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EFFECT OF DIETARY PHOSPHORUS AND RESTRICTED FEEDING ON PERFORMANCE, EGG QUALITY AND SERUM BIOCHEMICAL TRAITS OF LAYING HENS AT THE SECOND PRODUCTION PHASE

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Abstract

The effect of available phosphorus and restriction of feeding on the performance, egg quality, serum biochemical and yolk fatty acid profiles in laying hens at 40 to 60 weeks of age was studied. A total 540 laying hens (Lohmann Brown) aged at 40 weeks were randomly placed in a 2×3 factorial arrangement of dietary treatments including two levels of available phosphorus (AP, 0.32 and 0.45%) and three restricted feeding levels (RFL, 90, 95 and 100%) having 5 replicates in each treatment, and the experiment were conducted for twenty weeks. The results showed that dietary interaction between AP and RFL had significant effects on egg production percentage ($P<0.01$), egg weight ($P<0.05$), daily egg mass ($P<0.01$) and feed conversion ratio ($P<0.01$). Production performance was significantly ($P<0.01$) affected by RFL; and both 95% and 100% RFL had showed similar mean value in egg production and egg weight. Egg quality traits: eggshell color and eggshell breaking strength were differed ($P<0.05$) among the treatments. Moreover, albumen height, Haugh unit and eggshell breaking strength significantly ($P<0.05$) increased in diet contained 0.45% AP. Dietary limitation of feed did not alter the egg quality except eggshell color. Serum albumin was influenced among the treatments and the mean value of albumin and total protein were same in sole effect of 95% and 100% RFL. Higher serum glucose was obtained in response to the primary effect of 0.32% AP. The total concentration of yolk fatty acid profiles was not assorted in the impacts of AP, RFL and in their interaction. Therefore, the results suggest that 95% feeding was sufficient to hens during second production period, for optimum production performance and egg quality as well as diet containing 0.45% inorganic phosphorus could have influenced positively on egg quality.

Keywords: Albumen, Egg mass, Egg quality, Feed restriction, Yolk fatty acid

Introduction

For raising commercial poultry industry, cost of production is considered to be a crucial factor since total expenses enormously influenced by feed price that around 65 to 75% of total cost of poultry production (Abdurofi *et al*., 2017). Rising costs of feed, labor

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and miscellaneous items without a corresponding increase in the price of eggs have made feed restriction one of the ways of reducing production costs in commercial layer farms. Productivity and profitability widely depend to a larger extent on the genetic make-up or constitution of an animal being reared for either meat or egg production. In addition, other important factors influencing productivity include prevailing environmental conditions, management practices such as feed and feeding management and the technical knowledge of the farmers (Bell and Weaver, 2002). Dietary feed restriction has, therefore, been carried out by limiting the amount of feed given to birds each day (Scott *et al*., 1999) or fixed time restricted for access to feed (Sandoval and Gernat, 1996). Reducing feed supply whether qualitative or quantitative is usually practiced to limit feed intake of birds in order to improve efficiency of feed utilization as well as reduce feed and production costs (Zhan *et al*., 2007), thereby, increased economic benefit (Olawumi, 2014). Moreover, control feeding program has been associated to keeping up body weight during laying period and also assured that birds do not make abundance fat in abdominal cavity (Kostal *et al*., 1992). Agreeing to Batonon *et al*., (2014) also reported that marginal reduction of feed when offered to hens during production period that was able to perform at an equivalent level of full fed control diet without any unfavorable effects with their performance.

Phosphorus is an essential mineral after that calcium in laying hen diets. It plays a key role in numerous body functions including bone development, cellular digestion system, cellular regulatory mechanism and formation of egg (Sohail and Roland, 2002). Laying hens fulfill their requirements from the formulated corn and soybean-based diet. Various concentration of plant based feed ingredients are used for ration formulation therein present approximately two thirds of total phosphorus as insoluble complex form (phytic acid), which cannot utilize by poultry due to the lack of insufficient of phytase enzyme in the digestive tract (Singh, 2008). Although, phosphorus requirement of laying hens varies depending on the composition of diet (Skrivan *et al*., 2010; Kim *et al*., 2017) and calcium level (Pelicia *et al*., 2009), vitamin D3 (Keshavarz, 1996) and phytase enzyme availability (Kim *et al*., 2017). The level of phosphorus either over or low in diet can negatively be influenced on the egg production and egg quality. As a result, required dietary phosphorus according to hen ages must be maintained in diets for ideal production and good management. Swiatkiewicz *et al*., (2010) reported that eggshell thickness and breaking strength were decreased by reducing the dietary levels of calcium and phosphorus. On the other hand, Li *et al*., (2007) shown that production performance of layers fed diet containing available phosphorus of 2.9 and 4.0 g/kg diet from 23 to 47 weeks of age were similar. However, the establishment of available phosphorus requirement for commercial layers is a persistent challenge that might be due to the fundamental fact of instability of variable amount of phytate phosphorus in the diet and moreover, research information is very scanty on relation between available phosphorus and egg quality of hens at older ages. Therefore, the main focus of this study was therefore to know the egg quality, serum and yolk fatty acid composition of laying hens at second phase production period with different dietary available phosphorus and restricted feeding levels.

Materials and Methods
Birds and experimental design

A total of 540 commercial laying hens (Lohmann Brown) 40 weeks of age were arbitrarily assigned to six treatments with five replicates and 18 hens in each replicate, the experimental period was 20 weeks from 40 to 60 weeks of age. Before starting the experiment, hens were selected based upon their similar body weight and egg production. And, hens were subjected to seven days adaptation period, provided mash feed (115g/hen/day) according to the breeders' manual. Treatments were assigned in a completely randomized deign 2 × 3 factorial arrangement, consisting of two AP (0.32 and 0.45) and three RFL were set at 90, 95 and 100%. Hens were housed in 3-tier A-shaped metal cages stand totaling 6 lines with two hens per cage. Mortality was recorded properly and all dead birds were replaced from spare hens and kept up identical treatments.

Table 1. Ingredients and nutrient composition of diet

Ingredients	Percent (%)
Corn	67.23
Soybean meal (44%)	17.38
Corn gluten meal	4.24
Limestone	9.42
Calcium-phosphate	0.94
Salt	0.38
L-Lysine	0.05
DL-Methionine	0.03
Vitamin premix	0.18
Mineral premix	0.15
Calculated nutritional levels	
Metabolizable energy (ME, kcal/kg)	2800.00
Crude protein (CP, %)	16.00
Lysine (%)	0.74
Methionine (%)	0.32
Calcium (%)	3.8
Available phosphorus (%)	0.32

[1]Contains per kg: vit. A 5500 IU; vit. D3 1100 ICU; vit. E 11 mg; vit. B12 0.0066 mg; vit. K3 1.1 mg; riboflavin 4.4 mg; pathothenic acid 11 mg (calciumpantothenate:1.96 mg); choline 190.96 mg; folic acid 0.55 mg; pyridoxine 2.2 mg; biotin 0.11 mg; thiamine 2.2mg; ethoxyquin 125 mg. [2]Contains per kg: Cu 10 mg; Fe 60 mg; I 0.46 mg; Mn 120 mg; Zn 100 mg.

Experimental diet

The basal diet content 2800 Kcal/kg Metabolizable energy (ME), Crude protein (CP) 16%, Calcium (Ca) 3.8%, AP 0.32% and other dietary nutrient contents in diet showed in Table 1. According to the treatment, experimental diets were prepared with level of Ca 4.0%, AP 0.32 and 0.45%. Fine limestone as Ca source that contained 38.5% Ca and monodicalcium phosphate (MDCP) that comprised 18% Ca and 21% phosphorus (P) were added in diet for the adjustment of Ca and AP levels. In the entire experimental period, house temperature was maintained at 22±3^{o}C with photoperiod was fixed at 16 h light and 8 h dark.

Production performance and egg quality of laying hens

Throughout the experimental period, the number of eggs laid, cracked and broken eggs per replica in each group were registered daily evening at 5.00 pm and egg production was expressed as percentage of hen day egg production. The egg weight was taken every two days interval excluding cracked and abnormal eggs. Feed conversion ratio (FCR) was expressed as the ratio of gram of feed consumed and divided by daily egg mass. It was calculated by multiplying the average hen day production percentage by the average weight of eggs. Cracked and broken eggs were recorded daily in proper spreadsheets, and were evaluated as the total number of cracked or broken eggs divided by the number of produced eggs, and the result was multiplied by 100. Thirty eggs from each group were collected at the termination of every four weeks to determine exterior and interior egg quality. Eggshell strength was measured using egg multi tester instrument (QC-SPA, Technical Services and Supplies, TSS, UK). The shell thickness was measured at different three points using micrometer (Digimatic micrometer, series 293-330, Mitutoyo, Japan) after removing the inner shell membrane. An average of three different thickness measurements of an egg was described as eggshell thickness. Albumen height, Haugh unit, eggshell and yolk color were measured using semi-automated egg multi tester equipment (QCM+, TSS, UK).

Serum biochemical analysis

At the termination of the experimental period, blood samples were collected from the wing vein of two hens per replication and transferred into non-heparinized tubes. The collected blood samples were centrifugation at 3000 rpm for 15 minutes at 4^{o}C, serum was transferred into eppendorf tube and kept at -20^{o}C until the laboratory analysis. Serum albumin, triglycerides, cholesterol, high density lipoprotein (HDL), total protein, glucose, AST and ALT were measured by Konelab 20 analyzer (Thermo Fisher Scientific, Vantaa, Finland) following the manufacturer's instructions.

Yolk fatty acids profile

Towards the end of the experiment, eight eggs from each group were picked randomly in order to determine their fatty acids profile. Yolks from collected eggs were separated and 0.5g of fresh yolk was weighed into test tube. Analysis of the fatty acid composition of the total lipid was carried out by 7683B Series Injector and using 1µl 6890N Network GC system (Agilent Technologies, Santa Clara, USA) gas chromatography equipped.

Statistical analysis

The data were analyzed by two-way analysis of variance (ANOVA) using General Linear Models (GLM) Procedure of SAS (Statistical Analysis System, version 9.1, 2002) included the main effects of AP, RFL and the interaction of these effects. Duncan's multiple-range test was used to detect significant probability value (P<0.05).

Table 2. Effect of available phosphorus and restricted feeding levels on production performance of laying hens

AP (%)	RFL (%)	Egg Production (%)	Egg weight (g)	Daily egg mass (g)	FCR	Broken egg (%)	Cracked egg (%)
---- 40-60 weeks ----							
0.32	90	89.21^{c}	60.86^{c}	54.29^{b}	1.91^{c}	0.01	1.09
	95	89.97^{b}	61.48^{abc}	55.31^{ab}	1.98^{b}	0.18	1.47
	100	90.33^{a}	62.16^{ab}	56.15^{a}	2.05^{a}	0.18	1.18
0.45	90	88.92^{c}	61.19^{bc}	54.41^{b}	1.90^{c}	0.16	1.16
	95	89.85^{b}	61.89^{abc}	55.50^{a}	1.97^{b}	0.16	1.07
	100	89.83^{b}	62.66^{a}	56.29^{a}	2.04^{a}	0.12	0.81
Main effects							
AP (%)	0.32	89.83	61.50	55.25	1.98	0.13	1.25
	0.45	89.53	61.91	55.40	1.97	0.15	1.02
RFL (%)	90	89.06^{b}	61.02^{b}	54.35^{c}	1.91^{c}	0.09	1.13
	95	89.91^{a}	61.68^{ab}	55.40^{b}	1.98^{b}	0.17	1.27
	100	90.08^{a}	62.41^{a}	56.22^{a}	2.05^{a}	0.15	0.99
SEM		0.11	0.19	0.20	0.01	0.02	0.10
P value							
AP x RFL		<.0001	0.04	0.002	<.0001	0.30	0.68
AP		0.16	0.28	0.73	0.81	0.68	0.27
RFL		<.0001	0.005	<.0001	<.0001	0.32	0.59

AP, available phosphorus; RFL, restricted feeding levels; FCR, feed conversion ratio; SEM, standard error of the means; ^{a,b,c} means in the same column with different supercripts differ (P<0.05).

Results

Egg production, egg weight, feed conversion and broken eggs

The production performance of layers fed different levels of AP and access of feeding was presented in Table 2. Significant (P<0.05) difference shown in egg production, egg weight, daily egg mass and FCR in response to the main effects of RFL and the dietary interaction between AP and RFL treatment groups. The egg production of laying hens tended higher between the interaction of 0.32% AP and full-fed control (100%) group than that of other dietary treatments. As for main effect of AP, production performances of layer were not significantly affected but marginally decline egg production percentage and increase egg weight, daily egg mass were observed in high AP level. Comparable mean value was found in egg production percentage between 95 and 100% access of feeding group as well as egg weight in 90% and 95%; 95 and 100% feeding group. Egg production had significantly (P<0.01) inferior in 90% feeding group. Increasing level of restricted feed had linear effect on daily average egg mass and feed conversion ratio. Percentage of broken and cracked eggs in the present study was not influenced by the effect of AP or RFL and in their interaction.

Albumen height, haugh unit, yolk color and eggshell breaking strength

No significant (P>0.05) interaction was observed in egg quality traits between the restricted feeding and the AP levels without exception of eggshell color and eggshell breaking strength (Table 3). Hens fed diet containing 0.45% AP significantly (P<0.5) increased albumen height, haugh unit and eggshell breaking strength (P<0.01) than hens fed of 0.32% AP. Besides, egg yolk color and eggshell thickness was tended to be increased numerically in the main effect of high AP in diet. Restricted feeding level did not influence the egg quality traits except egg shell color (P<0.05).

Table 3. Effect of available phosphorus and restricted feeding levels on egg quality of laying hens at 60 weeks

AP (%)	RFL (%)	Eggshell color	Albumen height (mm)	Haugh Unit	Yolk Color	Eggshell breaking Strength (kg/cm^2)	Eggshell thickness (mm)
0.32	90	26.73^{a}	8.18	89.48	4.03	2.74^{b}	0.369
	95	25.37^{ab}	7.92	88.12	4.17	2.87^{b}	0.366
	100	25.73^{ab}	8.20	89.54	4.10	3.03^{ab}	0.3726
0.45	90	27.07^{a}	8.64	91.90	4.27	3.39^{a}	0.372
	95	26.27^{ab}	8.27	90.34	3.93	3.16^{ab}	0.370
	100	24.57^{b}	8.50	90.92	4.27	3.47^{a}	0.377
Main effects							
AP (%)	0.32	25.94	8.10^{b}	89.05^{b}	4.10	2.88^{b}	0.369
	0.45	25.97	8.47^{a}	91.05^{a}	4.16	3.34^{a}	0.373
RFL (%)	90	26.90^{a}	8.41	90.69	4.15	3.07	0.371
	95	25.82^{ab}	8.09	89.23	4.05	3.02	0.368
	100	25.15^{b}	8.35	90.23	4.18	3.25	0.375
S EM		0.25	0.09	0.50	0.05	0.06	0.002
P value							
AP x RFL		0.04	0.24	0.33	0.21	0.004	0.719
AP		0.96	0.04	0.04	0.54	0.0003	0.359
RFL		0.02	0.30	0.48	0.46	0.30	0.365

AP, available phosphorus; RFL, restricted feeding levels; SEM, standard error of the means; ^{a,b} means in the same column with different supercripts differ (P<0.05).

Serum biochemical indices

The effects of dietary available phosphorus and restricted feeding on serum biochemical parameters of laying hens had been shown in Table 4. None of the serum biochemical parameters were unaffected except albumin, glucose and total protein in the study. As the effect of dietary AP, higher glucose concentration was found in 0.32% AP content in diet. Serum albumin and total protein were differed significantly (P<0.05) by the sole effect of access of feeding levels. However, statistically similar difference was noticed in between 95 and 100% feeding group. In addition, serum AST and ALT level were not altered by the dietary AP, RFL and their interaction.

Table 4. Effect of available phosphorus and restricted feeding levels on serum characteristics of laying hens aged at 60 weeks

AP (%)	RFL (%)	Alb (g/dl)	Chol (mg/dl)	Glu (mg/dl)	AST (IU/l)	ALT (IU/l)	HDL (mg/dl)	TP (mg/dl)	TG (mg/dl)
0.32	90	1.95^{b}	134.11	276.59	167.99	0.46	6.54	5.94	1338.71
	95	2.07^{ab}	157.33	273.81	181.65	0.90	10.15	6.25	1925.22
	100	2.15^{a}	173.94	263.48	166.08	1.14	11.72	6.62	2094.23
0.45	90	1.97^{b}	152.02	250.83	160.65	0.49	9.75	6.20	1804.16
	95	2.08^{ab}	152.97	264.14	167.64	0.75	10.63	6.50	1785.39
	100	2.12^{a}	139.50	263.40	165.38	0.59	7.71	6.67	1620.75
Main effects									
AP (%)	0.32	2.06	155.13	271.29^{a}	171.91	0.83	9.47	6.27	1786.05
	0.45	2.06	148.16	259.46^{b}	164.56	0.61	9.36	6.46	1736.76
RFL (%)	90	1.96^{b}	143.07	263.71	164.32	0.47	8.15	6.07^{b}	1571.43
	95	2.08^{a}	155.15	268.98	174.64	0.82	10.39	6.38^{ab}	1855.30
	100	2.13^{a}	156.72	263.44	165.73	0.86	9.72	6.65^{a}	1857.49
SEM		0.02	6.12	2.79	3.28	0.08	0.71	0.10	121.16
P value									
AP x RFL		0.02	0.52	0.10	0.59	0.12	0.29	0.20	0.60
AP		0.98	0.58	0.03	0.27	0.18	0.94	0.33	0.84
RFL		0.02	0.62	0.67	0.39	0.11	0.42	0.05	0.55

AP, available phosphorus; RFL, restricted feeding levels; SEM, standard error of the means; [a,b] means in the same column with different supercripts differ (P<0.05).

Fatty acid composition of egg yolk

The effect of AP and RFL on fatty acid composition of egg yolk had been summarized in Table 5. The egg yolk lipid profile was not significantly (P>0.05) differed in the interaction of AP and feed restriction groups. The significantly (P<0.05) more

Table 5. Effect of available phosphorus and restricted feeding levels on yolk fatty acid composition of laying hens at 60 weeks

AP (%)	RFL (%)	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1	C20:4	C22:6	MUFA	PUFA	UFA	SFA	UFA/SFA
0.32	90	0.49	27.30	3.50	9.78	45.18	13.05	0.18	0.29	0.17	0.07	48.97	13.46	62.43	37.57	1.66
	95	0.46	27.54	3.58	9.55	47.14	11.03	0.16	0.30	0.17	0.08	51.02	11.44	62.45	37.55	1.68
	100	0.44	27.15	3.54	9.61	45.33	13.26	0.15	0.27	0.18	0.07	49.14	13.65	62.80	37.20	1.70
0.45	90	0.48	27.18	3.55	9.58	45.20	13.34	0.16	0.30	0.15	0.07	49.05	13.72	62.77	37.24	1.69
	95	0.44	27.06	3.50	9.46	46.57	12.28	0.14	0.33	0.16	0.06	50.39	12.64	63.04	36.96	1.71
	100	0.47	26.97	3.36	9.55	45.05	13.87	0.14	0.34	0.19	0.07	48.75	14.27	63.02	36.99	1.71
Main effects																
AP (%)	0.32	0.46	27.33	3.54	9.65	45.88	12.44	0.16	0.29[b]	0.17	0.07	49.71	12.85	62.56	37.44	1.68
	0.45	0.46	27.07	3.47	9.53	45.61	13.17	0.15	0.32[a]	0.17	0.07	49.39	13.54	62.94	37.06	1.70
RFL (%)	90	0.48	27.24	3.52	9.68	45.19	13.20	0.17	0.30	0.16[b]	0.07	49.01	13.59	62.60	37.40	1.68
	95	0.45	27.30	3.54	9.50	46.86	11.66	0.15	0.31	0.16[b]	0.07	50.71	12.04	62.75	37.26	1.69
	100	0.45	27.06	3.45	9.58	45.19	13.56	0.15	0.30	0.18[a]	0.07	48.94	13.96	62.91	37.09	1.70
SEM		0.01	0.16	0.08	0.14	0.34	0.36	0.004	0.008	0.004	0.002	0.356	0 .36	0.258	0.26	0.02
P value																
AP × RFL		0.27	0.95	0.99	0.99	0.35	0.25	0.09	0.15	0.12	0.59	0.36	0.26	0.98	0.98	0.97
AP		0.98	0.44	0.67	0.67	0.69	0.32	0.06	0.02	0.67	0.18	0.66	0.34	0.47	0.47	0.51
RFL		0.15	0.83	0.91	0.87	0.06	0.07	0.10	0.63	0.04	0.95	0.07	0.07	0.89	0.89	0.83

AP, available phosphorus; RFL, restricted feeding level; SEM, standard error of the means; [a,b] means in the same column with different supercripts differ ($P<0.05$).

eicosenoic acid (20:1) was found in the sole level of 0.45% AP content diet and arachidonic acid (20:4) in 100% full-fed control group, and comparable mean value was obtained in 90 and 95% restricted feeding group. Linoleic acid (C18:2) increased 5.68% when hens given 0.45% AP content diet than 0.32% AP that level was 12.22%. Furthermore, the total proportion of polyunsaturated fatty acids (PUFA) 5.37% and unsaturated fatty acids (UFA) 0.61% were increased in 0.45% AP containing diet as well as decreased 1.01% saturated fatty acids (SFA) as compared that of diet containing 0.32% AP.

Discussion

The present results showed that there is no significant difference on performance of laying hens as the effect of AP, which conforms to the result obtained by Boling *et al*. (2000) who reported that hens fed diet containing AP levels from 0.15 to 0.45% had no altered production performance during the entire experimental period (20 to 70 weeks). The results were also consistent with previous findings of (Ceylan *et al*., 2003). Another study, it has been found that production performance was not significantly high when laying hens fed a diet containing either levels of 2.5g/kg or 3.5g/kg AP at 21 to 61 weeks (Hughes *et al*., 2008). Thus, several reports indicate that productivity of laying hens had not changed when non-phytate phosphorus was higher than 2.0g/kg in corn soybean based diet even neither phosphorus deficiency symptoms (Mansoori and Modirsanei, 2015; Kozlowski and Jeroch, 2011). Francesch *et al*., (2005) found that egg weight was not affected by AP levels but reducing AP level resulted in a decrease egg weight which was partly in accordance with our results. Production performances of layer in the present study had greatly reduced with increasing level of feed restriction, which is in agreement to the previous study (Scott *et al*., 1999; Olawumi, 2014) who noted that levels of feed restriction either short-term or long term has significant effect on productive performance parameters during laying period. They also proposed that moderate quantitative feed restriction could not have negative effect on egg production performance, egg weight and it can be used for reducing feed and production costs and also get profit maximization. However, during the laying stage, the energy is the most important factor to ensure optimal production rates (Costa *et al*., 2009).Therefore, the reduction of production performance in laying hens in this study may be attributed due to the daily imposition of restricted feeding, which might be related to the limited daily energy, calcium, phosphorus and other nutrient intake of laying hens.

The results of egg quality traits are in agreement with the findings of Englmaierova *et al*., (2012) who reported that high level of non-phytate phosphorus (4g/kg) in diet significantly increased albumen height, haugh unit and eggshell thickness as well as other quality traits (yolk color, eggshell breaking strength and shell thickness) were positively influenced. Moustafa *et al*., (2015) also reported that high dietary AP level numerically increased eggshell thickness as compared to low. It was also shown that high AP in diet decreased haugh unit, which was opposed with our present result. In another study of Park *et al*., (2009) found that egg quality parameters were tended to be increased in aged laying hen when dietary levels of non-phytate phosphorus were 0.30 to 0.40%. However, the results obtained in the present study indicate that high dietary AP

(0.45%) improved egg quality, it possibly due to the fact that phosphorus may have influenced metabolic process and blood phosphorus concentration in aged hens. As to the effects of RFL, there was no effect of restriction level in egg quality traits that findings were similar with Osman *et al.*, (2010) who observed that feed restriction during laying period had no impact on egg quality. Nevertheless, significant effect was obtained in egg shell color, albumen height and eggshell breaking strength in dietary interaction between AP and the RFL treatment groups; it may be associated due to insufficient nutrient elements for restriction of feeding.

Kaya *et al.*, (2014) reported that laying hens fed diet containing 0.37% AP supplemented with organic acid mixture had significantly increased serum glucose concentration than that of control. It could be attributed to the dietary acidification that might stimulate glycogenesis pathway through the conversion of glucose 6-phosphate (G-6-P) to glycogen when the glucose in blood was high (Fushimi *et al*., 2001). Furthermore, it can be associated with stress on account of dietary restricted feeding may be elevated secretion of plasma corticosterone hormone. Previous study revealed that effect of energy restriction in poultry reduced serum total cholesterol and high-density lipoproteins (Chen *et al*., 2012). Rajman *et al*., (2006) stated that quantitative feed limitation in poultry had an effect on plasma biochemistry and also appeared that protein, albumin and triglyceride concentration were decreased in blood. On the other hands, Mellouk *et al*., (2018) observed that total cholesterol and triglyceride concentration was decreased when the quantitative restriction feed given to birds. According to Ei-Far (2014) conducted a study to investigate the effects of feed restriction on ducks and found that serum triglyceride and total cholesterol was significantly lower in restricted feeding groups that was partly same trend to the current study, although our results did not show significant effect. However, it revealed that restriction of diet could be modified the plasma lipids concentration and reduced the basal metabolism (Weyenberg *et al*., 2008). The turnover of adipose tissue triglyceride is responsive to change in macronutrient intake in which fasting increases lipolysis (Varady *et al*., 2007).

Generally, yolk fatty acid profiles vary depending on number of factors including improvement of nutrients in diet and management strategies and/or dietary manipulation which have been carried out different dietary oil sources supplementation in layer diets such as flaxseed, garlic, black cumin, fish and vegetable oil that those have given some promising effects. Several studies have previously shown that lipid composition of eggs yolk is fairly easy to change with dietary additives (Sing and Sachan, 2010), farming conditions (Rizzi *et al*., 2007) and housing system (Hidalgo *et al*., 2008), and also influenced by the laying hen breed (Kiani and Gharooni, 2016). The results of the present study cannot be comparable with the finding of previous literature since there is no distinct illustrative related evidence of the effects of AP on yolk fatty acids in laying hens. Linoleic acid was higher ($P<0.05$) in the egg yolk of hens fed 0.45% AP in our current study. According to the findings of Fendri *et al*., (2012) the linoleic acid of egg yolk increased by the supplementation of natural mineral (zeolite) 1 or 2% in layer diets from that of control. It was shown that selenium supplementation from 0.15 to 0.30 mg/kg in diet of laying hens significantly increased percentage of total unsaturated fatty acids, in this way increasing or decreasing the concentration of n-3 or n-6 in the total fatty acids pool (Zdunczyk *et al*., 2013). A significant increase ($P<0.05$) of arachidonic acid (20:4) was found 12.5% higher in full-fed control (100%) group. It could be due to the activity of delta-6 desaturase enzyme that promotes the desaturation of linoleic acid into arachidonic acid (Vidal *et al*., 2013). Although, the present results were not comparable with Li *et al*., (2016) who reported that higher phosphorus level in diet was significantly reduced fatty acid content of broilers meat than deficient and normal phosphorus diet. Therefore, the present investigation revealed that results indicating that higher level of dietary phosphorus influenced the long chain fatty acids profile of egg yolk.

Conclusion

The current study demonstrated that 95% feeding could be followed in hens during second production period without any adverse effects statistical change on the performance and egg quality. Dietary inorganic phosphorus at the level of 0.32% was sufficient to maintain the production performance, whereas 0.45% for egg quality.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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