J.Natn.Sci.Foundation Sri Lanka 2022 50 (4): 745 - 754
DOI: http://dx.doi.org/10.4038/jnsfsr.v50i4.10664

RESEARCH ARTICLE

Poultry science

Influence of different bone meal particle size induced calcium specific appetite on performance and egg quality parameters of layer chickens†

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Submitted: 23 August 2021; Revised: 01 April 2022; Accepted: 05 April 2022

Abstract: The effects of free choice feeding of bone meal (BM) of different particle sizes on performance and egg quality traits of layer chickens were assessed. A total of 128 Hy-line White layers (56 wks old, BW ± SD: 1500 ± 18 g) were assigned randomly into 16 cages of 8 birds each. Four dietary treatments based on BM particle sizes (Layer Diet/Control; Layer diet + 1–2 mm BM; Layer diet + 2–3 mm BM; Layer diet + 3–4 mm BM) were choice fed for five weeks. Dry matter (DM), calcium (Ca), total phosphorus (TP), ash content, particle size distribution and *in vitro* solubility of BM were tested. The birds' performance and external and inernal egg quality traits were examined over a 5 wk period. The DM, Ca, TP and ash content of BM were 893, 79, 44 and 476 g/kg, respectively. The geometric mean diameter (GMD) and the geometric standard deviation (GSD) of BM were 1.7 and 1.6 mm, respectively. *In vitro* solubility of 1–2 mm, 2–3 mm and 3–4 mm BM particles were 56.3, 47.5 and 39.8%, respectively. Bone meal, when choice fed, increased BM intake, Ca intake, and laying percentage, and reduced damaged egg percentage. Birds fed with BM having 2–3 mm particles performed best in improving the performance and egg quality. Bone meal significantly ($p < 0.05$) improved the egg shell thickness with maximum thickness recorded from birds fed with BM of 2–3 mm particle size. BM with 2–3 and 3–4 mm particles improved ($p < 0.05$) shell ratio and unit surface shell weight. The present study concluded that feeding with BM with 2–3 mm particle size is the best to improve the overall performance and egg quality traits of layer chickens.

Keywords: Bone meal, calcium, egg shell, egg quality, free choice, particle size.

INTRODUCTION

Calcium (Ca) is the most copious mineral in an animal's body. In poultry, Ca serves as the major element of skeletal structure. Its role in the regulation of acid-base balance and blood clotting is also well known (McDonald *et al*., 2002). Ca is the major mineral of the egg shell. A chicken egg has 2.2 g of Ca, that exists mainly in the form of $CaCO_3$ in its eggshell (Pelicia *et al*., 2009).

The recommended Ca intake of white egg layers is 3.25 g/hen/day (NRC, 1994). However, many researchers have shown that the daily Ca intake in layer chickens should be above this recommended value (Zhang *et al*., 2017). The dietary recommendation of Ca for Hy-line layers ranges between 4.00 and 4.65 g per hen per day (Hy-line, 2016) or 4.0–4.6% of the total feed volume (Bradbury *et al*., 2014; Wilkinson *et al*., 2014b). Layers have high demand for Ca, especially during peak egg production period (Saunders-Blades *et al*., 2009). This high demand for Ca is largely met by the use of Ca supplements or grits in layer diets. Commercially available layer rations are mostly formulated to contain

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† Presented in part in Abstracts of 7th International Conference of Sabaragamuwa University of Sri Lanka, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka, 14-15 November 2019.

grains and their by-products, which have very low Ca content (Peixoto & Rutz, 1988), and the most of the layer rations available in the local market do not contain the recommended dietary Ca levels of 4.0-4.6%. Therefore the use of an external Ca source is recommended to avoid serious shell quality issues. It has been estimated that about 6–8% of the total eggs laid worldwide are either cracked or broken before reaching the hands of consumers (Englmaierová *et al*., 2017). However, with the resulting profit loss and health risks associated with cracked shells, eggshell quality remains one of the major concerns of the global poultry industry.

In the commercial poultry industry, supplementation of layer feed with external Ca sources is related in a straightforward manner to the grit feeding practices. Feed manufacturers have used oysters shell as a source of Ca for more than 100 years (Roland & Bryant, 1999; Saunders-Blades *et al*., 2009). However, limestone has become the major supplemental Ca source used in layer rations due to its abundance, low price and easy application (Çath *et al*., 2012). Oyster shells and limestone provide Ca in the form of $CaCO_3$, and each contains about 38% Ca (Saunders-Blades *et al*., 2009). Bone meal (BM) is an excellent dietary source of Ca and phosphorus (P) (Orban & Roland, 1992). Bone meal contains considerable levels of Ca ranging from 354 to 362 g/kg, depending on the species used (Orban & Roland, 1992). Studies that investigated the potential of BM as a Ca supplement to feed free choice in commercial layers are highly limited and the study by Rathnayaka *et al*. (2020) concluded that use of BM when choice fed, improves the shell thickness in layers, compared to those who were fed oyster shells and lime stone.

The best particle size of Ca supplements for layer chickens has been a controversial topic for almost a century (Saki *et al*., 2019). The particle size of Ca sources used in rations may have an influence on its availability to the laying hens. According to Zhang and Coon (1997), large limestone particle sizes (> 0.8 mm) with low *in vitro* solubilities (30–50%), were retained for a prolonged time in the gizzard, increasing *in vivo* solubility to higher than 94%. Eggshell is known to be synthesized during the night, when layers are off feed. Therefore, using larger particle sizes slows passage through the gastrointestinal tract (Tunç & Cufadar, 2015). This allows dietary Ca to be available for eggshell formation, with lower mobilization of bone Ca by the layer chickens (Saki *et al*., 2019). A number of research projects have been conducted to assess the effect of limestone, oyster shells, and egg shells as sources of Ca in layer diets and the effect of their particle sizes on egg shell quality (Saunders-Blades *et al*., 2009; Olgun *et al*., 2015; Tunç & Cufadar, 2015). However, no literature is available on the optimal particle size of BM to improve the egg shell quality parameters of layers.

As for other nutrients like lysine, methionine and selenium, poultry exhibit a special appetite for Ca (Wilkinson *et al*., 2014a). The existence of a special appetite in broilers (Wilkinson *et al*., 2013; 2014a) and layers (Bradbury *et al*., 2014) for Ca has been therefore widely tested by much research. This research used limestone as the primary Ca source. Therefore, the objective of the present study was to investigate the effect of free choice feeding of three different BM particle sizes on improving performance and egg quality characters of layer chickens.

MATERIALS AND METHODS

The experiment was reviewed and approved (ERC/A/04/2019/03) by the Institutional Animal Care and Use Committee of Animal and Research Ethics, Sabaragamuwa University of Sri Lanka. All birds were reared and cared for under the guidelines of the Hy-line layer management guide (Hy-line, 2016)

Experimental site

The study was conducted for a period of 5 weeks in a large-scale commercial layer farm in the Wennappuwa region (geographical coordinates: 7° 20' 48" N, 79° 50' 12" E), located in the North Western Province of Sri Lanka.

Birds

Fifty-six weeks old Hy-line white layers were individually weighed (1500 ± 18 g), and a total of 128 birds with uniform weight were assigned to 16 cages of 8 birds each so that the weight variation among cages was minimum. Each of four dietary treatments was randomly assigned to 4 cages. Each bird was provided 550 cm^2 cage space. The cages were housed in an environmentally controlled room (21 ± 1ºC) and a 20L:4D lighting schedule was provided. According to the Hy-line Management Guide (Hy-line, 2016), the layer feed (except the Ca supplements) was provided and water was available all the time.

Processing bone meal

Broiler thigh bones were separated and boiled to remove the meat parts. Bones were washed and were sterilized at 120ºC for one hour using an autoclave (VARIO

3028, Dixons Surgical Instruments Ltd, Wickford, UK). Sterilized bones were oven dried (180ºC) for 3 h. On completion of drying, the bones were ground into small pieces.

Particle size distribution of bone meal samples

Representative BM samples were tested in duplicates to determine the particle size distribution. A set of sieves sized 1.00, 2.00, 3.00, 4.00 mm and the particles were separated into different fractions through shaking for 10 min. The amounts of particles retained on each sieve was weighed and the Geometric Mean Diameter (GMD) and Geometric Standard Deviation (GSD) were calculated (Baker & Herman, 2002). The calculations were based on the assumption that the weight distributions of the BM samples were logarithmically normal (Martin,1985).

$$d_i = (d_u \times d_0)^{1/2} \quad \ldots..(1)$$

$$GMD = \log^{-1} [\sum (W_i \log d_i)/\sum W_i] \quad \ldots..(2)$$

$$GSD = \log^{-1} [\sum W_i (\log d_i - \log GMD)^2 / \sum W_i]^{1/2} \quad \ldots..(3)$$

where,

d_i = Geometric mean diameter of particles on ith sieve in the sieve stack,

d_u = Diameter of sieve openings of the ith sieve,

d_0 = Diameter of openings in next larger ith sieve in set,

W_i = Weight fraction on ith sieve in the stack.

Dietary treatments

A commercially available formulated layer feed was used as the basal diet. Bone meal having three different particle size ranges (*i.e.,* 1–2 mm, 2–3 mm and 3–4 mm particle sizes) were used as the test ingredients. Thigh bones from a commercial meat processing plant were processed into BM. Bone meal was separated into three fractions and the particles retained on 1.00, 2.00 and 3.00 mm sieves set was considered as the test ingredients. Bone meal separated based on three particle size ranges was introduced to its respective treatment in separate feeders, and offered *ad libitum* in accordance with the classical choice feeding method. The treatment fed only the layer feed with no supplemental BM served as the control.

Chemical analysis

The layer diet was analyzed in duplicate for its proximate composition (AOAC International, 2005). Bone meal was analysed in duplicates for moisture and ash contents (AOAC International, 2005). Dry matter (DM) contents of the layer diet and BM were analyzed by drying samples at 105°C overnight in a pre-weighed dried crucible in a convection oven (Model No: YCO – 010, Gemmy Industrial Corp, Taipei, Taiwan). Ash content in the layer diet and BM was determined by igniting the sample in a muffle furnace (FH- 12, Daihan Scientific Co. Ltd, Gangwon-do, Korea.) at 600 ºC for 6 hours. Nitrogen was determined by the Kjeldahl method. Ether extract (EE) was determined by Soxhlet extraction. Calcium in layer diet and BM were determined by Inductively Coupled Plasma Mass Spectroscopy (Agilent ICP-MS 7900, Agilent, USA) following to microwave digestion with undiluted nitric acid (Fleischer *et al*., 2014). Total P in layer diet and BM were determined colorimetrically (UV-Vis-1900 Shimadzu Corp., Kyoto, Japan) (AOAC International, 2005). The gross energy (GE) content of feed samples was measured using bomb calorimetry (Model No: IKA C 200, IKAWerke GmbH & Co. KG, Staufen, Germany). Three different particle sizes of BM were analysed in duplicate for their *in vitro* solubility by weight loss method (Cheng & Coon,1990; Anwar *et al*., 2016).

Performance parameters

Daily Ca intake, body weight gain (BWG), feed conversion ratio (FCR), weekly egg production, and weekly damaged egg percentage (%) were recorded over 5 weeks. Daily Ca intake of birds were derived from summing up both the dietary and BM Ca intakes from each respective treatment. Feed conversion ratio was calculated as the amount of feed (kg) required to produce 12 eggs. Mortality was recorded over the experimental period.

Egg quality parameters

A total of 80 eggs (5 eggs per replicate) were collected on every 4th day of the week for analysis. Damaged eggs were identified through visual observation and candling. The eggs that had gross cracks or hair-line cracks were considered damaged eggs. The egg weights were measured with an electronic balance (Model No: AS III R2, Radwag Elektroniczne, Poland) to the nearest 0.01 g. A digital caliper was used to measure egg length and width to the nearest 0.01 mm. 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 egg albumen and the weight was measured. Egg shells were washed gently to remove the retained albumen. Egg shells were air dried for 24 hours and the shell weight was measured. The shell thickness was measured from three points. Calculations to determine external and internal egg quality parameters were done using the formulae given below, described by Kul and Seker (2004) and Duman *et al*. (2016).

$$Shape\ Index\ (\%) = \frac{Width\ (cm)}{Length\ (cm)}\ x\ 100$$

$$Shell\ Ratio\ (\%) = \frac{Shell\ weight\ (g)}{Egg\ weight\ (g)}\ x\ 100$$

$$Egg\ Surface\ Area\ (S) = 3.9782\ W^{0.7056}$$

Where;
S = Egg surface area (cm^2)
W = Egg weight (mg)

$$Unit\ Surface\ Shell\ Weight\ (\frac{mg}{cm^2}) = \frac{Shell\ weight\ (mg)}{Egg\ surface\ area\ (cm^2)}$$

$$Albumen\ Index\ (\%) = \frac{Albumen\ height\ (mm)}{[Albumen\ length\ (mm) + Albumen\ width (mm)]/2}\ x\ 100$$

$$Albumen\ Ratio\ (\%) = \frac{Albumen\ weight\ (g)}{Egg\ weight\ (g)}\ x\ 100$$

Haugh Unit (HU) = $100 \log (H + 7.57 - 1.7W^{0.37})$

Where;
H = Albumen height (mm)
W = Egg weight (g)

$$Yolk\ Index\ (\%) = \frac{Yolk\ height\ (mm)}{Yolk\ diameter\ (mm)}\ x\ 100$$

Statistical analysis

Data were subjected to one-way ANOVA in a Completely Randomized Design and were analyzed using the GLM procedure of SAS (SAS, 2002). Differences between means were separated using Duncan's Multiple Range Test at a significant level of 0.05.

RESULTS AND DISCUSSION

Particle size distribution

The Geometric Mean Diameter (GMD) and Geometric Standard Deviation (GSD) of BM were 1.70 mm and 1.63 mm, respectively (Figure 1). Bone meal particles that passed through the 4.00 mm sieve and were retained on the 2.00 mm sieve were considered coarse. The particles that passed through the 2.00 mm sieve were considered as fine particles. The relative proportion (59.6%) of fine particles (0–2 mm) was higher than the proportion of coarse particles (40.4%).

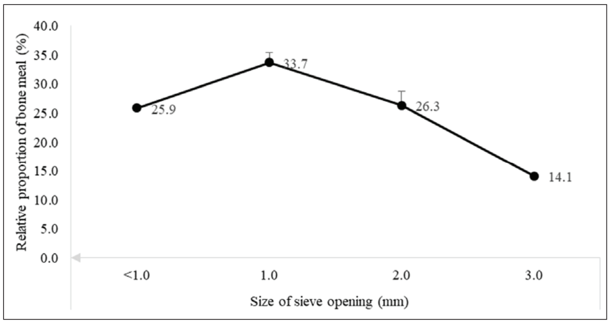

Figure 1: Particle size distribution of raw bone meal used in the experiment

Analysed composition of the layer feed and bone meal

According to NRC (1994) and Hy Line (2016), Crude Protein (CP) levels for layer rations could range from 155 to 170 g/kg (Table 1). The analysed CP content of the layer feed is 183.2 g/kg (Table 1). Layer diets are formulated to contain 40–46 g/kg Ca (Bradbury *et al*., 2014; Wilkinson *et al*., 2014b). The analysed Ca content in the diet (31 g/kg) was lower than that of the recommended dietary Ca level for layer chickens (Table 1).

Table 1: The analyzed composition of the layer feed (as received basis)

Item	Dry matter (g/kg)	Ash (g/kg)	Crude protein (g/kg)	Crude fat (g/kg)	Calcium (g/kg)	Total phosphorus (g/kg)	Gross energy (kJ/kg)
Layer feed	908.9	118.0	183.2	144.0	31.0	7.5	4040.3
Bone meal	892.8	476.0	-	-	79.0	44.0	-

According to NRC (1994), BM is supposed to contain 298 g/kg of Ca and 125 g/kg of total P. The analysed Ca (79 g/kg) and P (44 g/kg) content in BM used in the present study were much lower than those of reported in NRC (1994). Differences in Ca and P contents of BM have been reported. The calcium content of BM was found to range from 193 to 370 g/kg (Orban & Roland, 1992; Field, 2000; Phiraphinyo *et al*., 2006; Khalil *et al*., 2017). Phosphorus in BM ranged from17.7 to 193.3 g/kg (Orban & Roland,1992; Khalil *et al*., 2017). However, Ca and P contents of bones are known to be affected by the species, age, nutrition and sampling site (Orban & Roland,1992; McDonald *et al*., 2002; Phiraphinyo *et al*., 2006; Khalil *et al*., 2017). However, the Ca and P contents of BM produced from thigh bones in the present study are lower than those reported by Rathnayaka *et al*. (2020) for BM (Ca 101.6 g/kg and P 59.8 g/kg) of thigh bone origin.

In vitro solubility test results

According to Rao & Roland (1989), Ca supplements with larger particle sizes had a higher *in vivo* solubility but they were less soluble under *in vitro* conditions. In the present study, fine particle sizes (1–2 mm) recorded the highest solubility (56.25%) (Table 2). Some researchers have found no relationship between Ca solubility *in vitro* and Ca solubility *in vivo* when two Ca sources of the same particle sizes were compared (Rao & Roland, 1990). Orban and Roland (1992) revealed that the *in vitro* solubility of coarse (3.3 mm), granular (2.2 mm) and fine (0.8 mm) chicken BM were 13, 53 and 32%, respectively.

Table 2: *In vitro* solubility of bone meal (BM)samples used in the experiment

Particle size (mm)	*In vitro* solubility (%) ± SE[1]
1.0–2.0	56.25 ± 0.45
2.0–3.0	47.45 ± 0.32
3.0–4.0	39.80 ± 0.30

[1] Standard error

The particle size of an ingredient may be a factor influencing digestibility of Ca and P. Larger feed particles are digested over a longer time in the gizzard than fine feed particles, making Ca more available during the period of shell formation throughout the night (Scott *et al*., 1971). According to Hyline (2016) feeding layers over 63-weeks of age with 65% of the coarse limestone (2 and 4 mm in diameter) effectively maintains egg quality. Pizzolante *et al*. (2009) revealed that feeding coarse limestone to the layers produced fewer cracked eggs.

Performance parameters

No mortality was detected during the entire experimental period. Bone meal intake, Ca intake, damaged egg percentage and laying percentage were affected significantly ($p < 0.05$) by particle sizes of the BM. However, FCR and weight gain of layers were not affected ($p > 0.05$) by the particle size of BM (Table 3).

Bone meal intake and Ca intake

The highest daily BM intake (37.39 g/b/day) ($p < 0.05$) was observed in the birds fed with BM having 2–3 mm particle size (Table 3). The lowest BM intake (21.85 g/b/day) was observed in birds fed with BM having 1–2 mm particle size. The birds fed with BM having 2–3 mm particle size showed the highest ($p < 0.05$) daily Ca intake (5.74 g/b/day) (Table 3). The birds fed with the control diet showed the lowest Ca intake (2.79 g/b/day). It is noteworthy that all three BM diets were able to fulfil the recommended daily dietary Ca requirement of layers. This could be due to the layers' ability to select Ca when BM was fed in separate feeders. This Ca-specific appetite has been previously reported by Wilkinson *et al*. (2014a) for broilers when choice-fed with limestone.

Damaged egg percentage

The highest damaged egg percentage (9.34%) was reported from the group fed with the control diet (Table 3). Feeding BM to the layers significantly ($p < 0.05$) reduced the damaged egg percentage (1.28–1.57%). However, feeding BM with different particle sizes had no effect ($p > 0.05$) on the damaged egg percentage. The finding of this study is comparable to that reported by Rathnayaka *et al.* (2020) who found that free-choice feeding of BM reduced the damaged egg percentage (1.01%) in layers as compared to those fed with a control diet alone, limestone, and oyster shells. This effect of BM supplementation to reduce damaged egg percentage may be due to (i) the birds' ability to meet their daily Ca requirement through BM intakes and (ii) the availability of BM Ca to the birds for shell formation.

Feed conversion ratio (FCR)

Choice feeding of BM has no effect on FCR ($p > 0.05$) (Table 3). According to Auttawong *et al.* (2013), coarse feed improved FCR when birds were fed *ad libitum*. However, when feeding time was limited or restrictedly, the coarse feed effect disappeared. Throughout this experiment the birds were fed based on Hy-line nutrition guidelines (Hy-line, 2016) with *ad libitum* water. This can be the reason for the insignificant effect among the treatments for FCR.

Laying percentage

The laying percentage was highest ($p < 0.05$) in birds fed with BM having 2–3 mm particle size (90.07%) (Table 3). Feeding with the control diet reduced ($p < 0.05$) the laying percentage. However, Rathnayaka *et al*. (2020) found that feeding BM to the commercial layers has no effect on laying percentage and the laying percentages observed during their study were not affected by the source of Ca. In contrast, Ahmed *et al.* (2013) found that the laying percentage increased when fed with limestone.

Weight gain

The weight gain of birds was similar ($p > 0.05$) among treatments (Table 3). The non-significant effect of Ca on weight gain of layers has been previously reported (Pelicia *et al*., 2009; Rathnayaka *et al*., 2020).

External egg quality parameters

The effect of feeding three different particle sizes of BM on external egg quality parameters in commercial layers is presented in Table 4. No BM particle size effect was

Table 3: Effect of different treatments on layer performance[1]

	Bone meal intake per bird per day (g)	Ca intake per bird per day (g)	Egg damage (%)	FCR (kg feed/12 egg)	Laying percentage (%)	Weight gain (g/bird/day)
Control	0.0^{d}	2.79^{d}	9.34^{a}	1.29^{a}	68.44^{c}	40.0^{a}
1–2 mm	21.85^{c}	4.52^{c}	1.57^{b}	1.28^{a}	85.00^{b}	42.0^{a}
2–3 mm	37.39^{a}	5.74^{a}	1.28^{b}	1.32^{a}	90.07^{a}	38.0^{a}
3–4 mm	36.68^{b}	5.69^{b}	1.35^{b}	1.29^{a}	84.99^{b}	40.0^{a}
SEM[2]	0.14	0.01	0.76	19.35	1.29	11.92
Probability	***	***	***	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.

$^{a-d}$ Means in a column not sharing a common superscript are significantly different at $p < 0.05$.

Table 4: Effect of three different particle sizes of bone meal on external egg quality characteristics commercial layers[1]

	Egg weight (g)	Shape index (%)	Shell ratio (%)	Unit surface shell weight (mg/cm^{2})	Shell thickness (mm)
Control	65.04^{a}	73.72^{a}	12.03^{b}	86.02^{b}	0.412^{c}
1–2 mm	66.54^{a}	74.49^{a}	11.90^{b}	86.37^{b}	0.434^{b}
2–3 mm	66.58^{a}	72.44^{b}	12.34^{a}	88.43^{a}	0.47^{7}a
3–4 mm	66.26^{a}	73.64^{a}	12.51^{a}	89.55^{a}	0.435^{b}
SEM[2]	0.534	0.350	0.136	0.890	0.006
Probability	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.

$^{a-c}$Means in a column not sharing a common superscript are significantly different at $p < 0.05$.

found for egg weight ($p < 0.05$). Shape index, shell ratio, unit surface shell weight (USSW), and shell thickness were affected significantly ($p < 0.05$) by different BM particle sizes.

Egg weight

Egg weights of birds among treatments were similar ($p > 0.05$) (Table 4). According to Amornthewaphat *et al*. (2007), the particle size of the corn feed did not influence the egg weight. Guinotte and Nys (1991) demonstrated that the layers fed with coarser limestone particles produced heavier eggs as compared to those consuming pulverized limestone.

Shape index

The egg shape index of birds was affected significantly ($p < 0.05$) by the different particle sizes of BM. A significant reduction ($p < 0.05$) of the shape index was observed when the birds were fed with 2–3 mm particle size (72.4%), as compared to 1–2 mm (74.5%) or 3–4 mm (73.64%) particle sizes of BM and the control diet (73.7%). As defined by Duman *et al*. (2016), all the eggs produced during the present experiment can be considered oval (72–76%). According to Alkan *et al*. (2010), a significant negative correlation exists between the egg shape index and shell thickness. Similarly, the birds fed with BM having 2–3 mm particle size reported the lowest shape index and the highest shell thickness (0.477 mm).

Shell ratio and unit surface shell weight (USSW)

The birds fed with BM having 2–3 mm and 3–4 mm particle sizes recorded the highest shell ratio (12.3% and 12.5%, respectively). According to Ozcelik (2002), the shell ratio depends on egg weight, shell weight, and shell thickness. However, it is evident that no proportional increase exists between egg weight and shell weight, and therefore the shell ratio (Kul & Seker, 2004). This may be a reason for observed differences in shell ratios in the present study although the egg weights were similar.

Feeding with BM having 2–3 mm and 3–4 mm particle sizes resulted the highest ($p < 0.05$) unit surface shell weight (USSW). As shown by Zhang *et al*. (2017), USSW was significantly increased with higher Ca levels and lower limestone solubility. According to these researchers, the highest USSW was obtained at 4–5 g/day Ca intakes and 30.1–39.8% limestone solubilities. The birds fed with BM having 2–3 mm and 3–4 mm particle sizes in the present study reported the highest Ca intake per day while exhibiting comparatively lower *in vitro* solubilities of the BM. Higher retention time in gizzards due to lower *in vitro* solubilities may be the reason for the highest USSW reported in birds fed BM supplements having 2–3 mm and 3–4 mm particle sizes.

Egg shell thickness

Egg shell thickness of birds differed significantly ($p < 0.05$) among treatments. The highest egg shell thickness (0.477 mm) was reported for the birds fed with BM having 2–3 mm particle size. The lowest egg shell thickness (0.411 mm) was recorded in birds fed with the control diet. Dietary Ca level is known to have an effect on the egg shell thickness. The layer diet used for the present experiment contained 31 g/kg Ca. The birds fed the control diet received Ca solely from the layer diet. This may be the reason for having the lowest egg shell thickness in birds fed with the control diet.

According to Scott *et al*. (1971), Ca supplements with large particle size are expected to be retained for a prolonged period in the gizzard making Ca more available during the period of shell formation. This may be a reason for the highest shell thickness recorded in the birds fed with BM having 2–3 mm particle size .

Internal egg quality parameters

The effect of feeding three different particle sizes of BM on internal egg quality parameters in commercial layers is presented in Table 5.

All the internal egg quality parameters tested were not affected ($p > 0.05$) by the particle sizes of BM. The result of the present study for internal egg quality parameters are in agreement with the findings of Rathnayaka *et al*. (2020), who found that feeding layers with either limestone, oyster shells or BM did not improve internal quality traits of chicken eggs. Moreover, the Haugh unit was not altered when Hy-Line Brown layers were fed with diets ground by roller and hammer mill to different particle sizes (Pérez-Bonilla *et al*., 2014). The present study also confirmed that different particle sizes had no effect on the Haugh unit. According to Wang *et al*. (2014) particle sizes of Ca sources have no effect on the egg yolk colour of Longyan ducks, which is in agreement with the results of the present study.

Table 5: The effect of feeding three different particle sizes of bone meal on internal egg quality characteristics of commercial layers[1]

	Albumen index (%)	Albumen ratio (%)	Haugh unit	Yolk colour	Yolk index (%)	Yolk ratio (%)
Control	5.50^a	60.08^a	59.78^a	12.04^a	42.61^a	27.59^a
1–2mm	5.54^a	60.76^a	60.11^a	12.09^a	42.53^a	27.21^a
2–3mm	5.69^a	60.42^a	59.99^a	12.01^a	42.69^a	27.22^a
3–4mm	5.69^a	60.35^a	60.28^a	12.02^a	42.41^a	27.16^a
SEM[2]	0.07	0.33	0.50	0.05	0.10	0.23
Probability	NS	NS	NS	NS	NS	NS

NS = not significant.

[1] Each value represents the mean of four replicates (8 birds/replicate).

[2] Pooled standard error means.

[a-b] Means in a column not sharing a common superscript are significantly different at $p < 0.05$.

CONCLUSION

The present study demonstrated that free-choice feeding of BM improves the egg shell thickness and reduces damaged egg percentage in commercial layers. Bone meal containing 2–3 mm particles improves BM intake, Ca intake, laying percentage and shell thickness more than other sized particles. Neither BM nor its particle sizes have positive effects on feed conversion ratio, weight gain, egg weight, or internal egg quality parameters tested. The present study concludes that BM particle size influences the Ca specific appetite of layer chickens and feeding BM having 2–3 mm particle size is the best in improving overall performance and egg quality parameters.

Conflict of interest

The authors disclose that they have no conflicts of interest concerning this article.

Acknowledgement

The authors acknowledge the assistance of the technical staff of Maxies and Company (Pvt.) Ltd, Wennappuwa, Sri Lanka and the technical staff of the Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University, Belihuloya, Sri Lanka.

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