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EFFECT OF PHYTASE ENZYME SUPPLEMENTATION IN DEFICIENT PHOSPHATE DIET ON BROILER PERFORMANCE AND SOME BLOOD TRAITS

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ABSTRACT
The experiment was conducted to determine the effect of microbial phytase in Ca deficient and available aP diets on broiler performance and some blood traits. Three hundred day-old chicks (Ross 308) were distributed into five equal groups with two replicates. The control diets (T_1) contained Corn-SBM, 0.9% Ca and 0.40% aP without enzyme, whereas T_2, T_3, T_4 and T_5 contained Corn-SBM, 0.60% Ca and 0.30% aP+1000 phytase/ kg; T_2+ 2000 phytase /kg; Corn-SBM, 0.30% Ca and 0.20% aP + 1000 phytase /kg; T_4 + 2000 phytase/ kg respectively. All diets were formulated to be iso-caloric (3150 kcal/ kg) and iso-nitrogenous (20.2%).Supplemented diets with 2000 units phytase kg resulted in a significant decrease in feed consumption ($P \leq 0.05$) with the lowest for (T_5), while no significant effects on weight gain, carcass dressing, carcass cuts, and Giblets. In addition, none of the biochemical and hematological parameters tested differed significantly for all treatments ($P \leq 0.05$). Phytase supplementation to diets did not influence calcium, phosphorus, and iron contents with no detrimental impact on blood parameters of broiler chicks.

KEY WORDS: Broiler, Phytase, Performance, Blood parameters, Poultry, Nutrition.

INTRODUCTION

In recent years, there has been a concerted effort to improve the nutritive worth of feed stuffs using exogenous enzymes. Large proportion of phosphorus in plant materials exist as phytate form (salts of phytic acid) and about 70% is not available to be used by monogastric animals (Rezaei *et al*., 2007). Grains constitute of 50-70% of poultry diets with 28.2% phosphorus found in phytic acid molecule which is concentrated in most plant seeds.Phytate is a strong acid, it may forms ionic linkage between proteins and some minerals such as Ca, Mg, Cu, Zn, Fe, and K in aqueous medium of the intestines (Sebastian *et al*., 1997). The vulnerable portability of poultry to exploit phosphorus phytate in grains (Ravindran *et al*., 2006; Wu *et al*., 2003; NRC, 1994) attributed to decrease secretion of phytase in the intestines of birds. Large amount of phosphorus in bird droppings, cause environmental pollution in areas with intensive poultry production (Mondal *et al*., 2007). Phytase has the ability to break down undigested phytic acid in grains and oilseeds releasing digestible calcium and phosphorus in monogastric animals (Ravindran *et al*., 1999; Todd *et al*., 2004). Microbial phytase positively influence nutrients such as amino acids and phosphorus to be advantageous (Yi *et al*., 1996; Namkung and Leeson, 1999). It may reduce the degree of foam formation, thus alter the utilization of energy of fats in the intestines (Ravindran *et al*., 2001). It may increases the availability of phosphorus in corn-soybean diets by 15-45% and decreases it in the waste to about one third without affecting productive performance (Kornegay, 1999). Addition of phytase to low phosphorus diets led to an increase in body weight with significant increase in phosphorus and calcium balance (Mondal *et al*., 2007). However, Akyurek *et al*. (2005) claimed no effect of phytase on productive performance and growth of broilers as well as the level of calcium and phosphorus in the blood. Yan *et al*. (2006) indicated that the level of 0.7% of calcium is enough to maintain the performance of broilers properly. Most studies were performed on the second week of starter period with the emphasis on performance and the relationship between phosphorus and calcium in diet and body. Variable results and conclusions were obtained by various workers. This study aimed to investigate the effect of different levels of phytase on the performance, some blood traits and carcass characteristics of broiler chicks fed diets containing decreasing levels of dicalcium phosphate during the first four weeks of growth, period (2 – 6) weeks of age.

MATERIALS & METHODS

Experimental Design

This experiment performed during the period from 13th of November 2012 to 25th of December 2012.A number of 300 one-day old unsexed broiler chicks (Ross 308) were purchased from a commercial hatchery, Babylon, Iraq. Chicks were transported to the poultry farm of College of Veterinary Medicine, University of Kufa. Ten pens were used to accommodate the chicks, each pen contain 30 birds. They were fed starter ration during the first 2 weeks before the commencement of the experiment, then subjected to the experimental diets for 4 weeks. Chicks were equally divided into 5 groups and distributed randomly to pens with 2 replicates each.

Animals and Management Program

Chicks were housed on cleaned and disinfected floor covered with sawdust in a suitable size pens (2x1.5x2) m. Each pen was equipped with one feeder and drinker to allow *ad libitum* consumption of food and water. Light

was provided 24 hours. Gas incubators were used to maintain the temperature within 34-35 C° during the first week, and decreased 2 degrees for every successive week of age. Chicks were vaccinated according to the veterinary vaccination program from the first day to the termination of the experiment. The initial average weight of chicks was 42 g. Feed consumption, body weight and mortality rate were weekly recorded

Feeding Program

Five experimental diets T_1, T_2, T_3, T_4 and T_5 were formulated to be iso-caloric (3150 Kcal/ Kg) and iso-nitrogenous (20.2%) to meet the nutrient requirement of broiler (NRC, 1994) as follows:

T_1- control (Corn-SBM, 0.9% Ca and 0.40% aP without enzyme). T_2- (Corn-SBM, 0.60% Ca and 0.30% aP+1000 phytase /kg).T_3- (T_2+ 2000 phytase/ kg). T_4- (Corn-SBM, 0.30% Ca and 0.20% aP + 1000 phytase/ kg) & T_5- (T_4 + 2000 phytase/ kg).

Phytase enzyme produced by Mainland Company, Beijing, China, was used to supplement the diets. The data on percent composition and percent chemical contents in diets are given in Table 1.

TABLE 1: Diets composition and chemical analysis

Treatments / Ingredients %	Control(T_1)	T_2	T_3	T_4	T_5
Corn	60.00	61.40	61.40	63.80	63.80
Soybean meal	30.00	30.00	30.00	30.00	30.00
Protein.conc.*	4.00	4.00	4.00	3.00	3.00
Corn oil	4.30	3.80	3.80	3.10	3.10
Di-calcium Phosphate	0.70	0.30	0.30	0.10	0.10
Limestone	1. 00	0.50	0.50	------	----
Phytase enzyme	---	+**	++ ***	+**	++ ***
Total	100	100	100	100	100
Chemical analysis%					
ME Kcal /Kg ****	3150	3150	3150	3150	3150
Crude protein	20.20	20.20	20.20	20.20	20.20
Crude fiber	3.60	3.60	3.60	3.60	3.60
Lysine	1.08	1.08	1.08	1.08	1.08
Methionine	0.41	0.41	0.41	0.41	0.41
Meth.+cys.	0.65	0.65	0.65	0.65	0.65
Total Calcium	0.90	0.60	0.60	0.30	0.30
A. Phosphorous	0.40	0.30	0.30	0.20	0.20
C:P ratio	156.0	156.0	156.0	156.0	156.0

*WAFI(Holland, Company)Contain per kg: ME 2150Kcal/ kg, CP 40%, Fat 5%, CF 5%, Ca 5.6%, A phosphorous2.65%, Methionine3.70%, Meth.+cys.4.10%, Lys.3.85%, Tryp.0.4%, Thr.1.29%, Vitamin A 220000 iu, Vitamin D3 60000 i.u, Vitamin E 60mg, Vitamin B1 60mg, Vitamin B2 140mg, Vitamin B6 80mg, Vitamin B12 700mg, Vitamin K3 50mg, Niacin 800mg,Biotin 2000mg,Pantothenic acid 320mg,Copper 200mg,Iron 1000mg,Manganese 1600mg,Zinc 1200mg, Iodine 20mg.

** Added 1000 phytase/ kg: phytase unit is the activity of phytase that generates 1 micromole of inorganic phosphorus per minute from an excess of sodium phytate at pH 5.5 and 37 degrees Celsius (Zyla, et al., 1995).

***:Added 2000 phytase/ kg

****:Diets contains according to NRC(1994).

Blood Samples Collection and Laboratory Analysis

At the end of the experiment, 20 chicks were taken randomly from each feeding group for preparation of serum samples. Five ml of blood was collected in sterile test tubes without anticoagulant from each chick and placed in slanting position at room temperature to clot. Blood samples were centrifuged at 3000 rpm for 10 minutes, and then serum was collected and stored at -20 C°. Total protein, triglycerides, cholesterol, glucose, uric acid, calcium, and phosphorus in serum were determined by Chemistry Analyser Model Cyan-Belgium using available commercial kits. The following hematological studies, Hb, PCV%, RBCs, WBCs, ratio of heterophiles to lymphocytes(H/L) were performed within two hours after blood collection (Shastry, 1983) using Hematology Analyser Model C60Gex for measurement.

Carcass measurements

At day 42th of age birds were slaughtered according to the routine practices adopted in commercial broiler slaughter house after fasting period of 10 hours, then eviscerated and the weight of liver, heart, and gizzard were measured. Total clean carcass weight was recorded and then each carcass was split into its cuts (breast, thighs, drumstics, back, neck, and wings) according to the method indicated by North (1984) and then their weights were recorded.

Statistical Analysis

Completely randomized design was applied. Data were tabulated and subjected to one-way analysis of variance (ANOVA) using statistical computer program (SAS Institute, 2001). Duncan's multiple rang test with 0.05 level of significance was applied for statistical analysis to compare the differences among treatment means (Duncan, 1955). The following statistical model was used:

$Xij = \mu + ti + eij$

Where Xij denotes the jth observation in ith treatment, μ is the population mean, ti represents the effect of treatment, and eij is the random experimental error.

RESULTS & DISCUSSION

The data on weight gain feed consumption and feed conversion ratio for 4 and 6 weeks of age are given in Table 2.

TABLE 2: Average weight gain, feed consumption and feed conversion ratio for 4 and 6 weeks of age

Traits \ Treat.	T_1 Control	T2	T3	T4	T5
Initial BW g/bird (2wk)	400.0±14.2	390.0±10.4	410.0±12.4	400.0±14.3	395.0±15.1
Weight gain g/bird (4wk)	740.0±25.3	700.0±24.8	720.0±23.4	715.0±26.1	750.0±22.8
Weight gain g/bird (6wk)	710.0±37.6	720.0±36.6	750.0±39.1	730.0±37.3	770.0±38.2
Feed cons. g/bird (4wk)	1258.0±29.6	1260.0±31.4	1224.0±28.5	1240.0±32.7	1200.0±30.4
Fee cons. g/bird (6wk)	1562.0± 19.9[a]	1368.0±18.5 [b]	1350.0± 18.9[b]	1400.0±17.8[b]	1320.0±17.5 [b]
Feed conv. ratio (4wk)	1.70± 0.06	1.80±0.05	1.70±0.06	1.73±0.05	1.60±0.09
Feed conv. ratio (6wk)	2.20±0.04 [a]	1.90±0.06 [b]	1.80±0.05 [bc]	1.91±0.07 [b]	1.71±0.06 [c]

*Rows of different letters are significantly different (P<0.05)

Weight gain was not affected significantly in all diets for both 4 and 6 weeks of age. This result is close to that claimed by Mondal *et al*. (2007) in which supplementation of at least 500 units of phytase/ kg in starter diets did not affect broiler body weight significantly. Feed consumption was decreased significantly (P≤ 0.05) for all diets with the least (1320 g / bird) for the diet supplemented with 2000 phytase/ kg at only 6 weeks of age. This might be due to the increase in proteins, lipids and carbohydrates digestibility in diets resulted in the fulfillment of the nutrients' requirement of birds with less amount of diet, or enhancement in the metabolism of available energy (Radeliffe, 2002). Phytase enzyme might release phosphorus from phytin which is utilized in growth process and gives better results comparing to addition of dicalcium phosphate without phytase. Feed conversion ratio was significantly better for all diets at 6 weeks of age with the best for diet supplemented with 2000 phytase/ kg (1.71). In spite of insignificant differences among all diets in weight gain, it seems to be negatively correlated with feed conversion ratio. These findings are accord with the results obtained by several researchers(Qian *et al*., 1997; Huff *et al*., 1998; Namkung and leeson, 1999; Zyla *et al*., 2000; and Bozkurt *et al*., 2006), who indicated that addition of phytase to low phosphorus diets produce better feed conversion and better weight gain. The data on dressing percentage, clean carcass, and edibles of birds slaughtered at 6 weeks of age are given in Table 3.

TABLE 3: Dressing percentage, clean carcass, and edibles of birds slaughtered at 6 weeks of age

	Percentage of carcass cuts %				
Traits	T_1 Control	T_2	T_3	T_4	T_5
Breast	29.6±1.0	30.4±0.9	30.5±0.8	30.8±0.8	31.0±0.9
Thighs	17.8±0.5	17.9±0.4	17.8±0.6	17.5±0.8	18.0±0.9
Drumstick	14.4±0.6	13.9±0.5	14.2±0.8	14.0±0.7	14.6±0.7
Back	17.5±0.5	16.8±0.4	16.8±0.5	16. 6±0.6	16.2±0.9
Neck	8.2±0.6	7.4±0.8	7.3±0.9	7.1±0.9	8.0±0.7
Wings	12.0±0.7	12.8±0.5	12.9±0.4	12. 7±0.6	12.0±0.6

No significant differences detected among treatments.

The comparisons among averages of all traits (dressing percentage, clean carcass, and edibles) at 6 weeks of age for all diets are statistically similar. This means that phytase has no effect on any investigated trait. However, calculated dressing percentage was raised with the use of phytase in diets comparing with the result obtained by (Khawaja, 2003) on broiler fed commercial diet. Our results are consistent with (Moharrey and Mohammadpour 2005) who claimed that diets supplemented with phytase did not affect the weight of heart and gizzard. Liver weight showed some increase perhaps due to increase in metabolic activity of the liver. The data on the percentage of carcass cuts are given in Table 4.

TABLE 4: Means of some productive traits of broiler slaughtered at 6 weeks of age

Traits \ Treat.	T_1 Control	T_2	T_3	T_4	T_5
Final body weight(g)/bird	1850.0±25.5	1810.0±24.8	1880.0±26.7	1845.0±25.2	1915.0±42.4
Dressing percentage	72.0±2.8	73.0±3.1	75.0±3.9	74.0±4.3	76.0±4.9
Clean carcass weight(g)without edibles	1332.0±110.1	1321.0±90.7	1410.0±89.4	1365.0±112.4	1455.0±121.4
Heart weight(g)	9.0±0.08	9.2±0.09	9.3±0.07	9.0±0.08	9.2±0.09
Liver weight(g)	65.0±2.9	65.0±2.3	70.0±5.4	68.0±4.2	72.0±4.9
Gizzard weight(g)	60.0±1.7	57.0±3.1	58.0±2.4	59.0±1.4	59.0±1.6

No significant differences detected among treatments.

All percentages of carcass cuts (breast, thighs, drumstick, back, neck, and wings) for all diets did not show any significant difference. These results were agreed with previous finding by Akyurek *et al*. (2005) who reported that phytase supplementation had no effect on carcass cuts percentages. However, the general trend of weight increase in carcass cuts, seem to be direct proportional with the amount of phytase supplementation, cleaned carcass, and dressing percentage.

Table 5 showed that all diets supplemented with phytase had no significant influences on all biochemical parameters investigated in this experiment (P≤ 0.05). However, there was a slight increase in glucose, Cholesterol, triglyceride, and HDL. On other hand, a decrease was recorded in LDL, uric acid, and total protein in the blood of birds. These results confirmed previous result reported by Shakmak (2003) and Al-Harthi (2006). They found that phytase supplementation had no adverse effect on biochemical constituents of broiler plasma. All diets supplemented with phytase also showed insignificant influences on all hematological parameters investigated in this experiment (P≤ 0.05). However, there was a slight increase in all hematological parameters and minerals, H/L ratio was decreased slightly in the blood of birds fed diets supplemented with phytase (Table 6). This might be due to the enhanced of immune system indicated by increase in lymphocyte count. These results are in close with the finding of Woyengo and Nyachoti, (2011). They explained that improvements in hematological parameters could be due to inhibition of phytic acid by phytase which caused an increasing in the availability of nutrients for efficient absorption. Woyengo and Nyachoti, (2011) found similar results concerning levels of calcium, phosphorus, and iron in blood serum. The increase in Fe levels also agreed with Paik *et al*. (2000) and Stahl *et al*. (1999).

TABLE 5: The level of biochemical parameters in blood serum

Parameters	T1 Control	T2	T3	T4	T5
glucose(mg dL^{-1})	231.00±3.5	233.80±2.9	234.00±2.7	235.00±2.5	237.80±4.2
cholesterol(mg dL^{-1})	141.00±1.5	141.70±1.7	142.60 ±1.6	142.00±1.6	142.30±1.7
Triglyceride(mg dL^{-1})	36.50±2.4	37.10±2.7	38.00±2.8	37.30±2.9	39.60±3.1
HDL(mg dL^{-1})	54.10±1.5	54.60±1.7	54.80±1.8	54.70±1.8	55.00±1.9
LDL(mg dL^{-1})	65.40±1.8	65.10±1.7	64.10±1.8	64.90±2.1	63.80±3.2
Uric acid(mg dL^{-1})	166.00±2.1	163.70±3.2	161.20±4.1	158.00±7.5	156.10±10.3
Total protein(g dL^{-1})	2.95±0.2	2.83±0.1	2.63±0.4	2.80±0.3	2.50±0.4

No significant differences detected among treatments

TABLE 6: Hematological parameters and some minerals' in blood serum

Treat. / Traits	T1 Control	T2	T3	T4	T5
Hb (g dl^{-1})	9.95±1.2	10.13±0.9	10.88±1.3	9.95±0.9	11.20±1.7
PCV (%)	32.00±0.9	32.33±0.9	35.00±3.1	33.10±1.5	34.40±2.5
(RBC X 10)^{6} /µL	2.87±0.8	3.01±0.7	3.20±0.3	3.09±0.6	3.30±0.4
(WBC X 10) ^{3} /µL	22.40±0.4	22.60±0.3	22.90±0.4	22.70±0.3	23.00±0.5
H/L ratio	0.36±0.05	0.34±0.04	0.31±0.02	0.32±0.03	0.29±0.05
Calcium mg dL^{-1}	5.26±1.6	6.08±0.8	5.34±1.4	6.76±0.9	7.45 ±1.5
Phosphorus mg dL^{-1}	2.90±0.6	3.10±0.5	3.50±0.4	3.30±0.3	3.60±0.3
Iron µg dL^{-1}	143.30±5.9	146.10±3.6	149.20±2.7	147.30±3.1	151.10±2.5

No significant differences detected among treatments

CONCLUSION

It can be concluded that supplementation of corn-soybean grower diets with 2000 of microbial phytase/ kg increases the bioavailability and compensate inorganic calcium and phosphorus with 66% and 50% of organic calcium and phosphorus respectively. Obviously, this supplementation must be recommended to reduce the cost of diets and improve productive performance of broiler without adverse effects on biochemical and hematological parameters**.**

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