Effect of Supplementation with High Levels of Phytase Enzyme Compared with Commercial Dose in Corn-Soybean based Diets on Productive and Physiological Performance in Broiler

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EFFECT OF ADDING HIGH LEVELS OF PHYTEASE ENZYME TO CORN-SOYBEANS BASED DIETS IN THE PRODUCTION AND PHYSIOLOGICAL PERFORMANCE OF BROILERS

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ABSTRACT : This study was conducted at the Poultry Research Station of the Livestock Research Department / Agricultural Research Department, Ministry of Agriculture in Abu Ghraib. During the period from 19 September 2017 to 30 October 2017. In this study, two hundred forty male of ROSS 308 broilers were randomly distributed into four treatments with 3 replicates per treatment and 20 chicks per replicate. The birds were fed on equal diets in the percentage of protein and metabolic energy according to the age requirements referred to in the Ross 308 index. The experimental treatments were involved: T1 was a positive control treatment with a complete nutrient requirement. T2 was negative control (with a deficient requirement for phosphorus available 0.17 aP), T3 was negative control with the addition of 1250 unit/kg of phytase enzyme, T4 negative control with the addition of 2500 unit/kg of phytease enzyme. The results of the present study showed a significant increase (P <0.05) in the body weight, average weight increase and the feed consumption rate of T1 compared to the negative control treatment. A significant increase (P <0.05) was observed in the mentioned characteristics of the two treatments. The results of the study also showed a significant increase (P <0.05) in the feed conversion coefficient of the negative control treatment (NC) compared to the positive control treatment (PC). The two treatments of the phytase enzyme were significantly (P <0.05) reduced FCR in NC and there was no significant difference between the addition coefficients and PC. As for the percentage of mortality, the results of the study did not show any significant difference between the experimental treatments. The higher concentration of the ALP enzyme in NC compared to PC was noted as well as ALP was significantly (P<0.05) reduced in T3 while a significant decrease was detected in T4 compared to the other treatments. As for the level of phosphorus in the blood, the percentage of thigh ash and the percentage of ash phosphorus showed a significant decrease (P <0.05) in the NC compared to the PC. A significant increase (P <0.05) in T3 and T4 to a level close to the PC was revealed in addition, there are no negative effects of addition of the enzyme in the production and physiological performance of broilers.

Key words : Phytease enzyme, metabolic energy, body weight, broilers.

INTRODUCTION

Phosphorus is one of the most important nutrients to be supplied in sufficient quantities in poultry diets (Sommerfeld *et al*, 2018) and plays a major role in bones growth and development, energy metabolism and cell building (Tayyab and MC Lean, 2015). Preservation of phosphorus sources is expected to be one of the determinants of its use as it is expected that the global reserves of phosphate rocks will be fully depleted during 50-100 years. As a result of this decline, the prices of phosphorus have increased, resulting in an increase in the cost of production of poultry diets (Shastak *et al*, 2012; Rodehutscord and Shastak, 2013).

Yellow corn is the main source of poultry in America and Brazil. The United States alone produces about 41% of global corn production (FAO, 2009). Approximately 60-90% of the total phosphorus found in feedstocks of vegetable origin used in poultry diets is in the form of phytic acid (Mayoenositol hexafosphate) and its mixed salts are called flavidates or alvites (Rodehutscord *et al*, 2016; Adeola, 2018) where the benefit from phosphorus is low in single-stomach animals, including poultry, and is limited to 40% due to the lack of phytoase enzyme necessary to dissolve phytite and release phosphorus as it is dumped with waste, causing environmental problems (Wang *et al*, 2017). Phytic acid also plays a role as an anti-feeder by binding with ions minerals positive charge and the formation of insoluble complexes with starch, fats, proteins and inhibition of the effectiveness of certain enzymes (Lee *et al*, 2015; Singh *et al*, 2015; Kumar *et al*, 2016). Recently, it is common to use phytoase (the hexafosphate meowinositol) to gradually extract phosvitide groups from the phytic acid molecule and to release the inorganic phosphate, myoinocytol and nutrients, as well as increase their metabolism and absorption (Truong *et al*, 2017; Gautier *et al*, 2017). The high-level phytase concept has attracted the attention of many researchers over the past few years (Farhadi *et al*, 2017; Walk *et al*, 2018; Gautier *et al*, 2017). These high levels of the enzyme are believed to accelerate the extraction of phosphit to reduce the negative effects of phytite as an anti-nutritional supplement (Wealleans *et al*, 2016). Near-complete decomposition of phytite and phytic acid esters and significant improvement in body weight (Walk *et al*, 2018). The aim of this study was to find out the effect of adding two high levels of the phytoase enzyme from Buttiauxella to the broiler diet to determine the best level of addition and the effect on the production and physiological performance of the broilers.

MATERIALS AND METHODS

This study was conducted at the poultry research station of the Livestock Research Department, Agricultural Research Department, Ministry of Agriculture in Abu Ghraib during the period from 19 September 2017 to 30 October 2017. Two hundred forty males of ROSS 308 broilers were randomly distributed into four treatments with 3 replicates per treatment and 20 chicks per replicate. Chicks were used at the age of one day at an initial weight of 40 g/birds. The chicks were distributed randomly to the replicates after weighing 20 birds per group. The chicks were raised in semi-enclosed system and the halls were divided into pins with dimension of 2 x 3 meters (length X width) for each pin which represented one of the replicates of the experiment. The experimental treatments were involved: T1 was a positive control treatment with a complete nutrient requirement. T2 was negative control (with a deficient requirement for phosphorus available 0.17 aP), T3 was negative control with the addition of 1250 unit/kg of phytase enzyme, T4 negative control with the addition of 2500 unit/kg of phytease enzyme. The pins were designed and provided with requirements and maintained the ideal temperature by using electric and gas incubators with a thermal regulator. The feed and water were provided ad libitum throughout the experiment. The continuous lighting system was used for the first seven days of life and was reduced to 20 hours/day by two hours every 12 hours (from 5 to 7) using electronic clocks from the eighth day to three days before the end of the experiment.

One way Analysis was conducted. The trend included the effects of the four treatments, following the General Linear Model and using the SAS Statistical Standard (SAS, 2012). Significant differences between mean values were tested using the Duncan Multilevel Test (Duncan, 1955) at the mean of 0.05 and 0.01 according to the mathematical model:

$$Yij = ì + Ti + Eij.$$

The cholesterol level was estimated according to the instructions in the kit and according to Richmond (1973). The method of enzymatic analysis of glucose was followed by Asatoor and King (1954) using the kit kit manufactured by Biosystems-Spain. The concentration of phosphorus in blood plasma was measured by Fisk and Subbarow (1925). The activity of the ALP Alkaline Phosphatsas enzyme was estimated using the Biomerieux-France kit and the absorption spectroscopy spectroscopy at 546 nm based on the King and Armstrong method (1943). The concentration of phosphorus was measured in bone ash according to the method specified by John (1970).

Phytic enzyme was obtained from Danisco UK LTD from Amman / Irbid, commercial name PHY @ 10000 Axtra. This enzyme is produced from Buttiauxella bacteria, expressed in Trichoderma Reesei mushroom, which is made in the form of fine spherical granules which was stable at a high temperature degree 95 ^{o}C and this feature is very important during the process of manufacturing of feedstuffs and this enzyme was relatively high efficiency in the low pH (2.5 - 5.5), which allows it to work at the beginning of the digestive tract.

RESULTS AND DISCUSSION

Productive attributes

Average body weight and weight gain

Table 2 showed a significant increase (P <0.05) in the live body weight ratio of positive control PC (758.97 g) for 1-3 weeks compared to negative control NC (504.37 g). The addition of phytease at levels of 1250, 2500 FTU/kg to NC treatment resulted in a significant increase (P <0.05) in the mean body weight (757.17, 800.9 g) respectively and at a close level of positive control treatment. In the sixth week of the experiment, the mean increase in the body weight ratio of the positive control treatment PC (2426.47) compared to the treatment of NC (1598.73) and the addition of 1250, 2500 FTU/Kg to the NC treatment was observed significant increase (P <0.05) (2447.1), 2541.2 g and did not differ significantly from the treatment of PC.

Average weight gain

The results of the study shown in Table (3). A significant increase (P <0.05) in the weight increase of the positive control treatment PC (718.8 g) for the period 1-3 weeks, compared to the negative control treatment NC (464.76 g). The addition of phytease at levels 1250, 2500 FTU/Kg to NC treatment showed a significant increase (P <0.05) in the weight gain (717.03, 760.73 g) respectively and at a close level of PC. Similarly, in the period from 4 to 6 weeks, the increase in the rate of increase of the positive control ratio of PC (1667.5 g) was observed compared to the NC negative control (1094.34 g). The addition of phytease at levels of 1250, 2500 FTU/Kg to the treatment of NC to a significant increase (P <0.05) at (1649.93, 1740.84 g), respectively, and at a close level of PC. For the overall weight increase (1-6 weeks), the positive control treatment (PCR) was significantly higher (P <.05) compared to NC (1559.1). The addition of phytease at levels 1250, 2500 FTU/Kg to NC treatment showed a significant increase (P <0.05) in the average weight gain (2366.97, 2501.57) and similar to PC treatment.

The results were consistent with Gautier et al., (2018) who indicated a significant increase (P<0.05) in the supplemented treatments compared to non-supplemented treatments. Pieniazek *et al* (2017) pointed (1-42 w) a significant decrease (P <0.05) was observed in the body weight ratio of the NC treatment (0.17) compared with the PCF (completed requirements of phosphorus) in addition, adding phytease enzyme resulted in a significant increase compared to NC. The results were also consistent with those indicated by Walk *et al* (2018) where a significant increase (P <0.05) was observed in the daily weight increase with the increase in the addition of the enzyme to the control diets. The results for the weight increase were also consistent with Taheri *et al* (2015), who revealed a significant increase (P <0.01) was observed in the PC treatment compared to the NC treatment (npp = 0.2) and the addition of the phytease to the negative control treatment improved (P <0.05) compared to the NC without addition, with no differences between the addition treatments and the PC treatment. The results were not consistent with finding of Momeneh *et al* (2018). There was no significant difference (P<0.05) in the body weight and the weight gain of the broiler at the addition of the 0,500, 2500 FTU/kg to full dietary requirements.

Feed consumption rate

The results shown in Table 4 showed a significant increase (P <0.05) in the feed consumption ratio (FI) of PC treatment PC (979.9 g) for the 1-3 week period compared to the NC treatment (719.57 g). The addition of phytease at levels of 1250, 2500 FTU/Kg to NC treatment showed a significant increase (P <0.05) in feed consumption rate (956.2, 1001.77 g) respectively and to a similar level of positive control treatment. A significant increase (P <0.05) was observed in the feed consumption ratio of the PC treatment (3083.47 g) for the period of 4-6 weeks compared to the NC treatment (2240.83 g), which resulted in the addition of phytease at levels 1250, 2500 FTU/(P <0.05) to (3057.5, 3167.5 g), respectively and at a close level of PC treatment. In the experimental period (1-6 weeks), PC treatment (4063.31 g) compared with NC treatment (2962.15 g) was significantly improved with the addition of phytease at levels 1250, 2500 FTU/Kg to NC (4005.43, 4169.24 g) which did not differ significantly (P <0.05) from PC.

These results were consistent with Pieniazek *et al* (2017). The higher feed consumption ratio for full positive PC control was observed compared to NC negative (0.17 ap) at a significant level (P <0.05). The addition of the enzyme at the level of 500 and 2000 FTU/Kg to the NC treatment has significantly increased (P <0.05) than the feed consumption ratio compared to the NC treatment. (P <0.05) in the feed consumption ratio of the positive control treatment PC compared to the NC negative control treatment. The addition of the enzyme to the NC treatment was significantly increased (P <0.05) average feed consumption ratio compared to the NC treatment to a comparable level of PC. The results are consistent with finding of Walk *et al* (2018), who noting that there was a significant increase in the average feed consumption in PC compared with NC and NC with addition of the enzyme at different levels. The results were not consistent with those indicated by Momeneh *et al* (2018). There was no significant difference (P<0.05) in the feed consumption rate when adding phytase enzyme by 0, 500, 2500 FTU/kg to full dietary requirements for 1 - kg.

Feed Conversion Coefficient

The results shown in Table 5 indicated a significant increase (P <0.05) in the dietary conversion coefficient of NC (1.55) for 1-3 weeks compared with PC (1.36). The addition of phytase at levels of 1250, 2500 FTU/Kg was significantly (P <0.05) reduced the feed conversion coefficient in NC treatment (1.33, 1.31 respectively) and at a close level of PC treatment. The results were similar for 4-6 weeks where a significant increase (P <0.05) was observed in the dietary conversion coefficient of NC (2.05) compared to PC (1.85). The addition of phytase at levels 1250, 2500 FTU/Kg to NC treatment showed a significant decrease (P <0.05) where it reached (1.85, 1.82 respectively) and did not differ significantly from the treatment of PC. As a final result, the feed conversion coefficient was increased during the total duration of the experiment for NC (P <0.05), where it was (1.90) compared to the treatment of PC (1.70) with a significant decrease in FCR when adding the levels of 1250, 2500 FTU/Kg to the NC as well as there were significant differences between the addition treatments and PC

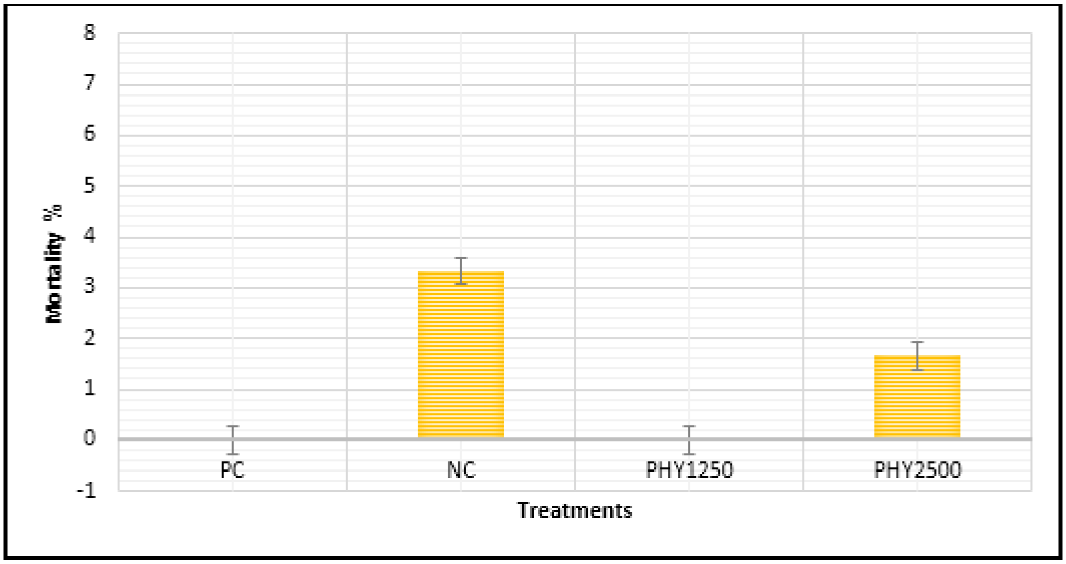

Fig. 1 : The effect of adding different levels of Phytase on the mortality percentage.

treatment. The results were consistent with Pieniazek *et al* (2017), who pointed that a significant decrease in FCR in the positive PC control treatment compared to NC negative control. The addition of the phytase enzyme by 500, 2000 resulted in a significant decrease (P <0.05) compared to NC treatment and at a close level of PC treatment. Additionally, the present results in agreement with finding of Taheri and Taherkhan (2015), who revealed a significant increase (P <0.01) in the feed conversion coefficient of NC (npp = 0.2) compared to the PC. The addition of the phytase enzyme to the NC diet led to a significant decrease (P <0.05) compared to the negative control ratio, with no differences between addition coefficients and positive control treatment. Results were not consistent with finding of Walk (2018) and Truong *et al* (2017), who detected insignificant differences in the feed conversion coefficient between the experimental treatments was noted.

Mortality

Fig. 1 showed no significant differences (P<0.05) in the mortality ratio between the experimental treatments. These results were consistent with Farhadi et al. (2017) who demonstrated that insignificant difference (P<0.05) in the percentage of mortality during the experiment period (1-42 days) between the experimental treatments PC, NC (deficiency of 0.15 Ap), NC with addition of different levels of enzyme. The results are not consistent with Pieniazek *et al* (2017) and for the period (1-42 days), where the increase in the percentage of mortality in the NC treatment was observed to be deficient (nPP0.17) and the addition of the phytase by 2000 FTU/kg was capable of removing this effect. The results also correlated with Liu *et al* (2016) in their 1 to 21 day gestational age observed a significant increase in the percentage of losses in NC (aP, 0.18) compared to PC positive control (aP, 0.48).

Physical characteristics

The results in Table 6 showed no significant differences between the experimental treatments for the concentration of glucose and cholesterol in the blood while a significant increase (P <0.05) in serum phosphorus concentration for PC (5.13) compared with NC (4.13). Addition of 1250, 2500 FTU/kg to NC treatment resulted in a significant increase (P <0.05) in the concentration of phosphorus in blood. Furthermore, a significant increase (P <.05) at the level of ALP in the blood for NC negative control (1490.6) compared to (1281.33) for the treatment of PC. The addition of 1250, 2500 FTU/Kg to NC treatment showed a significant decrease (P <0.05) in the concentration of ALP (1294.0, 1231.3). The addition treatment of 1250 FTU/Kg was similar to PC treatment. A significant decrease (P <0.05) 2500 FTU/Kg compared to the rest of the experiment treatments. There was a significant decrease (P <0.05) for phosphorus for NC deficiency compared to the PC control and adding enzyme levels showed the possibility of compensating for the shortage of phosphorus to the treatment in full needs by the dissolution of phytic acid and the disengagement with phosphorus to release it which represent an evident by the laboratory results of the level of phosphorus in the serum. As for ALP, its activity or high blood level is associated with abnormalities in the bone (Brenes et al., 2003), which can be associated with phosphorus deficiency. A decrease in ALP activity was observed in the addition of phytase enzyme to the diets, which can be attributed to the lower concentration of this enzyme due

Table 1 : Feed components and calculated chemical composition of maize strains used in the experiment.

Content	Starter diet 1-10 day		Grower diet 11-21		Finisher diet 22-42	
	PC	NC	PC	NC	PC	NC
Yellow corn	58.12	58.78	61.92	62.55	64.15	64.8
Soybean meal	35.55	35.45	30.7	30.6	28.2	28.1
Mix of minerals and vitamins	1	1	1	1	1	1
Plant oil	1.55	1.35	2.95	2.75	3.5	3.3
Calcium di phosphate	1.8	0.85	1.6	0.67	1.4	0.5
Limestone	1.2	1.8	1.05	1.65	1.1	1.65
Methionine %	0.24	0.23	0.23	0.23	0.18	0.18
Lysine%	0.24	0.24	0.25	0.25	0.17	0.17
salt	0.3	0.3	0.3	0.3	0.3	0.3
Total	100%	100%	100%	100%	100%	100%
Crude protein %	22	22	20	20	19	19
ME kcal/kg	2954	2956	3089	3090	3152	3153
Methionine and cysteine%	1.04	1.04	0.98	0.98	0.91	0.91
Lysine %	1.39	1.39	1.26	1.26	1.13	1.13
Calcium %	1	1	0.88	0.88	0.84	0.84
Phosphorus%	0.45	0.28	0.41	0.24	0.37	0.2

* The mixture of minerals and vitamins used in the manufacture of broiler Premix 1% produced by the company Intraco LTD Belgian-free of calcium and phosphorus, ** Soya bean used from the source of Argentine crude protein ratio of 48%, and the metabolic energy 2440 kcal/kg, *** Chemical composition of other feedstocks based on (NRC 1994).

Table 2 : Effect of addition of different levels of Phytease enzyme on the mean body weight (g) at 42 days.

Weeks	Treatments			
	PC	NC	NC+1250 FTU/kg	NC+2500 FTU/kg
3	a 758.97± 38.04	b 504.37 ± 21.51	a 757.17 ± 5.26	a 800.90± 50.32
6	a 2426.47 ± 129.11	b 1598.72 ± 62.14	a 2407.1 ± 10.93	a 2541.20 ± 161.87

* Values represent the mean ± standard error, ** The different small letters within one row indicate significant differences between the coefficients, at a significant level (P<0.05).

Table 3 : Effect of addition of different levels of Phytase enzyme on the average weight gain (g).

Weeks	Treatments			
	PC	NC	NC+1250 FTU/kg	NC+2500 FTU/kg
1-3	a760 73 ± 38.04	b464 76 ± 21.42	a717.03 ± 5.35	a 760.73 ± 50.30
4-6	a1667 50 ± 91.08	b1094 34 ± 40.69	a1649m93 ± 6.54	a 112.07 ± 1740.84
1-6	a2386.30 ±129.11	b1559.10 ± 62.08	a2366.97 ± 11.01	a2501.57 ±162.37

*Values represent the mean ± standard error, ** The different small letters within one row indicate significant differences between the coefficients, at a significant level (P<0.05).

to increased availability of phosphorus released from the phytite complex (Catala-Gergori *et al*, 2006). Therefore, the decrease in ALP activity in both treatments can be attributed to the increased availability of phosphorus released from the decomposition of the phytite complexes in addition to these high levels of enzyme.

These results were consistent with results of Islam *et al* (2017), who reported for deficient content of nPP without enzyme supplementation or 1500 FTU/kg addition at 42 days with no significant differences in cholesterol and glucose levels. The results also agreed with Amer (2014) who detected that there was no significant difference in the level of cholesterol, glucose, between PC and NC1 and NC2, supplemented with 1000, 2000 FTU/kg. Moreover, the present results were in agreement with previous work of Farhadi *et al* (2017), who noted a significant increase (P <0.05) in the serum phosphorus level for PC compared with NC treatment (0.15 aP deficiency), and the addition of different levels of phytase enzyme led to an increase (P <0.05) in phosphorus concentration in the serum with no significant difference compared to PC treatment. The results were consistent with Taheri and Taherkhani (2015) in a trial of 22-42 days with a significant increase (P <0.05) in serum

Table 4 : The effect of feeding different levels of phytase in the average feed consumption (g).

Weeks	Treatments			
	PC	NC	NC+1250 FTU/kg	NC+2500 FTU/kg
1-3	b 979.90 ± 55.20	a719.57 ± 26.26	b956.20 ± 17.08	b1001.77 ± 68.65
4-6	b 3083.47 ± 166.98	a 2240.83 ± 83.49	b 3057.50 ± 40.02	b 3167.50 ± 249.05
1-6	b 4063.31 ± 221.75	a2962.15 ±108.14	b 4005.43 ± 36.60	b 4169.24 ± 316.80

*Values represent the mean ± standard error, ** The different small letters within one row indicate significant differences between the coefficients, at a significant level (P<0.05).

Table 5 : The effect of feeding different levels of phytase on the feed conversion coefficient (g).

Weeks	Treatments			
	PC	NC	NC+1250 FTU/kg	NC+2500 FTU/kg
1-3	b 1.36 ± 0.01	a 1.55 ± 0.02	b 1.33 ± 0.01	b 1.32 ± 0.02
4-6	b 1.85 ± 0.00	a2.05 ± 0.01	b 1.85 ± 0.02	b 1.82 ±0.02
1-6	b1.70 ± 0.00	a 1.90 ± 0.01	b 1.69 ± 0.01	b1.66 ± 0.02

*Values represent the mean ± standard error, ** The different small letters within one row indicate significant differences between the coefficients, at a significant level (P<0.05).

Table 6 : The effect of adding different levels of phytase enzyme on the blood characteristics and chemical properties at the age of 42 days.

Traits	Treatments			
	PC	NC	NC+1250 FTU/kg	NC+2500 FTU/kg
Glucose mg/ml	a206.33 ± 3.28	a 203.67 ± 2.73	a199.67 ± 0.33	a203.33 ± 3.76
Cholesterol mg/ml	a214.00 ±4.93	a208.67 ± 5.92	a214.00 ± 7.02	a211.67 ± 10.14
Phosphorus mg/ml	a5.13 ± 0.12	b4.13 ± 0.07	a5.16 ± 0.12	a5.23 ± 0.12
ALP UNIT/L	b 1281.33 ±7.05	a 1490.6 ± 18.52	b 1294.00 ± 5.29	c1231.3 ± 11.21

*Values represent the mean ± standard error, ** The different small letters within one row indicate significant differences between the coefficients, at a significant level (P<0.05).

Table 7 : The effect of addition of different levels of phytase in ash and phosphorus of the thigh at 42 days.

Traits	Treatments			
	PC	NC	NC+1250 FTU/kg	NC+2500 FTU/kg
ASH	a 48.93 ± 0.37	b46.87 ± 0.12	a48.80 ± 0.35	a49.10 ± 0.26
PASH	a 18.70 ± 0.15	b 17.10 ± 0.57	a18.57 ± 0.09	a18.77 ± 0.09

*Values represent the mean ± standard error, ** The different small letters within one row indicate significant differences between the coefficients, at a significant level (P<0.05).

phosphorus concentration for PC control treatment (0.43 aP) compared to NC treatment (0.15: aP). The addition of 4000 FTU/kg to the NC treatment resulted in a significant increase (P <0.05) in serum phosphorus concentration and near PC positive control. These results are consistent with what Farhadi *et al* (2017) reported in his 42-day trial of an increase in the level of serum ALP in the serum (NC 0.15) compared with PC treatment and that the addition of phytase was significantly reduced (P <0.05)) at the level of ALP to levels comparable to PC treatment. In addition, Tanay bilal *et al* (2015) reported a significant increase (P <0.05) in the serum ALP concentration in the NC treatment (0.15 ap) compared to the PC as well as, adding 600 FTU/kg of phytase enzyme to NC treatment significantly reduced (P <0.05) concentration of serum ALP with significant difference compared with PC. The results were not consistent with Summerfeld *et al* (2018) who revealed that insignificant difference in the level of phosphorus in blood plasma when adding the phytase to the full phosphorus needs of 500, 1500, 3000 FTU/kg. Also, these results were not consistent with finding of Momeneh *et al* (2018), who pointed insignificant differences between diet with complete needs and the addition of phytase by 0, 500, 2500 FTU/kg for the period 1-28 days.

Percentage of phosphorus and bone ash

The results of Table 7 in the sixth week of age showed a significant increase (P <0.05) for the percentage of thigh bone ash for PC (48.93%) compared to NC treatment

(46.86). The addition of phytase at levels of 1250, 2500 FTU/Kg to NC treatment resulted in a significant increase in the percentage of bone ash (48.8, 49.1), with no significant difference between them and PC treatment. A significant (P <0.05) increase in the percentage of thigh ash in PC (18.7) versus (17.1) for the NC treatment. A significant increase (P<0.05) was observed in the percentage of thigh phosphorus when adding phytase enzyme at levels 1250, 2500 FTU/Kg to NC treatment where it was (18.57, 18.77) and close to PC treatment. This increase in the percentage of ashes and phosphorus ash of the levels of addition is a good indication of the increased concentration of minerals in the bone due to the release of minerals such as Ca, P, Cu and Zn of the phytite complexes (Sebastian *et al*, 1996) and retention of K, Mg, P, Cu, Fe, S and Mn when phytzase was added to NC diets (Beeson *et al*, 2017). The standards for the ratio of minerals in bone ash are more sensitive to the phosphorus status of birds than the growth criteria (Taheri, 2015). Bone ash is the best variable to determine the response to enzyme activity within the body (Dos Santos, 2013).

The results were consistent with Farhadi *et al* (2017) who indicated a significant decrease (P <0.05) in the percentage of thigh ash and the percentage of thigh ash phosphorus in the treatment of NC deficiency compared to PC control. Moreover, adding phytase to NC at level of 6000 FTU/kg resulted in a significant improvement (P <0.05) in the percentage of thigh ash and the percentage of thigh ash phosphorus and near to PC treatment. Tanay Bilal *et al* (2015) showed a significant decrease (P <0.05) in thigh ash phosphorus in NC treatment compared with the PC. Gautier *et al* (2018) showed a decrease in thigh ash phosphorus in NC1 and NC2 compared with PC control treatment and that the decrease was less than attenuated when 1500 FTU/kg of enzyme was added. The results did not agree with those mentioned by Momeneh *et al* (2018), noting that there was no significant difference (P <0.05) in the percentage of thigh ash and the percentage of thigh ash phosphorus when using complete dietary needs and the addition of the phytase enzyme at the level of 2500, FTU/kg for a period of 1-28 days of the age of the broilers.

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