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Effect of Phytase Enzyme Supplementation in Low Energy-Protein Layer Diet on Tibial Mineral Contents

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ABSTRACT

An experiment was carried out using 200 hundred Athulya birds divided into 10 treatments (T1-T10) to find out the effect of phytase enzyme on tibial mineral contents. Phytase was supplemented at 0, 500 and 1000 units/kg in low energy, low protein and low energy- protein layer chicken diets containing available phosphorus of 0.30% from 21 to 40 weeks of age. A standard layer ration was offered to birds in T1. Experimental diets from T2 to T10 were formulated with two levels of crude protein (18 and 16 percent) and two levels of metabolisable energy (2600 and 2400 ME kcal/kg diet). After the laying period, four birds from each treatment were randomly selected, slaughtered and data on tibial mineral contents viz. tibial ash. calcium and phosphorus were measured. The tibial mineral contents viz. tibial ash. calcium and phosphorus showed significant ($P<0.01$) increase among phytase supplemented treatments.

Keywords: Phytase, low energy-protein, tibial minerals

Indian layer industry is growing annually at the rate of 5 to 7 per cent. Feed production and availability of feed ingredients are limited to meet the increasing demand of industry. Most of the cereals and their by-products used in poultry diet have phosphorus in the form of phytate which is considered as an anti-nutritional factor and not fully utilized by the birds. Phosphorus is an important major mineral nutrient in layer diet which played important role in bone formation and its strength. It is one of the costliest and essential nutrients required by poultry. About 70 to 75 percent of the total phosphorus in poultry feed ingredients present as phytate phosphorus and only 25 to 30 percent of phytate phosphorus is absorbed and the balance is excreted as waste. This research work has been aimed to increase the digestibility of phytate phosphorus by supplementation of exogenous phytase enzyme in low nutrients layer diet and to improve the tibial mineral contents.

MATERIALS AND METHODS

Two hundred, White Leghorn hybrid pullets at 16 weeks of age were selected and housed in individual cages. They were divided into ten treatments with four replicates each having five hens. A Standard Layer ration (CP-18 per cent, ME-2600 kcal/kg diet, available phosphorus-0.5 per cent) was formulated as per BIS (1992) and offered to birds in T1. Experimental diets from T2 to T10 were formulated with two levels of crude protein (18 and 16 percent), two levels of metabolisable energy (2600 and 2400 ME kcal/kg diet) and three levels of phytase (0,500 and 1000 units/kg) as detailed in Table 1. The available phosphorus level in all treatments except T1 was 0.3 per cent. The experimental rations viz., Standard layer ration (SLR), Low energy ration (LER), Low protein ration (LPR) and Low energy-protein ration (LEPR) were offered *ad libitum* from 21 to 40 weeks of age.

Table 1. Allocation of different dietary treatments to experimental birds

Treatments	Rations	Crude protein percent	Metabolisable energy kcal/kg	Available phosphorus percent	Phytase units/kg
T1	SLR	18	2600	0.5	0
T2	LER	18	2400	0.3	0
T3	LER	18	2400	0.3	500
T4	LER	18	2400	0.3	1000
T5	LPR	16	2600	0.3	0
T6	LPR	16	2600	0.3	500
T7	LPR	16	2600	0.3	1000
T8	LEPR	16	2400	0.3	0
T9	LEPR	16	2400	0.3	500
T10	LEPR	16	2400	0.3	1000

After the production period, four birds from each treatment were randomly selected and humanely slaughtered to find out the tibial mineral status. The left tibial bone from each of the bird subjected to slaughter studies was collected and dried overnight at 70° C. All the bones are boiled in 2 percent sodium hydroxide solution and defatted using the soxhlet apparatus. Subsequently all bones were ashed at 560 to 600° C for eight hours. The total ash content was recorded. Tibial bone samples were subjected to wet-ash digestion to determine its calcium and phosphorus content. Tibial calcium was analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model 3110) after wet digestion, using nitric acid and perchloric acid (2:1). Tibial phosphorus content of bone samples was analyzed by colorimetry (ANSA method, AOAC, 1990) using spectrophotometer (Spectronic 20D$^+$, spectronic instruments, USA).

All the data obtained in this study were subjected to statistical analysis as per the methods of Snedecor and Cochran (1994).

Table 2. Effect of phytase supplementation in low energy-protein diet on per cent tibial phosphorus content in Athulya layer+

Treatments	Tibial ash content**	Tibial calcium content**	Tibial phosphorus content**
T1	33.43 ^{bcd} ± 0.39	33.43^{c} ± 0.30	17.22 ^{bcd} ± 0.18
T2	32.45^{abc} ± 0.41	31.04^{ab} ± 0.73	15.48^{a} ± 0.26
T3	33.79^{cd} ± 0.36	32.19^{bc} ± 0.99	17.45^{cd} ± 0.22
T4	33.75^{cd} ± 0.40	33.65^{c} ± 0.61	17.48^{cd} ± 0.36
T5	32.16^{ab} ± 0.36	31.07^{ab} ± 0.37	16.47^{b} ± 0.20
T6	33.87^{cd} ± 0.37	32.69^{bc} ± 0.55	17.72^{d} ± 0.14
T7	34.32^{d} ± 0.67	33.42^{c} ± 0.39	17.98^{d} ± 0.33
T8	31.42^{a} ± 0.53	30.74^{a} ± 0.49	15.22^{a} ± 0.45
T9	33.13^{bcd} ± 0.25	33.51^{c} ± 0.67	16.73^{bc} ± 0.37
T10	34.19^{d} ± 0.59	33.90^{c} ± 0.33	17.45^{cd} ± 0.22
P-value	0.01	0.01	0.00

+Mean of four values with SE

Means bearing different superscripts within the same column differed significantly **($P<0.01$)

RESULTS AND DISCUSSION

The data on tibial ash, calcium and phosphorus contents as influenced by supplementation of phytase in various dietary treatment groups are set out in Table 2.

Tibial ash

Mean tibial ash content for various dietary treatment groups were 33.43, 32.45, 33.79, 33.75, 32.16, 33.87, 34.32, 31.42, 33.13 and 34.19% for treatment groups T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10 respectively. The highest tibial ash content of 34.32% was recorded in (T7) birds received LPR supplemented with phytase 1000 units/kg of feed and the lowest value of 31.42% was observed in LEPR fed (T8) control group.

Significantly (P<0.01) highest tibial ash content of 34.32 and 34.19% was observed in LPR (T7) and LEPR (T10) supplemented with phytase 1000 units/kg of feed respectively when compared with negative controls (T2, T5 and T8) and was comparable with other experimental rations which added with different levels of phytase and SLR fed group.

Similar finding was recorded by Carlos and Edward (1998), Punna and Roland (1999), Punna and Roland (2000), Sukumar (1999), Musapuor *et al*. (2005), Panda *et al. (*2005) and Tofuko *et al. (*2010).

Tibial calcium content

Mean tibial calcium content for various dietary treatment groups were 33.43, 31.04, 32.19, 33.65, 31.07, 32.69, 33.42, 30.74, 33.51 and 33.90% for treatment groups T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10 respectively. The highest tibial calcium content of 33.90% was recorded in (T10) birds received LEPR supplemented with phytase 1000 units/kg of feed and the lowest value of 30.74% was observed in LEPR fed (T8) control group. Significantly (P<0.01) lowest tibial calcium content of 31.04, 31.07 and 30.74% was observed in LER (T2), LPR (T5) and LEPR (T10) fed birds respectively when compared with all the phytase supplemented rations and SLR fed treatment groups. Irrespective of levels of phytase supplementations, the entire enzyme added groups showed highest tibial calcium content values and was statistically comparable within them and SLR fed groups.

Tibial phosphorus content

Mean tibial phosphorus content for various dietary treatment groups were 17.22, 15.48, 17.45, 17.48, 16.47, 17.72, 17.98, 15.22, 16.73 and 17.45% for treatment groups T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10 respectively. The highest tibial phosphorus content of 17.98% was recorded in (T7) birds received LPR supplemented with phytase 1000 units/kg of feed and the lowest value of 15.22% was observed in LEPR fed (T8) control group. Significantly (P<0.01) lowest tibial phosphorus content of 15.48 and 15.22 was observed in LER (T2) and LEPR (T8) fed control treatments respectively when compared with all other dietary treatment groups. Birds fed LPR without phytase showed intermediate tibial phosphorus content value of 16.47% which was comparable with birds received SLR and LEPR supplemented with phytase 500 units/kg of feed. Significantly highest tibial phosphorus content was noted in T6 (17.72%) and T7 (17.98%) birds when compared with all the negative controls and LEPR supplemented with phytase 500 units/kg of feed and was comparable with T1, T3, T4 and T10 dietary treatments.

The present finding is in agreement with Musapuor *et al*. (2005) who reported that supplementation of phytase at 1000 units/kg in low phosphorus diet of laying hens significantly improved the tibial phosphorus content. Panda *et al. (*2005), Lei *et al.* (2011) and Lan *et al. (*2012) also reported similar research findings.

Addition of phytase might have reduced chelate formation of phytate with calcium which in turn resulted better absorption of calcium. It may be a reason for higher tibial calcium content in all the phytase-supplemented rations fed birds. Overall, improvement in tibial mineral contents was observed in all the phytase supplemented groups may be due to best utilization of dietary calcium and phytate bound phosphorus. From this study, it may be concluded that addition of commercial phytase enzyme in low nutrients layer diet improves tibial mineral contents which may decrease bone problems in layer birds during the course of laying period.

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