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Impact of using phytase enzyme with different levels of calcium and phosphorus on broiler chickens' performance, carcass traits and blood parameters

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ABSTRACT

A five week feeding study was carried out to assess the effects of phytase enzyme with different level of calcium and available phosphorus on Ross 308 broilers on performance, carcass traits and deposition of calcium and phosphorus on serum and tibia of broiler chickens. One hundred and sixty-eight one day-old Ross 308 broiler chicks were used. The birds were weighed and assigned to equal six treatment groups with fixed calcium to phosphorus ratio 2:1. Each two treatments have the same value of available phosphorus according feeding stage but one of them with supplementation of Hiphos GT (100g/ton phytase enzyme unit FTU/Kg) and the other one without supplementation in a completely randomized design. Each treatment had four replicates and each replicate contains seven chicks. The results showed that phytase enzyme supplementation with available phosphorus 0.5% and 0.45% significantly elevated ($P < 0.05$) body weight, body weight gain and feed intake, and reduced ($P < 0.05$) feed conversion ratio in starter period. All parameters in grower, finisher period and overall showed non-significant for all groups. Moreover, the highest significant value from all other groups in liver enzymes Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, founded in broilers fed the diet with phytase enzyme and available phosphorus 0.40% and without phytase enzyme in creatinine. Calcium value with available phosphorus 0.35% with phytase enzyme showed high significance. In Phosphorus we found that usage of phytase enzyme with available phosphorus 0.30% and 0.35% as same as 0.35% without phytase. In weight, eviscerated and dressing usage of phytase enzyme with available phosphorus 0.4% revealed the highest significant difference from all groups. Thigh, breast and drumstick value showed non-significant difference from all other groups. It can be concluded that supplementation of phytase enzyme in treatments with low calcium and available phosphorus improve performance, kidney liver function test, mineralization of bone and carcass traits.

Introduction

Chicken meat is an important source of animal proteins and organic and inorganic substances of high biological value for human (Lee *et al.*, 2012). Poultry industry in developing countries is facing many problems due to high costs of feed ingredients (Abd El-Hack *et al.*, 2015). Poultry nutrition is targeting methods to improve mineral and nutrient digestibility, for example, by using of diverse techniques of feed treatment or exogenous enzymes application (Humer *et al.*, 2015). Digestive enzyme in poultry diet play an important role in enhances economic cost of the diets (Attia *et al.*, 2008).

Nutrition programs in poultry industry were important to achieve right body weight gain and high-quality meat in the context of public health (Jha and Berrocoso, 2015). Service and improvement for chickens to grow at their best and have improved feed efficiency, their gut health is crucial (Yadav and Jha, 2019).

Calcium and phosphorous are couple of essential minerals which are involved in many physiological functions of the body of living poultry (Underwood and Suttle, 2001). In animal bodies, calcium predominates, although phosphorus is the second most prevalent mineral. The two main minerals that make up bone are calcium and phosphorus. The bone is where the majority of the calcium and phosphorus in the body are found (McDonald, 2002). Calcium and phosphorus are essential minerals for the metabolic and structural processes involved in the production of bone and eggshells (Pelicia *et al.,* 2009). Thesis minerals present in bones as 95 calcium and phosphor-us 80-90% (Hirsch and Baruch, 2003). Excess of calcium in diet reduces phosphorus absorption due to the formation of insoluble complexes in intestinal lumen (Rama *et al.*, 2006). Jadhav *et al.* (2016) reported that Calcium and phosphorus in the diet increase body weight gain and FCR.

The metabolic pathways depend heavily on phosphorus, an essential nutrient (Shastak *et al.*, 2012). Due to its function as the cell's energy currency and regulator of cellular mechanisms, phosphorus is crucial for cellular metabolism (Musilova *et al.*, 2014). Phosphorus enhances growth performance (Hamdi *et al.* 2017). Phosphorus is crucial for growth and bodily function, according to Williams *et al.* (2000). Growth, cellular and membrane function, energy metabolism, and acid-base balance all depend on calcium (Fleming, 2008). Calcium is essential for metabolism, blood coagulation, enzyme activation, neuromuscular function, muscle contraction, cell adhesion, and intracellular signaling, according to Weglarz and Angel (2013).

Broiler body weight and feed efficiency increased when feed's calcium content was increased (Prabowo *et al.*, 2019). Salts of phytic acid are known as phytase and have antinutrient characteristics that prevent absorption of phosphorus and several other cations (including magnesium, manganese, zinc, iron, calcium, potassium, copper and cobalt) (Cosgrove and Irving, 1980). Phytase is enzyme hydrolyses Phytate to inositol and inorganic phosphate (Thacker *et al.*, 2009).

Phytase is an enzyme act in bonds of phosphate group of phytate leading to release of phosphorus (Cromwell and Coffey, 1991). Phytase enzyme plays an important role in catalysis the stepwise hydrolysis of phytate to inorganic phosphates and inositol (Greiner and Konietzny, 1999). Phytase enzymes reduce antinutritional effect of phytate by improve phosphorous, calcium; amino acids digestibility and energy beside reduce the excretion of inorganic phosphorous (Cowieson and Ravindran, 2007). Phytase is one of exogenous enzymes used in monogastric animal

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and birds' nutrition (Ptak *et al*., 2015). Low dietary calcium from 1% to 0.5% increasing phytate phosphorus consumption by 15%, dietary calcium and phosphorus levels have an impact on phytate phosphorus use (Mohamed *et al*., 1991). Phytate lowers pH of cecal digesta and increases body weight, weights of cecum, and weights of cecal and cecal digesta (Alaeldein, and Abudabos, 2012). Hens require high phytate phosphorus in growth stage for increasing body weight (Rutherfurd *et al*., 2012). Indicated that phytate affects avian performance by lowering the digestibility of amino acids, decreasing mineral absorption, and raising endogenous losses. Yan *et al*. (2001) stated that phytase enzyme in diet induces release of phytate bound phosphorus and decreases phosphorus excretion, release of phytate bound phosphorus and decreases phosphorus excretion, Exogenous phytases improve the availability and utilization of minerals, amino acids, and energy while also enhancing the performance of the birds in chicken diets by direct hydrolytic effects on Phytase (Pirgozliev *et al*.,, 2008).

The aim of the present study was to investigate the effect of phytase enzyme on body Performance, bone mineralization and Carcass traits as well as some biochemical parameters in broilers.

Materials and methods

Ethical approval

The research was conducted in accordance with the institutional guidelines for the care and use of experimental animals approved by the Institutional Animal Care and Use Committee, Zagazig University, Egypt (Approval No. Zu- IACUC/2/F/270/2023).

Birds and management

One hundred and sixty-eight one day chicks (Ross 308 broiler) were purchased from a producer of commercial chicks. Chicks were housed in a conventional house using a battery system. Upon arrival, they were weighed and randomly allocated equally to six treatment groups, each containing four replicates with seven chicks for each one. Broilers were vaccinated against Newcastle (on the 4th and 14th days) and Gumboro diseases (on the 7th and 22nd day).

Experimental design and diets

For the experiment, isocaloric and isonitrogenous broiler diets were offered in mash form, fed according to the rearing phase (Table 2), and formulated to meet the nutrient requirements set by Ross 308 Broiler Nutrition Specification (2022). Each two treatments have the same value of available phosphorus according to the feeding stage, but one of them supplemented with Hiphos GT (100gm/ton phytase enzyme unit FTU/Kg) and the other one without supplementation as showed in Table 1.

Sampling, procedures, and laboratory Analysis

The following parameters were measured: body weight, weight gain, feed intake and feed conversion ratio for the different stages of age starter (day 10), grower (day 24), and finisher (day 35). Feed conversion ratio (FCR) was calculated according to Wanger *et al*. (1983).

At the end of experiment (35 days), five birds were selected from each group and slaughtered for collection of blood samples, which were collected from the wing vein under aseptic conditions into a sterile syringe without anticoagulants and then centrifuged at 3,000 × g for 15 min. The sera were stored at −20°C until usage to determine calcium, phosphorus, ALT, AST, and creatinine.

Eviscerated carcass, dressing percentage, thigh, drumstick, and breast were weighed at the end of the experimental period by selecting three birds from each group that fasted overnight, weighed, and then slaughtered by a sharp knife to complete bleeding. Subsequently, their feathers were plucked, and evisceration was performed; then, the right tibia was collected for aching. A total of three birds per treatment at days 10, 23 and 35 were euthanized by slaughter and were burned in a muffle furnace at 600°C; then, Ca and P were analyzed according AOAC (1990) (methods 927.02 & 965.17, respectively). Minerals were determined by method of Nation and Robinson (1971).

Statistical analysis

Results were reported as mean ± SEM (Standard Error of Mean). In order to assess the influence of the six treatment groups on the different biochemical parameters, one-way analysis of variance (ANOVA) by Duncan multiple test as post hoc test were used. The value of $P < 0.05$ was used to indicate statistical significance. All analyses and charts were done using Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) and Graph Pad prism 8.0.2 (Graph Pad Software, Inc).

Results

Performance Parameters

The results of the current study (Tables 3) showed that phytase enzyme supplementation significantly increased body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). Group with available phosphorus 0.5% with phytase enzyme and group with available phosphorus 0.45% with phytase enzyme showed significantly higher performance reading value during starter period for body weight (BWT) and body weight gain (BWG) but showed non-significant increase from group with available phosphorus 0.5% without phytase enzyme and group with available phosphorus 0.4% with phytase enzyme, G1 and G6 showed non-significant reading from all other groups (group with available phosphorus 0.45% without phytase enzyme and group with available phosphorus 0.4% without phytase enzyme in BWT and BWG). Regarding feed intake (FI); group with available phosphorus 0.5% with phytase enzyme showed highly significant difference from all groups except group without available phosphorus 0.5% with phytase enzyme and group with available phosphorus 0.45% with phytase enzyme, these later group showed non-significant difference from all groups (group with available phosphorus 0.45% with phytase enzyme, group with available phosphorus 0.4% without phytase enzyme and group with available phosphorus 0.4% with phytase enzyme), Feed conversion ratio (FCR) in

Table 1. Experimental Design.

Groups	Ca: AP	Starter 0 to10 day age	Grower 11 to 23 days age	Finisher 24 to 35 days age
G1	Ca: AP (2:1) without phytase enzyme	1% Ca + 0.5% AP	0.9% Ca - 0. 45% AP	0.8% Ca - 0. 4% AP
G2	Ca: AP (2:1) with phytase enzyme	1% Ca + 0.5% AP	0.9% Ca - 0. 45% AP	0.8% Ca - 0. 4% AP
G3	Ca: AP (2:1) without phytase enzyme	0.9% Ca + 0.45% AP	0.80% Ca - 0.4% AP	0.7% Ca - 0.35% AP
G4	Ca: AP (2:1) with phytase enzyme	0.9% Ca + 0.45% AP	0.80% Ca - 0.4% AP	0.7% Ca - 0.35% AP
G5	Ca: AP (2:1) without phytase enzyme	0.8% Ca + 0.4% AP	0.70% Ca - 0.35% AP	0.6% Ca - 0.3% AP
G6	Ca: AP (2:1) with phytase enzyme	0.8% Ca + 0.4% AP	0.70% Ca - 0.35% AP	0.6% Ca - 0.3% AP

Table 2. Composition of the experimental diet from day 1 to 35 /100 kg (with Phytase enzyme).

Ingredients	Feeding stages																	
	Starter stage						Grower stage						Finisher stage					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
Yellow corn	50.1	50.62	50.8	51.32	51.5	52.02	54.55	55.07	55.3	55.82	56	56.52	57.61	58.13	58.6	59.12	59.2	59.75
Soybean meal	36.6	36.6	36.6	36.6	36.6	36.6	32.2	32	32	32	32	32	28.6	28.6	28.5	28.5	28.5	28.5
Corn gluten, 60%	5	5	5	5	5	5	4.5	4.5	4.5	4.5	4.5	4.5	4.2	4.2	4.1	4.1	4.1	4.1
Soybean oil	3.5	3.5	3.19	3.19	2.9	2.9	4.5	4.5	4.2	4.2	3.9	3.9	5.7	5.7	5.3	5.3	5.1	5.1
Monocalcium phosphate	1.9	1.38	1.66	1.08	1.4	0.88	1.7	1.18	1.45	0.93	1.2	6.8	1.5	0.98	1.26	0.74	1	0.48
Calcium carbonate	1.4	1.4	1.25	1.25	1.1	1.1	1.3	1.3	1.1	1.1	0.95	0.95	1.1	1.1	0.94	0.94	0.8	0.8
L-lysine Hcl 98%	0.32	0.32	0.32	0.32	0.32	0.32	0.29	0.29	0.29	0.29	0.29	0.29	0.27	0.27	0.27	0.27	0.27	0.27
DL-Methionine, 99%	0.31	0.31	0.31	0.31	0.31	0.31	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.27	0.27
L-threonine 98.5%	0.11	0.11	0.11	0.11	0.11	0.11	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7
Sodium bicarbonate	0.28	0.28	0.28	0.28	0.28	0.28	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Common salt	0.18	0.18	0.18	0.18	0.1.8	0.18	0.3	0.3	0.3	0.3	0.3	0.3	0.31	0.31	0.31	0.31	0.32	0.32
phytase	0	0.1	0	0.1	0	0.1	0	0.1	0	0.1	0	0.1	0	0.1	0	0.1	0	0.1
Premix	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Calculated composition																	
ME(Kcal/kg)	3001.3	3001.3	3000	3000	3001	3001	3098	3098	3099	3100	3100	3100	3202	3202	3198	3198	3202	3202
CP	23.64	23.64	23.69	23.69	23.74	23.74	21.53	21.53	21.58	21.58	21.64	21.64	20.01	20.01	19.97	19.97	20.02	20.02
Ca	0.10	0.99	0.90	0.89	0.81	0.80	0.91	0.91	0.80	0.80	0.71	0.71	0.8	0.8	0.70	0.70	0.61	0.61
Available P	0.50	0.49	0.45	0.44	0.40	0.39	0.45	0.45	0.40	0.40	0.35	0.35	0.40	0.40	0.35	0.35	0.30	0.30
Lysine	1.44	1.44	1.44	1.44	1.44	1.44	1.29	1.29	1.29	1.29	1.29	1.29	1.19	1.19	1.19	1.19	1.19	1.09
Methionine	0.69	0.69	0.69	0.69	0.69	0.69	0.63	0.63	0.63	0.63	0.63	0.63	0.59	0.59	0.60	0.60	0.60	0.60
Threonine	0.10	0.10	1.00	1.00	1.00	1.00	0.89	0.89	0.89	0.89	0.89	0.89	0.82	0.82	0.82	0.82	0.82	0.82

Mineral and vitamin premix: Each1kg diet contain Vit.A (12000 IU), Vit.D3 (5000 IU), Vit.E (80 IU), Vit.k3 (3.2mg), Vit. B1 (3.2 mg), Vit.B2 (8.6 mg), Vit.B6 (4.3mg), pantothenic acid (20 mg), Vit. B12 (0.017 mg), niacin (65 mg) ,folic acid (2.20 mg), biotin (0.22 mg), fe (20mg), Mn (120 mg), Cu (16 mg), I (1.25 mg), Se (0.30 mg) and Zn (110 mg).

Table 3. The effect of adding phytase enzyme with different levels of calcium and phosphorus on growth performance in broilers from days 1 to 35.

	Starter			
	BWT (g)	BWG (g)	FI (g)	FCR
G1 Ca :AP (2:1) AP0.5 without phytase enzyme	266±16.06238^{ab}	225.5±16.58312^{ab}	254.75±14.244882^{ab}	1.130±0.0216^{abc}
G2 Ca :AP (2:1) AP 0.5 with phytase enzyme	283.5±12.1518^{a}	243.498±12.134^{a}	274.25±13.326^{a}	1.12638±0.0085^{abc}
G3 Ca : AP (2:1) AP 0.45 without phytase enzyme	244.5±17.13671^{b}	204±16.86495^{b}	231.75±16.23525^{b}	1.13750±0.01893^{ab}
G4 Ca :AP (2:1) AP 0.45 with phytase enzyme	270.5±3.316^{a}	230.253±3.00^{a}	255.25±5.25^{ab}	1.10849±0.0928^{bc}
G5 Ca :AP (2:1) AP0.40 without phytase enzyme	243±10.48809^{b}	203±10.488^{b}	234±6.324555^{b}	1.155±0.02645^{a}
G6 Ca :AP (2:1) AP0.40 with phytase enzyme	258.75±6.0208^{ab}	218.4±6.307^{ab}	239±11.04^{b}	1.09402±0.0258^{c}
G1 Ca : AP (2:1) AP 0.45 without phytase enzyme	1207.5±55	941.5±49.69574	1174.5±72.890329	1.24750±0.04991
G2 Ca :AP (2:1) AP 0.45 with phytase enzyme	1265±26.467	981.5±19.347	1237.5±29.38	1.26147±0.04936
G3 Ca :AP (2:1) AP 0.40 without phytase enzyme	1212.5±71.35592	968±60.40419	1202.5±63.568865	1.2425±0.03304
G4 Ca :AP (2:1) AP 0.40 with phytase enzyme	1255±20.8166	984.5±20.27	1265±69.62	1.28444±0.0528
G5 Ca :AP (2:1) AP 0.35 without phytase enzyme	1175±98.82645	932±90.31427	1167.75±87.868745	1.2550±0.03696
G6 Ca :AP (2:1) AP 0.35 with phytase enzyme	1215±37.859	956.25±40.82	1218±31.696	1.2744±0.02138
G1 Ca :AP (2:1) AP 0.40 without phytase enzyme	2212.25±64.132035	1004.75±14.93039	1498±53.435319	1.495±0.07416
G2 Ca :AP (2:1) AP 0.40 with phytase enzyme	2245.5±69.09	980.5±73.259	1419.5±91.25	1.44899±0.0417
G3 Ca :AP (2:1) AP 0.35 without phytase enzyme	2206.75±127.10462	994.25±63.55247	1438.75±50.940325	1.4475±0.06344
G4 Ca :AP (2:1) AP 0.35 with phytase enzyme	2280.25±108.95	1025.25±111.54	1505.25±114.68	1.47223±0.0511
G5 Ca :AP (2:1) AP 0.30 without phytase enzyme	2201.5±178.06834	1026.5±81.45551	1490±89.431538	1.4525±0.03594
G6 Ca :AP (2:1) AP 0.30 with phytase enzyme	2299.75±73.086	1084.75±48.84	1575.75±54.579	1.45327±0.0260
G1 Ca :AP (2:1) without phytase enzyme		71.85±64.44328	2927.25±100.70212	1.3483±0.04814
G2 Ca :AP (2:1) with phytase enzyme		2205.4975±69.08244	2931.25±119.29341	1.329±0.03148
G3 Ca :AP (2:1) without phytase enzyme		2166.25±127.31225	2873±112.86866	1.3278±0.04284
G4 Ca :AP (2:1) with phytase enzyme		2240.0025±109.12	3025.5±159.61307	1.3506±0.02067
G5 Ca :AP (2:1) without phytase enzyme		2161.475±178.04934	2891.75±180.61077	1.3396±0.03259
G6 Ca :AP (2:1) with phytase enzyme		2259.4±73.58388	3032.75±62.51600	1.3427±0.02078

starter period had the lowest reading in group with available phosphorus 0.4% with phytase enzyme which showed highly significant decrease from group with available phosphorus 0.45% without phytase enzyme and group with available phosphorus 0.4% without phytase enzyme (non-significant to each other). G3 and G5 showed the highest reading group, G3 showed non-significant difference from all groups except G6, G4 showed significant difference from all groups except G5. All other groups were non-significant to each other. All parameters in grower, finisher period and overall showed non- significant for all groups.

Serum analysis

Our results (Tables 4) showed that phytase enzyme supplementation with low phosphorus level showed decreases of liver enzymes and creatinine, and increase of calcium and phosphorus in serum. Concerning ALT in group with available phosphorus 0.4% with phytase enzyme showed the highest significant activity of ALT from all other groups, and all other group show non-significant from each other. Group with available phosphorus 0.4% with phytase enzyme in AST showed the highest significant increase in AST activity from all other groups followed by group with available phosphorus 0.4% without phytase enzyme then group with available phosphorus 0.35% with phytase enzyme showed significant increase also from other groups. Group with available phosphorus 0.35% without phytase enzyme showed the lowest reading significantly from all groups except G5 and G6, in creatinine test, group with available phosphorus 0.4% without phytase enzyme showed the highest significant increase in reading value from all other group and all other groups is non-significant to each other.

At the end of the experiment the group with available phosphorus 0.35% with phytase enzyme showed highly significant calcium deposition value except with group with available phosphorus 0.30% with phytase enzyme, G4 followed by G6 (non-significant to each other), Group with available phosphorus 0.40% without phytase enzyme and group with available phosphorus 0.30% without phytase enzyme (non-significant to each other) showed significant decrease from G4 with available phosphorus 0.35% with phytase enzyme, group with available phosphorus 0.35% without phytase enzyme showed the lowest significance Ca deposition reading, however when showed phosphorus result, we found the G3 with available phosphorus 0.35% without phytase enzyme, group with available phosphorus 0.35% with phytase enzyme and with available phosphorus 0.30% with phytase enzyme (non-significant to each other) showed the highest P value from all other groups which showed non-significance from other.

Minerals Deposition

Results (Table 5) showed that during the starter, grower, and finisher stages; G6showed significant increases in Ca, P deposition and Ca, P ratio in bone ashing, During all periods These results showed that when availability of P decrease with phytase , significantly more Ca, P deposition and Ca. P ratio followed by lower availability without phytase that showed in all periods as in P level that showed significantly gradually decrease in value from G6 (the lowest availability with phytase) to G4 (the medium availability with phytase) and G2 (higher availability with phytase) followed by G5 (the lowest availability without phytase), G3 (the medium availability without phytase) finally G1 (higher availability without phy-

Table 4. The effect of adding phytase enzyme with different levels of calcium and phosphorus on Kidney, liver function and Ca, P in serum of broilers on day 35.

Calcium levels (Ca: P)	ALT	AST	Creatinine	Calcium	Phosphorus
G1 Ca :AP (2:1) AP 0.40 without phytase enzyme	11.375±0.47871[b]	359.5±16.62328[b]	0.3475±0.01893[a]	7.6675±0.06344[c]	6.6625±0.094[b]
G2 Ca :AP (2:1) AP 0.40 with phytase enzyme	13.7± 0.57155[a]	393.25±7.0887[a]	0.228±0.0098[b]	6.8375±0.309[d]	6.4225±0.2943[b]
G3 Ca :AP (2:1) AP 0.35 without phytase enzyme	11.175±0.56789[b]	270.25±10.30776[de]	0.2778±0.01266[b]	8.5955±0.28757[b]	7.53±0.18129[a]
G4 Ca :AP (2:1) AP 0.35 with phytase enzyme	10.7±0.4761[b]	303.25±6.0759[c]	0.2625±0.0221[b]	9.055±0.1436[a]	8±0.3554[a]
G5 Ca :AP (2:1) AP 0.30 without phytase enzyme	10.55±0.97125[b]	282.75±6.0208[d]	0.2455±0.03485[b]	7.885±0.26615[c]	6.5178±0.19774[b]
G6 Ca :AP (2:1) AP 0.30 with phytase enzyme	10.915±0.10630[b]	253.75±8.46[e]	0.251±0.0311[b]	8.73±0.1257[ab]	7.5675±0.3183[a]

Table 5. The effect of adding phytase enzyme with different levels of calcium and phosphorus on ashing of tibia in broilers on days10, 24 and 35.

Calcium levels (Ca: P)	Starter		
	Ca	P	Ca, P ratio
G1 Ca :AP (2:1) AP0.5 without phytase enzyme	2.8325±0.0055[f]	1.82±0.00816[c]	1.5525±0.0055[e]
G2 Ca :AP (2:1) AP 0.5 with phytase enzyme	3.49±0.0216[c]	2.0±0.0216[a]	1.7475±0.025[c]
G3 Ca : AP (2:1) AP 0.45 without phytase enzyme	3.09±0.02449[e]	1.9375±0.005[b]	1.595±0.01291[d]
G4 Ca :AP (2:1) AP 0.45 with phytase enzyme	3.8525±0.01258[b]	2.0125±0.025[a]	1.915±0.0173[b]
G5 Ca :AP (2:1) AP0.40 without phytase enzyme	3.2725±0.01258[d]	2.02±0.0216[a]	1.62±0.01826[d]
G6 Ca :AP (2:1) AP0.40 with phytase enzyme	4.04±0.02944[a]	2.0225±0.01708[a]	1.9975±0.005[a]
G1 Ca : AP (2:1) AP 0.45 without phytase enzyme	2.8575±0.0096[f]	2.145±0.01291[b]	1.3325±0.005[f]
G2 Ca :AP (2:1) AP 0.45 with phytase enzyme	3.76±0.0230[c]	2.1425±0.005[b]	1.755±0.0057[c]
G3 Ca :AP (2:1) AP 0.40 without phytase enzyme	3.1675±0.0206[e]	2.0975±0.005[d]	1.51±0.00816[e]
G4 Ca :AP (2:1) AP 0.40 with phytase enzyme	4.0825±0.033[b]	2.20±0[a]	1.855±0.0129[b]
G5 Ca :AP (2:1) AP 0.35 without phytase enzyme	3.53±0.00816[d]	2.12±0[c]	1.6625±0.005[d]
G6 Ca :AP (2:1) AP 0.35 with phytase enzyme	4.23±0.0258[a]	2.2025±0.005[a]	1.92±0.0141[a]
G1 Ca :AP (2:1) AP 0.40 without phytase enzyme	3.825±0.01732[f]	2.2325±0.005[e]	1.7125±0.0957[f]
G2 Ca :AP (2:1) AP 0.40 with phytase enzyme	4.26±0.0294[e]	2.3875±0.005[b]	1.7825±0.01258[e]
G3 Ca :AP (2:1) AP 0.35 without phytase enzyme	4.4925±0.09465[d]	2.335±0.00577[d]	1.9225±0.03594[d]
G4 Ca :AP (2:1) AP 0.35 with phytase enzyme	5.4125±0.0805[b]	2.4125±0.005[a]	2.245±0.0310[b]
G5 Ca :AP (2:1) AP 0.30 without phytase enzyme	4.99±0.05477[c]	2.3725±0.005[c]	2.1025±0.025[c]
G6 Ca :AP (2:1) AP 0.30 with phytase enzyme	6.285±0.0331[a]	2.42±0[a]	2.6±0.0141[a]

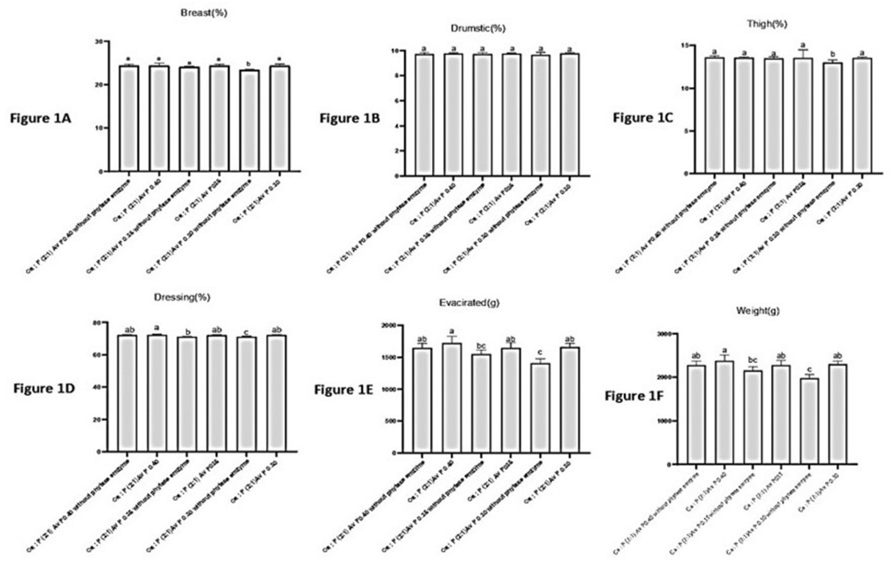

Fig. 1. The effect of adding phytase enzyme with different levels of calcium and phosphorus on carcass quality in broilers on day 35.

tase). All significant from each other, while in P level showed the same result of Ca level except in starter period, that G6(the lowest availability with phytase), G4 (the medium availability with phytase), and G2 (the highest availability with phytase) showed non-significant to each other with G5 (the lowest availability without phytase), and in grower period showed that G6 (the lowest availability with phytase) and G4 (the medium availability with phytase) non-significant to each other and significantly higher than G2 (the highest availability with phytase) which showed the same level of P availability with G1(the highest availability without phytase). but in finisher period G6 (the lowest availability with phytase) and G4 (the medium availability with phytase) non-significant to each other, then our conclusion is that the lower availability with phytase the more P deposition followed by lower availability without phytase (i.e.: G6 and G4 followed significantly by G2, G5,G3,G1). Ca , P ratio showed the same result in Ca deposition but different in two things, first one in starter period we found G5 (the lowest availability without phytase) non-significant to G3 (the medium availability without phytase), and the second one in finisher G6 (the lowest availability with phytase) and G4 (the medium availability with phytase) had the highest value ,followed by G5 (the lowest availability without phytase) then G3(the medium availability without phytase), G2 (the highest availability with phytase), finally G1 (the highest availability without phytase).

Carcass Quality Traits

Our results (Figure 1) showed that supplementation with phytase enzyme and phosphorus level 0.40% had a great effect on carcass traits. Also weight, eviscerated and dressing the same result which said that group with available phosphorus 0.40% with phytase enzyme the highest significant difference from all groups except group with available phosphorus 0.40% without phytase enzyme, group with available phosphorus 0.35% with phytase enzyme and group with available phosphorus 0.30% with phytase enzyme which showed non-significant difference from each other, group with available phosphorus 0.30% without phytase enzyme showed the lowest significant reading in these three parameters except with group with available phosphorus 0.35% without phytase enzyme in weight and eviscerated value only, group with available phosphorus 0.35% without phytase enzyme show non-significant value from all groups except group with available phosphorus 0.4% with phytase enzyme in theses parameters, with one only exception in dressing value showed significant increase from group with available phosphorus 0.30% without phytase enzyme and significant decrease from group with available phosphorus 0.40% with phytase enzyme, Thigh and breast value showed significant lowest value in with available phosphorus 0.30% without phytase enzyme from all other groups which showed non-significant among each other, drumstick value showed non-significant between each other.

Discussion

Phytase enzyme supplementation significantly increased BW, BWG, FI and FCR. Phosphorus level 0.5% and 0.45% with phytase enzyme significantly increased in BW and BWG in starter period, Also, there were no significant increase from group with phosphorus level 0.40% and phytase enzyme which showed the lowest reading of FCR. All parameters in grower, finisher period and overall showed non-significant for all groups. So, we can use phytase enzyme with low level of phosphorus in ration for good performance of broiler chicken.

Comparing our findings with the results related to phytase enzyme and available phosphorus level separately. The improvement in BW and BWG in this study due to phytase enzyme supplementation supported the hypothesis that phytase enzyme would improve the performance of broiler chickens by enhancing digestibility of nutrient.

Same results were reported by Motawe *et al*. (2012) who mentioned that addition phytase in level of 500 U to broilers diet induces significant improved BWG and. FCR. These results are comparable with results obtained previously Peter and Velmurugu (2000) stated that phytase enzymes in poultry ration improve in body weight gain due increase in P availability and feed intake beside improve nutrient utilization. These results agree with those of Lei and Stahl (2001) reported that phytases enzymes play an important role in release phosphate from phytate. In addition, another report by Salem *et al*. (2008) concluded that phytase improved body performance and carcass yield due to increase in nutrient availability for tissue growth. our result agreed with result recorded by Dilger *et al*. (2004) stated that phytase enzymes broilers ration reducing phosphorus excretion besides improving nutrient digestibility and growth performance. Increase in body weight and reduction in fed conversion rate in broilers received phytase enzymes were observed previously by Sun *et al*. (2011) reported that phytase enzymes improved in body performance dueto elevation in nutrient digestibility. The above mentioned results were supported by previous studies of Pirgozlev *et al*. (2011) found that broilers fed ration contain high levels of phytase enzymes induced better feed conversion rate. The obtained results are compatible with those reported by Rashid and Ismail (2019) mentioned that quail received different levels of phytase enzymes revealed significant increase in feed intake, weight gain and feed converter ratio. Our observed results were previously recorded by Wang *et al*. (2021) who stated that increasing phytase linearly increased broiler BWG and FCR.

Our results are confirmed previously by Larbier and Leclercq (1994) stated that calcium and phosphorus in broiler ration led to elevation in BW and BWG. Close similarity was seen between these findings and those obtained by Hurwitz *et al*. (1995) indicated that presence calcium and phosphorus in ration induces increase in growth rate. Our results go hand in hand with those reported Li *et al*. (2012) reported that balance between dietary calcium and phosphorus induces increase in growth rate in poultry. Birds fed low calcium and phosphorus diet (0.30% P and 0.6% Ca) induce decrease in body weighed less than those fed the control diet (0.45% P and 0.9% Ca) at 18 days of age (Yan *et al*., 2005). Same changes in body weight, weight gain and feed conversion rate were observed by Sakr *et al*. (2014) in quail received phytase enzymes.

Usage of phytase enzyme improve liver enzymes (AST- ALT), creatinine. group with available phosphorus 0.4% with phytase enzyme showed the highest significant ALT reading from all other groups, and all other group show non-significant from each other. Group with available phosphorus 0.4% with phytase enzyme in AST showed also the highest significant reading from all other groups followed by group with available phosphorus 0.4% without phytase enzyme then group with available phosphorus 0.35% with phytase enzyme show significant increase also from other groups, Group with available phosphorus 0.35% without phytase enzyme show the lowest reading significantly from all groups except G5 and G6, in creatinine test, group with available phosphorus 0.4% without phytase enzyme show the highest significant increase in reading value from all other group and all other groups is non-significant to each other.

Calcium and phosphorus in were increased during using phytase enzyme. calcium in group with available phosphorus 0.35% with phytase enzyme showed highly significant except with group with available phosphorus 0.30% with phytase enzyme, G4 followed by G6 (non-significant to each other), group with available phosphorus 0.40% without phytase enzyme and group with available phosphorus 0.30% without phytase enzyme (non-significant to each other) show significant decrease from G4 with available phosphorus 0.35% with phytase enzyme, group with available phosphorus 0.35% without phytase enzyme showed the lowest significance Ca deposition reading, however when showed phosphorus result, we found the G3 with available phosphorus 0.35% without phytase enzyme, group with available phosphorus 0.35% with phytase enzyme and with available phosphorus 0.30% with phytase enzyme (non-significant to each other) show highest P value from all other groups which showed non-significance from other also.

Our results were revealed that phytase enzymes improved liver enzymes (AST- ALT) creatinine, calcium and phosphorus which supported by the results of Church *et al*. (1984) stated that phytase enzymes improved in liver enzymes. These results in accordance with that obtained by Shakmak (2003) found that phytase enzymes revealed no adverse effect on serum liver enzymes of broilers. Our result was supported by the result of El-Ghamry *et al*. (2005) reported that phytase enzyme induced significant effect of liver enzymes activity; Our observed data are fit with those reported by Jalali and Babaei (2012) stated that birds fed dietary enzyme show increase in GOT and GPT. Phytase induce insignificant effect in serum calcium, creatinine, AST and ALT (Shehab *et al*., 2012).

This finding is disagreed with the result of Van Straalen *et al*. (1991) who reported that low calcium and phosphorus contents in ration reduced serum calcium, phosphorus and liver enzymes. Similar changes in serum calcium, phosphorus and liver enzymes were recorded by Zhang *et al*. (2020) in broiler chickens received ration low calcium and phosphorus. Broilers fed on low phosphorus levels in ration showed significant increase in plasma ALT, AST, creatinine associated with significant decrease in plasma calcium and phosphorus (Mahmoud *et al*., 2021).

Supplementation with phytase enzyme during the starter, grower, and finisher stages with low available phosphorus increased Ca and AP deposition, G6 group showed a significant increase in Ca, P deposition and Ca, P ratio in bone ashing, during all periods. These results revealed that decrease the availability P with phytase, resulted in significantly more Ca, P deposition and Ca. P ratio followed by lower availability without phytase that showed in all periods as in P level that showed significantly gradually decrease in value from G6 (the lowest availability with phytase) to G4 (the medium availability with phytase) and G2 (higher availability with phytase) followed by G5 (the lowest availability without phytase). All significant from each other, while in P level showed the same result of Ca level except in starter period, that G6 (the lowest availability with phytase), G4 (the medium availability with phytase), and G2 (the highest availability with phytase) showed non-significant to each other with G5(the lowest availability without phytase, and in grower period showed that G6 (the lowest availability with phytase) and G4 (the medium availability with phytase) non-significant to each other and significantly higher than G2 (the highest availability with phytase) which showed the same level of P availability with G1(the highest availability without phytase). but in finisher period G6 (the lowest availability with phytase) and G4 (the medium availability with phytase) non-significant to each other, then our conclusion is that the lower availability with phytase the more P deposition followed by lower availability without phytase (i.e.: G6 and G4 followed significantly by G2, G5, G3, G1). Ca , P ratio showed the same result in Ca deposition but different in two things, first one in starter period we found G5 (the lowest availability without phytase) non-significant to G3 (the medium availability without phytase) , and the second one in finisher G6 (the lowest availability with phytase) and G4 (the medium availability with phytase) had the highest value ,followed by G5 (the lowest availability without phytase) then G3 (the medium availability without phytase),G2 (the highest availability with phytase), finally G1 (the highest availability without phytase).

Our results were revealed that phytase enzymes improved in tibial calcium and phosphorus ash (Liem *et al*., 2008) who proved that Phytase enzymes induce increase in tibia ash. On a similar ground Francesch and Geraert (2009) reported that dietary phytase addition had a positive effect on the amount of tibia ash, calcium and phosphorus. These results agree with findings of Abdalnoor (2010) reported that phytase induced significant increase in calcium and phosphorus on tibia ash content. Phytase increased the eviscerated carcass ration (Wang *et al*., 2013). Our finding coordinates with those recorded by Adeola and Walk (2013) stated that phytase enzymes in ration increased bone mineralization. These recorded results were similar to these recorded Li *et al*. (2015) stated that phytase enzyme increased tibia ash weight and ash %. Our results were supported by result of Gautier *et al*. (2018) recorded that phytase increased tibia ash content and minerals. Previous findings in accordant with the results obtained by Leyva *et al*. (2019) mentioned that broilers received phytase enzyme showed improved bone mineralization. Similar results were recorded previously by Panel *et al*. (2019) recorded that phytase enzymes in broiler ration improved bone mineralization and tibia mineralization. Previous findings in accordant with the results obtained by Lu *et al*. (2019) reported that phytase enzyme in broiler diets induce bone parameters. Same results were observed Wang *et al* (2021) stated that increasing phytase linearly increased bone mineralization. This change was recorded by Shanmugam and In (2022) stated that phytase supplementation in broilers induced improvements on tibia ash.

Bone mineral content and ash % were linearly decreased as the level of dietary calcium (Onyango *et al*., 2003). Our results are agreed with earlier findings of Watson *et al*. (2006) mentioned that broiler received ration low in calcium and available phosphorus revealed reduction toe and tibia ash percentage. Similar results were recorded by Mondal *et al*. (2007) indicated that the tibia ash, calcium, and P were decreased in the birds fed low- phosphorus diets. Obtained result agrees with Wulandari *et al*. (2012) stated that low calcium and phosphorus in ration induce decrease in bone formation represented by low tibial ash content. Same observation was recorded by Díaz *et al*. (2019) reported that broilers fed adjusted calcium: available phosphorus (2:1) diets revealed maximum weight of tibia ash and weight of phosphorus in tibia ash were observed at available phosphorus levels of 0.40%, 0.52%, and 0.53% and at available phosphorus levels of 0.63%, 0.62 %, 0.59% in broilers fed the variable calcium: available phosphorus diets. Our results were agreement with earlier reports of Fallah *et al*. (2019) stated that normal level of calcium and phosphorus induced increase in tibial calcium ash content in broilers. Same results were observed by Zeyad *et al*. (2020) found broilers received diet contain 30% available phosphorus showed decreased in tibia ash, calcium, and phosphorus Broilers fed ration low in calcium and available phosphorus revealed decrease in ash and phosphorus content of tibia (Wang *et al*., 2021).

Our results (Figures 1) showed that Supplementation with phytase enzyme and phosphorus level 0.40% had a great effect on carcass traits. Also weight, eviscerated and dressing the same result which said that group with available phosphorus 0.40% with phytase enzyme the highest significant difference from all groups except group with available phosphorus 0.40% without phytase enzyme, group with available phosphorus 0.35% with phytase enzyme and group with available phosphorus 0.30% with phytase enzyme which showed non-significant difference from each other, group with available phosphorus 0.30% without phytase enzyme showed the lowest significant reading in these three parameters except with group with available phosphorus 0.35% without phytase enzyme in weight and eviscerated value only, group with available phosphorus 0.35% without phytase enzyme show non-significant value from all groups except group with available phosphorus 0.4% with phytase enzyme in theses parameters, with one only exception in dressing value showed significant increase from group with available phosphorus 0.30% without phytase enzyme and significant decrease from group with available phosphorus 0.40% with phytase enzyme, Thigh and breast value showed significant lowest value in with available phosphorus 0.30% without phytase enzyme from all other groups which showed non-significant among each other, drumstick value showed non-significant between each other.

Same results were observed by Dilger *et al*. (2004) stated that broilers received ration contain phytase showed increased in organ weight. Same results were reported by Watson *et al*. (2006) when broilers fed nutritionally adequate diets with phytase enzymes showed improved carcass traits. In addition, Motawe *et al*. (2012) stated that addition phytase enzymes to broiler diets increased internal organs. Chicks supplemented with phytase enzyme had a great effect on carcass traits (Sakr *et al*., 2014).

Our result was reported previously by Jadhav *et al*. (2016) stated that broiler chickens received calcium and phosphorus premix with synergistic phytase enzyme in improving carcass quality. This finding fitted closely with the data previously obtained by Gautier *et al*. (2018) who mentioned that broiler chickens feed in ration contain phytase improved carcass quality. The obtained data about the body weight agree with Rashid and Ismail (2019) mentioned that supplementation phytase enzyme improved carcass traits. Our results coordinate with those reported by Zeyad *et al*. (2020) reported that chicken fed ration contain calcium and phosphorus restriction: improved, carcass traits. Mahmoud *et al*. (2021) recorded those growing Egyptian geese received calcium and phosphorus levels improved carcass characteristics. In addition, Shanmugam and In (2022) stated that broilers Phytase in ration improved carcass characteristics and organ weight.

Conclusion

Supplementation of phytase enzyme could be effective and showed better results in some parameters when supplemented with low phosphorus ration in broiler chickens fed on adequate phosphorus diet.

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Conflict of interest

The authors declare that they have no conflict of interest.

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