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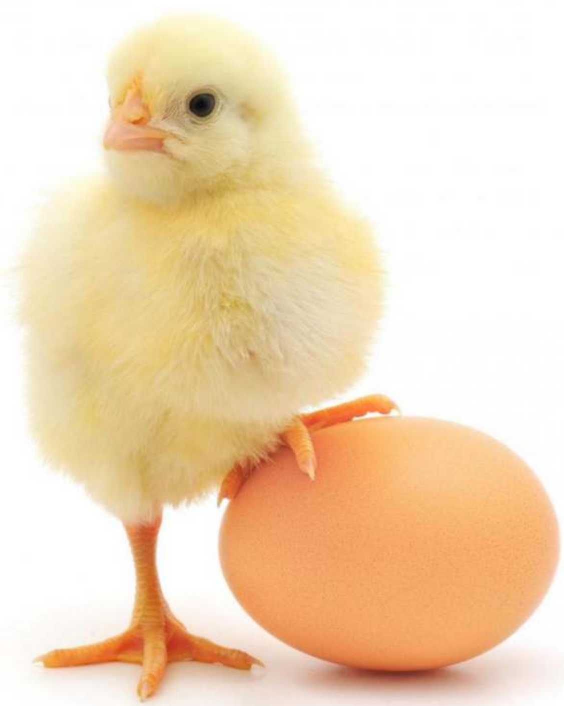

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Effect of Adding Different Dietary Calcium to Available Phosphorus Ratios on Productive Performance and Eggshell Quality of White Egg Layer Hens

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Abstract: To establish the optimum dietary calcium to available phosphorus ratio for the best productive performance and eggshell quality of layer hens, a total of 160 laying hens, 44-wk-old were used to study the effect of adding four different dietary ratios (13:1, 7.6:1, 5.17:1 and 3.87:1) of calcium to available phosphorus on the productive performance and eggshell quality of layer hens. Each treatment was replicated ten times with 4 hens each. Initial and final body weights, egg production, egg weight, egg mass, feed consumption, feed conversion ratio per egg mass, egg specific gravity, eggshell weight and eggshell thickness, eggshell percentage, eggshell weight per surface area Haugh unit and yolk colour were measured. The results obtained from the present study showed no significant differences among all the dietary calcium to available phosphorus ratios on body weight, egg production, egg weight, egg mass, feed consumption, feed conversion ratio per egg mass, egg specific gravity, eggshell thickness, eggshell percentage, eggshell weight per surface area, Haugh unit and yolk colour for the entire experimental period (from 44 to 52 wk of age) of layer hen hens. Based on the results obtained from the present study, it was possible to conclude that the greatest calcium to available phosphorus ratio 13:1 (the highest calcium level, 4.29 and 0.33% the lowest available phosphorus level) was sufficient to maintain the productive performance and eggshell quality at the lowest cost of white egg layer hen from 44 to 52 wk of age.

Key words: Available phosphorus, calcium, eggshell quality, layer hen, productive performance

INTRODUCTION

Calcium and phosphorus were considered the two very important macro-minerals in the layer hen diets due to their great contribution in productive performance and eggshell quality (Underwood and Suttle, 2001; Schreiweis *et al.*, 2003). The calcium and phosphorus represented about 99 and 80% of poultry bone ashes, respectively. These minerals have many important metabolic and fundamental roles in egg productive performance and eggshell quality for layer hens (McDowell, 1992). Furthermore, phosphorus was recognized as the third most expensive nutrient in poultry diet after energy and amino acids (Sari *et al.*, 2012).

The requirement of calcium and available phosphorus levels for layer hens have been changed due to the endless advances in genetic enhancement, nutrition, environment and management. These two macro-minerals are interdependently required in poultry diets and their functions are closely related to each other (Rama Rao *et al.*, 2006). While calcium is necessary to add at large quantity, available phosphorus is very important to add at low quantity. Some studies indicated the need to reduce dietary available phosphorus level to enhance internal and external eggshell quality for layer hens.

In general, available phosphorus requirements decrease as calcium requirements increase in the poultry diets. The explanations for these contrasting directions are unclear, but might be attributed to the fact that high dietary calcium levels reduced available phosphorus. Ballam *et al.* (1985) noted that excess dietary phosphorus reduced the availability of other minerals and subsequently increased the environmental pollution. Also, Hartel (1990) reported that high calcium levels in layer hen diets decreased phosphorus utilization. He also noted that the excess of phosphorus in layer hen diets inhibited the release of bone calcium and the adequate mineralization of eggshell. Anderson *et al.* (1995) reported that the excess of calcium consumption decreased the phosphorus utilization due to calcium to phosphorus ratio changes. These authors also reported that the excess of phosphorus levels in layer hen diets resulted in calcium deficiency. Furthermore, increasing dietary calcium level decreased the dietary energy content through the chelation of lipids (Driver *et al.*, 2005). In addition, several studies noted that the excess of calcium in layer hen diets reduced the availability of phosphorus, magnesium, manganese, copper, iron, zinc and other micro minerals and initiated secondary deficiencies (Selle *et al.*, 2009). Pelicia *et al.*

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(2009a) verified an elevating in the calcium excretion as dietary calcium levels increased when brown egg layer hen fed diets containing 3.0, 3.5, 4.0 and 4.5% calcium from 59 to 70 wk of age.

In contrast, insufficiency of phosphorus in poultry diets resulted in loss appetite, reduced growth in growing chicks and increased body weight loss in adult birds (Christensen *et al*., 2003; Narcy *et al*., 2009). On the other hand, some studies noted that high or low level of available phosphorus in layer hen diets adversely affected the productive performance and the eggshell quality (Harms, 1982; Bar and Hurwitz, 1984). Hartel (1990) observed a significant reduction in productive performance and high mortality rate for layer hen fed diets containing low phosphorus and high calcium levels. They also found that the deficiency of calcium and phosphorus produced bad eggshell quality and decreased egg size and egg production.

Gilbert *et al*. (1981) found that 3.0% calcium level was insufficient and 4.5% calcium must be supplemented in layer hen diets to keep reasonable eggshell quality. Mathur *et al*. (1982) mentioned that the available phosphorus level required for caged layer hens was higher than those reared on litter (Mathur *et al*., 1982). Daghir *et al*. (1985) recommended 0.25% available phosphorus as the minimum level required for layer hen diets. Hartel (1990) suggested that minimum daily requirement of available phosphorus about 360 mg for layer hen, whereas, phosphorus at the level of 0.4% was considered inadequate for layer hen diets by Vandepopuliere and Lyons (1992). Although, the National Research Council (NRC) in 1994 estimated that the minimum daily requirement of available phosphorus about 250 mg for each layer hen (NRC, 1994). This value was significantly lower than the previous recommendation of 350 mg available phosphorus reported by the NRC in 1984 (NRC, 1984). Also, the dietary calcium requirement was reduced from 3.75 to 3.25%. Pelicia *et al*. (2009b) found that adding 0.25% available phosphorus into layer hen diet was sufficient to maintain the productive performance and the eggshell quality after molting in the second egg laying cycle. Vieira *et al*. (2011) recommended 3.41% calcium as the minimum level required in the diet of layer hens. The dietary calcium and phosphorus have been studied extensively to reduce excessive phosphorus excretion of poultry. Pastore *et al*. (2012) noted that the excreta mineral contents elevated linearly as the dietary calcium levels increased. They attributed that for the bird ability to retain only the necessary calcium amount and to eliminate the excess in the excreta. They concluded that the bird kept using the same calcium amount regardless of the dietary calcium levels and eliminated the excess in the excreta.

Because the requirements of calcium and available phosphorus of layer hens have continuously changed, more consideration has been drawn to evaluate the calcium to available phosphorus ratio for layer hen diets. Thus, besides calcium and available phosphorus levels, their ratio in layer hen diets must be corrected to get the optimal productive performance and eggshell quality. Hence, the identifying the calcium to available phosphorus ratio requirements of commercial layer hens is very important concern for both poultry nutritionists and egg producers.

Rosol and Capen (1997) confirmed that monogastrics have the capacity to develop mechanisms of adaptation which result in increased absorption of phosphorus when it was supplemented at low dietary levels. They noted that the lower level of phosphorus promoted by using the higher calcium to phosphorus ratio was enough to meet the requirements of layer hens. Therefore, calcium to available phosphorus ratio was as important as formulating the diet itself. Van Eekeren *et al*. (1999) observed that calcium to phosphorus ratio up to 6:1 was suitable and necessary for layer hen diets. However, Boorman and Gunaratne (2001) found that the dietary calcium to phosphorus ratio was very important because high dietary phosphorus levels interfered with the calcium absorption reduced eggshell quality. Lukic *et al*. (2009) noted that the discussion of the calcium was always associated with the phosphorus in formula calcium to phosphorus ratios. Therefore, Pelicia *et al*. (2009b) suggested that 4.5% calcium and 0.25% available phosphorus with 18:1 of calcium to phosphorus ratio in the diet for the best productive performance for brown egg layer hens. The lower calcium to phosphorus ratios in the diet caused an excess of phosphorus, which decreased its absorption and increased the losses through feces (Pelicia *et al*., 2011). On the other hand, Pastore *et al*. (2009) noted that the daily consumption of 3.51 g of calcium and 289 mg of available phosphorus per layer hen fed diets containing diets containing 3.9% calcium with 12.12:1 calcium to available phosphorus ratio were enough requirements of calcium and available phosphorus for obtaining satisfactory productive performance and eggshell quality of white egg layer hens from 42 to 58 wk of age.

There were very little research had evaluated the effects of the different dietary calcium to available phosphorus ratios on the productive performance and eggshell quality of layer hens. In addition, there was little information on the dietary calcium to available phosphorus ratios of layer hens during the 2nd phase of the first laying cycle. Therefore, to determine the optimum calcium to available phosphorus ratio for the best productive performance and eggshell quality for white egg layer hens, this study was carried out to evaluate the effects of adding different four dietary calcium to available phosphorus ratios (13:1, 7.6:1, 5.17:1 and 3.87:1) on productive performance and eggshell quality of layer hens during the 2nd phase of the first laying cycle from 44 to 52 wk of age.

MATERIALS AND METHODS

Experimental design: The present study was conducted at the Agriculture Research Center belonged to King Faisal University, Al Hassa city, Kingdom of Saudi Arabia, in the period from September to October, 2014. A total of 160 layer hens (Hisex White®, 44-wk-old) during the 2nd phase of the first egg laying cycle over a period of 8-wk from 44 to 52 wk of age were used. Layer hens were weighed and randomly distributed in battery group cages (50 x 30 x 30 cm^3) separated by a 1.0 m aisle, equipped with galvanized-iron trough feeders covering the entire front length of metal cages and nipple drinkers. Hens fed four different dietary calcium to available phosphorus ratios (13:1, 7.6:1, 5.17:1 and 3.87:1) with ten replicates of four layer hens each. Hens were managed and fed according to the description and recommendations described in the Hisex white management guide, North American edition, 2008. The layer diets used in this study were calculated to be isocloric contained 2654 Kcal metabolizable energy and isonitrogenus contained 16.24% crude protein per kg of feed as shown in Table 1. At 44 wk of age, hens started to receive the experimental diets, all with the same amount of energy and nutrients, except for the calcium to available phosphorus ratios. The calcium levels were obtained by using limestone (50% fine and 50% coarse) while the available phosphorus levels were achieved by adding dicalcium phosphate (medium texture). Each hen fed 120 g once daily at 8 h and water was provided to all layer hens *ad libitum* during all the experimental period. All hens received a 16L:8D photo program for the duration throughout the whole experimental period.

Measurements: Initial and final body weight, egg production, egg weight, egg mass, feed consumption, feed conversion ratio per egg mass (kg feed per kg egg), egg specific gravity, eggshell weight, eggshell percentage, eggshell thickness, eggshell weight per surface area, Haugh unit and yolk colour were measured.

Initial body weight at the beginning and the final body weight at the end of the experiment for layer hens were measured and the average body weight gain was calculated by the differences between the two body weights. The experimental period had duration of 8 wk, divided into four sub periods of 2 wk each and concluded when hens completed 52 wk of age. At the end of each sub period, the egg production, egg weight, egg mass, feed consumption, feed conversion ratio per egg mass, egg specific gravity, eggshell weight, eggshell thickness, eggshell percentage, eggshell weight per surface area, Haugh unit and yolk colour were measured. Each 2 wk the feed leftovers from feeders were weighed and the feed consumption was measured. Feed consumption and egg number produced per hen were recorded on daily basis. Egg weight, egg mass, eggshell weight and eggshell thickness, egg specific gravity, eggshell percentage, eggshell weight per surface area, Haugh units and yolk colour for each replicate were calculated at the last 3 consecutive d of each sub period. For the calculation of egg mass, the average egg production was multiplied by the average egg weight divided by 100. The feed conversion ratio per egg mass was obtained and calculated as kilograms by the ratio between total feed consumed per hen/total egg mass produced per hen.

Collected eggs were stored overnight in the same room before egg specific gravity was determined. Egg specific gravity was determined as described by Harms *et al.*, (1990) by immersing the eggs in graded saline solutions of density ranged from 1.055 to 1.100 g/cm^3 with interval increments of 0.005 g/cm^3 between them. After determining egg specific gravity, the same eggs were subsequently broken, their components were separated and then eggshell with shell membranes were washed and left to dry in the air before being individually weighed.

The eggshell thickness including its membranes was a result from the three readings performed at three different sites of the equatorial region of the same eggshell by using a digital caliper (pachymeter) with 0.01 mm precision and calculating the average among the three sites. Eggshell weight per surface area (ESWSA) expressed in mg/cm^2 was determined according to Abdullah *et al.* (1993). The following formula was used:

$$\text{ESWSA} = \{\text{EGW}/[3.9782 \times (\text{EW}0.7056)]\} \times 1000$$

Where, EGW: eggshell weight, EW: egg weight

Albumen height was measured with an Ames micrometer (model S-6428, Ames, Waltham, MA) at a point halfway between the yolk and the edge of the widest expanse of albumen according to USDA (2000). Haugh unit was calculated as follows:

$$\text{Haugh unit} = 100 \times \log (H + 7.57 - 1.7W^{0.37})$$

Where, H is albumin height (mm) and W is egg weight (Panda, 1996). The yolk colour of eggs was measured using Roche colourimetric fan with scores varying between 1 and 15.

Statistical analysis: The results data obtained were subjected to one-way ANOVA using the GLM procedure of a statistical software package (SPSS 18.0, SPSS Inc., Chicago, IL). Experimental units were based on cage averages and the treatment means were expressed as mean±standard error of means (SEM) and separated and compared by the F test ($p \leq 0.05$) using the Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

To formulate the experimental diets, all feed ingredients used in the present study were the same percentages among all the experimental dietary treatments expect limestone to dicalcium phosphate ratios. The dicalcium phosphate is an expensive source of calcium and phosphorus in poultry diets. On the other hand, limestone considered as the principal supplemental calcium source used due to its abundant natural reserves, low cost and easy incorporation into layer hen diets. Therefore, limestone was sold at relatively cheaper cost than dicalcium phosphate.

Limestone to dicalcium phosphate ratios of 89.7:10.3, 80.2:19.8, 69.9:29.1 and 60.3:39.7 were used in the present study to obtain 4.29,4.12,3.93 and 3.75% calcium and 0.33, 0.54, 0.76 and 0.97% available phosphorus with keeping calcium to available phosphorus ratios at 13:1, 7.6:1, 5.17:1 and 3.87:1, respectively as shown in Table 1. In the present study, each layer hen daily consumed about 120 g layer diet obtaining about 396, 648, 912 and 1164 mg available phosphorus and 5.148, 4.944, 4.716 and 4.500 g calcium daily.

The results obtained from the present study showed that no significant differences of all the dietary calcium to available phosphorus ratios on productive performance parameters such as body weight, egg production, egg weight, egg mass, feed consumption and feed conversion ratio per egg mass were detected as shown in Table 2.

No effect on productive performance parameters when layer hens fed diets containing calcium levels in the range used in the present study were reported by different studies (Frost and Roland, 1991; Clunies *et al*., 1992; Leeson *et al*., 1993). Keshavarz and Nakajima (1993) noted no effect on egg weight when layer hens fed diets containing calcium levels ranged from 2.80 and 4.40%. Safaa *et al*. (2008) also observed no significant effects on body weight gain, egg weight and feed consumption for brown egg layer hens fed diets containing 3.5 or 4.0% calcium with keeping the calcium to available phosphorus ratio of 12:1 from 58 to 73 wk of age. The results obtained for the productive performance parameters in the present study were also similar to those found by Pelicia *et al*. (2009a) who noted no effect on productive performance parameters such as body weight, feed consumption, egg production, feed conversion ratio, egg weight and egg mass when layer hen fed diets supplemented with 3.0, 3.5, 4.0 and 4.5% calcium and 0.25, 0.30, 0.35 and 0.40% available phosphorus levels at different calcium to available phosphorus ratios after molting in the second egg laying cycle from 90 to 108 wk of age. Similarly, Rosa *et al*. (2011) observed no effect on feed consumption, egg production, feed conversion ratio per egg mass and egg weight from the dietary calcium to phosphorus ratios with 8:1; 11:1 and 14:1 of brown egg layer hens from 28 to 36 wk of age. El-Ghamry *et al*. (2011) reported no significant differences in egg production, egg weight and egg mass when Fayoumi layer hens fed diets containing four levels of calcium ranged from 2.4 and 3.3% from 45 to 57 wk of age. In addition, Salama *et al*. (2012) found that body weight gain, egg weight and feed conversion ratio per egg mass were not significantly affected by calcium levels ranged from 3.0 and 4.0% in the diets of commercial layer hens from 24 to 36 wk of age. Pastore *et al*. (2009) showed no effect for the calcium levels and the calcium to phosphorus ratios on body weight, egg production, egg weight, egg mass, feed consumption and feed conversion ratio when fed layer hen diets containing three levels of calcium (3.9, 4.2 and 4.5%) and three calcium to phosphorus ratios (12.12:1; 10.53:1; and 9.30:1) from 42 to 58 wk of age.

However, some studies reported a significant improvement in egg production as calcium levels were increased from 2.2 to 3.9% and as available phosphorus levels increased from 0.1 to 0.3% in layer hen diets (Hartel, 1990; Abdullah *et al*., 1993). Frost and Roland (1991) noted a significant linear increased in feed consumption as dietary calcium level decreased. Gordon and Roland (1998) found that increasing dietary available phosphorus levels from 0.1 to 0.3% resulted in increased feed consumption by about 8.9%. In addition, Safaa *et al*. (2008) observed improvements in the egg

Table 1: Composition experimental diets[1]

	--- Calcium to available phosphorus ratios ---			
Feed ingredients (%)	13:1	7.6:1	5.17:1	3.87:1
Dietary treatments				
Yellow corn	62.00	62.00	62.00	62.00
Wheat bran	5.00	5.00	5.00	5.00
Soybean meal (48.5%) CP	21.00	21.00	21.00	21.00
Limestone	10.40	9.30	8.10	7.00
Dicalcium PO_4	1.20	2.30	3.50	4.60
DL-Methionine	0.10	0.10	0.10	0.10
Vitamin-mineral Premix[2]	0.10	0.10	0.10	0.10
Salt	0.20	0.20	0.20	0.20
Calculated nutritional composition				
Energy (Kcal ME/kg feed)	2654	2654	2654	2654
Crude protein (%)	16.24	16.24	16.24	16.24
Crude fat (%)	2.72	2.72	2.72	2.72
Crude fiber (%)	2.83	2.83	2.83	2.83
Linolenic acid (%)	1.63	1.63	1.63	1.63
Calcium (%)	4.29	4.12	3.93	3.75
Available phosphorus (%)	0.33	0.54	0.76	0.97
Limestone: Dicalcium phosphate	89.7:10.3	80.2:19.8	69.9:29.1	60.3:39.7
Feed cost ($)	238.7	246.7	255.2	263.2

[1]Calculated analysis of the diets was as follows: 16.24% crude protein; 2,654 kcal metabolizable energy; 2.72% crude fat, 3.83% crude fiber, 1.63% linolenic acid, 0.44% methionine, 1.25% lysine, 0.77%; threonine, 0.28%; tryptophan/kg feed

[2]Vitamin-mineral premix added at this rate yields: 149.60 mg Mn, 16.50 mg Fe, 1.70 mg Cu, 125.40 mg Zn, 0.25 mg Se, 1.05 mg I, 11,023 IU vitamin A, 46 IU vitamin E, 3,858 IU vitamin D_3, 1.47 mg minadione, 2.94 mg thiamine, 5.85 mg riboflavin, 20.21 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 478 mg choline, 16.50 µg vitamin B_{12}, 45.93 mg niacin and 7.17 mg pyridoxine per kg diet

production, egg mass and feed conversion ratio per egg mass when layer hens fed a diet contained 4.0% calcium compared to those fed a diet contained 3.5% calcium. Also, Pelicia *et al.* (2009b) noted that feed conversion ratio per dozen eggs improved (1.73) when dietary calcium level increased from 3.0 to 3.47%; however, feed conversion ratio per dozen eggs worsened (1.76) when calcium levels was added into diets between 3.47 and 4.21%, but improved thereafter. Therefore, these authors suggested that the diet containing 4.5% calcium improved feed conversion ratio per dozen eggs.

Similarly, Silva *et al.* (2008) observed that the increase in the calcium to phosphorus ratios from 9:1 to 14:1 in diets for layer hens improved egg production linearly. These authors also noted significant effect from the calcium to phosphorus ratios on egg weight, egg mass and feed conversion ratio per egg mass. Therefore, they concluded that the calcium to phosphorus ratio of 14:1 (4.2% calcium and 0.3% available phosphorus) would guarantee satisfactory productive performance of brown egg layers from 28 to 44 wk of age. Murata *et al.* (2009) recorded an increase in egg production when layer hens fed diets containing 4.05% calcium and linear significant improvements in egg weight and feed conversion ratio per egg dozen of white egg layer hens fed diets supplemented with 3.75, 4.15, or 4.55% calcium at 57 wk of age.

On the other hand, Owings *et al.* (1977) observed a reduction in egg production when available phosphorus levels were reduced from 0.22 to 0.11% in layer hen diets at 55 wk of age. These authors studied lower available phosphorus levels than those used in the present study, which might explain the differences in egg production between these two studies. Also, Hartel (1990) observed a significant reduction in productive performance parameters and increasing in mortality rate when layer hen consumed less than 360 mg available phosphorus daily in their diets. Some studies noted that the deficiency of calcium and phosphorus levels in poultry diets increased mortality rate and elevated loss body weight in adult birds and reduced body weight in growing chicks (Underwood and Suttle, 2001; Christensen *et al.*, 2003; Narcy *et al.*, 2009).

Eggshell quality

A-Egg external quality: The external eggshell quality was recognized as the most accurate and commonly measurement used indicators of calcium and phosphorus metabolism in layer hens. Determination of egg specific gravity was useful because it was related to eggshell thickness and eggshell percentage. Measuring eggshell weight was a more labor-intensive method for measuring calcium and phosphorus metabolism in layer hens, but can be used to validate egg specific gravity results.

In the present study, no significant differences of dietary calcium to available phosphorus ratios on egg external quality parameters such as egg specific gravity, eggshell thickness, eggshell weight, eggshell percentage and eggshell weight per surface area as shown in Table 2. These results were in agreement with those findings reported by Keshavarz and Nakajima (1993) who noted no effect of 3.5 or 4.5% dietary calcium on egg specific gravity. Also, Chowdhury and Smith (2002) recorded no effect of the dietary calcium levels ranged from 2.5 and 4.0% on eggshell weight. The results for eggshell weight and egg specific gravity were similar to those obtained by Murata *et al.* (2009), who also observed no effect on eggshell weight and egg specific gravity when white layer hen fed diets containing 3.75, 4.15 and 4.55% calcium levels at 57 wk of age. Similarly, Pelicia *et al.* (2009a) found no significant differences in eggshell thickness and egg specific gravity for brown egg layer hens fed 3.0, 3.5, 4.0, or 4.5% calcium levels in the second egg laying cycle. Pelicia *et al.* (2009a) found no significant effects of calcium and available phosphorus levels on egg specific gravity and eggshell thickness. They also showed that dietary calcium levels affected eggshell percentage and eggshell weight per surface area.

These results were consistent with the results obtained from the present study and with those found by Abd El-Maksoud (2010) and Salama and EL-Sheikh (2012), who found that eggshell quality was not significantly affected by different levels (3.25 up to 4.0 and 0.30 up to 0.40%) of calcium and available phosphorus, respectively. The same results were recorded by Pastore *et al.* (2009) who reported no effect of the calcium levels and the calcium to phosphorus ratios on egg specific gravity, eggshell weight and eggshell thickness when layer hens fed diets containing three levels of calcium (3.9, 4.2 and 4.5%) and three calcium to phosphorus ratios (12.12:1; 10.53:1 and 9.30:1) from 42 to 58 wk of age.

However, some studies noted an improvement in egg specific gravity and eggshell weight as dietary calcium levels increased from 2.5 to 3.1% and available phosphorus from 0.1 to 0.5% (Ousterhout, 1980; Hartel, 1990; Frost and Roland, 1991; Abdullah *et al.*, 1993, Gordon and Roland, 1998). Daghir *et al.* (1985) reported an enhancement in eggshell thickness when layer hen fed diets containing 0.35% or lower available phosphorus levels. Also, Clunies *et al.* (1992) found an increase in eggshell weight and egg specific gravity when layer hen fed diets containing 3.5 and 4.5% calcium. Sohail and Roland (2002) observed that the lowest dietary available phosphorus level produced the lowest egg specific gravity when layer hens were fed diets containing 0.1 to 0.7% available phosphorus levels. Safaa *et al.* (2008) showed an improvement in eggshell thickness for brown egg layer hens fed diets containing 4.0% calcium compared to those fed diets

Table 2: Productive performance and eggshell quality parameters of Hisex white egg layer hens fed diets with four calcium to available phosphorus ratios from 44 to 52 wk of age

	Calcium to available phosphorus ratios			
Parameters	13:1	7.6:1	5.17:1	3.87:1
Dietary treatments				
Productive performance				
Initial body weight (g)	1517.857±21.737	1478.333±35.418	1533.000±55.082	1499.167±30.995
Final body weight (g)	1472.143±37.526	1435.556±34.129	1415.000±37.417	1351.111±14.490
Body weight gain (g)	15.714±13.382	39.722±34.961	1533.000±55.082	1499.167±30.995
Egg production (number)	42.250±1.430	41.375±1.634	37.900±2.325	41.958±2.342
Egg production (%)	75.446±2.553	73.884±2.918	67.679±4.151	74.926±4.182
Egg weight (g)	63.850±0.839	61.183±0.333	60.920±0.729	62.879±1.137
Egg mass (kg)	2.697±0.092	2.532±0.105	2.311±0.152	2.639±0.158
Feed consumption (kg)	5.685±0.046	5.361±0.235	5.371±0.161	5.441±0.121
Feed conversion ratio (kg feed/kg egg)	2.122±0.065	2.130±0.112	2.357±0.141	2.091±0.103
External eggshell quality				
Egg specific gravity (g/cm^3)	1.090±0.001	1.090±0.001	1.090±0.001	1.092±0.001
Shell thickness (mm)	0.453±0.048	0.388±0.024	0.402±0.008	0.398±0.015
Eggshell weight (g)	11.286±0.184	10.500±0.342	10.800±0.200	10.667±0.333
Eggshell percentage	17.638±0.119	17.720±0.238	17.128±0.152	17.254±0.166
Eggshell weight per surface area (mg/cm^3)	14.890±0.134	14.850±0.234	14.660±0.094	14.530±0.194
Internal eggshell quality				
Egg Huagh unit	8.718±0.374	8.567±0.312	8.945±0.244	8.513±0.198
Egg yolk colour	5.464±0.184	5.083±0.211	5.050±0.200	5.583±0.230

Means±standard error of mean within a row that do not share a common superscript are significantly different ($p \leq 0.05$)

containing 3.5% calcium with keeping the calcium to phosphorus ratio at 12:1 from 58 to 73 wk of age. Also, Pelicia *et al*. (2009b) showed that increasing the dietary calcium level up to 4.5% enhanced the eggshell percentage and eggshell weights per surface area as well as 0.25% available phosphorus without improvements were detected as dietary phosphorus level increased. Therefore, these authors suggested that the diet containing 4.5% calcium improved feed conversion ratio per dozen eggs and eggshell quality.

Conversely, Gilbert *et al*. (1981) noted a decline in the eggshell percentage when layer hens fed diet containing 3.0% calcium as compared to those fed diet containing 4.5% calcium. Miles *et al*. (1983) showed that egg specific gravity was inversely proportional to dietary available phosphorus levels. These authors determined that total phosphorus levels higher than 0.5% (0.7, 1.5 and 2.3%) were inversely related to egg specific gravity and that calcium levels below 0.3% (0.23 and 0.17%) produced the worst results. Also, Junqueira *et al*. (1984) reported a reduction in the egg specific gravity when layer hens fed 0.3% total phosphorus as compared to those fed 0.6% total phosphorus.

On the other hand, Vieira *et al*. (2011) mentioned that the 3.9% calcium level was enough to meet the requirements of the layers to obtain the best efficiency of calcium and to maintain the eggshell quality when studied the effect of the calcium levels of 2.8, 3.3 and 3.8% in the diet of brown egg layers at 40 wk of age. Pastore *et al*. (2009) mentioned that diets containing calcium at 3.9% and a calcium to available phosphorus ratio of 12.12:1, corresponding to a daily consumption of 3.51 g of calcium and 289 mg of available phosphorus per layer hen, were enough for obtaining satisfactory productive performance and eggshell quality for commercial white egg layer hens from 42 to 58 wk of age.

B-Egg internal quality: In the present study, no significant differences of dietary calcium to available phosphorus ratios on egg internal quality parameters such as Haugh unit and yolk colour as shown in Table 2. The results were in agreement with Pelicia *et al*. (2009b) who noted that no significant effects of dietary calcium and available phosphorus levels on Haugh units, but were in disagreement with their findings that the intensity of orange in the yolk colour increased with increasing dietary calcium levels when layer hen fed diets containing four calcium (3.0, 3.5, 4.0 and 4.5%) and four available phosphorus levels (0.25, 0.30, 0.35 and 0.40%) after molting from 90 to 108 wk of age.

Although was known that the calcium and phosphorus levels of a diet for layer hens were directly related to the productive performance and the eggshell quality of layer hen. There were no effects of the calcium to phosphorus ratios productive performance and the eggshell quality parameters of layer hen, the increase of calcium and the decrease of phosphorus level (higher calcium to available phosphorus ratio) did not affect the productive performance and eggshell quality parameters of the layer hens.

Ceylan *et al*. (2003) observed that as the calcium to phosphorus ratios were reduced in the diet characterized by the high levels of phosphorus, the concentration of phosphorus in the excreta increased when white egg layer hens fed diets containing 0.40, 0.35, 0.30, or 0.25% available phosphorus and 3.8% calcium from 20 to 40 wk of age. From nutritional, economical and environmental view, it is very important to produce more-economical diets appropriate for layer hen, to promote less excretion of minerals especially phosphorus in the feces and to reduce the negative effect of poultry production on the environment. Reducing the phosphorus in the diet without effects on productive performance and eggshell quality of layer hens might decrease the phosphorus excreted in the faces and reduced the environmental phosphorus polluting.

In the present study, the feed cost for the experimental dietary treatments containing 13:1, 7.6:1, 5.17:1 and 3.87:1 of the calcium to available phosphorus ratios were 238.7, 246.7, 255.2 and 263.2 $/ton as shown in Table 1. Therefore, the results obtained from the present study indicated that using diet containing 13:1 calcium to available phosphorus ratio saved about 24.5 $/ton feed without affect the productive performance and eggshell quality parameters of Hisex white layer hen from 44 to 52 wk of age.

The results obtained from the present study indicated that there were no deficiencies of calcium and available phosphorus levels used in layer hen diets. This might explain why no significant differences among all the dietary calcium to available phosphorus ratios were detected in all the productive performance and eggshell quality parameters measured in the present study compared with some other studies. This might be attributed into the minimum requirements of calcium and available phosphorus levels used in the present study were more than those reported in several studies.

Conclusion: The lack of effects of the all dietary calcium to available phosphorus ratios used in the present study on productive performance and eggshell quality of layer hens might be probably due to the fact that the marginal level of calcium and the available phosphorus and the calcium to available phosphorus ratios utilized were enough to meet the requirements or were not suboptimal or below the recommendations of layer hens. Based on the results obtained from the present study, it was possible to conclude that the greatest calcium to available phosphorus ratio 13:1 (the highest calcium level, 4.29 and 0.33% the lowest available phosphorus level) was sufficient to maintain the productive performance and eggshell quality at the lowest cost of white egg layer hen from 44 to 52 wk of age. Thus, it was possible to formulate a diet of lower cost, more appropriate to layer hen, with lower excretion of minerals, especially phosphorus and thus reducing the negative effect of layer production on the environment. Further studies are required to identify and to determine the calcium and phosphorus retention in the blood and excretion resulted from different calcium to available phosphorus ratios.

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