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RESEARCH ARTICLE

Cumulative effect of phytase and vitamin D supplementation on performance and bone mineralization in broiler

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Fitaz ve vitamin D ilavesinin broylerlerde performans ve kemik mineralizasyonu üzerine kümülatif etkisi

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Öz

Amaç: Çalışmanın amacı düşük düzeyde fosfor içeren rasyonlarla beslenen broylerlere fitaz ve vitamin D ilavesi yapılmasının performans ve kemik mineralizasyonu üzerindeki etkinliğinin belirlenmesidir.

Gereç ve Yöntem: Çalışmada toplam 240 adet günlük yaşta civciv, her birinde 10 adet civciv bulunan 3 tekerrürlü 8 gruba ayrıldı (A, B, C, D, E, F, G ve H). Grup A içerisinde normal oranda fosfor bulunan temel rasyonla beslendi (Kontrol), Grup B inorganik fosfor ve fitaz ilavesi yapılmayan rasyonla beslendi (Negatif kontrol). Grup C, D ve E'nin rasyonları ise sırasıyla yalnızca 3 düzeyde fitaz içerirken (250, 500, 750 fitaz birimi (FTU)/kg yem); F, G ve H grupları yine sırasıyla 3 düzeyde fitazın yanı sıra (250, 500, 750 FTU/kg yem), vitamin D_3'ü (4000, 5000, 6000 IU/kg yem) de içerdi.

Bulgular: Çalışmada fitaz ve vitamin D_3 ilave edilen gruplarda (özellikle, 500 FTU/kg fitaz + 5000 IU/kg vitamin D_3 ilave edilen grup) A ve B gruplarından daha fazla canlı ağırlık artışı, karkas randımanı, sternum ile bacak uzunlukları, ayak ve tibia kül yüzdesi ile kalsiyum ve fosfor düzeyi belirlenirken ($P<0.05$); yemden yararlanma oranının bu gruplarda geliştiği ($P<0.05$) tespit edildi. Fitaz ve vitamin D_3 ilavesinde 500 FTU/kg + 5000 IU/kg düzeyinin, maliyet/kg canlı ağırlık açısından bakıldığında 250 ve 750 FTU/kg düzeylerine göre daha ekonomik olduğu belirlendi.

Öneri: Düşük fosfor içeren rasyonlara 500 FTU/kg fitaz + 5000 IU/kg vitamin D_3 ilavesinin broylerlerde büyüme performansı, karkas ağırlığı ve kemik mineralizasyonunu geliştirdiği ifade edilebilir.

Anahtar kelimeler: Fitaz, vitamin D, broiler, büyüme performansı, fosfor

Abstract

Aim: The aim of the study was to determine the efficacy of phytase and vitamin D supplementation in low phosphorous diet on performance and bone mineralization in broiler.

Materials and Methods: A total of 240 one-day-old broiler chicks were divided into 8 groups (A, B, C, D, E, F, G and H) with 3 subgroups in each, comprising 10 birds in each subgroup. Group A was fed normal basal diet having normal phosphorous ratio (Control) and group B was fed diet without inorganic phosphorous and phytase supplementation (Negative control). Group C, D E were offered diet containing only three levels of phytase (250, 500, 750 phytase unit (FTU)/kg feed) and group F, G and H were offered diets having three levels of phytase (250, 500, 750 FTU/kg feed) in combination of vitamin D_3 (4000, 5000, 6000 IU/kg feed) respectively.

Results: The phytase and vitamin D_3 supplemented groups (particularly, supplementation of 500 FTU/kg plus 5000 IU/kg) showed increased ($P<0.05$) live weight gain, dressing percentage, keel and shank length, toe and tibia ash percentage, calcium and phosphorous level in blood plasma; improved ($P<0.05$) feed conversion ratio as compared to the group A and B. Moreover, supplementation of phytase and vitamin D_3 at 500 FTU/kg plus 5000 IU/kg was most economical as cost/kg live weight than 250 and 750 FTU/kg diet supplementation.

Conclusion: It may be concluded that 500 FTU/kg phytase + 5000 IU/kg vitamin D_3 supplementation to low-P diet improved growth performance, carcass weight and bone mineralization in broilers.

Keywords: Phytase, vitamin D, broiler, growth performance, phosphorous

Introduction

Poultry diets contain ingredients of plant origin in which about 70% phosphorous is present in the form of Phytate Phosphorous (PP) (Mohammad et al 1991). Phytate is formed when phytic acid forms complexes with the cations and consequently, reduces availability of certain nutrients in birds (Cufadar and Bahtiyarca 2004). PP complex in poultry feed contains bounded phosphorous from plant source which is not available to poultry (Rezaei et al 2007). Phytate disturbs the activity of digestive enzymes. For these properties, phytate is included in category of anti-nutritional factors. Poultry has less ability to utilize phytate phosphorous because of less amount of phytase production in intestine of poultry (Ravindran et al 2006), that lead to excessive excretion of phosphorous in feces which cause environmental pollution (Waldroup et al 2000). During last decade supplementation of phytase enzyme in poultry diets has increased, it hydrolyses phytate molecule and releases phosphorous which is present in bound form. 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). Therefore, it is necessary to add exogenous synthetic phytase to enhance the release of PP because it reduces inorganic phosphorous requirement (Angel et al 2007). For these reasons, exogenous phytase enzyme is supplemented in poultry rations to reduce loss in terms of economic gain and phosphorous excretion in the environment (Dilger et al 2004, Onyango et al 2005).

Moreover, absorption of PP and Ca is increased by use of vitamin D which stimulates the hydrolysis of PP (Mohammed et al 1991). As a result of vitamin D supplementation, there is increase in the permeability of mucosal cells to carry minerals by increasing their deposition in bones and it also causes an increase in the bird weight gain and feed conversion ratio (Waldroup et al 2000). Supplementation of vitamin D in diets of broiler increases calcium and phosphorous level in plasma because it reduces rickets (Edwards 2002). In previous studies better results were obtained by using combination of phytase and vitamin D3 in diets of broiler chicks fed low level of Ca and P (Angel et al 2007).

Table 1. Dietary protocol.

Groups	Diets
A	Basal broiler commercial feed*
B	Basal diet without DCP and Phytase**
C	250 FTU/kg Phytase
D	500 FTU/kg Phytase
E	750 FTU/kg Phytase
F	250 FTU/kg Phytase + 4000IU/kg Vitamin D_3
G	500 FTU/kg Phytase + 5000IU/kg Vitamin D_3
H	750 FTU/kg Phytase + 6000IU/kg Vitamin D_3

FTU is the quantity of enzyme that releases 1µ 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 and IU=0.025µg/kg), *Control, **Negative control.

Table 2. Ingredients composition of experimental diets (%).

Ingredients	Starter	Finisher
Maize	37.88	37.23
Wheat	8	9
Rice tips	4	4
Rice polish	6	6
Cotton seed meal	2	2
Corn gluten meal, 60%	2	2
Soya bean meal	8	8
Canola meal	16	15
Fish meal	3	2
Sunflower meal	8	9
Molasses	2	2
Limestone	1.25	1.25
Vitamin mineral-premix*	0.02	0.02
Vegetable oil	1	2
Salt	0.5	0.5
L-Lysine	0.15	0
DL-Methionine	0.2	0
Chemical Composition		
Crude protein, %	22	20
ME, kcal/kg	3000	3200
Calcium, %	1.0	0.90
Available phosphorous, %	0.45	0.35

*Vitamin Premix contained all the required minerals, except for phosphorus source and vitamin D in diet B.

The main purpose of the study was to study the cumulative effects of phytase and vitamin D on the performance of broiler, hence to compensate major loss of bone and leg deformities in later stages of life due to heavier weight of birds and less availability of Ca and P by supplementing phytase and vitamin D.

Materials and Methods

The trial was conducted at Poultry Research and Training Center (PRTC) of the University of Veterinary and Animal Sciences (UVAS), after approval from the ethical committee of university. A total of 240 (one-day-old) broiler chicks were bought from the hatchery, weighed and divided into 8 dietary treatment groups (A, B, C, D, E, F, G and H) in completely randomized design. Each group consisted of 30 broiler chicks with 3 replicates having 10 chicks in each (8×3×10=240

birds). Eight diets were formulated according to the standards described by NRC (1994). All diets were iso-caloric and iso-nitrogenous. Birds were kept on starter feed for 2 weeks and for 4 weeks on finisher. Diet A was basal diet (Control) and diet B was formulated without dicalcium phosphate (DCP) and phytase supplementation (Negative control). Diet C, D and E were formulated with different levels of phytase 250, 500 and 750 phytase units (FTU)/kg, respectively. While diet F, G and H were having 250 FTU phytase plus 4000 IU/kg vitamin D, 500 FTU/kg phytase plus 5000 IU/kg vitamin D and 750 FTU/kg phytase plus 6000 IU/kg vitamin D (Table 1). The feed and fresh water were offered ad libitum throughout the experiment. The ingredient and chemical composition of diets are shown in Table 2.

Feed sample were collected and analyzed according to AOAC (2000) and metabolisable energy was determined by using Leeson and Summers (2005) method. Weekly feed intake was recorded. Chicks were weighed at the 1st day of their arrival 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 42 days for dressing percentage calculation. The dressing percentage was determined by following formula:

Dressing percentage = Dressed weight (g) / Live weight (g)

At 42 days age 2 live birds from each replicate were randomly taken to estimate shank length and keel bone length. Shank length and keel bone length were measured by using measuring tape. Samples of toe/tibia of 3 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 6 hours at 650°C for ash estimation which expressed as dry weight of toes samples (Sheideler 2000). Similar procedure was adopted for tibia ash. Mortality of each group was recorded separately.

Blood samples of slaughtered birds were collected in heparinized tubes and samples were brought to laboratory at 4°C. Plasma was separated by centrifugation at 4000 rpm for 15 minutes. Digestion of samples was done by using 10% TCA. After digestion and dilution, Samples were analyzed for Ca and P by using spectrophotometer. At the end of experiment, feed cost per kg live weight for each group was calculated to determine the economics of use of phytase and vitamin D_3.

Data obtained were analyzed by analysis of variance (ANOVA) under Completely Randomized Design (Steel et al 1997) and comparison of means was done by using Duncan's Multiple Range test (Duncan 1955). A significance level of $P<0.05$ was used.

Results

Results showed that live weight gain was highest in group G compared to control and other groups ($P<0.05$). There was no significant difference in feed intake of different groups ($P>0.05$); however it was numerically higher in group G. FCR decreased ($P<0.05$) in treatment group G and H, it was lowest in group G (Table 3). Mortality in group B was 6% and in group A was 4% while in other groups C, D, E, F, G and H was less than 2%.

Carcass weight was highest in group G ($P<0.05$) and lowest in group B as compared to control group. Dressing percentage was highest ($P<0.05$) in group G and lowest in group B as

Table 3. The effect of phytase and vitamin D supplementation on live weight gain, feed intake, FCR, carcass weight, dressing percentage, keel bone and shank length in broilers (0-6 weeks).

Groups	Live Weight gain (g)	Feed intake (g)	FCR	Carcass weight (%)	Dressing (%)	Keel bone length (cm)	Shank length (cm)
A	2075.67±29.7^{bc}	4420.0±41.6^{a}	2.12±0.016^{ab}	1215.00±45.70^{cd}	62.6±0.675^{b}	14.08±0.200^{c}	6.91±0.131^{bcd}
B	1999.33±45.6^{c}	4400.0±11.5^{a}	2.16 ±0.029^{a}	1055.00±19.20^{d}	59.95±0.652^{c}	12.86±0.333^{d}	6.16±0.213^{d}
C	2104.67±61.3^{bc}	4433.3±48.4^{a}	2.10± 0.04^{abc}	1300.83±25.22^{bc}	63.9±0.538^{ab}	14.50±0.238^{bc}	6.56±0.135^{bcd}
D	2143.3±18.5^{ab}	4466.6±29.1^{a}	2.08±0.005^{bcd}	1228.83±43.13^{c}	63.07±0.286^{ab}	14.41±0.316^{bc}	6.50±0.092^{cd}
E	2161.67±36.5^{ab}	4460.0±45.8^{a}	2.06±0.015^{bcd}	1434.17±67.89^{ab}	63.4±0.629^{ab}	15.20±0.333^{ab}	7.28±0.098^{b}
F	2181.67±11.6^{ab}	4490.0±23.1^{a}	2.05±0.011^{bcd}	1357.50±79.28^{bc}	63.1±0.760^{ab}	15.00±0.387^{abc}	6.98±0.258^{bc}
G	2243.33±32.8^{a}	4503.3±26.0^{a}	2.003±0.018^{d}	1574.17±71.40^{a}	65.2±1.401^{a}	15.58±.416^{a}	8.33±0.333$_{a}$
H	2193.33±35.2^{ab}	4476.6±42.5^{a}	2.03±0.018^{cd}	1310.80±59.05^{bc}	63.9±0.510^{ab}	14.40±0.359^{bc}	6.75±0.432^{bcd}

a, b, c, d: Values with different superscripts in a column differ significantly ($P<0.05$).

Table 4. The effect of phytase and vitamin D supplementation on apparent availability of minerals (Ca and P), toe and tibia ash in broilers (0-6 weeks).

Groups	Ca (mg/dL)	P (mg/dL)	Toe ash (%)	Tibia ash (%)
A	9.03±1.843^{bc}	6.50±0.655^{bc}	11.13±0.766^{d}	52.26±1.320^{cd}
B	6.50±0.854^{c}	5.10±1.650^{c}	8.66±0.664^{e}	50.00±1.154^{d}
C	9.40±0.832^{bc}	6.93±0.371^{bc}	12.50±0.228^{cd}	54.83±1.301^{bc}
D	10.06±1.030^{ab}	7.93±2.400^{abc}	13.33±0.333^{bcd}	56.00±0.577^{bc}
E	10.67±0.881^{ab}	8.90±1.184^{abc}	14.33±0.881^{bc}	56.93±0.581^{a}
F	10.76±0.935^{ab}	9.30±0.781^{abc}	14.50±0.763^{abc}	58.83±0.440^{b}
G	13.40±0.709^{a}	11.6±0.808^{a}	16.66±0.881^{a}	66.00±3.055^{a}
H	10.80±0.416^{ab}	10.2±1.322^{bc}	15.33±0.881^{ab}	63.00±0.577^{b}

a, b, c, d, e: Values with different superscripts in a column differ significantly (P<0.05).

Table 5. Economics of experimental diets.

	A	B	C	D	E	F	G	H
Cost/kg diet	40	37	37.096	37. 192	37.288	37.126	37.232	37.338
Cost/kg Live weight	85.204	81.809	78. 159	77.507	76.957	76.430	74.746	76.208

compared to control group (Table 3). Keel bone and shank length was highest (P<0.05) in group G and lowest in group B as compared to control group (Table 3). Highest level (P<0.05) of Ca and P was presented in group G and lowest level of Ca and P was in group B. Highest toe and tibia ash were found (P<0.05) in group G and lowest toe and tibia ash percentage were present by group B (Table 4). Economics of experimental diets is summarized in Table 5. Numerically maximum feed was consumed by group G. Feed cost/kg of live weight was calculated, excluding cost of day-old chick. It was revealed that birds of treatment group G fed with phytase 500 FTU/kg and vitamin D_3 5000 IU/kg were reared at lowest cost and diet was more economical.

Discussion

The results showed that supplementation of phytase and vitamin D increased weight gain in group G significantly. Weight gain of broilers fed with different levels of phytase and vitamin D was highest for group G. The findings of present study are similar with findings of Qian et al (1997), Sebastian et al (1997) and Cabahug et al (1999). They conducted the experiments and reported that improvement in growth performance was due to phytase supplementation. Similarly Fritts et al (2003) conducted a trial to investigate the effect of different levels of phytase and vitamin D on weight gain and bone development. They used vitamin D_3 (cholecalciferol) and 25-hydroxy cholecalciferol (25-OH-D_3) with different levels 125, 250, 500, 1000, 2000 and 4000 IU/kg, respectively. They concluded that increase in weight gain of broilers was due to addition of phytase and vitamin D. Our results are also similar with the study conducted by Bozkurt et al (2006) who reported that weight gain and FCR of broilers fed low P diets containing phytase were comparable. In present study phytase and vitamin D supplementation in P deficient diets resulted in better performance as exogenous supplemented phytase can break the bonds in PP complex and releases the bound P as well as other nutrients in that complex those otherwise unavailable for utilization. Santos et al (2008) presented the similar results that phytase supplementation in P deficient diets improved feed intake and better nutrient utilization which ultimately resulted in better performance.

Feed intake of broilers in all groups was non-significant but in group G it was increased numerically due to addition of phytase and vitamin D in the rations. The results of present study are in line with findings of Richter (1994) who performed an experiment to evaluate the growth performance, tibia mineralization and phosphorous excretion in broilers with total phosphorous 0.4-0.5% and different phytase levels 300 and 700 FTU/kg feed and concluded that feed intake was increased due to phytase supplementation. Similarly Biehl et al (1997), Broz et al (1997) conducted same trials on broilers and used different levels of phytase enzyme. They concluded that feed intake was increased due to supplementation of phytase enzyme. In another study Boling et al (2001) conducted an experiment and fed broiler chicks with low phosphorous diets plus different levels of fungal phytase 0, 125, 250 and 500 PU/kg. They concluded that increasing intake of phytase supplementation significantly increased feed intake of broilers. Present study results are also in agreement with the results of Mondal et al (2007). They concluded that broi-

lers fed with P-deficient diets supplemented with phytase at levels higher than 500 PU/kg feed increased the feed intake of broilers.

The results showed that supplementation of diets with phytase and vitamin D has increased FCR. Results showed that better FCR was observed in group G, H and poor in group B. Results of present study are similar with the results of Sebastian et al (1997) and Namkung and Leeson (1999) who conducted experiments to study the effect of phytase on growth performance of broilers fed with phytase, low in Ca and P. They concluded that phytase addition increased FCR of broilers at marketing weight compared to low P diets. Results of present study are also similar with Kwon et al (1995) and Kies et al (2001). They conducted experiments and concluded that FCR was better with addition of phytase and vitamin D. Similarly Aksakal and Bilal (2002) performed an experiment to investigate the effect of phytase and Vitamin D on absorption of minerals. They used 144 day old broiler chicks and fed them with 2 different Ca levels and vitamin D (5 microgram/kg) and 600 FYT/kg phytase. They reported that absorption of Ca, Zn, P, Mn and Cu was increased with increase of body weight and feed conversion ratio due to phytase and Vitamin D. In another study conducted by Shafey et al (1990) who conducted an experiment to evaluate the effect of phytase on broiler. They used 3 levels of phytase (0, 0.75 and 1.5 kg/ton) in feed with six treatments. They concluded that improvements in FCR of broiler were greater on low non-phytate phosphorous (NPP) diets which were due to phytase supplementation.

Highest carcass weight was observed in group G and lowest in group B. The results of present study are similar with results of Atia et al (2000) and Scheideler (2000). They studied the effect of phytase in diets with different levels of zinc and low P contents on broiler performance, carcass characteristics and bone mineralization. They concluded that carcass weight was increased due to phytase supplementation. Cufadar and Bahtiyarca (2004) studied effect of phytase in diet with different levels of phytase and vitamin D with low P contents on broiler growth performance and carcass characteristics. They concluded that carcass weight was increased due to phytase and vitamin D supplementation. Results are also similar with the results of Ahmed et al (2004), Pillai et al (2006) and Angel et al (2007). They reported that carcass weight was increased due to supplementation of phytase and vitamin D.

Highest dressing percentage was observed in group G and lowest in group B. The results of present study are in agreement with results of Manangi et al (2006). They reported that dressing percentage was increased with the use of phytase and vitamin D.

Results showed that supplementation of diets with phytase and vitamin D to birds increased keel bone length and shank length. It is an indication of improvement in skeleton muscle development which increased muscles, weight gain better FCR and dressing percentage in broilers. The findings of present study are in line with findings of Leesen and Summers (1984), Lilburn et al (1989). The results of present study are different with the results of Saylor et al (2005) and Lim et al (2001). They concluded that phytase and vitamin D had no effect on keel bone, shank length and shank weight in broilers.

In this study highest calcium level was observed in group G (13.4 mg/dl) and lowest level calcium was observed in group B (6.53 mg/dl). Results of present study are similar with the findings of Mohammad et al (1991). They conducted an experiment and used different levels of phytase and cholecalciferol. They reported that change in calcium and phosphorous levels were due to addition of phytase and vitamin D affecting the absorption of minerals. They concluded that Vitamin D increased calcium absorption. The results of present study are also in agreement with the results studied by Edwards (2002), Dilger et al (2004) and Ravindran et al (2006). They conducted experiments and stated that supplementation of phytase and vitamin D had significant effect on calcium absorption. Results showed that supplementation of diets with phytase and vitamin D to birds increased phosphorous in plasma. Results showed that highest phosphorous concentration was observed in group G. The results of present study are similar with results observed by Denbow et al (1995). They concluded that phytase increased plasma phosphorous contents in broilers. Onyango et al (2005) conducted an experiment to check efficiency of phytase and vitamin D for increase in the utilization of calcium and PP in broilers fed with diets containing soybean and corn which resulted in increased calcium and phosphorous utilization. They concluded that phytase increased phosphorous contents in plasma.

Toe ash percentage of broilers fed diets containing different levels of phytase and vitamin D was highest in group G and lowest in group B. Tibia ash percentage was highest in group G. The results of present study are in line with findings of Lim et al (2001). They reported that phytase and vitamin D increased toe and tibia ash percentage. Similarly Bedford et al (2005) reported that addition of phytase and vitamin D had significant effect on toe and tibia ash percentage. Supplemented group G showed highest bone mineralization as shown in terms of higher toe and tibia ash (Tang et al 2012). In other studies by Ahmad et al (2000) and Watson (2006) it was reported that P deficient diets result in decreased bone ash percentage and supplementation of phytase improved the bone ash contents. Driver et al (2006) performed an experiment to evaluate tibial strength with toe and tibia ash by supplementation of different phytase levels in feed. They reported that microbial phytase addition in diets increased toe and tibia ash. Bone strength depends upon Ca and P level in

tibia ash. Keel bone length/shank length of broilers fed diets containing different levels of phytase and vitamin D was highest in group G and low in group B.

Group G had lowest cost of production when compared with cost of other treatment groups. Group A had the highest cost of production than other treatment groups.

Conclusion

It is concluded that use of phytase enzyme in broiler diets at 500 FTU/kg of feed along with vitamin D supplementation at 5000 IU/kg of feed resulted in better growth performance and carcass weight, improved toe/tibia ash and Ca/P availability in blood of broiler. It is recommended to use phytase which may result in overall better profitability of farm operation.

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