



## Short Communication

# Effect of Vitamin D and Phytase on Growth, Blood Mineral Level and Slaughter Parameters of Broilers

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## ABSTRACT

Current study investigated the dietary effect of vitamin D and phytase on performance, blood mineral level and slaughter parameters in broiler chicken. Diet A was without vitamin D and phytase supplementation (control). Diets B, C, D, E, F and G were supplemented with phytase at the rate of 400 FTU/kg, 800 FTU/kg, vitamin D 4000 IU/kg, 8000 IU/kg, phytase 400 FTU/kg + vitamin D 4000 IU/kg and phytase 800 FTU/kg + vitamin D 8000 IU/kg of feed, respectively. Each diet was fed to 30 broiler birds from post hatching to day 35. Feed intake was higher ( $P < 0.05$ ) in birds offered diet B and D, while the weight gain and feed conversion ratio were better ( $P < 0.05$ ) in those birds offered diet F. Serum calcium contents were more ( $P < 0.05$ ) in groups D and G, while higher ( $P < 0.05$ ) phosphorous contents were found in those birds fed diet C and G. The highest ( $P < 0.05$ ) dressing percentage was observed in group F, and highest ( $P < 0.05$ ) tibial ash contents were found in groups F and G. Keel bone length was improved ( $P < 0.05$ ) in those birds receiving ration F and G, while highest ( $P < 0.05$ ) shank length was observed in those birds receiving diet G. Based on these results, it was concluded that phytase and vitamin D, at the level of 400 FTU/kg and 4000 IU/kg, cumulatively improved the weight gain, feed conversion ratio, dressing percentage, keel bone length and bone mineralization.

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## Authors' Contribution

MS and FA designed the study. AR carried out the experiments. AAK and MS helped in analysis and proof reading, MA and AUH wrote the manuscript.

## Key words

Vitamin D, Phytase, Growth, Minerals, Chicken

Minerals play an important role in animal production, reproduction, health and growth. Calcium and phosphorous are vital for physiological processes, body functions, bones, muscles, tissue formation and nerve transmission (Yatoo *et al.*, 2013). Phosphorous (80% in bones and 20% in body fluids) maintains body functions. About 70% phytate phosphorous is present in ingredients of plant source (Rezaei *et al.*, 2007).

The action of digestive enzymes is disturbed by phytate. Phytase is used to overcome these problems. It reduces the economic losses and as well as environmental phosphorous excretion (Dilger *et al.*, 2004). Phytase enzyme hydrolyses phytate molecule and releases phosphorous present in bound form. Poultry cannot utilize the phytate phosphorous due to low production of intestinal phytase in poultry (Abbasi *et al.*, 2019).

Calcium is major part of skeletal tissues and bones. Its deficiency leads to rickets, osteoporosis, osteomalacia and osteodyschondroplasia. Bones are shorter in length

and have reduced mass and minerals (Onyango *et al.*, 2005). A large quantity of Ca present in diet interferes with the availability of many minerals, such as P, Mg, Mn, and Zn. Feed intake was disturbed when high levels of Ca are added in poultry diet. Vitamin D regulated the dietary calcium which is converted into 1, 25-dihydroxy-vitamin D in liver and kidney. Calcium binding proteins increase calcium absorption from the intestine (Zyla *et al.*, 2000).

Vitamin D<sub>3</sub> is produced in animal body upon irradiation of 7-dehydro-cholesterol in skin which is more effective than vitamin D<sub>2</sub> plant steroid ergosterol (Saylor *et al.*, 2005). Vitamin D increases the absorption of Ca by increasing the permeability of cells of mucosa to minerals and enhances their deposition in bones. Vitamin D induces formation of osteocalcin (bone protein) (NRC, 1994). Due to deficiency of vitamin D, growing birds develop hypocalcaemia, stunts skeletal development of long bones and weakened shafts. Black coloration of feathers is due to vitamin D deficiency (Panda *et al.*, 2007). Plasma levels of Ca and P were increased when broilers diets were supplemented with vitamin D and phytase (Edwards, 2002). Broilers fed low levels of Ca and P gave significant results when their diets were supplemented with combination of

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vitamin D<sub>3</sub> and phytase (Angel *et al.*, 2007).

There is paucity of data on cumulative effect of vitamin D and phytase. So, this research has been planned to study the mutual effect of vitamin D and phytase on broiler growth, slaughter parameters and calcium, phosphorus absorption in blood.

#### Material and methods

The trial was conducted at Raja Muhammad Akram Research Center, University of Agriculture, Faisalabad. The duration of trial was 35 days. All the experimental procedures were approved by the university ethics committee, University of Agriculture, Faisalabad.

Two hundred and ten day-old broiler chicks were weighed on very first day of experiment and were divided into 7 dietary treatment groups under completely randomized design. A basal ration (CP 21% and M.E 2950 kcal/kg) was formulated (NRC, 1994). Diet A was without vitamin D and phytase supplementation (control). The control diet A has maize grain 61.97%, canola meal 6.55%, soybean meal 24.54%, corn gluten meal (60%) 2.23%, soya oil 1%, di calcium phosphate 1.57%, limestone 1.33%, vitamin premix 5%, l-lysine HCl 0.15%, DL-methionine 0.1%, L-threonine 0.06%. Diets B, C, D, E, F and G were supplemented with phytase at the rate of 400 FTU/kg, 800 FTU/kg, vitamin D 4000 IU/kg, 8000 IU/kg, phytase 400 FTU/kg + vitamin D 4000 IU/kg and phytase 800 FTU/kg + vitamin D 8000 IU/kg of feed, respectively. FTU is the quantity of enzyme that releases 1 μ mol orthophosphate/min from 0.0051 mol/L sodium phytate at pH 5.5 and a temperature of 37 °C (1 FTU=0.00024 g/Kg, IU=0.025 μg/kg). The basic dosage levels of phytase and vitamin D were picked from previous studies (Angel *et al.*, 2007), however, for final selection particularly for combined use these were chosen after a preliminary trial (unpublished data). The control diet (A) had maize grain 61.97%, canola meal 6.55%, soybean meal 24.54%, corn gluten (60%) 2.23%, soya oil 1%, di calcium phosphate 1.57%, limestone 1.33%, vitamin premix 0.5% l. lysine HCl 0.15%, DL. Methionine 0.1%, L- threonine 0.06%. FTU is the quantity of enzyme that releases 1 μ mol orthophosphate/min from 0.0051 mol/L sodium phytate at pH 5.5 and a temperate of 37 °C (1 FTU=0.00024 g/kg, IU= 0.025 μg/kg). The phytase and vitamin D were obtained from commercial source (Sigma-Aldrich, USA). Each group consisted of 30 broiler chicks with 3 replicates of 10 chicks each (7×3×10=210 birds). The birds were provided florescent light with standard conditions of pH, humidity and temperature recommended for broilers. Feed was offered *ad libitum* and there was free access to fresh and clean water throughout the experiment.

Chicks were weighed on 1<sup>st</sup> day of their arrival. Then, they were weighed weekly to estimate weekly body weight

gain. The weekly average weight gain was calculated by subtracting the previously week weight gain. Total body weight gain was recorded at end of the trial.

A weighed quantity of feed was offered to each group throughout the week and feed refused was weighed at the end of each week to estimate weekly feed consumption. Feed consumption of each group was calculated by subtracting feed orts from each feeder to the feed offered.

Data recorded, regarding weight gain and feed intake were used to calculate weekly feed conversion ratio. The FCR was calculated by

$$\text{Feed Conversion Ratio(FCR)} = \frac{\text{Feed Consumed(g)}}{\text{Weight Gain(g)}}$$

Two birds were randomly selected at the end of trial and slaughtered for dressing percentage. The dressing percentage was determined by using following formula:

$$\text{Dressing Percentage} = \frac{\text{Dressed Weight (g)}}{\text{Live Weight (g)}} \times 100$$

At the end of experiment, 2 birds from each replicate were randomly taken to estimate shank length and keel bone length. Shank length and keel bone length was measured by using measuring tape. Similarly, samples of toe / tibia were collected at the end of experiment. Tibia bones were cleaned and skin was removed. The bones were then kept in hot air oven for 24 h at 65°C for drying and then weighed. After drying, weighed samples were kept in muffle furnace for 4 h at 650°C for ash estimation which expressed as dry weight of tibia bone.

Blood samples of slaughtered birds (2/replicate) were collected in syringes which were kept in vertical position for 24 h. After this the blood serum was collected in vacutainers. The samples were taken to the laboratory (Ripha International Laboratories, Lahore, Pakistan) for the serum calcium and phosphorous determination using a kit method (Crest biosystems, Japan).

The data collected during this experiment were analyzed using GLM procedure of SAS 9.1 (SAS, 2009) and means were compared using Tukey's Test (Steel *et al.*, 1997). The level of significance was considered at 5% probability.

#### Results and discussion

Feed intake of broilers was significantly affected when various levels of vitamin D and phytase were added in the ration separately or in combination. Table I shows that highest feed intake was observed in groups B and D, while lowest in groups F and G. So, feed intake was improved due to vitamin D and phytase supplementation added separately.

Considerable difference ( $P \leq 0.05$ ) on weight gain was observed in broilers when supplemented various concentrations of vitamin D + phytase (Table I). Highest weight gain (1766) was seen in group F and the lowest

in group G (1490) at 35 days. Addition of vitamin D and phytase showed significant difference ( $P \leq 0.05$ ) in feed conversion ratio. Results showed that best feed conversion ratio was observed in treatment F while, poorest FCR was observed in groups A, B, C and G (Table I).

Table II shows the effect of phytase and vitamin D administered separately and in combination on dressing percentage, toe/tibia ash percentage, and keel bone and shank length of broilers. Dressing percentage was found to be highest in treatment F and lowest in treatment C. Dressing percentage was improved after vitamin D and phytase addition. The highest tibia ash was found in treatments F and G while the lowest tibia ash percentage was observed in treatments A, B, C, and D. Keel bone length was highest in groups F and G and the lowest keel bone length was observed in groups A, B and C. The highest shank length was observed in group G and lowest shank length was observed in groups B, D, E and F.

Supplementation of various levels of vitamin D and phytase significantly ( $P \leq 0.05$ ) affected the serum calcium and phosphorus contents in broilers fed experimental rations as A, B, C, D, E, F and G. The highest level of calcium was present in group D and G and lowest level of calcium was observed in group A (Table II), Whereas, the highest serum phosphorus level was observed in groups C and G, while the lowest level of phosphorus was observed in groups A, B, D, E and F (Table II).

A large body of evidences are accumulated that obviously indicates the efficacy of exogenous enzymes in diets to improve the availability of nutrients that results

significant effects on animal performance (Beigh *et al.*, 2018). Likewise, vitamins play a key role in animal performance, growth, bone mineralization health and welfare (Zhang *et al.*, 2019). Though, in commercial poultry vitamins are being used according to recommendations of NRC (1994), however, there is several reports that have shown deficiency of vitamins and/or minerals (like calcium, zinc, copper etc.) in poultry that led to poor performance and meat quality defects (Richards *et al.*, 2010). Our dietary regimens (phytase and Vit. D) have expressively improved the performance parameters (feed intake, body weight and FCR) of broilers.

The results of present study showed that body weight gain was significantly different among all treatment groups. The findings of this study were similar to the findings of Fritts *et al.* (2003) who studied the effect of different concentrations of 25-hydroxy cholecalciferol (25-OH-D<sub>3</sub>) and vitamin D<sub>3</sub> (cholecalciferol). They reported that improvement in weight gain of broiler birds was due to supplementation of vitamin D and phytase. The results were similar to those performed by Bozkurt *et al.* (2006), who reported that weight gain was improved in broiler birds offered phosphorus deficient rations having phytase. Similarly, Aksal and Bilal (2002) studied the effect of vitamin D and phytase on absorption of minerals. They showed that absorption of Ca, P, Mn, Zn and Cu was increased with better feed conversion ratio due to vitamin D and phytase supplementation. All these studies collectively support the hypothesis that phytase could improve the P bioavailability from phytic acid molecules of feedstuffs

**Table I. Effect of different levels of phytase and vitamin D on feed intake, body weight, feed conversion ratio, dressing percentage, tibia ash percentage, and shank and keel lengths in broilers at 35 days age.**

Parameters	Treatments*							SEM**
	A	B	C	D	E	F	G	
Feed intake (g)	3239 <sup>ab</sup>	3339 <sup>a</sup>	3132 <sup>ab</sup>	3250 <sup>a</sup>	3175 <sup>ab</sup>	3056 <sup>b</sup>	3076 <sup>b</sup>	38.5
Weight gain (g)	1574 <sup>bcd</sup>	1619 <sup>bcd</sup>	1553 <sup>cd</sup>	1690 <sup>ab</sup>	1639 <sup>abc</sup>	1766 <sup>a</sup>	1490 <sup>d</sup>	34.52
Feed conversion ratio	2.06 <sup>a</sup>	2.06 <sup>a</sup>	2.01 <sup>a</sup>	1.92 <sup>ab</sup>	1.93 <sup>ab</sup>	1.73 <sup>b</sup>	2.06 <sup>a</sup>	0.05
Dressing percentage	67.11 <sup>ab</sup>	70.50 <sup>ab</sup>	64.43 <sup>b</sup>	68.28 <sup>ab</sup>	70.38 <sup>ab</sup>	73.08 <sup>a</sup>	69.75 <sup>ab</sup>	1.05
Tibia ash percentage	92.71 <sup>b</sup>	92.74 <sup>b</sup>	93.99 <sup>b</sup>	93.46 <sup>b</sup>	96.08 <sup>ab</sup>	97.88 <sup>a</sup>	97.93 <sup>a</sup>	0.87
Shank length (cm)	8.23 <sup>ab</sup>	7.60 <sup>b</sup>	8.50 <sup>ab</sup>	6.80 <sup>b</sup>	7.63 <sup>b</sup>	6.90 <sup>b</sup>	9.53 <sup>a</sup>	0.36
Keel bone (cm)	11.87 <sup>b</sup>	12.13 <sup>b</sup>	12.03 <sup>b</sup>	13.43 <sup>ab</sup>	13.60 <sup>ab</sup>	14.52 <sup>a</sup>	14.37 <sup>a</sup>	0.42

\*Treatments A was control, without supplementation of phytase and vitamin D. Diets B, C, D, E, F and G were supplemented @ phytase 400 FTU/kg, 800 FTU/kg, vitamin D 4000 IU/kg, 8000 IU/kg, phytase 400 FTU/kg + vitamin D 4000 IU/kg and phytase 800 FTU/kg + vitamin D 8000 IU/kg of feed, respectively; \*\*Indicates Standard Error mean.

**Table II. Effect of different levels of phytase and vitamin D on serum calcium and phosphorus in broilers at 35 days age.**

Parameters	Treatments*							SEM**
	A	B	C	D	E	F	G	
Serum Calcium (mg/dl)	5.85 <sup>b</sup>	6.83 <sup>ab</sup>	6.87 <sup>ab</sup>	8.63 <sup>a</sup>	6.87 <sup>ab</sup>	6.70 <sup>ab</sup>	8.80 <sup>a</sup>	0.41
Serum Phosphorous (mg/dl)	4.17 <sup>b</sup>	4.06 <sup>b</sup>	6.43 <sup>a</sup>	4.43 <sup>b</sup>	4.67 <sup>b</sup>	4.49 <sup>b</sup>	6.24 <sup>a</sup>	0.37

For explanation of \* and \*\* and statistical detail, see Table I.

(Bozkurt et al., 2006), and this increased availability of P have beneficial effects on weight gain and feed: Gain ratio in chickens. However, it is also been hypothesized that phytase supplementing animals have better performance because of positive effects of enzymes on utilization of metabolizable energy (Ravindran et al., 2006).

Results of present study are also in line with findings of Adebisi et al. (2009), who examined the effect of adding maize based rations with various concentrations of microbial phytase on bioavailability of phosphorous in serum. They stated that phytase addition in rations enhanced P, Ca, and Cu bioavailability in broilers serum significantly, thus cause the strengthening of bones and mineral deposition in smooth muscles (Woyengo et al., 2010). They offered basal rations (recommended concentrations of vitamin D and Ca) as control and suboptimal levels of calcium and non-phytate phosphorous. They reported that plasma phosphorous was increased. Vitamin D increased the absorption and retention of phosphorous in blood serum.

The effects of vitamins on carcass parameters like dressing percentage is still controversial (Zhang et al., 2019). Our results have demonstrated that addition of rations with vitamin D and phytase to broilers ration improved dressing percentage. Maximum effects were observed when birds were fed vitamin D 4000 IU/kg in combination with 400 FTU/kg of phytase (group F). Our results have demonstrated that combined supplementation of vitamin D and phytase improved the tibial ash percentage. These results were in accordance with Driver et al. (2006) who investigated tibial strength and tibial ash contents by addition of various concentrations of phytase in ration. They showed that microbial phytase supplementation in rations enhanced the tibial ash percentage.

Our dietary regimens vitamin D and phytase have also significantly affected the keel bone and shank length of broilers. However, the findings of present research trial were opposed by Saylor et al. (2005), who reported that vitamin D and phytase had no effect on keel bone and shank length in broiler birds.

#### Conclusion

From the present study, it is concluded that combined supplementation of phytase and vitamin D, at the level of 400 FTU/kg and 4000 IU/kg respectively improved the weight gain, feed conversion ratio, dressing percentage, keel bone length and bone mineralization in broilers.

#### Statement of conflict of interest

The authors have declared no conflict of interest.

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