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Effect of Inulin Inclusion in Low Phosphorus Diets on Some Hematological, Immunological Parameters and Broiler Chickens Performance

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Abstract: A feeding trial was conducted to study the effect of inulin inclusion in low phosphorus diets on some hematological, immunological parameters and broiler chickens performance. After sexing, 224 day-old male Ross 308 broiler chicks were randomly allotted to 4 treatments with 4 replicates of 14 chicks each. The birds were offered the following diets for 42 days: (T1) positive control, (T2) negative control, (T3) negative control+0.5% inulin and (T4) negative control+1% inulin. The experimental diets were formulated based upon corn-soybean meal. Body weight gain, livability and EPEF were not affected by dietary inulin in low phosphorus diets throughout the experiment ($p>0.05$). Feed Conversion Ratio (FCR) significantly improved when diet supplemented with 0.5 and 1% inulin compared with the controls. Indices of main immune organs were not affected in birds fed diets with inulin supplement in comparison with the controls. Total anti-SRBC and IgM titers of broiler chickens significantly increased at 35 days of age when birds fed low phosphorus diets. Inulin supplementation significantly increased total anti-SRBC and IgG in broiler chickens at 35 days of age. 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 were significant ($p<0.05$) at 42 days of age. In conclusion, dietary inulin supplementation in low phosphorus diets significantly improved FCR and may enhance immune response in broiler chickens.

Key words: Broiler chickens, inulin, phosphorus, humoral immune response, performance

INTRODUCTION

Poultry feed is mainly constituted of corn-soybean meal that poultry cannot digest 15-25% of its nutrients. Approximately two thirds of phosphorus in cereal grains and oilseed meals are present in the form of phytic acid and some other minerals such as Zn, Cu, Co, Fe and Ca to form phytic phosphorus which are not available for poultry and most of them are excreted in the litter. It can increase environmental P pollution and feed costs. Phosphorus is one of the most important and expensive macro minerals required by poultry and plays a vital role in the development and maintenance of the skeletal system and in numerous biochemical reactions in the body. The role of phosphorus and determination of its requirement in broiler nutrition has received a great deal of attention and continues to do so, especially in the context of pollution from phosphorus run-off from agricultural operations (Kies *et al*., 2001; Adeola and Sands, 2003). Feeding reduced P diets supplemented with non digestible oligosaccharides have been shown to promote mineral absorption in the large intestine of both humans and rats. During the last decade, several alternative methods for reducing the negative impacts of phytate P on the environment and poultry performance have been recommended. Feeding the birds with diets formulated closer to their Non Phytate Phosphorus (NPP) requirements, coupled with use of microbial phytase are among the most successful l strategies that have attracted scientific and practical attention (Sohail and Roland, 1999; Karimi *et al*., 2011).

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; Bosscher, 2009). 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). Inulin is able to improve uptake of Ca, Mg, Cu, Zn and Fe but the effects of P have been more variable.

Information on the effect of inulin supplementation in reduced P diets on the immune system, haematological and serum biochemical parameters of broiler chicks is still very scanty in literature. The objectives of this study were to evaluate the effect of inulin inclusion in low phosphorus diets on some hematological, immunological parameters and performance of broiler chickens.

MATERIALS AND METHODS

Birds and housing: A total of 224 male, day old Ross 308 broiler chicks were obtained from a commercial hatchery with an average body weight of 41.38 g and randomly allocated to 4 treatments of 4 replicate pens of 14 birds each. Each pen was 1.28 m^2. This research was conducted from 25 May 2011 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 the chicks were provided with 24 h of light after which 18L: 6D was supplied until the end of the trial. Temperature was kept at about 32°C during the first 3 days and then gradually was reduced according to the normal management practices until a temperature of 21°C was achieved at day 24, subsequently maintaining this temperature. 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: Four experimental diets were used in this trial including positive control (NPP level equal Ross Broiler Nutrition Specification, 2007) negative control (NPP level 30% less than Ross Broiler Nutrition Specification, 2007), 0.5 and 1% inulin supplementation in negative control. The inulin used in this study was a commercial product (Orafti® GR, BENEO-Orafti B 3300, Tienen, Belgium), obtained from chicory roots with a Degree of Polymerisation (DP) of carbohydrates ≥10, 92% inulin content, 10% sweetness, according to the manufacturer data sheet. Diets were isocaloric and isonitrogenous based on Ross Broiler Nutrition Specification in 2007 recommendation and were offered in mash form. The composition and nutrient concentration of the diets is shown in Table 1.

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:

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

Hematological and serum analysis: At the 24 and 42 days of ages, following 6 h 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. Hb was estimated according to Dacie and Lewis. 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

Table 1: Composition and nutrient concentration of diets

	Starter (inulin %)				Grower (inulin %)				Finisher (inulin %)			
Composition	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Ingredients												
Corn grain	50.24	54.01	52.89	51.77	46.38	45.49	45.83	46.62	50.89	50.69	50.89	50.57
Soybean meal	38.82	38.04	38.63	38.85	35.90	34.93	34.41	35.96	30.34	30.24	30.44	30.64
Wheat grain	1.00	0.00	0.00	0.00	6.73	10.11	8.50	6.40	7.97	9.26	7.88	7.10
Soybean oil	5.16	4.09	4.48	4.88	7.00	6.43	6.73	7.00	7.00	7.00	7.48	7.88
Limestone	1.44	0.99	0.99	0.98	1.11	0.84	0.83	0.83	1.06	0.77	0.77	0.77
Dicalcium phosphate	1.77	0.94	0.94	0.95	1.63	0.93	0.94	0.94	1.53	0.83	0.85	0.83
Salt	0.40	0.40	0.40	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	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	0.25	0.25	0.25
DL-Methionine	0.33	0.33	0.33	0.33	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15
L-Lysine HCl	0.34	0.34	0.34	0.34	0.17	0.19	0.18	0.17	0.16	0.16	0.16	0.16
Inulin	0.00	0.00	0.50	1.00	0.00	0.00	0.50	1.00	0.00`	0.00	0.50	1.00
Calculated analysis												
ME (kcal kg^{-1})	3025.00	3025.00	3025.00	3025.00	3150.00	3150.00	3150.00	3150.00	3200.00	3200.00	3200.00	3200.00
Protein (%)	22.00	22.00	22.00	22.00	21.00	21.00	21.00	21.00	19.00	19.00	19.00	19.00
Ca (%)	1.05	1.05	1.05	1.05	0.90	0.90	0.90	0.90	0.85	0.85	0.85	0.85
Available P (%)	0.50	0.35	0.35	0.35	0.45	0.32	0.32	0.32	0.42	0.29	0.29	0.29
Na (%)	0.17	0.17	0.17	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	1.43	1.24	1.24	1.24	1.24	1.09	1.09	1.09	1.09
Met+Cys (%)	1.02	1.02	1.02	1.02	0.85	0.85	0.85	0.85	0.77	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 , 9.1 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; T1 = Positive control (NPP level equal Ross Broiler nutrition specification); T2 = Negative control (NPP level 30% less than Ross Broiler nutrition specification); T3 = Negative control+0.5% inulin; T4 = Negative control+1% inulin

(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 was centrifuged for 15 min at 2000 rpm, the supernatant throw away and SRBC was washed three times in Phosphate Buffered Solution (PBS) and was 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. 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 (Freed, 1997) (East Lansing, USA) Software package. Means were compared using Duncan's multiple-range test. A difference with a probability of $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Body Weight (BW) gain, Feed Intake (FI), Feed Conversion Ratio (FCR), livability and EPEF are shown in Table 2. No significant differences were seen among treatments for BW gain, livability and EPEF by inulin inclusion in low phosphorus diets throughout the experiment ($p>0.05$). Inulin inclusion had no significant effect on FI during the starter (1-10 days), grower (11-24 days) and finisher (25-42 days) but in overall periods (1-42 days) birds that fed low phosphorus diets without inulin had higher ($p<0.05$) FI in comparison with birds fed 1% inulin supplementation. FCR significantly improved when diet supplemented with 0.5 and 1% inulin.

Table 2: Body weight gain, feed intake and feed conversion ratio of broiler chickens

	Treatments				
Parameters	T1	T2	T3	T4	SEM
Body weight gain (g/chick) (days)					
1-10	225.90	208.50	215.20	218.00	5.747
11-24	652.50	640.60	618.20	621.20	12.76
25-42	1226.40	1258.40	1298.30	1312.20	26.39
1-42	2104.80	2103.00	2131.90	2151.50	23.34
Feed intake (g/chick) (days)					
1-10	231.80	218.00	222.90	225.70	7.424
11-24	1064.40	1068.60	1055.60	1074.70	20.07
25-42	2467.70	2570.10	2478.20	2430.20	37.39
1-42	3763.90^{ab}	3855.00^{a}	3756.80^{ab}	3714.90^{b}	35.27
Feed conversion ratio (g/g)					
1-10 (days)	1.029	1.049	1.036	1.037	0.042
11-24 (days)	1.633	1.681	1.711	1.730	0.045
25-42 (days)	2.015^{ab}	2.046^{a}	1.910^{bc}	1.853^{c}	0.039
1-42 (days)	1.789^{ab}	1.835^{a}	1.762^{b}	1.727^{b}	0.002
Livability (%)	92.430	98.210	94.640	96.430	1.928
EPEF	270.400	268.100	272.400	286.500	7.741

T1 = Positive control (NPP level equal Ross Broiler nutrition specification); T2 = Negative control (NPP level 30% less than Ross Broiler nutrition specification); T3 = Negative control+0.5% inulin, T4 = Negative control+1% inulin; SEM = Standard Error Mean; means in the same row with an uncommon letter are significantly different at $p \leq 0.05$; EPEF = European Production Efficiency Factor

Junior *et al.* (2010) reported the reduction of calcium and available phosphorus levels of the feed to 0.55 and 0.275% respectively, does not interfere on the performance of the broilers. Karimi *et al.* (2011) used different levels (%) of dietary NPP fed from 0-20 days (0.45, 0.40, 0.35, 0.30, 0.25 compared with feeding 0.20 NPP with and without 500 F.T.U. of phytase per kg of diet) and from 21-36 days of age (0.414, 0.364, 0.314, 0.264, 0.214 compared with 0.164 NPP with and without 500 F.T.U. of phytase per kg of diet) were evaluated using a total of 588 days old commercial broiler chicks. They reported that optimum FCR and mortality was supported at lower levels of NPP (0.25%). Between 21 and 36 days, 0.364% was enough to optimize BW, FI whilst only 0.314% or greater was needed to support optimum FCR and survivability. The available information concerns the effect of inulin on broiler performance shows conflicting results. Rebole *et al.* (2010) and Verdonk and van Leeuwen (2004) showed that inulin may increase BW gain. Wu *et al.* (1999) found Fructooligosaccharide (FOS) to increase BW and improve FCR and Catala-Gregori *et al.* (2008) found FOS to increase BW gain in broilers.

However, other studies did not find inulin or FOS to increase growth (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 ($p>0.05$) BW gain, FI and FCR of broiler chickens. This variability in the effectiveness of inulin may be due to the effect of different factors such as:

Table 3: Effect of various levels of inulin on relative main immune organ weights of broiler chicks at day 24 and 42 of age

	Treatments				
Parameters (days old)	T1	T2	T3	T4	SEM
Thymus					
24	0.41	0.51	0.53	0.41	0.050
42	0.31	0.38	0.40	0.40	0.052
Spleen					
24	0.09	0.10	0.11	0.12	0.016
42	0.09	0.13	0.11	0.12	0.016
Bursa of fabricius					
24	0.25	0.30	0.33	0.30	0.045
42	0.08	0.11	0.09	0.08	0.016

T1 = Positive control (NPP level equal Ross Broiler nutrition specification); T2 = Negative control (NPP level 30% less than Ross Broiler nutrition specification); T3 = Negative control+0.5% inulin; T4 = Negative control+1% inulin; SEM = Standard Error Mean; Means in the same row with an uncommon letter are significantly different at $p \leq 0.05$; Organ weight; Percentage of live body weight

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).

Indices of main immune organs were not affected ($p>0.05$) in birds fed low phosphorus diets and also in birds fed inulin supplements in comparison with positive control group (Table 3). 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.

The effect of inulin supplementation on total anti-SRBC, gG and IgM titers of broiler chickens at 28 and 35 days of age are shown in Table 4. Decreasing dietary available P concentration increased ($p<0.05$) antibody responses (total anti-SRBC and IgM) to sheep red blood cells in broiler chickens at 35 days of age. Inulin supplementation significantly increased total anti-SRBC and IgG in broiler chickens at 35 days of age (14 days after injection). Kegley *et al.* (2001) reported similar results but in pigs. Kegley *et al.* (2001) used four corn-soybean meal-based treatment diets in pigs that were formulated to contain 0.16, 0.24, 0.32 or 0.40% available P, they reported increasing dietary available P concentration decreased antibody responses to sheep red blood cells and there was a tendency for a linear P effect ($p<0.10$) on the change in immunoglobulin G antibody response 7 days after injection of sheep red blood cells. There was also a tendency for a cubic ($p<0.10$) P effect on days 14. Pigs fed the 0.4% available P diet developed the fewest immunoglobulin G antibodies against sheep red blood cells. There is evidence that 1, 25-dihydroxyvitamin D3 which would be increased in the treatments with low available P has a stimulatory influence on monocytes and may enhance antigen presentation (Manolagas *et al.*, 1985). Lemire *et al.* (1984) showed that 1, 25-dihydroxyvitamin D3 decreased proliferation and antibody production by human peripheral blood mononuclear cells.

Table 4: Effect of inulin supplementation on total anti-SRBC, IgG and IgM titers of broiler chickens at 28 and 35 days of age

Parameters (days old)	Treatments T1	T2	T3	T4	SEM
Total anti-SRBC (mg dL^{-1})					
28	366.5^{b}	390.8^{ab}	382.0^{ab}	423.8^{a}	14.09
35	446.0^{c}	610.0^{b}	689.0^{a}	660.0^{a}	11.02
IgG (mg dL^{-1})					
28	308.0	325.0	316.3	351.8	13.93
35	323.0^{c}	350.0^{c}	483.0^{a}	396.5^{b}	10.30
IgM (mg dL^{-1})					
28	58.5^{b}	65.8^{a}	66.5^{a}	72.25^{a}	2.224
35	123.0^{b}	260.0^{a}	251.0^{a}	262.3^{a}	6.890

T1 = Positive control (NPP level equal Ross Broiler nutrition specification); T2 = Negative control (NPP level 30% less than Ross Broiler nutrition specification); T3 = Negative control+0.5% inulin; T4 = negative control+1% inulin; SEM = Standard Error Mean; Means in the same row with an uncommon letter are significantly different at p≤0.05

In another study, 1, 25-dihydroxyvitamin D3 suppressed interleukin-2 production and inhibited the proliferation of Phytohemogglutinin (PHA) stimulated human lymphocytes (Tsoukas *et al*., 1984). These could explain the higher antibody response seen in birds fed low dietary available P. Total anti-SRBC and IgG significantly increased when diets supplemented with inulin compared to other birds at 42 days of age. 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 immunos-timulating 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; Macfarlance and Cummings, 1999).

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 5. Haematological parameters had not shown significant difference among treatments at 24 days of age. Total proteins, albumin and globulins significantly increased in birds fed low phosphorus diets with or without inulin supplementation only at 24 days of age. No significant differences were seen in total proteins, albumin and globulins among treatments at 42 days of age. These results agree with findings of Farzinpour *et al*. (2011) who showed that the reduced nPP level diets had no significant effect on albumin and total protein at 42 days of age. Inulin supplementation had no significant effect (p>0.05) on Hb and RBCs throughout the experimental period. WBCs, heterophils and heterophils to lymphocytes ratio significantly increased p<0.05) in birds fed low phosphorus diets with or without inulin supplementation at 42 days of age. The addition of inulin to the diet may inhibit the nutritional stress or any stress

Table 5: Haematological and serum biochemical parameters of 24 and 42 days old broiler chicks

Parameters (days old)	Treatments T1	T2	T3	T4	SEM
Hb (g dL^{-1})					
24	13.030	13.00	12.930	12.900	0.164
42	12.530	12.50	12.630	12.950	0.262
RBC (10^{6} dL^{-1})					
24	3.100	3.075	3.025	3.050	0.055
42	2.925	2.975	3.025	2.975	0.076
WBC (10^{4} dL^{-1})					
24	2.132	2.100	2.075	2.250	0.071
42	2.500^{b}	2.750^{a}	2.800^{a}	2.825^{a}	0.006
Heterophils (%)					
24	27.000	27.50	27.000	28.500	0.842
42	21.750^{b}	31.75^{a}	33.250^{a}	34.250^{a}	1.233
Lymphocytes (%)					
24	60.750	63.50	63.000	62.500	1.028
42	65.250^{a}	55.25^{b}	51.750^{b}	56.000^{b}	2.171
H/L ratio					
24	0.445	0.445	0.430	0.457	0.022
42	0.335^{b}	0.580^{a}	0.642^{a}	0.630^{a}	0.039
Total proteins (g dL^{-1})					
24	5.225^{b}	6.175^{a}	6.425^{a}	6.600^{a}	0.247
42	5.575	6.050	5.875	6.350	0.324
Albumin (g dL^{-1})					
24	2.375^{b}	2325^{b}	2.675^{a}	2.750^{a}	0.088
42	3.175	3.200	3.525	3.475	0.165
Globulins (g dL^{-1})					
24	2.775^{b}	3.850^{a}	3.475^{ab}	3.750^{a}	0.295
42	2.400	2.850	2.350	2.875	0.313

T1 = Positive control (NPP level equal Ross Broiler nutrition specification); T2 = Negative control (NPP level 30% less than Ross Broiler nutrition specification); T3 = Negative control+0.5% inulin, T4 = Negative control+1% inulin; SEM = Standard Error Mean; Means in the same row with an uncommon letter are significantly different at p≤0.05; H = Heterophils; L = Lymphocytes

which causes an increase in lymphocytes ratio because the stress can stimulate the adrenal gland to produce some hormones such as estrones which has a direct effect to lyze a lymphatic cell (Gross and Siegel, 1983). Because few reports are available regarding this topic, more studies may be needed to verify the effects of inulin inclusion in low phosphorus diets in poultry.

CONCLUSION

The results of the present study showed that BW gain, livability and EPEF were not affected by reducing P diets and inulin supplementation significantly improved FCR and slightly increased BW gain, compared with the controls. Inulin supplementation in reduced P diets may enhance immune response in broiler chickens.

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