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Effects of Dietary Available Phosphorus Levels and Phytase Supplementation on Performance, Egg Quality and Serum Biochemical Parameters of Hy-Line Brown Laying Hens from 40 to 60 Weeks of Age

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ABSTRACT This study was performed to evaluate the effects of available phosphorus (AP) levels with or without supplemental phytase on the performance, egg quality, and serum biochemical parameters of laying hens. A total of 540 laying hens (40-week-old) were housed in cages and assigned to 6 dietary treatments with 5 replicates each, for 20 weeks. The treatments consisted of 0.20%, 0.25%, and 0.30% AP diets with or without phytase supplementation. During the 20-week period, egg production was lowest in hens fed the 0.20% AP diet; however, phytase supplementation in the diet completely corrected the adverse effect (P<0.05). No consistent difference was observed in egg production between hens fed the 0.25% and 0.30% AP diets and those fed the 0.20% and 0.30% AP diets with phytase supplementation. Similarly, egg mass was lowest in the 0.20% AP diet-fed group, and no difference in egg mass was observed in the 0.25% and 0.30% AP diet as well as the phytase-supplemented diet groups; however, egg mass was improved in the phytase-supplemented diet groups(P<0.05). Egg quality traits did not differ with dietary treatments. Serum alkaline phosphatase level showed a linear decrease (P<0.05) in the phytase-treated groups with increasing AP levels; moreover, a numerically linear increase (P<0.05) in serum Ca and P levels was observed in the phytase-treated groups. The results of this study indicate that phytase supplementation in the diet of laying hens could increase egg production and may lead to greater mineral absorption.

(Key words: performance, egg quality, serum biochemical, available phosphorus, phytase)

INTRODUCTION

Phytic acid (myo-inositol hexakis-dihydrogenphosphate, IP6) is a ubiquitous compound that is abundant in plant source feed ingredients (cereal grains and oilseeds), serving as the major storage form of phosphorus (P). According to the literature, about two-thirds of the total P in these plant source materials is present as the form of phytate which are salts of phytic acid and act as an anti-nutritional element (Jing et al., 2018). Phytic acid is an unwanted dietary agent and carries a strong negative charge thereby cause it make easily chelates form with these nutrient elements not only macro-micro minerals such Ca, Fe, Zn, Mg, Mn, Cu and Co but also some other organic components like proteins and carbohydrates (Cowieson et al., 2009). Due to chelating nature with nutrients phytate limits the availability of minerals and other dietary nutrients in monogastric animals because of which poor phytate hydrolyzing enzymes called phytase present in the gastrointestinal tract; therefore, the effect of phytase supplementation in the diet of monogastric animals is being extensively investigated (Adeola and Cowieson, 2011). The beneficial effects of phytase as an additive in the diet which can hydrolyze phytate-bound components as a result may have improve performance and nutrient utilization by increasing metabolic activity and digestibility of monogastric animals (Butani and Parnerkar, 2015). In recent years, a series of studies have shown that phytase has a potent ability to liberate the anti-nutritional effect of phytate and improve the digestibility of phytate-bound P, Ca amino acids and energy as well as minimize of excess P excretion to the environment (Dersjant-li et al., 2015). Additionally, observed results of previous study on laying hens indicated that the supplemen-

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tation of phytase in the diet of laying hens had improved egg production, egg quality, nutrient digestibility, and retention of minerals (Panda et al., 2005; Gao et al., 2013; Englmaierova et al., 2014; Rojas et al., 2017). Moreover, the use of exogenous phytase as a feed additive to poultry diet may partially or completely minimize nutrient variability of feedstuffs by reducing the cost of nutritional inputs, as example, by replacing inorganic source such as dicalcium phosphate and increase the accuracy of feed formulation (Sousa et al., 2015), as well as leads to reduction of P excretion in the environment (Slominski, 2011). However, although a number of studies have already been carried out to investigate the potentiality of phytase use as feed additive in laying hens diet to extended persistency of lay after end of peak but there were no general resemble till indicated among the research findings about concerning between the dietary AP requirements and addition of phytase on laying hens, even, there have very sparse information concerning the effect of phytase addition in laying hens diet on the serum biochemistry variables. Thus, the purpose of this study was to investigate the effect of AP levels with or without supplemental phytase on performance, egg quality and serum biochemical parameters of Hy-line brown laying hens from 40 to 60 weeks of age.

MATERIALS AND METHODS

1. Experimental Design and Diets

The experimental procedure was performed following the guidelines for the use of animals in experimentation, as provided by the Jeonbuk National University, Republic of Korea. A total of 540 Hy-line brown laying hens aged at 38 weeks of age, obtained from a commercial source, were placed in a poultry house in conventional type three-tier cages (two hens per cage). The cage dimensions were 30 cm × 40 cm, equating to 1,200 cm^2 floor space per cage. During the pre-experimental period from 38 to 40 weeks of age, hens were kept in the house for the acclimatization and were fed a common conventional layer mash diet and, the egg production recorded daily and eggs were weighed two times in a week.

Thereafter, at the age of 40 weeks, the hens (similar mean egg production of hens) were randomly assigned into 1 of 6 dietary treatments, each comprising five replicates and eighteen hens in each replicate in a completely randomized design. A replicate consisted of three adjacent cages in upper and alike in middle and bottom to minimize the cage level effect. The cages were equipped with trough feeder and nipple drinker line. A continuous feed trough was divided as the replicate as if hens were not able to consume feed assigned to the adjoining replicate. Three isoenergetic (2,750 kcal/kg ME) and isonitrogenous (16.0% CP) diets were formulated to contain three levels of 0.20, 0.25 and 0.30% AP respectively, and with or without phytase (1,000 FTU per kg diet, Ronozyme Hiphos-L, *Aspergillus oryzae* 6-phytase) was supplemented. A constant level of 4.0% Ca was maintained in all dietary treatments and other dietary nutrients were fulfilled as the requirement of laying hen by the following specification of NRC (1994). During the experiment, hens under all treatment groups were given free access to mash type feed and water, and were exposed to a 16L: 8D lighting schedule. The mortality was replaced by spare hens kept upon on identical treatments throughout the experimental period. The composition of ingredients and chemical analysis of the basal diets are presented in Table 1.

2. Performance of Laying Hens

Laying performance of hens was determined at the end of every four weeks by monitoring egg production, egg weight, egg mass, feed intake and feed conversion ratio. Egg production was recorded daily which was expressed on a hen-day basis (% hen-day) at the end of each four weeks. Eggs were collected two times in a week and the weight was taken to calculate mean egg weight. Egg mass (g/hen/day) was calculated by multiplying egg weight by egg production. Feed intake was determined at the end of four weeks as the replicate basis of each treatment by subtracting the remaining feed from the original amount of feed that was provided. Feed conversion ratio was calculated on the basis of amount of feed intake in gram divided by egg mass in gram.

3. Egg Quality

Thirty eggs from each dietary treatment group were arbitrarily selected at 44, 48, 52, 56 and 60 weeks of age to

Table 1. The ingredient and nutrient composition of experimental diets

Items	% of available phosphorus (AP) in the diet		
	0.20	0.25	0.30
Ingredient (%)			
Corn	55.700	55.550	55.400
Wheat HRW	3.820	3.555	3.290
Wheat bran	10.220	10.505	10.790
Soybean meal (48%)	19.370	19.370	19.370
Monocalcium phosphate (Ca 18%, P 21%)	0.630	0.875	1.120
Limestone (Ca 38.5%)	9.660	9.545	9.430
Iodized salt	0.300	0.300	0.300
DL-Methionine (99%)	0.100	0.100	0.100
Vitamin premix	0.100	0.100	0.100
Mineral premix	0.100	0.100	0.100
Total	100	100	100
Calculated nutrient composition			
Metabolizable energy (kcal/kg)	2,750	2,750	2,750
Crude protein (%)	16.00	16.00	16.00
Calcium (%)	4.00	4.00	4.00
Total phosphorus (%)	0.59	0.64	0.70
Available phosphorus (%)	0.20	0.25	0.30
Lysine (%)	0.80	0.80	0.80
Methionine (%)	0.36	0.36	0.36
Cysteine (%)	0.29	0.29	0.29
Arginine (%)	0.99	0.99	0.99

[1] Vitamin supplement provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500IU; vitamin E, 20 IU; vitamin B_1, 1.5 mg; vitamin B_2, 5.0 mg; vitamin B_6, 0.15 mg; vitamin B_{12} 15.0 mg; choline, 300 mg; pantothenate, 12 mg; nicotinic acid, 50 mg; biotin, 0.15 mg; folic acid, 1.5 mg.

[2] Mineral supplemented provided per kilogram of diet: Fe, 60 mg, Cu, 10 mg; Zn, 80 mg; Mn, 110 mg; Iodine, 0.48 mg; Se, 0.40 mg.

estimate egg quality parameters. The egg quality parameters in terms of egg shell breaking strength, egg shell thickness, albumen height and Haugh unit were measured. Egg shell breaking strength was measured by egg shell strength tester (QC-SPA shell strength analyzer, TSS, UK) and the maximum force required to crack the shell surface was recorded, and expressed as unit of shell surface area (kg/cm^2). The egg shell thickness was measured from the three different parts of shell in each egg (air cell, equator and sharp end) after removing the inner shell membrane using a micrometer screw gauge (Digimatic micrometer, series 293- 330, Mitutoyo, Japan) and the mean value was taken as thickness in millimeter. Haugh units were measured automatically by the formula of Haugh ($HU = 100 \log [(\text{albumen height} + 7.57) - (1.7 \text{ egg weight}^{0.37})]$)using the multi egg tester (Technical Services and Supplies, TSS, UK) including QCBi digital balance and QCH

albumen height gauge. Table 1. The ingredient and nutrient composition of experimental diets.

4. Serum Biochemical Components

At the end of the experiment (60 weeks of age), blood samples were collected from the wing vein of arbitrary selected ten hens from each treatment by 3 mL sterile syringe. Immediately transfer into non-heparinized blood collection tubes. The collected blood samples were centrifuged at 3,000 rpm for 15 min at 4℃, and serum was separated into eppendorf tubes. Thereafter, it stored at −20℃ until required for the analysis of biochemical parameters, including albumin (ALB), total protein (TP), cholesterol (CHOL), high-density lipoprotein (HDL), glucose (GLU), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP), that were assessed using commercially available diagnostic kits by Konelab 20 Analyzer (Thermo Fisher Scientific, Vantaa, Finland) following the manufacture's instruction guidelines.

5. Statistical Analysis

Experimental data were analyzed by ANOVA procedures for one-way completely randomized design using general linear model (GLM) procedure of SAS (SAS 9.1, 2009). Differences among the treatments were tested using Duncan's multiple range tests. A statistical value of $P<0.05$ was regarded as a significant difference and a value of $P<0.10$ was considered indicative of a tendency.

RESULTS

1. Performance of Laying Hens

Effects of dietary AP with or without phytase supplementation on production performances of laying hens over the period from 40 to 60 weeks of age are presented in Table 2. Egg production rate was not affected at the end of each four weeks from 40 to 44, 45 to 48 and 49 to 52 weeks of age except for the period at 53 to 56 weeks ($P<0.05$) and the entire period of 40 to 60 weeks of age ($P<0.01$). During the entire experiment (40 to 60 weeks), significantly lower egg production was found in hens fed a diet containing 0.20% AP while the value was higher in the 0.25% AP diet with

Table 2. Effect of dietary available phosphorus levels with or without supplementation of phytase on productive performance of laying hens

Parameters	% of AP without phytase			% of AP with phytase			SEM	*P*- value
	0.20	0.25	0.30	0.20	0.25	0.30		
Egg production (%)								
40~44 weeks	88.66	88.94	89.43	90.03	89.73	89.56	0.31	0.859
45~48 weeks	85.63	86.38	87.19	87.30	88.06	87.12	0.30	0.229
49~52 weeks	85.44	86.64	86.90	87.22	87.93	87.33	0.34	0.419
53~56 weeks	84.61^{b}	86.21^{ab}	86.66^{a}	87.18^{a}	87.86^{a}	86.88^{a}	0.30	0.023
57~60 weeks	83.19	84.64	84.86	86.10	86.97	84.89	0.40	0.095
40~60 weeks	85.51^{c}	86.56^{bc}	87.01^{ab}	87.59^{ab}	88.11^{a}	87.16^{ab}	0.23	0.013
Egg weight (g)								
40~44 weeks	61.08	61.22	61.58	61.39	61.66	61.23	0.12	0.784
45~48 weeks	61.29	61.23	61.04	60.98	61.10	61.33	0.12	0.957
49~52 weeks	61.24	61.21	60.82	61.44	60.83	61.02	0.14	0.801
53~56 weeks	60.42	60.62	61.01	60.81	60.55	60.04	0.18	0.737
57~60 weeks	61.21	60.73	60.82	61.17	60.70	61.05	0.15	0.879
40~60 weeks	61.05	61.00	61.05	61.16	60.97	60.93	0.07	0.974

Table 2. Continued

Parameters	% of AP without phytase			% of AP with phytase			SEM	*P*- value
	0.20	0.25	0.30	0.20	0.25	0.30		
Egg mass (g/h/d)								
40~44 weeks	54.16	54.44	55.06	55.26	55.32	54.84	0.22	0.632
45~48 weeks	52.48	52.89	53.22	53.30	53.80	53.43	0.20	0.538
49~52 weeks	52.32	53.03	52.85	53.58	53.49	53.28	0.22	0.635
53~56 weeks	51.11	52.26	52.87	53.01	53.19	52.16	0.23	0.081
57~60 weeks	50.93	51.40	51.61	52.66	52.79	51.81	0.23	0.130
40~60 weeks	52.20^{b}	52.80^{ab}	53.12^{ab}	53.56^{a}	53.72^{a}	53.10^{ab}	0.15	0.020
Feed intake (g/h/d)								
40~44 weeks	111.40	111.83	111.43	111.09	111.74	111.48	0.17	0.877
45~48 weeks	112.12	112.79	113.19	112.53	113.20	112.20	0.33	0.911
49~52 weeks	112.86	115.09	114.18	114.56	113.75	115.68	0.60	0.844
53~56 weeks	113.47	114.55	114.35	113.80	114.64	113.52	0.37	0.919
57~60 weeks	113.78	113.49	113.41	114.98	114.03	114.46	0.29	0.652
40~60 weeks	112.72	113.55	113.31	113.39	113.47	113.47	0.16	0.744
Feed conversion ratio (g of feed consumed/g of egg mass)								
40~44 weeks	2.06	2.05	2.02	2.01	2.02	2.03	0.01	0.546
45~48 weeks	2.14	2.13	2.13	2.11	2.10	2.10	0.01	0.843
49~52 weeks	2.16	2.17	2.16	2.14	2.13	2.17	0.01	0.951
53~56 weeks	2.22	2.19	2.16	2.15	2.16	2.18	0.01	0.278
57~60 weeks	2.23	2.21	2.19	2.18	2.16	2.21	0.01	0.320
40~60 weeks	2.16	2.15	2.14	2.12	2.11	2.13	0.01	0.135

SEM, Standard error of the mean; AP, available phosphorus; $^{a\sim c}$ Means in the same row bearing different superscript differ significantly; Ronozyme HiPhos-L, 6-phytase produced by the strain of *Aspergillus oryzae* was used to supply 1,000 FTUkg^{-1} of feed.

supplemental phytase when compared that of other dietary treatments. However, there was no consistent difference observed in egg production between hens fed on diets different dietary AP concentrations with phytase. The tendency effect (P<0.10) was found in egg mass by the dietary treatments at 53 to 56 weeks of age and the higher value (53.19 g) was obtained at 0.25% AP diet with phytase but this effect failed to attain statistical significance. Overall period from 40 to 60 weeks of age, significantly (P<0.05) lower egg mass value obtained when hens were fed 0.20% AP diet and the higher value was in diets containing 0.20 and 0.25% AP with phytase. Egg weight, feed intake and feed conversion ratio were not affected among the dietary treatments at the end of each four weeks and over the entire duration of the experiment, regardless of AP levels or phytase supplementation to the diets.

2. Egg Quality

Effect of dietary AP levels with or without supplementation of phytase on egg quality traits are presented in Table 3. Statistical analysis of the obtained data revealed that there was no effect on egg shell thickness, egg shell breaking

Table 3. Effect of dietary available phosphorus levels with or without supplementation of phytase on egg quality of laying hens

Parameters	% of AP without phytase			% of AP with phytase			SEM	*P*- value
	0.20	0.25	0.30	0.20	0.25	0.30		
Egg shell thickness (mm)								
44 weeks	0.353	0.355	0.349	0.349	0.354	0.354	0.002	0.943
48 weeks	0.364	0.369	0.365	0.362	0.360	0.357	0.002	0.583
52 weeks	0.345	0.364	0.356	0.352	0.361	0.354	0.002	0.340
56 weeks	0.342	0.346	0.356	0.348	0.351	0.349	0.001	0.423
60 weeks	0.349	0.364	0.361	0.358	0.354	0.359	0.002	0.408
Egg shell breaking strength (kg/cm^2)								
44 weeks	2.66	2.80	2.75	2.74	2.74	2.63	0.04	0.872
48 weeks	2.68	2.62	2.81	2.74	2.67	2.58	0.04	0.551
52 weeks	2.47	2.53	2.56	2.40	2.47	2.32	0.05	0.701
56 weeks	2.48	2.43	2.68	2.35	2.44	2.61	0.04	0.246
60 weeks	2.82	2.87	2.92	3.09	2.94	3.07	0.07	0.832
Albumen height (mm)								
44 weeks	8.46	8.32	8.57	8.51	8.22	8.20	0.08	0.708
48 weeks	8.09	8.04	8.20	8.03	7.93	8.19	0.08	0.927
52 weeks	7.84	7.44	7.83	7.48	7.76	7.43	0.09	0.489
56 weeks	8.80	8.85	8.83	8.75	8.80	8.35	0.08	0.425
60 weeks	8.21	8.02	8.28	8.47	8.22	8.03	0.09	0.738
Haugh unit score								
44 weeks	91.20	90.10	91.73	90.74	89.64	90.01	0.44	0.744
48 weeks	88.52	88.63	89.46	87.65	88.02	89.17	0.45	0.872
52 weeks	88.06	85.61	87.29	85.44	87.67	85.95	0.50	0.503
56 weeks	92.69	92.88	93.20	93.26	93.94	91.24	0.44	0.630
60 weeks	89.62	88.60	89.66	91.08	89.94	87.93	0.52	0.596

SEM, Standard error of the mean; AP, available phosphorus; Ronozyme HiPhos-L, 6-phytase produced by the strain of *Aspergillus oryzae* was used to supply 1,000 $FTUkg^{-1}$ of feed.

strength, albumen height and Haugh unit by dietary treatments when evaluated at the end of every four weeks during the study for twenty weeks.

3. Serum Biochemical Components

The effect of experimental diets on serum biochemical parameters of laying hens are shown in Table 4. In this study, there was no consistent effect of dietary AP concentrations either between with or without supplemental phytase on ALB, TP, GLU, CHOL, HDL, TG, ALT and AST levels in serum observed. However, the addition of phytase to the AP containing diets did not significantly affect serum Ca and P concentration but it did increase linear numerically for dietary levels of AP from 0.20 to 0.30% with phytase when evaluated without supplemental phytase groups. The ALP level in serum had a tendency ($P<0.10$) to decrease linearly by the addition

Table 4. Effect of dietary available phosphorus levels with or without supplementation of phytase on serum biochemical components of laying hens at 60 weeks

Parameters	% of AP without phytase			% of AP with phytase			SEM	*P*-value
	0.20	0.25	0.30	0.20	0.25	0.30		
ALB (mg/dL)	2.06	2.19	1.96	2.14	2.06	2.02	0.04	0.744
TP (mg/dL)	5.97	6.27	5.59	6.98	5.92	5.92	0.19	0.383
CHOL (mg/dL)	161.94	155.18	152.55	159.35	151.12	146.42	6.42	0.989
HdL (mg/dL)	9.26	7.68	9.01	7.14	7.79	8.25	0.55	0.891
GLU (mg/dL)	259.25	263.81	279.18	254.75	273.44	268.40	4.25	0.607
ALT (IU/L)	1.27	1.03	0.86	0.89	1.33	0.85	0.14	0.898
AST (IU/L)	175.38	185.53	171.67	166.51	190.21	161.32	5.01	0.565
TG (mg/dL)	1,877.33	1,579.06	1,463.60	1,721.28	1,456.62	1,450.97	93.69	0.742
P (mg/dL)	5.36	5.87	6.02	6.17	6.56	7.03	0.25	0.536
Ca (mg/dL)	16.54	17.07	17.38	17.91	18.89	19.07	0.42	0.459
ALP (IU/L)	455.01	418.41	397.56	392.87	370.83	358.21	10.25	0.066

SEM, Standard error of the mean; AP, available phosphorus; ALB, albumin; TP, total protein; CHOL, cholesterol; HDL, high density-lipoprotein; GLU, glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglycerides; Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; Ronozyme HiPhos-L, 6-phytase produced by the strain of *Aspergillus oryzae* was used to supply 1,000 FTUkg^{-1} of feed.

of phytase into the diets when did compare that were not supplemented phytase.

DISCUSSION

Phytase enzymes are being extensively used in diet as dietary feed additives in order to increase the digestibility of phytate P and positively influence the digestibility of other nutrients. The beneficial effect of phytase supplementation on layers has been studied by numerous investigations but it is still going discussion into the researchers because there could not be found general resemble from related literature regarding the dietary level of AP with inclusion level of phytase in laying performance. As a result, in this study, we have explored the impact of dietary variation levels of AP with or without phytase supplementation in commercial layer diets during several age periods at the second production phase. The results of the current experiment showed that the addition of phytase into the basal diet containing levels of 0.20 to 0.30% AP increased egg production of laying hens in comparison to respective AP diets that were not supplemented phytase during overall period of the experiment from 40 to 60 weeks of age. Although, there was no consistent difference obtained in egg production between 0.25 to 0.30% AP diet and supplementation of phytase in 0.20 to 0.30% AP diets but numerically higher egg production was obtained due to hens fed 0.25% AP diet with phytase when compared with all other dietary treatments. This finding is in consistent with the findings of Englmaierova et al. (2014) who showed that the dietary level of 0.17% AP or 0.21% AP diet with phytase supplementation significantly improved egg production of laying hens than that of without phytase from 37 to 49 weeks of age, whereas similar to those obtained for the diet of 0.30% AP with or without phytase. Silversides and Hruby (2009) reported that the egg production increased with the supplementation of phytase into the laying hens diet when hens were fed either 0.19% AP or 0.22% AP diet from 45 to 60 weeks of age as compared with performance of hens fed a control diet containing 0.34% AP. Moreover, similar to the current experiment, it has shown that phytase supple-

mentation to the control diet 0.45% AP and the low 0.30% AP diet significantly increased egg production from 75.49 to 77.96% and from 64.59 to 76.54% respectively (Cabuk et al., 2004). In the present study, it also showed that egg production of hens receiving 0.20% AP was significantly lower than those given higher AP diet (0.25 or 0.30%). It demonstrates that 0.20% AP was insufficient to provide the daily P requirement of laying hens. This adverse effect was completely corrected by phytase supplementation that may have helped to increase absorption of nutrients resulting that corrected egg production. Previous studies have reported that supplementing a diet containing 0.12 to 0.20% AP with phytase resulted in a significant improvement in laying egg production when compared with the same diet that was not supplemented with phytase (Hughes et al., 2008; Yan et al., 2009; Rojas et al., 2017). It is conceivable therefore that the improvement in egg production of laying hens which might have been due to increased availability of P and eliminating the anti-nutritive effect of phytate on digestibility of other nutrients by phytase (Lei et al., 2011). Egg weight is the elementary trait to assess egg quality. In the present study, egg weight was not affected by AP concentrations and supplementation of phytase into the diet, which implies that the dietary lowest level of 0.20% AP was adequate to maintain egg weight during the period of 40 to 60 weeks of age, even in supplementation of phytase. Similar to previous findings, no significant effect of AP concentration and phytase supplementation on egg weight was reported in laying hens between 32 to 48 or 62 weeks of age (Panda et al., 2005; Meyer and Parsons, 2011; Englmaierova et al., 2014). In the present study at 40 to 60 weeks, egg mass was significantly lower for hens fed 0.20% AP diet without phytase, whereas diet with 0.20% AP plus phytase had equal egg mass to the 0.25 and 0.30% AP diets with or without phytase. Similar to these findings, Englmaierova et al. (2014) reported that the addition of phytase to the diet containing 0.21% AP improved egg mass of laying hens which was alike to the diet containing 0.30% AP with or without phytase. The results regarding the egg mass was also agreement with those obtained by Wu et al. (2006) reported that phytase supplementation significantly improved egg mass in the diets containing 0.11% AP, but had no effect on egg mass for the diet containing 0.26% AP. Thus, the improvement of egg mass value during the entire experiment in this study which may have associated with increase egg production rate of hens due to supplemental phytase into the diets. The present study demonstrated that feed consumption of laying hens by dietary AP concentrations had similar to those fed same diets that were supplemented phytase and hence the lowest concentration of 0.20% AP diet, which may be optimum during this period. Results regarding the feed intake of laying hens is in agreement with those obtained by Hughes et al. (2008) who reported that hens from 21 to 61 weeks of age fed diets containing 0.15 to 0.25% AP with supplemental phytase did not affect the feed consumption of laying hens that was similar to those of hens fed a diet containing 0.35% AP. Baghban-Kanani et al. (2020) who found that there was no significant effect on feed consumption of laying hens in response to phytase supplementation in the diet containing 0.30% AP from 56 to 65 weeks of age. Also concurrent of these observations, Meyer and Parsons (2011) and Wang et al. (2013) did not find significant effect of phytase supplementation on feed consumption in laying hens. There was no difference in feed conversion ratio due to addition of phytase in the diets. The feed utilization efficiency of laying hens is closely related to feed intake, egg mass output, egg weight and egg production. Although in this study during the whole experimental period there was significant difference in the egg production and egg mass output but did not affect other performance traits in laying hens. It is suspected that the addition of phytase in the diets having the same effect in helping the absorption of proteins and lipids in the digestive tract, so that the resulting weight of the eggs had almost the same between treatments. Egg weight ratio similar to feed consumption did not provide a significant effect on feed utilization efficiency variations. The results regarding the feed conversion ratio are in agreement with those obtained by Wang et al. (2013) who reported that the addition of phytase into the laying hen diet containing level of 0.16 to 0.26% AP had not differed feed conversion that were similar to those of hens fed a diet containing 0.36% AP where phytase was not added, from 44 to 55 weeks of age. Similarly, Meyer and

Parsons (2011) reported that the feed utilization efficiency of laying hens fed diets containing either 0.20% AP or 0.11% AP supplemented with phytase was not significantly different from that of hens fed a diet containing 0.45% AP from 32 to 62 weeks of age.

No improvement in egg quality traits as observed in this study which was unexpected. Regarding this fact, earlier studies have shown that incorporation of phytase into the diet improved egg shell quality, especially egg shell thickness and egg shell breaking strength which could probably be associated for the consequence of increased divalent mineral utilization such Ca and P retention through the metabolic process by reducing the formation of insoluble mineral phytate complexes (Lim et al., 2003; Liu et al., 2007; Zyla et al., 2011). However, the current study findings were concomitant with Kim et al. (2017) reported that egg quality traits including egg shell thickness and egg shell breaking strength were not influenced for hens fed diet containing 0.26% AP supplemented with phytase which was similar to those obtained either with 0.26% AP or 0.38% AP where did not supplement phytase, and suggesting that which might be due to adequate concentrations of Ca and P present in the diets. Similarly, Wang et al. (2013) reported that there was no significant difference between the dietary treatments for egg shell thickness and egg shell breaking strength when hens were fed diets containing 0.16% to 0.26% AP supplemented with phytase and the diet containing 0.36% AP. Thus, it may be speculated that based on the observation for similar feed intake in diets either with the presence or absence of phytase, Ca and P concentrations in these diets were sufficient to support proper egg shell formation during this phase.

In the current study, serum Ca and P level had linear numerically increased trend in laying hens due to the phytase addition with dietary AP concentrations but did not reach significant level among the treatments. The numerically increase these values in serum which could probably be attributed by the hydrolysis of phytate due to addition of phytase in the diets, which might have helped to digest and absorb Ca and P that might be correlated with egg production. The results regarding the serum P which partly agreement with the findings of Silversides et al. (2006) who reported that phytase supplementation in laying hens diet increased serum P level from 5.17 mg/dl (with no phytase addition to diets) to 6.3 mg/dl with addition of phytase. Similarly, Rama-Rao et al. (1999) who reported that the P increased linearly in response to phytase supplementation. The results regarding the serum Ca are being supported by data obtained by Hassanien and Elnagar (2011) who reported that serum Ca concentration increased with the supplementation of phytase and indicating that phytase can improve Ca bioavailability in laying hens. As the scientific literature it is noted that, a higher phytate might be responsible for the higher ALP activity in the plasma is associated with intestinal lesions, skeletal disorder or liver dysfunction and also may be related to Ca or P deficiency or excess Ca and P ratio in diet. In this study, a linear tendency to decrease ALP concentration in serum due to phytase addition with the dietary AP levels observed, which might be reflected in the increased availability of P. In a way, in agreement with the current study, it has been found that supplemental phytase to the diets caused a decrease serum ALP concentration in laying hens (Musapuor et al., 2005) and broilers (Farhadi et al., 2017), thereby might have increased P availability. In the previous study, Attia et al. (2010) who reported that the serum concentration of total protein, cholesterol, aspartate amino transferase, alanine amino transferase, cholesterol and triglyceride were not influenced by phytase supplementation into the diets of laying hens, which were concomitant with the results of current study.

On the basis of these results, it is concluded that supplementation of phytase into the diet of laying hens have positively significant influence effect on egg production, egg mass output and has a tendency effect to decrease serum ALP concentration which may indicate increase P availability, also led to being numerically increased Ca and P in serum. However, with respect to egg production, the diet containing 0.20% AP with phytase is sufficient to maintain optimum egg production during the second production period of laying hens. Moreover, further follow up studies are required in this regard to investigate the layer performance, egg quality parameters, serum biochemical traits, mineral

retention and cell-mediated immunity of egg-type birds.

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