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Effect of afternoon feeding of particulate limestone on layers' performance, egg and bone quality

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Abstract

An experiment was conducted to investigate the effect of split feeding of calcium on the laying performance of hens in the first production cycle, and to study the egg and bone quality of end-of-lay hens. A total of 7700 Hendrix ISA Dekalb 15-week-old white pullets were divided into two groups containing an equal number of birds (3850 in each). The control group was conventionally fed with 3.9% calcium in the pellets, with a 2:1 ratio of coarse to fine limestone particles, and with a 0900, 1100, 1400, and 1700 feeding regime. The feed of the test group contained 1.6% calcium. The test group received their remaining calcium as particulate limestone at 1400 and 1700 feeding hours. Egg quality from 64, 68 and 72-week-old hens and bone quality at the end of production were measured. A significant increase in egg weight was observed in the test group. Treatment had no effect on the eggshell quality traits such as eggshell thickness, eggshell weight, shell percent and eggshell breaking strength, and also no effect on bone quality parameters such as length, breadth, weight, and ash content. This study hints at the potential of implication of split feeding, however, it is essential to more conclusively determine a true assessment of laying performance.

Key words: split feeding, pellet feed, calcium, limestone, egg quality, bone quality

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Abbreviations

g	gram
mm	milieter
FCR	Feed Conversion Ratio
ANOVA	Analysis of Variance
R	Statististical programming

1. Introduction

Eggs are a nutritionally and commercially valuable food commodity. Eggs contain about 16 % protein, which is nutritionally complete meaning it contains all of the essential amino acids that human body cannot synthesized (IDAHOR, 2017). Biological value of eggs is 100 (Hoffman & Falvo, 2004) which is higher than other meat products, implying the protein is more available to the body. Due to this nutritional significance, egg is popular food among the consumers all over the world. Egg consumption is also high in Norway: 68 million kg of eggs was produced to be consumed in 2016 (Taylor, 2018). High demand encourages high production; however the quality of eggs is also important. Commercially, eggs are also graded according to external qualities such as size. Generally larger eggs are expensive because edible percentage in larger eggs is comparatively higher (Jacob et al., 2000).

Another important criterion for the egg producers is eggshell quality. As the eggs in the production chain are exposed to physical and environmental stress during handling, packaging, and transportation, the incidence of eggshell cracking increases if the quality of eggshell is poor. Micro cracks can also lead to microbial contamination which might incur health hazard among consumers (Hunton, 2005). Beside, a broken egg also means loss of nutrient loss.

Quality of eggs can be influenced by the feeding system. In a conventional feeding system, hens daily rations contain the same nutrient concentration. However, when offered free choice hens show different pattern of nutrient consumption. Protein consumption is higher early in the day while calcium consumption is higher later (Chah & Moran Jr, 1985). Mongin and Sauveur (1974) reported that consumption of oyster shells sharpedly increased from 1600-2000 h when fed with low calcium diet. The variation in nutrient consumption can be largely explained by the physiological need for specific nutrients depending on the egg formation stage.

Hens ovulate one yolk at a time and each egg passes through the sequential steps of egg formation in the oviduct before it is shelled and laid out. While yolk is synthesized prior to ovulation (Redshaw & Follett, 1972), albumin and shell mineral are synthesized after ovulation. During the first 3-4 hours of ovulation, albumin and shell membrane are synthesized (Roberts, 2004). The main site for the albumin protein synthesis is the magnum (Hiramoto et al., 1990). The next phase is eggshell formation which takes place in the eggshell gland (uterus). This is the longest step in egg formation cycle and lasts for about 20 hours (Keshavarz, 1998b). Most

of the eggshell calcification occurs during dark hour when the hen has stopped eating. Very little (if any) calcium is stored in the oviduct, thus calcium has to be mobilized through the blood (Driggers & Comar, 1949). When the dietary calcium becomes deficient the hen depends on its bones to provide the necessary calcium (Saunders-Blades et al., 2009). If this continues bone quality declines, and dependency on bone mobilization can also be negatively impact eggshell quality (Farmer et al., 1986).

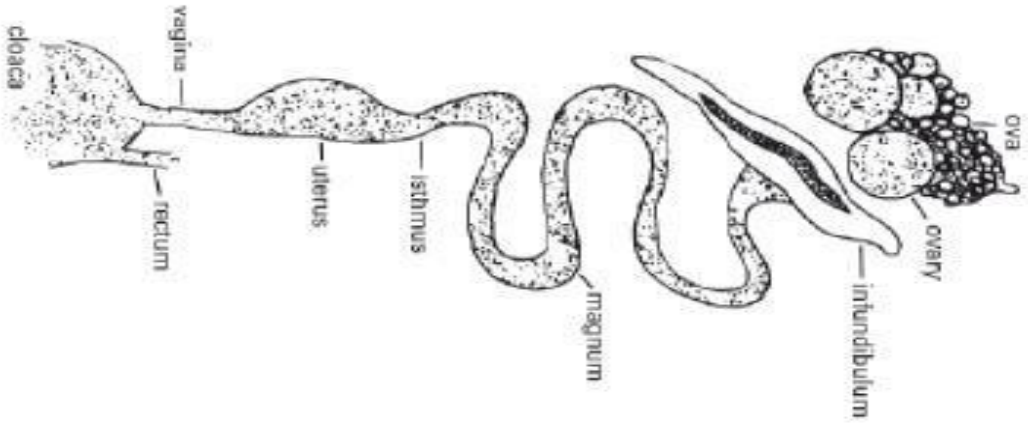
As hens age, eggshell quality generally declines due to decreasing intestinal absorption (Bar & Hurwitz, 1987). Availability of dietary calcium during eggshell formation can be increased by feeding coarse particles of calcium source. Larger particles persist in the gizzard and dissolve slowly serving as calcium reserve for eggshell calcification (Guinotte et al., 1995). Several studies revealed that larger particles improve eggshell quality (Koreleski & Świątkiewicz, 2004; Lichovnikova, 2007; Pavlovski et al., 2003), and bone quality (Oliveira et al., 2013).

Since the choice feeding method was shown to have positive impact on the egg production and eggshell quality (Chah & Moran Jr, 1985; Olver & Malan, 2000), the feeding pattern can be adapted in commercial feeding regime, as a split feeding system, by varying the calcium concentration and particle size in the feed.

The objective of this thesis is to study the effect of afternoon feeding of calcium, in the form of limestone particles, on laying performance, and eggshell and bone quality of old age hens.

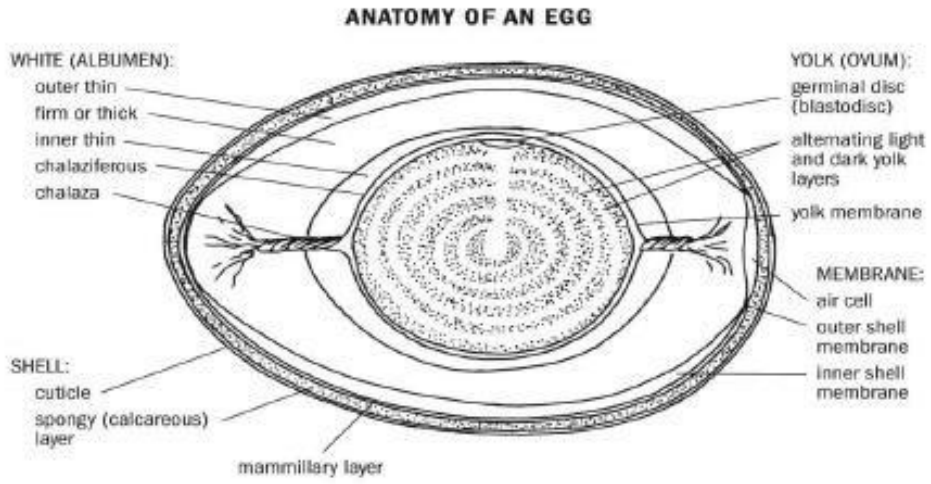
2 Literature Review

2.1 Ovulatory sequence and egg formation



(IDAHOR, 2017)

Figure 1 Reproductive tract of a bird showing ovary, oviduct and uterus



(Roberts, 2004)

Figure 2 Cross sectional structure of egg

The ovary of a hen contains a substantial number of follicles at different developmental stages at any given time (IDAHOR, 2017). The mature follicle containing yolk is released (ovulated) from the ovary to the oviduct and is laid out as a fully formed egg. The eggs are laid in sequence and after continual laying there is a pause of one day in commercial hens before new sequence begins (IDAHOR, 2017). The number of eggs in sequence depends on the clutch size of the

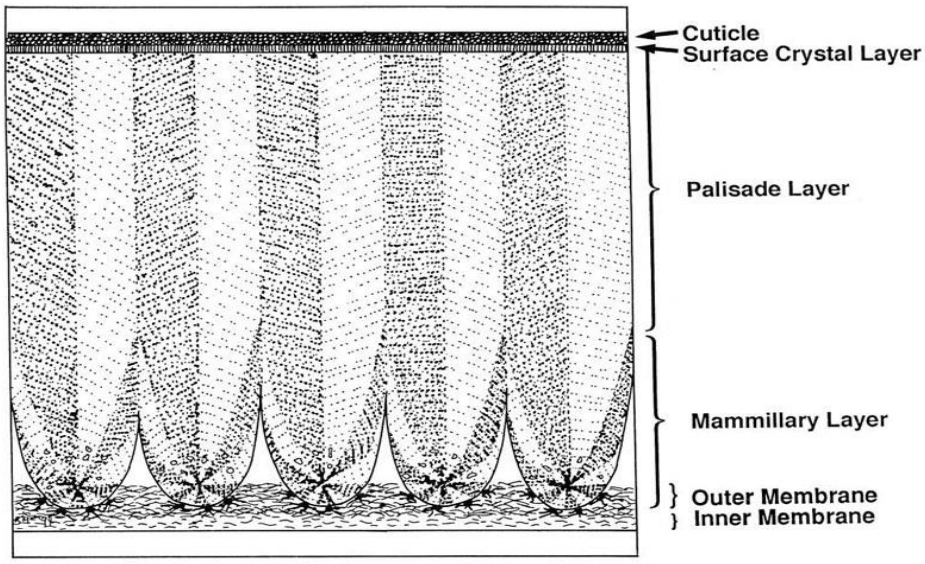
hens. Clutch size of commercial hens can be large (up to 200 eggs), however generally hens in peak production lay 9 eggs in sequence (Etches, 1987). The first egg of the sequence is ovulated from the left ovary to the left oviduct (the right ovary and oviduct are not functional in hens) (Roberts, 2004) within few hours of the morning or after a light is turned on (Lague et al., 1975). This results in oviposition in the next morning. Next ovulation follows oviposition by 1 hour and the subsequent oviposition and ovulations occurs later in the day (Lague et al., 1975). Consequently, the laying cycle tends to be slightly greater than 24 hours and ovulation and oviposition always occur during the daylight.

Egg formation is a complex process in which specific nutrients are accumulated from the specific regions of the oviduct. First the egg is engulfed by the funnel shaped infundibulum where previtilline membrane and chalazae are formed within 15 minutes of residence period (Fig 2). The egg then passes to the magnum where egg albumin is synthesised. Albumin proteins are produced by tubular cells in the magnum and the synthesis is continuous throughout the 4 hours when the developing egg is present (Moran Jr, 1987). It then enters into the isthmus where inner and outer shell membranes are formed. Next, in the distal isthmus (red isthmus), organic aggregates, known as mammillary core, are deposited on the outer surface of the shell at randomly located sites (Hincke et al., 2012). These sites are the also the nucleation sites of calcium carbonate crystals (Hincke et al., 2012).

Shell mineralization takes place 6 hours after ovulation, in the uterus (Roberts, 2004). At this stage, the egg enlarges to its maximum size by the entrance of fluid containing water and salt into the albumin (Taylor, 1970). The shell membrane of swelled albumin is bathed in uterine fluid which is supersaturated with calcium ions (6-10mM) and bicarbonate ions (70mM) (Hincke et al., 2012). On the egg's surface the solution quickly precipitates as calcite, which is the stable polymorph of calcium carbonate (Hincke et al., 2012).

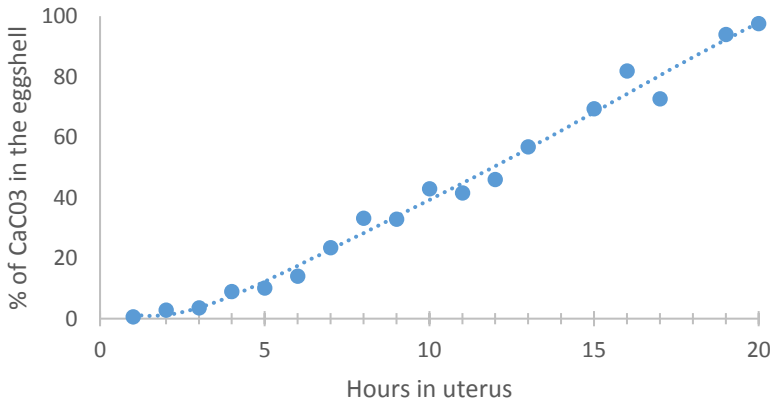
The process of eggshell calcification is the longest step in egg formation and lasts for about 20 hours. It is composed of three stages. The first 3-5 hours is the initiation period which is characterized by slow mineralization. During this period the mammillary core is nucleated with radial growths of micro-crystals of calcite, which gives rise to the cone-like structure known as the mammillary knobe (Hincke et al., 2012; Nys et al., 2004). The mammillary knob as a whole forms the mammillary layer (fig 3). The layer is anchored to the outer shell membrane by the embedding of fibers of outer shell membrane into the knobs (Nys et al., 1999; Nys et al., 2004).

Rapid calcification takes place in the second stage (fig 4) during which a thickly calcified region known as the palisade region is formed. This region is a columnar structure of calcite crystals formed by the outwardly growing crystals competing for space (Nys et al., 2004) and extends from the mammillary knob to the transitional vertical crystal region (Hincke et al., 2012; Nys et al., 1999). The final stage is the termination stage which takes place in the last hour before oviposition (Nys et al., 2004). In the final stage the cuticle is deposited on the top of the shell. Cuticle is an organic material, however the inner layer of the cuticle also contains a thin layer of hydroxyapatite (Dennis et al., 1996).



(Roberts, 2004)

Figure 3 Structure of eggshell



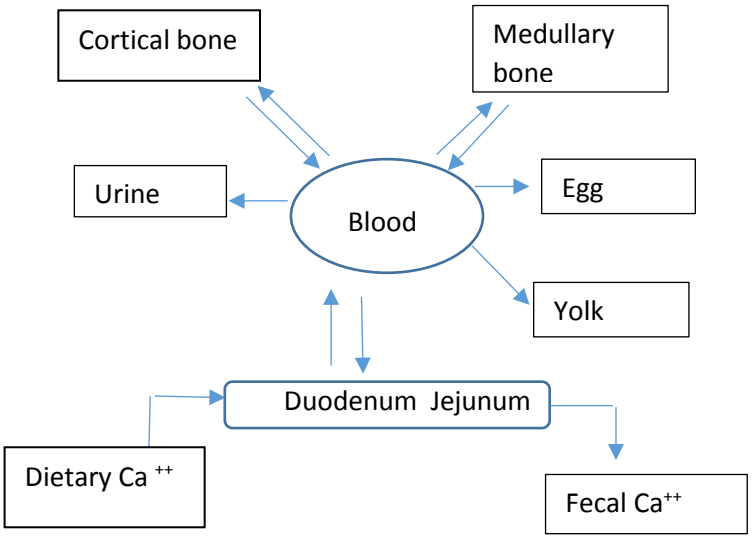
(Burmester, 1940)

Figure 4 Hourly $CaCO_3$ deposition in the shell of the egg in uterus until oviposition

2. Calcium homeostasis in the laying hens

Of total body calcium of mammals, 99% is stored in bones and the remaining is shared by intracellular and extracellular calcium (de Matos, 2008). Extracellular calcium constitute 0.1 % of total calcium and is comprised of ionic calcium and bound calcium. Ionic calcium is the biologically active type of calcium which is used for various physiological functions. In birds, ionic calcium represents only 20-60% of the extracellular calcium (de Matos, 2008). Therefore, calcium homeostasis is maintained by tight regulation of extracellular calcium, particularly ionic calcium.

In non-laying birds, calcium metabolism is similar to other animals in that calcium is excreted in feces and urine. However, movement of huge amount of calcium during egg formation involves various physiological compartments (fig 5). During egg formation, calcium is mostly utilized for shell production and excretion is reduced (Mueller et al., 1964; Taylor & Kirkley, 1967). Intensive shell calcification puts severe pressure on the extracellular pool of calcium. In this process which lasts at least 15 hours, 1.5-2 g of calcium is deposited in eggshell. The average plasma volume and plasma calcium concentration is 100 ml and 25mg/100 ml respectively. Meaning that calcium is drawn from the blood at the rate of 100 mg/hour (Hertelendy & Taylor, 1961) This would reduce plasma Ca^{2+} concentration to zero within 15 minutes (Etches, 1987) unless advanced metabolism does not exist.



(Etches, 1987)

Figure 5 Movement of calcium into the different physiological compartments

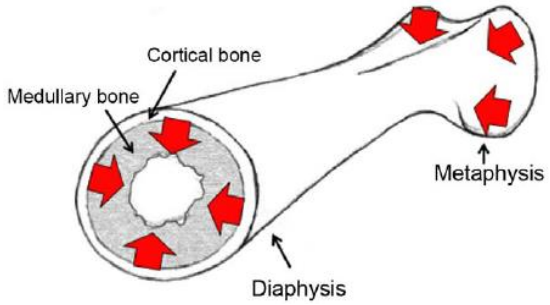
2.2.1 Role of intestinal absorption in maintaining calcium homeostasis

One of the consequences of the discrepancy in the timing of physiological needs and availability of calcium for eggshell calcification is increased intestinal absorption. Calcium is absorbed in the duodenum and upper jejunum (Hurwitz & Bar, 1965; Hurwitz et al., 1973). Intestinal absorption is greatly increased during the period of eggshell calcification compared to when the eggshell gland is empty. Hurwitz and Bar (1969) reported that 70% absorption of dietary calcium intake is during eggshell calcification but only 40% absorption when the shell is not being formed. However, it is argued that despite the increase in intestinal absorption, this cannot support shell calcification process completely. This is indicated by the fact that calcification takes place for long hours during the night while intestinal lumen was found to be empty of calcium 4-5 hours after the hen consumes the evening feed (Bar, 2009). Therefore, it is suggested that there is an alternative source of calcium, which is considered to be bone.

2.2.2 Role of bone in maintaining calcium homeostasis

2.2.2.1 Bone types in laying hens

Bone formation in chicken is outlined in detail by (Whitehead, 2004). Structural bone of laying pullets fully develops during the rearing period. The bone elongates by the process of mineralization of a cartilaginous precursor known as endochondral ossification. Mineralization occurs when osteoblasts (bone forming cells) form bone matrix and raises CO^{2+} and PO_4^{3-} concentrations. Osteoclasts (bone destroying cells) couple with osteoblasts in the remodeling process of bone ossification. As the bone widens, due to secretion of concentric layer of mineral, cortical bone is formed (fig 6). When the hen becomes sexually mature and laying is imminent, the development of structural bone is stopped, and instead a spongy type of bone, medullary bone, is formed and laid under structural surface of legs and wings bones namely, tibia, humerus and femur (Hurwitz, 1965).



(Johnsson et al., 2012).

Figure 6 Structure of long bone showing Cortical bone and Medullary bone

2.2.2.2 Medullary bone as a source of calcium

Rapid eggshell calcification requires a labile source of calcium. Several studies confirm that the medullary bone has a high turn over rate (Hurwitz, 1965; Kerschnitzki et al., 2014) According to (Hurwitz, 1965), the turn over rate of medullary bone is at minimum 10-15 times the cortical bone. In the medullary bone, osteoblastic and osteoclastic activities are regularly present during the laying period. However, as the shell calcification process is underway osteoclastic activities predominate and hydroxyapatite structure is dissociated to release Ca^{2+} and PO_4^-. Ca^{2+} is transported to the eggshell gland and the excess phosphorus is excreted (Kerschnitzki et al., 2014). When the egg shell gland is inactive, i.e. before the egg reaches the eggshell gland, osteoblast predominates which accrue dietary Ca^{2+} and it regains its structure. Thus the rapid turnover rate makes the medullary bone a labile source of calcium (Hurwitz, 1965; Kerschnitzki et al., 2014) As much as 25-40% of the eggshell calcium is contributed by medullary bone (Driggers & Comar, 1949).

2.3 Dietary calcium requirement of laying hens

Growing pullets require calcium for normal growth and bone mineralization. Excessive calcium at this stage is also detrimental as high calcium and low phosphorus can cause kidney lesions (Wideman Jr et al., 1985). Birds' need for calcium increases when new medullary bone has to be formed (Bar A., 2009), therefore increasing dietary calcium level 7 to 10 days prior to when the first egg is laid is beneficial (Roland, 1994) cited in (Roberts, 2004). An egg-laying hen has a remarkably high demand for calcium for eggshell calcification while the phosphorus requirement remains low (Bar et al., 2002) as the eggshell contain little phosphorus (Ahmad & Balander, 2004). The recommended calcium level for laying hens is 3.6-4% (Bar, 2009).

2.4 Calcium deficiency disorder

Serum calcium deficiency or hypocalcemia in birds occurs due to dietary deficiency of calcium, deficiency of Vitamin D_3, and imbalanced Ca:P (de Matos, 2008; Rath et al., 2000). Phosphorus interacts with calcium, and a high level of phosphorus decreases intestinal calcium absorption and bioavailability. Vitamin D_3 plays an important role in calcium homeostasis and is essential to maintaining serum calcium. Various metabolic bone diseases arise in chickens due to hypocalcemia.

According to de Matos (2008), growing pullets suffer stunted growth and bowed legs due to insufficient bone mineralization, which is also known as rickets. Adult hens respond to short term calcium deficiency by producing defected eggs such as thin shelled eggs and shell-less eggs, and laying stops if the deficiency continues. Extended deficiency results bone fracture and deformities, known as osteomalacia. Osteoporosis occurs due to resorption of bone matrix as the bird ages. Birds also show poor feather condition and feather peaking behavior due to calcium deficiency. To prevent the calcium-related disorders and diseases, the diet of the bird should be adjusted to the level recommended according to the strain and age (de Matos, 2008).

2.5 Effect of Particle size on eggshell

In general, the effect of particle size is as follows,

(Rao & Roland Sr, 1990; Zhang & Coon, 1997).

According to (Zhang & Coon, 1997) larger particles are retained for longer period in the gizzard. Particle size should be greater than 0.8 mm if it is to be retained by the gizzard. Due to increased retention, calcium from the gizzard is released at a slow rate and therefore absorbed completely. Small particles, on the other hand, quickly release calcium ions into the digestive tract and thus may exceed intestinal absorption capacity, causing calcium to be excreted (Zhang & Coon, 1997).

Due to longer retention time of limestone it is able to provide calcium during long hours of dark period, for shell calcification, thus reducing bone mobilization. Nys et al. (1999) suggested particle size should be 1-4 mm to have positive effect on eggshell quality. Several studies revealed that a mixture of fine and coarse limestone gives better eggshell quality than only fine

limestone as calcium source (Guinotte et al., 1995; Koreleski & Świątkiewicz, 2004; Pavlovski et al., 2003) Lichovnikova (2007) and Scheideler (2004) respectively recommended 2/3rd and 1/3rd of the mixture should be large particles for better eggshell quality. However, Cordeiro et al. (2017) and De Witt et al. (2009) observed no influence of large particles on eggshell quality. Cufadar et al. (2011) reported large particles have a positive influence on eggshell quality only when dietary calcium is low.

2.6 Studies on the effect of calcium split feeding

Mozos et al. (2012) reported hens that received low protein and high calcium 8 and 10 hours post-oviposition consumed relatively less calcium, protein, and energy without negatively effecting eggshell quality, in comparison to the control group that received the same diet throughout the day. Similarly, in another study on brown hens of 57-65 weeks, decreasing calcium concentration in the morning and increasing calcium in the evening feed by 45% and 15% did not reduce eggshell quality (De los Mozos et al., 2014)

Keshavarz (1998a) observed no difference in laying performance and eggshell quality by supplementing 2.5% Ca in the morning and 4.5% Ca in the afternoon as pulverized limestone compared to a control feeding regimen with 3.5% calcium each time. However, the author reported that high calcium in the morning and low calcium in the evening reduced the eggshell quality. In contrast, in a 10 week trial on 41-week- old brown hens, Lin et al. (2018) found that feeding high calcium (3.6%) in the morning and low calcium (3.2%) in the afternoon significantly improved FCR of the hen. However, eggshell quality was not determined in the study.

In a recent study, Molnár et al. (2017) studied the effect of split feeding on non-molted old age brown hens (72 to 83 weeks) by supplementing different ratios of coarse and fine limestone in the afternoon diet of the test group and compared them to a conventionally fed control group. They found split feeding, in which all the calcium was supplied in afternoon diet was, inferior to conventional feeding in terms of bird performance and egg quality. Furthermore, supplementing 100 % coarse particles in the afternoon (average particle size 2.05 mm) had an adverse effect. In another study, Molnár et al. (2018) reported supplementing 50% fine limestone in the morning and 50% coarse limestone in the afternoon improves the eggshell quality of eggs form old aged brown hens.

Waldroup and Hellwig (2000), concluded that offering morning and evening diets differing in calcium have no benefit in comparison to conventional feeding, where calcium is supplied in the same proportion throughout the feeding regimen.

3. Materials and Methods

The experiment was conducted by Fiskå Mølle in a commercial farm in Stavanger, Norway. The experiment was carried out from November 1, 2016 to January 17, 2018.

3.1 Hens housing

A total of 7700 Hendrix ISA Dekalb 15-week-old white pullets of 15 weeks of age were divided into two groups containing an equal number of birds (3850 of each) and were assigned to two equal sections of the farm separated by the wire fence in the middle. Birds were raised in multi-tier barn under identical conditions. Both sides were facilitated with three tier iron perches, along with private nests for the birds. Each tier had one feeding belt running through it. Feed and water were provided ad libitum in automatic feeding belts and nipple and cup drinker respectively. A photoperiod of 15 hours was maintained using artificial light. Lights were turned on at 03:00 and turned off at 18:00.

3.2 Experimental procedure

Experimental treatment commenced once the birds were at peak lay, which was at 25 weeks, and the treatment groups consisted of one 'Control' and one 'Test' group. The laying percentage of Control group and Test group were 97% and 96% respectively at the beginning of experiment. Until 25 weeks of age both groups received identical diets: pre-lay diet from 15 to 18 weeks and light layer diet from 18 to 24 weeks, after which experimental diets were fed.

3.3 Diet

The feeding regime was set to feed four times a day at 9:00, 11:00, 14:00, and 17:00, and the birds were maintained with pelleted feed. The feeding course included 4 phases with changes in diets at 49, 58 and 63 weeks. Iso-caloric and iso-porteic diets based on wheat, soyabean meal, and corn gluten were formulated following the nutrient recommendation from the dekalb manual (ISA, 2009), differing only in the calcium content. The nutrient composition of the diets after analysis is given in Table 1. The control group received a standard diet containing coarse and fine limestone at a ratio of 2:1 (percentage inclusion 6% and 3%) throughout the feeding

regime while calcium content in the diet of the Test group was reduced to 1.4% with coarse and fine limestone at 1:1 (Table 2). Calcium content of both the coarse and fine limestone was 37.6%. Particle size distribution of the limestone is given in Table 1. The particle size of the limestone potentially reduces after pelleting, however, the particle size after pelleting was not analyzed.

In this study the Test group received the remaining calcium as separate limestone particles, the same coarse limestone that was included in the feed, supplemented at 14:00 and 17:00 feeding schedule (Table 3). To achieve this a small silo was installed into the hen house and was connected to the main feeding silo where limestone particles were mixed with feed before feeding to the feeding belt. Each time 27.7 kg of limestone was mixed with 140-160 kg feed which was estimated to supply 7.28 g of limestone per hen. It was calculated that this would supply 4.2% available calcium in total (including 1.4 % from the feed) to the birds. A slight oversupply was estimated to overcome potential technical loss.

Limestone particles with a particle size of 3-6 mm were provided to both groups in a feed box placed at the front of the rearing area on both sides. Approximately, 7.5 kg of this limestone was supplied each day. However, this was unintentional and was not included originally in experimental design.

Table 1 Analyzed nutrient composition of the diets

Analysis	25-48 weeks			49-57 weeks	
	Control	Test		Control	Test
NE, KJ/Kg	897.39	886.46	NE, KJ/Kg	895.74	887.73
Crude protein %	17.11	17.24	Crude Protein %	16.97	17.04
Lysine %	0.84	0.84	Lysine %	0.84	0.83
Methionine %	0.41	0.41	Methionine %	0.42	0.41
MET+CYS %	0.76	0.77	MET+CYS %	0.77	0.77
Calcium %	3.90	1.40	Calcium %	3.70	1.40
Phosphorus %	0.54	0.55	Phosphorus %	0.54	0.56
Ca:P	6.83	2.54	Ca:P	6.88	2.52
Analysis	58-62 weeks			63-77 weeks	
	Control	Test		Control	Test
NE, KJ/Kg	896.38	885.96	NE,KJ/Kg	891.33	879.10
Crude Protein %	16.98	17.49	Crude Protein %	16.10	16.75
Lysine %	0.84	0.83	Lysine %	0.78	0.88
Methionine %	0.42	0.41	Methionine %	0.39	0.39
MET+CYS %	0.77	0.78	MET+CYS %	0.71	0.72
Calcium %	4.00	1.40	Calcium %	3.80	1.40
Phosphorus %	0.51	0.56	Phosphorus %	0.41	0.45
Ca:P	7.87	2.49	Ca:P	9.36	3.19

New feed was introduced in 49, 58, and 63 weeks.

NE = Net energy

Table 2 Particle size distribution of the the limestone types

Sieve size (mm)	Fine limestone	Coarse limestone
0 - 0.5	73.80%	1.24%
0.5 - 1.0	25.69%	2.94%
1.0 - 1.5	0.18%	27.72%
1.5 - 2.0	0.10%	27.75%
2.0 - 2.5	0.12%	33.78%
2.5 - 3.0	0.06%	6.50%
3.0 - 3.5	0.05%	0.07%

Coarse limestone = limestone used in feed as well as separately fed to Test group.

Table 3 Experimental Treatments

	Control			Test			
Feeding regime	Calcium in feed	CL: FL in feed	Seperate Limestone/quantity	Calcium in feed	CL: FL in feed	Seperate limestone/ quantity	
9:00	3.9%	2:1	-	1.6%	1:1	-	
11:00	3.9%	2:1	-	1.6%	1:1	-	
14:00	3.9%	2:1	-	1.6%	1:1	x	
17:00	3.9%	2:1	-	1.6%	1:1	x	Total 27.7 kg

CL = Coarse Limestone (same limestone was used in feed and given separately to the Test group), FL = Fine Limestone, x = Separate limestone feeding included; total 27.7 kg was mixed including 1400 and 1700 feeding which was estimated to supply 7.28 gram of limestone per hen and 4.2 % calcium/hen.

3.4 Variables measured

3.4.1 Laying performance

Feed and water consumption, number of eggs, dirty eggs, cracked eggs, eggs weight and weight of the bird were recorded daily. Everyday 40-60 birds were weighed from each group and weight of an individual bird was estimated to be the average weight of the birds. Dead birds were removed and mortality was recorded each day. Dirty and cracked eggs were counted manually by visual inspection and their respective percentage was calculated as the percentage of the total eggs. In addition, reports of cracked eggs from the meat and egg processing company 'Nortura' were received every week. Number of eggs, weight of individual eggs, laying percent, egg mass, feed conversion per egg mass and mortality percent were calculated as follows:

Number of eggs= Number of crates × eggs in each crate

$$\text{Individual egg weight} = \frac{(\text{Weight of crates + eggs}) - \text{Weight of crates, (g)}}{\text{Total number of eggs}}$$

$$\text{Laying percent} = \frac{\text{number of eggs}}{\text{live birds}} * 100\%$$

$$\text{Egg mass} = \frac{\text{Total egg weight , (g/bird/day)}}{\text{Number of live birds}}$$

$$\text{Feed conversion per egg mass} = \frac{\text{Feed intake by individual bird}}{\text{Egg mass}}$$

$$\text{Mortality percent} = \frac{\text{total number of bird that died from the start (15 week)} * 100\%}{\text{Number of live birds}}$$

3.4.2 Eggshell quality

Egg quality of old age hens was determined by randomly selecting 30 eggs per group every 4 weeks starting from 68 weeks. In total three sampling was done three times and 180 eggs were collected from each group during this period. Eggs were placed in paper crates and packed in a cardboard box and posted to the Norwegian university of Life Sciences for analysis. Thirty eggs per treatment were considered as replicates. However, some of the eggs could not be used for analysis after they were found broken or with visual cracks.

Quality evaluation criteria included egg weight, egg length, egg diameter, eggshell breaking strength, eggshell thickness and eggshell weight. Eggs were stored at 4^0C for 7 days before analysis. An Electronic Digital Caliper with an accuracy of 0.01 milimeters was used to measure the length and breadth of the egg (fig 7). Length of the egg was determined by measuring end to end of the egg and breadth of the egg was determined by measuring at the broadest section. Egg weight was determined using a digital scale (AG204 DeltaRange) (fig 8) to the nearest 0.0001g. The breaking strength of the eggshells was measured using a Tinius Olsen Texture Analyzer HK5T with load cell of 250 N (fig 8). The eggs were compressed between two stainless steel plates and the breaking force in Newton was recorded at the cracking point.

Eggshell thickness was measured after the eggs were cracked for breaking strength. The eggs content was removed and the eggshell (with membrane intact) was rinsed with water. Shell thickness was determined as an average of six readings taken at three points (two readings each at the middle, narrow end and broad end) using a Digital micrometer MS25LP with an accuracy of 0.001 millimeters (fig 7). All the pieces of eggshell belonging to each individual egg were collected and dried at 105^0C overnight and eggshell weight was measured after cooling in a silica desiccator for 30 minutes, using a digital scale (AG204 DeltaRange) to the nearest 0.0001 g. Eggshell percent was calculated using (eggshell weight/egg weight) x 100%.

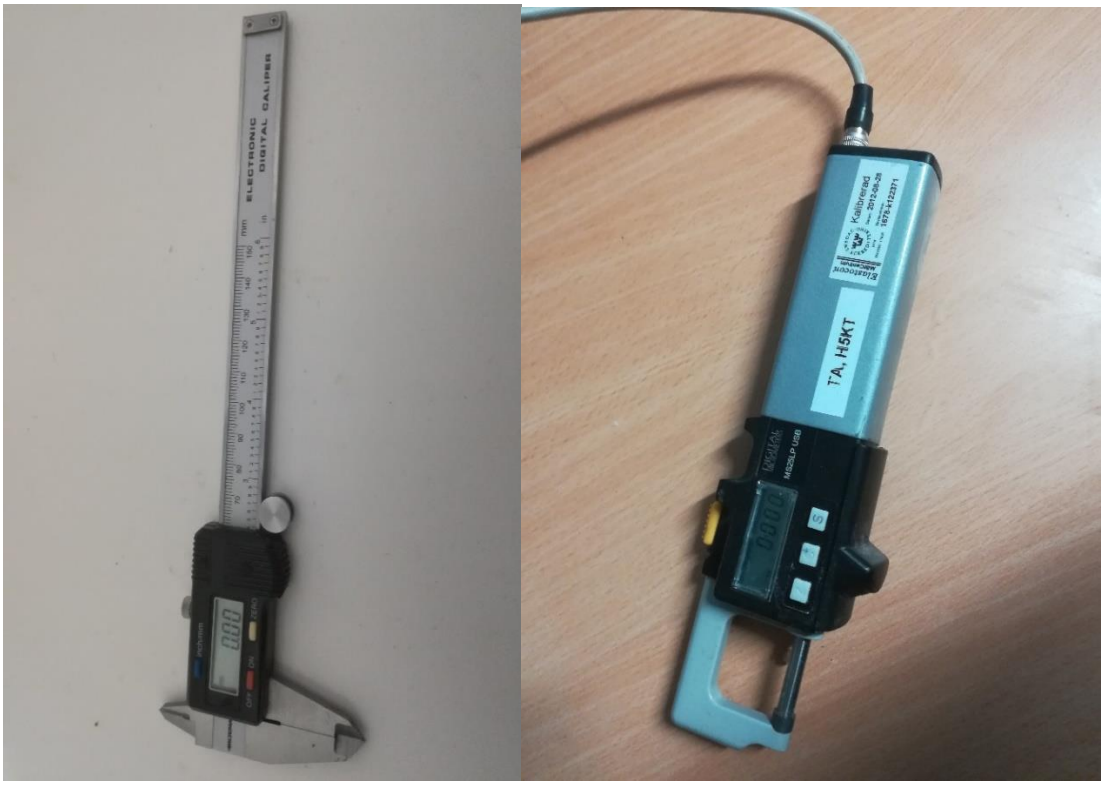


Figure 7 Measuring instruments: left, Electronic caliper used for egg length and breadth; right, digital micrometer used for eggshell thickness

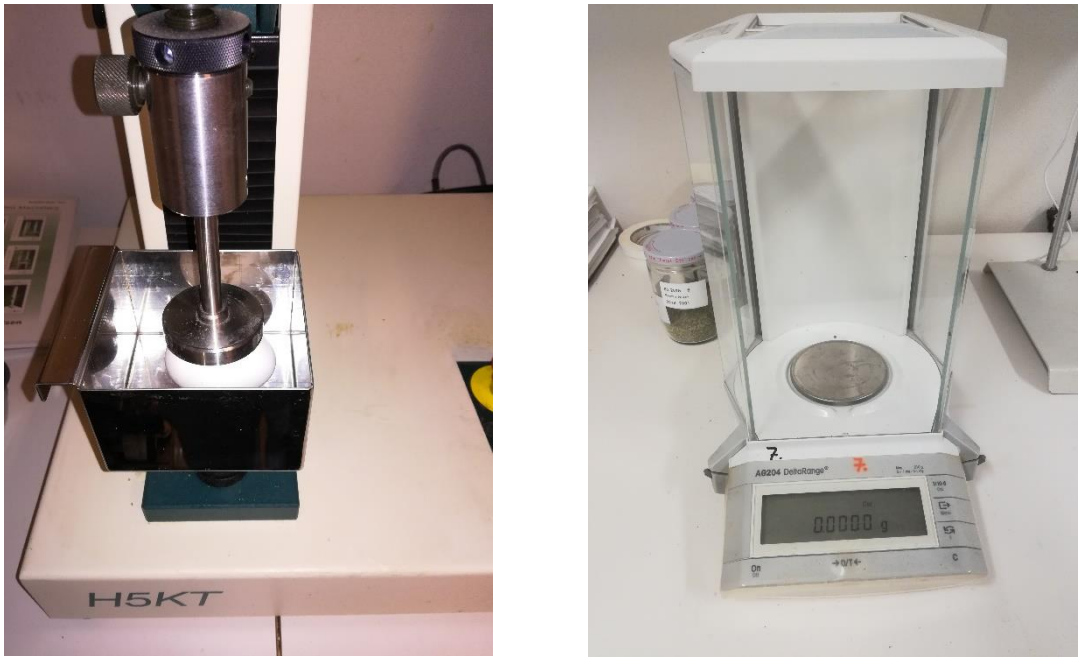


Figure 8 Measuring instruments: left, Texture analyser used for breaking strength; right, digital scale for egg weight and shell weight.

3.4.3 Bone quality

At the end of the experiment, the hens (now 77 weeks of age) were sacrificed by CO_2 asphyxiation and 30 hens from each group were selected to collect tibia for bone quality analysis. Each hen was considered as a replicate. However, 2 bones from the test group were erroneously placed in the control group, thus in total 32 bones from the control group and 28 bones from the test group were analyzed. The whole right leg of hens was separated, footpad clipped, and defleshed after boiling in water for 10 minutes as described by (Cordeiro et al., 2017). The tibias were separated and the adhered muscles were removed using hand, knife and scissors, along with the knee cap and fibula (fig 9). Samples were packed in ice and sent to the Norwegian University of Life Sciences. They were stored at -20^{0}C in plastic bags until analysis.

Bones were dried at 104^{0}C for 24 hour. The length of the bone was measured from end to end using the Electronic Digital Caliper. The diameter of the bone was measured by rotating the bone to measure the largest and smallest diameter at the middle of the shaft. Diameter of the bone was defined as the average of the two measurements. After taking length and breadth measurements the bones were crushed into small pieces using a clipper and a mortar and pestle. Samples were ashed at 550^{0}C for 15 hours. Ash content was determined by weighing on the digital scale after cooling in a silica desiccator for 1 hour. Bone mineral density as Seedor index was calculated by dividing the weight of the bone by the length of the bone (Seedor et al., 2005). Ash content per dry matter of the bone was calculated as the ratio of the ash divided by the dry bone weight in kg.

Figure 9 Bones:Left, Cleaned bone ready for drying; Right, Crushed bone ready for ashing

3.5 Statistical Analysis

Statistical analysis was done in R 3.4.3. A two factor interaction model was fitted with week and Treatment to test the eggshell quality. An ANOVA analysis was done for testing main and interaction effect of these factors. Further, to test the pairwise between week and Treatment, TUKEY test was used. Bone quality was analyzed using a T-test. As there was no replicates significance tests were not run for laying performance.

4. Results

4.1 Laying Performance

Laying percent was at peak, above 96%, until 45 weeks, after which there was a steady decline. After 60 weeks (old hens), the average laying percent in the Test group was 2% lower (fig: 10, top). Mortality percent in each group was similar. By the end of the experiment, 2% of birds died in each group (fig: 10, bottom).

The percentage of cracked eggs in the farm was less than 1%. In the packaging industry, on average, there were slightly more than 2% eggs cracked in each group. Percentage of cracking increased accordingly with age. Old hens of the Test group had almost 1% more cracked eggs than the Control group at the time after packaging. In week 48, there was a sudden increase in cracking, the reason of which is unknown. About 2% and 5% in each group cracked in the farm and the packaging industry respectively (fig 11, top and bottom).

Egg weight increased from 55 g from the beginning of the experiment to 65 g until the end. After 35 weeks, the weight of the eggs was already above 60 g (fig 12, bottom). On average, the test group eggs were 0.6 g heavier than the Control group. Average egg mass for both groups was about 59 g, however, in the old hens, the Test group average was about 0.7 g lower (fig 11, top).

Average feed intake for the Control group was 118 g; for the Test group it was 2% higher (fig 13, bottom). FCR per egg mass for both groups was similar at 2. Old hens had a high FCR (fig 13, top). At the beginning of the experiment, at 25 weeks, the weight of the hens in both the groups was about 1725 grams. Body weight increased until 52 weeks, after which it was fairly stable (fig 14). The weight of the Control group at 67 and 68 weeks and the Test group at 71

and 72 weeks was the lowest at 1500 and 1400 g respectively. This may be due to variation in individual bird. By the end of the experiment, the weights were 1800 g for the Test group and 1900 g for the Control group.

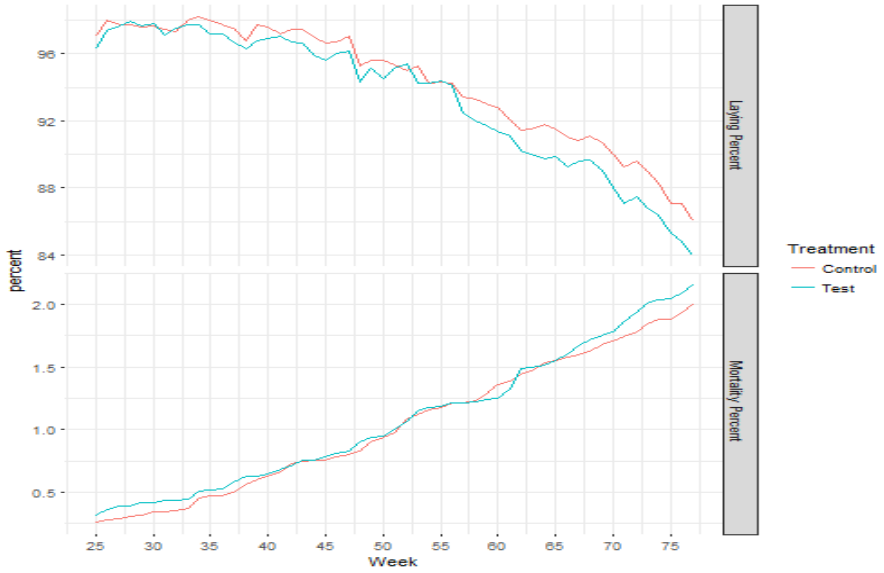


Figure 10 Effect of split feeding of calcium on laying and mortality percent. Top - Laying percent. Bottom- Mortality percent.

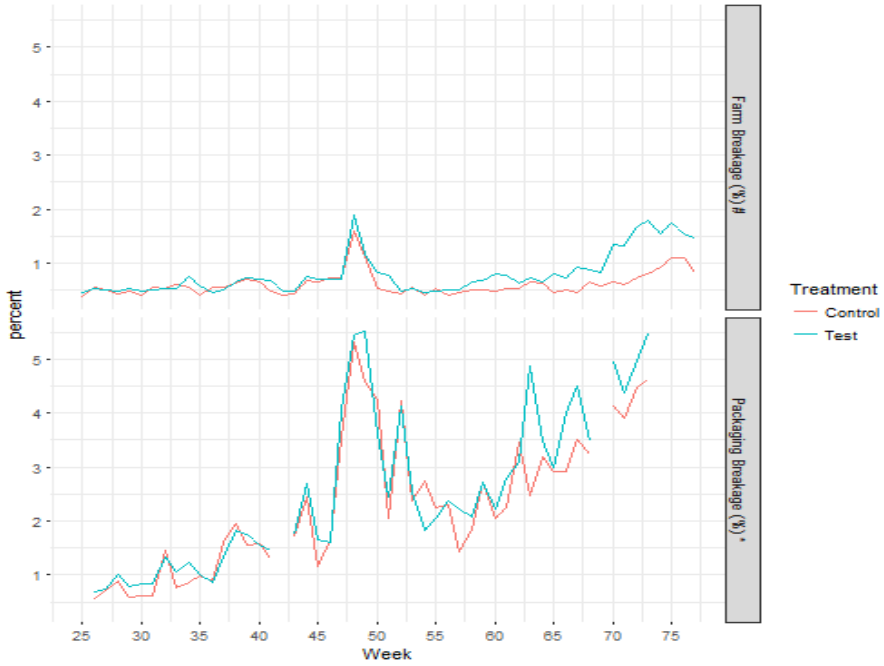


Figure 11 Effect of split feeding of calcium in eggs cracking %.(Top)- Percentage of cracked eggs in the farm. (Bottom)- Percentage of cracked eggs at the eggs packaging house "Nortura"; figure is based on the data send by the company every week.

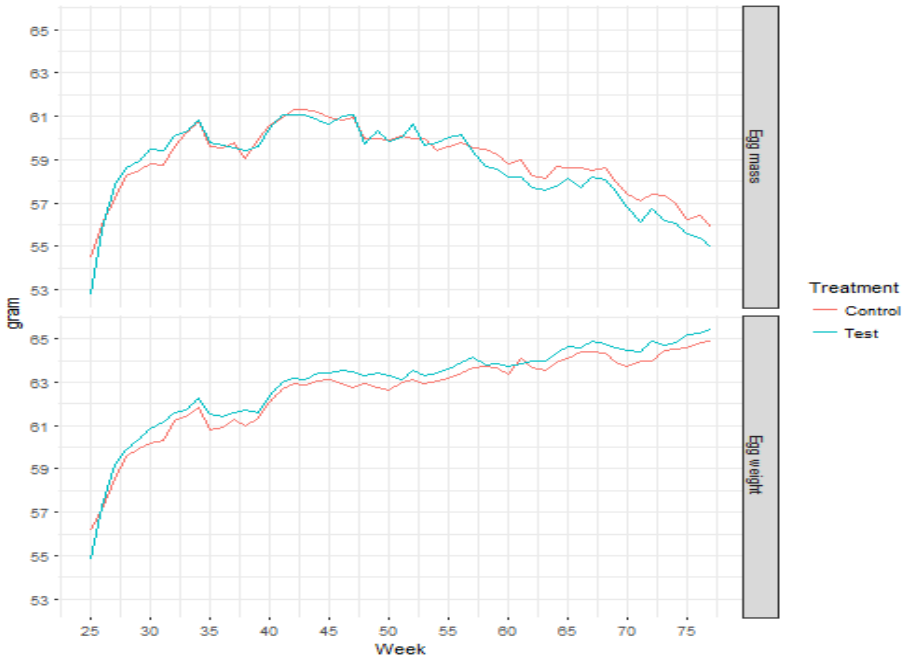


Figure 12 Effect of split feeding of calcium on egg weight and egg mass. (Top)-Egg mass. Calculated as total egg weight per day divided by total live birds (g/bird/day). (Bottom) - Egg weight

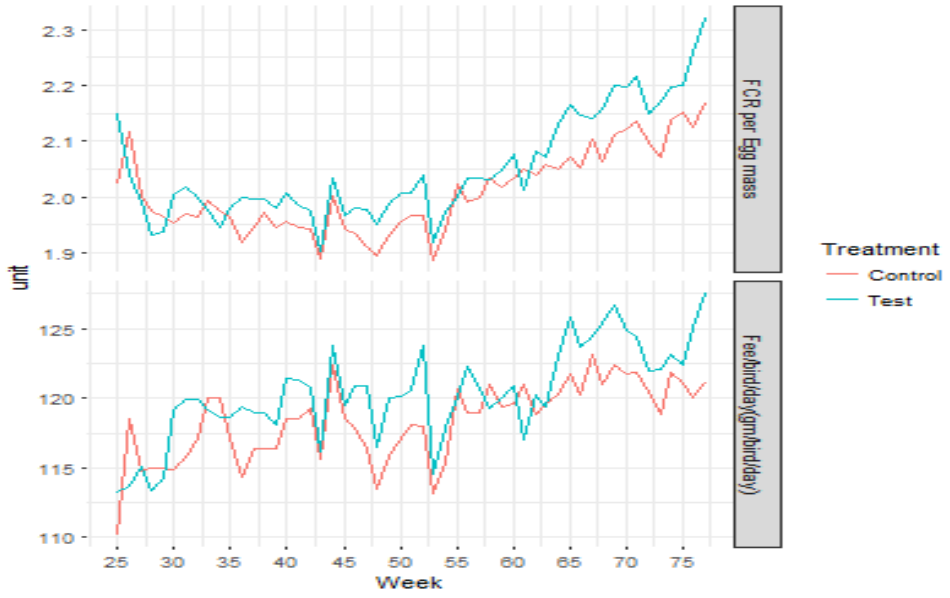


Figure 13: Effect of split feeding of calcium on feed intake and FCR per egg mass. (Top)- Feed Conversion Ratio per egg mass. Calculated as daily feed intake divided by egg mass. Egg mass was calculated as total egg weight per day divided by total live birds. (Bottom)- feed intake (gram per bird per day).

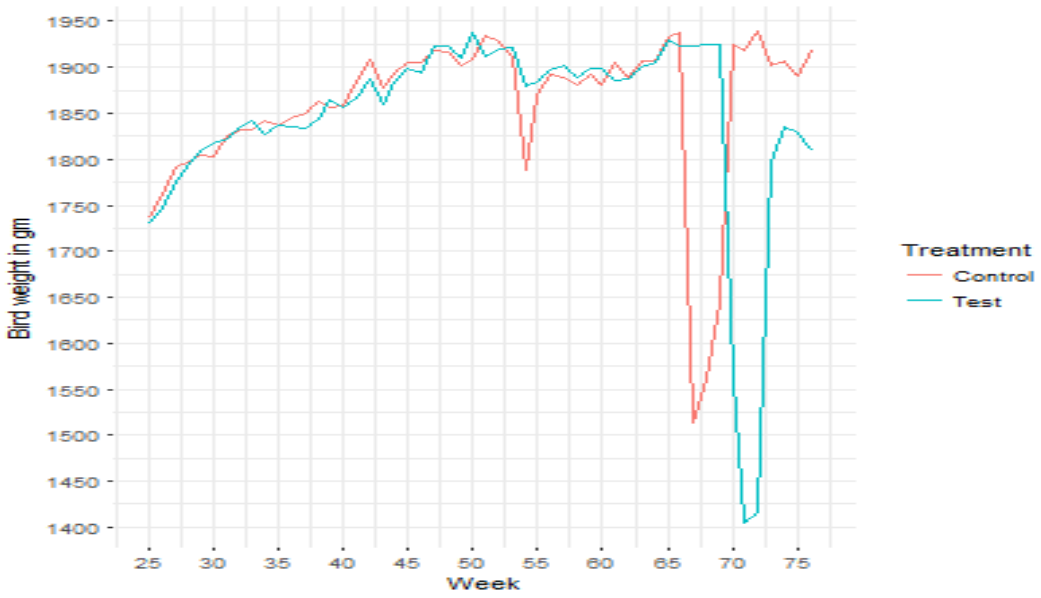


Figure 14 Effect of split feeding of calcium on the body weight.

4.2 Egg Quality

4.2.1 Egg quality

The results of the egg and bone quality parameters are presented in the boxplots. The box represents the middle 50% of the sample. The line inside the box represents the median. The red 'dot' inside the box shows the mean. The upper and lower whiskers represents the upper and lower 25 % of the sample. Symmetrical data would be represented by the presence of median at the middle of the box with the whisker length almost equal to the length of the box. The long length of the box represents the spread of the data. Unequal whisker lengths hints at skewness in the data. Values not within the range of the whiskers represent extreme values.

4.2.2 Egg length

Average egg lengths of the Control (C) and Test (T) groups were 58.2 mm and 58.6 mm (Table 4). Length of the eggs was less variable within the Treament group and the week as is revealed by the boxplot (fig 15). Egg length was not significantly affected by Treatment, week or their interaction. However, there was increasing tendency of the effect of the Treatment ($P = 0.09$) and week as represented by ($P = 0.09$) as represented by $P< 10$.

Table 2 Effect of split feeding of calcium on the egg quality.

Parameters	Treatment	Mean	SEM	P (<0.05)
Length	Control	58.16 mm	0.19	NS
	Test	58.64 mm	0.21	NS
Breadth	Control	44.34 mm	0.12	NS
	Test	44.43 mm	0.11	NS
Weight	Control	63.08 g	0.42	a
	Test	64.29 g	0.37	b
Breaking Strength	Control	32.04 N	1.03	NS
	Test	32.2 N	0.01	NS
Egg Shell Thickness	Control	0.37 mm	0.003	NS
	Test	0.36 mm	0.003	NS
Egg Shell Weight	Control	6.06 g	0.05	NS
	Test	6.10 g	0.05	NS
Egg Shell Percent	Control	9.62 %	0.07	NS
	Test	9.48 %	0.06	NS

a,b = column with different subscripts are significantly different. NS = Not significant.

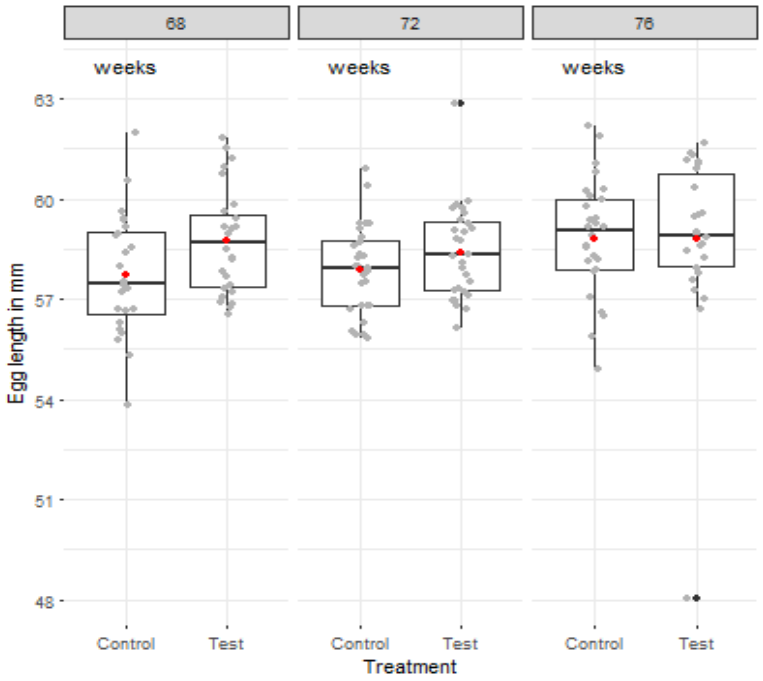


Figure 15 Effect of split feeding of calcium on the length of the egg.

4.2.3 Egg breadth

Breadth of the egg was not affected by the treatment and the week (fig 16). There was an effect of interaction of week and treatment on the egg breadth but not significant (P = 0.06).

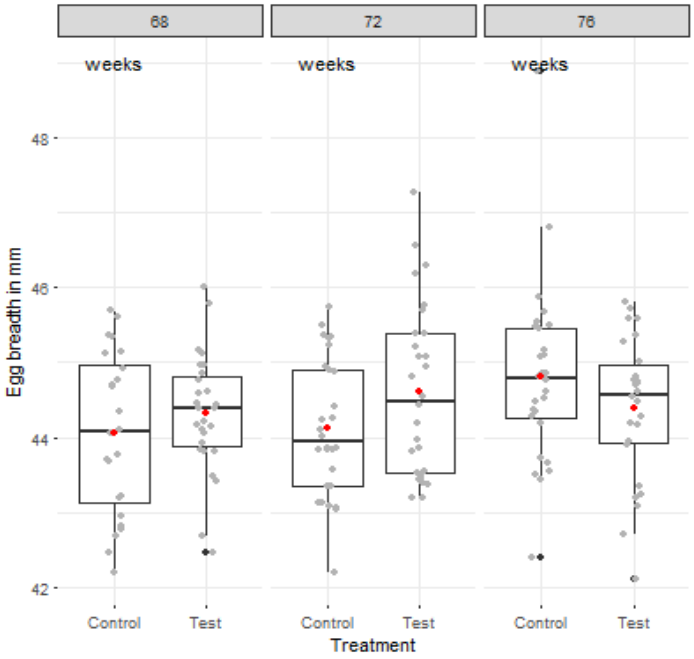


Figure 16 Effect of split feeding of calcium on the breath of the egg

4.2.4 Egg weight

The mean egg weight of C and T was 63 gram and 64 gram respectively . There were large variations in the egg weight. Treatment had a significant effect (P<0.05) on the egg weight. Week had a significant effect (P<0.01) on the egg weight and the mean egg weight increased in subsequent weeks (fig 17). Control group at week 76 (C76) was significantly different (p<0.05) than C68 and C72. There was no difference in Test groups between different weeks. Within the same week there was no effect of Treatment.

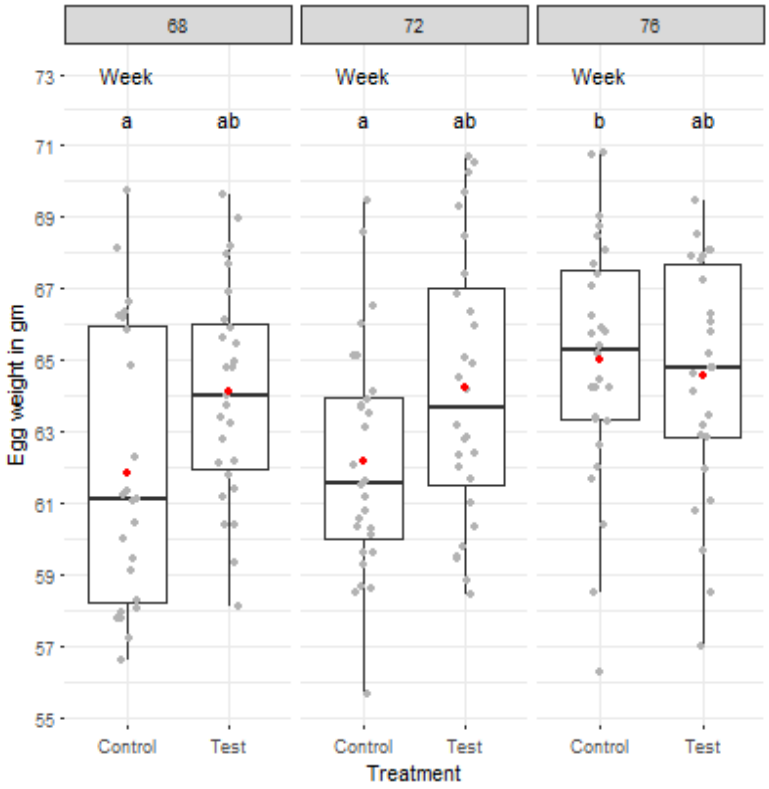


Figure 17 Effect of split feeding of calcium on the weight of the egg

4.2.5 Eggshell Breaking Strength

Eggshell breaking strength declined with age. However, this was not significant, except for the point C72 where mean eggshell breaking strength significantly declined ($p<0.05$ compared to week 68 (fig 18). The breaking strength of T72 was higher than T68 but this effect was not significant ($P = 0.08$).

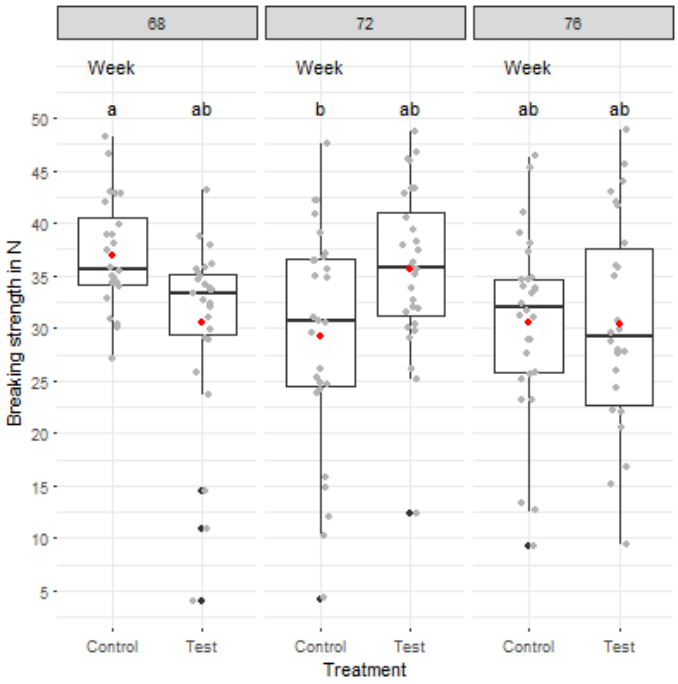


Figure 18 Effect of split feeding of calcium on the breaking strength of the eggshell

4.2.6 Eggshell Weight

Eggshell weight was not affected by age, Treatment or their interaction. Mean eggshell weight for both groups was 6.1 g of the entire sample (fig 19).

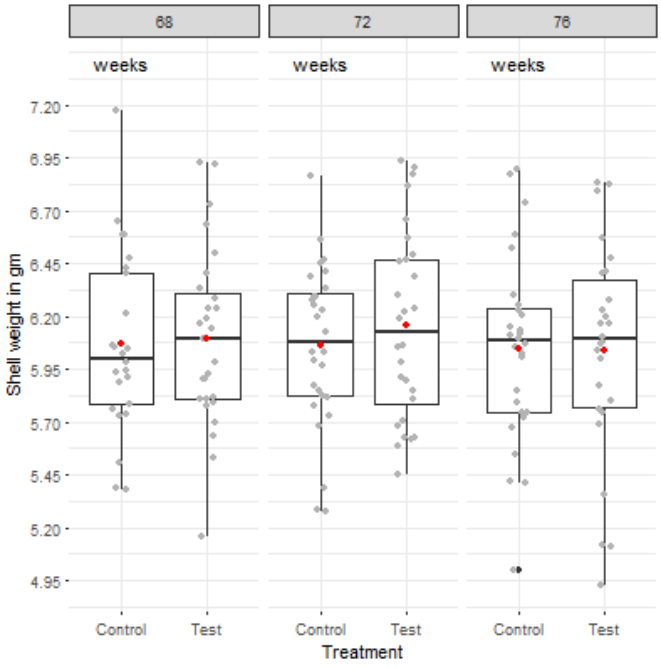


Figure 19 Effect of split feeding calcium on the eggshell weight.

4.2.7 Eggshell Thickness

Eggshell thickness was significantly affected by the age (P<0.001). Eggshell thickness at 72 and 76 weeks was significantly lower (P<0.001) than week 68 (fig 20). C72 and C76 were significantly lower (P<0.01) than C68. T76 was also significantly lower (P<0.01) than T68. Within the same week, C68 was signifcantly higher (P<0.01) than C68.

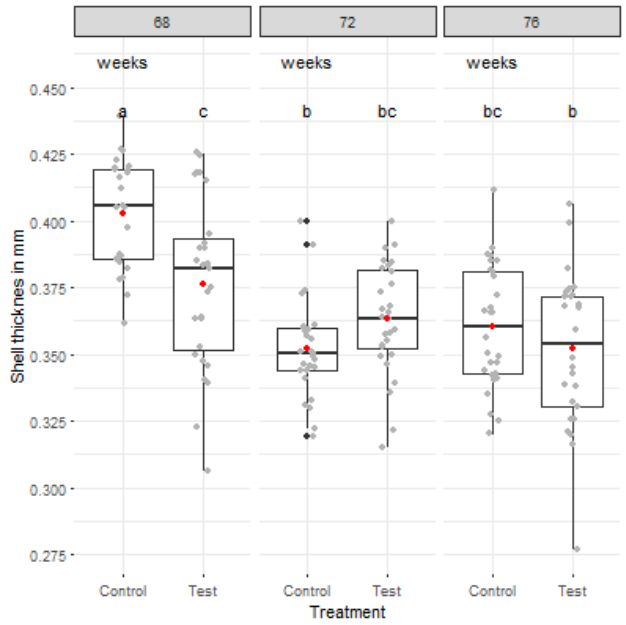


Figure 20 Effect of split feeding of calcium on eggshell thickness

4.2.8 Eggshell percent

Week had a significant effect on eggshell percent. C76 and T76 had significantly lower (P<0.01) eggshell percentages compared to C68 and C72 (fig 21).

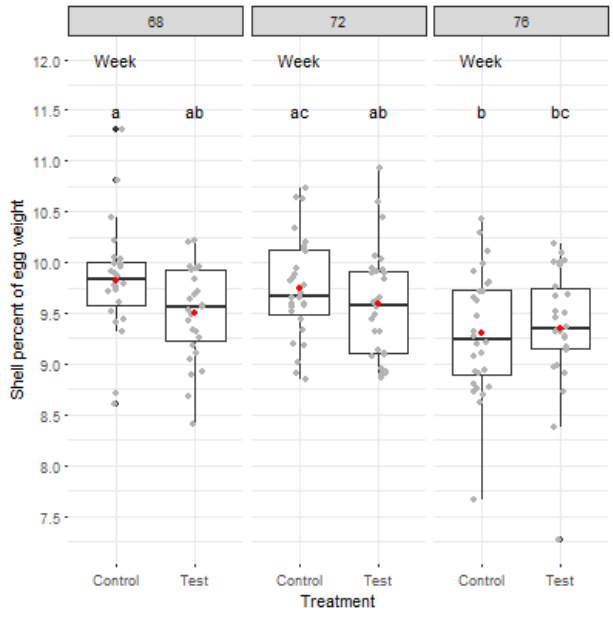


Figure 21 Effect of split feeding of calcium on eggshell percent

4.3 Bone quality

4.3.1 Bone Length

The mean lengths of bone in the Control and Test group were 114.27 g and 113.56 g respectively (fig 22). There was no effect of Treatment on the bone length.

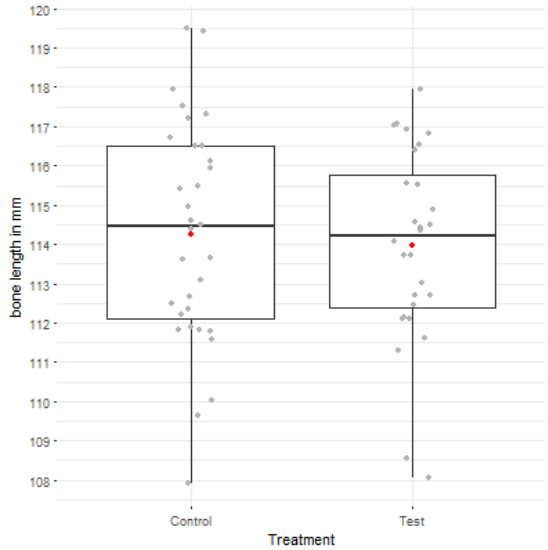


Figure 22 Effect of split feeding of calcium on length of the bone

4.3.2 Bone Diameter

The mean diameter of the bone in the Control and Test groups was 6.4 mm (Fig 23). There was no effect of Treatment on the diameter of the bone

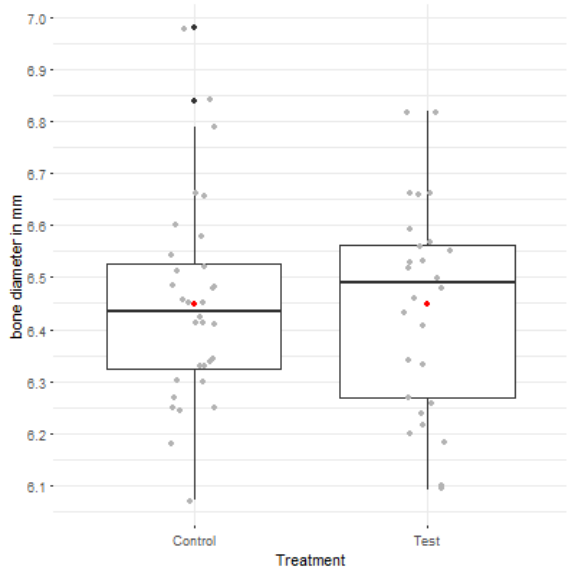


Figure 23 Effect of split feeding of calcium on the diameter of the bone.

4.3.3 Bone Weight

The mean weights of the bone in the control and Test group were 5.8 g and 5.9 g (fig 24). There was no effect of Treatment on the weight of the bone.

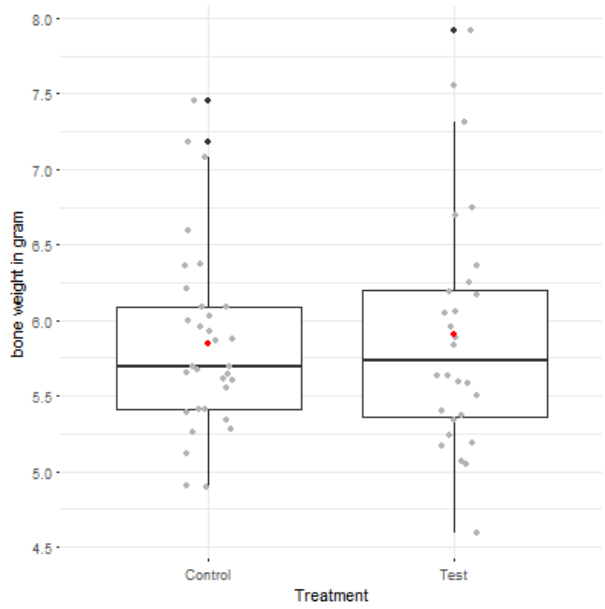


Figure 24 Effect of the split feeding on the weight of the bone.

4.3.4 Seedor Index (weight/length)

The Seedor index for the Control and Test group was 51.17 mg/mm and 51.78 mg/mm respectively (Fig: 25). There was no effect of Treatment on the Seedor index

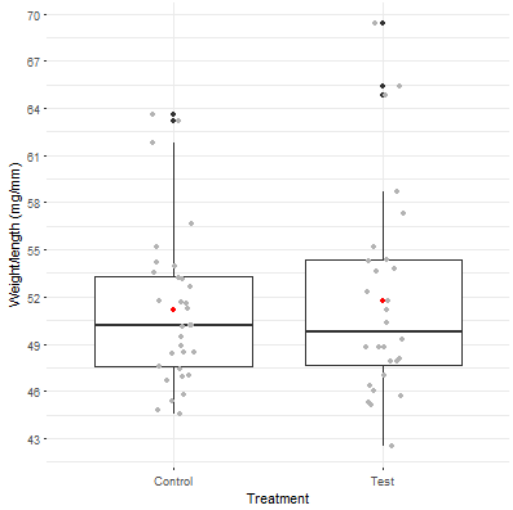


Figure 25 Effect of split feeding of calcium in the Seedor index.

4.3.5 Ash

Mean ash content of the Control and Test group was 3.04g and 3.16g respectively (fig 26). There was no effect of Treatment on the ash content.

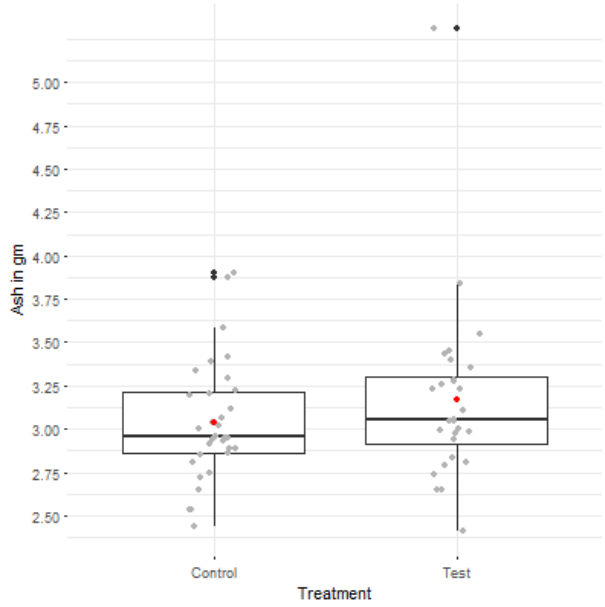


Figure 26 Effect of split feeding of calcium in the ash content

4.3.6 Ash/Kg dry bone

Mean ash/Kg dry bone for the Control and Test groups was 520.21 g/kg and 537.14/g/kg (fig 27). There was no effect of Treatment on the Ash/Kg dry matter of the bone

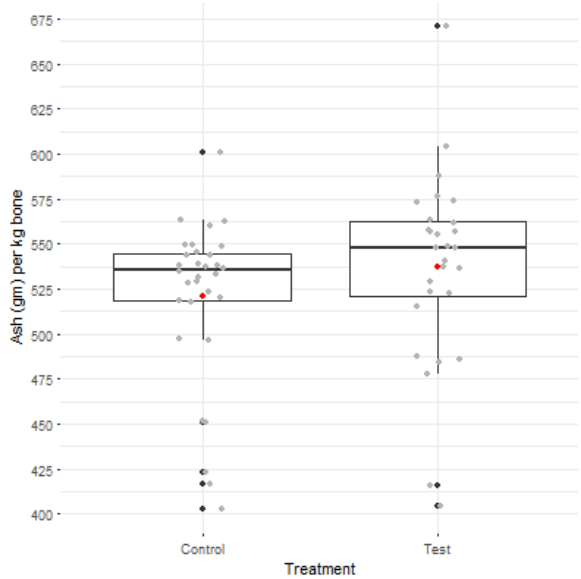


Figure 27 Effect of split feeding of calcium in the Ash/Kg dry bone

5. Discussion

It was expected that split feeding would increase egg weight and size and maintain eggshell quality. A significant increase in the weight of the eggs in the Test group was observed without affecting eggshell quality. Increase in egg weight by feeding low calcium level (0.8%) in the morning and high calcium level (3.2 % or 6.5%) in the evening was observed by (Waldroup & Hellwig, 2000). De los Mozos et al. (2014) found no effect on egg weight and feed intake by reducing morning calcium level by 45% and increasing evening calcium level by 15%. The increase in egg weight could be due to increase in feed intake which was higher (by 2%) in the Test group or due to the decrease in laying rate which was about 2% less in old hens of Test groups. The noticeable difference between this and the other experiments is that pelleted feed was used in this experiment and mash feed was used in most other studies.

Laying hens were found to increase their feed intake when calcium level was reduced (Roland Sr & Bryant, 1994). However, in split feeding, low calcium level in the morning did not increase in feed intake (Waldroup & Hellwig, 2000). It is possible that digestibility of the afternoon feed in the Test group might have been higher because the coarse limestone can reduce the transition rate of feed in the digestive tract of the birds so that it can be better digested (Guinotte et al., 1995). The coarse particles in the pellets of the Control group might be reduced by pelleting. In contrast, Lichovnikova (2007) reported that egg weight was reduced when 70% of the fine limestone was replaced by coarse limestone.

The rationale behind split feeding of calcium is that in the morning required calcium is low while in the evening, it is mostly required for eggshell calcification. The Laying hens that are offered free choice exhibit specific apetite for the calcium (Chah & Moran Jr, 1985; Olver & Malan, 2000) with calcium intake mostly concentrate in the late afternoon or evening (Hetland et al., 2003). For the first 10 hours calcium intake is as low as 11 (Chah & Moran Jr, 1985) to 12 % of the total intake (Mongin & Sauveur, 1974), reviewed in (Etches, 1987). The afternoon-peak of calcium intake minimises the risk of excretion before being deposited into the eggshell (Graveland & Berends, 1997). Hens can detect the deficiency of calcium and increase their calcium intake (Hughes & Wood-Gush, 1971). Even the commercial hens that are genetically selected for high performance can sense calcium deficiency if the calcium in the diet is low and increase the intake of an additional calcium source (Bradbury et al., 2014).

In the present study the calcium concentration of the Test group feed was reduced to 1.6% and the remaining calcium was provided as limestone particles in the afternoon feeding (1400 and 1700). Control group was fed 3.9% calcium all the four time. From the feed intake (assuming 50% intake at both halves of the feeding period) and analysed calcium in the feed and the limestone it can be calculated that both the groups were receiving 4.3 grams of calcium daily. The Test group may have received slightly less calcium, assuming loss while supplying limestone particles.

The calcium intake of the hens can be reduced by split feeding without affecting eggshell quality. De los Mozos et al. (2014) reported that the calcium level was reduced below the recommended value when morning calcium was decreased by 45% and the afternoon feed was increased by 15%. Egg production performance and eggshell quality traits were not affected by this reduction. Keshavarz (1998b) reported that hens fed with 2% calcium in the morning and 3.8% calcium in the afternoon did not show difference in egg mass and shell quality traits, such as shell weight, shell percent, and shell thickness while the dietary intake of the calcium was significantly reduced than the control group that received 3.8% calcium throughout.

Supply timing of calcium has significant importance in reducing the calcium requirement while maximising its utilisation. When the egg is in eggshell gland calcium requirement is high and the intestinal absorption of calcium is double than the time when eggshell gland is empty (Hurwitz & Bar, 1969). The requirement can be lowered by reducing excretion if the calcium is supplied during this period. Mozos et al. (2012), by the observation of movement of oviposition, found that the hens that received calcium rich diet 8 to 10 hours post oviposition consumed less calcium, in total, without decreasing eggshell quality.

Since the significant portion of the eggshell calcification takes place during the dark period the time to exploit through feeding in the afternoon or evening period. The concern is how much afternoon calcium level is needed on to maintain the eggshell quality. Waldroup and Hellwig (2000) observed that when the mornning calcium level was reduced to 1% and the evening calcium was maintained at 3.25%, there was a significant reduction in the eggshell breaking strength. However, when the evening calcium was raised from 3.25 to 6.5 % shell quality was not affected. Similar result was reported by Keshavarz (1998a) found no effect in eggshell quality when the calcium level of the afternoon feed was increased from 3.5% to 5.5% while

the morning calcium was at 1.5%. Thus the normal calcium level (3.5%) in the afternoon cannot maintain eggshell quality when coupled with a very low morning calcium level.

According to (Keshavarz, 1998a) limiting factor is feed intake. Feed intake in layers hens have been shown not to be stimulated by calcium level. Regardless of calcium concentration, evening feed consumption has been found to be limited to 50-60% of the total intake (Keshavarz, 1998a; Molnár et al., 2018; Waldroup & Hellwig, 2000). This limitation can be overcome by increasing calcium level in the evening feed. (Lee & Ohh, 2002), cited in (Molnár et al., 2018), even reported that reducing calcium level in the morning feed to 0.5% and increasing the level in afternoon feed by 9.5 % and 12.5% increases eggshell breaking strength. In our case, coarse limestone particles (90% of the particles were between 1-3 mm) were mixed on the top of the afternoon feed. It was expected the hens consume all the limestone regardless of feed consumption to meet their minimum requirement. This can be expected as hens can identify the coarse limestone particles and consume them. This was also demonstrated by Hetland et al. (2003), who observed that consumption of shellmeal, at 14-16 hours of post-oviposition, was higher than the whole wheat (whole wheat was included in the pellets).

Hens are mostly fed with calcium carbonate as a calcium source, however, for the eggshell calcification they use ionic calcium. Calcium carbonate is brokedown into ionic calcium by the acid produce in the proventriculus that is released into the gizzard, resulting in calcium solubilization (Guinotte et al., 1995). Activity of the gizzard in the laying hens was found to increase in conjunction with dilation of the crop (Mongin, 1976) as cited in (Guinotte et al., 1995). In parallel to the increased activity of the gizzard, the digestive tract of the hen also has low transition rate for the passing calcium. Calcium absorption is increased as a result of these phenomena (Guinotte et al., 1995; Zhang & Coon, 1997). Absorption is mainly completed in the upper duodenum and jejunum (Hurwitz & Bar, 1969). This is in contrast to chicks in which transition rate is high, so calcium absorption mainly takes place in the distal intestine, and also calcium absorption is also not as high as in laying hens (Guinotte et al., 1995).

Fine and coarse particles are absorbed differently in the digestive tract. Fine particles dissolves quickly so that absorption is rapid in the beginning. According to (Roland Sr et al., 1973) absorption is high for the first few hours after which it is still continuous but slow. Coarse particles larger than 0.8 mm are selectively retained by the gizzard (Zhang & Coon, 1997). Due to the accumulation in the gizzard and slow dissolution, calcium is slowly metered out (Zhang

& Coon, 1997) during the night. This provides calcium for the eggshell formation during the night when hen has not been eating which saves the hen from using the body calcium reserve. High dietary calcium level is also associated with a decrease in absorption and increase in excretion. It is possible that the effect is more pronounced in fine particles because they transfer rapidly in the digestive tract compared to coarse particles, and if the limit of intestine is exceeded they can be flushed out (Zhang & Coon, 1997). Absorption of a relatively high proportion of calcium when dietary calcium level is low in comparison to when dietary calcium level is high (Hurwitz & Bar, 1969), may be the reason for this. Coarse particles are also pushed out if consumed in excess without solubilisation (Zhang & Coon, 1997).

Intestinal absorption is not the only way hens obtain calcium during eggshell mineralization. A unique feature of female avian is that they have labile medullary bone. Involvement of the medullar bone in eggshell calcification is substantiated by various studies (Clunies et al., 1993). Exactly when during the eggshell calcification the medullary bone participates is also contentious. According to Clunies et al. (1993), it is 12-18 hours of post- oviposition that the bone resorption takes place. In contrast, Kerschnitzki et al. (2014) reported that medullary bone is involved once the eggshell gland is active.

Several studies reported the importance of particle size on the eggshell and bone quality. Koreleski and Świątkiewicz (2004) reported a 20-90% inclusion of coarse limestone of particle size 2-4 mm increased eggshell breaking strength. Cufadar et al. (2011) observed improved eggshell quality and bone quality in old hens when fed with limestone of 2-5 mm particle size. Similarly, Skřivan et al. (2010), reported a significant increase in the eggshell quality by the inclusion of coarse particles in the feed. However, others (Cordeiro et al., 2017; De Witt et al., 2009) observed no effect of particle size on the egg shell quality. Pizzolante et al. (2009) did not observe any significant effect of particle size on eggshell quality.

Fleming et al. (1998) observed high bone volume and breaking strength in laying hens that were fed particulate limestone (particle size 2.5 mm to 4 mm) instead of ground limestone. The authors concluded feeding particulate limestone may help to combat osteoporosis in laying hens. Saunders-Blades et al. (2009) reported that Tibia weight and breaking strength of old hens were higher when 1/3rd of the fine limestone was replaced by coarse particle (particle size (~ 1.5 mm to 3 mm). Similarly, (Oliveira et al., 2013) also observed improvement in bone quality when more than 60% of the fine limestone (0.23 mm) was replaced with limestone having mean

particle size of 0.6 mm. However, according to (Zhang & Coon, 1997) particles less than 0.8mm are not retained in gizzard. Keshavarz et al. (1993) observed no effect of particle size on the bone weight and ash content.

The effect of particle size with split feeding of calcium in egg quality have been studied by (Molnár et al., 2017) and (Molnár et al., 2018). For, the purpose of comparision, percentage contribution of morning and evening calcium in this experiment, is calculated. In the present trial, the afternoon feeding provided 78% of total in the test group, of which 60% was coarse limestone. The morning feed provided the remaing calcium as 20% fine limestone. In the control group initially, the ratio of coarse and fine limestone was 2:1. However, after pelleting, proportion of fine should have increased.

Molnár et al. (2017), observed that the in split feeding, where morning calcium was depleted completely was inferrior to conventional feeding. In this study, the author concluded that the split system should not have either 50% or 100% coarse limestone. 50% coarse limestone probably cannot provide enough calcium during the night while 100% coarse limestone could not provide enough calcium for the early calcification due to slow solubilisation. In such a split feeding system, 70% coarse and 30% fine limestone was reccommended. In our study, morning calcium for the Test group was reduced but not depleted, which suggest that morning calcium level was enough for the medullary bone to regain its structure if there had been any mobilisation.

(Molnár et al., 2018) further substatiated that morning calcium is important and best split feeding is the one which provides 50 % of the total calcium intake as fine limestone in the morning while afternoon limestone should provide 50% of the total intake in the coarse form. The eggs oviposited in the morning will have benefited by the morning calcium for the late calcification. In our experiment, 6 hours had already passed before the first feeding is commenced. It seems morning calcium for the calcification had less importance in the present experiment because most of the eggs might have been oviposited before first feeding. Interestingly, in that study the conventional group fed with 25% fine and 25% coarse limestone was reported to have very poor production performance, the reason for which was largely unknown (Molnár et al., 2018).

The conventional system in the present case was as good as the split system in terms of eggshell quality. There was no difference in the bone quality in both the groups. The possible reason for this could be the balance in coarse and fine limestone in the pellets. Both the Control and the Test group must have benefited by the presence of additional limestone that was erroneously provided to both the groups. The particle size of this limestone was 3-6 mm and approaximately 7.5 kg daily was supplied to both sides.

Laying performance such as egg production, feed intake, feed conversion and mortality are all important criteria for the successful commercial farming. Unless these criteria are assessed, the potential of a feeding system cannot be judged. However, due to the lack of statistical test these criteria were not compared. An important aspect worth noticeable in this experiment is the cracking of eggs which, specially from old hens, was much higher during packaging than in farm. Meaning the loss to the farmer due to eggshell cracking is still high. Thus a successful feeding system is the one that improves eggshell quality.

6. Conclusion

The present study hints the potential of split feeding of calcium to the layers by feeding particulae limestone on the top of afternoon pellet feed. Increase in egg weight by this feeding system can be expected without affecting eggshell quality and bone quality. However, laying performance must be assessed before claiming its potential.

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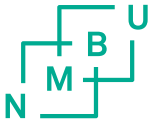

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