hb and RBCs throughout the experimental period. Inulin supplementation significantly increased WBCs at the end of grower and finisher periods. Inulin supplementation did not have significant effect on heterophils, lymphocytes, heterophils to lymphocytes ratio at 24 day of age. Percent of heterophils and heterophils to lymphocytes ratio were significantly increased but lymphocytes decreased at 42 days of age when the diets were supplemented with inulin. Serum biochemical parameters (total proteins, albumin and globulins) are shown in Table 6. Total proteins, albumin and globulins significantly increased when diets were supplemented with inulin at the end of grower period. There were not significant differences in total proteins, albumin and globulins among treated birds at the end of the experimental period.

DISCUSSION

The available information concerns the effect of inilin on broiler performance shows conflicting results. Rebole *et al*. (2010), Verdonk *et al*. (2004) showed that inulin may increase Body Weight Gain (BWG). Tianxing *et al*. (1999) found Fructo Oligo Saccharide (FOS) to increase BW and improve feed conversion ratio and Catala-Gregori *et al*. (2008) found FOS to increase BWG in broilers. However, other studies did not find inulin or FOS to increase growth (Geier *et al*., 2009; Janardhana *et al*., 2009; Rehman *et al*., 2008; Biggs *et al*., 2007; Koksal *et al*., 2011). Ortiz *et al*. (2009) and Alzueta *et al*. (2010) stated dietary inclusion of inulin up to 20 g kg^{-1} did not significantly affect ($p>0.05$) body weight gain, feed intake and feed to gain ratio of broiler chickens. This variability in the effectiveness of inulin may be due to the effect of different factors such as: inulin source, inclusion rate, type of diet, animal characteristics, degree of hygiene, husbandry condition and environmental stress (Patterson and Burkholder, 2003; Verdonk *et al*., 2005). The current study showed that the live body weight gain at 42 days of age was significantly increased when diet was supplemented with 1% inulin. The dietary inulin did not influence feed intake in comparison to control group. Similarly, Rebole *et al*. (2010) found inulin to improve BWG of broilers when fed at 1.0 and 2.0%. Catala-Gregori *et al*. (2008) found inulin to improve BWG in broilers but not FI. Lack of broiler response to FI has commonly been observed from inulin supplementation (Rebole *et al*., 2010; Geier *et al*., 2009; Janardhana *et al*., 2009; Rehman *et al*., 2008). The present study showed that 1% inulin supplementation significantly improved FCR ($p<0.05$). The improved FCR and BWG may be related to improve in apparent ileal digestibility of crude protein and crude fat (Alzueta *et al*., 2010). Also, there was a significant effect of inulin on the digestibility of most amino acids and major fatty acids such as oleic and linoleic acids (Alzueta *et al*., 2010). In parallel, the release of Short Chain Fatty Acids (SCFA) from fermentation of non-digestible carbohydrates may provide extra energy which was otherwise unavailable to the birds (Lan *et al*., 2005). On the other hand, Rehman *et al*. (2007) found that the supplementation of inulin resulted in an increase in the villi height of jejunum on broiler chickens and it is speculated that may increase the absorptive area and subsequently enhanced digestion and absorption. These changes may be resulted in a better FCR and BWG when the diets supplemented with 1% inulin. Bird mortality was not affected by dietary supplementation in our trial. As prebiotics are most useful in improving bird health under conditions where a challenge is present (Bailey *et al*., 1991) it may be that there was not sufficient challenge in this trial to result in mortality differences.

Relative weight of thymus, spleen and bursa of fabricius were not changed with inulin supplementation at 24 and 42 days of age with the exception of bursa of fabricious at 42 days of age. The lack of effect on immunological organ weights seems to indicate that production of Ig and lymphocytes were not increased with dietary inulin supplementation. This may be due to a lack of challenge as the birds were used in this trial and thus more isolated from pathogen presence than in a conventional production houses.

Total anti-SRBC and IgM antibody titers were significantly altered when diet supplemented with inulin at 35 days of age (14 days after injection). The total antibody titer was significantly higher in birds fed 0.5% inulin compared to other birds (Table 5). By promoting the growth of lactic acid producing bacteria, prebiotics have an indirect, beneficial effect on the immune system of the host. These bacterial populations produce immunostimulating substances that react with the immune system at different levels including the production of cytokines, mononuclear cells and macrophage phagocytosis as well as the induction of synthesis of large amounts of Ig (Yasui and Ohwaki, 1991; Macfarlane and Cummings, 1999). Immunoglobulins are produced in B-cells in bone marrow and the biological characteristics of IgG, IgA and IgM in poultry are similar to those of immunoglobulins in mammals (Sang-Oh and Byung-Sung, 2011). The increased serum IgG in turkeys treated with MOS (Savage *et al*., 1996; Cetein *et al*., 2005) and the increased serum IgG and IgM in broiler chickens treated with microencapsulated inulin (Sang-Oh and Byung-Sung, 2011) support the results of the present study.

CONCLUSION

In the present study, inulin supplementation significantly increased WBCs and FCR at the end of grower and finisher periods. Heterophils and heterophils to lymphocytes ratio were significantly increased but lymphocytes decreased at 42 day of age when the diets supplemented with inulin. Addition of inulin to diet may inhibits the nutritional stress or any stress which causes an increase in lymphocytes ratio because the stress can stimulates the adrenal gland to produce some hormones such as estrones which has a direct effect to lyze a lymphatic cell (Gross and Siegel, 1983). Inulin seems not to have a direct or in direct effect on the blood total protein, albumin and globulin.

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