ejpmr, 2018,5(6), 321-330

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFICACY OF PHYTASE SUPPLEMENTATION TO LOW PHOSPHORUS AND LYSINE BROILER CHICKENS DIET ON PERFORMANCE, BLOOD PARAMETERS AND NUTRIENTS DIGESTIBILITY

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Article Received on 11/04/2018 **Article Revised on 01/05/2018** **Article Accepted on 22/05/2018**

1. ABSTRACT

To assess the effects of the dietary phytase on the growth performance, and apparent nutrient digestibility of broiler chickens fed low available phosphorus and lysine diets, a total of 240 one-day-old Cobb 500 chicks were randomly allocated into six groups, with four replicates (40 birds/ group). The chicks were fed the starter (first two weeks), grower (second two weeks) and finisher (last week) diets supplemented with 0 or 1000 FTU/kg phytase activity. Six mash diets were formulated for the experimental groups which consisting of group 1 (control group, G1) fed corn soybean based diet without phytase supplementation. group 2 (G2) fed on lower NPP by 0.1% than required; group 3 fed (G3) on diet with 0.05% lower lysine than required; 4^{th} , 5^{th} and 6^{th} group (G4, G5 and G6) fed on the same diets of the previous groups respectively, but supplemented with phytase enzyme at a level of 0.2 g/kg diet. All birds received control diet during the first week and fed the experimental diet from the second week till the end of the experimental period. The highest BW was for the group fed the basal diet supplemented with phytase (the on top) that had increased feed consumption (1.6%; $P \leq 0.05$) with similar FCR and PER to the control. Addition of phytase to low NPP diet (G4) non significantly increased FBW and TWG, but showed significant ($P \leq 0.05$) decrease in TFI and FCR with higher PER and economic efficiency ($P \leq 0.05$) comparing with the group fed low NPP without phytase. The birds of G5 and G6 had higher CP digestibility than those of G2 and G3 respectively. Moreover, significant ($P \leq 0.05$) improvements in lysine digestibility (7.2%) and phosphorus (P) availability were observed with phytase supplementation. Further that, phytase supplementation had no adverse effect on haematological indices and biochemical constituents of serum, liver and kidney functions. It was concluded that phytase supplementation achieved better growth performance and higher economic efficiency. In addition, dietary phytase supplementation could replace 0.1% available phosphorous and 0.05% lysine.

KEYWORDS: Broilers, Microbial phytase, performance, P availability, lysine digestibility.

2. INTRODUCTION

Feed is the highest single expense in any system of poultry production, which accounting for up to 70% of total production cost per bird. Reducing feed cost is the main purpose for using feed enzymes. Feed enzymes participate in improving the efficiency of the production via targeting specific anti-nutrients in certain feed ingredients, allowing poultry to extract more nutrients from the feed and so improve feed efficiency and reducing costs by reformulating feed to contain lower levels of these nutrients.[1] In raw materials of plant origin commonly used in poultry diets such as soybean and corn, 60- 80% of the P is in the form of phytate phosphorus (The salt form of phytic acid).[2] Phytate is known to be an anti-nutrient which forms complexes with minerals (such as phosphorus and calcium) and proteins, making them unavailable for absorption.[3] Typically, it is considered that only 30% of the P of plants is actually available for the birds.[4] Also, the bond between phytate and proteins (usually between phytate and basic amino acids such as lysine when gut pH is less than the isoelectric point of proteins reducing their digestibility.[5] Poultry are unable to utilize phytate phosphorus present in feed as a result of the lack or low levels of gastrointestinal phytases (Phytate hydrolyzing enzymes), so they require exogenous phytases in their feed to counteract the adverse effect of such substances.[6] Microbial phytase is the most commonly used exogenous enzyme in the monogastric animals feed, where it reduce the antinutritional effect of phytate and improve the digestibility of phosphorous (P), calcium, amino acids and energy, as well as reduce the feed cost and the negative impact of inorganic P excretion to the environment.[7] Previous studies showed that, different levels of inclusion of phytase improved the performance of broilers fed phosphorus deficient or adequate nutrient diets.[8-11] Phytase supplementation to low phosphorus diets improved body weight and significantly reduced feed intake and feed conversion, which become better with the supplementation.[12] However, there was no

effect of phytase on hemato-biochemical parameters of broilers.[13] Microbial phytase supplementation has also been shown to increase crude protein and amino acids digestibility of in broiler chickens, including lysine digestibility.[14] The aim of this study was to investigate the effect of microbial phytase on performance, economic efficiency, digestibility coefficient of some nutrients and certain related blood parameters of broiler chickens fed corn- soy bean based diets during 5 weeks of age.

3. MATERIAL AND METHODS

3.1 Bird Management, Husbandry, Experimental Design and Diets

The present study is affirmed by the Committee of the Ethics of Animal Experiments of Damanhour University. Egypt. A total of 240 one-day-old broiler chicks (Cobb 500 strain) of mixed sex were randomly allotted by weight to 6 treatment groups, with four replicates of ten birds per replicate. The birds were housed in cages for 35 days. The fluorescent lights were on for 24 h each day. Diets and water were provided *ad libitum*. Six mash diets were formulated for the experimental groups which consisting of group 1(G1) (control group) fed corn soybean based diet without phytase supplementation. group 2 (G2) fed on lower NPP by 0.1% than required; group 3 (G3) fed on diet with 0.05% lower lysine than required; 4th, 5th and 6th group (G4, G5 and G6) fed on the same diets of the previous groups respectively, but supplemented with phytase enzyme (Quantum^{TM} Blue 5 G 6-phytase (EC3.1.3.26, from *Trichodema ressei*) 5000 FTU/g, AB vista company) with inclusion rate 0.2 g/kg feed. All birds received control diet during the first week and fed the experimental diet from the second week to 5th week. Starter diet was given in the first two weeks of the study, grower diet was given at third and fourth weeks and finisher diet was given at the last week. The diets were formulated according to recommendation book of Cobb 500 (2015). The chemical analysis of the basal diet was calculated according to tables of NRC (1994).[15] Ingredient composition and calculated chemical analysis of the basal diet are presented in Table 1.

3.2. Growth measurements and nutrients digestibility: Performance parameters including body weight at 1 and 35 days of age, feed consumption, feed conversion ratio , and protein efficiency ratio were measured weekly throughout the experimental period.[16] Economical evaluation for all experimental diets was made.[17] The digestibility trial was carried out at the end of the experiment to determine apparent digestibility of crude protein, ether extract, crude fiber, calcium, phosphorus, lysine and methionine, using total collection method.[18] Five birds from each treatment were taken in separate cages, fastened overnight and received the experimental diets daily with fresh water all the times. The excreta were quantitatively collected for 3-days period during which feed consumption data were also recorded. The excreta was dried in hot air oven at 60 ^{0}C for 3 days then at 105 ^{0}C for 3 hours, then finally ground and stored until chemical analysis and nutrient digestibility.

3.3 Blood sampling and some related blood parameters: Six blood samples were collected from each group at 35th days of age for the biochemical tests, and fresh blood samples were collected in clean dry vials containing anticoagulant (0.1 ml of 4% sodium citrate solution /1 ml blood) for determination of haematological indices and differential leukocytic count. Clear sera were obtained by allowing the blood samples to coagulate at room temperature, separated by centrifugation at 3000 rpm for 10 minutes then frozen at -20 C until used. The haematological indices determined include; Erythrocyte count (RBCs**)**, leukocytic count (WBCs) using a haemocytometer and Haematocrite value (PCV) were determined.[19] Hemoglobin content (Hb) determined according to Sahli´s method.[20] Blood constants: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were determined using the appropriate formula[21] and differential leukocytic count.[22] The serum biochemical include: calcium, phosphorus, alkaline phosphatase, total protein, albumin, globulin, AST, ALT, creatinine and uric acid concentration according to[23,24,25,26,27,28,29,30] respectively.

3.4 Analytical methods: Analytical DM contents of feed samples were determined by oven-drying at 105^{o}c overnight.[31] Crude protein of feed samples determined following Kjeldahl procedure.[31] The fecal nitrogen was determined following the procedures outlined by Jakobsen et al.(1960).[32] Ash content of feed and fecal samples was determined by incineration at 550C for 6 hrs. Calcium of feed and fecal sample was determined by flame photometer,[33] Phosphorus in these samples determined by colourimeteric procedure.[34] To analyze amino acid (AA), samples were ground to pass a 0.5-mm screen; feed samples as well as bag residues were acid-hydrolyzed with 6*N* phenol-HCl for 24 h at 110^{o}C [35] and AA concentrations of the hydrolysates were determined by the isotope dilution method.[36]

Briefly, 2 mL of the hydrolysate was diluted with 3 mL of ultrapure water and 1 mL of this solution was then combined with 200 µl of a mixture of labeled AA (13C and 15N AA isotope standards; CDN Isotopes, Pointe-Claire, Quebec; Cambridge Isotope Laboratories Inc., Andover, MA), which served as an internal standard. The solution was eluted through a poly-prep chromatography column (resin 100-200 mesh H; Bio-Rad, Hercules, CA), then derivatized with *N*-(*tert*-butyl dimethylsilyl)-*N*-methyl trifluoroacetamide and dimethyl formamide 1:1 (394882, 27.0547; Sigma-Aldrich) according to the method of.[37] Amino acids were quantified using GC-MS (Hewlett-Packard Model GC6890-MS5973; Agilent Technologies, Wilmington, DE) and a mass selective detector (Hewlett-Packard, Palo Alto, CA). Lysine was analyzed separately by subjecting the samples to performic acid oxidation, followed by HCl hydrolysis;[35]

this AA were analyzed with a Biochrom 20 AA analyzer (Amersham Pharmacia Biotech, Piscataway, NJ).

3.4 Statistical analysis

Data obtained in this work were statistically analyzed for analysis of variance (ANOVA) and Duncan's multiple range tests using the SPSS programming tool (IBM SPSS. 20) to assess significant differences.

4. RESULTS

4.1 Performances of broiler chickens: The response of the Cobb broilers growth performance to phytase supplementation is presented in Table (2) .The reduction of NPP (0.1%) (0.45 vs 0.35 %) showed non significant depression of the final weight and total weight gain in addition to TPER. However significant rising in feed consumption (1.3%; $P \leq 0.05$) was found leading to inferior feed conversion ratio (4.7%; $P \leq 0.05$) when compared with the control group. In spite of insignificant difference in weight gain and PER with the lysine reduction (0.05%), it showed significant ($P \leq 0.05$) increase in feed consumption by 2.6%, with deceitful feed conversion ratio ($p \geq 0.05$) in comparison with the control. The final weight and weight gain of the group fed the basal diet were significantly ($p \geq 0.05$) similar with those fed phytase supplemented diets (G 4, G5 and G6). However, the highest BW (103.1%) was for the group fed adequate nutrient diet supplemented with phytase (G4, the on top) that had increased feed consumption (1.6%; $P \leq 0.05$) with similar FCR and PER to the control. Addition of 1000 FTU phytase /kg feed to low NPP diet (G5) non significantly increased FBW and TWG, but showed significant ($P \leq 0.05$) decrease in TFI (1.5%) and FCR (6%) with higher PER (4.8% ;$P \leq 0.05$) and economic efficiency ($P \leq 0.05$) comparing with the group fed low NPP without phytase (G2). The birds of G6 had improved weight gain with lower ($P \leq 0.05$) feed consumption and FCR 2.6%, 4.2% respectively compared to those of G3. The European production efficiency factor (EPEF) was insignificantly ($P > 0.05$) improved in phytase supplemented groups. Viability percentages were 100% in all phytase supplemented groups similarly the non supplemented groups. The current result indicated better growth performance and higher economic efficiency with phytase supplementation.

4.2 Apparent digestibility of nutrients

Crude protein, fat, crude fiber, lysine and methionine digestibility, phosphorus and calcium availability of birds at the end of the experiment as influenced by the dietary treatments are presented in Table 3. No significant effect ($P > 0.05$) of dietary phytase supplementation on fat and crude fiber digestibility was found. However insignificant improvement was observed in G5 compared to G2. Concerning protein and amino acid digestibility, the apparent digestibility of crude protein (CP) was statistically similar in different experimental groups, but numerical differences were found. The birds of G5 and G6 had numerically higher CP digestibility by 3.2% and 4.5% than those of G2 and G3 respectively. A significant ($P \leq 0.05$) improvement in lysine digestibility (7.2%) was observed with phytase supplementation to low lysine diet (G6) compared to G3. The apparent digestibility of methionine non significantly ($p \geq 0.05$) improved in all phytase supplemented diets. The highest methionine digestibility was for the group received low lysine diet with phytase (G6). Finally, lysine reduction with 1000FTU bacterial phytase /kg diet had better response to the digestibility of the examined amino acids. In the current study insignificant improvement of Ca and P availability was observed with NPP reduction (0.1%). Comparing to the control group, P availability showed a significant increase ($P \leq 0.05$) with phytase supplemented groups (G5 and G6) by 30.7%, 28.4% respectively than the control. In spite of insignificant differences among all diets in Ca availability, a numerical increase in Ca and P availability was observed in birds received low NPP diet supplemented with phytase than the non supplemented group.

4.3 Blood analysis: The result of haematology of birds as influenced by the experimental diets is shown in Table (4). NPP reduction badly affect some blood indices of the chickens, which significantly reduced the values of Hb, PCV and MCH by 32%, 31.3% and 10% respectively when compared with the control. On the other hand, PCV and MCV were significantly ($p \leq 0.05$) reduced by 21.4% and 22.5% respectively in G3 when compared with G1. Values of Hb, RBC count, PCV and MCH showed significant ($p \leq 0.05$) improvement by 51%, 39.2%, 38.8% and 9% respectively in G4 when compared with G2. Haematological values of (Heterophil, Eosinophil, Basophil, Lymphocyte, and Monocyte) were statistically similar. The statistical analysis of the obtained data from Table (5) and revealed that all diets supplemented with phytase had no significant effects on the constituents of blood serum including total protein, albumin, globulin, uric acid, and creatinine, liver enzymes Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Serum alkaline phosphatase (ALP) at 35 day of age. Concerning calcium and phosphorus levels of blood serum, the results showed birds fed low NPP diets had lower serum Ca and P than control, which improved ($p \geq 0.05$) by phytase supplementation. The lowest calcium level was in the birds receiving diet supplemented with phytase as on top. However it had the highest phosphorus level.

Table 1: Ingredient composition and calculated nutrients of the basal diet (as fed basis).

Stage / Ingredient	Starter %	Grower %	Finisher %
Yellow corn, ground	58.2	62.72	64.59
Soybean meal (CP 44%)	30.6	27.5	24.5
Corn gluten (CP 60%)	5.1	3	4
Soybean oil	2.05	2.93	3.5
Mono calcium Phosphate*	1.5	1.4	1.2
Limestone	1.4	1.31	1.2
Lysine-HCL*	0.3	0.27	0.19
DL-Methionine*	0.13	0.15	0.1
Pre-mix*	0.3	0.3	0.3
Salt (NaCl)	0.3	0.3	0.3
Antimycotoxin*	0.1	0.1	0.1
Anticoccidial drug*	0.01	0.01	0.01
Anticlostridial drug*	0.01	0.01	0.01
The calculated nutrients (%)			
ME (Kcal/kg diet)	3002	3084	3167
Crude Protein	21.82	19.53	18.92
Ether extract	4.48	5.39	6
Calcium	0.9	0.84	0.76
Av.Phosphorus	0.45	0.42	0.38
Lysine	1.32	1.19	1.05
Methionine	0.5	0.48	0.43
Therionine	0.88	0.79	0.76

* The premix used was Hy-mix produced by Misr feed additives company and composed of (per 3 kg) vitamin A 12000000 IU, vitamin D3 4000000 IU, vitamin E 60000 mg, vitamin K3 3000 mg, vitamin B1 2000 mg, vitamin B2 6500 mg, vitamin B6 5000 mg, vitamin B12 20 mg, niacin 45000 mg, biotin75 mg, folic acid 2000 mg, pantothenic acid 12000 mg, choline chloride 1000 gm manganese 100 gm, zinc 80 gm, iron 45 gm, copper 10 gm. , iodine 1gm, selenium 0.2 gm and cobalt 0.1 gm.

* Mono calcium phosphate was hi-phos 23 composed of 22.7 %phosphorus and 17 % calcium. Produced by Rotem Kimyevi madder SAN.VETIC A.S.

* Lysine was L-lysine monohydrochloride 99% pure, produced by Ajinomoto Company, Brasil.

* Methionine was Met Amino, DL methionine grade 99%, produced by Evonik Degussa Antwerpen.

* Anticlostridial feed additive was MAX-CURE produced by Probyn international inc, USA. Consisted of esterfied mannose oligosaccharides (MOS), Bacillus subtilis (C3201) and Bacillus lechniforms extract on silica dioxide as carrier.

*Anticoccidial drug was Maduramix produced by Delta vet trading company.

Table 2: Effect of dietary phytase supplementation on broiler performance at 35 day of age.

Item/Group	Phytase supplementation					
	Without			With		
	G1	G2	G3	G4	G5	G6
[1]IBW (g)	43.39±0.58	42.57±0.68	42.72±0.6	42.72±0.62	42.77±0.85	41.83±0.68
[2]FBW (g)	1673.57±15.27^{ab}	1626.3±19.44^{b}	1671.09±20.55^{ab}	1725.4±18.21^{a}	1678.24±18.16^{ab}	1695.36±21.11^{a}
[3]TWG(g/bird/week)	1629.86±15^{ab}	1581.81±19.58^{b}	1628.85±20.14^{ab}	1683.5±17.97^{a}	1633.91±17.98^{ab}	1653.79±20.58^{a}
[4]TFI(g/bird/week)	2752.5±10.17^{c}	2788.23±12.45^{b}	2824.54±6.51^{a}	2796.28±8.16^{b}	2744.2±14.33^{c}	2753.4±6.73^{c}
[5]TFC	1.69±0.02^{bc}	1.77±0.02^{a}	1.74±0.02^{ab}	1.67±0.02^{c}	1.68±0.02^{bc}	1.67±0.02^{c}
[6]TPER	3.17±0.03^{ab}	3.06±0.04^{b}	3.11±0.04^{ab}	3.21±0.04^{a}	3.22±0.03^{a}	3.23±0.04^{a}
[7]EPEF	282.5±3.98^{abc}	268.36±4.63^{c}	276.15±5.19^{bc}	295.99±3.16^{a}	283.25±5.95^{abc}	290.7±3.32 ^{ab}
Eco. Efficiency%	39.31±0.89^{a}	34.49±1.78^{b}	38.19±2.15^{ab}	42.75±0.85^{a}	41±1.73^{a}	41.5±0.87^{a}

Statistical analysis was done ANOVA followed by Duncan's multiple range tests

Values are given as mean ±Standard error (SE) of four replicates, each replicate contained 10 birds.

Any two means for a performance parameter bearing different superscript letters in a row are significantly ($P < 0.05$) different from each other. Therefore, ``aº and ``bº letters in the same row are significantly different, while ``abº and ``bº are non-significantly different.

[1]Initial body weight, [2]final body weight, [3]total weight gain, [4]total feed intake, [5]total feed conversion ratio, [6]total Protein efficiency ratio

[7]European production efficiency factor= [(viability % × body weight Kg / age (d) × FCR)] ×100.

G1= basal diet; G2= low NPP diet; G3 low lysine diet; G4, G5 and G6 were fed on the same diets of the previous groups supplemented with phytase enzyme.

Table 3: Effect of dietary phytase supplementation on availability (%) of calcium and phosphorus and digestibility (%) of some nutrients in broiler chickens.

Item (%)/ Group	Phytase supplementation					
	Without			With		
	G1	G2	G3	G4	G5	G6
Crude protein	87±1	83.33±3.84	82±3	81.5±2.5	86±1	85.67±2.96
Fat	87±1.73	86±1.73	85.67±0.33	85.67±1.2	88±1.15	85.67±0.67
Crude fiber	46.33±3.48	33.67±4.67	42±9.81	33.33±3.33	41.33±2.33	39.33±4.26
Lysine	87.67±0.88^{ab}	88±2.65^{ab}	83±2.31^{b}	86.67±0.67^{ab}	92±1.15^{a}	89±1.73^{a}
Methionine	87.33±0.67	83±6.43	84±3.61	89±2.31	87.67±3.67	90±1.73
Phosphorus	58.67±3.93^{b}	69.67±3.38^{ab}	67.67±2.85^{ab}	67.33±4.18^{ab}	76.67±2.85^{a}	75.33±3.18^{a}
Calcium	64.67±1.2	70.33±3.18	67.67±4.7	68±1	74.33±2.85	65.33±3.48

Mean values with different letters in the same row differ significantly at $P<0.05$. Values are expressed as means ±standard error.

G1= basal diet; G2= low NPP diet; G3 low lysine diet; G4, G5 and G6 were fed on the same diets of the previous groups supplemented with phytase enzyme.

Table 4: Effect of dietary phytase supplementation on haematological indices of 35 day old broilers

Item/Group	Phytase supplementation					
	Without			With		
	G1	G2	G3	G4	G5	G6
Hb(g/100ml)	9.38±0.69[a]	7.1±0.42[b]	9.25±0.66[a]	9.38±0.75[a]	10.75±0.6[a]	10.5±0.17[a]
RBC(106/cmm3)	3.29±0.29[ab]	2.73±0.1[b]	3.27±0.22[ab]	3.31±0.21[ab]	3.8±0.25[a]	3.73±0.05[a]
PCV%	44±1.08[a]	33.5±2.02[c]	36.25±2.9[bc]	42±2.8[ab]	46.5±1.71[a]	41±0.71[ab]
MCV	135.93±8.68[a]	123.05±6.2[ab]	110.93±3.1[b]	127.4±7.66[ab]	123.03±3.39[ab]	110.1±1.69[b]
MCH (Ug)	28.6±0.54[a]	26±0.8[b]	28.33±0.43[a]	28.2±0.59[a]	28.35±0.41[a]	28.18±0.5[a]
MCHC (%)	21.23±1.11[b]	21.25±0.89[b]	25.6±0.82[a]	22.4±1.45[b]	23.08±0.54[ab]	25.63±0.13[a]
WBC(103/cmm3)	21550±1524.52[a]	22700±2547.88[a]	26400±2986.08[a]	25975±3837.83[a]	23800±1844.36[a]	22700±1503.88[a]
PLT*1000	266±19.65[ab]	274.75±27.87[ab]	300.75±6.43[a]	244.5±14.03[ab]	235±16.73[b]	225.75±15.85[b]
Lymphocytes (%)	34.25±2.69a	40±1.47[a]	38.5±1.19[a]	38.5±1.32[a]	35.25±2.72[a]	37.5±2.5[a]
Monocytes (%)	4.75±1.25a	4.25±0.85[a]	4±1.41[a]	4.5±1.26[a]	3.25±0.75[a]	4.75±1.25[a]
Eosinophils, (%)	1.25±0.25a	1±0.41[a]	2±0.41[a]	1±0.41[a]	1.25±0.25[a]	1±0.41[a]
Basophils, (%)	0±0	0±0	0±0	0±0	0±0	0±0

Means with different superscript in the same column differ significantly ($p<0.05$).

Hb- haemoglobin; RBC- Red blood cells; PCV- Packed cell volume; MCV- Mean cell volume; MCH- mean cell haemoglobin; MCHC- mean cell haemoglobin concentration. WBC's=white blood cell.

G1= basal diet; G2= low NPP diet; G3 low lysine diet; G4, G5 and G6 were fed on the same diets of the previous groups supplemented with phytase enzyme.

Table 5: Effect of dietary phytase supplementation on serum metabolites of 35 day old broilers

Item /Group	Phytase supplementation					
	Without			With		
	G1	G2	G3	G4	G5	G6
T. protein(g/dl)	4.5±0.33[a]	4.28±0.18[a]	4.89±0.26[a]	4.83±0.66[a]	5.04±0.44[a]	4.77±0.38[a]
albumin(g/dl)	2.82±0.12[a]	2.96±0.17[a]	3.27±0.21[a]	2.95±0.22[a]	3.42±0.2[a]	3.06±0.16[a]
globulin(g/dl)	1.68±0.25[a]	1.32±0.26[a]	1.62±0.31[a]	1.88±0.74[a]	1.63±0.56[a]	1.71±0.43[a]
Calcium (mg/dl)	11.88±1.15[a]	11.08±0.74[a]	12.46±0.88[a]	10.86±0.74[a]	12.29±0.34[a]	12.37±0.35[a]
Phosphorus (mg/dl)	4.83±0.32[ab]	3.93±0.31[b]	4.55±0.4[ab]	5.76±0.57[a]	4.73±0.32[ab]	4.27±0.4[b]
Urea(mg/dl)	27.57±1.22[a]	26.79±0.86[a]	26.12±1.27[a]	26.63±0.11[a]	27.63±1.44[a]	27.58±2.34[a]
Creatinine	0.39±0.1[a]	0.41±0.03[a]	0.42±0.05[a]	0.28±0.13[a]	0.4±0.12[a]	0.44±0.06[a]
[1]ALT	12.87±0.74[a]	10.46±0.4[a]	11.1±0.86[a]	12.8±1.8[a]	12.53±0.54[a]	11.56±0.87[a]
[2]AST	32.18±2.36[a]	33.44±2[a]	32.82±4.74[a]	33.05±8.05[a]	33.1±3.18[a]	28.56±3.56[a]
[3]ALP (iu/l)	412.39±22.95[a]	384.33±40.49[a]	464.55±74.95[a]	459.32±3.59[a]	367.48±28.13[a]	466.28±52.28[a]

[3] SEMs bearing different superscript letters are significantly (P <0.05) different from the other values within the same column.

G1= basal diet; G2= low NPP diet; G3 low lysine diet; G4, G5 and G6 were fed on the same diets of the previous groups supplemented with phytase enzyme

Abbreviations

[1]Alanine aminotransferase

[2]Aspartate aminotransferase

[3]Alkaline phosphatase.

5. DISCUSSION

5.1 Performance measurements: The lower weight gain with higher feed consumption and feed conversion ratio indicated the adverse effect of reducing NPP level on broiler performance. The growth depression resulted from phosphorus reduction associated with the important functions of this mineral in the body as it performing important roles in energy regulation, protein and amino acid synthesis and fat transportation in the body.[38] Increasing feed consumption of birds fed phosphorus deficient diets may be a result of increasing dietary Ca: P ratio that stimulating higher appetite,[39] which supported by the finding of Shaw et al (2010) who reported an increase in FI ($P < 0.001$) in broilers fed low-NPP (0.35 %) corn-soybean meal diets in the grower period.[40] The chicks fed diet containing lower NPP (0.07%) levels had significantly higher feed conversion ratio during 21-40d.[41] In contrast, low non-phytate phosphorus (NPP) mash diets (2.3 g/kg and1.5 g/kg) in the grower phase (d 22–42) had no effect on FI, but depressed feed efficiency.[42] The final live weight and total weight gain of birds received enzyme incorporated diets were insignificantly greater than the birds fed diet without enzyme incorporation however the similarity to the control. The improvement in weight and weight gain of adequate P diet supplemented with phytase may be related to the extra-phosphoric 'effect of the enzyme. Where the digestion and disappearance of both protein and starch were increased by phytase addition.[43] The increased feed intake and the release of nutrient from the phytate-mineral complex may be the causes of increased weight gain of the birds fed the on top diet.[3,44] The results are in harmony with the finding of Emami et al(2013) who claimed that, FBW of 28 day old broilers fed low NPP (0.1 g/kg) supplemented with phytase, non significantly differ from control,[45] and confirmed previous finding that, phytase supplementation to corn soy bean based diet containing available phosphorus (0.19%) for six weeks had no significant effect on FBW.[13] Also, addition of phytase to adequate Ca and NPP diets non significantly increased BW of broilers fed from 1 to 25 d of age,[46] higher levels of phytase were required to maximize AA, energy, and growth responses in broilers. Moreover, phytase supplementation to adequate P finisher broiler diet (21- to 38-d-old) non significantly improved body weight gain.[47] Reduction of feed consumption resulted from phytase supplementation to low NPP diet may attributed to the satisfaction of the chicks nutrients' requirement with less amount of diet as a result of increasing digestibility of proteins, lipids and carbohydrates, or enhancement in the metabolism of available energy.[48,49] On the other hand, a significant ($p \leq 0.05$) increase in feed consumption of broilers fed corn-soybean meal-barley based diet with low Av P level (70% of required) supplemented with phytase when compared with low available phosphorus diet without phytase.[50] However, phytase supplementation to adequate nutrient diet (G4) significantly increased feed intake which supported by previous reports; as the inclusion of 600 phytase units/kg of diet increased the intestinal transit time in broilers fed nutritionally adequate diet resulting in higher feed consumption and weight gain.[48,51] Improvement of feed conversion ratio of phytase supplemented groups (G5, G6) which related to the lower feed intake with better growth may be due to some factors,[52] liberation of the minerals from the phytate mineral complex; Utilization of inositol (final product of the dephosphorylation of the phytic acid) by animals; Increase in the digestibility of the starch and increase in protein availability. In addition to the indirect effect on the available energy for the birds where the enzyme interfered the reaction of saponification between the lipids and the minerals of the phytate mineral complex.[53] In the current study, feed efficiency of G4 was not affected by phytase supplementation due to simultaneous increases in weight gain and feed consumption.[54]

5.2 Nutrient digestibility: The improvement of nitrogen and amino acids (lysine and methionine) digestibility in response to phytase supplementation has been attributed to the liberation of proteins and amino acids when phytate is hydrolyzed by phytase. Released proteins and amino acids are then available for digestion or absorption.[55] Moreover, phytate promotes the transition of Na+ into the small-intestinal lumen and this suggests that phytate may interfere with glucose and amino acid absorption by compromising Na+-dependent transport systems and the activity of the Na pump (Na+-K+-ATPase). While the phytate was degraded to more innocuous ester by the exogenous phytase, the negative influences were attenuated, finally increasing AA digestibility occurred[56] It was found that supplementation of 500 FTU phytase/ kg to P adequate maize based diet significantly increased digestibility coefficients of lysine and protein digestibility.[43] Who interpreted his finding as phytate interferes with not only protein digestion but also impede the absorption of amino acids via Na-dependent transport systems.[57] At the end of the growing period, chicks fed low NPP diet had insignificantly higher Ca and P availability than in the control as a result of partial adaptation to phosphorus deficiency. This ability was achieved by increased ileal absorption of P and Ca, increased ileal phytate P disappearance[58] or increasing the intestinal phytase activity in response to low dietary P.[59,60] The significant responses in P digestibility to phytase supplementation pursuant to the hydrolysis of dietary phytate and the liberation of phytate-bound P were anticipated outcomes.[5,9,47,61,62] In addition, inclusion of microbial phytase significantly improved the P retention coefficient in broilers fed the Low NPP diet.[9,47,61] Concerning to Ca availability, no significant responses to 1000 FTU/kg phytase for digestibility coefficients or retention of calcium were found,[9,43,63] but Ca retention significantly increased following the inclusion of phytase in diets with reduced nutrient specification.[9]

5.3 Blood parameters: The improvement in Hb , RBC and PCV of birds received the phytase supplemented diets on account of increasing nutrient available for hematopoiesis and erythropoiesis resulting from the release of nutrient from the phytate–mineral complex.[48] The results about the blood indices and differential leukocytic count are in line with the report of Shehab et al.(2012)[64] who claimed that phytase supplementation influenced worthily on the haematological constituent of Japanese quail diet does not adversely affect the birds. Also agree with the researches in ducklings[65] and broilers,[13,66] which stated that, RBC and haemoglobin concentration significantly raised by phytase inclusion while total leucocyte count and differential leukocytic count didn't affected. In the current study, improvement of serum Ca and P levels in the birds of G5 was supported by the report of Ahmed et al.(2018) who found the same result in layers.[67] Finally phytase supplementation had no deleterious effect on biochemical constituents of serum and liver or kidney functions of broiler chicks. These findings are accord with the results obtained in Japanese quails[64] and in broilers[13,68] that, phytase supplementation had no adverse effect on biochemical constituents of blood.

6. CONCLUSION

It can be concluded that the supplementation of adequate nutrients diet or diets containing 1 g/kg of available P or 0.5 g/kg of lysine with exogenous phytase can improve the performance and the nutrient digestibility of the chicks during 5 weeks of age with higher economic efficiency. Further that, phytase supplementation had no adverse effect on haematological indices and biochemical constituents of serum, liver and kidney functions, which indicated that bacterial phytase addition to adequate and low NPP diets didn't affect the broiler chickens health.

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