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Effect of phytase supplementation in low energy-protein layer diet on availability of calcium and total phosphorus

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Abstract

An experiment was carried out using two hundred Athulya birds divided into10 treatments (T1-T10) to find out the effect of phytase enzyme on calcium and total phosphorus availability. 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 per cent 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 to conduct a metabolism trial and data on availability of calcium and total phosphorus were determined. The availability of calcium and total phosphorus in phytase supplemented groups showed significant ($P<0.01$) improvement among treatments when compared with negative controls.

Keywords: Phytase, low energy-protein, Calcium and Phosphorus

Introduction

Indian layer industry is continuously growing at the rate of 5 to 10 per cent in the last three decades. Feed production and availability of feed ingredients are limited to meet the demand of poultry 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. Calcium and phosphorus are important major mineral nutrients in layer diet which played important role in bone formation and its strength. Phosphorus 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 availability of calcium and phytate phosphorus by supplementation of exogenous phytase enzyme in low nutrients layer diets.

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) . The available phosphorus level in all treatments except T1 was 0.3 per cent. The experimental rations (Table. 1) 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. During the experimental period, the birds were offered standard layer diet (SLD-positive control) T1, low energy diet (LED) T2, LED supplemented with phytase 500 and 1000 units/kg (T3 and T4), low protein diet (LPD) T5, LPD with phytase 500 and 1000 units/kg (T6 and T7), low energy-protein diet (LEPD) T8 and LEPD with phytase 500 and 1000 units/kg (T9 and T10). Treatments T2, T5 and T8 were termed as negative controls.

At the end of production period, a metabolism trial was conducted using forty birds (one bird from each replicate) selected randomly. Birds were housed in individual metabolism cages with facilities for feeding, watering and excreta collection. Water was provided *ad libitum*. Excreta were collected for three consecutive days over 24 hour period using total collection method as described by Summers *et al.* (1976). Excreta collected daily from each bird was weighed and representative samples were taken after thorough mixing. The total amount of feed consumed and excreta voided for each individual bird were recorded.

The excreta samples were dried in the oven at 100˚C overnight and ground prior to the estimation of minerals and rest of the proximate principles. The calcium contents of both feed and faecal samples were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model 3110) after wet digestion, using nitric acid and perchloric acid (2:1). Phosphorus contents of both feed and faecal samples were analyzed by colorimetry (ANSA method, AOAC, 1990) using spectrophotometer (Spectronic 20D$^+$, spectronic instruments, USA) to determine availability of total phosphorus and phytate phosphorus. Data collected on various parameters were statistically analyzed by Completely Randomized Design (CRD) as described by Snedecor and Cochran (1994).

Table 1. Per cent ingredient composition of experimental diets

Ingredients	SLD	LED	LPD	LEPD
Yellow maize	58.00	46.00	58.50	47.00
Soya bean meal	28.35	27.00	22.10	21.00
Wheat bran	2.00	4.10	4.00	5.10
De oiled rice bran	2.00	13.00	5.50	17.00
Dicalcium phosphate	2.00	0.75	0.75	0.75
Shell grit	7.00	8.50	8.50	8.50
Salt	0.20	0.20	0.20	0.20
Merivite[1]	0.015	0.015	0.015	0.015
DL-methionine	0.100	0.100	0.100	0.100
Tefroli[2]	0.100	0.100	0.100	0.100
Meriplex[3]	0.015	0.015	0.015	0.015
Choline chloride[4]	0.120	0.120	0.120	0.120
Ultra TM[5]	0.100	0.100	0.100	0.100
Total	100	100	100	100

Merivite[1]: A+B_2+D_3+K (Wockhardt Ltd., Mumbai) : Each gram contains : Vitamin A: 82,500 IU, Vitamin B_2:52 mg, Vitamin D_3:12,000 IU, Vitamin K: 10mg, Calcium: 166 mg, Phosphate: 395 mg. (Vetroquinol India Animal Health Pvt.Ltd. Mumbai.)

Tefroli[2] : Liver tonic powder. Dr. Herbs India, Vellore, Tamil Nadu.

Meriplex[3] : Each gram contains: Vitamin B_1: 8mg, Vitamin B_6: 16mg, Vitamin B_{12}: 80mcg, Vitamin E: 80mg, Niacin: 120mg, Folic acid: 8mg, Calcium pantothenate : 80mg, Calcium: 86mg. (Vetroquinol India Animal Health Pvt.Ltd. Mumbai.)

Choline chloride[4] : NB group Co-Ltd. Mumbai.

Ultra TM[5]: Each gram contains: Manganese: 54mg, Zinc: 52 mg, Iron: 20mg, Copper: 2mg, Iodine: 2mg and Cobalt: 1mg.

Results and Discussion

Calcium availability

The statistical analysis of data revealed significantly ($P<0.01$) higher calcium availability in all phytase supplemented LED, LPD and LEPD fed birds when compared with negative control diets fed groups. However, calcium availability of birds fed SLD was comparable with phytase-supplemented groups.

Jalal and Scheideler (2001) observed that addition of phytase at 250 units/kg in corn soya layer diets significantly increased the per cent calcium availability. Aureli *et al.* (2011) found that supplementation of phytase at 500, 1000 and 2000 units/kg in low phosphorus (0.30 per cent) corn soya broiler diet significantly improved the calcium utilization. The present finding clearly reveals a beneficial effect of phytase supplementation in layer diet in improving utilization of calcium.

Phosphorus availability

The data on phosphorus availability as influenced by supplementation of phytase in various dietary treatment groups is set out in Table 2. Mean phosphorus availability values for various dietary treatment groups were 58.97, 54.25, 61.58, 62.64, 54.47, 60.94, 63.99, 54.28, 60.31 and 62.21 per cent for treatment groups T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10 respectively. The highest phosphorus availability value of 63.99 per cent was recorded in the phytase (1000 units/kg) supplemented LPD fed (T7) treatment group and lowest availability value of 54.25 was observed in LED fed (T2) control group.

Statistical analysis of data revealed significant ($P<0.01$) difference among various dietary treatments. Significantly lower phosphorus availability was observed in LED (T2), LPD (T5) and LEPD (T8) control diets fed groups when compared with all phytase supplemented diets and SLD fed groups. However, phosphorus availability value of 63.99 per cent (T7) was significantly higher when compared with all other treatments except T4 and T10. Birds which received the supplemental phytase at 1000 units/kg showed higher phosphorus availability values when compared with controls . Experimental diets which were supplemented with 500 units/kg of phytase showed significantly better phosphorus availability when compared with their negative controls and were comparable with SLD fed group except T3. When graded levels of phytase were added to LPD and LEPD, phosphorus availability also increased significantly. However, addition of 500 and 1000 units of phytase in birds fed LED did not reveal any significant difference within them. Overall, supplementation of phytase in low phosphorus diet significantly improved the phosphorus availability when compared with their negative controls. On perusal of data, it clearly explains that addition of phytase to low phosphorus diet improved the availability of phosphorus from 6.03 to 9.52 per cent in different experimental diets fed birds. The addition of exogenous phytase in low phosphorus, energy and protein layer feed showed a tremendous potential to increase availability of phosphorus. The present finding is in close agreement with that of Kannan (2004), Liu *et al*. (2007), Zhou *et al*. (2008) and Aureli *et al.* (2011).

Phytate phosphorus utilization

The data on phytate phosphorus availability as influenced by supplementation of phytase in various dietary treatment groups is set out in Table 20. Mean phytate phosphorus availability values for various dietary treatment groups were 46.95, 45.83, 51.38, 52.91, 44.44, 50.95, 53.67, 47.30, 53.09 and 54.56 per cent for treatment groups *viz*., T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10 respectively. The highest phytate phosphorus availability value of 54.56 per cent was recorded in the phytase (1000 units/kg) supplemented LEPD fed (T10) treatment group and the lowest availability value of 44.44 was observed in LPD fed (T5) control group.

Statistical analysis of data showed significant ($P<0.01$) difference among various dietary treatments. Significantly higher phytate phosphorus availability was observed in all phytase supplemented groups except T6 when compared with all the negative and positive control diets fed treatment groups. However, phytate phosphorus availability of T6 (LPD supplemented with phytase 500 units/kg of feed) was comparable with T8 and other phytase supplemented treatments and was significantly higher than that of T1, T2 and T5 control diets fed groups. When birds were fed diet containing high phytate content (0.50 per cent) without phytase, correspondingly phytate utilization also increased marginally. Ravindran *et al.* (2006) also found that addition of phytase at 500, 750 and 1000 units/kg in broiler diet containing varying level of phytate content significantly improved the ileal phytate phosphorus utilization. Ramin *et al.* (2011) and Rutherfurd *et al.* (2012) recorded similar findings in broilers.

Table.2. Effect of phytase supplementation in low energy-protein diet on per cent calcium, phosphorus and phytate phosphorus availability in Athulya layers+

Treatments	Calcium availability**	Phosphorus availability**	Phytate phosphorus availability**
T1	$61.93^{b} \pm 0.36$	$58.97^{b} \pm 0.51$	$46.95^{a} \pm 2.14$
T2	$57.82^{a} \pm 0.44$	$54.25^{a} \pm 0.91$	$45.83^{a} \pm 1.61$
T3	$60.61^{b} \pm 0.58$	$61.58^{c} \pm 0.81$	$51.38^{c} \pm 1.75$
T4	$60.35^{b} \pm 0.48$	$62.64^{cd} \pm 1.07$	$52.91^{c} \pm 1.05$
T5	$57.26^{a} \pm 0.96$	$54.47^{a} \pm 0.41$	$44.44^{a} \pm 0.99$
T6	$61.80^{b} \pm 0.30$	$60.94^{bc} \pm 0.83$	$50.95^{bc} \pm 1.27$
T7	$61.32^{b} \pm 0.55$	$63.99^{d} \pm 0.63$	$53.67^{c} \pm 1.09$
T8	$57.25^{a} \pm 0.41$	$54.28^{a} \pm 0.47$	$47.30^{ab} \pm 0.43$
T9	$60.79^{b} \pm 0.68$	$60.31^{bc} \pm 0.39$	$53.09^{c} \pm 0.71$
T10	$61.8^{b} \pm 0.51$	$62.21^{cd} \pm 0.98$	$54.56^{c} \pm 0.86$

+Means of four values with SE

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

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