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Original Research Article

Efficacy of dietary phytase supplementation on laying performance and expression of osteopontin and calbindin genes in eggshell gland

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ABSTRACT

This study was conducted to evaluate the effects of different levels of dietary phytase supplementation in the layer feed on egg production performance, egg shell quality and expression of osteopontin (*OPN*) and calbindin (*CALB1*) genes. Seventy-five White Leghorn layers at 23 weeks of age were randomly divided into 5 groups consisting of a control diet with 0.33% non-phytate phosphorus (NPP) and 4 low phosphorus (P) diets: 2 diets (T1 and T2) with 0.24% NPP + 250 FTU/kg laboratory produced phytase or commercial phytase and another 2 diets (T3 and T4) with 0.16% NPP + 500 FTU/kg laboratory produced phytase or commercial phytase with complete replacement of inorganic P. The results indicated that there were no significant differences ($P > 0.05$) in egg production performance and quality of egg during the first 2 months of trial. However, in next 2 months, a significant drop in egg production and feed intake was observed in birds fed diets with low P and 500 FTU/kg supplementation of laboratory produced phytase. Osteopontin gene was up-regulated whereas the *CALB1* gene was down regulated in all phytase treatment groups irrespective of the source of phytase. The current data demonstrated that 250 FTU/kg supplementation of laboratory produced phytase with 50% less NPP supplementation and 500 FTU/kg supplementation of commercial phytase even without NPP in diet can maintain the egg production. The up-regulation of *OPN* and down regulation of *CALB1* in egg shell gland in the entire phytase treated group birds irrespective of the source of enzymes is indicative of the changes in P bioavailability at this site.

1. Introduction

Phosphorus (P) is a major and essential mineral for all animals and is involved directly or indirectly in all physiological functions and also a component of large co-enzymes (Anselme, 2003). To reduce excess P excretion and better utilization, phytase enzyme is supplemented for maintaining optimum economical poultry production. Laying birds require high calcium (Ca) level for egg shell deposition and bone maintenance (Pizzolante et al., 2009). Both minerals are most crucial during the laying period (De Vries et al., 2010). The Ca and P levels in the diet also influence the eggshell formation which in turn linked with quality of egg in the laying birds.

The levels of Ca and P control the expressions of genes and these products involved in physical and chemical parameters of the calcification processes. The osteopontin (OPN) comprises of a polyaspartic acid sequence, sites of Ser/Thr phosphorylation that mediate hydroxyapatite binding, and 2 highly conserved tripeptide arginyl-glycyl-aspartic acid (RGD) motifs in the chicken that mediate cell attachment/signaling (Sodek et al., 2000). Additionally, OPN shows other post-translational modifications such as glycosylation and sulfation for metabolism and other functions. This phosphoprotein is found in the eggshell and the gene expressed in egg shell gland (ESG) of chicken (Fernandez et al., 2003; Mann et al., 2007). In the chicken, uterine expression of

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the *OPN* gene is temporally associated with eggshell mineralization through a coupling of physical expansion of the uterus with *OPN* gene expression (Lavelin et al., 2000). Calbindin (CALB1) on the other hand is a 28 kDa calcium binding protein (Chard et al., 1993), which was found in the ESG, and predominantly a Ca^{2+} dependent protein and related to eggshell quality (Nys et al., 1989; Bar et al., 1992). The presence of *CALB1* in the shell gland mucosa of laying hens increases with the onset of egg production, and it decreases as egg production ceases (Corradino et al., 1968).

Keeping the above background in mind the current study was designed to understand the comparative efficacy of low P diets supplemented with laboratory produced phytase derived from fungal and commercially available phytase which is derived from microbes especially recombinant bacteria on egg production and egg shell quality; and the effects of different diets on expression of the *CALB1* and *OPN* genes in layer chicken. The study was also to test whether laboratory produced phytase was of similar efficacy as that of commercial phytase.

2. Materials and methods

2.1. Experimental design and diets

Seventy-five White Leghorn layer birds at 15 weeks of age were procured from a commercial layer farm, housed in individual laying cages fitted with individual feeder and water supply. The cages were illuminated with 24-h lighting. Birds were fed standard diets till 22 weeks of age and then shifted to experimental diets by 23 weeks of age when all the birds were in peak egg production. Birds were randomly assigned to 5 treatment groups and each treatment group had 15 replicates. Dietary treatment groups consisted of a control diet with 0.33% non-phytate phosphorus (NPP) and 4 low P diets: 2 treatment diets (T1 and T2) with 0.24% NPP + 250 FTU/kg laboratory produced phytase or commercial phytase and another 2 treatment diets (T3 and T4) with 0.16% NPP + 500 FTU/kg laboratory produced phytase or commercial phytase with complete replacement of inorganic P. All the birds were offered feed *ad libitum* during the entire 17-wk experimental period. The composition of diet and the nutrient content for the experimental layers are given in Table 1. The experimental diet contained 2600 ME (kcal/kg), 17% CP and 3.5% Ca in all the groups.

Table 1
Ingredient and nutrient composition of the experimental layer diet (DM basis).[1]

Item	Control	T1 and T2	T3 and T4
Ingredient, %			
Maize	57.75	57.75	57.95
Soybean meal	23	23	23
De oiled rice bran	10	10	10
Limestone	7.61	8.11	8.41
Salt (NaCl)	0.30	0.30	0.30
$CaHPO_4$	1.0	0.5	–
$_{DL}$-methionine	0.09	0.09	0.09
Trace mineral and vitamin premix[2]	0.25	0.25	0.25
Nutrient composition, %			
ME,[3] kcal/kg	2,614	2,614	2,620
CP	17.1	17.1	17.1
Ca	3.53	3.50	3.42
Total P	0.632	0.552	0.472
Phytate P	0.308	0.308	0.308
Available P[3]	0.32	0.24	0.16
Lysine	0.74	0.74	0.74
Methionine	0.34	0.34	0.34
Threonine	0.62	0.62	0.62

[1] Control = 0.33% non-phytate phosphorus (NPP); T1 = 0.24% NPP + 250 FTU/kg laboratory produced phytase; T2 = 0.24% NPP + 250 FTU/kg commercial phytase; T3 = 0.16% NPP + 500 FTU/kg laboratory produced phytase; T4 = 0.16% NPP + 500 FTU/kg commercial phytase.
[2] Trace mineral premix, 1 g/kg; vitamin premix, 1 g/kg and choline, 0.5 g/kg. Trace mineral premix supplied mg/kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. Vitamin premix supplied per kg diet: vitamin A, 8250 IU; vitamin D_3, 30 mg; vitamin K, 1 mg; vitamin E, 40 IU; vitamin B_1, 2 mg; vitamin B_2, 4 mg; vitamin B_{12}, 0.01 mg; niacin, 60 mg; pantothenic acid, 10 mg.
[3] Calculated values.

2.2. Phytase for feeding trail

Laboratory produced phytase was produced by immobilization of *Aspergillus awamori* NCIM 885 strain procured from National Collection of Industrial Microorganisms, Pune following the technique of Lalpanmawia et al. (2014). The crude phytase enzyme obtained from fungal fermentation was filtered, clarified by centrifugation at 5590 × *g* for 15 min and precipitated from the media by 90% saturation of ammonium sulfate salts. The pellet obtained was dissolved in 0.2 mol/L sodium acetate buffer and respective FTU in the required volume was added in the measured layer diet and mixed uniformly. The commercial phytase (*Escherichia coli* derived product, 5000 FTU/g) was used for the present study. Phytase activity was determined as per the protocol described by Kim and Lei (2005) and phytate as per Haug and Lantzsch (1983). The Ca and P in feed were analyzed by inductively coupled plasma–optical emission spectroscopy ICP–OES using a Perkin Elmer instrument.

2.3. Egg production, egg weight and egg shell quality

Egg productions were recorded daily on individual basis. Daily weighed amount of feed was added and residue was recorded every 4 wk interval. Eggs of each bird were taken and weighted twice a week whereas the eggshell weight and the thickness were determined individually at 2-week intervals. Thickness of the eggshells was measured at 3 different locations (middle, broad and narrow end) using a micrometer gauge.

2.4. Collection of eggshell gland tissue samples

The birds were daily monitored for any leg weakness, lameness, etc. At the end of the trial, 6 hens per treatment group were sacrificed after egg production by cervical dislocation. Egg shell glands from the birds were detached from the oviduct, the bulbous were opened, and the inner layer was scraped with sterile micro slides and collected in the micro centrifuge tube containing 0.5 mL RNA later (Sigma Aldrich Cat No. R0901-100ML). Tissue samples were stored at 4 °C overnight and then stored at −80 °C for future use.

2.5. Isolation of total RNA and quality assessment

Total RNA was extracted from minimum 3 randomly selected tissue samples of ESG from each group using TRI Reagent (Sigma Aldrich, TRI Reagent BD Cat #T9424) according to the manufacturer's instruction. About 60 to 100 mg tissue samples in 1 μL TRI Reagent were homogenized in 2 μL micro centrifuge tubes and processed. The total RNA yields and purity were determined using NanoDrop ND-2000 UV–Vis Spectrophotometer (Thermo Fischer Scientific, Czech Republic) by the absorbance at 260 and 280 nm. The integrity of denatured RNA was determined by 1% agarose gel electrophoresis in 0.2 mol/L 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (pH 7.0). One microgram of sample with one volume of 5 × denaturing buffer were mixed properly and denatured at 60 °C for 10 min.

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