Acta Scientiarum. Animal Sciences
ISSN: 1807-8672
Editora da Universidade Estadual de Maringá - EDUEM

Saki, Aliasghar; Rahmani, Abbas; Yousefi, Azam
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Acta Scientiarum. Animal Sciences, vol. 41, e42926, 2019
Editora da Universidade Estadual de Maringá - EDUEM

DOI: https://doi.org/10.4025/actascianimsci.v41i1.42926

Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v41i1.42926

Calcium particle size and feeding time influence egg shell quality in laying hens

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ABSTRACT. An experiment with Leghorn laying hens was undertaken to determine the effect of oyster shell particle size and feeding time on different production variables, calcium retention, plasma calcium content and egg internal and external quality. Two hundred Leghorn layers (40 weeks old old) were allocated in five dietary treatments with four replicates during ten weeks. Two particle size combinations (wherein 50% of calcium substituted by medium or coarse particles (1-2 mm and 2-4 mm respectively) and two feeding time (8-pm or 9-am) were compared against a control diet (100% ground, <1 mm which fed with meal). Egg number, egg production, egg mass and feed conversion ratio did not differ among treatments ($p > 0.05$). Hens fed diets containing coarse Ca had significantly greater feed intake and calcium content of excreta ($p > 0.05$), whereas medium particle size reduced feed intake compared to control. Coarse particle size and feeding time at 9-pm significantly increased the calcium content of egg shell, egg shell thickness, egg surface area (ESA) and shell weight per unit surface area (SWUSA) ($p < 0.05$). Plasma calcium concentration, gizzard digesta calcium content and egg specific gravity were not affected by treatments ($p > 0.05$). Providing of calcium at 9-pm resulted an increase of egg shell (%), shell weight and thickness ($p < 0.05$). The results have shown that substitution of fine oyster shell with 50% coarse particles (2-4 mm) and feeding time at 9-pm have better effects on egg shell quality.

Keywords: calcium retention; feeding time; oyster shell; particle size.

Received on May 21, 2018.
Accepted on September 5, 2018.

Introduction

Calcium (Ca) is a key mineral in laying hens, particularly during the laying period (Araujo et al., 2011). Dietary Ca is one of the primary factors affecting egg shell quality and shell strength (William, Horacio, Paulo, Luis, & Marcelo, 2006). Calcium constitutes approximately 1.5% and 40.0% of BW and eggshell weight respectively (Bölükbasi, Çelebi, & Utlu, 2005). It is usually supplied as calcium carbonate in the form of limestone or oyster shell, or from other sources, such as marine shells. The Ca availability of these dietary sources is a function of their chemical composition and their capacity to physical bind to other dietary components (McNaughton & Deaton, 1980).

Sá, Gomes, Albino, Rostagno, and D'Agostini (2004) have determined the relative Ca availability in dicalcium phosphate, calcitic limestone and dolomitic limestone to be 99%, 84%, and 75% respectively. Providing Ca in the form of oyster shell has shown higher digestibility and retention than the calcium carbonate fed as ground limestone (Roland, 1988). Particle size of calcium sources in layers' diets is an important factor affected calcium bioavailability. Acknowledging this importance, the identification of the Ca bioavailability of different sources and also the ideal Ca particle size (generally limestone) in laying hens have been received great interest by researchers for a long time (Araujo et al., 2011; De Witt, Kuleile, Van der Merwe, & Fair, 2008; Ekmay & Coon, 2011; Guinotte & Nys, 1991; Guo & Kim, 2012; Koreleski & Świątkiewicz, 2004; Lichovnikova, 2007; Pavlovski, Vitorović, Lukić, & Spasojević, 2003; Pelicia et al., 2011; Saunders-Blades, MacIsaac, Korver, & Anderson, 2009; Skřivan, Marounek, Bubancova, & Podsedníček, 2010).

Some researchers have shown that coarse particle size had, generally, a beneficial effect on egg shell quality (Koreleski & Świątkiewicz, 2004; Lichovnikova, 2007; Skřivan et al., 2010), egg specific gravity (Ekmay & Coon, 2011), and also bone strength (Guinotte & Nys, 1991). However, others have reported no effects of large particles of limestone on performance or egg quality, egg shell thickness, egg breaking strength and specific gravity (Keshavarz, 1998; Skřivan et al., 2010). Overall, there are inconsistent results of

particle size of Ca in laying hens diets. Differences in Ca sources solubility might be responsible for the contradictory findings.

Another strategy to improve eggshell quality is limited feeding or changing the time of feeding during the day (particularly from morning to afternoon) which is used to improve eggshell quality of broiler breeder hens (Bootwalla, Wilson, & Harms, 1983; Wilson & Keeling, 1991). Feeding in the late day work through supplying dietary Ca at times that correspond more closely to period of shell deposition, resulted in improved calcium utilization, which is usually manifested as an increased in egg specific gravity, shell quality, shell weight and thickness. However, effects of feeding time on performance are various. Some investigations have shown that afternoon feeding resulted in a higher rate of egg production (Bootwalla et al., 1983), whereas others reported no effect (Wilson & Keeling, 1991).

Implementing a good Ca feeding program is a method which permits the hen to adjust calcium intake to its needs. Although numerous studies have been carried out in this area, there are no reports on interaction between oyster shell particle size and time of feeding in laying hens in middle of production period. Considering the importance of obtaining more information on this subject, the objective of this study was to evaluate the effects of feeding time and oyster shell particle size on shell quality and calcium retention in Leghorn laying hens.

Material and methods

Calcium particles and dietary treatments

Dietary treatments consisted of three oyster shell particles sizes namely ground (<1 mm) as control diet which fed with meal; medium (1-2 mm) and coarse (2-4 mm) which fed at morning (8-am) and night (9-pm) for treatments 1,3 and 2,4 respectively. Calcium (7.8 %) was supplied in the form of powder with particle size less than 1 mm in control diet. In other treatments, 50 % of calcium was supplied by oyster shell particle sizes less than 1 mm and the residual (50 %) by particle sizes larger than 1 mm, include 1-2 mm (for first and second treatments) and 2-4 mm (for third and fourth treatments). The particle size of oyster shell was obtained by analysis in sieves shaker. The calculated and chemical composition of diets and *in vitro* solubility of oyster shell particles are presented in Table 1 and Table 2 respectively.

Experimental design

Trial was set up in a completely randomized factorial design, with five treatments, four replications and ten birds in each. A total of 200 Leghorn layers (40 weeks old) were randomly assigned to five dietary treatments (40 birds/ treatment) with four replicates (10 birds/ replicate) in a 10-wk trial. The hens were housed in a battery cage system (42×40×50 cm). Laying hens were individually reared in one floor adjacent cages which equipped with nipple drinker, and trough. Lights were controlled by time switches according to the recommended hours of lighting as per schedule supplied by the Leghorn breeding company.

Measurements

Feed intake (g/day/laying hen), egg number, egg weight (g), egg production (%) and egg mass were recorded weekly. The FCR was calculated as the amount of feed consumed per unit of BWG.

At the end of weeks 3, 6 and 9 of experiment, four eggs from each replicates were randomly selected for egg shell quality measurement. The shells were washed under slightly flowing water to remove adhering albumen according to Kul and Seker (2004) and wiped with a paper towel to remove excessive moisture. A micrometer sensitive in 0.001 mm was used for measuring the eggshell thickness. Variables i.e. egg index (EI), egg specific gravity (ESG), egg surface area (ESA), egg shell weight per unit surface area (SWUSA), and shell ratio (SR) were investigated by following equations:

$$EI = \frac{W}{L} * 100$$

$$EI = \frac{EW}{EW - EWW}$$

$$ESA(cm^2) = 3.9782W^{0.7056}$$

$$SR = \frac{SW}{EW} * 100$$

$$SWUSA(gr\ cm^{-2}) = \frac{SW}{ESA}$$

where: EI is egg index; W is egg width; L is egg length; EW is egg weight; EWW is weight of egg weighted in water; ESA is egg surface area; SR is shell ratio; ESG is egg specific gravity; SWUSA is shell weight per unit surface area; and SW is shell weight.

The *In vitro* solubility of the oyster shell was determined by the method described by Zhang and Coon (1997). Briefly, a 2.0 (g) oyster shell sample was poured into HCl solution (0.2 N) that was warmed at 42 Cº. After allowing 10 min for reaction, the undissolved limestone was filtered and weighed after drying finally was expressed as the percentage weight loss.

At the end of experiment three birds of each replication selected and a sample of trunk blood were collected quickly (30s) into vials containing 10 mg sodium heparin and placed immediately into an ice bath. The blood samples were centrifuged (in 5000 rpm under refrigeration at 4 ºC) for 15 min; then, the plasma was decanted and assayed in a way where in cresolphthalein complexone was employed as the color reagent and illustrates the Ca content of samples. In order to determining Ca content of gizzard, 2 birds from each replicate have been slaughtered 3 hours after feeding. Calcium content of gizzard content was measured by chromatography method. Excreta samples were taken daily, by plastic plates which have been placed under the cages. Then samples were weighed and a 10% aliquot was stored in a freezer at -10°C until laboratory analysis. The contents of total calcium in excreta were analyzed by spectrophotometer.

Table 1. Diet composition and chemical analysis.

Feedstuff	treatments	Control
Corn	57.66	57.66
Soybean meal	28.62	28.62
Oil	3.02	3.02
Powder oyster shell	3.93	7.8
Oyster shell	3.87	-
Dicalcium phosphate	1.48	1.48
Vitamin mix1	0.25	0.25
Trace mineral mix2	0.25	0.25
Salt	0.42	0.42
methionine	0.5	0.5
total	100	100
		Calculated Chemical composition
Metabolizable energy (kcal kg)	2900	2900
Protein (%)	16.5	16.5
Calcium(%)	3.5	3.5
Ava. Phosphorous(%)	0.3	0.3
Sodium	0.15	0.15
Potassium	0.71	0.71
Chloride	0.21	0.21
Digestible methionine	0.3	0.3
Digestible lysine	0.7	0.7
%Ingredient	Phosphorous (%)	Calcium (%)
Corn	0.305	0.01
Soybean meal	0.69	1.2
Oyster shell	0.041	38.86
Dicalcium phosphate	0.05	35

[1]Vitamins added per kg of diet: vitamin A, 7.2g; vitamin D3, 7 g; vitamin E, 14.6g; vitamin K3, 1.6g; thiamin, 0.72g; riboflavin, 3.3g; pyridoxine, 6.2mg; vitamin B12, 0.6mg; pantothenic acid, 12g niacin, 2.160g; biotin, 0.2 g.
[2] Minerals added per kg of diet: Mn, 64mg; Zn, 44mg; Cu, 16mg; Se, 8mg; Fe,100 mg; I, 0.64 mg; Co, 0.2 mg.

Table 2. In vitro solubility of oyster shell particle size distribution.

Particle size	<1 mm	1-2 mm	2-4 mm	P
Solubility (%)	57.69^{a}±2.57	51.95^{b}±0.93	45.83^{c}±1.68	0.0001

a,b,c Means values within a row with no common superscript are significantly differ ($p < 0.05$).

Statistical analysis

Two analyses include completely randomized design and repeated measurement were used. Data were analyzed in a 2×2+1 factorial designed by Statistical Analysis System (SAS, 2004) and means were tested by Duncan multiple range test. Statements of statistical significance are based on ($p < 0.05$).

Results

The effects of different particle sizes and feeding time on laying hens performance have shown in Table 3. As shown in the Table 3, feed intake was significantly ($p < 0.05$) increased by treatment 3 compared to the control. Egg number, egg production, egg mass and feed conversion ratio were not significantly ($p > 0.05$) influenced by treatments. But egg weight significantly decreased by treatments 1, 2 and 4 in comparison with control ($p < 0.05$).

Results of egg index, egg specific gravity, egg surface area (ESA) and Shell Weight per Unit Surface Area (SWUSA) have presented in Table 3. No statistical significant differences in egg index and egg specific gravity characteristics were detected. In contrast SWUSA was significantly increased ($p < 0.05$) by control and treatment 4 (coarse particle size at 9-pm) rather than other treatments.

There was no significant interaction ($p > 0.05$) between feeding time and oyster shell particle size on the shell weight, shell thickness, calcium concentration of plasma, excreta and gizzard digesta as shown in table 4. Whereas egg shell (%), shell weight and calcium content of excreta were significantly increased by main effects of large particle size (coarse *vs* medium) ($p < 0.05$). Also providing of Ca at 9-pm resulted in an increase of egg shell (%), shell weight and thickness ($p < 0.05$). Unlike egg shell percentage, egg shell weight, Ca concentration of plasma, gizzard digesta and excreta, in the case of egg shell thickness there is a significant increase ($p < 0.05$) in treatment 4 compared to the control.

Table 3. Effects of treatments on laying hens performance and egg external quality.

	Treatment 1[a]	Treatment 2[b]	Treatment 3[c]	Treatment 4[d]	Control	P value
Egg number	23.67± 4.58	24.15± 4.58	24.05± 3.67	23.55 ±4.3	24.52±4.45	0.9868
Egg weight (g)	63.45[c]± 1.57	65.34[b]± 2.64	66.80[a]± 1.83	65.40[b]± 1.99	66.86[a]± 1.54	0.0003
Egg production (%)	67.64± 12.63	69.00± 13.09	68.71± 10.47	67.28± 12.29	70.07±12.71	0.9868
Egg mass	42.95± 8.24	45.26± 9.30	45.87 ±6.68	44.04± 8.32	46.86±8.34	0.8295
Feed intake (g d^{-1} $bird^{-1}$)	77.25[c] ± 0.04	79[c]±0.05	84[a] ± 0.031	82.5[ab] ± 0.045	83.25[b] ±0.02	0.0042
FCR	2.169± 0.71	2.082± 0.48	2.166± 0.57	2.215± 0.46	2.081±0.43	0.9479
Egg index	74.97± 8.18	72.59± 2.34	74.00± 2.75	72.36± 2.71	74.27±4.40	0.6405
Egg specific gravity	1.07± 0.005	1.07± 0.005	1.07± 0.006	1.07± 0.005	1.07±0.004	0.7920
ESA(cm^2)[1]	90.4[b]±13.60	91.13[b]± 3.82	91.81[ab]±5.24	93.77[a]± 4.80	94.25[a]±3.25	0.047
SWUSA(gr cm^{-2})[2]	0.71[b] ± 0.01	0.70[b] ± 0.01	0.71[b] ± 0.01	0.72[a] ± 0.01	0.70[b]±0.01	0.040

a-Diet with particle size between 1-2 mm at 8-am. b- Diet with particle size between 1-2 mm at 9-pm. c- Diet with particle size between 2-4 mm at 8-am. d- Diet with particle size between 2-4 mm at 9-pm. 1.Egg surface area 2. Shell weight per unit surface area. [a,b]Means values within a row with no common superscript are significantly differ ($p < 0.05$).

Table 4. Effects of feeding program and particle size on calcium concentration in egg shell, plasma, excreta, gizzard digesta at the end of experiment.

		Egg shell, %	Plasma, mg dL^{-1}	Excreta, mg dL^{-1}	Gizzard digesta, mg dL^{-1}	Shell Weight, g	Shell Thickness, mm
Particle Size	1-2	35.74[b]±0.72	20.43±4.45	5.63[b]±1.31	3.63 ± 1.85	4.64[b]± 0.53	0.371[b] ± 0.033
	2-4	43.32[a]±0.69	23.30±6.34	7.66[a]±1.49	4.72 ± 1.07	4.94[a] ± 0.66	0.388[a] ± 0.048
P value		0.0001	0.1361	0.0201	0.1814	0.035	0.0714
Feeding Program	8am	36.26[b]±1.03	20.24±5.23	6.68 ± 1.90	4.42± 1.32	4.60[b]± 0.58	0.363[b]± 0.035
	9pm	36.79[a]±1.05	23.40±5.75	6.61 ± 1.64	3.94± 1.83	4.98[a]± 0.59	0.396[a] ± 0.043
P value		0.0025	0.1516	0.9177	0.5423	0.0103	0.0011
P value of Interaction		0.0001	0.2085	0.06193	0.3593	0.05140	0.9778
Treatment 1[a]		29.30±6.21	22.3 ±3.30	5.69 ± 1.13	4.28 ± 1.77	4.89 ± 0.58	0.382[ab]± 0.03
Treatment 2[b]		22.61±0.33	22.61±0.33	5.58 ± 1.67	3.87 ± 1.93	4.91 ± 0.45	0.388[ab]± 0.032
Treatment 3[c]		30.71±10.13	30.71±10.13	7.68 2.12	4.55 ± 0.95	5.03 ± 0.68	0.383[ab] ±0.04
Treatment 4[d]		24.81±3.17	22.42±6.23	7.64 ± 0.81	4.89 ± 1.30	5.10 ± 0.49	0.403[a] ± 0.04
Control		24.38 ± 6.18	21.93±3.60	4.47 ± 2.46	2.61 ± 0.85	5.03 ± 0.47	0.369[b]± 0.03
P value		0.665	0.383	0.242	0.1484	0.7668	0.0424

[a,b]Means values within a column with no common superscript are significantly differ ($P < 0.05$). a-Diet with particle size between 1-2 mm at 8 am. b- Diet with particle size between 1-2 mm at 9-pm. c- Diet with particle size between 2-4 mm at 8-am. d- Diet with particle size between 2-4 mm at 9-pm.

Discussion

The results of this study showed that feed intake of hens could significantly ($p < 0.05$) altered by different feeding treatments. In comparison with control, different feeding time couldn't affect feed intake. It has been shown that the longer retention time of large particle sizes of calcium in digestive tract, has improved of energy digestibility (Nam, Lee, Joo, Kim, & Kang, 1998), and reduce feed intake (Richards, 2003). This has justified the lower feed intake in treatments 1 and 2 rather than control. The more fine particles, the more excreting rate, which in turn has affected the feed intake. Lower feed intake in treatment 1 and 2 than control are in agreement with Guo and Kim (2012) who have reported the large particle size and mixture of two particle sizes of limestone have beneficial effects on feed intake, rather than small particles. Also Guinotte and Nys (1991) have found that calcium carbonate particles in range of 0.15 to 3.35 mm increased the feed intake, which is in agreement with results of treatment 3 and 4 (2-4 mm) in current study. Furthermore, Siegel Yo, Siegel, Guerin, and Picard (1997) have suggested that large particle size in hen rations, resulted in more selectivity and consequently feed intake may be increased.

Neither feeding time (treatments 1 and 3 or 2 and 4 *vs* control) nor Ca particle size (treatments 1 and 2 or 3 and 4 *vs* control) could affect egg number, egg production, egg mass and FCR. Results of FCR in this study are in accordance with Ito, Faria, Kuwano, Junqueira, and Araujo (2006) who also did not observe any difference in feed conversion ratio with different limestone particle sizes.

In current study larger particle size of oyster shell (coarse *vs* medium) in diet and feeding time (9-pm *vs* 8-am) improved the calcium content of egg shell which is in agreement with the results reported by Rao and Roland (1989). However, Skřivan et al. (2010) didn't observe any effect of particle size.

A slower solubility of sources of Ca would make it available during the time of the eggshell calcification and diminish bone Ca and P mobilization (Skřivan et al., 2010), which it could justify improvement in egg shell Ca content by feeding large particle size and nightly in present study. However, Saunders-Blades et al. (2009) concluded that calcium particle size and different sources have no effect on eggshell quality.

Such traits as breaking strength, thickness, pore density, and elasticity accounts for shell quality determinant, which simultaneously are correlated with the egg specific gravity (ESG) (Sooncharenying & Edwards, 1989). Egg surface area (ESA) is one of the shell quality characteristics (Narushin, 2001) and egg interior parameters (Narushin & Romanov, 2002). Egg specific gravity together with ESA and SWUSA may serve as indicators of egg shell and egg internal quality. Neither feeding time nor the Ca particle size could not affect the egg specific gravity in this experiment. The results of the effect of limestone particle size on egg shell quality have similarity with those reported by other researchers (Jardim Filho et al., 2005; Keshavarz, 1998; Murata, Santana, Jardim Filho, & Ariki, 2009; Saunders-Blades et al., 2009; Yo et al., 1997). But, Roland (1988) and Guinotte and Nys (1991) have stated that larger particles are superior to small or medium particles in improving the eggshell strength and weight. Different calcium sources solubility and/or the period in lay could be responsible for the contrary results.

With respect to feeding time, results are in accordance with other researchers[21]who have reported egg weight and egg specific gravity were not affected by feeding time.

In current study feeding time at (9-pm *vs* 8-am) improved shell weight, shell thickness and egg shell (%). But there is no difference in egg weight simultaneity with shell weight, egg shell (%) and shell thickness in birds fed at 9-pm. This suggests that feeding later in the day didn't affect oviducal transit times or egg formation time, as stated earlier (Backhouse & Gous, 2005). Therefore, feeding later in day influences the shell weight by providing a greater proportion of retained calcium in the gastrointestinal tract at the commencement of shell formation than morning-fed birds.[35] These results are in agreement with Backhouse and Gous (2005).

SWUSA showed a significant improvement by feeding oyster shell particle size 2-4 mm and nightly feeding. Egg Surface Area (ESA) followed by a reduction in treatments with Ca particle size 1-2 mm in comparison with control, suggesting the more potent effect of Ca particle size on these two characteristics rather than time of feeding. Increasing calcium concentration in egg shell refers to improve numerical calcium concentration in plasma (Table 4). Oyster shell with coarse particles (2-4 mm) *vs* medium (1-2 mm) increased calcium concentration in excreta (Table 4) which may be attributed to lower solubility. Accordingly, Zhang and Coon (1997) have reported that larger particle size significantly increased calcium concentration in excreta. Large particle size of oyster shell in diet could be lead to improve calcium release and absorption in body, therefore increase Ca turn over and consequently calcium concentration in excreta.

From these results, it has been arisen that Ca retention as a percentage of Ca ingestion decreased with increasing Ca particle size. Diets containing coarse particles (2-4 mm) numerically increased Ca concentration in gizzard digesta (Table 4). Egg shell quality improvement in these treatments may be attributed to lower solubility and gradually release of Ca in gastrointestinal tract by large particles. As it has been shown that larger particles of oyster shell and limestone have lower solubility than small particle size In-vitro (Saunders-Blades et al., 2009).

Overall, when finely particles of oyster shell are substituted partially with larger particle size releasing of Ca become slowly and steadily, which in combination with delay feeding could supply Ca more closely to shell formation time.

Conclusion

Egg shell quality and calcium retention in body increased by a dietary treatment containing 50% of total Ca as coarse particle size (2-4 mm) rather than medium and ground (1-2 mm and <1 mm respectively) of oyster shell. Feeding time at 9-pm increased the shell weight and shell thickness. Calcium content in egg shell increased by substituting coarse oyster shell at level of 50% of total Ca and feeding time at 9-pm.

Acknowledgements

The authors gratefully acknowledge Department of Animal Science of Bu-Ali Sina University for financial support.

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