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Free-choice Feeding of Three Different Dietary Calcium Sources and their Influence on Egg Quality Parameters of Commercial Layers

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ABSTRACT

Purpose: *The study reported herein was conducted to determine the effects of choice feeding three different calcium (Ca) sources on external and internal egg quality parameters of commercial layers.*

Research Method: *Three dietary Ca sources (oyster shells, limestone and bone meal) were choice fed ad libitumly along with a commercial layer feed. The birds fed only the commercial layer diet was used as the control. The experiment was conducted as a complete randomized design. A total of 128, 55 weeks old Hy-line white layer hens (1500 ±21g) were assigned into 16 cages of 8 birds each. Four replicate cages were randomly assigned to each of the four dietary treatments. Bird's performances were tested weekly. Twenty eggs from each treatment were collected weekly over five weeks period and were assessed for shell and internal quality parameters.*

Findings: *Daily Ca intakes of birds affected significantly ($P<0.05$) by the Ca source. The birds fed oyster shells and limestone showed the highest ($P<0.05$) daily Ca intakes. Feeding bone meal significantly ($P<0.05$) reduced damaged shell percentage. Weekly body weight gain, weekly egg production and feed conversion ratio among treatments were similar ($P>0.05$). Dietary supplementation of Ca significantly ($P<0.05$) improved the egg weight. Shape index and shell ratios among treatments were similar ($P>0.05$). Feeding bone meal significantly ($P<0.05$) improved unit surface shell weight (USSW) and the shell thickness. Calcium supplementation had no effect ($P>0.05$) on albumen and yolk quality parameters of eggs.*

Originality/value: *The present study concluded that feeding bone meal to commercial layers improves USSW, shell thickness thereby reducing egg damages. Calcium source has no influence in improving internal quality parameters of chicken eggs.*

Keywords: *Bone meal, Calcium, Hy-line white, Limestone, Oyster shell*

INTRODUCTION

The eggshell works as a "natural package" to the interior egg contents while protecting the embryo from physical damages. The eggshell quality continues to be one of the major concerns of the egg industry. Eggs with inferior shell quality incur serious economic losses to the poultry producers. It has been found that the average of eggs cracked prior to point of consumption varied from 13% to 20% (An *et al.*, 2016). The eggshell therefore, must be sufficiently strong enough to resist damages occur during egg laying, collection, grading, and transportation, and must be able to remain intact until it reaches the hand of consumers. Egg shell quality and its strength is therefore, a vital factor in poultry production as eggs with defective shells lead to great economic losses (Lavelin *et al.,* 2000). These defects may be either microscopic (*i.e.*

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hair-line cracks) or macroscopic (*i.e.* gross cracks) or may be presented as thin-shelled eggs or shell-less eggs. The damaged egg shells enhance penetration of micro-organisms into the egg thus reducing the interior egg quality. However, it is well known that damages to egg shells can be minimized by improving the egg shell thickness (An *et al*., 2016).

Calcium is an important macro-element that is required for optimum egg shell strength. The palisade layer of the shell, which is responsible for its mechanical strength, consists of more than 90% Ca in the form of Ca carbonate ($CaCO_3$). A linear improvement in eggshell quality has been observed with increasing dietary levels of Ca (An *et al*., 2016). Source, as well as the particle size, solubility and dietary inclusion level of Ca are known to influence the egg shell quality (Scheideler, 1998; Pizzolante *et al*., 2009). Calcium is also found to responsible for maintaining the internal egg quality (Ahmed *et al*., 2013). According to Pelicia *et al.,* (2007), feed intake can be influenced by dietary Ca concentration. In domestic fowl, Ca present in feed is mainly absorbed between the duodenum and lower jejunum (Hurwitz and Bar, 1970).

Commercial layer diets are typically formulated to contain 40-46 g/kg and 3.1-5 g/kg, total Ca and non-phytate P, respectively (Leeson and Summers, 2005). However, it has been found that the most of the layer rations available in Sri Lanka do not contain aforementioned dietary Ca levels (Ravindran, 1995) and the use of a supplementary Ca source is therefore frequently recommended by the feed manufacturers. As insufficient dietary Ca may lead to poor shell quality, the commercial layers must be provided with a supplementary Ca source which can fulfil their daily Ca requirement. Interestingly, as for other nutrients like lysine (Newman and Sands 1983), total protein (Forbes and Shariatmadari, 1994), methionine (Steinruck *et al.,* 1990) and selenium (Zuberbuehler *et al*., 2002) poultry have been shown to exert a special appetite for Ca (Wood-Gush and Kare, 1966; Joshua and Mueller, 1979). Existence of special appetite in poultry for Ca has been widely tested therefore, by numerous researches (Wilkinson *et al.,* 2014).

In commercial poultry production provision of external Ca sources is directly related with the grit feeding practices. Grit feeding supplies Ca required for the laying hens. It is recommended to start grit feeding at the onset of laying. Grit feeding is also important in the mechanical digestion which takes place in the gizzard. It is recommended to provide at least 3.5 – 4g of Ca for a hen per day (Hy-Line, 2016). Provision of good reliable, digestible and cost-effective Ca source will immensely help the poultry industry to keep eggs for prolong period without damages (Saunders-Blades *et al.,* 2009).

In commercial layer production, limestone and oyster shells have been commonly used as Ca supplements in Sri Lanka. In broiler processing, bones are left as a by-product which has a potential to be used as a Ca source. So far, number of studies have been conducted to investigate the effect of limestone (Safaa *et al.,* 2008; Saunders-Blades *et al.,* 2009; Ahmed *et al*., 2013), oyster shell (Safaa *et al.,* 2008; Saunders-Blades *et al.,* 2009; Ahmed *et al.,* 2013) and egg shells (Olgun *et al.,* 2015; Tunç and Cufadar, 2015) as sources of Ca in layer diets. However, to the author's knowledge, no research has been conducted to investigate the effect of bone meal as an external Ca source on egg shell quality parameters of layers. And the researches conducted based on free choice grit feeding in commercial layers are also limited. Therefore, the present study was carried out to investigate the effect of free choice feeding of three Ca sources (oyster shell, limestone, and bone meal) on external and internal egg quality characters of commercial layers.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted for a period of 5 weeks in a large-scale commercial layer farm in Wennappuwa (Geographical coordinates: 7° 20'

48" North, 79° 50' 12" East, Sri Lanka) situated in North Western province, Sri Lanka.

Birds

Fifty-five weeks old Hy-line white layers were individually weighed (1500±21g) and, a total of 128 birds of uniform body weight were selected and assigned to 16 cages of 8 birds each so that the weight variation among cages was minimum. Four cages were randomly assigned to each of the four treatments. Each bird was provided with 550 cm^2 floor space. The cages were housed in an environmentally controlled room (21 ± 1^{o}C) throughout the experiment period. A lighting schedule of 20L: 4D was provided. The layer feed, except the Ca sources was given as recommended by Hy-line Management Guide (Hy-line, 2016) and water was freely available throughout the experimental period.

Diet

Commercially available layer feed was used as the basal ration. Three different Ca sources, namely oyster shell, limestone and bone meal were used as the supplemental Ca sources. Oyster shell and limestone were purchased from local market while thigh bones left from a commercial meat processing plant were processed into a meal. Each supplemental Ca source was introduced to its respective treatment in separate feeders and offered *adlibitum* adopting classical choice feeding method. The treatment fed only the layer feed with no supplemental Ca source served as the control.

Processing Bone Meal

Broiler thigh bone was processed into a meal. Separated thigh bones were boiled until loosen the meat particles. Bones were washed to remove meat particles and were sterilized at 120°C for one hour using an autoclave (VARIO 3028, Dixons Surgical Instruments Ltd, Wickford, UK). Sterilized bones were sun dried and then crushed into desirable particle sizes.

Chemical Analysis

The commercial layer feed was analyzed in duplicates for its proximate composition (AOAC International, 1995). Three Ca sources were analysed in duplicates for moisture and ash contents (AOAC International, 1995). Dry matter (DM) was determined by drying samples at 105°C for overnight in a pre-weighed dried crucible in a convection oven (Model No: YCO – 010, Gemmy Industrial Corp, Taipei, Taiwan). Ash content was determined by igniting the sample in a muffle furnace (FH- 12, Daihan Scientific Co. Ltd, Gangwon-do, Korea.) at 600 ^{0}C for 6 hours. Nitrogen was determined by Kjeldahl method. Ether extract (EE) was determined by Soxhlet extraction. Calcium in feed and grit sources were determined by colorimetric assay (Flexor E, Vital Scientific NV, Spankeren/Dieren, the Netherlands) following digestion with 6M HCl to release Ca (AOAC International, 1995). Total P in feed and grit sources were determined colorimetrically (AOAC International, 1995). The samples were ashed and digested in 6M HCl to prepare for the colorimetric step. Phosphorus was determined colorimetrically (UV mini 1240 Shimadzu Corp., Kyoto, Japan) at 680 nm. Bomb calorimetry (Model No: IKA C 200, IKAWerke GmbH & Co. KG, Staufen, Germany) was used to determine the gross energy (GE) contents of samples.

Particle Size Distribution of Ca Sources

Representative grit samples were tested in duplicates to determine the particle size distribution. A set of sieves sized 0.225, 0.354, 0.4, 1.19, 2.38, and 4mm were used to separate particles into different fractions through shaking. The samples were passed through a set of sieves for 10 minutes. The amount of particles retained on each sieve was weighed. The geometric mean diameter (GMD) and geometric standard deviation (GSD) were calculated as described by Baker and Herman (2002) using the formula below. These calculations were based on the assumption that the weight distributions of the grit samples were logarithmically normal (Martin, 1985).

$d_i = (d_u \times d_o)^{0.5}$

GMD= $\log^{-1} [\sum (Wi \log di) / \sum Wi]$

GSD = $\log^{-1}[\sum W_i (\log d_i - \log d_{gw})^2 / \sum W_i)]^{0.5}$
Where,

d_i = diameter of i^{th} sieve in the stack,

d_u = diameter opening through which particles will pass (sieve proceeding i^{th}),

d_o = diameter opening through which particles will not pass (i^{th} sieve),

W_i = weight fraction on i^{th} sieve in the stack.

Performance Parameters

Daily egg production, weekly damaged egg percentage (%), daily feed intake, daily Ca intake, body weight gain (BWG), feed conversion ratio (FCR) were recorded over 5 weeks. Calcium intakes derived from both the diet and respective supplemental Ca source were used to calculate daily Ca intake of birds. Feed conversion ratio was calculated as the amount of feed (kg) to produce a dozen (12) eggs. Mortality was recorded throughout the experimental period.

Egg Quality Parameters

A total of 80 eggs were collected weekly for laboratory analysis. Visual observation and spot candling was performed to identify damaged eggs. The eggs had hair-line cracks and gross cracks were considered damaged eggs. The weight, length and width of each egg were measured. Eggs were broken individually on a white flat tile in order to measure the yolk height, yolk diameter, albumen length, albumen height and the yolk colour. The yolk was separated from albumen and the weight was recorded.

Egg shells were washed gently to remove the remained albumen. Egg shells were air dried for 24 hours. The shell weights were obtained and the average shell thicknesses were obtained from three points (Kul and Seker, 2004). Calculations to determine external and internal egg quality parameters were done using the formulae described by Kul and Seker (2004), and Olawumi and Ogunlade (2008).

Statistical Analysis

Data were subjected to one way-ANOVA procedures using the statistical software package SAS (2001). The means were separated using Duncan multiple range test. Differences were considered significant at P<0.05.

RESULTS AND DISCUSSION

Analysed DM, ash, crude protein (CP), EE, Ca, total P and GE contents of the layer diet used during the experimental period are presented in Table 01. Layer diets are typically formulated to contain 40-46 g/kg and 3.1-5 g/kg, total Ca and non-phytate P, respectively (Leeson and Summers, 2005). The analysed Ca content in the diet was lower than the recommended dietary Ca level for commercial layers. The analysed DM and Ca contents of three Ca sources are presented in Table 01. The highest Ca content (353.7g/kg) was present in limestone while the lowest (101.6 g/kg) was from the bone meal. According to NRC (1994), limestone contains about 380 g/kg of Ca on weight/weight basis. However, Ca content in limestone (353.7 g/kg) was lower than that of reported by NRC (1994). Variation in the Ca concentration of limestone (339.7-428 g/kg) has been previously reported elsewhere (Ajakaiye *et al*., 1997; Wilkinson *et al*., 2013; Olgun *et al.,* 2015; Anwar *et al*., 2016b). Analysed Ca content in oyster shells (271.5 g/kg) was lower than that of reported in NRC (1994). According to NRC (1994) oyster shells contain 380 g/kg of Ca and 1g/kg of P level (NRC, 1994). However, variation in the Ca content of oyster shells (320.9-370 g/kg) has been previously reported (Olgun *et al*., 2015; Anwar *et al*., 2017). Bone meal contains 298g/kg of Ca and 125g/kg of P (NRC, 1994). Calcium (101.6 g/kg) and P contents (59.8 g/kg) in bone meal used in the present experiment were lower than that of reported by NRC (1994). Variation in the Ca and P contents of bone meal has been previously reported. Calcium content of bone meal was found to be varied from 193 to 370 g/kg (Orban and Roland, 1992; Field, 2000; Phiraphinyo *et al*., 2006; Khalil *et al*., 2017). The analysed composition of bone meal P varied from17.7 to 193.3 g/kg (Orban and Roland,1992; Phiraphinyo *et al*., 2006; Khalil *et al*., 2017). Calcium and P contents of bones are known to be affected by the species', age, feed nutrition and site of sampling (Orban and Roland,1992; Phiraphinyo *et al*., 2006; Khalil *et al*., 2017). However, the P content of bone meal produced from thigh bones (59.8 g/kg) in the present study is more or less comparable to that of reported by Khalil *et al.,* (2017) for bone meal P of thigh bone origin (45.2 g/kg).

NRC (1994) and Hy line (2016), recommended CP levels for layer rations range from155 and 170 g/kg. The analysed CP content of the layer feed is within the recommended value. The resulted GE level is satisfactory and sufficient to provide energy to the layers.

Particle Size Distribution

Particle size distribution of three different Ca sources is presented in Fig. 01. The geometric mean diameter (GMD) of oyster shells, limestone and bone meal were 0.616, 2.23 and 0.465 mm, respectively. The geometric standard diameter (GSD) of oyster shells, limestone and bone meal were 2.44, 2.18 and 2.40, respectively. The relative proportion of the coarse particles (>2.3 mm) was higher in limestone (58.53%). Medium sized particles (0.354 – 2.38 mm) were higher in oyster shells (56.01%). The bone meal contained more fine particles (0.225mm or less) (52.80%) than other two sources. However, particle distribution in bone meal was found to be different than the other two sources.

The solubility of the oyster shells, limestone and bone meal depends on their particle size. Particle size may be a factor influencing digestibility of Ca and P. A study by Orban and Roland (1992) demonstrated that the solubility of chicken bone meal of coarse (3.3mm), granular (2.2mm) and fine (0.8mm) were 13, 53 and 32%, respectively. Therefore, dietary Ca levels may need to adjust based on solubility of the particles. Hy-line (2016) recommended to provide more (60%), coarse (2000–4000 Micron) particles to commercial layers and less amount (40%) of fine (0-2000 micron) particles. Therefore, periodic particle size evaluation is an essential component of a feed manufacturing quality assurance program (Baker and Herrman, 2002).

According to Pizzolante *et al.,* (2009), coarse limestone produced less cracked eggs. In contrast, a study conducted by de Araujo *et al.,* (2011), revealed that no effect due to limestone particle sizes on egg weight, egg dozen conversions, shell thickness, egg shell ratio and Haugh Unit. Pelicia *et al.,* (2009) showed that *in vitro* solubility of limestone decreased with increasing particle size and no significant effect of particle size distribution on egg production, egg weight and feed intake of layers. However, although the effects of particle sizes were not tested, the present results demonstrated that bone meal having fine particles was more effective in improving shell quality characters in commercial layers. But Anwar *et al.,* (2016a) concluded that the particle sizes with coarser particles are more digestible than finer limestone particles. Numbers of research suggested that larger particle size limestone led to increased gizzard and duodenal soluble Ca which may be beneficial in accommodating needs for eggshell formation (Ajakaiye *et al*., 1997; Zhang and Coon, 1997; Guo and Kim, 2012). Zhang and Coon (1997) clearly demonstrated that large particle-sized limestone has an inherent ability to retain prolong period in the gizzard of commercial layers. These authors further showed that the Ca retention in the gizzard varies with Ca source causing variations in Ca availability. However, in contrast, Guinotte *et al.,* (1991) reported increased Ca retention in broilers fed diets with fine $CaCO_3$ (0.15 mm) as compared to medium (0.6-0.8 mm) and coarse $CaCO_3$(>1.18 mm).

Table 01: The analyzed composition of the layer feed and calcium sources.

Item	Dry matter (g/kg)	Ash g/kg	Crude Protein (g/kg)	Ether extract (g/kg)	Calcium (g/kg)	Total Phosphorus (g/kg)	Gross Energy (MJ/kg)
Layer feed	892.4	105.1	172.8	63.8	36.0	7.8	14.7
Oyster shell	992.8	817.9	-	-	271.5	0.24	-
Limestone	996.7	881.5	-	-	353.7	0.14	-
Bone meal	899.4	458.5	-	-	101.6	59.8	-

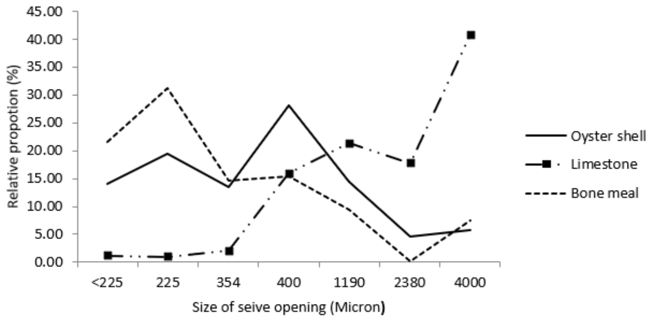

Figure 01: Particle size distribution of three Ca source

Performance Parameters

All birds remained healthy during the experimental period and no mortality was recorded. The influence of three Ca sources on bird's performance is presented in Table 02. Daily Ca intake per bird and damaged egg percentage were affected significantly (P<0.05) by Ca sources. However, FCR, weekly production and weight gain of birds were not affected by the Ca source (P>0.05).

Feed, Grit and Ca intake:

Daily feed intakes of birds fed the control diet alone, and the diets supplemented with oyster shells, lime stone and bone meal were 95.0, 95.7, 94.9 and 94.8 g/bird, respectively. Moreover, the present study revealed that the contribution of dietary Ca in fulfilling the daily Ca requirement of birds fed the control diet alone, and the diets supplemented with oyster shells, lime stone and bone meal were 3.42, 3.45, 3.42, 3.41g/bird, respectively. Grit intakes of birds fed control, oyster shells, lime stone and bone meal were 0.00, 9.26, 8.09 and 11.83 g/bird/day. Calcium intakes of birds between treatments were significantly different (P<0.05) (Table 02). The daily Ca intake of birds was significantly the highest (P<0.05) in birds fed oyster shells and limestone. (5.96 and 6.27 g/bird/day, respectively). The lowest Ca intake was observed in the birds fed control (3.42 g) diet. Bone meal treatment provided Ca intake of 4.61 g/bird/day.

Table 02: Effect of different calcium sources on layer performance[1].

	Ca intake (g/bird/ day)	Egg Damage (%)	FCR (Feed kg)/12egg)	Weekly Egg Production	Weight Gain (g)
Control	3.42^{c}	4.68^{a}	1.28	50.15	40
Oyster Shell	5.96^{a}	3.13^{a}	1.30	49.4	35
Limestone	6.28^{a}	3.79^{a}	1.32	48.95	35
Bone Meal	4.62^{b}	1.01^{b}	1.29	50.1	45
SEM[2]	0.161	0.645	0.019	0.601	11.85
Probability	***	**	NS	NS	NS

*NS = Not Significant; *P<0.05; **P<0.01; ***P<0.001.*

[1]Each value represents the mean of four replicates (8 birds/replicate).; [2]Pooled standard error mean. ; [a-b]Means in a column not sharing a common superscript are significantly different at P<0.05.

It is well known that the daily Ca intake of commercial layers must be maintained at 4.5g to fulfil the Ca requirement (Hy-line, 2016). This study confirmed that all three Ca sources used satisfied the daily Ca requirement of Hy-line layers.

Damaged Egg Percentage (%):

According to the collected data, bone meal treatment resulted in the lowest egg damage ($P<0.05$) percentage (1.01%). Damaged shell % in bird fed oyster shell (3.13%), limestone (3.79%) and the control (4.68%) were similar ($P>0.05$). Calcium availability is known to be affected by the Ca source and be related with its particle size. Moreover, it has been demonstrated that feeding Ca sources that containing P in their molecule may possibly result lower Ca bioavailability to poultry (Bessa, 1992) affecting on egg shell thickness.

***Feed Conversion Ratio*:**

Feed conversion ratio was not affected ($P>0.05$) by treatment (Table 02). Egg production and feed intakes of birds were not changed according to the treatments during experimental period. The layers were fed to a constant feed amount (95±1 g/b/d) and with *adlibitum* water availability (Hy-line, 2016). This may be the reason for preforming the birds in a same level. However, Ahmed *et al.,* (2013) reported low FCR in birds fed limestone.

Weekly Egg Production:

Weekly egg production of hens were not affected by treatments ($P>0.05$). As an average, birds fed oyster shells, lime stone, bone meal and control treatments produced 49.4, 48.95, 50.1 and 50.15 of eggs weekly (Table 02). Normally in commercial layers, hen day egg production ranges between 95 and 98%. Similarly, Scheideler (1998) reported that productive performance of layers was not affected by sources of dietary Ca. However, in contrast Ahmed *et al.,* (2013) reported that laying percentage increased with feeding limestone to layers.

Body Weight Gain:

Weight gain of layers during the experimental period was not significantly different ($P>0.05$) (Table 02). According to Pelicia *et al.,* (2009) Ca level had no effect on weight gain of the laying hens. Moreover, Sultana *et al.,* (2007) reported that the Ca source, their levels or their interactions did not cause any effect on initial live weight and final body weights of Japanese quail layers.

External Egg Quality Parameters

The effect of feeding different Ca sources on external egg quality parameters in commercial layers is presented in Table 03. Among the different criteria tested, egg weight, unit surface shell weight (USSW) and shell thickness were affected ($P>0.05$) by the treatments. However, shape index and shell ratio were not differed significantly ($P>0.05$).

Egg Weight:

During the experimental period the lowest egg weight ($P<0.05$) was seen among the birds fed the control diet (Table 03). A significant reduction of egg weight was observed when the birds were fed with control diet as compared to oyster shell, limestone and bone meal. Calcium supplementation improved the egg weight ($P<0.05$) in layers. However, the egg weights of birds fed three Ca sources were similar ($P>0.05$). Eggs from the layers fed the control diet had the lowest weight. It may due probably to absence of grit to facilitate mechanical digestion. Insufficient dietary Ca might reduce the egg weight in birds fed control diet. Saunders-Blades *et al.,* (2009) found that the egg weights did not differ among the Ca sources and particle sizes when fed to 51-70 weeks old layers.

Shape Index (%):

Shape index of eggs between treatments were similar ($P>0.05$). The egg shape index of birds fed oyster shell, limestone and bone meal and control diet were 76.41, 76.43, 75.60 and 76.54, respectively (Table 03).

Table 03: The effect of calcium sources on external egg quality parameters of commercial layers[1].

	Egg Weight (g)	Shape Index (%)	Shell Ratio (%)	Unit Surface Shell Weight (mg/cm^2)	Shell Thickness (mm)
Control	59.46^b	76.55	9.20	64.01^b	0.512^c
Oyster Shell	60.70^a	76.41	9.45	66.08^b	0.537^b
Limestone	61.63^a	76.44	9.605	65.84^b	0.500^c
Bone Meal	61.90^a	75.60	10.01	70.30^a	0.597^a
SEM[2]	0.437	0.323	0.272	1.198	0.005
Probability	***	NS	NS	**	***

*NS = Not Significant; *P<0.05; **P<0.01; ***P<0.001.*

[1]Each value represents the mean of four replicates (8birds/replicate).;[2]Pooled standard error mean.; [a-c]Means in a column not sharing a common superscript are significantly different at P<0.05.

Shell Ratio (%):

Shell ratio was not affected by treatments (P>0.05) (Table 03). Average shell ratios of the birds fed oyster shell, limestone and bone meal and control were 9.44, 9.60, 10.01 and 9.19%, respectively. It is evident that increase in egg weight is not accompanied by a proportionate increase in shell weight, thereby the shell ratio (Kul and Seker, 2004). This may be the reason for having similar shell ratios among treatments reported different egg weights in the present study.

Unit Surface Shell Weight (USSW):

The highest mean USSW (P<0.05) was given by the birds fed bone meal (70.3 mg/cm^2) compared to those fed oyster shell, limestone and control; 66.1, 65.8 and 64.0 mg/cm^2, respectively (Table 03).

Egg Shell Thickness:

Egg shell thickness was significantly (P<0.05) different among treatments (Table 03). Birds fed bone meal reported the highest (P<0.05) mean shell thickness (0.596 mm) which was significantly different from other treatments. Birds fed oyster shell reported the next highest (P<0.05) shell thickness (0.536 mm). Shell thickness of birds fed oyster shell was significantly different from other treatments. Shell thickness of the eggs of the birds fed limestone and control were similar (0.500 and 0.511 mm, respectively). It is well known that the birds are responsive to increased dietary Ca up to a certain limit and higher dietary Ca concentrations may impair shell thickness in birds (de Souza *et al*., 2016). Keshavarz (1998) observed that increasing daily Ca intake from 3.51 to 4.25 g/hen improved shell thickness from 0.372 to 0.386 mm. In contrast, de Souza *et al.,* (2016) revealed that the dietary Ca above 3.5% impaired shell thickness in Japanese quail layers. When Ca is in excess, intestinal absorption and consequently utilization might be impaired (de Souza *et al*., 2016). Two possible explanations may be provided for the observed result in the present study. First excessive Ca intake observed particularly in birds fed oyster shells and limestone, could probably result low Ca utilization in birds. In order to maintain optimum blood Ca levels, excess Ca will be excreted leaving some Ca available for metabolism. On the other hand, high dietary Ca can cause hypercalcaemia, which in turn reduces the secretion of parathyroid hormone and synthesis of $1,25(OH)_2D_3$, therefore lowering plasma Ca concentrations by decreasing its reabsorption from the kidney and absorption from the intestine (de Matos, 2008; Anwar, 2017). Similarly, higher plasma Ca concentrations also stimulate the secretion of calcitonin by ultimobranchial gland which reduces Ca resorption from bones and kidneys which leads to low plasma Ca concentrations (Anwar, 2017).

Table 04: The effect of calcium sources on internal egg quality parameters of commercial layers[1].

	Albumen Index	Albumen Ratio	Hough Unit	Yolk Colour	Yolk Index	Yolk Ratio
Control	9.92	61.8	86.46	12.00	42.88	28.99
Oyster Shell	9.93	62.9	86.23	12.02	43.53	27.61
Limestone	9.86	61.6	86.45	12.09	43.60	28.85
Bone Meal	9.72	62.3	85.98	12.03	43.72	27.67
SEM[2]	0.177	1.03	0.544	0.056	0.294	0.792
Probability	NS	NS	NS	NS	NS	NS

NS = Not Significant.

[1]Each value represents the mean of four replicates (8birds/replicate).; [2]Pooled standard error mean.;[a-b]Means in a column not sharing a common superscript are significantly different at P<0.05.

Second, increased Ca intakes increased the absorption of Ca through the passive pathway, while reducing its absorption through the active pathway keeping the overall effect as reduction in the percentage of Ca absorption.

Internal Egg Quality Parameters

None of the internal egg quality parameters were affected (P>0.05) by the treatments (Table 04).

Haugh unit:

Finding of the present study is in an agreement with the finding of Pizzolante, *et al.,* (2009) who found that HU was not affected by dietary Ca concentrations. However, HU is known to be differed according to the layer breed (Monira, 2003).

Yolk colour:

According to the present study, Ca source has no any relationship with the yolk colour. However, in contrast others (Guo and Kim, 2012, Tortuero and Centeno, 1977) reported that increasing dietary Ca level from 30 to 40 g/kg reduced egg yolk colour by approximately one unit on the Roche colour fan. A similar trend was observed by Jones (2007), who found that the yellow pigment of egg shells in ring-necked pheasants decreased with increasing dietary calcium. No difference (P>0.05) between treatments were observed for yolk index (Table 04).

CONCLUSION

The present study concluded that free choice feeding of bone meal to layers improves unit surface shell weight, shell thickness and thereby reduce egg shell damages. Feeding Ca sources did not change internal quality parameters of eggs. Among the Ca sources tested, bone meal is the best Ca source for commercial layers.

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