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Efficacy of Feeding Various Calcium Source and Concentration on Egg Quality, Some Blood Variables, and Performance of Aged Laying Hens

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Abstract

The primary aim of this study was to assay the influence of dietary Ca sources and levels on performance, egg quality indices, and selected blood variables of laying hens. A total of 192 Bovanz commercial layers were distributed to 6 dietary treatment groups with 4 replicates and 8 birds in each replicate. The experimental diets were iso-caloric and iso-nitrogenous, but they were different in the concentrations of available phosphorus and total calcium (0.29 and 3.8% or 0.31 and 4.0%, respectively) and in the origination of applied calcium (limestone A, limestone B, and oyster shell). Although the amounts of calcium and phosphorus were different among the diets, the ratio between them was the same in all diets. The results indicated that dietary treatments had not any significant effect on feed intake, feed conversion ratio, egg production, egg weight, egg mass, and body weight gain of hens. Egg quality indices were not influenced by dietary treatments in both egg sampling, except for eggshell weight and shell weight ratio, which decreased as a reduction of the dietary Ca level in the second period. The rate of broken, soft-shell and unmarketable eggs laid by the hens fed a diet containing lower Ca was increased. There was no significant effect of dietary Ca source and concentration on blood Ca and P, while serum ALP activity decreased significantly with increasing the amount of calcium in the diet. There was also, no interaction between Ca source and concentration for any of these parameters. Although all Ca sources applied in this experiment could supply the hens with sufficient Ca, the rate of unmarketable eggs decreased by using a higher concentration of Ca.

Introduction

Egg producers are frequently seeking to improve their profitability by expanding egg production and enhanced egg quality. Nearly eight percent of all losses in egg production is obtained due to the poor eggshell quality (Gheisari *et al.,* 2011). These losses have a prominent effect on the economics of commercial egg production. On the other hand, some factors such as nutrition, management, and environmental conditions can directly affect the quality of the eggshell (Emery *et al.,* 1984; Solomon, 1991). It's clear that understanding the mechanism of action and paying attention to these agents can have a significant impact on the economy of egg producer farms.

The age of the birds (Albatshan *et al.,* 1994) and dietary calcium (Clunies *et al.,* 1992) are the main factors affecting the eggshell quality. For this reason, the low quality of eggshells produced at the end of the laying period is a chief concern in poultry nutrition. It is already accepted this issue has been associated with egg weight growing with no corresponding increase of mineral deposition into the eggshell, which might due to decreased potency of mineral absorption and mobilization in the body with age (Roland, 1979).

Metabolism and the turnover of calcium in layers are complicated and amazing in comparison to mammals due to its vital function in the reproductively female birds in eggshell formation (Hester, 2017). Furthermore, Ca as the most abundant inorganic portion of the skeleton plays a key role in a

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wide range of biological processes. It's required for muscle contraction, the release of synaptic neurotransmitters, and bone integrity (Scanes, 2015). On the other hand, the two elements of calcium and phosphorus are discussed together because of their close association with metabolism. Insofar as differences in dietary calcium concentration can affect the rate of hydrolysis of phytate phosphorus in the small intestine of birds. Therefore, to achieve maximum efficiency of dietary calcium and phosphorus, it is important to pay attention to the levels of each of these two minerals and the ratio between them. Despite all that, because calcium sources, mostly oyster shell and limestone, are low-priced compared with other minerals, slight emphasis paid attention to determining the Ca requirement (Powell *et al.,* 2011). Control of Ca metabolism in hens is hugely effective and firmly regulated, necessary for the high desire of Ca, which are related to the eggshell calcification and fast growth rate of hens. Briefly, Ca is absorbed from the intestines and transported to the uterus through the blood, however, due to the great desire for Ca while eggshell formation, the body cannot receive the Ca speedy enough and this is when the birds turn to their skeletal reservoir as a subsequent source (Johnson, 2015). In the late stage of egg production, Ca metabolism is under strain when layers are less capable to absorb Ca (Albatshan *et al.,* 1994).

The Ca concentration in the diet involves both financial and nutritional implications. Shortages in Ca mostly, cause health and welfare implications including a reduction in eggshell quality and enhance the prevalence of leg health problems (Underwood and Suttle, 2001). Significantly, as a result of a decline in the dietary level of calcium, the potency of both exogenous and endogenous phytase may be improved and the proportion of excreted phosphorus may be reduced (Selle *et al.,* 2000). Besides, redundant dietary Ca fed to hens can consequence in urate deposits in the kidney (Crespo, 2014). Moreover, the increasing dietary energy content may be decreased by chelating of lipids due to the high amount of dietary Ca (Driver *et al.,* 2005). Egg producers mostly apply two dominant sources of Ca, include of oyster shell or limestone.

The majority of researchers, while evaluating two sources at similar particle sizes, deduced that limestone and oyster shell involve an identical value for the eggshell quality (Roland, 1986). Even regarding limestone, the composition of various limestone sources can be different and might be due to the region where it's mined. Sources might also vary as the Ca quantity and the existence of other nutrients, which could impact the usage of calcium source by the laying hens (Reid and Weber, 1976). The purpose of the present experiment, therefore, was to determine the efficacy of different sources and concentrations of Ca in diets on performance, egg quality indices and some blood parameters of aged laying hens.

Materials and Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Shahid Bahonar University of Kerman, Kerman, Iran.

Birds and experimental diets

In total, one hundred ninety-two, 94-week Bovanz white commercial layers in the second year of production (nearly one month following termination of the molting phase and the onset of re-laying) with similar weights and production rate were included in this experiment. Hens were distributed in factorial arrangement with completely randomized design into 6 dietary groups with 4 replicates of 8 birds each. The experimental diets were formulated to meet or exceed the nutritional needs of birds (Hendrix Genetics, 2010). The diets were isocaloric and isonitrogenous (Table 1), but with the same ratio of calcium and available phosphorus their concentrations (0.29 and 3.8% or 0.31 and 4.0%, respectively) as well as the origin of calcium (limestone A, limestone B, and oyster shell) was differed. The origin of Limestone "A" was Golestan province in the north of Iran while limestone "B" obtained from Fars province in the south-central part of Iran. Oyster shell prepared from the coastal city of Gilan, Iran.

All Ca sources were fed to laying hens at the identical particle size. All hens were kept in three-tiered cages (L× W× H= 74× 60× 46 cm) with 8 birds in each cage. Artificial lighting was provided so that the birds were exposed daily to 16 h light: 8 h dark. This project began in early July and lasted seventy days following 7 days of adaptation. Throughout the experimental periods, water was available *ad libitum* and identical management conditions were considered for all birds.

Data collection and procedures

To determine the body weight gain (BWG), all birds were weighed at the onset (94 weeks of age) and the end (104 weeks of age) of study. Eggs were counted and weighed daily and egg mass was computed based on grams of egg/hen/day. Daily egg production was evaluated on the hen-day basis. The egg loss was considered as eggs that were broken, cracked, or soft-shell. Feed intake (FI) was calculated on a cage basis by dividing the amount of weekly feed consumption by the number of hens at the end of the week. Feed conversion ratio (FCR) was stated as the grams of feed consumed per grams of egg produced.

At 99 and 104 weeks of age, eggs were taken from each treatment in three consecutive days, weighed and egg quality traits were measured. The flotation procedure (Hempe *et al*., 1988) was applied to

determine specific gravity with a range of salt solutions from 1.065 to 1.120 g/cm^3. After that, the egg index was calculated according to the procedure described by Shuttz (1953). Also, Wesley and Staldelmen, (1959) method was applied to the determination of the Haugh unit and yolk index. Yolk weight ratio was obtained by dividing the yolk weight by the total egg weight and expressed as a percentage (Salajegheh *et al.,* 2018). Shell weight was measured after cleaning adhering albumen and drying at laboratory temperature for 48 h. Shell thickness was measured at three different locations (sharp and blunt ends, and also a middle section of an egg) using a micrometer and the mean value was taken as thickness (Salajegheh *et al.,* 2018). The eggshell ratio was calculated using the following formula: eggshell ratio (%) = (shell weight/egg weight) × 100. Eventually, collected egg yolks were evaluated and scored using the Roche yolk color fan (1: light yellow; 15: orange) and then, the weight of yolk was registered and displayed as a% of egg weight (Salajegheh *et al.,* 2017).

Table 1. Ingredients and composition of experimental diets

Feed ingredients (g / 100 g diet)						
Ca source	Limestone A		Limestone B		Oyster shell	
level [a]	1	2	1	2	1	2
Corn	36.00	36.00	36.00	36.00	36.00	36.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00
Barley	5.94	5.94	5.94	5.94	5.94	5.94
Wheat	15.00	15.00	15.00	15.00	15.00	15.00
Wheat bran	4.00	4.00	4.00	4.00	4.00	4.00
Soybean oil	2.86	2.86	2.86	2.86	2.86	2.86
Dicalcium phosphate	1.08	1.18	1.08	1.18	1.08	1.18
Lime stone	9.21	9.68	9.21	9.68	-	-
Oyster shell	-	-	-	-	9.46	9.95
Common salt	0.17	0.17	0.17	0.17	0.16	0.16
NaHCO3	0.22	0.22	0.22	0.22	0.25	0.25
Vit. & Min. Premix [b]	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine-HCL	0.08	0.08	0.08	0.08	0.08	0.08
DL-Methionine	0.18	0.18	0.18	0.18	0.18	0.18
Natozim Plus [c]	0.07	0.07	0.07	0.07	0.07	0.07
Filler (Sand)	4.69	4.12	4.69	4.12	4.42	3.84
Calculated analyses						
ME_n (Kcal/kg)	2600	2600	2600	2600	2600	2600
Crude protein (%)	15.00	15.00	15.00	15.00	15.00	15.00
Ether extract (%)	5.00	5.00	5.00	5.00	5.00	5.00
Crude fiber (%)	3.36	3.36	3.36	3.36	3.36	3.36
Calcium (%)	3.80	4.00	3.80	4.00	3.80	4.00
Available P (%)	0.29	0.31	0.29	0.31	0.29	0.31
Lysine (%)	0.79	0.79	0.79	0.79	0.79	0.79
Methionine (%)	0.40	0.40	0.40	0.40	0.40	0.40
Met & Cys (%)	0.64	0.64	0.64	0.64	0.64	0.64

[a] Level 1: Available P and Ca (0.29 and 3.8%); level 2: Available P and Ca (0.31 and 4.0%).

[b] Mineral premix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4; Zn, 169.4 mg. 'Vitamins premix supplied the following per kg of diet: Vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitamin K; 4 mg; vitamin B\2, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.

[c] Natozim Plus: each kg provides: 10000000 units zylanase, 6000000 units cellulase, 700000 units beta-glucanase, 700000 units alpha-amylase, 70000 units pectinase, 500000 units phytase, 3000000 units protease and 30000 units lipase.

At the end of the trial, blood samples were obtained via the bronchial vein of two birds from each replicate. The samples were poured into tubes and then centrifuged at 3,000g for 10 min to harvest serum. The serum was elutriated into vials and kept at -20° C for more analysis. Ultimately, the serum concentrations of total Ca, P, and alkaline phosphatase (ALP) activity was evaluated by colorimetric assay using commercial kits (Pars Azmon, Iran).

Statistical analysis

The experiment was done as a completely randomized design in a 3 × 2 factorial arrangement with dietary Ca source as the first, and Ca concentration as the second factor. The data were analyzed by the GLM procedure of SAS Institute (2010). Tukey's multiple range test was applied to determine the difference among all treatments. The experimental unit for egg quality and performance

indices was a cage, whiles individual bird data were applied for serum parameters. All differences were considered statistically significant if the probability was less than 0.05.

Results

Productive performance of laying hens

The average results of the productive performance of layers are summarized in Table 2. Different sources and concentrations of calcium had no significant effect on the values of FI, FCR, egg production, egg mass, egg weight, and body weight gain of hens ($P > 0.05$). Interaction between concentration and source of calcium was not observed for these traits.

Table 2. Effect of calcium source and concentration on feed intake, egg production, feed conversion ratio, egg mass, egg weight, and body weight gain

Treatment	Feed intake (g/hen/day)	Hen-day production (%)	Feed conversion Ratio (g feed/g egg)	Egg mass (g/hen/ day)	Egg weight (g)	Body weight gain (g)
Ca source						
Limestone A	106.86	82.10	1.98	53.98	65.76	7.34
Limestone B	106.34	82.30	1.99	53.95	65.63	0.47
Oyster shell	109.21	83.42	1.99	54.94	66.02	11.04
Ca and P level *						
1	106.98	82.71	2.00	53.86	65.53	0.43
2	107.96	84.58	1.99	54.72	66.08	12.14
SEM	0. 638	1.008	0.022	0.728	0.151	3.214
			P-values			
Ca source	0.141	0.875	0.998	0.850	0.564	0.404
Ca level	0. 419	0.786	0.829	0.596	0.082	0.081
Ca source × level	0.217	0.891	0.309	0.861	0.713	0.849

* Level 1: Available P and Ca (0.29 and 3.8%); level 2: Available P and Ca (0.31 and 4.0%).
"There was no statistical difference"

Egg quality criteria

The egg quality characteristics at 99 and 104 weeks of age are presented in Table 3 and Table 4, respectively. Egg quality indices were not influenced by different Ca source and concentration in the first period, (Table 3). These parameters also were not affected by dietary treatments in the second period, except for shell weight and eggshell ratio, which diminished ($P < 0.05$) as a reduction in the dietary Ca level (Table 4). In other words, shell weight and shell weight ratio negatively influenced ($P < 0.05$) in hens received a diet with lower Ca and available P (Ca= 3.8% and P= 0.29). In both periods of the experiment, the interaction between Ca source and concentration was not statistically significant.

Unmarketable eggs

The effect of various calcium sources and concentrations on broken, shell-less and unmarketable eggs are presented in Table 5. In this experiment, the calcium source did not affect the rate of unmarketable eggs produced by the layers ($P > 0.05$). On the other side, the rate of soft-shell, broken and unmarketable eggs produced by the hens fed diets containing lower Ca and available P (Ca= 3.8% and P= 0.29) was increased ($P < 0.01$). There was not any interaction between Ca source and concentration on the percentage of broken, soft-shell and unmarketable eggs.

Blood variables

The effect of different Ca source and concentration on serum variables is illustrated in Table 6. Blood Ca and P did not differ by various dietary Ca source and concentration throughout the trail, whilst, serum ALP activity declined significantly with increasing dietary calcium ($P < 0.05$). The interaction between calcium source and the level was not statistically significant regard to these traits ($P < 0.05$).

Table 3. Effect of calcium source and concentration on egg index, yolk color score, yolk index, yolk weight ratio, Haugh unit, eggshell weight, eggshell thickness, eggshell weight ratio and egg specific gravity (at 99 wk of age) [a]

Treatments	Egg characteristics (1-5 Wk)								
Ca source	Egg index	Yolk color (Roch index)	Yolk index	Yolk weight ratio (%)	Hugh unit	Shell weight (g)	Shell thickness ($mm \times 10^{-2}$)	Shell weight ratio (%)	Egg specific gravity (unit)
Limestone A	73.06	6.87	40.27	27.71	74.51	9.13	36.66	14.19	1.07
Limestone B	71.45	6.87	40.06	28.01	79.12	8.66	36.35	12.85	1.06
Oyster shell	71.36	6.75	39.55	28.85	79.06	8.79	35.75	13.40	1.06
Ca and P level [*]									
1	71.65	6.92	40.06	28.03	79.12	8.80	35.43	13.05	1.06
2	72.26	6.75	39.87	28.35	76.00	8.92	37.08	13.91	1.07
SEM	0.358	0.078	0.494	0.417	0.753	0.119	0.778	0.292	0.002
					P-values				
Ca source	0.093	0.744	0.861	0.552	0.222	0.292	0.885	0.151	0.545
Ca level	0.378	0.288	0.864	0.718	0.207	0.642	0.294	0.123	0.795
Ca source × level	0.367	0.151	0.905	0.488	0.302	0.691	0.142	0.279	0.055

[*] Level 1: Available P and Ca (0.29 and 3.8%); level 2: Available P and Ca (0.31 and 4.0%).
"There was no statistical difference"

Table 4. Effect of calcium source and concentration on egg index, yolk color score, yolk index, yolk weight ratio, Haugh unit, eggshell weight, eggshell thickness, eggshell weight ratio and egg specific gravity (at 104 wk of age) [a]

Treatments	Egg characteristics (5-10 Wk)								
	Egg index	Yolk color (Roch index)	Yolk index	Yolk Weight ratio (%)	Hugh unit	Shell weight (g)	Shell thickness ($mm \times 10^{-2}$)	Shell weight ratio (%)	Egg specific gravity (unit)
Ca source									
Limestone A	71.54	7.19	40.60	27.37	74.99	8.77	37.85	13.47	1.07
Limestone B	70.74	7.12	40.05	27.57	77.92	8.74	36.00	13.09	1.07
Oyster shell	70.01	7.06	39.78	28.32	78.24	8.76	35.33	13.17	1.06
Ca and P level [*]									
1	70.86	7.17	40.00	28.06	77.16	8.56[b]	35.47	12.90[b]	1.06
2	70.66	7.08	40.29	27.44	76.94	8.95[a]	37.32	13.59[a]	1.07
SEM	0.361	0.054	0.311	0.386	0.783	0.092	0.705	0.157	0.001
					P-values				
Ca source	0.272	0.670	0.605	0.620	0.210	0.989	0.314	0.493	0.353
Ca level	0.797	0.470	0.672	0.465	0.894	0.042	0.189	0.020	0.696
Ca source × level	0.810	0.404	0.895	0.751	0.613	0.555	0.303	0.109	0.075

[*] Level 1: Available P and Ca (0.29 and 3.8%); level 2: Available P and Ca (0.31 and 4.0%).
[ab] Means within a column showing different superscripts are significantly different ($P < 0.05$).

Table 5. Effect of calcium source and concentration on broken, shell-less and unmarketable eggs

Treatment		Broken eggs (%)	Shell less eggs (%)	Unmarketable eggs (%)
Ca source				
	Limestone A	2.05	0.95	3.01
	Limestone B	2.17	1.07	3.23
	Oyster shell	1.92	0.87	2.79
Ca and P level *				
	1	3.11[a]	1.57[a]	4.67[a]
	2	0.99[b]	0.36[b]	1.35[b]
	SEM	0. 246	0.150	0.375
			P-values	
Ca source		0.690	0.588	0.436
Ca level		<0.01	<0.01	<0.01
Ca source × level		0.401	0.107	0.105

* Level 1: Available P and Ca (0.29 and 3.8%); level 2: Available P and Ca (0.31 and 4.0%).
[ab] Means within a column showing different superscripts are significantly different ($P < 0.05$).

Table 6. Effect of calcium source and concentration on Ca, P content and alkaline phosphatase (ALP) activity in blood

Treatment		Ca (mg/dL)	P (mg/dL)	ALP (U/L)
Ca source				
	Limestone A	25.00	6.75	329.70
	Limestone B	23.87	6.74	340.58
	Oyster shell	23.37	6.80	374.80
Ca and P level *				
	1	23.25	6.81	399.31[a]
	2	24.92	6.73	297.41[b]
	SEM	0. 545	0.159	22.908
			P-values	
Ca source		0.494	0.988	0.696
Ca level		0.155	0.827	0.035
Ca source × level		0.994	0.964	0.885

* Level 1: Available P and Ca (0.29 and 3.8%); level 2: Available P and Ca (0.31 and 4.0%).
[ab] Means within a column showing different superscripts are significantly different ($P < 0.05$).

Discussion

The data in Table 2 indicates that dietary treatments had an insignificant effect on the productive performance of hens. The findings of other studies regarding this issue have been inconsistent. Some researchers have found improvement in egg production (Ahmad and Balander, 2003; Ahmed *et al.,* 2013), while other authors observed no change (Guinotte and Nys, 1991; Grizzle *et al.,* 1992; Keshavarz and Nakajima, 1993; Scheideler, 1998; Safaa *et al.,* 2008; Swiatkiewicz *et al.,* 2015), which agrees with current observations.

FI and FCR were also similar among treatments in our experiment; therefore, it seemed that BWG was not influenced by different calcium sources and concentrations. This also applies to broiler chicks wherein many authors have shown that the bird performance was not negatively influenced by a slight decrement in dietary Ca level (Driver *et al.,* 2005; Ziaei *et al.,* 2008; Hamdi *et al.,* 2015). Our results indicated that all three sources of calcium used in this trial could satisfy the hen's requirement of Ca, to sustain desirable egg production level, somewhat they not to have to regulate feed intake to compensate for poor calcium bioavailability.

Many authors have reported that no differences were observed in the aforesaid factors when evaluating various Ca sources, such as limestone or oyster shell or a blend of these (Safaa *et al.,* 2008; Pelicia *et al.,* 2009; Catli *et al.,* 2012; Ganjigohari *et al.,* 2017). In contrast, some studies in the past, have reported that limestone as a Ca source can cause a superior FCR in laying hens in comparison to oyster shells (Ahmed *et al.,* 2013).

These disagreements among authors might be clarified by many factors such as strain, production cycle, age and nutrient characteristics of the diets applied. Besides, in our study, the hens which received various Ca source and concentration had similar egg mass and egg weight during the experimental period. In agreement with our findings, Miller and Sunde, (1975), and Guinotte and Nys,

(1991) demonstrated that egg weight and egg mass did not influence by calcium source when given to laying hens at the same dietary level. Other researchers, however, indicated that egg mass could be ameliorated by raising Ca in rations of birds (Safaa *et al.,* 2008; Pelicia *et al.,* 2009; Catli *et al.,* 2012).

Although there was no significant difference between Ca sources for any production parameters in our study, oyster shell numerically increased egg production, egg mass, egg weight and hens body weight in comparison to limestone. There is a preceding opinion which states limestone has a faster solubility rate in comparison to the oyster shell (Kuhl and Sullivan, 1977; Guinotte and Nys, 1991) and hence, the solubility of Ca source has an impact on the layer's capability to put upon it. These authors also stressed that oyster shell positively affects eggshell and laying hen's performance in comparison to smaller limestone particles.

As noted in Tables 3 and 4, Egg quality characteristics were not influenced by dietary treatments in both egg sampling, except for shell weight and eggshell ratio, which decreased as a reduction of the dietary Ca level in the second period. By increased intestinal absorption and resorption of the extremely labile reservoir found in the medullary bone developing in female hens in reaction to the activity of gonadal steroid, the Ca required for eggshell calcification is supplied (Klansing, 1998). It was previously known that eggshell quality can be improved by providing calcium as 'grit', particularly when the bird approaches the end of the laying period. Factors like the ability of the bird to store the particulate pieces in the gizzard and gradual release of calcium overnight have been cited as the reasons for this enhancement (Rao *et al.,* 1992). Published data in this respect, however, are contradictory with reports showing increase (Lim *et al.,* 2003; Rodrigues *et al.,* 2005; Safaa *et al.,* 2008), decrease (Amy, 2016) or do not have any explicit effects (Rao *et al.,* 2014; Souza *et al.,* 2016) on eggshell quality criteria. Therefore, the efficiency of various levels of Ca remains under discussion (Arpasova *et al.,* 2010; De Arauja *et al.,* 2011). Published data, however, are conflicting respect to the efficacy of unlike Ca sources on eggshell quality. Several authors have claimed that oyster shell could be superior to ground limestone for shell quality (Grizzle *et al.,* 1992; Keshavarz and Nakajima, 1993) while others pointed out that eggshell quality to be identical to birds received large particle limestone or those fed oyster shell (Miller and Sunde, 1975; Muir *et al.,* 1976).

The different findings in the literature probably resulted from the various calcium sources being applied within the dissimilar experiments as well as the form and size and also, concentration in which they were supplied for birds. It's possible that a part of these trivial discrepancies among authors' causes from the discrepancy in the strain, production cycle, age, and nutrient characteristics of the diets applied. Moreover, increasing the temperature will generally lead to a reduction in shell quality. This will be more serious when birds are raised at high altitudes above the sea level.

In the present study, Ca source had no significant effect on the rate of unmarketable eggs, but the percentage of broken, soft-shell and unmarketable eggs produced by the hens fed diets containing lower Ca and available P was increased. For the egg industry worldwide, the production of eggs with proper eggshell quality is pivotal to the economic viability of the industry. It is now well accepted that deficiency of Ca is generally recognized to cause a reduction in the eggshell quality indices. Our findings are in agreement with the findings of Safaa *et al.* (2008) who stated that two levels of dietary Ca (3.5 and 4%) influenced the rate of broken and shell-less eggs. These authors considered that birds fed the high-Ca diet had a lower rate of damaged eggs in comparison to those fed the low Ca diet. These results disagreed with data obtained by Lim *et al.* (2003) who stated that different levels of Ca (3 and 4%) in layers diet had not any significant effect on broken and soft eggs. As noted above, the differing reports can be resulted from the various Ca sources being applied within the dissimilar assessments as well as the form and level in which they were supplied. Although sufficient inclusion of Ca, vitamin D and P are predominantly effective during the laying period, another description of these results may simply be a result of the hens' aging. Numerous studies have exhibited that eggshell quality reduces as birds grow older (Roland, 1979; Albatshan *et al.,* 1994; Roberts and Ball, 2004). These authors deduced that old birds possibly were less efficient in absorbing calcium than younger ones.

In respect to serum variables, ALP activity significantly declined with the raising of dietary calcium. Many factors including calcitonin, vitamin D, ATPase, and intestinal ALP exist, can affect the solubility of Ca, and it's binding to proteins in the enterocyte and blood.

As a consequence, the absorption mechanism of Ca and P in the hen body is too complicated (De Matos, 2008). Our results are in agreement with the data obtained by Swiatkiewicz *et al.* (2015) who reported various Ca source and concentration had not any significant effect on Ca and P content in blood serum of laying hens. In contrast, numerous studies have been completed in laying hens claimed that increasing the amount of Ca (3.75%) in the diet resulted in a rise in blood Ca (Elaroussi *et al.,* 1994; Pelicia *et al.,* 2009 and 2011). Previous studies revealed that many interacting feedback loops such as Ca, P, parathyroid hormone, vitamin D_3, and calcitonin can regulate Ca homeostasis and

subsequently its concentration in the blood. In laying hens, sexual hormones also participate in Ca homeostasis. These mechanisms facilitate sustain blood concentration of Ca in a slight range for normal body function. Regards to ALP activity in the blood of layers, lower Ca level in the diet increased it when compared to the serum ALP activity of hens fed diets containing higher Ca. Alkaline phosphatase plays a key role in the mineralization process of eggshell and bone, and the observations of other trials have revealed an opposite relationship between dietary calcium and serum ALP activity. Rao *et al.* (2003) and Swiatkiewicz *et al.* (2015) demonstrated that ALP activity was enhanced in hens received the diets deficient in calcium.

Conclusion

For improving the eggshell quality of aged laying hens, it becomes prominent to advertise the levels of Ca and management conditions. The present study revealed that the rate of unmarketable eggs seen in old laying hens can be decreased by using an adequate concentration of Ca. Although all Ca sources examined in this study could satisfy the hen's requirement of Ca, the addition of 4% Ca to the diet in comparison to 3.8%, improved eggshell quality and decreased blood ALP activity in laying hens.

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