INFLUENCE OF LIMESTONE PARTICLE SIZE IN LAYER DIETS ON SHELL CHARACTERISTICS AT PEAK PRODUCTION

BY

PHIRINYANE TOBIN BOITUMELO

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Supervisor: Prof. H. J. van der Merwe

Co-supervisors: Prof. J.E.J. du Toit and Prof. J. P.Hayes

BLOEMFONTEIN

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DECLARATION

I hereby declare that this dissertation submitted to the University of Free State for the degree, MAGISTER SCIENTIAE (Animal Science), has not previously been submitted for a degree at any other University. I further cede copyright of the thesis in favour of the University of the Free State.

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CHAPTER 1

GENERAL INTRODUCTION

Poultry in general and eggs in particular are some of the cheapest sources, in terms of production input costs, of proteins and vitamins, especially in developing societies (Kuhl *et al.*, 1977). With increasing pressures on land and drives towards a sustainable framework for food production, poultry production in general and egg production in particular has attracted the attention of scientists, researchers and policy-makers as a viable and sustainable source of affordable and environmentally friendly proteins and vitamins. However, for poultry production in general and egg production in particular to provide the necessary food safety-net especially with regard to the provision of proteins and vitamins, there is a need to determine the optimal mix of nutrients that are needed for sustainable and affordable poultry and egg production.

Calcium plays a vital role in egg-production metabolic processes. Calcium is found in blood of chickens, as well as other animals, in three forms: bound to plasma proteins, bound to inorganic compounds and freely disassociated or ionised (Copp, 1969). The physiologically active component is ionised calcium, which has been firmly established to have a major role in many biochemical processes, including nerve conduction (Frankenhaeuser, 1957), muscle contraction (Colomo & Rachmaninoff, 1968), blood clotting (Olson & Suttie, 1977), and hormonal regulation of bone metabolism (Cohn & Hamilton, 1976). Consequently, the regulation of blood ionised calcium (Ca^{++}) within a narrow range is of great importance since this blood calcium fraction affects the function of the aforementioned biological processes.

It is generally accepted that gastric acid secretion is a predisposing process to the solubilisation process of calcium in the gastrointestinal tract, before calcium can be absorbed in the ionic state. In chicks the soluble fraction of calcium in the ventriculus and small intestine is dependant on pH, but this is not the case in laying hens. The duodenum and jejunum of the hens contain a large amount of solubilised calcium for use in egg shell formation during the laying stage in spite of the relative high pH of 4.5 compared to the pH 6.5 in the stomach. Hence, in the laying stage birds also have very high calcium retention (Mongin & Sauveur, 1977).

The increase of soluble calcium in the small intestine is the result of an increased gastric acid secretion at the beginning of the dark period, induced by the enlarging of the crop because of the increase in feed intake just before the beginning of the night (Mongin & Sauveur, 1977). High levels of calcium in the diet can also raise the pH of the ventriculus because of the buffering capacity of calcium. Nonetheless, the increase of calcium in the diet will increase the amount of solubilised calcium in the ventriculus and small intestine.

It has been demonstrated on numerous occasions that increasing the level of calcium in a laying diet will result in an improvement in eggshell quality (Balloun & Marion, 1962; Reddy & Sanford, 1963). However, in the above reports depression in production was noted at the higher levels of dietary calcium.

It is important that calcium should be provided in the right amounts to layers. The provision of calcium in the right amounts to layers serves two important purposes, i.e. ensuring that the eggs produced are of the right quality, both in terms of shell texture and nutrition value, and ensuring that layers do not resort to bone demineralisation in attempts to compensate for calcium deficiency. Bone resorbtion leads to layers with weak bones and this often leads to fractures that ultimately impact on production. Bone fractures in layers towards the end of lay are in fact a cause for concern. There are estimates that 30% of layers suffer bone fractures during their lifetime (Gibson & Roberfriod, 1995; Hurwitz & Bar, 1969).

Lower levels in the supply of calcium will lead to weaker shells, which will have serious consequences in terms of viability of the egg in hatcheries and also for supply of intact eggs to the consumer (Ahmad & Balander, 2003).

However, the importance of calcium in the egg-production metabolic processes in layers is not only underscored by the negative effects that can be occasioned by low levels in the supply of calcium, but also by an over-supply of calcium to layers: in this kind of scenario, the desired ratio of calcium to phosphorous (2:1) will be compromised and will inhibit the absorption of other minerals into the layers metabolic system, and consequently affect production

The foregoing underscores the importance of calcium in egg-production metabolism processes in layers. However, the challenge does not only lie in providing calcium to layers. The challenge with regard to calcium provision to layers relates to two aspects, namely: the provision of calcium to layers from environmentally sustainable sources; and the identification of the ideal calcium particle size that would optimise egg-shell quality at peak production, thereby reducing mechanical and nutrient related losses associated with weak egg-shells.

The importance of calcium to animals in general and layers in particular has been of interest to researchers for a long time. This interest in calcium varies from the search for the most optimal sources of calcium for layers in layer feeds, a search for the optimal or ideal calcium particle size in layer feeds, as well as the influence of calcium (positively and negatively) on egg-production metabolism and the quality of eggs.

Optimum particle size of calcium supplements for laying chickens has been a controversial subject for almost a century. Renewed interest in this subject was evident early in the 1970's as a result of several reports indicating markedly improved egg shell breaking strength when hen-sized oyster shell replaced a portion of the ground limestone in laying hen diets. Most of the past data indicate large particle size calcium supplements to be superior to ground or small particle supplements. Miller & Sunde (1975) present an excellent review of literature on this subject.

Other studies address the problem of egg-quality and how calcium affects overall egg-quality. Egg breakage still represents a large economic loss to the poultry industry. It was estimated that 13 to 20% of total eggs produced are cracked or lost before reaching their final destination (Roland, 1988). This may lead to the question of improving the eggshell quality. Numerous reports have been presented regarding the effectiveness of feeding various calcium sources in either the pulverised or the granulated form on eggshell quality. Scott *et al.* (1971) and Roland (1986), in their reviews have shown a positive effect of calcium with a coarse particle size on eggshell quality in half of the reported studies.

There has also been an increase in the number of studies that address themselves to the question of sources of calcium for layer diets, especially the best sources of calcium for layer diets, but there are varying views on what would constitute the best sources of calcium for layer diets.

Calcium is usually supplied as calcium carbonate from limestone in poultry rations, or from other sources, such as marine shells in diets for chicks (McNaughton *et al.*, 1974; Gerry, 1980) or in hens (Roland, 1986). The calcium sources differ in their origin (animal or mineral deposit) and their particle size; as a consequence, their physio-chemical characteristics are different (Mongin & Sauveur, 1977). In this regard, considerable attention has been given to laying hens and it has been shown that coarse particle size had, generally, a beneficial effect on egg shell quality (Roland, 1986), and on bone strength (Guinotte and Nys, 1990).

Scott *et al.* (1971) suggested that a large, particulate calcium source like oyster shells were metered out of the chicken gizzard at a slower rate than ground oyster shell. The same author stated that large particle size enables greater use of dietary calcium for eggshell formation during the night and resulted in a stronger shell. Research focusing on the effect of layer performance and shell quality of different limestone sizes has yielded conflicting results.

Roland *et al.* (1974) and Kuhl *et al.* (1977), reported that particle size has no effect on egg shell thickness, egg breaking strength and specific gravity, respectively, whereas others found shell quality measured as egg breaking strength was significantly improved (Meyer *et al.*, 1973; Watkins *et al.*, 1977). Therefore, limestone solubility differences might be responsible for the contradictory findings. Similarly, Rabon & Roland (1985) have shown that the solubility of limestone particles of similar size from different sources varied by 62%.

Other studies have addressed the question of the role of calcium in animal metabolic processes. Rao & Roland (1989) found that the *in vitro* solubility of coarse limestone particles (>0.8 mm) was about a third of that of finer particles (<0.8 mm) at 35% for the coarse particles and 95% for the fine particles. The amount of calcium solubilised by the hen (*in vivo*) was significantly larger for the coarser particles than the finer particles during the 24-27 hour trial period. The amount of calcium solubilised from the coarse particles was 20-30% more than that of the finer particles. According to these authors a longer retention time of the coarse particles in the crop and ventriculus of the chicken means that these organs are used as a reservoir for calcium in the body. This means that the calcium is made available in a more uniform fashion during the period of eggshell formation, during the night when these organs are used as a reservoir for calcium in the body.

Ehtesham & Chowdhury (2002) observed that poultry producers are always interested in high production at minimum expenditure on nutrients so as to economise their feeding practices. In addition, there is a recent trend to reduce unnecessary wastage of nutrients which are excreted through excreta and therefore become potential pollutants of the environment. By investigating what would be the ideal limestone [a naturally occurring source of calcium and one that is environmentally friendly] particle in layer diets, this study aims at making a noble contribution towards not only a sustainable and environmentally compatible poultry production in general and egg production in particular, but also towards a sustainable source of proteins and vitamins, on a continent facing many challenges in the feeding of its citizens.

From the literature it is evident that research on calcium constitutes an important undertaking for researchers interested in both establishing what would be the optimal sources of calcium in layer diets and understanding the role that dietary calcium plays in boosting egg production and egg quality in layers. However, a main a concern is that mineral calcium sources like limestone differ in their origin, purity and particle size. These differences could lead to a variation in bone strength and egg shell quality. In South Africa, limestone from Limpopo province of South Africa is mainly used as a calcium source in layer diets. Therefore research is urgently needed to identify the ideal particle size for this particular limestone source in layer diets.

However, the review of this body of literature is conscious of the fact that in all of the studies none of the researchers have ever addressed the specific problem of the ideal particle size of one specific limestone source which was the concern of the present study.

The aim of the study was to investigate the influence of limestone particle size and distribution in layer diets on egg production and egg quality at peak production.

The following hypotheses guided the study:

(i) Increasing particle size of limestone in layer diets have a positive effect on eggshell quality at peak production

(ii) Increasing the proportion of coarse particle size in layer diets have a positive effect on eggshell quality at peak production.

The dissertation is organised in six chapters. Chapter 1 is the general introduction Chapter 2 reviews the relevant literature. Chapter 3 presents a general overview of the research methodology used in the entire study. In Chapter 4 the influence of different particle sizes of limestone in layer diets on egg production and egg quality at peak production was investigated. The influence of particle size distribution of limestone in layer diets on egg production and egg quality at peak production was investigated and reported in Chapter 5. Chapter 6 outlines the general conclusions and recommendations. References relevant to a particular chapter are cited in a reference list at the end of each chapter. The dissertation is rounded off with an abstract (and an Afrikaans translation thereof).

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Studies on the importance of calcium in egg production have been of interest to nutritionists for a long time. Initially, use was made of correlation analysis to determine the relationship between various traits affecting the mode of inheritance and distribution of egg production. In particular, studies have focused on the role of calcium on egg production, egg shell thickness, egg weight, and body weight, egg breaking strength, feed intake and feed conversion ratio (Chen & Chen, 2004; Watkins *et al.*, 1977).

Many studies consider the following three factors, namely calcium source; particle size; and dietary calcium level, and how these three factors interplay with egg production, egg shell thickness, egg weight, and body weight, egg breaking strength, feed intake and feed conversion (Khurshid *et al.*, 2003).

The current chapter reviews the available literature on egg shell quality and egg production, with specific reference to literature on calcium source, particle size, and dietary calcium level, and how these three factors interplay with egg production, egg shell thickness, egg weight, and body weight, feed intake and feed conversion.

2.2 Occurrence of calcium (Ca)

Calcium is the most prevalent mineral in the body and is required in the diet in larger quantities than any other mineral (Elaroussi *et al.*, 1994; Siebrits, 1993). It is one of the key elements required for maintenance and egg production (Elaroussi *et al.*, 1994). Calcium plays a major role in a wide variety of biological functions in the body, of which the structuring of bones is the most important (Siebrits, 1993). It is therefore the most abundant inorganic component of the skeleton (Elaroussi *et al.*, 1994). The high calcium requirement of growing chickens is driven by the need for skeletal development. In laying hens, most calcium is used for shell formation (Highfill, 1998; Klasing, 1998). Calcium constitutes more than a third of the total mineral content of an adult bird (Klasing, 1998) and comprises about 1.5% of the bird's weight (Underwood, 1981; Highfill, 1998; Larbier & Leclercq, 1994). Calcium is the main component of the skeleton. For instance, skeleton contains 98-99 % of a bird's calcium, most of which is in the form of hydroxyapatite, ($Ca_{10}(PO_4)_6(OH)_2$), with a small amount of non-crystalline calcium phosphate and calcium carbonate (Klasing, 1998; Siebrits, 1993).

2.3 The effect of body weight on egg production and quality

If egg weight is considered, it is observed that a 1.36 kg hen at sexual maturity produces only 162 eggs out of a total of 212 eggs weighing 56.75 g or more. Du Plessis & Erasmus (1972) observed that for every extra 0.45 g of body weight, there is an increase of 25 eggs weighing more than 56.75 g per bird. The increase of eggs of 56.75 g or more continues until the bird reaches 2.04 kg body weight. The relationship between egg production, egg weight and mature body weight follows the same pattern as observed in the body weight at sexual maturity. The only difference that exists between the two breeds (Leghorn and New Hampshire) is that is in leghorns there no relationship between total egg production and body weight at sexual maturity. In case of the New Hampshire, hens weighing between 2.1 to 2.7 kg at mature are highest producers. The importance of calcium in the diets of layers is underscored by the fact that calcium is critical in determining the body weight of birds, and the weight of birds has an effect on egg production and quality.

2.4 Calcium absorption

Calcium salts are more soluble in an acid solution; hence, absorption occurs mainly in the upper small intestine (duodenum) where feed contents are still somewhat acidic following digestion in the stomach (Ensminger *et al.*, 1990). Some absorption also occurs in the lower intestine (Highfill, 1998; Perry, 1984; Underwood & Suttle, 1999). Calcium is absorbed by active transport when dietary calcium levels are low. Passive absorption in the jejunum and ileum is the major absorptive process when calcium intake is adequate or high (Bronner & Pansu, 1999; Klasing, 1998). According to Bronner & Pansu (1999), calcium that reaches the large intestine undergoes absorption by both active and passive process. The levels of parathyroid hormone (PTH) and 1.25-dehydroxy vitamin D_3 control the efficiency of absorption. High levels of these hormones occur when levels of blood ionised calcium (Ca^{2+}) are low (Klasing, 1998). The induction speeds the synthesis of calbindin, which binds calcium and facilitates transport across the intestinal epithelial cells (Klasing, 1998). As a result, sufficient level of vitamin D_3 in breeding diets is necessary to ensure absorption of calcium by the hen.

According to Thompson & Fowler (1990), the absorption of calcium is regulated to a greater extent by animal requirements and is inversely related to intake. Calcium absorption is a closely regulated process that involves the action of vitamin D_3, PTH, and calcitanin regulates (Thompson & Fowler, 1990; Postman, 1998). The absorption of calcium is by active transport, which requires energy, and by passive diffusion. Active calcium transport has four primary steps: (i) energy-dependent uptake of Ca^{2+} across the enterocyte membrane, (ii) binding of Ca^{2+} to calbindin within endocytic vesicles; (iii) fusion of vesicles with lysosomes; and (iv) movement of lysosomes along microtubules and exocytosis of the contents at the basal lateral membrane (Klasing, 1998). According to Fischer (1983), the average rate of absorption of calcium from the digestive tract is 83 mg per hour and the short-term demand for calcification is met partly from labile stores in medullary bone. Excess calcium or phosphorus interferes with the absorption of each other, a fact that helps to explain why a certain ratio between them in the diet is desirable (Maynard *et al.*, 1979).

Chen & Chen (2004) reported higher calcium levels in tibiae which might be due to the high serum levels of birds with probiotics supplementation. The supplementation improved calcium and phosphorus absorption. Kruger *et al.* (2003) reported that improved calcium absorption decreases the occurrence of bone fractures and osteoporosis. The observation is that 10% of production is cracked or broken eggs between oviposition and retail sale (Zeidler 2001, Naber *et al.*, 1963) and eggshell quality, especially shell strength, decreases with age of hens (Rodriguez-Navarro *et al.*, 2002). Increasing the dietary calcium content from 2.5 to 5% resulted in higher calcium content in eggshell weight, eggshell percentage and eggshell strength. The results showed increased calcium content in both the bones and the eggshell of the birds as compared to control. Eggshell strength is improved because of the consequence of the increased mineral absorption. Due to insulin, calcium contributes to improved eggshell quality which may result in reduces breaking of the shells and improved productivity of the laying hens, especially with birds of relatively old age (50 weeks).

2.5 Calcium requirements

The most common calcium supplements used in poultry feeding include ground limestone, oyster shells, bone meal, calcite, chalk and marble (Ensminger, 1992). Bone meal, dicalcium phosphate, defluorinated phosphate, and raw rock phosphate are used where both calcium and phosphorus are needed in the diet. High concentrations of $CaCO_3$ and calcium phosphate render the diet unpalatable (Ensminger, 1992).

Previously, Ahmad *et al.* (2003) reported that egg production linearly increased with increasing calcium levels from 2.5% to 5% and this was observed as early as the second week of experiments. There was a 7% average production difference (75.3% *vs*. 82.3%) between the lowest 2.5% and the highest 5% calcium intake levels respectively. Average feed consumption for the six experimental diets ranged between 111g – 114 g per hen per day. Hens fed calcium deficient diets generally over-consumed to alleviate this problem. However, due to the feeding threshold levels in chicken (i.e. hens can only take as much feed at a time) these hens did not get the required calcium from the calcium deficient diet and production in these hens declined. Two sets of conclusions were established from this study; first, dietary calcium level has a significant effect on egg production, and secondly, dietary calcium level has no significant effect on feed consumption.

Ahmad & Balander (2003) fed Hyline hens a diet containing three different calcium sources (limestone, oyster shell and marine sea shells) and reported that the average production were not significantly different.

The amount of dietary calcium required to maximising bone or eggshell mineralisation and the strength is greater than that needed for other functions. Requirement levels are based on the premise that all of the calcium consumed has a bioavailability similar to that of $CaCO_3$ (Klasing, 1998). Laying hens require higher dietary levels of calcium than non-layers, as calcium is required for eggshell formation. By providing calcium over a greater part of each 24 hour period, the need for bone resorption would be decreased (Hill, 1998). To ensure maximum shell quality, it is recommended that hens consume a minimum of 3.75g calcium/hen/day (Roland, 1986). Grau & Roudybush (1987) reported that the calcium requirement of laying hens is 100 times greater than that of non-layers, while Saunders & Hayes (2000) quoted it to be 20 times greater. The metabolism of such large quantities of calcium in the laying hen is therefore intense, and represents a transfer rate of 155 mg Ca^{2+} per hour from the blood to the uterus for almost 20 hours, during which the shell is deposited around the egg (Saunders & Hayes, 2000).

In tropical areas, the calcium level in the feed has to be higher during hot periods because of lower feed intake during such periods (Voeders, 1994). The calcium balance is maintained by the absorption from the digestive tract of about 83 mg Ca^{2+} per hour, and the utilisation of between 4 to 5g of calcium stored in medullary bone (Saunders & Hayes, 2000). According to Singh & Panda (1996), the hen absorbs about 100 mg of calcium per hour for eggshell formation, which is a very high rate, considering the size of the birds. On the other hand, Roland & Farmer (1984) states that the hen needs 125 mg dietary calcium every hour for 16 hours to form an eggshell. According to Roland (1986), the average calcium requirement for eggshell formation within a population of hens is greatest at approximately peak production. However, because the amount of calcium deposited on the shell can increase slightly with the age of hen and production might not be a factor in the individual hen's daily requirement, the calcium requirement for an individual hen for a particular egg on a particular day could increase with age. As hens age, the average quantity of calcium deposited on the eggshell per day (percent production x calcium content of eggshell) declines. Calcium deposition in eggshell prior to peak production is at least 5% less compared to the quantity of calcium deposited in eggshell after peak.

The calcium requirement for maximum shell thickness is greater than that for maximum egg production and as shell thickness is related to strength, the requirement quoted is for maximum or near maximum shell thickness (Hill, 1998). Dietary calcium content of 3.25 to 3.75% is believed to be desirable for laying hens. Scott *et al.* (1971) reported that calcium level of 5% caused a decline in feed consumption, while it did not improve eggshell quality above that obtained with 3.5% calcium. These researchers suggested that if the hen retains 50% of the calcium she eats for egg and eggshell production, she will need to consume 1.16 kg of Ca^{2+} per annum.

Guinotte & Nys (1990) studied the effect of source (oyster shell *vs* limestone) and particle size (particulate *vs* ground) in White Leghorn and reported that egg production, egg mass and feed efficiency were not modified, either by the origin or the particle size of calcium. Hens fed particulate oyster shells had a higher feed intake than those on all the other treatments ($P<0.01$). Hens supplemented with particulate limestone consumed more feed than those fed the ground oyster shells. These results demonstrated that particulate supplements calcium resulted in higher ($P< 0.01$) feed consumption.

Both calcium and phosphorus play a role in egg production and hatchability of fertile eggs. Marley *et al.* (1980) conducted two experiments in which diets containing two levels of dietary calcium (2.5 and 3.5%) and three levels of dietary phosphorus (0.3, 0.4 and 0.5%) were fed to turkey hens. In both experiments increasing calcium in the diet of turkey hens, resulted in a slight numerical increase in egg production. Hens that received 3.5 % dietary calcium laid at a rate of 2.5% points higher than hens receiving 2.5% calcium. Similar observations were made by Ahmad *et al.* (2003) who reported that increasing dietary calcium level from 2.5 to 5.0 in Bovines hens increased egg production from 75.3 to 82.4% and egg specific gravity from 1.078 to 1.083 units. It was also observed that calcium level had no effect on feed consumption or egg weight. In addition, birds receiving high calcium diet in another experiment laid at a rate of 0.9% point higher than those on the lower calcium diets.

Watkins *et al.* (1977) used Leghorn hens to investigate the effect of calcium level, sources and particle size on production. They reported that the particles either ground or hen-sized did not show any significant difference in effect on egg production. The egg production and feed conversion ratio were poor for groups of birds fed 1.75% calcium diets. Hens fed 3.25% calcium diets produced more eggs of better quality and required less feed than hens fed 1.75% or 2.5% calcium diets. Production was improved with increasing dietary calcium level from 2.5 -3.5%.

The results of previous study by Hurwitz & Bornstein (1963) showed that feed consumption tended to be somewhat lower for birds fed the high calcium diets as compared control diet. The difference in feed conversion was more pronounced (although not significant) with supplementary calcium carbonate (irrespective of source) resulting in an improved feed conversion in all cases. Of all the sources of calcium carbonate used in the diets, precipitated salt resulted in lowest feed consumption and most efficient feed utilisation. Hens fed other diets gained less weight than those on the control diet, and those fed the precipitated calcium carbonate even lost weight during the trial.

Hurwitz *et al.* (1969) conducted several experiments on the interaction effect of fat and calcium on feed intake. Three levels of fat were used (1, 4 and 7%) in the form of acidulated soapstock, each with two levels of dietary calcium (3.0 and 4.5). The results showed that neither production nor egg size were significantly (P>0.05) influenced by any of the dietary variables. Feed intake was significantly depressed by dietary calcium (P<0.05) and supplementary fat ($P<0.01$). Feed conversion was improved by both calcium ($P<0.05$) and fat ($P<0.01$). Body weight gain tended ($P>0.05$) to increase with the fat supplementation, whilst calcium significantly ($P<0.01$) depressed body weight gain.

2.6 Calcium sources and particle size

Solubility of calcium carbonates depends on the particle size and also on the source origin (Guinotte & Nys, 1990). Small particle sources such as pulverised $CaCO_3$ passes quickly through the digestive tract and the bird may not be able to efficiently extract enough to meet its needs. Additionally, the finely ground limestone is absorbed by the hen during the day when the hen is eating but during the hours of darkness a metering of calcium occurs in the digestive tract from the gizzard, because of the breakdown of the shell grit or limestone chips (Woolford, 1994). On the other hand, large particle sizes of the same compound (e.g. $CaCO_3$ in the form of coarse limestone or oyster shell) will be retained in the gizzards for a longer period of time (Korver, 1999; Keshavarz, 2001; Woolford, 1994). This situation allows for a gradual release of calcium from the gizzard to the small intestine for absorption, resulting in increased time over which the hen receives dietary calcium. This is inconsistent with the findings of Roland *et al.* (1972a,b) who reported that very little calcium is metered out of the gizzard during the night. Anderson *et al.* (1984) reported lower weight gains and bone ash values with powdered $CaCO_3$ (=147µ) than with the medium sized particles, when calcium levels were increased from 0.9 to 1.5%. In their study, limestone particle size did not affect hen weight gain to feed ratio, indicating that it is not advantageous to use a fine calcium source. Guinotte & Nys (1990) reported higher feed consumption and improved percentage ash and tibia breaking strength by feeding laying hens coarse particles sizes of calcium sources.

According to Dekalb (1998), one third of the laying diet dietary calcium should be supplied in large particle form (2 – 5 mm). When the calcium requirement in the feed is divided between small and large particle forms, pullets can consume calcium according to their needs. Those pullets, which are the first to come into lay, can consume needed large particles of calcium source, while immature pullets can avoid unnecessary calcium intake. These results support the concept that larger particle size or lower *in vitro* solubility may increase calcium retention for layers. According to Zhang & Coon (1997), the limestone retention of calcium in the gizzard of laying hens for improving shell quality may be dependent upon particle size, porosity of the calcium source, and overall *in vivo* solubility of the calcium source.

Limestone and oyster shells, which are good sources of calcium, can be fed to chickens separately or as a mixture. It is claimed (Sreenivas, 1997) that separate feeding of calcium improves feed consumption, egg production and shell quality. El-Agguory *et al.* (1989) observed that pullets that were fed calcium in the form of two thirds limestone plus one third oyster she ll consumed a greater amount of feed (22.5 kg) than those that were provided with one third limestone plus two thirds oyster shell (21 kg). It was concluded that adding oyster shell to limestone at a ratio of 1:2 increases the palatability of feed. Another study by Scott *et al.* (1971) and Watkins *et al.* (1977) also revealed that egg breaking strength was improved by feeding two thirds calcium supplements as hen-sized oyster shell and one third pulverised limestone. Eggs from birds receiving only pulverised limestone as calcium supplement were also reported to be inferior compared with eggs from birds fed two thirds of calcium supplement as hen sized oyster shell and one third? pulverised limestone (Scott *et al.*, 1971). Eggs with breaking strength (BS) value less than 2.25 kg broke easily compared to those with values greater than 2.7 kg.

In a related study, Guinotte *et al.* (1991) observed that pulverised limestone (less that 0.15 mm particles) improved calcium retention, 4-week body weight (BW) and feed conversion compared to medium (6 to 1.18 mm) and coarse particles (>1.18 mm) of calcium. Additionally, fine particle calcium (limestone) increased intake and weight gain. In that experiment the origin of the calcium sources hardly affected calcium utilisation. McNaughton (1981) reported that 20 to 60 United States Bureau of Standards (USBS) particle-sized $CaCO_3$ when fed to 1 to 21 day old broiler chicks produced higher body weight (BW) than either the USBS 12 to 20 or 100 to 200 particle-sized $CaCO_3$ sources. Particle -sized $CaCO_3$ was fed to broilers, both bone ash and BW increased by feeding at least 0.25% available phosphorus and 0.70 dietary calcium. In contrast, when pulverised (12 to 20) and course (100 to 200) particles were fed, bone ash and BW were maximized by feeding at least 0.30% available phosphorus. Increased tibia ash values were also obtained by feeding the medium particle-sized (16-50) commercial oyster shell product in the chick's diet.

According to Guinotte & Nys (1990), a larger beneficial effect of coarse particles size on percentage ash of the tibia was observed but no significant difference stemming from origin of calcium sources for this criterion. Bone measurements were not affected by an interaction between origin and size of calcium sources. Ionised blood calcium and pH of hens fed the calcium sources were not significantly different. Total calcium was increased by the use of sea shells during eggshell formation and inorganic phosphorus was higher in hens fed on the ground limestone diet. Metabolic energy, nitrogen retention or calcium retention values were not different on different sources. The solubility of calcium carbonate depends on the size of the particle but also on the source origin.

The above cited studies underscore the important effect of calcium level, sources and particle size on production. However, the important effects of calcium level, sources and particle size are not confined to production only. These variables also impact on egg quality. The literature on the effects of calcium level, sources and particle size on egg quality is reviewed in the following section.

2.7 Effect of calcium level, sources and particle size on metabolic processes

Scott *et al.* (1982) also suggested that due to the slow movement of large particles in the digestive tract they are exposed to an acidic environment to dissociate the calcium carbonate into ionic calcium, resulting in calcium available for absorption. Hens require ionic calcium for intestinal absorption. Despite the conflicting results from various studies on the beneficial effect of large particles, calcium or with low *in vitro* solubility on eggshell and bone state (Scott *et al.*, 1971). Roland (1986) states a positive effect of large particles staying longer in the digestive tract and therefore being exposed to acidic conditions and thereby releasing calcium.

Studies of Cheng & Coon (1987) and Zhang & Coon (1997) demonstrated that a low *in vitro* solubility compared to a higher *in vitro* solubility of limestone is superior for eggshell quality and bone status. The low *in vitro* solubility of calcium supplements allows for increased gizzard retention and *in vivo* solubility that is required for utilization.

2.8 Conclusions

From the literature reviewed, it is evident that calcium plays an important role in egg production in layers. However, from the literature, no study has been done on the ideal particle size and/or distribution of specifically limestone as a source of calcium in layer diets, and how it will influence egg production and quality. Furthermore, no study has underscored the importance of limestone as a readily available viable source of calcium for layer feeds, without replacing it with other calcium sources such as oyster shells. This study seeks to fill this hiatus in research and in the literature.

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CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

The present chapter provides a general overview of the materials and methods used in the study. Specific materials and methods used are detailed in chapter 4 and chapter 5 respectively.

3.2 Materials

The materials used in the present study were as follows:-

(a) Experimental pullets.
(b) Battery cage system.
(c) Feed.
(d) Measuring devices.

Details pertaining to each of the above materials are discussed below.

3.2.1 Experimental pullets

The pullets used in the study were 17 weeks old Lohmann Silver pullets obtained from a commercial layer pullet producer, namely Nulaid farm (Paardefontein).

3.2.2 Battery cage system

The pullets were housed in a battery cage system as shown in Figure 3.1. Birds were individually housed to monitor the feed intake and production of each hen. The hens were placed in individual cages and housed in a building with natural ventilation. Lights were controlled by time switches according to the recommended hours of lighting as per schedule supplied by the breeding company. All cages were fitted with feed troughs, water nipples and perches.

Figure 3.1 Battery cage systems

3.2.3 Feeding

Following the weeks of adjustment from 17 to 18, the hens were fed test diets. The dietary treatments consisted of the feeding of three limestone particles sizes namely <1 mm; 1-2 mm and 2-3.8 mm (in the first experiment; chapter 4), as well as different ratios of fine and coarse particle sizes (in the second experiment; chapter 5) to supply a dietary calcium level of 3.8%. The ratios were 100 fine: 0 coarse, 0 fine: 100 coarse, 75 fine:25 coarse, 50 fine:50 coarse and 25 fine:75 coarse. The birds had free access to water and feed.

A basal layer feed (Table 3.1 and 3.2) diet was fed containing 0.6% calcium from an amorphous limestone. This diet was supplemented with the test sources of calcium to a final level of 3.8% Ca. The test sources consisted of limestone of three different particle sizes namely <1 mm, 1-2 mm, >2-3.8 mm. These were included singly and in all combinations to form six dietary treatments all having the same calcium level but coming from different grit sizes and combinations of sizes.

An amorphous limestone source, halfway between Dwaalboom and Northam in the Limpopo province of South Africa was used. The 90 % $CaCO_3$ limestone source contained 36 % calcium. The physical and calculated chemical composition of the feed is presented in Table 3.1 and 3.2.

Figure 3.2 Limestone particle size distributions in layer diets

The pullets were fed *ad libitum* and feed intake of each pullet determined as described in paragraph 3.3.1.

Table 3.1 Physical composition of the basal layer diet on an air dry basis

Raw materials	Percentage (%)
Yellow maize	60.09
Maize gluten	1.38
Wheat bran	1.81
Full fat Soya	5.0
Soya oil cake	7.24
Sunflower oil cake	10.0
Fish meal	3.27
Calcium carbonate	9.58
Mono-calcium phosphate	0.74
Fine salt	0.37
Natuphos 500 (phytase 500 high inclusion)	0.06
Sodium hydro carbonate	0.04
Choline powder	0.01
Methionine	0.02
Mineral/vitamin Premix	0.4

Table 3.2 Calculated chemical composition of the basal layer diet on an air dry basis

Component	%
Moisture	10.00
Crude Protein	17.0
Fat	4.16
Ash	13.37
Neutral-detergent. fibre	10.00
Acid-detergent fibre	4.95
Fibre	3.75
Calcium	3.6
Phosphorus	0.56
Available phosphorus	0.29
Chlorine	0.3
Sodium	0.18
Potassium	0.57
Magnesium	0.24
Metabolisable energy (MJ/kg)	11.48
Arginine	1.08
Isoleucine	0.68
Lysine	0.79
Methionine	0.36
Threonine	0.61
Tryptophan	0.18
Methionine + Cystine	0.68

3.2.4 Measuring devices

Measuring devices are shown in Figures 3.3, 3.4, 3.5, 3.6 and 3.7. Accurate calibrated scales were used to measure the weight of the feed, birds and eggs. Scales to measure the weight of the feed and birds were 0.01g sensitive (Figure 3.6 and 3.7), while the scale used for eggshell weight was 0.001g sensitive (Figure 3.4). An eggshell thickness meter (Figure 3.3 and 3.5) sensitive to 0.01 mm was used for measuring shell thickness.

Figure 3.3 Egg thickness meter

Figure 3.4 Scale used to measure eggshell weight

Figure 3.5 Measurement of shell thickness

Figure 3.6 Scale used to measure egg weight

Figure 3.7 Scale used to measure body weight of birds

3.3 Methods

The following data were collected in the present study namely; weekly feed intake per pullet; daily egg weight and number of eggs; eggshell thickness, egg weight and body weight. A general discussion of these methods is provided in the following sections.

3.3.1 Determination of weekly feed intake per pullet

A 20 l plastic bucket with approximately 5 kg of feed for each pullet was accurately weighed, i.e. a bucket for each of the pullets. Each day each bird was fed approximately 100g of feed in the morning and more feed was added in the afternoon if necessary. After seven days, the feed intake was determined by weighing the bucket and remaining feed as well as the residues in the feed trough. Feed disappearance was determined by difference and considered to be the weekly feed intake.

3.3.2 Recording of daily egg weight and number of eggs

From 18 weeks of age, eggs were collected from each of the laying birds and egg numbers and weight recorded and summarised on weekly basis throughout the experimental period (i.e.18-28weeks). Abnormal eggs, which were shell-less and those with defective shells were recorded for production calculations.

3.3.3 Determination of eggshell thickness and weight

Five eggs of each hen in the six groups were randomly collected at week 24 to determine the shell quality. Following the measurement of egg weight, egg was broken and shell thickness and shell weight (including membranes) determined. The shells were washed under slightly flowing water to remove adhering albumen (Kuhl & Seker, 2004; Nordstrom & Ousterhout, 1982; Strong, 1989) and wiped with a paper towel to remove excessive moisture. A thickness meter sensitive to 0.001 mm was used for measuring the eggshell thickness. Three thickness measurements were made on the sharp, blunt and equator parts of the shell and the average calculated for each. This method was developed by Ikeme *et al.* (1983) and adapted by Ehtesham & Chowdhury (2002).

The following variables, i.e. egg surface area; egg contents; egg volume; egg shell weight per unit area; and shell percentage were investigated in the process of determining eggshell thickness and weight. The formulae used to obtain these variables were as follows:

(a) Egg surface area (ESA) = $3.9782W^{0.7056}$ (Carter, 1974,1975)

(b) Egg contents (EC) = EW (Egg weight) – SW (Shell Weight) (Narushin, 1977; and Arad & Marder, 1982.

(c) Egg volume:=a W b where, a = 0.7608 (constant)
b = 1.0474 (constant)
W = egg weight
(Carter., 1974, 1975).

(d) Shell percentage = SW/EW*100

(e) Shell weight per unit surface area (SWUSA) = SW/ESA

(f) Egg output = % Egg production x egg weight

3.3.4 Monitoring of body weight

Individual body weights of birds were recorded at week 18, 20, 24 and 28 as illustrated in Figure 3.7.

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CHAPTER 4

EFFECT OF PARTICLE SIZE OF LIMESTONE ON EGG PRODUCTION AND SHELL QUALITY OF LAYERS AT PEAK PRODUCTION

4.1 Introduction

A major concern in poultry production is egg breakage which still represents a large economic loss to the poultry industry. It was estimated that 13-20% of total eggs produced are cracked or lost before reaching their final destination (Roland, 1988). Due to the losses brought about by egg breakages a lot of research has been conducted in the past to increase eggshell strength. There are many factors involved in egg shell formation and shell quality. However, of all these factors, calcium, as a major constituent of the eggshells, features as the most prominent factor affecting shell quality. The macro factors include, but are not limited to, the source and level of calcium in the diet, phosphorus level in the diet, temporal intake of these minerals and particle size of calcium supplements. Less than optimum calcium can cause demineralisation of the bone, low serum calcium levels and subsequently low egg production of thin-shelled eggs and consequently high egg breakage.

Optimum particle size of calcium supplements for layers has been a controversial subject for many years (Miller & Sunde, 1975). Research focusing on the effect of different limestone particle sizes in layers diets on layer performance and shell quality has yielded conflicting results. Roland *et al.* (1974), Muir *et al.* (1975) and Kuhl *et al.* (1977) report that particle size of a calcium source has no effect on eggshell thickness, egg breaking strength and specific gravity. In a series of studies Scott *et al.* (1971), Roland,. (1986) and Guinotte *et al.* (1991) established a positive effect on egg shell quality when calcium sources with coarse particle size was included in layer diets. Scott *et al.* (1971) and Ahmad & Balander (2003) attributed the improved eggshell strength obtained from feeding hen-sized oyster shell to the longer retention time in the gizzard which allowed the calcium to be "metered out" into the intestines during the time of maximum need for shell calcification.

It seems that differences in calcium solubility might be responsible for the contradictory findings. Rabon & Roland (1985) have shown that the solubility of limestone particles of similar size from different sources varied by as much as 62%. The fact that mineral calcium sources like limestone differ in their origin, purity, and particle size is a major concern. In South Africa, amorphous limestone from Limpopo province a single source is mainly used for supplementing layer diets. As far as could be established there is no published information available whether the different particle sizes that are marketed by this supplier are equally suitable to support high egg yields and good shell quality. This research is especially relevant with the advent of the modern high-producing layer strains. The aim of this study was therefore to investigate the influence of different particle sizes from a specific limestone supplier on egg production and eggshell quality at peak production.

4.2 Materials and Methods

Ninety-nine layer pullets at 17 weeks of age were obtained from a commercial layer-strain breeder. The pullets were randomly allocated to three groups (n= 33/group). Pullets in each group received individually the same layer diet, composition as in Table 3.2.3, except that the calcium was supplied one of three different commercial limestone particle sizes:

(a) less than 1.0 millimetre
(b) between 1.0 and 2.0 millimetre
(c) between 2.0 and 3.8 millimetre

These particle sizes are manufactured by screening the limestone through several sieves of different diameter. The source consists of an amorphous limestone as described in Chapter 3 and contains 90 % $CaCO_3$ and thus 36 % calcium.

The hens were randomly placed in individual cages within a common room for all treatments. Cages were fitted with feed troughs, water nipples and perches. The birds had individually free access to water and feed. Feed intake was recorded weekly. At arrival (week 17) the hens were subjected to a sixteen (16) hour light and an eight (8) hour darkness regime, regulated by a timer.

Eggs numbers were recorded daily and summarised on weekly basis throughout the experimental period (i.e.18-28weeks). Abnormal eggs, which were shell-less and those with defective shells were recorded for production calculations. Percent lay on a daily basis was calculated using the formula given by North (1984). Individual egg weights were recorded for all the eggs produced by each hen on a daily basis.

Five eggs of each hen in the three groups were randomly collected at week 24 to determine the shell quality. Following the measurement of egg weight, eggs were broken and shell thickness and shell weight (including membranes) determined. The shells were washed under slightly flowing water to remove adhering albumen (Kuhl & Seker, 2004; Nordstrom & Ousterhout, 1982; Strong, 1989) and wiped with a paper towel to remove excessive moisture. A thickness meter sensitive to 0.001 mm was used for measuring the egg thickness. Three measurements were made: three on the sharp end, three on the blunt and three on the equator parts of the egg and the averages of each used in the statistical analyses of the data. This method was developed by Ikeme *et al.* (1983) and adapted by Ehtesham & Chowdhury (2002).

Some external quality traits of the egg were also calculated, namely surface area = ($3.9782W^{.7056}$), where W is the egg weight in gram; shell weight per unit surface area (SWUSA) = (mg/cm^2); egg volume according to the procedure described by Carter (1974; 1975), Narushin (1977) and Arad & Marder (1982). Egg output was calculated as % egg production x egg weight. (North & Bell, 1990).

4.2.1 Statistical analysis

Data were subjected to ANOVA using the general linear model procedure (SAS Institute, 1999) to determine the effect of particle size and age on response variables relating to egg production. The same procedure was followed to determine the effect of the particle size on response variables (shell thickness, shell weight, shell percentage, SWUSA, egg surface area, egg volume and egg contents).

4.3 Results and Discussion

4.3.1 Feed intake

The weekly feed intake of the hens on the diets with different particle sizes is shown in Table 4.1 and Figure 4.1. It seems that the particle size of limestone in the diet did not significantly (P>0.05) influenced the feed intake of hens. These results are in agreement with that of Guinotte *et al.* (1991) and El-Agguory *et al.* (1989) who found that a particles range 0.15 to 3.35 mm did not affect feed intake in White leghorn hens. An average daily feed intake of 118 g was calculated for the total experimental period across the treatments. The low initial feed intake at 18 weeks of age in the different treatments could be attributed to stress and adaptation to the new surroundings.

Table 4.1 The effect of dietary limestone particle size on the weekly feed intake (g) of layers

week	**Particle size**			**Significance (P)**	**CV**
	1 mm	1-2 mm	>2-3.8 mm		
18	716	665	769	0.1682	23.6
19	770	748	803	0.5618	18.0
20	740	729	749	0.9330	14.0
21	717	732	734	0.9583	12.5
22	740	747	751	0.1767	9.7
23	762	745	755	0.7155	9.4
24	775	741	749	0.3524	9.7
25	771	759	773	0.2956	10.1
26	777	772	802	0.1556	8.9
27	737	715	729	0.2923	8.0
28	748	734	734	0.8497	7.8

CV = coefficient of variance

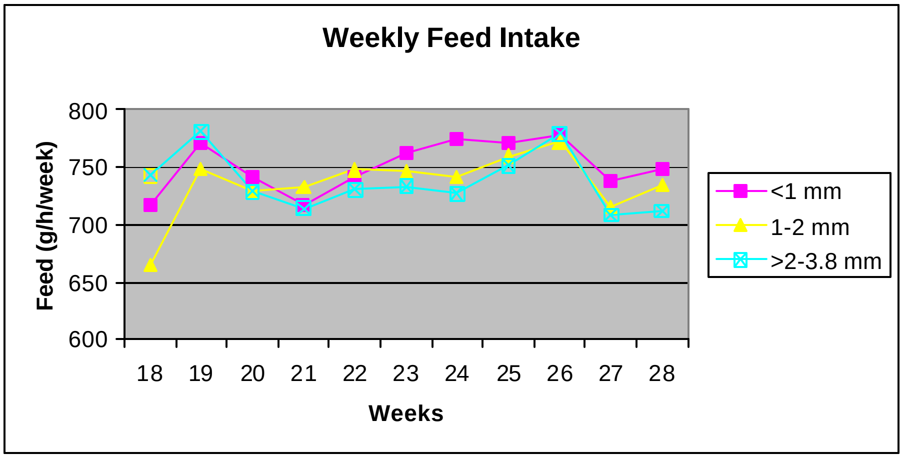

Figure 4.1 Effect of dietary limestone particle size on the feed intake of layers

4.3.2 Body weight

The body weight pattern of layers as outlined in Table 4.2 and Figure 4.2 followed the same trend as that of feed intake as shown in Figure 4.1. No significant (P>0.01) influence of particle size on the body weight of the birds could be detected. Anderson *et al.* (1984) reported lower weight gains and bone ash values when layers (22-32 weeks) were fed a diet with powdered limestone compared to medium sized particles. Guinotte *et al.* (1991) observed no effect of calcium origin nor particle size on live weight of hens, but those fed coarse particles had higher body weights (P<0.01). Factors like origin, purity and solubility of limestone as well as age of layers and diet could contribute to these varying findings.

A statistically significant (P<0.001) increase in body weight of layers occurred from week 18 to 28. The average body weight of hens for treatments with <1 mm, 1-2 mm and >2-3.8 mm particle sizes at 19 weeks were 1.78 kg, 1.78 kg and 1.77 kg respectively. During the entire experimental period the hens receiving these diets showed a weight gain of 145 g, 155 g and 71 g respectively. Layers receiving the coarsest particles consumed significantly less feed than the other two groups. Accordingly Hurwitz *et al.* (1969) found no significant differences in body weight although control birds fed mash had higher weight gain.

Table 4.2 Body weight (g) changes of layers

week	Particle size			Significance (P)	CV
	<1 mm	1-2 mm	>2-3.8 mm		
18	1784	1789	1828	0.8186	10.2
20	1844	1852	1867	0.9934	8.5
24	1873	1897	1860	0.8513	7.1
28	1929	1944	1900	0.8104	7.2

CV = coefficient of variance

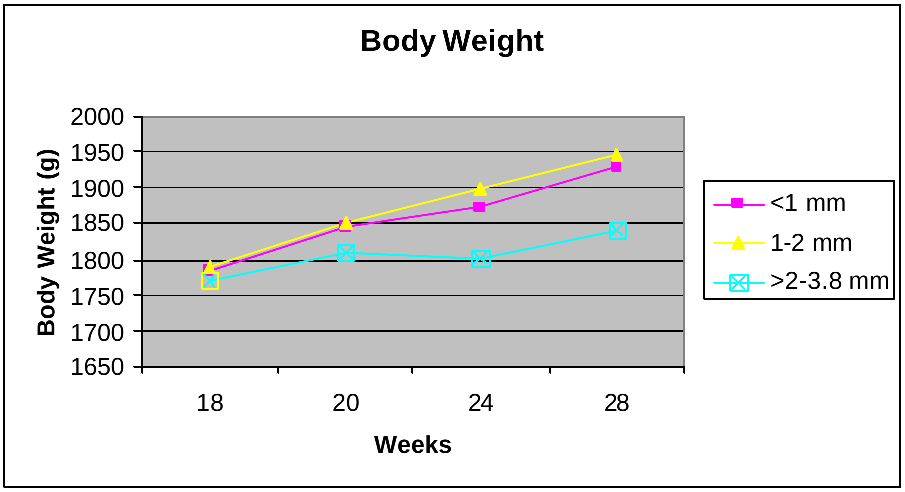

Figure 4.2 Body weight changes of layers

4.3.3 Egg production

The influence of limestone particle size on egg production is set out Table 4.3 and Figure 4.3. It is clear that the physical size of limestone did not significantly (P> 0.05) influence the rate of egg production of layers. The results were in agreement with that of Hurwitz *et al.* (1969), Guinotte & Nys (1990) and Watkins *et al.* (1977) who found that production is not affected by particle size in Leghorn hens fed ground and hen-sized particle sizes.

A significant (P<0.001) increase in egg production occurred over the entire duration of the experimental period. The largest (P<0.05) increase occurred from week 18 to 22. Thereafter egg production was more or less constant (P>0.05) up to week 28. Accordingly Sreenivas (1997) found that egg production remains constant just around peak production in order to lengthen the egg formation time.

Cracked and shell-less eggs represented 11% of the total egg production during the experimental period week 19 to 24 and week 28. This was lower than the 13-20 % reported by Guinotte & Nys (1990) for the period (20-30 weeks). Most of the cracked or shell-less eggs were recorded at the treatment with fine particles. Watkins *et al.* (1977) stated that ground limestone produce poor egg shells oppose to coarse ones.

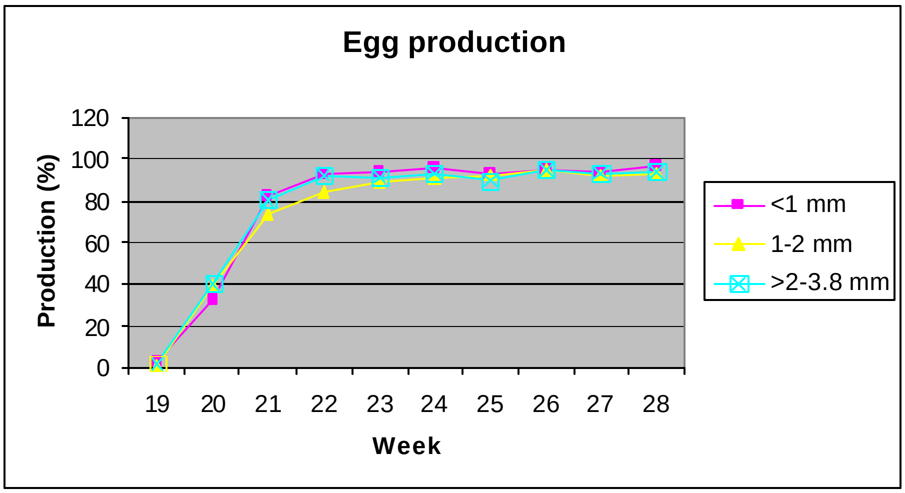

Figure 4.3 Effect of dietary limestone particle size on egg production

Table 4.3 The influence of limestone particle size on egg characteristics at peak production (week 24)

Parameter	Particle size			Significance (P)	CV%
	< 1 mm	1-2 mm	>2-3.8 mm		
Egg. production (%/h/d)	79.82	74.87	79.73	0.1114	14.0
Egg weight (g/egg)	43.87	49.36	51.69	0.2159	12.9
Egg output (g)	56.72	51.97	56.00	0.2388	22.3
Egg volume (ml)	41.53	38.16	38.94	0.1114	17.0
Egg contents (g)	38.63	44.35	46.45	0.2317	13.3
Egg surface area (cm^2)	57.33	62.31	64.37	0.1011	16.4
Shell weight (g)	5.24	5.01	5.24	0.4710	17.1
SWUSA (mg/cm^2)	91.40	80.40	81.40	0.2099	18.0
Shell percentage (%)	10.42	10.15	10.14	0.2229	22.3
Shell thickness (mm):					
Sharp end	0.432	0.422	0.432	0.1335	4.1
Equator	0.442	0.432	0.422	0.7994	14.5
Blunt end	0.432	0.422	0.422	0.4613	4.5

SWUSA = Shell weight per unit surface area

CV = Coefficient of variation

4.3.4 Egg weight

From Table 4.3 it is evident that the particle size of limestone in layer diets did not significantly influence egg weight (P=0.2159) as well as egg output (p=0.2388) at peak production (24 weeks). Average egg weight only tended (P=0.2159) to be heavier when coarse limestone was included in the diet. In contrast with these results Watkins *et al*. (1977) reported that the inclusion of coarse particle sizes of limestone in layer diets improved egg weight.

Age and body weight are primary factors that influence egg weight. From Figure 4.4 it is evident that egg weight increased (P<0.0001) from week 19 to 28. These findings confirm previous observations that egg weight is the lowest at the beginning of the production cycle and increase throughout the laying period (Leeson & Summers, 1982; McDaniel, 1983).

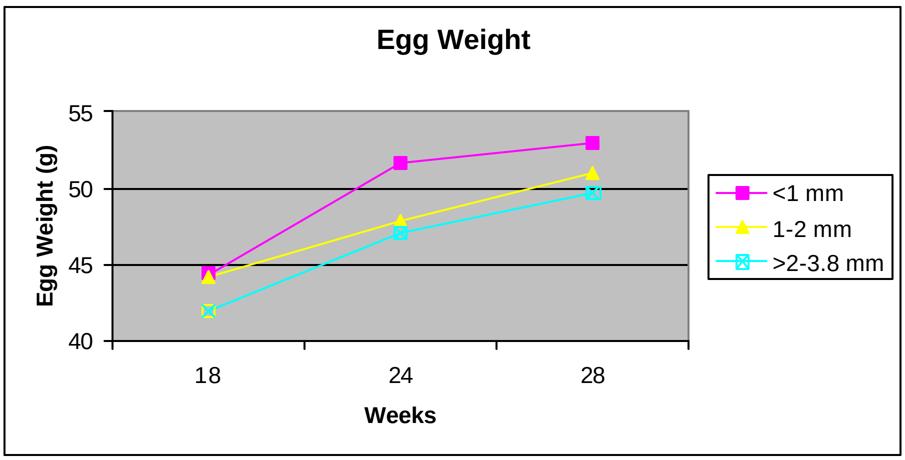

Figure 4.4 Effect of age of layers on egg weight

4.3.5 Egg shell quality

The term "shell quality" is frequently used as a synonym for shell strength and denotes the ability of eggshells to withstand externally applied forces without cracking or breaking (Hamilton, 1982). Egg shell quality can be defined by variables such as egg specific gravity through its relationship to shell porosity as shown by the positive correlation with pore concentration (Peebles & Brake, 1987). Factors that affect the strength of eggshells are heredity, clutch position, rate of production (Hammerle, 1969), age, health status (disease), season, temperature, nutrition (Hammerle, 1969; Woolford., 1994), strain of hen, oviposition, egg shell ultra structure (Hamilton *et al.*, 1979a; 1979b). Other factors are housing system, length of lay and neuro-humoral reproductive control mechanisms (Woolford, 1994). The most common physical properties associated with egg shell strength are shell thickness, shell weight, shell percentage per unit surface area (SWUSA) and may be classified as shell quality measurements.

In Table 4.3 data on egg volume, egg contents, egg surface area, SWUSA, shell percentage and shell thickness are presented. No statistical significant differences in any of these egg characteristics could be detected. According to these results limestone particle size in layer diets has no influence on eggshell quality. Accordingly Zhang & Coon (1996) and Keshavarz & Nukajima (1993) and Keshavarz (1998) reported no statistically significant influence of limestone particle size on eggshell thickness and weight. Roland (1986 and 1988), Miller & Sunde (1975), Scott *et al.* (1971) and Guinotte & Nys (1990), however, stated that larger particles are superior to small or medium particles in improving the eggshell strength and weight. Rabon and Roland (1985) have shown that the solubility of limestone particles of similar size from different sources varied by 62 %. Therefore solubility differences could also be responsible for the contrary results. The contrary results may be attributed to the source of calcium. The period in lay could probably also be responsible for the varying results.

4.4 Conclusions

The results of the present trial are not in agreement with the views held by some nutritionists that coarse particles in layer diets improve egg production and egg shell quality. It might be that the practice to supply a portion of the Ca requirements as grit could have a beneficial effect towards the end of the laying year and not during peak production when pullets still have large supplies of Ca as medullary bone. This could be the subject for future investigations.

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CHAPTER 5

EFFECT OF DIFFERENT RATIOS OF COARSE AND FINE LIMESTONE PARTICLES ON PRODUCTION AND SHELL QUALITY OF LAYERS AT PEAK PRODUCION

5.1 Introduction

Any deficiency in the supply or problem in calcium metabolism will lead to weaker eggshells. This will have serious consequences on hatching egg quality as well as on the production of table eggs. According to Roland (1986), the average calcium requirements for eggshell formation within a population of hens are the greatest at approximately peak production and that was the reason why the present investigation was limited to that period only.

There are several factors involved in egg shell formation of which calcium as a major constituent of the eggshell feature prominently. In this regard not only the source and level of calcium is important but also the particle size of the calcium source. Several researchers (Roland, 1986; 1988, Miller & Sunde, 1975; Scott *et al*., 1971; Guinotte & Nys, 1990) are of the opinion that larger particles are superior to small or medium in improving egg shell strength and weight. In contrast with these researchers, results of Cheng & Coon (1987), Keshavarz & Nakajima (1993) and Keshavarz, (1998), Roland *et al*. (1972a) and the results of Chapter 4, no influence of large particle size on egg shell thickness and egg weight could be detected. Therefore the question arises whether the distribution of different particle sizes of a specific calcium source in layer diets is not an important factor in the egg shell formation. Solubility of calcium carbonate depends on the particle size and also on the source origin (Guinotte & Nys, 1990). The ultimate aim should be to supply fine and coarse limestone particles in such a ratio in layer diets that calcium is frequently available for egg shell formation. Small particle sources such as pulverised $CaCO_3$ passes quickly through the digestive tract and the bird may not be able to sufficiently extract enough to meet its needs. On the other hand, ground limestone could be absorbed by the hen during the day when the hen is eating, but during the hours of darkness metering of calcium occurs in the digestive tract from the gizzard because of the breakdown of the shell grit or limestone chips (Woolford, 1994). Larger particle sizes of the same compound (e.g. $CaCO_3$ in the form of coarse limestone or oyster shell) will be retained in the gizzard for a longer period of time (Korver, 1999; Keshavarz, 2001, Woolford, 1994). The situation allows for a gradual release of calcium from the gizzard to the small intestine for absorption, resulting in increased time over which the hen receives dietary calcium. According to Farmer *et al.* (1986) the aim is to offer the bird a constant supply of calcium to improve the shell characteristics and not an excess since it lowers production. The essence is to find an alternative avenue of combining both calcium particles with a rapid passage that is a readily available calcium source and those that release the calcium slowly so to have a constant supply of calcium during the entire day for shell formation and egg contents.

The aim of the study was therefore to investigate the effect of particle size distribution from a specific limestone source in a layer diet on egg production and egg quality at 24 weeks (peak production) of age.

5.2 Materials and Methods

One hundred and sixty seven point-of-lay pullets at 17 weeks old of the same batch that were used for Experiment 1 (chapter 4) were obtained from a commercial pullet rearer. All the pullets received the same layer diet except for the particle size distribution of the calcium supplement that was in the diets during lay. The composition of the diet was as shown in paragraph 3.2.3.

The pullets were randomly allocated to five groups of thirty three pullets per group. Pullets in each group received one of five different ratios of fine (less than 1.0 mm) and coarse (between 2.0 and 3.8 mm) limestone particles namely 100, 75, 50, 25 and 0% fine or coarse particles.

The two particle sizes of limestone grit were obtained from a commercial supplie r of limestone to the poultry industry. These were classed as Fine, (F) (particle size < 1.0 mm) and Coarse, (C) (particle size 2.0 – 3.8 mm). The two types were mixed in the following ratios 100F: 0C, 75F: 25C, 50F: 50C, 25F: 75C and 0F: 100C. There were

thus five dietary treatments with 34 individual hens in single cages serving as replicates for each treatment.
Limestone was screened through sieves to obtain samples with appropriate diameters. An amorphous limestone as described in Chapter 3 and Chapter 4 was used. The limestone source contained 90 % $CaCO_3$ and 36 % calcium.

Cages were fitted with feed troughs, water nipples and perches. The individual birds had free access to water and feed. Feed intake was recorded weekly. At arrival (week 17) the hens were subjected to a sixteen (16) hour light and an eight (8) hour darkness regime, regulated by a timer.

Individual egg weights were recorded for all the eggs produced by each hen on daily a basis. Eggs numbers were recorded daily and summarised on a weekly basis throughout the experimental period (*i.e.*18-28weeks). Abnormal eggs that were shell-less and those with defective shells were also recorded for production calculations. Percent lay on a daily basis was calculated using the formula given by North (1984).

Five eggs of each hen were collected at week 24 to determine the shell quality. Following the measurement of egg weight, an egg was broken and shell thickness and shell weight (including membranes) determined. The shells were washed under slightly flowing water to remove adhering albumen (Kuhl and Seker, 2004; Nordstrom & Ousterhout, 1982; Strong, 1989) and wiped with a paper towel to remove excessive moisture. A meter sensitive to 0.001 mm was used for measuring the eggshell thickness. Three measurements were made on the sharp, blunt and equator part of an egg and average thickness obtained for individual location (see Table 5.2). This method was developed by Ikeme *et al.* (1983) and adapted by Ehtesham & Chowdhury (2002).

Some external quality traits of the egg were calculated using formula on the above-mentioned measures, i.e. surface area ($3.9782W^{.7056}$), where W is the egg weight in gram , shell weight per unit surface area SWUSA = (mg/cm^2), and egg volume were calculated according to procedure described by Carter (1974; 1975) Arad & Marder (1982) and Narushin (1997). Egg output was calculated as in Chapter 4 by multiplying % egg production x egg weight (North & Bell, 1990).

5.2.1 Statistical analysis

Data were subjected to ANOVA using the general linear model procedure (SAS Institute , 1999) to determine the effect of particle size distribution and age on response variables relating to egg production. The same procedure was followed to determine the effect of particle size distribution on response variables (shell thickness, shell weight, shell percentage, SWUSA, egg surface area, egg volume and egg contents).

5.3 Results and Discussion

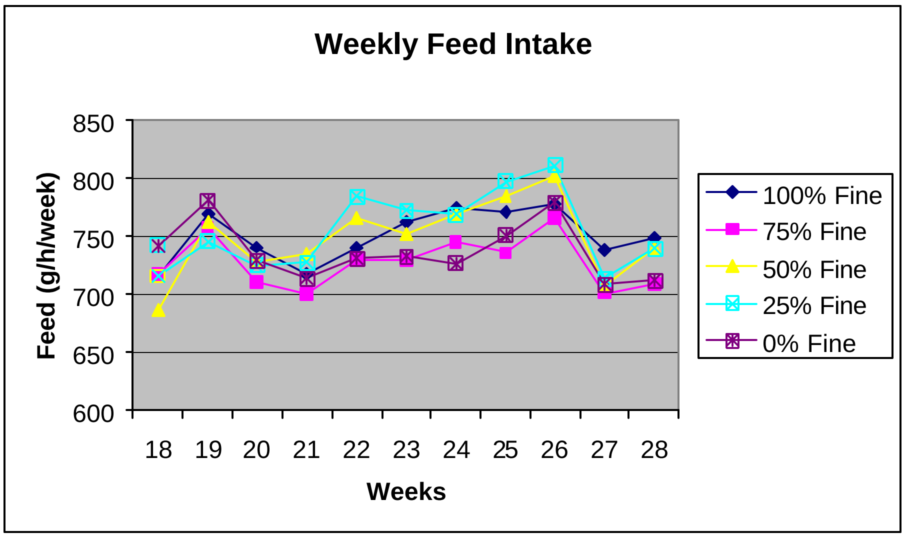
5.3.1 Feed intake

The weekly feed intake of the hens on the diets with different particle sizes is shown in Table 5.1 and Figure 5.1. It seems that the different particle size distributions of limestone in the diet did not significantly (P>0.05) influenced the feed intake of hens. Watkins *et al.* (1977) observed that particle size distribution did not affect feed intake significantly. An average daily feed intake 119 g of was calculated for the total experimental period across treatments. In accordance with Chapter 4 the lower feed intake recorded at arrival (week 18) compared to week 19 for the hens in the different treatments could probably be attributed to stress and adaptation to the new surroundings. Variation in temperature could probably explain the fluctuations in feed intake during the experimental period. The lower intakes of hens at weeks 26 and 27 could be attributed to higher prevailing temperatures. A highly significant (P<0.001) treatment x age interaction for intake occurred. According to statistical analysis feed intake significantly (P<0.001) increased over time. These results are in agreement with a trial conducted by Guinotte & Nys (1990).who found that significant increases occurred in feed intake in Leghorns from 66 to 77 weeks , when hens were fed particulate limestone were supplemented with coarse particles of limestone .

Table 5.1 Effect of limestone particle size distribution on weekly feed intake (g) of layers

Week	**Particle size ratios (% fine: coarse)**					**Significance (P)**	**CV**
	100	**75**	**50**	**25**	**0**		
18	716	739	686	716	769	0.1682	23.6
19	770	780	762	746	803	0.5618	18.0
20	740	732	727	724	749	0.9330	14.0
21	717	722	734	727	734	0.9583	12.5
22	740	751	766	784	751	0.1767	9.7
23	762	752	751	772	755	0.7155	9.4
24	775	768	770	768	749	0.3524	9.7
25	771	758	785	797	773	0.2956	10.1
26	777	789	801	811	802	0.1556	8.9
27	737	722	707	714	729	0.2923	8.0
28	748	731	739	739	734	0.8497	7.8

CV = coefficient of variance



Fine <1.0 mm, Coarse >2.0-3.8 mm

Figure 5.1 Effect of limestone particle size distribution on weekly feed intake of layers

5.3.2 Body weight

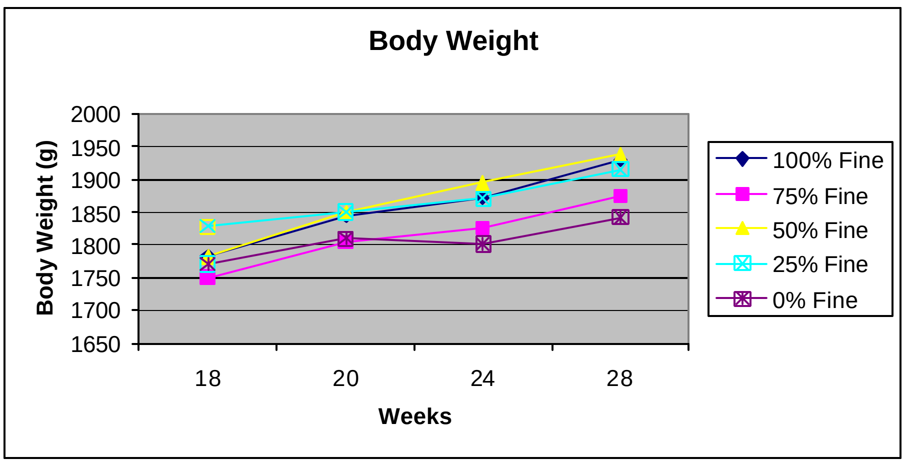
The changes in body weight of layers are outlined in Table 5.2 and Figure 5.2. In accordance with feed intake, no significant ($P>0.05$) influence of particle size distribution on the body weight of the birds could be detected. A statistically significant ($P<0.001$) increase in body weight of layers occurred from week 18 to 28. The average body weight of hens with the 100, 75, 50 25 and 0 fine limestone particles in the diet were 1.78 kg, 1.77 kg, 1.75 kg, 1.78 kg and 1.83 kg respectively.

During the entire experimental period the hens receiving the diets with 100, 75, 50 25 and 0 fine limestone particles showed a weight gain of 146 g, 71 g, 124 g, 156 g and 86 g respectively.

Table 5.2 Body weight (g) changes of layers

Week	Particle size ratios (fine: coarse)					Significance (P)	CV
	100	**75**	**50**	**25**	**0**		
18	1784	1805	1782	1828	1828	0.8186	10.2
20	1844	1861	1850	1850	1867	0.9934	8.5
24	1873	1883	1895	1871	1860	0.8513	7.1
28	1929	1933	1938	1915	1900	0.8104	7.2

CV = coefficient of variance



Fine <1.0 mm, Coarse >2.0-3.8 mm

Figure 5.2 Effect of limestone particle size distribution on the body weight in layers

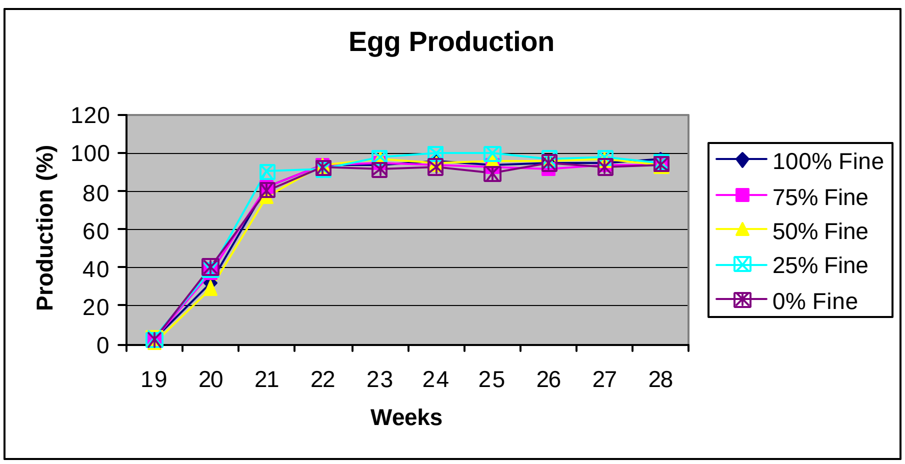
5.3.3 Egg production

From Table 5.3 and Figure 5.3 it seems that different ratios of limestone particle sizes did not influence (P = 0.3041) egg production. Similarly Watkins *et al.* (1977) observed no influence of particle size distribution on egg production. The results are also in agreement with that of Hurwitz *et al.* (1969) and Guinotte & Nys (1990) found that production is not affected by particle size using ISA Brown hens fed ground and particulate sizes.

Table 5.3 The influence of limestone particle size on egg characteristics at peak production (Week 24).

Parameter	Particle size ratios (fine: coarse)					Significance (P)	CV %
	100%	75%	50%	25%	0%		
Egg production (%)	79.82	80.09	77.40	81.30	79.68	0.3041	9.0
Egg weight (g)	49.54	48.87	48.13	48.80	47.77	0.4558	8.4
Egg output (g)	39.0	38.0	38.0	37.5	39.5	0.5066	15.6
Egg volume (ml)	41.53	39.73	39.95	40.56	38.92	0.1310	10.2
Egg contents (g)	44.38[a]	44.02[a]	42.89[b]	43.42[b]	42.98[b]	0.0001	14.4
Egg surface area (cm^2)	62.5	61.87	61.21	61.81	60.88	0.1393	9.2
Shell weight (g)	5.16[a]	4.85[b]	5.24[a]	5.38[a]	4.79[b]	0.0017	13.4
Shell percentage (%)	10.44[ab]	9.95[a]	10.98[ab]	11.06[b]	10.12[ab]	0.0001	19.1
SWUSA (mg/cm^2)	82.65	78.39	85.61	87.04	78.68	0.0142	14.3
Shell thickness (mm):							
Sharp end	0.432	0.422	0.432	0.432	0.422	0.1429	4.6
Equator	0.442	0.432	0.432	0.432	0.452	0.3314	11.7
Blunt end	0.432	0.422	0.432	0.432	0.432	0.2468	4.2

Means within rows with different superscripts differ at P<0.05, SWUSA = Shell weight per unit surface area, CV = Coefficient of variation



Fine <1.0 mm, Coarse >2.0-3.8 mm

Figure 5.3: Effect of different ratios of limestone particles on egg production in layers

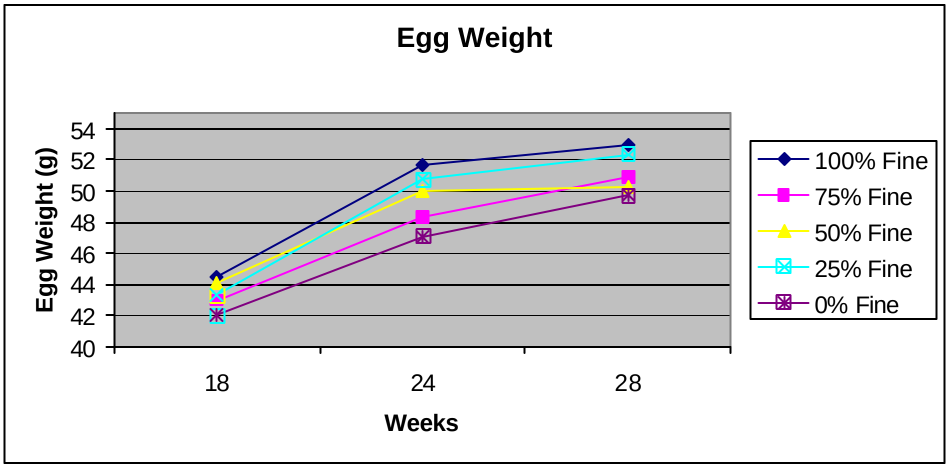
Figure 5.3 illustrates that there was a significant (P<0.05) increase in production from week 18 to 21. Thereafter egg production remained constantly high. In accordance Leeson & Summers (1982) and McDaniel (1983) found a non significant (P> 0.05). increase in production for hens fed oyster shell for the period 21 to 30 weeks.

A significant (P<0.001) increase in egg production occurred over the entire duration of the experimental period. An average production percentage of 80 % was observed up to Week 28. This corresponded well with the 78 % calculated in Chapter 4. This is in agreement with Sreenivas (1997) who found a constant egg production occurred at peak.

Cracked and shell-less eggs represented 9% of the total egg production during the experimental period, week 19 and 28. This was lower than the 13-20 % reported by Guinotte & Nys (1990) for the period 20 to 30 weeks. Most of the cracked or shell-less eggs were recorded at the treatment with fine particles. Watkins *et al*. (1977) is of the opinion that ground limestone produce poor egg shells opposed to coarse ones.

5.3.4 Egg weight

Egg weight (P = 0.4558) as well as egg output (P= 0.5066) was not significantly influenced by limestone particle size in the diet (Table 5.3). These findings are in agreement with Cheng & Coon (1987), who concluded that switching from ground limestone to coarse oyster shell resulted in no significant differences in egg weight. From Figure 5.4 it is evident that egg weight increased (P<0.05) from week 19 to 28. It can be seen from Figure 5.4 that the egg weights of all the rations began around ±40g and increased to ±50g by the 24th week and maintained the trend up to the 28th week. These findings confirmed the previous observations that egg weight is the lowest at the beginning of the production cycle and increase throughout the laying period (Leeson & Summers, 1982; McDaniel, 1983).



Fine <1.0 mm

Coarse >2.0-3.8 mm

Figure 5.4: Effect of dietary particle size distribution on egg weight

5.3.5 Egg quality

The term "shell quality" is frequently used as a synonym for shell strength and denotes the ability of eggshells to withstand externally applied forces without cracking or breaking (Hamilton, 1982). The most common physical properties associated with egg shell strength are shell thickness and shell specific gravity. Richards & Staley (1967) suggested that shell thickness, shell weight, shell percentage and shell weight per unit surface area (SWUSA) may be classified as shell quality measurements, as these variables are highly significantly (P<0.01) correlated with each other.

In Table 5.3 the influence of limestone particle size distribution on egg volume, egg contents and egg surface area can be observed. No significant differences occurred in egg volume (P=0.1310) and egg surface area (P=0.1393). The highest (P<0.001) egg contents was recorded where 100 and 75 % fine limestone particles were included in the diet. Although significant differences for shell weight (P<0.0017) and shell percentage (P< 0.0001) occurred, no clear influence of particle size distribution on these characteristics could be detected.

From Table 5.3 it further seems that SWUSA was significantly (P<0.0142) different amongst treatments but this was not confirmed by Tukey's test. In accordance with

SWUSA no significant (P> 0.05) different in eggshell thickness occurred. The findings of this trial are in contrary to the findings of Watkins *et al.* (1977) who observed that replacement of two-thirds of fine calcium particles with hen size particles of improved egg-shell strength. Accordingly Dekalb (1998) is of opinion that one third of the layer dietary calcium should be supplied in large particle form (2-5 mm). Factors like the source of calcium and the time of laying period could probably explain these contrary results. Zhang & Coon (1997) stated that the limestone retention of calcium in the gizzard of laying hens for improving shell quality may be dependent upon particle size, porosity of the calcium source and overall *in vitro* solubility of the calcium source.

5.4 Conclusions

From the results it seems that the ratio of fine (<1.0 mm) and coarse (>2.0-3.8 mm) limestone particles in a layer diet does not influence egg production and egg shell quality (shell thickness, egg weight, egg output, egg surface area, shell percentage and SWUSA) at 24 weeks of age. These results apply however, only for the specific limestone used in this study and for peak production. The influence of dietary limestone particle size distributions at a later stage of the laying period on egg production and egg quality warrants further research.

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CHAPTER 6

GENERAL CONCLUSIONS

Calcium plays a vital role in bone and shell formation. It is important that calcium should be provided in the right amounts to ensure that strong shells are produced and that bone demineralisation does not occur in an attempt to compensate for calcium deficiencies during egg shell formation. In this regard not only the calcium intake is of importance, but other factors like calcium source, particle size of the calcium source, distribution of the particle size in the diet, origin of the calcium source, purity of calcium source and age of the hen could influence bone structure and shell formation. In the present study the effect of particle size and distribution of a specific amorphous limestone source on feed intake and weight changes of layers as well as egg production and characteristics at peak production (24 weeks) were investigated.

It seems from the results that particle size of limestone (<1 mm, 1 - 2 mm or 2 - 3.8 mm) in layer diets does not influence feed intake or body weight gain of layers. Egg characteristics *viz* shell quality, egg size, shell weight and shell thickness were also not influenced by the different particle sizes.

The experimental diets were all supplemented to the same calcium level, namely 3.8% and no information was thus obtained on what the effect of particle size at higher or lower calcium levels would have been.

The influence of limestone particle size on bone characteristics was not addressed in the present study and the investigation was also only limited to the period of peak production (week 24). Bone fractures often occur at the end of lay and are indeed of concern regarding the welfare of layers at depletion. A more pronounced influence of dietary limestone particle size on egg production and especially shell formation might also be of more relevance at the end of lay and warrants further investigation. This probably also apply for the influence of dietary limestone particle size distribution on bone integrity.

From the results it could be concluded that dietary limestone sizes varying from <1 mm, 1 - 2 mm or 2 - 3.8 mm had no influence on egg production or egg quality characteristics at peak production (week 24).

ABSTRACT

A study was conducted to investigate the influence of different particle sizes of a specific amorphous limestone source (calcium carbonate) in a layer diet on egg production and egg quality at peak production (week 24).

Ninety nine layer pullets, 17 weeks old, were obtained from a commercial layer breeder. The pullets were randomly allocated to three groups. All the pullets received the same layer diet. The only differences among the treatment groups were that the pullets in each group received one of the three different particle sizes in the diet namely:-

(a) Less than 1.0 millimetre
(b) 1.0 and 2.0 millimetre
(c) 2.0 and 3.8 millimetre

The influence of the three particle sizes on the feed intake, body weight and egg production as well as egg characteristics was recorded.

The average weekly feed intake, body weight and egg production of layers from week 18 up to 28 were not (P> 0.05) different among the layer diets with the different particle sizes. The particle size of limestone in the layer diets did not influence egg weight (P= 0.2159) and egg output (P=0.2388) significantly at peak production. Average egg weight tended (P= 0.2159) to be heavier when coarse particles were fed. No statistical significant (P> 0.05) differences in egg volume, egg contents, egg surface area, shell weight per unit surface area, shell percentage and shell thickness occurred. According to these results limestone particle size in layer diets has no influence on eggshell quality.

In the second study the effect of particle size distribution of a specific limestone source in layer diet on egg production and egg quality at peak production (week 24) was investigated.

One hundred and sixty seven layer pullets, 17 weeks old, were obtained from a commercial layer breeder. The pullets were randomly allocated to five groups. All the pullets received the same layer diet. The only differences among the treatment groups were that the pullets in each group received one of five different ratios of fine (less than 1.0 mm) and coarse (between 2.0 and 3.8 mm) limestone particle size namely 100, 75, 50, 25 and 0 % fine or coarse. The influence of the five dietary limestone particle size distributions on the feed intake, body weight, and egg production of layers as well as egg characteristics mentioned in the first study were investigated.

No significant ($P > 0.05$) influence of dietary limestone particle size distribution was found on feed intake, body weight and egg production of layers. Accordingly no significant differences occurred in egg volume, ($P = 0.1310$) and egg surface area ($P = 0.1393$). The highest ($P < 0.001$) egg contents were recorded where 100 and 75% fine limestone particles were included in the diet. Although significant differences for shell weight ($P < 0.0017$) and shell percentage ($P < 0.0001$) occurred, no clear influence of particle size distribution on these characteristics could be detected.

In accordance with shell weight per unit surface area no significant differences ($P > 0.05$) in eggshell thickness occurred.

It was concluded that different dietary limestone particle sizes and distributions have no influence on eggshell quality at peak production (week 24). The influence of dietary limestone particle size and distribution during the later stages of the laying period on bone formation and egg quality needs further investigation.

OPSOMMING

’n Studie is uitgevoer om die invloed van verskillende partikelgroottes van ’n spesifieke amorfe kalksteenbron (kalsiumkarbonaat) in lêhendiëte op eierproduksie en eierkwaliteit by piekproduksie (24 weke) na te gaan.

Nege-en negentig, 17 weke oud lêhenne is van ’n kommersiële teler verkry. Die henne is ewekansig in drie groepe ingedeel. Al die lêhenne het dieselfde lêhendieet ontvang. Die enigste verskille tussen die behandelings was dat die lêhenne in elke groep een van drie verskillende partikelgroottes in die diet ontvang het naamlik:-

a) kleiner as 1.0 millimeter
b) tussen 1.0 en 2.0 millimeter
c) tussen 2.0 en 3.8 millimeter

Die invloed van die drie partikelgroottes op voerinname, liggaamsgewig en eierproduksie asook eiereienskappe is ondersoek.

Die gemiddelde weeklikse voerinname, liggaamsgewig en eierproduksie van lêhenne vanaf 18 tot 28 weke het nie betekenisvol ($P>0.05$) tussen die lêhendiete met verskillende kalksteen partikelgroottes verskil nie. Die partikelgroottes van voerkalk in die lêhendiete het nie eiergewig betekenisvol ($P=0.2159$) by piekproduksie beïnvloed nie. Gemiddelde eiergewig ($P = 0.2159$) en eiermassa (g eier/hen/dag) ($P= 0.2388$) het geneig om swaarder te wees indien growwe kalksteen verskaf is. Geen statisties betekenisvolle ($P>0.05$) verskille in eiervolume, eierinhoud, eieroppervlakte, dopgewig per eenheid oppervlakte, doppersentasie en dopdikte het voorgekom nie. Volgens hierdie resultate het kalksteen, partikelgrootte in lêhendiete geen invloed op eierdopkwaliteit nie.

In ’n tweede studie is die invloed van partikelgrootte verspreiding van ’n spesifieke kalksteenbron in ’n lêhendieet op eierproduksie en eierkwaliteit by piekproduksie (24 weke) ondersoek.

Eenhonderd sewe- en sestig 17 weke oud lêhenne is van 'n kommersiële teler verkry. Die lêhenne is ewekansig aan vyf groepe toegeken. Al die lêhenne het dieselfde lêhendieet ontvang. Die enigste verskille tussen die behandelings was dat die lêhenne in elke groep onderskeidelik een van vyf verskillende verhoudings van fyn (minder as 1.0 mm) en growwe (tussen 2.0 en 3.0 mm) kalksteenpartikels ontvang het naamlik 100, 75, 50, 25 en 0% fyn of grof. Die invloed van die vyf dieetkalksteen partikelgrootte verspreidings op voerinname, liggaamsgewig en eierproduksie van lêhenne asook die eiereienskappe genoem in die eerste studie, is ondersoek.

Geen betekenisvolle ($P>0.05$) invloed van dieetkalksteen partikelgrootte verspreiding op voerinname, liggaamsgewig en die eierproduksie van die lêhenne is gevind nie. Dienooreenkomstig is geen betekenisvolle verskille in eiervolume ($P = 0.1310$) en eieroppervlakte ($P = 0.1393$) gevind nie. Die hoogste ($P<0.001$) eierinhoud is gevind waar die 100% en 75% fyn kalksteen partikels in die dieet ingesluit is. Alhoewel betekenisvolle verskille vir dopgewig ($P<0.0017$) en doppersentasie ($P<0.0001$) voorgekom het, kon geen duidelike invloed van partikelgrootte verspreiding op hierdie eienskappe waargeneem word nie.

In ooreenstemming met dopgewig per eenheidsoppervlakte het geen betekenisvolle ($P>0.05$) verskille in eierdopdikte voorgekom nie.

Daar is tot die gevolgtrekking gekom dat verskillende dieetkalksteen partikelgroottes en verspreidings, geen invloed op eierdopkwaliteit by piekproduksie (24 weke) tot gevolg het nie. Die invloed van beide dieetkalksteen partikelgrootte en verspreiding gedurende die latere stadiums van die lêperiode op beenvorming en eierkwaliteit vereis verdere ondersoek.