Effects of Locally Extracted Phytase on Blood Profiles, Phosphorus in Excreta and Economic Benefits of Supplementation in the White Leghorn Chickens

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Abstract: A study was conducted to evaluate the performances of White Leghorn chicken that fed diets with or without phytase supplementation. The experiment was conducted at a poultry farm of Haramaya University at a distance of 510 km east of Addis Ababa. At 25 weeks of age, 168 White Leghorn hens were randomly divided into four experimental feeds (treatments). Phytase was extracted from germinated rye grains. The hens were fed treatment diets containing phytase at the level of 0 (T_1), 300 (T_2), 600 (T_3), and 1200 (T_4) FTU/kg. Each pen contained 12 hens and 2 cocks per replicate in complete randomized design (CRD) and the feeding experiment lasted for 90 days. There were no significant ($p>0.05$) differences in total serum, albumin, cholesterol, and low-density lipoprotein of hens that were fed diets contained phytase at different levels and hens fed the control diet. However, the level of high-density lipoproteins (HDLP) in hens at the different treatments showed significant differences ($p<0.05$) at T3 and T4 than other treatment groups. Among the treatments, maximum net income was recorded from the diet contained phytase at 1200 FTU/kg (T_4), followed by chickens raised at T_3, T_2, and T_1. Phytase supplementation of a diet has also a significant reduction on Ca and P levels in the hen excreta. Based on the results of this study, supplementation of hen's diet with phytase has improved high density lipoprotein of serum, net income and bioavailability of Ca and P to the body of layers of White Leghorn without affecting the hematology of layers.

Keywords: *Blood profiles, Excreta, Layer chicken, Locally extracted phytase*

Introduction

Phytase (phymyo-inositol hexaphosphate phosphohydrolase) belongs to a specific group phosphatase that is capable of hydrolysing phytate to a series of lower phosphatase esters of myo-inositol and inorganic phosphate (Tijskens *et al*., 2001). As more phosphorus is released from phytate leading to more breakdown of intact IP-6, the less able it is to bind or chelate minerals, starch or proteins directly or *via* ionic bridges (Selle and Ravindran, 2007). Reducing the phytate-bound compounds through the use of phytase may enhance the bioavailability and digestibility of phosphorus and divalent or trivalent cations but also indirectly increase energy and nitrogen utilization (Selle *et al*., 2003).

Higher elimination of phosphorus in the excretions considered as an environmental problem that resulted the contamination of rivers, lakes and underground water. Such problems can be even aggravated when the bird's manure is used as fertilizer since the amount of phosphorus added to the soil exceeds the plant's requirements for phosphorus and excess of it is easy to go to the groundwater, rivers, lakes and oceans and can lead to mortality of aquatic animals by stimulating algae growth. According to Syers *et al*. (1973), phosphorus and nitrogen are considered the limiting elements for plant growth because their excess causes an increase of the eutrophication process and consequently reduction of the water quality.

Phytase is an enzyme that makes the phosphorus from phytate available for animal digestion. Phytase can reduce the antinutritional effect of phytate and improve the digestibility of phosphorous (P), calcium, amino acids and energy, as well as reduce the negative impact of inorganic P excretion to the environment (Yueming *et al*., 2015). In the 1960's, a group of scientist working for international minerals and chemicals corporation recognized that the need to supplement the diets of monogastric animals with inorganic phosphorus could be decreased if phytate phosphorus could be made available to animals by treating the grains with phytase. Inclusion of phytase enzyme to monogastric diets reduces phosphorus excretion due to this it reduces reduce the amount of phosphorus concentration phosphorus from the environment (Jongbloed and Lenis, 1992).

For these mentioned reasons phytase is being used in poultry industry to enhance more nutrients utilization and reduce pollution problems emanating from phosphorus in faeces. Hematological parameters serve as indicators of the physiological and nutritional state of the birds (Chowdhury *et al*., 2005). Hematological indices are reflection of the effects of dietary treatments on the animals in terms of type, quality and amount of the feed ingested and were available for the

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animal to meet its physiological, biochemical and metabolic necessities (Ewuola and Egbunike, 2008; Pace, 2014). Fijabi *et al.* (2018) and Ahmed *et al.* (2017) reported that inclusion of phytase up to 1000 FTU/kg did not alter some blood parameters of broilers and layers, respectively. Therefore, the present study aimed to evaluate the effect of locally extracted phytase on hematology, serum metabolites, phosphorus and calcium contents in excreta of White Leghorn layers.

Materials and Methods

Description of the Study Area

The experiment was conducted at Haramaya University poultry farm at a distance of 510 km east of Addis Ababa, located at 42°3' east of longitude, 9°26'north latitude and an altitude of 1980 meter above sea level. The mean annual rainfall of the area is 780 mm and the average minimum and maximum temperature are 8.5 and 24.4°C, respectively (Mishra *et al.*, 2004).

Experimental Animals Management and Treatments

Before the commencement of the actual experiment, the experimental pens, watering and feeding troughs and laying nests were thoroughly cleaned, disinfected and sprayed against external parasites. One hundred sixty eight White Leghorn chicken breed with similar body weight (BW) (mean±SD) of 25 weeks age had taken from Haramaya University Poultry Farm and randomly distributed into four experimental rations containing phytase at the level of 0 (T_1), 300 (T_2), 600 (T_3), 1200 (T_4) FTU/kg. The birds were divided into three replicates with 12 layer hens and two cocks per replicate in complete randomized design (CRD) experiment and were fed experimental diets for ninety days of feeding trial. The birds were kept in deep litter floor housing, which was covered with sawdust litter material of about 7 cm depth. The wet litter has changed with dry, disinfected and clean sawdust whenever required. Birds in a pen were fed *ad libitum* in a group and feeds were offered twice a day at 8:00 and 4:00 hours throughout the experimental period. Water has been available at all the time. The house has 16 hours lighting across the experimental period. Birds were adapted the experimental diets for one week before the commencement of the actual data collection.

The experimental birds were fed on the diet supplemented with levels of phytase enzyme at 0.0 FTU/kg, 300 FTU/kg, 600FTU/kg and 1200FTU/kg on layers ration representing T_1, T_2, T_3 and T_4, respectively.

The phytase activity is expressed in phytase units (FTU). One phytase unit (FTU) is defined as the amount of enzyme that liberates 1 micromole of inorganic phosphorus per minute from 0.0051 mol/l sodium phytate at 37° and pH 5.50 under the conditions of the test (Engelen *et al.*, 2001). The inclusion rate became 0.1 g/kg for the experiment. The pH and temperature optima were around 4.5-6.0 and 45-60 ^{0}C. The enzyme was provided in the forms of powder and added to the diet before mixing. These procedures allow the enzymes to mix intimately with the dietary ingredients and this allows them, at least potentially, to react effectively with their substrates (Acamovic, 2001).

Table 1. Feed ingredients proportion of layers ration.

Ingredients	**Percentage (%)**
Maize	46.00
Wheat short	15.00
Soybean meal	13.39
Dicalcium phosphate	1.00
Nougseed cake	15.00
Salt	0.50
*Vitamin Premix	1.00
Limestone	7.00
Methionine	0.01
Lysine	0.10
Dicalcium phosphate	1.00
Total	**100.00**

** Vitamin premix 50 kg contains, Vit A = 2000000iu, Vit D3 = 400000 iu, Vit E = 10000 mg, Vit K3 = 300 mg, Vit B1 = 150 mg, Vit B2 = 1000 mg, Vit B3 = 2000 mg, Vit B6 = 500 mg, Vit B12 = 4 mg, Vitpp = 60000 mg, Folic acid = 160 mg, Choline chloride = 30000 mg, Anti-oxidant = 500gm, Manganese = 10000 mg, Zinc = 14000 mg, Iron = 9000 mg, Copper = 1000 mg, Sodium = 200 mg, Selenium = 80 mg, Calcium = 28.2%.*

Table 2. Chemical composition of feed ingredients used to formulate experimental ration**.**

Chemical components	**Ingredients**			
	Maize	SBM	NSC	WS
DM (%)	90.85	94.25	93	90.15
CP (% DM)	8.9	42.5	33.4	16.3
EE (%DM)	2.9	7.2	7.3	2
Ash (%DM)	7.5	6.3	11.3	11.9
CF (%DM)	3.9	5.1	16.2	7.4
Ca (% DM)	0.06	0.3	0.26	0.13
P (% DM)	0.32	0.65	0.67	0.89
ME(Kcal/kg)	3456 .83	3633.27	2450.14	2917.9

DM= Dry matter; CP= Crude protein; EE= Ether extract; CF = Crude fiber; Ca= Calcium; P= Phosphorus; ME = Metabolizable energy; SBM= Soybean meal; NSC= Noug seed cake; WS= Wheat short.

Based on chemical composition of feed ingredients the treatment ration was formulated (Table 1 and 2) to be iso-caloric and iso-nitrogenous with 2800-2900 kcal ME/kg DM and 16-17% CP to meet the nutrient requirements of layers (NRC, 1994).

Chemical Analysis

Representative samples of formulated feed were analyzed before starting the experiment for chemical composition. According to the proximate analysis method (AOAC, 2000) chemical analysis of experimental feeds were carried out for dry matter/DM, ether extract/EE, crude fiber/CF, ash and nitrogen content were determined by Kjeldahl procedure and crude protein/CP calculated as Nx6.25. Metabolizable energy of the experimental diets were determined by indirect method according to the formula given by Wiseman (1987) as follows:

ME (Kcal/Kg DM) =3951+54.4 EE-88.7 CF- 40.8 ash

Extraction Procedure of Phytase Enzyme from Rye

Rye grains were purchased from a local market of Harar town. The grains had been soaked for twelve hours with deionized water earlier than sprouting become taken into consideration underway. At 12 hours, the deionized soak water was poured off and one pint of deionized water was added to the jars. The grains were soaked for 5 seconds and the water was poured off. The excess water was shaken out of the jars before the jars were set up, mouth downward, to drain the seeds. Once a day the grains were rinsed with sterile water; after rinsing the water was removed completely. The extra water become shaken out of the jars earlier than the jars had been set up, mouth downward, to empty the seeds. The grains had been then allowed to germinate on sterile bins with inside the darkish at 20^0C. One hundred ml of 1.2% HCl was added to each flask. The flask was sealed with plastic wrap. The flasks were shaken at 200 rpm for two hours at 26°C. The samples were vacuum filtered with #1 Whatman Filter paper. The filtrate was stored, not more than one week, in the refrigerator at 1°C. Sample were weighed and placed in 250 ml Erlenmeyer flasks, then extracted by the procedures of (Harland and Oberleas, 1977). Then phytase was produced from the extracted germinated rye. The enzyme extraction was conducted at Biotechnology Laboratory of Haramaya University.

Hematological and Serum Biochemical Parameters

At the end of the experiment, blood samples (5 ml each) were collected from the wing vein of 12 hens from each treatment. Hematological and serum biochemical analysis was conducted at Haramaya University veterinary physiology laboratory and Haramaya University higher clinic laboratory, respectively. For, the blood analysis, 2.5 ml of blood was collected using EDTA (Ethylene diamine tetraacetic acid) tube while the remaining 2.5 ml was collected in a plain tube and left to coagulate. Blood samples were analyzed for total red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), total white blood cell (WBC), total protein (TP), and serum cholesterol concentration. RBC and WBC were determined by using an improved Neubauer hemocytometer chamber (Dacie and Lewis, 1991). Hemoglobin concentration was determined by using acid hematin or Sahli's methods. The packed cell volume (PCV) by micro-hematocrit (capillary) tubes method and centrifuged at 3000 rpm for 5 minutes (Dacie and Lewis, 1991). Finally, serum was harvested from blood collected in a plain tube which was transferred to an Endorphin tube and stored at -20°C and analyzed for serum chemistry parameters (serum total protein and albumin, total cholesterol count, HDL-C, and LDL-C) with an automated chemistry analyzer (Biotechnical, Targa 3000, US) using a commercial kit (Douglas *et al.*, 2010). The globulin value was determined as the difference between serum total protein and albumin (Doumas *et al.*, 1981).

Phosphorus and Calcium Analysis from Excreta

Excreta was sampled daily per pen and pooled per treatment for the entire experimental period for chemical analysis. Calcium and phosphorus content from excreta were analyzed by spectrophotometer (AOAC, 2000).

Partial Budget Analysis

The partial budget analysis was conducted to determine the profitability of the supplementation of phytase in layer ration. The analysis considered the cost of feed (variable cost) consumed by the birds, selling prices of eggs and other costs were assumed to be similar for all the treatments. The analysis involved calculation of the variable cost and benefits. Net income (NI) or Net return (NR) was calculated as the amount of money left when total variable costs (TVC) were subtracted from the total returns (TR) as follows:

NI (NR) =TR–TVC

The change in Net income (ΔNI) or Net return (ΔNR) were calculated by the difference between change in total return (ΔTR) and the change in total variable costs (ΔTVC) as follows:

ΔNI (**Δ**NR) = ΔTR-ΔTVC

The marginal rate of return (MRR) measures increases in net income (ΔNI) associated with each additional unit of expenditure (ΔTVC) and it was calculated as follows:

$$\Delta MRR = \frac{\Delta NR}{\Delta TVC}$$

Where,

MRR=Marginal Rate of Return
ΔNR=Changes in Net Return
ΔTVC=Changes in Total Variable Cost

Feed cost per dozen of egg was obtained by dividing feed cost to total egg production of each treatment and multiplying by twelve.

Egg sale per feed cost measures increase in return per unit of expenditure was calculated as:

$$\text{Egg sale per feed cost} = \frac{\text{Gross Income}}{\text{Feed cost}}$$

Statistical Analysis

All the data collected in this study were subjected to statistical analyses using SAS (2016). When the analysis of variance revealed the existence of significant differences among the dietary treatments, Duncan's multiple range tests was used to locate the treatment means that are significantly different at P<0.05.

Results and Discussion

Hematology and Serum Biochemical Parameters

Hematology and serum biochemical parameters are presented in Table 3. The results of hematological parameters in this study suggested that the test diets did not pose any severe effects on the health status of the experimental birds.

Table 3. Effect of phytase enzyme on hematological and serum biochemistry of White Leghorn layer chickens.

Parameters	Treatments					
	T_1	T_2	T_3	T_4	SEM	SL
Packed cell volume (%)	30.05	29.83	30.52	31.18	0.72	NS
Red blood cell (10^6 /µl)	3.00	2.87	3.19	3.29	0.02	NS
Hemoglobin (g/dl)	9.66	9.43	10.02	10.20	0.34	NS
Total serum protein (g/dl)	4.00	3.98	3.90	3.80	0.03	NS
Albumin (g/dl)	1.72	1.69	1.66	1.63	0.01	NS
Globulin (g/dl)	2.27	2.28	2.24	2.26	0.001	NS
Albumin/Globulin	0.75	0.74	0.76	0.75	0.001	NS
Total cholesterol (mg/dl)	164.90	174.73	166.92	174.27	3.65	NS
High density lipoprotein (mg/dl)	37.10[b]	39.73[b]	43.800[a]	44.867[a]	1.10	**
Low density lipoprotein (mg/dl)	99.33	96.67	94.45	93.38	1.42	NS
White blood cell (10^3 /µl)	6.50	5.00	7.50	5.83	3.20	NS

[ab]Means within the same row with different superscript letters are significantly different at P<0.05; NS= Non-significant, SEM= Standard error of the mean; SL= significance level; T_1= Control group, T_2= 300 FTU/Kg, T_3= 600 FTU/Kg, T_4= 1200FTU/Kg.

The results of the serum analysis from the hens blood revealed that there was no significant difference (P>0.05) in total serum, albumin, cholesterol and high-density lipoprotein that fed the phytase enzyme supplemented diet and the control diet. However, HDLP was significantly different (P<0.05) among treatments. HDL-cholesterol concentration was significantly high (P<0.05) at a high-level of T3 and T4. This result was in line with the finding of Pace (2014) who reported HDL-cholesterol concentration increased with on phytase supplemented treatment groups. The reason for the reduction of LDL-C and increase of HDL-C were increase utilization of polyunsaturated fat in specifically omega-3 and omega-6 fatty acids (Mohammed *et al.*, 2020). Similar research has shown that these essential fatty acids can lower bad cholesterol and prevent cardiovascular disease (Sateri *et al.*, 2017).

Treatment test shows that there were non-significant (P>0.05) difference between hens in the control diet and treated diet in average WBC. In Table 3, it was observed that average counts of WBC were from hens in the control and treated groups, respectively. The comparable WBC of birds suggests that the animals were apparently healthy (Ahamefule *et al.*, 2008) during the experimental period.

The findings in Table 3 show that average red blood cell counts varied in different treatments T1, T2, T3, and T4, respectively. According to Fudge (1999), the values obtained for RBC of layers fed phytase enzyme diets were between the range of 3.07 to 7.50 × 106/mm3. Red blood cells are responsible for the transportation of oxygen and carbon dioxide in the blood as well as manufacture of hemoglobin hence higher values indicate a greater potential for this function and a better state of health (Okeudo *et al.*, 2003).

Normal blood hemoglobin was in T1 (control) and in treated groups T2, T3, and T4, respectively (Table 3). In treated group T4 the maximum hemoglobin was counted 10.2 g/dl and in T1 average hemoglobin was counted 9.66 g/dl and showed a non-significant (P>0.05) difference between control group and treatment groups.

The average packed cells volume (PCV) from the hens in control and treated groups are showed on Table 3. Maximum and minimum PCV were counted in T4 (31.18 %) and T2 (29.83%). However, in the present findings, PCV counted was not significantly (P>0.05) different among the treatments. The same, results on the total protein (TP) showed a non-significant difference among the treatments.

Partial Budget Analysis of Phytase Supplementation

The prices of feed ingredients used were obtained from market. By recording total feed intake, feed cost, feed consumption and total egg production of laying chickens for each treatment during the study and calculating the economics associated with feed cost determined the benefits of supplementation (Table 4). Maximum net income was recorded at T_4 (1200 FTU/kg), followed by chickens raised at T_3, T_2 and T_1.

Base on the result T_4 chickens had the highest total revenue, increased revenue per unit spend, and a good ratio of egg sales to feed costs. This difference can be due to feed conversion ratio and feed intake, which leads to differences in egg production. The marginal rate of return (MRR) indicates that for each additional egg cost, 1 ETB of additional units yields an additional profit of -0.17, -0.21, and 0.15 ETB for T_2, T_3, and T_4, respectively. Among the treatments, T_4 was found to be more profitable when net return was taken into account. When taking MRR into account, T_4 also was more economical than others. As a result, diet from T_4 is selected based on net return. Therefore, the results of this study suggest that diet supplemented with 1200 FTU / kg phytase enzyme (T_4) could make more money than other supplemental levels.

Table 4. Partial budget analysis for layers which feed control and phytase supplemented diet.

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Cost/100kg diet	1140	1208	1276.8	1345.6
Total feed consumed (kg)	325.81	335.44	346.66	353.77
Total feed cost/treatment (Birr)	3714.27	4052.08	4423.38	4760.33
Total variable cost	4764.27	5102.08	5473.38	5810.33
Total egg produced	1349.784	1437.91	1532.84	1636.52
Gross income (total return) Birr	9448.48	10065.38	10729.88	11455.67
Net return (Net income) Birr	4684.22	4963.30	5256.50	5645.34
ΔTR	0.00	279.08	293.19	388.84
ΔTVC	0.00	337.81	371.30	336.95
ΔNR	0	-58.73	-78.10	51.89
MRR	-	-0.17	-0.21	0.15
Egg sale/feed cost	2.54	2.48	2.42	2.41
Feed cost/dozen egg(birr)	33.02	33.81	34.62	34.91

TFC= Total feed cost, TVC= Total variable cost, TR= Total return, NR= Net return, ΔTR= Change in Net return, ΔTVC= Change in Total Variable Cost, ΔNR= Change in Net return, MRR= Marginal rate of return, T_1= Control group, T_2= 300 FTU/kg, T_3= 600 FTU/kg, T_4= 1200FTU/kg.

Effect of Phytase on Phosphorus and Calcium Content in Hen Excreta

Effects of phytase supplementation on the excreta calcium and phosphorus are presented in Table 5. Treatments have significant ($P<0.05$) effect on excreta Ca and P. Significant reduction of Ca and P excretion was observed by phytase supplementation of diet ($P<0.05$). Phosphorus reduction was recorded among treatments supplemented with different levels of phytase enzyme and the control diet. However, Ca reduction did not recorded between birds fed 300, 600 and 1200 FTU/kg due to the level of phytase inclusion.

Table 5. Effects of phytase enzyme supplementation in the diets of layer chickens on their excreta concentration of Ca and P.

Treatments	Excreta Ca (%ash)	Excreta P (%ash)
Control	6.30[a]	0.79[a]
300	4.37[b]	0.60[b]
600	3.12[b]	0.48[c]
1200	2.495[b]	0.42[c]
SEM	0.577	0.029
P-level	0.007	0.003

[a-c]Means within a column with different superscript letters are significantly different (P<0.05); SEM=Standard error of the mean.

According to Um and Paik (1999), the reduction of P excretion was recorded with supplementary phytase. This results in line with the finding of Pace (2014) who reported that phytase improved availability of phytate phosphorus in layer diets. Our results agree with the finding of Gordon and Roland (1997) and Van der Klis *et al.* (1997) who reported that phytase definitely increases P utilization in corn-soybean meal diets for laying hens. The study of Qian *et al.* (1997) who stated that high Ca may suppresses phytase ability to hydrolyze phytate P by competing for the active sites of the enzymes. Further, phytate would be less likely to bind Ca and the efficacy of bacterial phytases would be influenced to a lesser extent by Ca-phytate complexes. In the present study Ca content of excreta in phytase supplemented groups were reduced as compared to control. The study of Plumstead *et al.* (2008) reported a linear reduction in ileal phytate P degradation by 71%, when increasing dietary Ca level from 4.7 to 11.6 g/kg in broiler diets. However, the present study results were in contrast to Shirley and Edwards (2003) and Rabie *et al.* (2015) who reported phytase inclusion did not affect P and Ca retention, respectively.

Conclusion

There was no significant difference in total serum, albumin, cholesterol and low-density lipoprotein among phytase supplementation and the control diet, but content showed a significant ($P<0.05$) difference among treatments with regard to HDLP. In the present study results on partial budget analysis, the maximum net income was recorded at T_4 (1200 FTU/kg), followed by chickens raised at T_3, T_2, and T_1. Phytase

supplementation of a diet has a significant reduction on excreta Ca and P. Based on the results of this study, inclusion of phytase enzyme in layer chicken diet has improved high density lipoprotein of serum, net income and bioavailability of Ca and P to the body of layers of White Leghorn without affecting the hematology of layers.

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Conflict of Interests

The authors declare that they have no competing interests.

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