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Comparative Effect of Microbial Phytase Supplementation on Layer Chickens Fed Diets with Required or Reduced Phosphorous Level

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ABSTRACT

An experiment was conducted to determinethe effect of microbial phytase supplementation on layer`s feed. One hundred and sixteen 23-week-old Lohman brown laying hens were used in a 8-weeks feeding trial. Hens were randomly allotted into four treatments where the group (1) (control group) was fed basal diet with normal non-phytate phosphorous (0.38%) without phytase, group (2) fed diet with normal NNP (0.38%) and supplemented with phytase, group (3) fed diet with NNP 0.32% and supplemented with phytase enzyme and group (4) fed diet with NNP 0.26% and supplemented with phytase. Results revealedthat egg weight showed significant (p<0.05) increasein all phytase supplemented groups. Egg shell weight increasedsignificantly (p<0.05)in all phyatse supplemented groups when compared with the control group also shell thickness increased significantly (p<0.05) in both group (2 &3). No significant (P≥0.05) difference was observed in serum Ca,P level while alkaline phosphatase was significantly (P<0.05) increased in group (3). Egg shell analysis showed increase in egg shell ashand egg shell calcium % % in group (3) and group (4) when compared with the control group. Egg shell phosphorous% was higher in all phytase supplemented groups than the control group.Dietary 0.26% NNP level supplemented with phytase enzyme significantly (p<0.05) increase Ca availability. Also dietary 0.32% and 0.26% NNP level supplemented with phytase enzyme significantly increase (p<0.05) P availability by 33.85% and 20.7%. Phytase supplementation in laying hens ration is not recommended without reduction of dietary NNP.

Key Words: Layers, Microbial phytase, Ca and P availability, Egg production and Egg charactristics

Introduction

Poultry production in Egypt has become one of the biggest agriculture industries and its improvement is one of the main objectives of both private and public sectors. Layers need special feeding care where corn and soybean meal are the major feedstuffs in their diets. Phytate is considered the major form of phosphorus in cereal grains, beans and oilseed mealswhich considered the primarily feed stuffs in poultry diets. Phytate phosphorous is poorly utilized by poultry birds due to lack of endogenous phytase enzyme **(Khan et al. 2013, Angel et. al 2002**). Phytate has antinutritional effects in poultry due to its ability to form insoluble complexes with essential minerals and proteins and also leads to more excretion of excess phosphorus into the environment which considered a serious cause of environmental pollution **(Diarraet. al. 2010)**. The inclusion of microbial phytase in poultry diets has widely increased in the last few decades in order to

decrease phosphorus pollution in the environment and to make phosphorus available to birds from phytate**(Khan et al. 2013)**.The use of phytase enzymes in poultry diet leads to liberating phosphorus and other phytate bound nutrients so it`s now more common to use and there are several commercial phytase enzymes available on the market. Microbial phytases may partially or completely replace inorganic P supplementation in poultry diets and this replacement can reduce P excretion by up to 50% and enhance bioavailabilities of Ca **(Emmenes 2014, Lei and Stahl 2000)**. Therefore, the goal of the study is toinvestigate the possible effect of dietary supplementation of phytase enzyme on productive performance parameters, some blood parameters and nutrient digestibility of layinghens.

MATERIAL AND METHODS

1. Birds, accommodation and management

The present study is affirmed by the Committee on the Ethics of Animal Experiments of Damanhour University, Egypt. A total of 116 Lohman Brown (LB), 23 weeks of age were obtained from a local private farm (Bassuin, Gharbia, Egypt), weighed individually and allocated into four groups (29 hens per group) separated from each other by tightly wire walls and each group was housed in 2.5x2.5 m with suitable feeder and waterer. Hens house were provided with 14 hours light and 10 hours dark in 24th week and with 15 hours light and 9 hours dark at the 25th week and 16 hours light and 8 hours dark from the 26th week till the end of experiment. The hens kept in the house for 15 days before the start of the experiment and fed layer diet.The firstthree weeks of the experiment the feed was offered as 110 g/bird/day in all experimental groups at the first three weeks of the experiment and from 4th week till the end of the experiment the offered feed was 120 g/bird/day**.**

2. Experimental design and feeding program

Hens were randomly allotted into four treatments where the group (1) (control group) was fed diet with non-phytate P (0.38%) without phytase, group (2) fed on diet with non-phytate P (0.38%) and supplemented with phytase, group (3) fed on diet with non-phytate P (0.32%) and supplemented with phytase and group (4) fed on diet with non-phytate P (0.26%) and supplemented with phytase. The laying hens were fed on the basal diet formulated from a corn, soybean meal based diets. The diets were formulated according to the recommendation book of LOHMAN BROWN®. The chemical analysis of the basal diet was calculated according to **NRC, 1994.**Ingredient composition (%) and calculated chemical analysis of the basal and experimental dietsare showed in Table (1).

3. Sample collection

Five blood samples were taken from each group from the wing vein and each sample was evacuated in sterilized glass tube and left to coagulate in room temperature and then put in the centrifuge at 3000 rpm for 5 minutes where the clear serum was separated. The serum then transferred into sterilized vials and kept in deep freezer until chemical analysis. At the end of experiment the daily feed intake and faeces voided were recorded from each experimental group (Five chicks in each group were housed with special modification) for 3 successive days, and the samples of feces was dried then kept for chemical analysis (calcium and phosphorus) and nutrient availability. Egg shell was dried and crushed and kept for chemical analysis (protein, dry matter, ash, calcium and phosphorous).

4. Blood parameters

Serum calcium, phosphorous,alkaline phosphatase, total serum protein, albumin, globulin and uric acid concentrations according to **AOAC, 1990**;**Fiske and Subba row, 1925;Bergmeyer, 1974;Doumas et al., 1981; Reinhold, 1953; Coles, 1974** and **Fossatti and Prencipe, 1980** respectively.

5. Digestibility coefficient:

Nutrient digestibility was calculated according to the following formula: Nutrient digestibility = 100 – (100x % acid insoluble ash in feed/% acid insoluble ash in feces) x %nutrient in feces/ % nutrient in feed)(**Goddard and McLean, 2001**)

6. Analytical methods:

Analytical DM contents of egg shell samples were determined by oven-drying at 105°c for 48 h (**AOAC, 1990**). Ash content of egg shell samples was determined by incineration at 550°c overnight. HCl insoluble ash was determined according to (**Hart and Fisher, 1971**). Calcium of fecal sample and egg shell were determined by flame photometer according to (**Slavin, 1968**), Phosphorus in fecal samples and egg shell were determined by colourimeteric procedure according to (**Geriche and Kurmies, 1952**). Eggshell thickness was measured by using a micrometer thickness gauge (Vernier Caliper, China).

7. Statistical analysis

Data were analyzed using GLM procedure of the statistical analysis system software (**SAS, 1996**) with dietary treatment as the mean effect. Means were separated using the least square means of the same program, and the level of significance was 0.05.

RESULTS

1- Effect of phytase on performance of laying hens:

The present data in table(2) showed that the body weight at the beginning of the experimental groups did not differ significantly and Phytase supplemented with normal NPP level non-significantly ($p\geq0.05$) reduced body weight change of laying hens throughout the experimental period by about 14.7% compared with the control group (**Table 4**). Moreover, it was observed that NNP reduction to 0.32% or 0.26% with phytase supplementation significantly ($p<0.05$) reduced body weight changes throughout the whole experimental periods by about 35.4% and 20.5% respectively compared with control. Reduction of NNP to 0.32% and 0.26% with phytase supplementation lead non-significant ($p\geq0.05$))increasein average egg number per hen per week, while supplementation of phytase without reduction of NNP lead to non-significant ($p\geq0.05$) decrease in egg number per hen per week. Regarding average egg production% throughout the whole experimental periods it was observed that phytase supplementation without reduction of dietary NNP non-significantly ($p\geq0.05$) reduced egg production% by about 1.6% compared with the control group. However NNP reduction to 0.32% or 0.26% with phytase supplementation non-significantly ($p\geq0.05$) increased egg production% by about 0.65% and 0.35% respectively compared with the control.

Throughout the whole experimental period it was observed that dietary 0.38, 0.32 or 0.26% of NNP with phytase supplementation significantly ($p<0.05$) increased egg weight by about 2.9%, 3.4% and 2.6% respectively

compared with control fed on basal diet without phytase supplementation.Regarding average egg mass throughout the whole experimental period it was observed that phytase supplementation with 0.38% NNP had no effect on average egg mass (51.68 g/hen/day) compared with laying hens group fed on the same diet without phytase enzyme supplementation (51.59 g). However, 0.32% or 0.26% of NNP with phytase supplementation non-significantly ($p\geq0.05$) increased average egg mass of laying hens about 2.9% and 1.7% respectively compared with control. There was a nonsignificant ($p\geq0.05$) differencein averagefeed intake among all experimental groups. The average of feed conversion ratio all over the experimental period showed nonsignificant ($p\geq0.05$) differences between all experimental groups but laying hens receiving phytase as a 25%replacement of monocalcium phosphate was the lowest numerically.

2- Effect of phytase supplementation on egg quality:

The data presented in Table (3)showed that egg width, egg length, egg shape index, yolk height, yolk width, yolk index, yolk weight and albumin /yolk ratio in all experimental groups were non significantly ($p\geq0.05$) different, while albumen weight in laying hens in group (2) andgroup(3)was higher than the control group. Albumen weight in laying hens in group (4)showed nonsignificant($p\geq0.05$) differencewhen compared with the control group and other phytase supplemented groups but was numerically higher than the control group. Shell weight in all laying hens receiving diets supplemented with phytase was higher than the control group.Shell thickness in laying hens in group (3) and group (4) was higher than the control group while the phytase on top group showed nonsignificant ($p\geq0.05$) difference than the control group.

3- Effect of phytaseon some blood parameters:

Results presented in table (4) showed that there wasnonsignificant($p\geq0.05$) increasein blood serum calcium and phosphorous levels in laying hens in group (3)and laying hens in group (4) than the control group while the lowest calcium level was in the laying hens receiving diet supplemented with phytase as on top. Serum alkaline phosphatase in laying hens in group (3)was significantly ($p<0.05$) increased than the other experimental groups while hens in group (4) showed nonsignificant($p\geq0.05$) difference than the other experimental groups. Serum total protein, serum albumin, serum globulin and serum albumin globulin ratio were nonsignificant($p\geq0.05$)differentin all experimental groups. Uric acid showed a significant ($p<0.05$) increase in both hens in group (3) and group (4)than the control group.

4- The effect of phytase on egg shell chemical analysis

The present results in table (5) showed no differences in egg shell protein % in all experimental groups.Reduction of NNP to 0.32% and 0.26% and supplemented with phytase showed higher egg shell ash by 2.2% and 2.8% than the control group while NNP 0.38% without phytase showed decrease in egg shell ash by 1.9% than the control group. Egg shell calcium% at the end of the experimental period was numerically higher in both laying hens fed in ration with NNP 0.32% and 0.26% and supplemented with phytase by (8.3% and 10.8%) when compared with the control group.
Egg shell phosphorous% at the end of the experimental period was numerically higher in

all phyatse supplemented groups (2, 3 and 4) by (10.1%, 15.1% and 12.7% respectively) when compared with the control one.

The effect ofphytase on phosphorous and calcium availability:

It was observed that dietary 0.26% NNP level supplemented with phytase enzyme significantly ($p<0.05$) increase Ca availability by 22.7% than the control. However, dietary 0.38% and 0.32% NNP supplemented with phytase enzyme non-significantly increase ($p\geq0.05$) Ca availability by 15.17% and 4.63%than the control. On the other hand dietary 0.32% and 0.26% NNP level supplemented with phytase enzyme significantly increase ($p<0.05$) P availability by 33.85% and 20.7% while dietary 0.38% NNP level supplemented with phytase enzyme showed non-significant ($p\geq0.05$) increase by 7.8% than the control.

Discussion

1- Effect of phytase on performance of laying hens:

Effect of phytase on body weight: it was observed that normal NPP level non-significantly ($p\geq0.05$) reduced body weight change of laying hens throughout the experimental period by about 14.7% compared with the control group (**Table 4**). Moreover, it was observed that NNP reduction to 0.32% or 0.26% with phytase supplementation significantly ($p<0.05$) reduced body weight changes throughout the whole experimental periods by about 35.4% and 20.5% respectively compared with controlthese results are disagree with(**Amin and Hamidi, 2013**) and(**Keshavarz, 2000**) who found that the effect of phytase on body weight was significant ($P < 0.05$) due to consistently greater BW in the presence of phytase than in the absence of phytase in the diet. Because the genetics of the layers which have a tendency to manufacture eggs rather than building up of body tissues particularly at the beak of production the effect of phytase supplementation on body weight is a secondary effect**(Amin and Hamidi, 2013)**.

Egg number per hen per week showed nonsignificant differences ($p\geq0.05$) between all experimental groups and also egg production% showed nonsignificant ($p\geq0.05$) differencein phytase supplemented groups and control group, these results are in agreement with (**Liebert et al., 2005**), (**Meyer and Parsons, 2011**), (**Wang et al., 2013**) and (**Jalal andScheideler, 2001**).

Phytase supplemented groups showed significant ($p<0.05$)higher egg weight than the control group,these results are in agreement with those obtained by (**Keshavarz, 2000**), (**Ahmadi et al., 2008**) and (**Peter, 1992**) who recorded that phytase generally had a favorable effect on egg weight and disagree with (**Hassanien and Elnagar, 2011**) and (**Panda et al., 2005**) who found that the phytase supplementation on egg weight was not significant ($p\geq0.05$). Egg mass per hen is showed nonsignificant ($p\geq0.05$) differencebetween all experimental groups, these results are in agreement with (**Meyer and Parsons, 2011**) who found that there was no significant($p\geq0.05$) differenceobserved among the dietary treatments in egg masswhile on the other hand the present results are disagree with the finding obtained by (**Jalal and Scheideler, 2001**) and(**Hassanien and Elnagar, 2011**) who found that, the supplementation of phytase in normal corn-soybean meal diets improved egg mass.

The nonsignificant($p \geq 0.05$) difference in average feed intake among all experimental groups was agreed with the finding obtained by (**Wang et al., 2013**), (**Zaghari, 2009**), (**Keshavarz, 2003**) and (**Meyer and Parsons, 2011**) who found that there was no significant ($p \geq 0.05$) differencein average daily feed intake also agree with (**Hassanien and Elnagar, 2011**) who found that the addition of phytase increased feed consumption but the difference not significant ($p \geq 0.05$). The average of feed conversion ratio all over the experimental period showed nonsignificant ($p \geq 0.05$) differences between all experimental groups, these results are in agreement with (**Hassanien and Elnagar, 2011**) who found that the addition of phytase increased feed conversion ratio but the difference not significant ($p \geq 0.05$) and also disagree with(**Jalal and Scheideler, 2001**) who found that supplementation of phytase in normal, corn-soybean meal diets improved feed conversion.

2- The effect of phytase on egg quality:

The results of the present experiment of the egg shape parameters, yolk index and yolk weightwhich showed nonsignificant ($p \geq 0.05$) differences are in agreement with (**lucky et al., 2014**) who found shape index, yolk index, yolk percent had no relation with dietary exogenous phytase. Also agree with (**Harsini et al., 2009**) who found that the supplementation of phytase had no significant ($p \geq 0.05$) influence on yolk index and egg shape index andagree with (**Ahmadi et al., 2008**) who found that phytase supplementation did not affect yolk weight, although albumen and shell weight were significantly affectedalso agreement with (**Narahari and Jayaprasad, 2001**) and (**Metwally, 2006**) they found a beneficial effect of phytase supplementation on shell quality.

3- The effect of phytaseon blood parameters

The Previous studies have stated that the serum P concentration seems to be less indicative of phytase efficacy than total tract P digestibility and retention of dietary P (**Yi and Kornegay, 1996** and **Jongbloed and Mroz, 1999**). The obtained results of nonsignificant ($p \geq 0.05$) difference in blood calcium and phosphorous levels among all experimental groups.These results are in agreement with Lan et al. (2002) showed that phytase had no significant effect on plasma Ca. On the other hand the present results are disgreewith (**Yan et al., 2009**) and (**Attia et al., 2001**) who reported that a significant increase in plasma Ca and P was noticed due to phytase addition. Alsodisagree with(**Musapuor et al., (2005**) reported that dietary phytase caused a significant ($p<0.05$) decrease in plasma alkaline phosphatase activity.

4- The effect of phytase on egg shell chemical analysis

Beneficial effect of phytase was found in egg shell ash, egg shell calcium and egg shell phosphorous especially in both laying hens in group (3) and group (4) In general, eggshell quality increases concurrently with the increase in digestibility that occurs in response to phytase supplementation (**Yan et al.,2009**). The phytase on top group was the lowest egg shell Ca % this may be due to the excess P liberated from phytase addition leading to calcium phosphorous imbalance. These results are disagreewith (**Zaghari,2009**)and(**Harsini et al., 2009**)who

found that there was no significant differences in egg shell ash, Ca and P content among different dietary treatments.

5- The effect of phytase on phosphorous and calcium availability:

Phytase effect on Ca and Pavailability was obviously found in both laying hens in group (3)and group (4). The beneficial effects of microbial phytase supplementation of P-deficient diets in poultry have been well documented (**Wu et al., 2006**), which suggests that phytase supplementation can release phytate-bound nutrients and consequently improve nutrient utilization. These results are in agreement with the finding obtained by (**Wu et al., 2006**) and (**Wang et al., 2013**) who found that the phytase had significantly ($p<0.05$) reduced excreted P than the control diet. The results are also in agreement with (**Sobolewska et al., 2015**) and (**Panda et al., 2005**) who found that the adding of phytase to diet significantly ($P<0.05$) enhanced phosphorus retention. Also (**Liu etal., 2007**) found that the supplementing of phytase can improve the digestibility of Ca and P.

Conclusion

From the results of this study, it could be concluded that reduction of NNP levels to 0.32% and 0.26% with supplementation of phyatse lead to increase egg weight, egg shell quality (shell weight, shell thickness, shell ash, shell calcium% and shell phosphorous%), calcium and phosphorous availability and also lead to numerically increase in egg number, egg mass, blood calcium and blood phosphorous levels. Phytase supplementation in laying hens ration is not recommended without reduction of dietary NNP. Reduction of NNP levels to0.32% and 0.26% with supplementation of phyatse was the best economic efficiency

Table1:Ingredient composition(%) and calculated chemical analysis of the basal and experimental diets.

Ingredient	Diet			
	Group 1	Group	Group 3	Group 4
Yellow corn, ground	55.5	55.5	55.9	56.1
Soyabean meal (44% CP)	30.2	30.2	30.1	30.1
Ground limestone	9.32	9.32	9.45	9.575
Monocalcium phosphate	1.1	1.1	0.825	0.55
Vegetable oil	2.83	2.82	2.665	2.615
Salt (Nacl)	0.2	0.2	0.2	0.2
Vitamin-trace mineral mixture*	0.3	0.3	0.3	0.3
DL-Methionine	0.1	0.1	0.1	0.1

Bicarbonate sodium	0.225	0.225	0.225	0.225
Choline chlorid	0.1	0.1	0.1	0.1
Antitoxin	0.125	0.125	0.125	0.125
Phytase	-	0.01	0.01	0.01
Analyzed and calculated composition				
Crude protein %	17.11	17.11	17.10	17.11
ME (K cal /kg diet)	2774	2773	2771	2773
Cal. / protein ratio	162.13	162.07	162.05	162.07
Calcium%	3.73	3.73	3.73	3.73
Ph., available%	0.38	0.38	0.32	0.26
Total P%	0.61	0.62	0.52	0.45
Linolinic acid%	1.34	1.34	1.35	1.35
Lysine%	1.02	1.02	1.02	1.02
Methionine %	0.41	0.41	0.41	0.41
Methionine +cysteine%	0.70	0.70	0.70	0.70
Sodium %	0.16	0.16	0.16	0.16
Chloride %	0.16	0.16	0.16	0.16

*vitamin & mineral mixture produced by multi vita co. for animal nutrition. Each 3 kilogram contains: Vitamin A12000000 i.u, Vitamin D3 3500000i.u, Vitamin E 25000mg, Vitamin K3 3000 mg, Vitamin B1 1000mg, Vitamin B2 6000 mg, Vitamin B6 3000 mg, Vitamin B12 20 mg, Niacin 30000 mg, Biotin 100 mg, Folic acid 1000 mg, Pantothenic acid 10000 mg, Zinc 70000 mg, Manganese 100000 mg, Iron 35000 mg, Copper 10000 mg, Iodine 1000 mg, Cobalt 300 mg, Sellinum 250 mg, Calcium carbonate up to 3 kg. Mono calcium phosphate analysis was 16.71% calcium and 22.43% phosphorous.

Table 2: The effect of dietary phytase supplementation on initial, final body weight, Average egg number, egg production%, egg mass (g/group/day), egg mass (g/hen/day), egg weight (g), feed intake (g/hen/week) and feed conversion ratio of laying hens in different groups throughout the experiment:

Item	Group 1	Group 2	Group 3	Group 4
Initial body weight	1815.4±28.53^{a}	1810.6±32.53^{a}	1832.2±27.30^{a}	1826.4±30.23^{a}
Final body weight	1934.0±31.45^{a}	1912.0±36.43^{a}	1909.3±20.51^{a}	1920.4±28.02^{a}
Weight gain	119.32±10.1^{a}	101.76±11.52^{ab}	77.10±8.23^{c}	94.85±9.59^{bc}
Egg number	5.99±.12^{a}	5.89±0.07^{a}	6.03±0.08^{a}	6.01±0.04^{a}

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Egg production %	85.62±1.68[a]	84.25±0.95[a]	86.18±1.15[a]	85.92±0.60[a]
Egg mass (g/hen/day)	51.69±1.18[a]	51.68±0.66[a]	53.19±1.06[a]	52.56±0.57[a]
Egg weight (g)	59.66±0.54[b]	61.44±.54[a]	61.66±.57[a]	61.21±0.44[a]
Feed intake(g/hen/day)	111.74±2.15[a]	112.49±1.94[a]	115.40±2.12[a]	114.2±2.24[a]
Feed conversion ratio%	2.15±0.08[a]	2.21±0.06[a]	2.14±0.04[a]	2.18±0.04[a]
Survival %	86.21	86.21	93.1	93.1

Values are means ± Standard error.
Means within the same row carrying different superscripts are significantly different at p≤ 0.05.

Table 3:The effect of dietary phytase supplementation on average values of some egg parameters recorded at the end of experimental period for different groups:

Item	Group 1	Group 2	Group 3	Group4
Egg width (cm)	4.42±2.79[a]	4.45±3.10[a]	4.47±2.21[a]	4.46±2.09[a]
Egg length (cm)	5.66±5.86[a]	5.68±4.24[a]	5.76±4.57[a]	5.62±8.36[a]
Egg shape index	0.78±1.14[a]	0.78±9.65[a]	0.78±7.51[a]	0.79±1.42[a]
Yolk height (cm)	1.68±1.36[a]	1.70±2.42[a]	1.68±3.37[a]	1.70±4.68[a]
Yolk width (cm)	3.87±6.14[a]	3.90±2.94[a]	3.90±3.64[a]	3.84±3.4[a]
Yolk index	0.43±9.69[a]	0.44±6.44[a]	0.43±1.04[a]	0.44±1.30[a]
Yolk weight (g)	16.54±0.31[a]	16.49±0.25[a]	16.65±0.38[a]	17.15±0.38[a]
Albumen weight (g)	33.99±0.51[b]	35.96±0.50[a]	36.06±0.88[a]	35.56±0.36[ab]
Yolk / albumin ratio	0.49±1.22[a]	0.46±8.94[a]	0.47±2.16[a]	0.48±1.36[a]
Shell weight (g)	7.27±0.12[b]	8.03±0.18[a]	8.02±0.17[a]	8.03±0.15[a]
Shell thickness (mm)	7.20±2.13[b]	7.20±1.53[b]	8.00±3.58[a]	7.95±1.74[a]

Values are means ± Standard error.
Means within the same row carrying different superscripts are significantly different at p≤ 0.05.

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Table 4: effect of dietary phytase supplementation on some blood parameters of laying hens in different groups at the end of the experiment:

Item	Group 1	Group 2	Group 3	Group 4
Calcium (mg/dl)	16.29±3.51[a]	14.78±1.65[a]	20.44±1.09[a]	19.79±1.96[a]
Phosphorus (mg/dl)	7.60±0.5[a]	7.67±0.61[a]	7.81±0.46[a]	8.38±0.77[a]
Alkaline phosphatase	467.42±21.56	478.22±45.82[b]	605.27±80.29[a]	517.45±107.7
Total protein (g/dl)	6.43±0.55[a]	5.52±0.29[a]	5.59±0.24[a]	5.93±0.37[a]
Albumin (g/dl)	2.64±0.16[a]	2.79±0.12[a]	2.9±0.48[a]	2.29±0.63[a]
Globulin (g/dl)	3.78±0.70[a]	2.72±0.25[a]	2.69±0.29[a]	3.64±0.94[a]
A/G ratio	0.77±0.19[a]	1.04±0.09[a]	1.14±0.32[a]	0.86±0.45[a]

Values are means ± Standard error.
Means within the same row carrying different superscripts are significantly different at p≤ 0.05.

Table 5: Effect of dietary phytase supplementation on Egg shell analysis of laying hens in different groups at the end of the experiment:

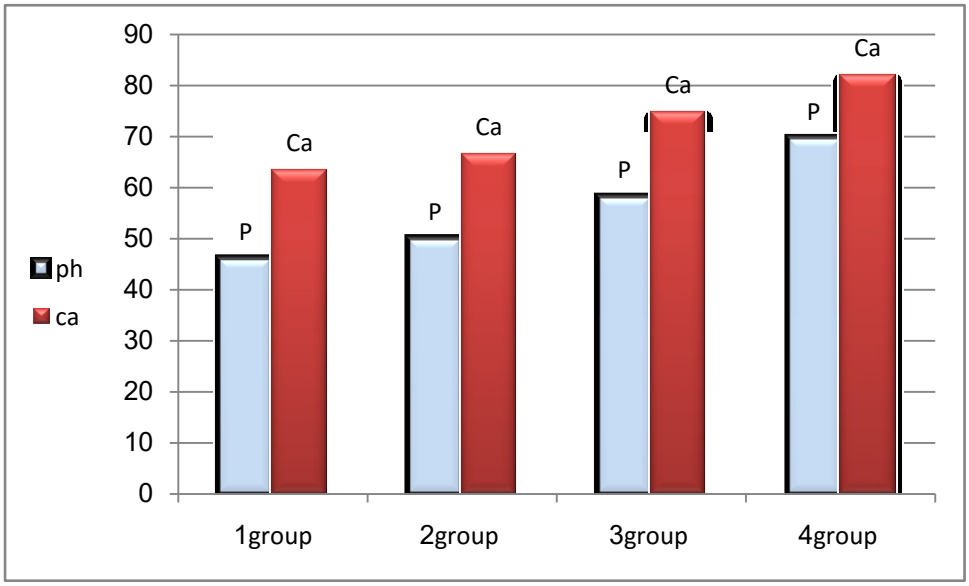
Item	Group 1	Group 2	Group 3	Group 4
Egg shell Protein%	6.0	6.0	6.0	6.0
Egg ash%	84.19	82.56	87.43	86.11
Egg shell moisture%	1.23	1.29	1.37	1.30
Egg shell Dry matter%	98.77	98.71	98.63	98.70
Egg shell calcium%	33.85	31.79	36.92	37.95
Egg shell phosphorus%	0.62	0.69	0.73	0.71

Table 6: Effect of dietary phytase supplementation on Phosphorous and calcium availabilityof laying hens in different groups at the end of the experiment:

Item	Group 1	Group 2	Group 3	Group 4
Phosphorus Availability %	46.09±5.19[c]	49.99±6.77[bc]	58.14±8.37[b]	69.63±4.01[a]
Calcium Availability %	63.43±4.60[b]	66.51±5.28[b]	74.77±2.82[ab]	82.09±0.97[a]

Values are means ± Standard error.
Means within the same row carrying different superscripts are significantly different at p≤ 0.05.

Figure 1: Phosphorous and calcium Availability of different groups at the end of experiment:



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