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SYNERGISTIC EFFECT OF DIETARY PHYTASE AND VITAMIN-D SUPPLEMENTATION ON PERFORMANCE, BLOOD INDICES AND BONE MINERALIZATION IN BROILER CHICKENS

By

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ABSTRACT

A feeding trial was conducted to evaluate the impact of dietary thermo - stable phytase and new analogue of vitamin D3 (1α-OH D3) supplementation on growth performance, blood parameters, and bone mineralization in broiler chicks. Four hundred and eighty, unsexed, 1-d-old Ross 308 broilers were distributed in a randomized experimental design, with four groups, 3 replicates each and 120 birds per experimental unit. The whole experimental period was 33 days. The four dietary groups were : Group A served as positive control and fed diet containing an adequate level of Av. P (available phosphorus) and calcium, while, Group B fed on diet low in Av.P and calcium (negative control), However, Group C, fed on diet containing the same level of Av.P and calcium as in the negative control one but supplemented with Thermo stable-phytase (500FTU/kg diet), while Group D was fed on diet containing the same level of Av.P and calcium as in the negative control but supplemented with 500FTU/kg phytase plus 5 ppb of 1α-OH D3. The combination of phytase and 1α-OH D3 were significantly improved ($P < 0.05$) broiler chicken body weight, feed intake, feed conversion rate, bone ash, and serum Ca and P compared with those fed the negative and positive control groups.

KeyWords:

Broiler chicken, phytase, 1α-OH D3, Growth performance, Bone quality.

INTRODUCTION

Poultry diets are mainly plant based, and composed of maize and soya bean meal. About 70% of phosphorus in plant materials exists as Phytic acid **(Rezaei *et al.,* 2007)**. Phytic acid is a

strong acid and creates complexes with multivalent cations such as (Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}) and nutrients (proteins, starch and lipids) forming insoluble salts rendering them unavailable for absorption **(Afinah *et al.*, 2010)**. Phytate disturbs the activity of digestive enzymes. For these properties, phytate is considered as anti-nutritional factor. Furthermore, the monogastric animals like poultry birds are unable to utilize this phytate phosphorus, as they lack endogenous phytase that lead to excessive excretion of phosphorous in feces which cause environmental pollution **(Yu *et al*, 2004)**. This lead to addition of inorganic chemicals such as MCP (monocalcium phosphate) and DCP (Diclcaium phosphate) to poultry diets in order to meet up with the phosphorus requirements of poultry. **Hasan *et al.*, (2012)** explained that the dietary addition of feed phosphates not only increases the feed and production costs, but may also lead to an increase of soluble P in the litter resulting in the potential for water contamination from excess P in the soil. Therefore, it is important to increase the bioavailability of P in the gastrointestinal tract of monogastric animals. One of the most practical and effective methods to achieve this objective is the addition of exogenous phytase enzyme and Vitamin D. During last decade, supplementation of phytase enzyme in poultry diets has used to release phosphates from phytate making it available for absorption and utilization **(Greiner and Konietzny, 2011)**. Phytase enzyme is very effective when Ca and non-phytate phosphorous (NPP) concentration is reduced in poultry rations and phytase increases the availability of bounded Ca and P **(Watson *et al.*, 2006)**. For these reasons, exogenous phytase enzyme is supplemented in poultry rations to reduce loss in terms of economic gain and phosphorous excretion (**Onyango *et al*., 2005)**. Moreover, absorption of PP (phytate phosphorus) and Ca is increased by vitamin D supplementation which stimulates the hydrolysis of PP **(Garcia *et al.*, 2013)**.Vitamin D supplementation decreases the incidence of bone disorders because vitamin D is involved in many physiological processes, including the absorption of calcium and phosphorus, bone mineralization and mobilization **(Kasim *et al*., 2006)**. Better results were obtained by using combination of phytase and vitamin D3 in diets of broiler chicks fed low level of Ca and P **(Han *et al*., 2009)**. The main purpose of the study was to study the synergistic effects of dietary phytase and vitamin D supplementation on the growth performance, bone characteristics and blood parameters in broiler chicken.

MATERIAL AND METHODS

The Experiment was conducted at the Poultry and Animal Research Center, Faculty of

Veterinary Medicine, Cairo University, after approval from the ethical committee of the university. A total of 480 (day-old) broiler chicks were weighed and divided into 4 dietary groups (A, B, C and D) in a completely randomized design. Each group consisted of 120 broiler chicks with 3 replicates having 40 chicks in each (4×3×40=480 birds). Four diets were formulated according to the Manual standards of broiler Ross 308 breed. Birds were fed starter, grower and finisher diets. The whole experimental period was 33 days. Group A was fed the basal diet (Positive Control) and Group B was fed diet which formulated with low phosphorous and calcium diet (contains 70 % of the recommended levels of Ca and P) and without phytase supplementation (Negative control). Group C was fed a negative control diet which supplemented with 500 FTU/kg diet. While Group D was fed a negative control diet which supplemented with 500 FTU/kg diet in addition to 5 ppb vitamin D3. The feed and fresh water were offered ad libitum throughout the experiment. The ingredient and chemical composition of diets are shown in (Table 1). Weekly feed intake was recorded. Chicks were weighed at the 1stday of the experiment and at the end of each week regularly to estimate weekly body weight gain. Total live weight gain was recorded at end of the trial. Data recorded for weight gain and feed intake were used to calculate weekly feed conversion rate (FCR). FCR= Feed intake (g) / Weight gain (g). Three birds from each subgroup were randomly selected and slaughtered at the age of 33 days for dressing percentage calculation. The dressing percentage was determined by following formula: Dressing percentage= dressed weight (g) / live weight (g) at the age of 33 days, Samples of toe/tibia of 9 slaughtered birds were collected. Middle toe was taken by severing joint between 2nd and 3rd tarsal bones. Toes were cleaned, nails and skin were removed. The toes then were put in hot air oven for 24 hours at 100°C for drying and then weighed. After drying, weighed samples were put in muffle furnace for 8 hours at 550°C for ash estimation which expressed as dry weight of toes samples **Sheideler (2000)**. Similar procedure was adopted for tibia ash. Blood samples were collected on sterile tubes contained no anticoagulant and serum was then separated by centrifugation at 1500 r.p.m for 10 minutes then kept at -18^{0}c Samples were analyzed for Ca and P by using spectrophotometer and diagnostic kits. Data obtained were statistically analyzed using SAS Software and comparison of means was done by using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

Growth performance parameters and carcass characteristics:

The impact of dietary fortification of both thermo-stable phytase and new analogue of vitamin D3 (1α-OH D3) on bird's growth performance (Body weight gain, feed intake and FCR) and Carcass characteristics (Carcass weight and Dressing %) are showed in (Table 2). The results revealed that, there was significant ($P<0.05$) improvement in growth performance in group D (the negative control diet supplemented with both phytase and vitamin D3) except for feed intake. Dietary supplementation of 500 FTU/kg phytase in group C significantly ($p<0.05$) improved Feed intake (FI). Carcass weight and Dressing percentage were significantly ($P<0.05$) improved in chicks fed the negative control diet supplemented with both phytase and vitamin D3 than other groups. These findings confirm most of the current available studies reported by **(Onyango, 2005; Bingol *et al*., 2009 and Bin Baraik, 2010)** whom stated that, the improvement in growth performance was due to phytase supplementation. In the same manner, these results confirm the findings of **Aureli *et al*., (2011)** who found that weight gain and FCR of broilers fed low P diets containing phytase were comparable. Moreover, our findings are in agreement with **Shaw *et al.,* (2011)** who concluded that phytase supplementation in P deficient diets improved feed intake and lead to better nutrient utilization which ultimately resulted in better performance. The positive impact of dietary fortification of both thermo-stable phytase and 1α-OH D3 was noticed in birds can be explained on the basis that exogenous supplemented phytase can lead to improvement in phytin phosphorus utilization in the chicks' intestine, and hydrolysis of phytin improves the overall nutritive value of the diet through better utilization of protein, essential amino acids, trace elements, energy and carbohydrates for bird growth. In addition, similar results were observed by many researchers **Ghasemi, (2006) and Bin- Baraik, (2010)** who concluded that FCR was better with addition of phytase and vitamin D. Also, our results are in agreement with **Han *et al*., (2016)** who reported that body weight and feed conversion ratio was increased due to addition of Vitamin D. Additionally **Vieira *et al*., (2015)** reported that, supplementation of Citrobracter braakii phytase in low non-phytate phosphorous (NPP) corn-soya diet had significant improvements in FCR of broiler. Our results are similar with the results of **Brito *et al*., (2010)** who studied the effect of phytase and vitamin D supplementation in diets low in Ca and P contents on carcass characteristics. They concluded

that carcass weight was increased due to phytase and vitamin D supplementation. Also, **Han *et al.*, (2015)** studied the effect of vitamin D in diet with different levels of P content on broiler carcass characteristics; they concluded that carcass weight was increased due to vitamin D supplementation. Results are also similar with the results of **(Pillai *et al*., 2006 and Angel *et al*., 2007)**. They found that carcass weight was increased due to phytase and vitamin D supplementation. Also our results agree with **Bingol, (2009)** who reported that, the dressing percentage was increased significantly with the use of phytase and vitamin D.

Serum biochemical indices and bone parameters:

Data concerning serum biochemical indices and bone ash percentage are shown in (Table 3). The data revealed that Serum levels of Ca and P ($P<0.05$) in addition to toe and tibia ash were significantly increased in group D than other groups. Our findings are supported with **Ravindran *et al*., (2006**) who stated that supplementation of phytase and vitamin D had significant effect on calcium absorption. In addition, the results of the experiment are in accordance with results observed by **Kim *et al*., (2011)** who concluded that phytase increased plasma phosphorous contents in broilers. Also, our data are in agree with **Onyango *et al.*, (2005)** who conducted an experiment to evaluate the efficiency of phytase and vitamin D in the utilization of calcium and PP in broilers fed diets containing soybean and corn which resulted in increased calcium and phosphorous utilization. They concluded that phytase increased phosphorous contents in plasma. Our findings are in agreements with findings of **Han *et al*., (2009)** who reported that phytase and vitamin D increased tibia ash percentage. Similarly **Tang *et al*., (2012)** reported that addition of phytase and vitamin D significantly increased toe and tibia ash percentage. Our results are supported with **Han *et al*., (2015)** who performed an experiment to evaluate tibias strength with tibia ash by supplementation of vitamin D in feed. He found that, that vitamin D supplementation in diets increased tibia ash. Bone strength depends on Ca and P in tibia ash.

CONCLUSION

From this experiment, we can conclude that, the use of phytase enzyme in broiler diets at 500 FTU/kg of feed along with vitamin D supplementation at 5ppb resulted in better growth performance and carcass weight, improved toe/tibia ash and Ca/P levels in the blood of broiler chicken.

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Table (1): Physical and chemical composition of the basal diet.

Ingredients	Starter (1-11)	Grower (12-24) day	Finisher (25-33)
Yellow corn	56.33	59.36	63.45
Corn gluten meal 60%	4.00	3.00	2.5
Soybean meal 47%	35.00	32.60	28.20
Soy oil	____	0.8	1.8
Monocalcium phosphate	1.55	1.30	1.2
Limestone	1.80	1.70	1.6
Common salt	0.40	0.40	0.40
DL-Methionine	0.14	0.12	0.12
L-Lysine	0.17	0.11	0.12
L-Threoinine	0.05	0.05	0.05
Toxin binder	0.10	0.10	0.10
Lysoforte	0.025	0.025	0.025
Betaine	0.075	0.075	0.075
Galpro protect	0.05	0.05	0.05
Econase XT	0.01	0.01	0.01
Broiler premix	0.30	0.30	0.30
chemical analysis			
ME(kcal/kg)	3025.86	3100.83	3200.24
Crude protein%	23.05	21.53	19.55
Crude fat%	2.54	2.60	2.68
Crude fiber%	2.33	2.29	2.22
Calcium%	1.02	0.93	0.87
Available phosphorus%	0.5	0.44	0.41

***per Kg premix: 1200000 IU vitA,350000 IU vit.D3,4000 mg vit.E,250mg vit.B1,800 mg vit.B2,600 mg vit.B6,3.2mg vit.B12, 450 mg vit.K3,4.5g nicotinic acid,1.5g Ca pantothenate,120 mg folic acid, 5mg biotin, 55 mg choline chloride,3g Fe,2 g Cu, 10 g Mn, 8 g Zn,120 mg I, 40 mg Co.**

Table (2): The effect of Dietary supplementation of phytase and vitamin D on growth performance and carcass traits in broiler chicken.

Groups	Live weight gain(g)	Feed intake (g)	FCR	Carcass weight (g)	Dressing %
A	2230±3.46^{b}	3570.50±186.20^{bc}	1.59±0.08^{b}	1850±21.79^{c}	75.64±0.51^{b}
B	2100±2.31^{c}	3483.50±76.50^{d}	1.66±0.09^{a}	1780±55.68^{d}	74.00±0.21^{c}
C	2280±35.80^{bc}	3680.5±67.55^{a}	1.62±0.01^{a}	1880 ±76.71^{bc}	75.00± 0.80^{bc}
D	2350±34.64^{a}	3500.5±40.13^{c}	1.48±0.01^{c}	1996±50.29^{a}	76.58±0.65^{a}

^{a,b,c,d} **Values with different superscripts in a column differ significantly (P<0.05).**

Table (3): The effect of Dietary supplementation of phytase and vitamin D on the serum minerals and bone ash in broiler chicken (0-5 weeks).

Groups	Ca (mg/dL)	P(mg/dL)	Toe ash %	Tibia ash (%)
A	9.42±0.55^{bc}	6.5±0.58^{b}	13.1±0.12^{bc}	41.84± 1.17^{b}
B	8.23±0.45^{c}	5.02±0.84^{c}	10.5±0.51^{d}	38.68±1.7^{d}
C	10.74 ±0.07^{b}	6.93±0.12^{b}	13.4±1.84^{b}	41.15±1.9^{bc}
D	14.5±0.54^{a}	10.09±1.26^{a}	15.68 ±1.5^{a}	46.5±0.88^{a}

^{a,b,c,d} **Values with different superscripts in a column differ significantly (P<0.05) .**