# THE CALCIUM REQUIREMENT OF COMMERCIAL LAYER PULLETS IN THE PRE-LAYING PERIOD

by

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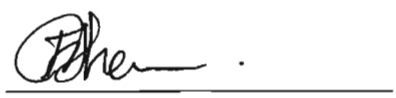
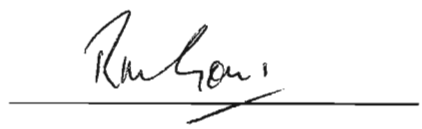
## DECLARATION

I hereby declare that the research reported in this thesis does not contain material which has been accepted for the award of any other degree or diploma in another person, except where due reference is made in the text.





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# ABSTRACT

Specifications for the amount of calcium to be included in feeds for pullets and laying hens vary considerably in the scientific literature. Much of this variation is due to the fact that genetic selection has changed the growth rate, age at sexual maturity and potential egg production of laying hens over the years, and these changes will continue in the future. Husbandry techniques have also changed, so that pullets are now sometimes encouraged to start producing eggs at an earlier age with the use of appropriate lighting programmes. The supply of calcium to pullets in the pre-laying period depends critically on the age at which the pullets reach maturity and start to lay eggs, as pullets should be given an opportunity to deposit calcium in their medullary bone just before laying commences. The difficulty is knowing how much calcium should be included in the feed before sexual maturity, and for how long before this event a higher calcium content should be included, in order to minimise problems associated with mineral deficiencies and excesses during the laying period.

The aim of the present study was to determine whether, by offering pullets a choice of two feeds varying in calcium content, the choice they made in the period leading up to sexual maturity could be used to determine the amount of calcium that should be fed to them during this period. Two experiments were conducted with Hyline pullets: in the first, 384 pullets were used, starting at 14 weeks of age, and in the second, 144 birds were used, starting at 10 weeks of age. In both experiments, equal numbers of Hyline Brown and Hyline Silver pullets were used.

The first experiment consisted of four dietary treatments: Two basal diets were formulated to contain high (30 g calcium/kg) and low (8 g calcium/kg) calcium contents, with all other nutrients being the same. These two basal diets were fed alone and as a 1:1 blend to produce an intermediate calcium diet (19 g calcium/kg). The fourth treatment consisted of the low calcium basal and limestone grit as a choice diet. At 18 weeks of age six pullets from each treatment were killed for analysis of tibia breaking strength and 144 of these pullets (72 Silver and 72 Brown) were selected randomly and kept on the same treatment as before, but individually so that age at sexual maturity could be determined. There was no significant difference observed in age at sexual maturity or mortality, but pullets that were on the low calcium feed

consumed significantly more feed and consequently, attained higher body weight gain than the other treatments. The opposite occurred for pullets that were on the high calcium diet. There was no significant effect of dietary calcium content on tibia breaking strength at 17 weeks.

For the second experiment, pullets (n = 144) were reared on a lighting regime of 8L:16D to 10, 14 or 18 weeks, at which ages the photoperiod was increased to 14 hours. This had the effect of altering the age at sexual maturity, so that the effects of age and attainment of sexual maturity could be separated when determining the choice made by pullets in the amount of calcium consumed in the pre-laying period. In all cases, pullets increased their intake of calcium approximately two weeks before attaining sexual maturity, this increase being independent of the age of the pullets at the time.

The study revealed that commercial laying-type pullets increase their intake of calcium, when given the opportunity to do so, approximately two weeks prior to the onset of lay. Where they do not have a choice between two sources of calcium this increased requirement for calcium causes pullets on low calcium feeds to increase their intake of feed and consequently simultaneously increase the intake of all nutrients other than calcium, resulting in an increased body weight. Where birds are fed a high calcium feed only, food intake does not increase to the same extent during this period, but the increase observed is likely to be to satisfy the increased demand for nutrients other than calcium in this pre-laying period.

On the basis of the choices made by pullets in this study, these birds should be reared on low calcium feeds until two weeks before the onset of lay, at which stage the calcium should be increased to enable the pullets to deposit calcium in their medullary bone in preparation for the increased demand for calcium in the laying period.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# GENERAL INTRODUCTION

The importance of calcium in relation to egg production is obvious. It can influence the number and size of eggs as well as shell thickness, but the calcium content of the feed may also influence the food intake. Therefore, dietary calcium can contribute substantially to the profitability of a poultry enterprise. Calcium is one of the essential minerals for normal functioning of essential biochemical processes in the body (e.g. blood clotting, shell formation, muscle contraction and transmission of nerve impulses). Supplying sufficient calcium in the diet means providing sufficient bioavailable calcium to maximize economically important processes such as growth and reproduction. This can be accomplished by providing adequate calcium in the diet or supplying supplementary limestone grit to the feed, since it is known that pullets have the ability to consume calcium to meet their requirement for maintenance and production.

The effect of calcium on shell quality and bone strength is actually a complex interaction of many mechanisms. Some mechanisms depend on the reproductive and hormonal status of the bird, some on the intestinal pH, the amount of feed consumed or the calcium source and form. Calcium is the most important nutrient to be considered when shell quality or bone problems occur, although it is known that functioning of hormones like parathyroid hormone, deficiency of vitamin $D_3$, calcitonin and other minerals, such as phosphorous, also affect shell quality. The calcium status of pullets and laying hens is co-regulated by parathyroid hormone, calcitonin and vitamin $D_3$, acting directly and indirectly on the intestine, bone and kidney, with the overall scheme of chemical, hormonal and target tissue interaction.

Controversy exists regarding the feeding of pullets prior to sexual maturity with little consensus as to whether or at what stage the dietary calcium content should be increased prior to the onset of laying. Genetic improvement of breeds and strains has altered the laying performance, bodyweight and age at sexual maturity of modern strains and these improvements, together with the latest environment interventions (lighting programme), have added to the confusion regarding the feeding of calcium prior to onset of lay.

Recommendations regarding the feeding of calcium to pullets prior to sexual maturity differ markedly: some suggest that calcium may be increased some weeks before the

onset of lay with no adverse affect on laying performances, whilst others point out that by doing so, the parathyroid gland becomes inactive resulting in a reduction in the ability of the bird to metabolise calcium leading to bone weakness and thinning of eggshells. The aim of the present study was to determine whether the pullets themselves could resolve the issue by making a rational choice in the daily amount of calcium consumed during the period leading up to sexual maturity, when presented with two feeds differing in calcium content. Because birds are known to have a calcium appetite, increased requirement for calcium would elicit high intake of calcium. In this way it was hoped that the controversy could be resolved.

# CHAPTER 1

# LITERATURE REVIEW

## 1.1 INTRODUCTION

Egg production is the ultimate goal when rearing a layer pullet. Laying performance is the integration of the genetic capability of the hen, the feed offered and the environmental conditions that prevail. It is well known that potential laying performance can be reduced by physiological alterations to the growing bird, due to the application of certain feeding and lighting strategies during rearing. Such alteration might result in distinctive changes in body characteristics during the pre-lay period, and may even alter processes relating to organ and tissue development. A nutrient having such an influence is calcium. If nutrient supply is not limited, each body organ or tissue will follow its own distinctive maturation curve. As a consequence, there will be a variation in the nutritional demand of every organ or tissue in the course of time, related to the development of that respective body structure. This means that the supply of nutrients (e.g. calcium) for certain organs may be critical at particular stages of immature growth. Ignoring such critical periods might influence subsequent performance negatively. Nutritional programs for pullets therefore have to be adjusted to take into account the stages of development of important body structures (e.g. the ovary and oviduct of the laying hen) (Kwakkel, 1993).

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There are many reasons for the scientific interest in calcium. One obvious reason involves the enormous agricultural economic importance of the domestic species that provides a significant part of our food supply. The low cost of calcium, together with the fact that calcium is a crucial factor in formation of eggshells, formation of blood clots, muscle contraction and transmission of nerve impulse conduction, results in excessive use of this nutrient in the diets of laying hens. Calcium is also involved in the regulation of heartbeat, can act as an activator or stabilizer of enzymes and is involved in hormone secretion.

Calcium metabolism in egg laying birds is amazing when compared with all other classes of vertebrates in the animal kingdom. The turnover of calcium in female birds during reproduction is many times more rapid than in mammals. A hen that lays 250

eggs a year secretes a quantity of calcium in the form of shell, corresponding to about 20 times the calcium content of her entire body (Sturkie, 1976). The production of the first egg is a very important event in the bird's metabolism: the body has to release 2g of calcium for shell formation, the main sources of which are feed and medullary bone reserves (Leeson and Summer, 1997). During the laying period, the bird utilizes large amounts of its medullary bone reserves to supplement its diet supply when shell is being formed.

The bird must maintain effective homeostatic mechanisms for storing the calcium in the medullary bone until it is required, then for removing calcium from storage, and transporting it to the uterus for shell formation. The process of shell formation is complex, and for this reason it is likely that things could go wrong, leading to poor eggshell quality, weaker bones and possibly even death. So it is vital to know the factors that influence the efficiency of utilization of calcium in the body. By understanding all the factors, management strategies and feeding strategies can be optimized to avoid some of the problems over which we have control. The nutrition, environment, and genotype affect the metabolism of calcium. But it is not only important to consider these factors once the birds are in lay, because the factors responsible for calcium metabolism can be manipulated before sexual maturity. In this review, factors concerned with calcium metabolism in the pullet and laying hen will be discussed.

## 1.2 THE ROLE OF CALCIUM IN LAYING HENS

Calcium is one of the most abundant minerals in the body and is often the major cation in the diet. Ninety nine percent of the body's calcium is located in the skeleton and the remaining one percent is distributed throughout the extracelluallar and intracellular fluids. When there are bone or shell problems, calcium is the nutrient that is mostly considered, although in actual fact many hormones and minerals can influence bone and shell quality.

Blood is the transport medium by which calcium is moved from the gastrointestinal tract to other tissues for utilization. Because the shell gland does not store enough calcium for shell formation, the ion must be extracted continuously from the blood. All

blood calcium is in the plasma and can be separated into two components: an ultrafiltrable fraction, which is primary ionic calcium, and a non-ultrafiltrable fraction, which is protein bound. The most obvious functions of calcium are to provide structural integrity to the skeleton and to contribute to the maintenance of blood calcium levels through on-going resorption and deposition. Calcium in bone tissue is not in a steady state, it is constantly being mobilized and deposited as bone growth. During the last 15 hours of shell formation, calcium moves across the shell gland of the hen at a rate of 100-150 mg/h. Because shell formation involves the removal of 100-150 mg of calcium /h, the concentration of calcium in blood would be zero within 8-18 minutes if it were not replenished constantly through intestinal absorption and mobilization from the bone (Sturkie, 1976).

Calcium is also important for controlling the excitability of nerves and muscles. Low calcium ($Ca^{2+}$) concentrations result in increased excitability of pre- and postganglionic nerve fibers and muscle. Calcium is also essential for normal blood-coagulation and for muscle relaxation because it stimulates adenosine triphosphatase. Calcium regulates the level of phosphorylation of endogenous protein in the nervous system (Georgievskii, 1982).

## 1.3 MEDULLARY BONE IN BIRDS

Just prior to the onset of sexual maturity, pullets develop unique labile and dynamic reserves of calcium in the form of non-structural bone, and because it is easily observed in the marrow cavity of the long bones, it is called medullary bone. It is believed that most of the increase in the pullet skeletal weight before sexual maturity is caused by the formation of this bone material (Miller, 1992). Hurwitz and Bar (1987) reported that medullary bone formation is a primary function of hormones rather than a result of the increased accumulation of calcium in the body due to increased absorption. The formation of medullary bone starts from the endosteal surface of the cortex in the form of interlacing spicules, which eventually fill the entire marrow cavity. Blood supply in the marrow cavity increases, which facilitate mobilization of the bone mineral during shell formation as well as its subsequent replacement, so the supply of blood plays a significant part in medullary bone formation. During calcium reduction, medullary bone calcium is maintained despite the exhaustion of calcium from structural bone, indicating

that medullary bone is not a simple reservoir of calcium: it also serves to buffer acute interruptions in calcium absorption, preventing hypo-or hypercalcaemia situations through hormonal control.

Dietary calcium content had a significant ($P < 0.05$) effect on total medullary bone calcium, as measured (Table 1.1) in an experiment conducted by Clunies *et al.* (1992). However, dietary calcium level appeared to have no significant ($P > 0.05$) effect on the individual medullary bones. The largest reserves of medullary bone calcium were found in the femur, followed by the tibiotarsus, regardless of level of calcium fed. Also, all other long bones (the carpus, radius, ulna, tarsus, and clavicle) when combined did not contain as much medullary bone calcium as the femur and the tibiotarsus separately. Birds fed diets containing 45 g calcium/kg had intermediate levels of medullary bone calcium that were not significantly different from those fed diets with 25 and 35 g calcium/kg. In an experiment conducted by Parks *et al.* (1992) an increase in dietary calcium to 45 g calcium/kg increased shell weight, but in the trial by Clunies *et al.* (1992) it was found that 45 g calcium/kg did not increase medullary calcium deposition. Therefore, high dietary calcium content might increase shell weight but do not have the same effect on medullary bone. Birds fed diets containing 45 g calcium/kg had the lowest amount of medullary bone calcium, while the birds fed 35 g calcium/kg had the highest. So increasing the dietary concentration to 45 g calcium/kg did not increase the deposition of calcium into medullary bone.

TABLE 1.1 The effect of calcium level on the medullary bone calcium reserves of laying hens (Clunies *et al.*, 1992).

| Calcium | Medullary bone calcium | | | | |
|---|---|---|---|---|---|
| Level (g/kg) | Femur (g) | Tibiotarsus (g) | Humerus (g) | Other[1] (g) | Total (g) |
| 25 | 0.226 | 0.204 | 0.54 | 0.116 | $0.60^{B}$ |
| 35 | 0.294 | 0.236 | 0.06 | 0.152 | $0.74^{A}$ |
| 45 | 0.246 | 0.228 | 0.07 | 0.138 | $0.68^{AB}$ |
| | NS | NS | NS | NS | * |
| SD | 0.044 | 0.044 | 0.03 | 0.031 | 0.08 |

$^{A,B}$means within columns with no common superscripts are significantly different ($P < 0.01$). [1]Includes the carpus, radius, scapula, tarsus, and ulna. *Significant ($P < 0.05$)

How medullary bone metabolism is affected by differences in dietary calcium levels remains controversial. Clunies *et al.* (1992) found that the relationship between medullary bone and dietary calcium was not linear, and an optimal level of dietary calcium was required to maximize medullary bone calcium reserves in active laying hens. However, Hurwitz and Bar (1966) found that low medullary bone calcium in laying hens was a result of low dietary calcium, not optimal levels of dietary calcium. Sohail and Roland (2000) reported that increasing dietary calcium from 31 to 37 g calcium/kg increased bone mineral content from 0.165 to 0.174 g/cm$^2$ and bone density from 0.252 to 0.274 g/cm$^2$. For each increase of 3.5 g calcium/ kg diet there is approximately 5% increase in bone mineral content and bone density. Dietary calcium had an effect on bone breaking strength: increasing calcium from 31 g calcium/ kg to 37 g calcium/ kg increased bone breaking strength from 16.5 to 21.5 kg. For every 3 g calcium/kg increase there was approximately 10% increase in bone breaking strength. Frost and Roland (1991) found that feeding three levels of dietary calcium (27.5, 37.5 and 42.5 g calcium/kg) to layers at 31 weeks of age had a significant effect on tibia breaking strength. Tibia ash and bone mineral content increased significantly with increasing dietary calcium. Birds that were fed 27.5, 37.5, and 42.5 g calcium/kg had 6.74, 7.14 and 7.48 kg bone strength respectively. They concluded that hens at peak production require at least 45.3 g calcium/kg per day for maximum bone strength.

The size of the medullary calcium pool also plays a critical role in the metabolism of calcium. Hurwitz and Bar (1969) reported that the overall ability of the hen to direct calcium from medullary bone to the eggshell depends on the amount of calcium stored in the medullary bone. An inadequate intake of calcium during the prelay period results in lower calcium stores at the onset of production. Hurwitz and Bar (1971) believed that during the early stage of egg production the calcium absorption mechanism is not fully developed.

In conclusion, dietary calcium deficiency or over- supply during the prelaying period will adversely affect bone development, and will therefore influence skeletal integrity. A deficiency or inappropriate levels of diertary calcium will lead to rickets and other disorders as well as impaired laying performance.

## 1.4 METABOLISMS AND HORMONAL CONTROL OF CALCIUM

### 1.4.1 Hormones involved in calcium regulation

There are three major hormones that control calcium in the body, these being parathyroid hormone (PTH), calcitonin and 1,25-dihydroxycholecalciferol ($1,25(OH_2)D_3$, the active metabolite of vitamin $D_3$), but prostaglandins, reproductive steroids and other hormones also play a role. Increased secretion of oestrogen and androgen at the beginning of follicle maturation stimulates the development of medullary bone. Changing levels of oestrogen may also regulate some of the actions of PTH on bone (Miller, 1992). Only the major hormones that are involved in calcium homeostasis will be discussed in this section.

#### 1.4.1.1 Parathyroid hormone

The parathyroid hormone is a protein or a polypeptide with a molecular weight of 9500, which contains 84 amino acid residues (Georgievskii, 1982). Parathyroid hormone regulates calcium metabolism, which is essential for eggshell formation, muscular contraction, blood clotting, enzyme systems, calcification of tissue, neuromuscular regulation, and the maintenance of a constant level of calcium in the blood (Georgievskii, 1982). The principal targets of parathyroid hormone are bone cells for mobilization of calcium and the renal tubules for tubular excretion of phosphate. Thus, parathyroid hormone fulfils its function by intensifying the excretion of phosphate in urine, as a result of increased secretion of phosphate anions in the distal segments of the renal ducts. This results to an increased level of calcium ions in the blood serum, so the greatest effect of parathyroid hormone is when the concentration of phosphate changes. The parathyroid hormone maintains the correct Ca:P ratio, is implicated in the absorption of calcium from the intestine, and stimulates the reabsorption of calcium in the convoluted tubules when calcium in the serum is low (Miller, 1992).

#### 1.4.1.2 Calcitonin

Calcitonin is synthesised by the ultimobranchial glands, which are just distal to the parathyroid glands. It is a polypeptide hormone with a molecular weight of 3000-4500 consisting of 32 amino acid residues with certain specificity (Georgievskii, 1982). The main function of calcitonin is the protection of the bones or skeleton from excessive resorption of calcium during the egg-laying period.

1.4.1.3 Vitamin D

Vitamin D is the collective name for a family of compounds having antirachitic properties. The most important of these compounds is ergocalciferol (Vitamin $D_2$) and cholecalciferol (Vitamin $D_3$). The form that affects calcium metabolism in birds is cholecalciferol. Consequently more emphasis will be given to this form throughout the review. The general function of vitamin $D_3$ is to raise plasma calcium and to maintain the level of phosphorous to the ratio that maximises bone mineralization. Parathyroid hormone, together with vitamin $D_3$, prevents tetany by increasing plasma calcium concentration and by stimulating the active transport of calcium and phosphorous across the intestinal epithelium (Georgievskii, 1982).

1.4.2 Calcium metabolism in birds

The maintenance of relatively constant levels of plasma ionic calcium is called calcium homeostasis. In the body, calcium homeostasis takes priority over other processes involving calcium, such as bone and eggshell calcification. Calcium homeostasis is essential in the regulation of important biological processes, such as cellular information transfer, hormonal biosynthesis and release, and cellular replication (Miller, 1992).

The body makes use of many homeostatic mechanisms, which are designed to maintain a constant level of circulating calcium plasma. The mechanisms act directly on the intestine, bone and kidney. The kidney serves as a safety valve, operating on a time-to-time basis to excrete excess calcium and phosphate. The quantities of calcium and phosphorous excreted in urine are determined by the cumulative rates of three processes: glomerular filtration, tubular reabsorption and tubular secretion with the help of hormones in order to maintain the right amount of calcium in the body (Wideman, 1986).

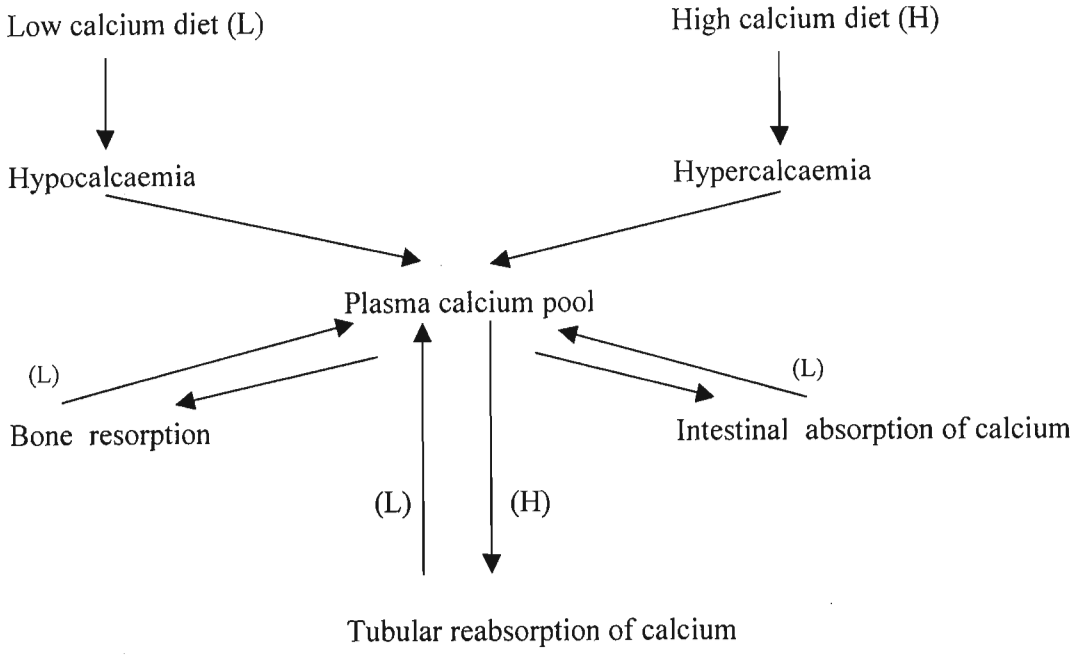


Figure 1.1. The effect of low (L) and high (H) calcium intake on calcium homeostasis (Shafey, 1993)

When a low calcium diet is fed the condition that results is called hypocalcaemia. A decline in plasma calcium concentration triggers the release of parathyroid hormone, which stimulates the biosynthesis of the metabolically active form of vitamin $D_3$ [1,25-$(OH)_2D_3$] by the kidney. This in turns increases calcium absorption from the gastrointestinal tract and re-absorption of calcium from the bone and the renal tubules (Figure 1.1). When the kidney increases calcium reabsorption it also decreases phosphate reabsorption, resulting in increased retention of calcium in the body (Shafey,1993).

When a high calcium diet is fed the resultant condition is called hypercalcaemia. An increase in plasma calcium concentration triggers the parafollicular cells in the thyroid to release calcitonin, which depresses plasma calcium by inhibiting bone resorption. Thus the amount absorbed from the gastrointestinal tract depends on the amount ingested and the proportion absorbed. As the percentage of calcium in the diet increases the proportion absorbed tends to decline. This is related to the fact that calcium absorption is an active process under the control of a calcium binding protein, which is vitamin $D_3$ dependent. In a vitamin $D_3$ ddeficiency, calcium absorption is reduced

because of impaired calcium binding protein formation, so skeletal and shell abnormalities can occur even in the presence of enough dietary calcium (Shafey, 1993).

## 1.5 FACTORS AFFECTING THE ABSORPTION OF CALCIUM

### 1.5.1 Source and form of calcium

Bioavailability of calcium is important in explaining the difference between the total amount of calcium in a feed ingredient and the amount that is used by the bird consuming the feed ingredient. The bioavailability of calcium is determined by measuring growth rate, ash percentage of the bone or the composition of the bone ash (Shafey, 1993). There are several types of calcium supplement on the market, but not all of them are created equal. There are inorganic and organic sources, which are also different in calcium bioavailability, since both the solubility and the concentration of calcium in these sources differ.

*Inorganic Sources of calcium*

Inorganic sources of calcium are found in limestone, gypsum, rock phosphate, calcium gluconate, mono- and dicalcium phosphate, meal and dolomite. All these supplements have different solubilities and concentrations of calcium.

*Organic Sources of calcium*

Organic sources of calcium such as that found in fishmeal are more readily available to birds than calcium from dicalcium phosphate and limestone (Shafey, 1993).

Research has indicated that improvements in shell quality can be obtained by feeding part of the dietary calcium as oyster shell or limestone chips. The hen's requirement for calcium is relatively low except at the time of the day when eggshell formation is taking place. Calcium is the major nutrient involved in shell calcification but researchers have questioned whether to use limestone or oyster shell. These calcium sources can vary widely in price. Oyster shell and limestone were probably the first, and are still the most common, concentrated sources of calcium fed to laying hens, and most experiments have been based on them (Roland, 1986).

A good quality limestone or oystershell should contain 380 to 390 g calcium/ kg. To ensure maximum shell quality it is recommended that hens consume a minimum of 3.75g calcium per day, and that in older hens or hens with shell quality problem, the calcium intake should be increased by as much as 1 g/d depending on the severity and type of shell quality problem (Roland, 1986). How limestone or oystershell should be fed to poultry is still not clear: some researchers believe that feeding limestone or oystershell continuously on a free choice basis, or on top of a diet which contains the full calcium requirement is not recommended, because egg shells show some chalky deposits and rough ends, and some soft shelled eggs are produced as a result. These unusual conditions are due to a deficiency of phosphate, because much of the phosphate ingested is excreted as soluble calcium phosphate (Leeson and Summers, 1997).

### 1.5.2 Particle size

The particle size of the calcium source plays a role in ensuring whether or not calcium is available in the gut when needed. It was reported by Coon *et al.* (2001) that larger particle size limestone results in increased weight gain, feed efficiency and calcium availability to the poultry at low dietary calcium levels. He suggested that the increased availability to laying hens of larger particles is the result of a slower rate of passage through the digestive tract of the hen, allowing absorption when needed for eggshell formation. Roland (1986) reported that larger particles are retained in the gizzard during the day. This means that large particles of calcium are released more slowly and this may be important for continuity of shell formation, especially in the dark period when birds are reluctant to eat or when there is no feed. The rate and percentage of calcium absorption may also depend on the rate of passage of food, which is quite variable in the various intestinal segments (Hurwitz and Bar, 1986)

### 1.5.3 Intestinal pH

A major effect of high calcium concentration in poultry may be a reduction in the bioavailability of other minerals. Phosphorous and calcium absorption is optimal at pH 6. When the pH is higher than 6.5 absorption of phosphorous is markedly decreased and calcium tends to precipitate from the solution (Shafey, 1993). Excess free fatty acids in the diet can cause the pH to decrease and therefore interfere with calcium and phosphorous absorption. Shafey *et al.* (1991) found that high dietary calcium concentration increased the pH of the intestinal crop contents, but did not influence the

pH of the contents in the rest of the gastrointestinal tract. They also reported that increased intestinal pH reduced the soluble fraction of minerals, and consequently their availability for absorption also decreases.

### 1.5.4 Calcium and phosphorous ratio

High calcium levels in the intestine reduce the absorption of phosphorous, zinc and manganese, and increase the pH in the gut. High plasma phosphorous decreases both calcium absorption from the gut and mobilization from the bone. When the concentration of phosphate in the serum increases, the hormones encourage an increase of calcium by excreting phosphate through the kidney in order to maintain the correct concentration of calcium and phosphorous (Hurwitz, 1976).

Hartel (1987) reported that the amounts of calcium and phosphorous required by laying hens at a given stage of production vary not only from day to day but also through the day. Shell calcification improves as the supply of dietary calcium increases, whereas it declines with increasing dietary phosphorous, therefore good shell quality is assumed to result from feeding diets high in calcium and low in phosphorous.

### 1.5.5 Amount fed

The amount of dietary calcium given to birds is one of the essential factors that affect calcium absorption. Usually the calcium requirement for birds is calculated on the basis of calcium retention. Morris (1972) used the data of MacIntyer *et al.*(1963) to calculate the calcium rentention of hens fed diets ranging in calcium from 1 to 6 % over a period of 280 days. The formula for calculating retention is: (calcium output in the shell/calcium intake in the diet) x 100. This formula ignores changes in skeletal calcium; but the data from which calcium rentention was derived covers a period of 280 days, during which the output of the shell is about 450 g/bird and the skeletal calcium is unlikely to have exceeded 4 g/bird. It is commonly believed that laying hens retain 50 percent of dietary calcium and it makes use of only this amount to maintain all the calcium requiring processes. However, practically the amount of calcium retained depends on the amount of calcium included in the diet as shown in Figure 1.2 (Morris, 1972).

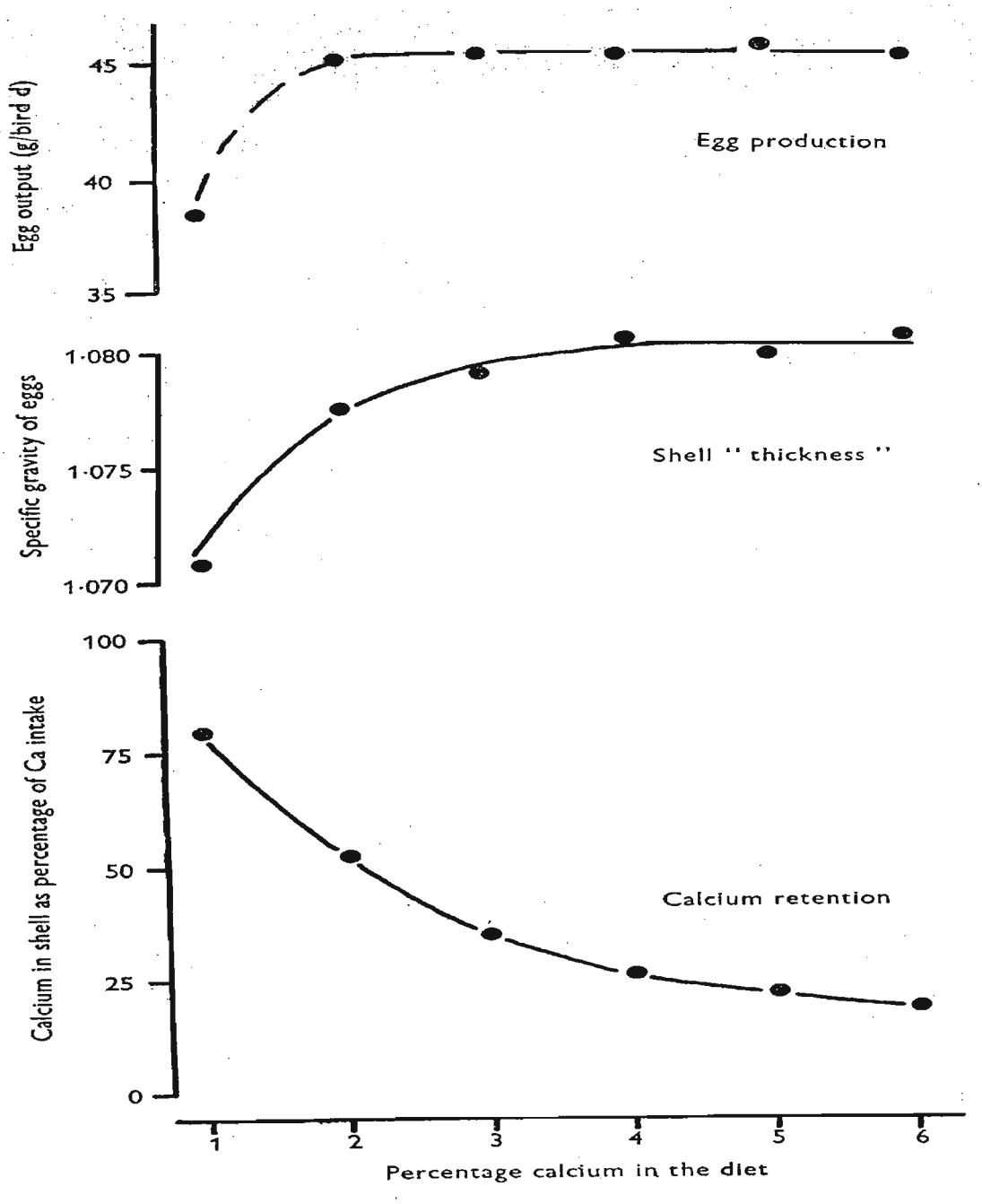


**Figure 1.2:** The effect of dietary calcium level on egg output, shell thickness and calcium retention (Morris, 1972) (data from MacIntyre *et al.*, 1963). Calcium output in form of eggshell has been estimated from the data reported for the rate of lay, egg weight and specific gravity of the eggs.

From the graph it can be seen that, when feeding a low calcium diet (10 g calcium /kg), a high efficiency of utilization of calcium results, but it does not give good shell

thickness or high egg production, which are important aspects in the egg production industry. The production of fewer eggs is in accordance with the findings by Morris and Taylor (1967), who reported that calcium influenced egg production, either in terms of egg number or egg size or both, by influencing food intake. When hens were fed diets with high calcium (30 g calcium/kg), voluntary feed consumption was about 25% greater on egg formation. From Figure 1.2, calcium intake of at least 4g per bird per day appeared to be necessary for best eggshell quality.

From the results in Figure 1.2 it can be concluded that it is economical and more sensible to feed high calcium diets to hens in the laying stage to obtain high egg production and thicker shells, using calcium sources that are relatively inexpensive and in which the calcium is readily available.

## 1.6 EFFECTS OF FEEDING CALCIUM IN EXCESS OR BELOW THE REQUIREMENT ON PERFORMANCE OF PULLETS

Many attempts have been made to reduce the incidence of poor shell and bone problems in laying hens through supplementation of the diet with additional calcium. In attempting to solve these problems in this way the added calcium has been shown occasionally to interfere with some of the body mechanisms, which result in different outcomes. If the diet fed to birds is deficient in calcium or contains excess calcium, it does not only affect the kidney and the hormones concerned. It also affects egg production, bone strength, egg quality, appetite, growth and sexual maturity. Hartel (1987) reported that an excess of dietary calcium interferes with the availability of other minerals, such as phosphorous, magnesium, manganese and zinc, and diets of high calcium content are detrimental to egg weight and sometimes to rates of lay.

In terms of calcium metabolism in laying hens, early introduction of high calcium diets to pullets prior to the onset of lay would appear to be beneficial in order to optimise the calcium balance in the body. But it can be argued that feeding very high levels of calcium prior to lay imposes unnecessary stress on the bird's kidneys. The stress is because birds try to maintain the right amount of calcium by excreting excess calcium. In a study by Shane *et al.* (1969), it was found that feeding diets containing 30 g

calcium/kg to chickens between 8 and 12 weeks of age resulted in higher levels of visceral urate deposits, mortality, nephrosis (kidney degeneration) and a smaller parathyroid gland. High levels of calcium during the growth period will interfere with the proper development of the parathyroid gland by increasing gut pH, which will decrease absorption of calcium (Shafey, 1993). The parathyroid gland is central in the hormonal control of calcium and is activated when pullets mature.

Scott *et al.* (1976) found that 10 g calcium/kg was inadequate to maximize bone mineralization prior to lay and suggested that higher (>10 g calcium/kg) levels should be fed during the time of medullary bone development. It has been found that excess calcium can reduce body weight prior to lay, delay sexual maturity and increase laying period mortality. They therefore suggested that the dietary calcium content should take into account the different stages of pullet development.

Hughes and Wood-Gush (1971) reported that diets with lower concentrations of calcium were more palatable than diets with high calcium concentration, which might explain why birds fed excess calcium had lower body weight. To confirm the findings of Scott *et al.*(1976), Roland (1986) indicated that feeding early maturing pullets diets containing 10 g calcium /kg caused the birds to compensate for the deficiency by eating extra feed to get more calcium. Over- consumption of other nutrients can occur when feed contain too little calcium because birds fed diets containing such diets increase food intake in an attempt to obtain more calcium, which results in an increase in body fat content. Body weight and body condition of birds around the time of maturity are important criteria that will influence layer performance. Each strain of bird has a characteristic mature body weight that must be reached for adequate egg production and egg mass output. In the experiment conducted by Bar *et al.* (1998) it was found that dietary calcium had a significant effect on body weight of layers at sexual maturity. Pullets that were given 22 g calcium /kg had higher body weight at sexual maturity than pullets that were feed 39 g calcium/kg. So it can be concluded that pullets fed high calcium levels would have reduced feed intake, while hens fed the low levels would have increased feed intake. Inadequate dietary calcium has an adverse effect on feed consumption and production.

Nevalainen (1969) found that a low calcium (9 g calcium/kg) diet was inadequate for ovary and oviduct development, that means low calcium (9 g calcium/kg) diets delay sexual maturity. On the other hand, Leeson *et al.* (1986) found that there was no significant effect of different levels of dietary calcium on sexual maturity. They found that a calcium concentration of 5.8g calcium/kg was adequate for ovulation but not for shell quality, and that calcium concentration had to be less than 0.5 g calcium/kg to inhibit ovulation. This indicates that prelay calcium concentration had little effect on maturity

In conclusion, the amount of calcium included in the feed and the rate of metabolism of calcium in the body are closely interconnected. Calcium homeostasis is disturbed to a larger extent by the presence of high dietary calcium levels, and the adaptation of birds to such levels depends primarily on the ability of the bird to reduce the efficiency of calcium absorption. So before deciding how much calcium to feed the criteria for adequacy of calcium should be considered, since excess calcium intake in the late rearing period has been shown to reduce both the subsequent performance and egg quality.

## 1.7 CHOICE FEEDING OF CALCIUM

Birds would have a better chance of meeting their nutrient requirement if they were offered simultaneously two feeds differing in nutrient content than if only one feed was offered. Laying hens, pullets and growing broilers have been reported to have the ability to select an adequate diet from a choice of two or three feeds that are individually inadequate (Forbes, 1995). One of the best-known examples of a specific appetite in farm species is the calcium appetite of laying hens. Research has clearly shown that the domestic fowl has the ability to selectively consume calcium to meet requirements for maintenance and production. They achieve this by visual or taste cues (Irving *et al.*, 1981). Hughes and Wood–Gush (1971) found that although a calcium appetite is present in growing chickens, it develops slowly (2-4 days) whereas it develops far more rapidly (half an hour) in laying hens where the calcium requirement are much higher. Calcium intake is regulated on an hour-to-hour basis by the needs of egg formation and other body mechanisms that require calcium. To substantiate this, Hughes and Wood-

Gush (1971) reported that when a low calcium diet was fed, the hens increased their feed intake. The high intake of food on the days during which an egg is being formed might be due to the higher calcium requirement on those days, but it might also be a response to amino acid or energy demand.

Some research (Classen and Scott, 1981) has indicated that a marked improvement in shell quality may be obtained by feeding dietary oyster shell or limestone grit. Where no additional grit is offered, the hen has no choice as to the time of the day calcium intake may be increased. In an experiment conducted by Classen and Scott (1981), pullets were given a choice of diets containing 0.89 or 3.5 g calcium per kg feed and grit containing 380 g calcium/kg. Pullets chose diets that contained 11.8, 20.8 and 35.0 g calcium per kg during the growing, prelaying (medullary bone formation) and egg laying phases, respectively. It was found that growing pullets had the ability to regulate their calcium intake better than laying hens. Holcombe *et al.* (1975) reported that both old and young hens have the ability to adjust their calcium intake, but to a different degree. On the other hand, Taher *et al.* (1984) reported that although laying hens possess a special appetite for calcium, they don't necessarily regulate calcium intake. Forbes (1995) reported that when a bird is given free access to limestone, it might have a problem in deciding how much limestone grit to eat. Another possibility is that the bird might eat an excess of a low calcium diet in order to get sufficient calcium from the diet. Sometimes they tend to choose familiar feed even when these are deficient in calcium.

Calcium intake is higher just before dark (at the time when egg shell formation is proceeding) than earlier in the day. When limestone grit was supplied separately in the trial by Classen and Scott (1981) there was an improvement in shell thickness, which was attributed to the steady release of calcium from the grit in the gizzard during the night when absorption of calcium incorporated in the feed had ceased, thus reducing the need to mobilize skeletal reserves of calcium.

If the calcium choice feeding system were to be practiced it would no longer be necessary to ascertain how much calcium should be included in the diet, as the birds would have the option of meeting its changing requirement for calcium by selecting the appropriate amount of limestone each day. There are different ways of meeting the

calcium requirement of pullets. Choice feeding would be the method most likely to succeed as long as pullets can be shown to make the appropriate choice when presented with food in this way.

## 1.8 DISCUSSION

The varying results from different authors demonstrate the difficulty of determining the calcium requirement of birds at different stages of production. It may be assumed that the calcium requirement for layers has been changing over the years because of the improved rates of lay brought about by genetic selection, which has contributed to the different values that are quoted for maximum production and good shell quality. The problem becomes more confusing and complex when a producer seeks advice from other producers, feed suppliers or researchers: the recommendations or suggestions one obtains in many cases are not only different but often exactly opposite. From the review it appears that there are some legitimate reasons why different levels of dietary calcium are recommended, some of these being:

1. The continued improvement in egg production potential.
2. Interrelation of calcium with other minerals like phosphorous, magnesium, and zinc.
3. Solubility of different particle sizes of limestone, oyster shell or other calcium sources.
4. Ability of the breed to adjust feed intake to meet calcium requirement.
5. Calcium requirement has been reported by many workers in terms of percent in the diet without taking into account the variation in feed intake.

The correct supply of calcium depends on the rate of inclusion of calcium in the diet, on the availability of the calcium source and on the amount of food consumed. The formation of the shell imposes considerable strain on the mechanisms used to provide calcium because the quantity of calcium in the shell is equivalent to 10% of the total body calcium. In order for hens to remain in a positive calcium balance they have evolved highly efficient physiological mechanisms for regulating calcium balance during shell formation. Deficiencies and excess levels of calcium play major roles in the development of leg abnormalities, shell problems and laying performance. However,

due to genetic and environmental differences, the severity of occurrences can be expected to vary.

The aim of this chapter was to review the relevant research conducted on the calcium requirements of pullets and layers, thereby gaining an understanding of the factors and mechanisms involved. Considerable work has been done on the amount of calcium required by pullets and laying hens, but less work has been done on the requirement for calcium during the pullet/layer transition period, when the pullet is building up reserves of calcium in the body in anticipation of the need for this mineral in the shell calcification process. The work reported in this thesis was an attempt to address this lack of information of the calcium requirement of the pullet in the weeks prior to attaining sexual maturity.

# CHAPTER 2

# RESPONSE OF PULLETS TO DIETARY CALCIUM PRIOR TO SEXUAL MATURITY

## 2.1 Introduction

Considerable efforts have been made to minimize poor shell quality and weak bones in laying hens. In spite of this research effort there is still considerable confusion about the amount of calcium that should be given to birds prior to sexual maturity. During the laying cycle the hen utilizes its medullary bone reserves to augment its readily available supply of calcium when a shell is being formed, but this medullary bone needs to be accumulated prior to the first egg being laid. The production of the first egg places a major strain on the metabolism of a pullet, as it has to depart with 2 g of calcium from the body, most of this being derived from the medullary bone (Clunies *et al.*, 1992). So building up the medullary reserves before sexual maturity is of utmost importance. However, it is still unclear whether the amount of calcium in a normal grower diet (about 10g calcium per kg) would be sufficient to accomplish this, given the ability of a pullet to regulate the efficiency of utilization of dietary calcium, especially during sexual maturation.

There are two opposing viewpoints regarding the amount of calcium that should be included in pullets feed prior to sexual maturity. In one case, evidence suggests that feeding high levels of calcium to pullets is harmful, whereas others have presented evidence to the contrary. Shane *et al.* (1969) and Hartel (1987) found that feeding pullets diets containing 30g calcium/kg resulted in higher levels of visceral urate deposits, mortality, nephrosis, low egg weight, low rate of lay and the interference with the availability of other minerals. In contrast, Keshavarz (1987) reported that feeding pullets a diet containing 35g calcium /kg increased medullary bone reserves, but did not affect kidney weight or plasma calcium concentration up to the age of 60 weeks. Bar *et al.* (1998) indicated that feeding pullets with a prelaying diet containing 39 to 40g calcium/kg would be harmless in practice, and suggested that the use of diets with a high calcium content (30– 40g calcium /kg) before sexual maturation may overcome the practical difficulties consequential from the need to replace, in time, a prelaying diet containing less than the required content of calcium for laying. Roland (1986) suggested

that low calcium diets (10 – 20g calcium/kg) are harmful when fed to early maturing pullets, because the pullets try to compensate for the deficiency by eating extra feed to get more calcium and this results in overweight pullets. It is difficult, from the above evidence, to formulate a judgment or hypothesis of the correct means of feeding calcium to pullets just prior to sexual maturity. One method of resolving the issue is to allow the pullets to choose the amount of calcium for themselves.

In trials designed to determine nutrient requirements it is sometimes useful to introduce a choice feeding treatment, because choice feeding is a system that enables each bird to determine for itself the amount of a nutrient that will maximize its biological performance. Research by Joshua and Mueller (1979) clearly indicated that domestic fowl have the ability to selectively consume calcium to meet maintenance and production requirements. So it is likely that choice feeding treatments would help to resolve the dilemma of how much calcium to include in feeds for pullets as they approach sexual maturity.

In the previous research directed towards determining the correct method of feeding calcium in the prelaying period, different methods have been employed to monitor the effects of high versus low calcium intakes, e.g. bone strength, egg shell thickness, rate of lay ,etc. In the present experiment, bone strength was used to monitor the effect of the different dietary treatments, but the trial was terminated once the pullets had reached sexual maturity, as the objective was to determine the amounts of calcium that pullets would consume up to that stage.

EXPERIMENT 1 (PULLETS; 14 – 24 WEEKS OF AGE)

## 2.2. Materials and methods

### 2.2.1 Birds and management

Hy-Line Brown and Silver pullets were raised from day old at Ukulinga Research Farm on a lighting programme of 8L:16 D. Three hundred and eighty four (384) Hy-Line pullets (192 Silver 192 Brown) at 14 weeks of age were randomly selected from the main group. These pullets were weighed and allocated to 24 cages, with 16 birds of the same strain per cage (12 cages of Silver and 12 cages of Brown). The growing cages measured 100, 45, and 55 cm in length, height and width respectively (i.e. 0.55m$^2$ of floor space). Eighteen (18) cages were supplied with a feeder measuring 100 cm long and 10 cm wide, while six cages were allocated two troughs for choice feeding purposes. In the latter case the two troughs were each of the same length, of 48 cm. At 18 weeks of age, one bird from each cage (six birds from each treatment) was selected randomly and then killed for analysis of bone breaking strength before sexual maturity. At 18 weeks of age 144 of the remaining pullets (72 Silver and 72 Brown) where selected randomly and kept on the same feeding treatments as before, but in individual rather than group cages so that the age at sexual maturity could be determined. Each cage of (measuring 43, 41, and 29 cm in length, height and width respectively) was supplied with a nipple drinker and either one or two feeders depending on the feeding treatment.

### 2.2.2 Treatments and feeds

Pullets were fed (*ad libitum*) a commercial pullet starter mash from day old to six weeks and pullet grower mash from seven to 14 weeks of age. Two basal diets were formulated to contain high (30g/kg) or low (8g/kg) calcium contents (Table 2.1) but with all other nutrients the same. Table 2.2 shows the formulated and analysed nutrient contents of these two basal diets. The experiment consisted of four dietary treatments as shown in Table 2.3. A 1:1 blend of the high and low calcium basal feeds were made to produce the medium calcium feed. Limestone grit was provided as the calcium source in the choice feeding treatment.

**Table 2.1** Composition (g/kg as fed) of the two basal feeds used in the experiment

| Ingredients | Low calcium | High calcium |
|---|---|---|
| Maize | 664 | 664 |
| Wheat bran | 1001 | 1001 |
| Soybean 46 | 82 | 82 |
| Sunflower 37 | 58 | 58 |
| Filler (sunflower husk) | 61 | 0 |
| Vitamin and Mineral premix | 1.5 | 1.5 |
| Limestone | 13.5 | 74.5 |
| Salt | 2.6 | 2.6 |
| Monocalcium Phosphate | 14.7 | 14.7 |
| Sodium bicarbonate | 2.9 | 2.9 |

**Table 2.2:** Calculated and analysed nutrient contents (as fed) (g/kg) of the experimental feeds

| | Feed | | | | | |
|---|---|---|---|---|---|---|
| | Low Calcium | | Medium Calcium | | High Calcium | |
| Nutrients | Calc | Actual | Calc | Actual | Calc | Actual |
| AME (MJ/kg) | 10.88 | 11.25 | 10.88 | 11.16 | 10.88 | 10.73 |
| Protein | 131.1 | 135.1 | 131.1 | 129.4 | 131.1 | 125.9 |
| Phosphorus | 5.0 | 7.0 | 5.0 | 6.9 | 5.0 | 7.3 |
| Calcium | 8.0 | 9.4 | 19.0 | 21.8 | 30.0 | 30.7 |

Calc = calculated during formulation Actual = observed from laboratory analysis

**Table 2.3:** Feeding Treatments

| Treatment | Feeding regime | Abbreviated name |
|---|---|---|
| 1 | Low calcium diet | Low |
| 2 | Medium calcium diet | Medium |
| 3 | High calcium diet | High |
| 4 | Low calcium diet + Limestone grit separatly | Choice |

### 2.2.3 Chemical Composition

A rapid *in vivo* technique was used in which adult roosters were forced-fed with a measured quantity of test sample following a 24 hours fasting period during which, the birds were given glucose in water (50g: 50 ml) per bird to ensure that they do not become dehydrated or lacking energy. After feeding, excract were colleted over the following 48 hours, dried, weighed and analysed for energy to determine the digestibility of the energy consumed. A correction was made for endogenous energy losses (EEL), as well as for nitrogen that may either have been retained or lost during the assay period. The resultant AME value does not include a correction for EEL. Protein was measured by the method described by AOAC (2000). Calcium and phosphorous were determined according to AOAC (2000) using a Varian Spectra AA-200 Atomic Absorption Spectrophotometer (Varian Inc., Palo, Alto, C.A., USA) and a Technicon Autoanalyser II (Technocon Instruments, NY, USA), respectively.

### 2.2.4 Feeding Procedure

From 14 to 18 weeks of age, 24 buckets that could hold up to 20 kg of feed were assigned to the 24 cages in which only one feed was to be offered, and each was filled with the feed allocated to that pen and weighed. Feed was transferred from the buckets to the troughs when necessary. At the end of each week feed was transferred from the trough to the bucket, and each bucket with feed was weighed before adding more feed for the following week and the buckets with feed were weighed again. Feed intake was calculated by subtracting the amount remaining from the amount allocated and dividing by the number of birds and days between. The same procedure was used with the troughs containing limestone grit.

2.2.5 Bone strength procedure

From the 24 pullets sampled at 18 weeks of age, the right tibias were removed and cleaned of the connective tissue and muscle tissue. The tibias were kept frozen until bone strength was to be measured. The tibias were thawed for two hours at room temperature then placed in an oven at $70^0$ C for 24 hours. The bone strength instrument (Plate I) used to measure the tibia strength consisted of a platform made of two steel rods 50 mm long and 65 mm wide that were spaced 40mm apart. This distance is prescribed for measuring the breaking strength of tibia from pullets and adult birds. To ensure that the bone remained stationary during the measuring process the rods were coated with a layer of rubber about 2 mm thick onto which the bone was placed. Each tibia was placed on the platform such that the midpoint was centered between the rods. A blade was brought down onto the tibia by applying steadily increasing pressure using a hydraulic press. The maximum load (kg) applied to the bone at the point of fracture was displayed on an LED (light emittin diodes) screen.

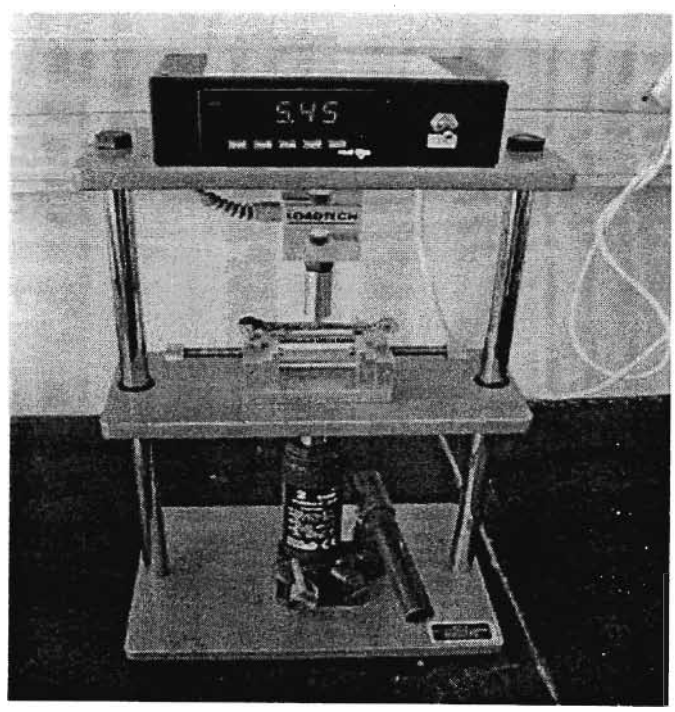


**Plate I :** The bone strength instrument used to measure the tibia strength

### 2.2.6 Measurements

Body weights were recorded at the beginning of the trial and at the end of each two week period. During the initial period, when pullets were housed in groups, they were weighed as a group and the mean weight was calculated, but in the next period birds were weighed individually. Feed and limestone intakes were measured once a week. Age, and body weight at sexual maturity were recorded for each pullet on the day that the first egg was laid.

### 2.2.7 Statistical analysis

The design of the experiment was 2 x 4 (strains and treatments respectively) factorial completely randomised design. The ANOVA procedure (using the statistical package GENSTAT, 1998) was used to analysis the interactive effect between feed intake, calcium intake, and body weight and bone strength.

## 2.3 Results

### 2.3.1 Feed intake.

The mean responses in feed intake by the two strains to four dietary calcium treatments are given in Table 2.4. Different dietary calcium levels had no significant effect on the amount of feed consumed at 15, 16, 18, 19 or 20 weeks of age, but it was significantly ($P < 0.05$) influenced by the dietary calcium content at 17 weeks. At this age feed intake decreased as the calcium content of the diet increased, with the highest intake on the low calcium diet and the lowest feed intake on the highest dietary calcium level. At 15, 16, 18 and 20 weeks of age the feed intake decreased with an increase in dietary calcium, though the differences were not significant. Table 2.10 indicates the avarage feed intake of two strains pullets for three-week intervals on the experimenta treatments. Figure 2.5 shows that from 14 to 17 weeks of age, increased in dietary calcium resulted in in the decrease in feed intake, but the opposite response was observed from 17 to 20 weeks of age. There were no significant differences between the strains for weeks 15, 16, 18 but there was a significant ($P<0.05$) different at 17 weeks of age, the silver strain had higher feed intake than the Brown strain. In all cases the birds consumed the most feed when the low calcium diet was offered. This was the case until 19 weeks of age when birds on high calcium diets increased intake to the same extent as those on the low

calcium, but the Brown strain at 19 and 20 weeks of age consumed significantly ($P<0.01$) more feed than the Silver strain, as shown in Figure 2.1. The results indicate that there is no significant interaction between strain and dietary calcium level in any of the weeks.

### 2.3.2 Calcium intake

Table 2.5 shows the calculated mean calcium intake of pullets fed different levels of dietary calcium. The calcium consumed by pullets on the choice treatment remained slightly above that of pullets on the low calcium treatment, until 18 weeks of age when birds from both strains started to consume increasing amounts of limestone grit to meet their calcium requirement prior to the first egg. Figure 2.3 indicates that when limestone grit was supplied as achoice, birds consumed the required limestone grit for each stage of development. Table 2.10 indicates the average calcium intake of two strains of pullets for three-week intervals on the experimental treatments. All the trends show (Figure 2.2) that calcium consumption for all the treatment increased significantly from 18 weeks of age, but this was in most cases because of an increase in feed intake. The highest increases in calcium intake were on the high calcium diets.

### 2.3.3 Body weight gain

The influence of dietary calcium level on body weight gain is shown in Table 2.6 and the response in feed intake of pullets of two strains to three dietary calcium levels at weekly intervals from 15 to 20 weeks of age is shown on Figure 2.6. From 14 to 20 weeks of age the change in body weight was not significantly different between treatments. The increase in body weight was highest in pullets on lower calcium diet, and lowest on the high calcium diet. The results indicate that there was no significant interaction between strain and calcium level in any of the weeks. From 16 to 18 weeks of age body weight gain decreased and from 18 to 20 weeks of age it increased, this trend corresponding with the change in feed intake. There was no significant effect on body weight gain when regressed against dietary calcium content from 18 to 20 weeks of age. The regression coefficient obtained was negative ($-0.710 \pm 0.825$ g/bird d).

### 2.3.4 Bone strength

The effect of dietary calcium on bone breaking strength is shown on Table 2.7. The dietary calcium treatments did not have a significant effect on bone strength at 18 weeks

of age, nor were there differences between the two strains. The trends are illustrated in Figure 2.7, the value of this variable as a measure of calcium adequacy is therefore questionable.

### 2.3.5 Bodyweight and age at sexual maturity

The effect of dietary calcium on age at sexual maturity and bodyweight at sexual maturity are shown on Table 2.8 and Figure 2.4. Body weight at sexual maturity was significantly ($P < 0.01$) affected by dietary calcium: pullets that were fed the low calcium diet had the highest body weight at sexual maturity, then followed by pullets on choice, medium dietary calcium and lastly high dietary calcium. Because Brown strain pullets had higher feed intake at 19 and 20 weeks of age, their body weight at sexual maturity was also significantly ($P < 0.0$) higher than that of the silver strain. Age at sexual maturity was not affected by the different rearing treatments and there were no significant differences between the strains in age at sexual maturity. From Figure 2.8 it can be seen that an increase in dietary calcium results in a decrease in body weight at sexual maturity.

**Table 2.4**: Feed intake of pullets during different weeks (ages) on the experimental treatments

| Age | Treatment | Feed Intake for two strains (g/bird d) Brown | Silver | Mean (g/bird d) |
|---|---|---|---|---|
| 15 weeks | Low | 80.1 | 80.5 | 80.3 |
| | Medium | 68.6 | 75.1 | 71.8 |
| | High | 71.0 | 72.3 | 71.6 |
| | Choice | 71.3 | 76.3 | 73.7 |
| | Mean (Strain) | 72.7 | 76.0 | |
| | SED: Strain = 2.75$^{ns}$ | Diet = 3.89$^{ns}$ | Strain x Diet = 5.51$^{ns}$ | |
| 16 weeks | Low | 70.1 | 70.8 | 70.5 |
| | Medium | 71.7 | 72.9 | 72.3 |
| | High | 70.7 | 72.4 | 71.5 |
| | Choice | 70.3 | 70.0 | 70.1 |
| | Mean (Strain) | 70.1 | 71.5 | |
| | SED: Strain = 1.16$^{ns}$ | Diet = 3.89$^{ns}$ | Strain x Diet = 5.51$^{ns}$ | |
| 17 weeks | Low | 69.4 | 73.3 | 71.4 |
| | Medium | 67.7 | 67.8 | 67.7 |
| | High | 62.9 | 66.3 | 64.6 |
| | Choice | 65.1 | 72.4 | 68.8 |
| | Mean (Strain) | 66.3 | 69.9 | |
| | SED: Strain = 1.25* | Diet = 1.773* | Strain x Diet = 2.50$^{ns}$ | |
| 18 weeks | Low | 67.6 | 72.7 | 70.2 |
| | Medium | 68.7 | 65.1 | 66.9 |
| | High | 66.4 | 61.0 | 63.7 |
| | Choice | 65.2 | 63.0 | 64.1 |
| | Mean (Strain) | 67.0 | 65.4 | |
| | SED: Strain = 2.33$^{ns}$ | Diet = 3.30$^{ns}$ | Strain x Diet = 4.67$^{ns}$ | |
| 19 weeks | Low | 92.8 | 82.4 | 87.6 |
| | Medium | 84.4 | 72.2 | 78.5 |
| | High | 94.6 | 83.5 | 89.0 |
| | Choice | 91.7 | 86.0 | 88.8 |
| | Mean (Strain) | 91.0 | 81.0 | |
| | SED: Strain = 3.25** | Diet = 4.60$^{ns}$ | Strain x Diet = 6.50$^{ns}$ | |
| 20 weeks | Low | 108.9 | 108.9 | 108.3 |
| | Medium | 110.9 | 106.5 | 108.0 |
| | High | 116.0 | 106.0 | 111.9 |
| | Choice | 120.3 | 109.3 | 115.0 |
| | Mean (Strain) | 114.1 | 107.3 | |
| | SED: Strain = 3.25** | Diet = 4.60$^{ns}$ | Strain x Diet = 6.50$^{ns}$ | |

* P< 0.05 ** P< 0.01 *** P< 0.001 $^{ns}$ P> 0.05

**Table 2.5**: Calcium intake of pullets on the experimental treatments

| Age weeks | Calcium intake (g/bird d) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Low | | Medium | | High | | Choice | |
| | Brown | Silver | Brown | Silver | Brown | Silver | Brown | Silver |
| 15 | 0.75 | 0.76 | 1.50 | 1.64 | 2.18 | 2.22 | 0.67 | 0.72 |
| 16 | 0.66 | 0.67 | 1.55 | 1.59 | 2.17 | 2.22 | 0.66 | 0.66 |
| 17 | 0.65 | 0.69 | 1.48 | 1.48 | 1.93 | 2.03 | 0.61 | 0.68 |
| 18 | 0.64 | 0.68 | 1.50 | 1.42 | 2.03 | 1.87 | 0.61 | 0.59 |
| 19 | 0.87 | 0.77 | 1.84 | 1.57 | 2.90 | 2.56 | 0.86 | 0.81 |
| 20 | 1.02 | 1.02 | 2.40 | 2.31 | 3.56 | 3.25 | 1.13 | 1.03 |

Calcium intakes were calculated on the basis of actual calcium content in each diet (9.4 g/kg for low and choice, 21.8 g/kg for medium, 30.7 g/kg for high)

**Table 2.6** Limestone and total calcium intake of pullets on the choice treatment

| Age weeks | Limestone intake (g/bird d) | | | | Total Ca intake |
|---|---|---|---|---|---|
| | Brown | Silver | Mean | S.E.D | (g/bird d) |
| 15 | 0.47 | 0.38 | 0.42 | 0.04 $^{n.s}$ | 1.12 |
| 16 | 0.45 | 0.38 | 0.42 | 0.04 $^{n.s}$ | 1.08 |
| 17 | 0.53 | 0.39 | 0.46 | 0.06 $^{n.s}$ | 1.11 |
| 18 | 0.55 | 0.53 | 0.54 | 0.06 $^{n.s}$ | 1.14 |
| 19 | 0.72 | 0.96 | 0.84 | 0.37 $^{n.s}$ | 1.68 |
| 20 | 1.29 | 0.93 | 1.11 | 0.55 $^{n.s}$ | 2.19 |

Total Ca intake is the sum of dietary calcium and calcium from limestone grit. Calcium from limestone grit was calculated on the basis of 38 % calcium.

**Table 2.5**: Calcium intake of pullets on the experimental treatments

| | Calcium intake from the experimental diets (g/bird d) | | | | | | | | Limestone grit intake (g/bird d) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | Low | | Medium | | High | | Choice | | | | | | |
| weeks | Brown | Silver | Brown | Silver | Brown | Silver | Brown | Silver | Brown | Silver | Mean | S.E.D | Total Ca intake |
| 15 | 0.75 | 0.76 | 1.5 | 1.64 | 2.18 | 2.22 | 0.67 | 0.72 | 0.47 | 0.38 | 0.42 | $0.04^{n.s}$ | 1.12 |
| 16 | 0.66 | 0.67 | 1.55 | 1.59 | 2.17 | 2.22 | 0.66 | 0.66 | 0.45 | 0.38 | 0.42 | $0.04^{n.s}$ | 1.08 |
| 17 | 0.65 | 0.69 | 1.48 | 1.48 | 1.93 | 2.03 | 0.61 | 0.68 | 0.53 | 0.39 | 0.46 | $0.06^{n.s}$ | 1.11 |
| 18 | 0.64 | 0.68 | 1.5 | 1.42 | 2.03 | 1.87 | 0.61 | 0.59 | 0.55 | 0.53 | 0.54 | $0.06^{n.s}$ | 1.14 |
| 19 | 0.87 | 0.77 | 1.84 | 1.57 | 2.9 | 2.56 | 0.86 | 0.81 | 0.72 | 0.96 | 0.84 | $0.37^{n.s}$ | 1.68 |
| 20 | 1.02 | 1.02 | 2.4 | 2.31 | 3.56 | 3.25 | 1.13 | 1.03 | 1.29 | 0.93 | 1.11 | $0.55^{n.s}$ | 2.19 |

Calcium intakes were calculated on the basis of actual calcium content in each diet (9.4 g/kg for low and choice, 21.8 g/kg for medium, 30.7 g/kg for high and 380 g/kg limestone grit). Total Ca intake is the sum of dietary calcium and calcium from limestone grit

**Table 2.6** Mean daily body weight gain of two strains over the two-week periods on four dietary treatments.

| Age (weeks) | Treatment | Body weight gain (g/bird d) Brown | Silver | Mean |
|---|---|---|---|---|
| 14 - 16 | Low | 9.53 | 10.90 | 10.22 |
| | Medium | 9.23 | 9.00 | 9.12 |
| | High | 8.73 | 8.77 | 8.75 |
| | Choice | 8.80 | 9.27 | 9.03 |
| | Mean (Strain) | 9.08 | 9.48 | |
| | SED: Strain = 0.392 $^{n.s}$ | Diet = 1.175 $^{n.s}$ | Strain x Diet = 1.662 $^{n.s}$ | |
| 16 - 18 | Low | 7.50 | 8.23 | 7.87 |
| | Medium | 7.00 | 6.53 | 6.77 |
| | High | 6.30 | 6.00 | 6.15 |
| | Choice | 7.37 | 7.53 | 7.45 |
| | Mean (Strain) | 7.04 | 7.08 | |
| | SED: Strain = 0.438 $^{n.s}$ | Diet = 0.619 $^{n.s}$ | Strain x Diet = 0.875 $^{n.s}$ | |
| 18 - 20 | Low | 17.3 | 16.4 | 16.9 |
| | Medium | 19.6 | 15.6 | 17.6 |
| | High | 15.6 | 15.3 | 15.1 |
| | Choice | 20.3 | 16.0 | 18.1 |
| | Mean (Strain) | 18.0 | 15.8 | |
| | SED: Strain = 2.59 $^{n.s}$ | Diet = 3.66 $^{n.s}$ | Strain x Diet = 5.18 $^{n.s}$ | |

* $P< 0.05$ ** $P< 0.01$ *** $P< 0.001$ $^{ns}P> 0.05$

Initial mean weight of pullets at 14 weeks was 1.89 kg for Brown and 1.92 kg for Silver strain.

**Table 2.7** Breaking strength of tibia from two strains of pullets at 18 weeks of age on four different dietary treatments

| Treatment | Breaking strength (kg) | | |
|---|---|---|---|
| | Brown | Silver | Mean |
| Low | 10.2 | 12.3 | 11.3 |
| Medium | 10.9 | 10.6 | 10.8 |
| High | 11.0 | 11.1 | 11.0 |
| Choice | 10.4 | 12.7 | 11.6 |
| Means (Strain) | 10.63 | 11.68 | |
| S.E.M: Diet = $0.981^{ns}$ Strain = $0.693^{ns}$ Diet x Strain = $1.387^{ns}$ | | | |

* P< 0.05 ** P < 0.01 ***P <0.001 $^{ns}$ P > 0.05

**Table 2.8** Body weight and age at sexual maturity of two strains of pullets on four dietary treatments

| Response variables | Treatment | Brown | Silver | Means |
|---|---|---|---|---|
| Body weight at sexual maturity (g/bird) | Low | 1801 | 1731 | 1766 |
| | Medium | 1721 | 1676 | 1698 |
| | High | 1718 | 1638 | 1678 |
| | Choice | 1723 | 1683 | 1703 |
| | Mean (strain) | 1741 | 1682 | |
| | SED Diet = 20.1** | Strain = 28.5* | Strain x Diet = $40.7^{ns}$ | |
| Age at sexual Maturity (days) | Low | 139 | 141 | 140 |
| | Medium | 142 | 146 | 144 |
| | High | 141 | 145 | 143 |
| | Choice | 140 | 142 | 142 |
| | Mean (strain) | 141 | 143 | |
| | SED Diet = $1.007^{n.s}$ | Strain = $0.7056^{n.s}$ | Strain x Diet = $1.4273^{n.s}$ | |

* P< 0.05 ** P < 0.01 ***P <0.001 $^{ns}$ P > 0.05

**Table 2.9** Average feed intake of two strains of pullets for three-week intervals on the experimental treatments

| Age (weeks) | Treatment | Feed intake (g/bird d) Brown | Silver | Means |
|---|---|---|---|---|
| 14 - 17 | Low | 73.26 | 74.92 | 74.01 |
| | Medium | 69.15 | 71.94 | 71.00 |
| | High | 68.22 | 70.36 | 69.30 |
| | Choice | 68.91 | 72.83 | 70.90 |
| | Mean (Strain) | 69.93 | 72.51 | |
| | SED: Strain = 1.015* | Diet = 1.436* | Strain x Diet = 2.030 $^{n.s}$ | |
| 17 - 20 | Low | 89.71 | 87.65 | 88.68 |
| | Medium | 88.11 | 81.27 | 84.69 |
| | High | 92.34 | 84.07 | 88.20 |
| | Choice | 92.54 | 86.08 | 89.31 |
| | Mean (Strain) | 90.67 | 84.77 | |
| | SED: Strain = 0.438 $^{**}$ | Diet = 0.619 $^{n.s}$ | Strain x Diet = 0.875 $^{n.s}$ | |

* P< 0.05 ** P< 0.01 *** P< 0.001 $^{ns}$P> 0.05

**Table 2.10** Average calcium intake of two strains of pullets for three-week intervals on the experimental treatments

| Age (weeks) | Treatment | Calcium intake (g/bird d) Brown | Silver | Mean |
|---|---|---|---|---|
| 14 - 17 | Low | 0.69 | 0.71 | 0.69 |
| | Medium | 1.51 | 1.57 | 1.54 |
| | High | 2.09 | 2.16 | 2.13 |
| | Choice | 1.13 | 1.07 | 1.10 |
| | Mean (Strain) | 1.35 | 1.38 | |
| | SED: Strain = .0066 $^{n.s}$ | Diet = 0.0933*** | Strain x Diet = 0.0623 $^{n.s}$ | |
| 17 - 20 | Low | 0.84 | 0.82 | 0.83 |
| | Medium | 1.91 | 1.77 | 1.84 |
| | High | 2.83 | 2.56 | 2.69 |
| | Choice | 1.72 | 1.62 | 1.67 |
| | Mean (Strain) | 1.83 | 1.69 | |
| | SED: Strain =0.213 $^{n.s}$ | Diet = 0.301*** | Strain x Diet = 0.425 $^{n.s}$ | |

* P< 0.05 ** P< 0.01 *** P< 0.001 $^{ns}$P> 0.05

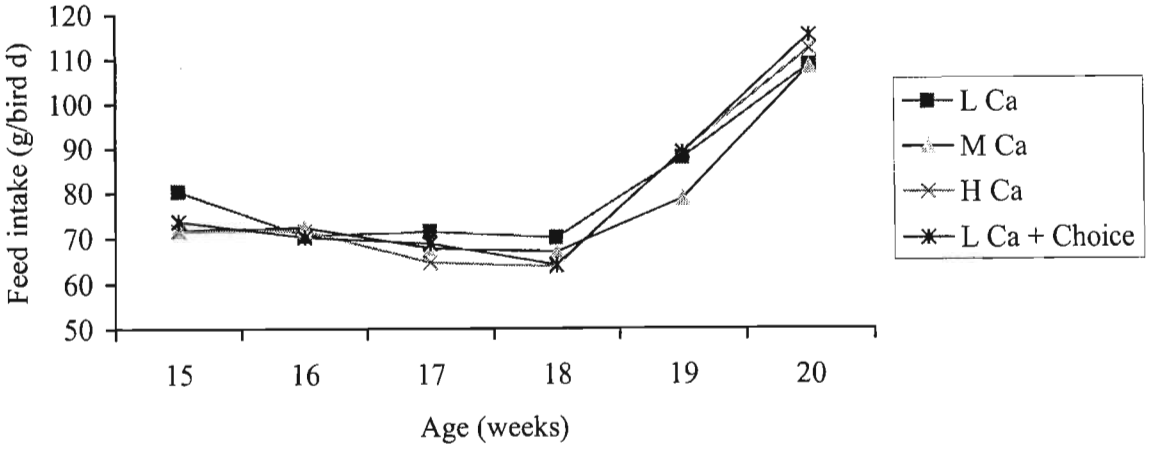


Figure 2.1: Daily feed intake of pullets at weekly intervals from 14 to 20 weeks of age: L Ca (low calcium diet), M Ca (medium calcium diet), H Ca (high calcium diet), and choice (limestone grit)

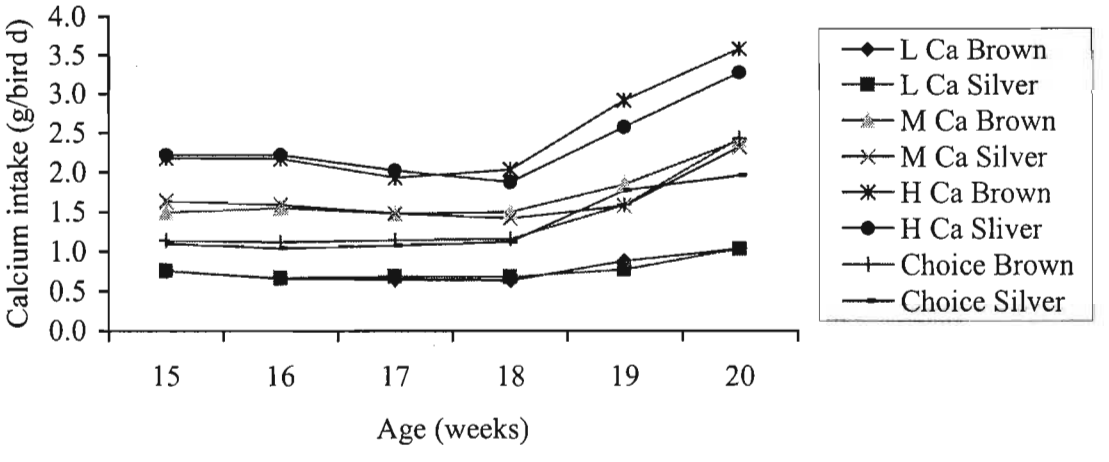


Figure 2.2: Daily calcium intake of pullets at weekly intervals from 14 to 20 weeks of age. L Ca (low calcium diet), M Ca (medium calcium diet), H Ca (high calcium diet), choice (limestone grit), Brown (Brown Strain) and Silver (Silver strain)

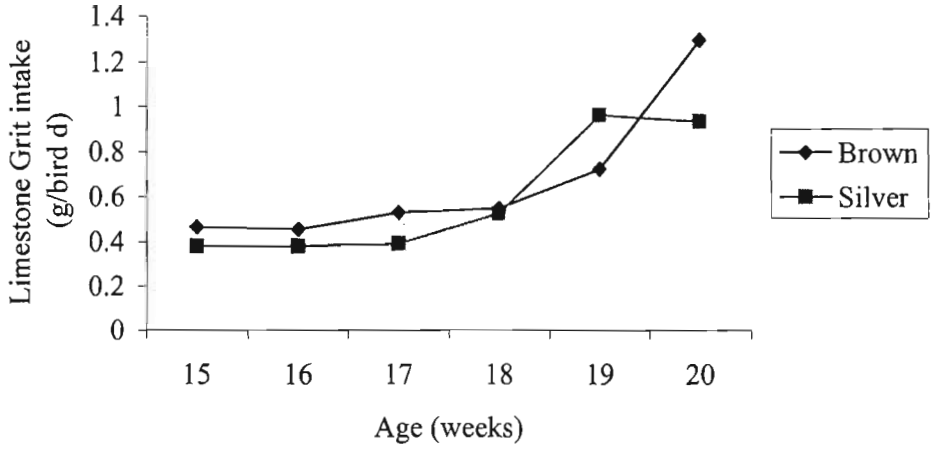


Figure 2.3: Limestone intake of pullets given a choice of a low calcium feed and limestone grit at weekly intervals from 14 to 20 weeks of age. Brown (limestone intake for Brown strain) and Silver (limestone intake for Silver strain)

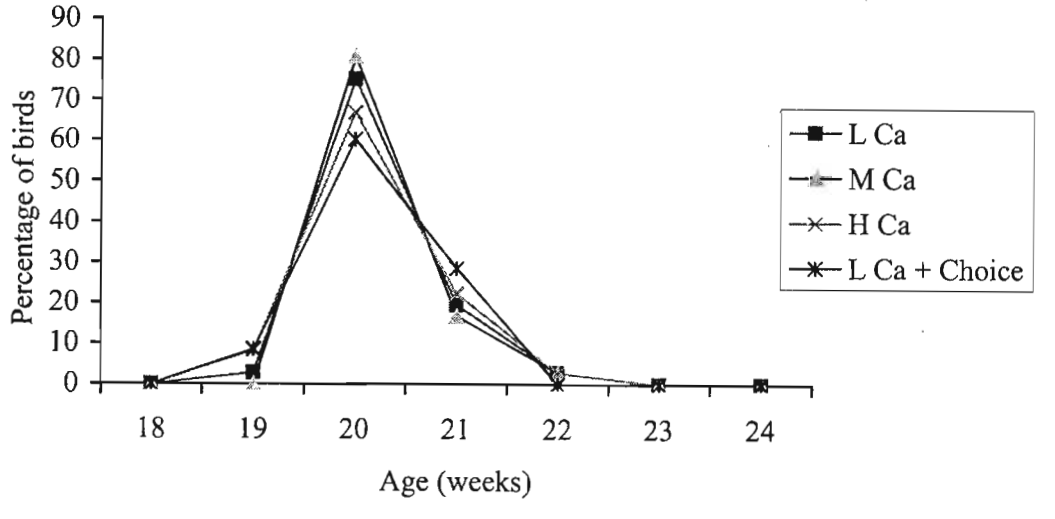


Figure 2.4: Proportion (percentage) of birds achieving sexual maturity each week. L Ca (low calcium diet), M Ca (medium calcium diet), H Ca (high calcium diet), and choice (limestone grit)

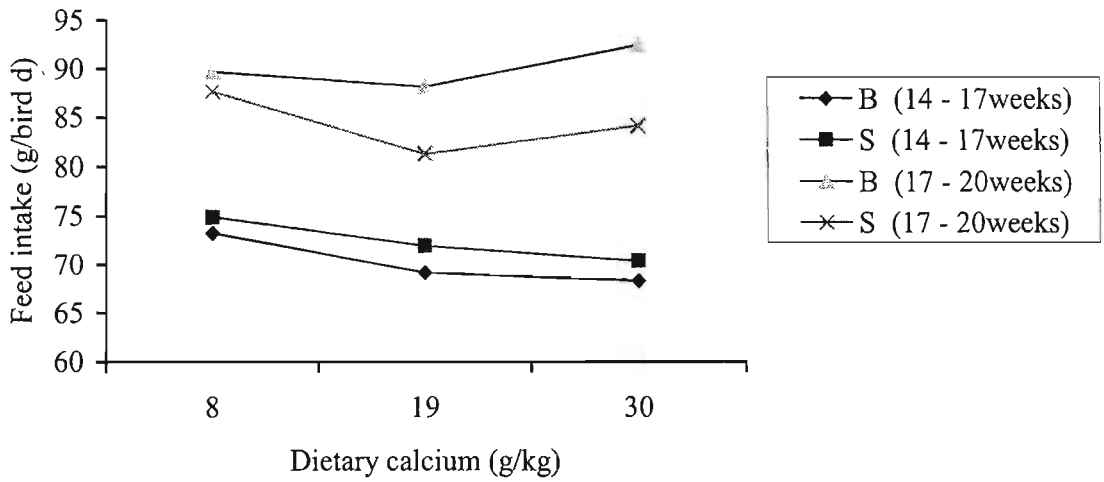


Figure 2.5: The response in feed intake of pullets of two strains to three dietary calcium levels at weekly intervals from 14 to 20 weeks of age. B (food intake for Brown strain) and S (food intake for Silver strain)

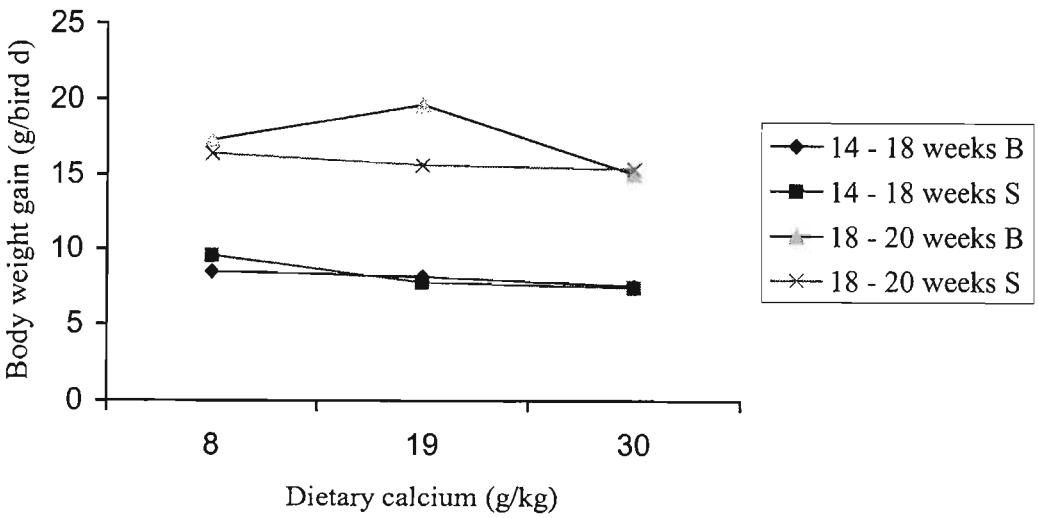


Figure 2.6: The response in bodyweight gain of pullets of two strains to three dietary calcium levels at weekly intervals from 14 to 20 weeks of age. B (Brown strain) and S (Silver strain).

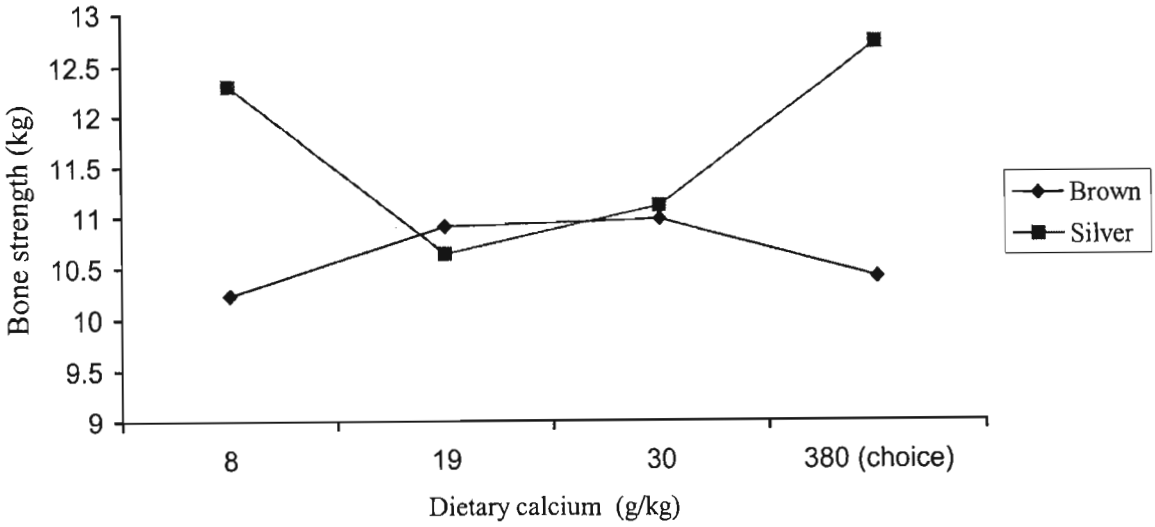


Figure 2. 7: The response in bone strength of pullets (at 18 weeks of age) to three dietary calcium levels

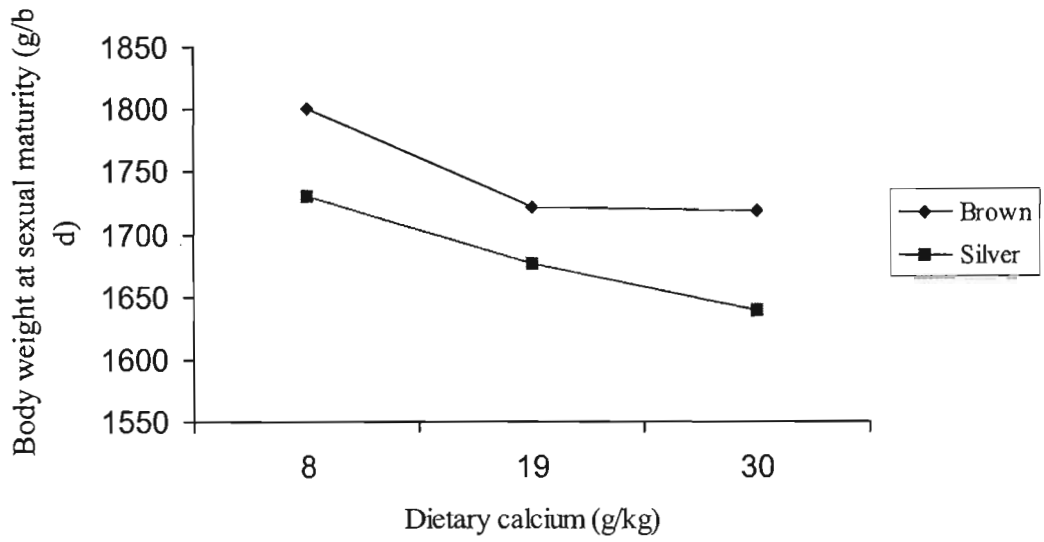


Figure 2.8: The response in bodyweight at sexual maturity of pullets to three dietary calcium levels

## 2.4 Discussion

The results show the response of two strains of pullets to low, medium and high levels of calcium in the diet and to a low calcium diet fed simultaneously with limestone grit as a choice source of calcium. These feeding treatments, applied before sexual maturity, affected most of the variables measured, although frequently the effect was not significant. The results of this experiment indicated that when low dietary calcium feed was given to birds, with limestone grit as a choice, birds consume enough feed with respect to their requirements for all essential nutrients, and they consumed separately the required limestone grit for each stage of development (Figure 2.3). This tends to refute the findings of Taher *et al.* (1984) who reported that when birds are offered limestone grit as a choice they tend to eat more than they require; in the present case intake increased as the need increased. So these data demonstrate the ability of pullets to adjust their own limestone grit intake with respect to the state of physiological development. It is for this reason the choice feeding treatment will be used as a base of comparison for other the three dietary calcium levels throughout the discussion.

Generally from 14 to 17 weeks of age, an increase in dietary calcium resulted in a decreased feed intake (Figure 2.5). The high feed intake on low calcium feed might have been due to the fact that pullets were trying to compensate for calcium deficiency by eating extra feed or because a low calcium diet is more palatable (Hughes and Wood-Gush, 1971), which implies that a high level of calcium in the diet tends to inhibit feed intake.

Assuming that pullets on the choice feeding treatment consumed enough feed to meet their requirements for all other nutrients and limestone grit for calcium requirements, the average feed intake of pullets on the low dietary calcium treatment is higher (>3 g/bird d) than that required and that on the high calcium diet is lower (< 1.6 g/bird d). Between 14 and 17 weeks of age, it was only the medium calcium pullets that had feed intakes that were the same (71 g/bird d) as those observed on the choice feeding treatment (Table 2.9). The amount of calcium that pullets on each treatment consumed depends on the amount of calcium in the diet. Assuming that pullets on the choice treatment consumed the required amount of calcium throughout the period, which was assumed to be 1.1g/bird d. Pullets that were on the high calcium diet consumed 2.13

g/bird d, which was 1.03 g bird d higher than the needed amount, and despite the fact that the feed intake of medium calcium diet was similar to that on the choice feeding treatment, the calcium consumed by pullets at this stage was also more (> 0.44 g/bird d) than required (Table 2.10). It can be clearly seen that the low calcium diet did not provide enough calcium, since the calcium intake of the pullets on this treatment was lower (< 0.403g/bird d) than on the choice feeding treatment, even though the feed intake was higher. It could be assumed that feed intake was increased in an effort to increase calcium intake. Perhaps feeding high calcium diets at this age is not crucial, owing to the fact that it is before the stage of bone development and it is possible that excess calcium might even lead to some other complications like visceral urate deposits, nephrosis and even interference with availability of other minerals as reported by Shane *et al.* (1969) and Hartel (1987). On the other hand the low calcium diet was also not adequate at this stage of growth. The results of this trial suggest that the required calcium intake should be around 1.1 g/bird d during the 14 – 17 weeks of age period. The change in body weight gain and the response that is shown in Figure 2.6 corresponds to the differences in feed intakes were that observed at this age.

The responses to the dietary treatments from 17 to 20 weeks of age were mostly not the same as those from 14 to 17 weeks of age, mainly because pullets were approaching sexual maturity during this phase. In this period the pullets are building up their reserves of calcium in the medullary bone as well as developing their ovary and oviduct in preparation for the production of eggs. Although feed intake for all treatments increased considerably during this period the average increase in intake by birds on the low calcium treatment was not sufficient, it was 0.84 g/bird d less than 1.67 g/bird d that was assumed to be the required intake of calcium by pullets on the choice feeding treatment, which was used as the base of reference (Figure 2.3). These results are in accordance with Scott *et al.* (1976) who found that 10g/kg dietary calcium is not enough to maximise bone mineralization prior to sexual maturity. Therefore, the low dietary calcium content is not adequate at this stage of development. The considerable increase in feed intake by pullets on the high calcium treatment was unlikely to have been due to an increase in calcium requirement, because these pullets consumed far more (>1.02g/bird d) calcium than was consumed by the pullet on the choice treatment (Figure 2.3). It is likely that pullets on the high calcium treatment increased their feed intakes in order to meet the requirement for other nutrients than calcium, such as protein

or energy, used in the egg formation process. The intersection of the medium calcium graph and the choice feeding treatments shown in Figure 2.2 indicates that the calcium content supplied by the medium calcium diet (1.84 g/bird d) is adequate at this stage of development, compared with 1.67 g/bird d from the choice treatment diet. This means that a dietary calcium content of around 19g calcium/kg is sufficient during the two weeks before sexual maturity.

Generally, pullets that were on the low calcium diet gained more weight (Table 2.7) than those on the choice treatment and as a result body weight at sexual maturity was excessive compared with that on the choice treatment. Conversely, the mean body weight gain for pullets on the high calcium treatment was less than of pullets on the choice treatment (Table 2.6). It was only the medium calcium diet that produced body weights that were close to those on the choice treatment. Figure 2.6 shows that despite the high feed intake that occurred on the high calcium diet, the body weight gain from 18 to 20 weeks of age did not correspond with the intake, indicating an inefficiency in this process.

Scott *et al.*,(1976) reported that high levels of calcium delay sexual maturity by influencing ovary and oviduct development. The results of the present study are not in agreement with his findings. Table 2.8 and Figure 2.4 show that dietary calcium level had no effect on age at sexual maturity. It is possible that there may be an effect if the different calcium contents are fed for longer than was the case in this trial, e.g. for the entire growing period rather than just four weeks.

The amount of calcium retained by laying hens depends on the amount of calcium included in the diet and on the amount required by the hen (Morris, 1972). So it does not follow that high calcium diets will necessary produce stronger bones than would medium or low calcium diets. Dietary calcium was shown to have an effect on bone breaking strength of laying hens by Sohail and Roland (2000), an increase in calcium concentration from 31 to 37g/kg increasing bone breaking strength from 16.5 to 21.5kg. But this was not the case with pullets at 17 weeks of age in the present trial, where there was no significant effect on bone strength (Table 2.6 and Figure 2.8). This might be because of a low calcium requirement before 18 weeks of age, or because pullets have the ability to regulate calcium absorption or that the different calcium contents have to

be fed for longer periods before differences can be detected. Hurwitz and Bar (1971) found that the poor absorption of calcium by pullets at this stage might be because the calcium absorption mechanism is not fully developed. In retrospect it would have been more useful to measure bone-breaking strength in the pullets at the time they reached sexual maturity than only three weeks after they had been placed on the experimental diets.

On average, the brown strain consumed more feed than the silver strain. Consequently this strain consumed more calcium and had greater body weight gain and a higher body weight at sexual maturity. Nevertheless, there was no difference in age at sexual maturity between strains (Table 2.8). Had the Brown strain reached sexual maturity much earlier than the Silver strain, this could have explained the increased need for calcium. So it can be concluded that the significant difference that existed in feed intake between strains was not related to calcium requirement, but perhaps to the higher maintenance requirement of the heavier strain.

The results of this trial suggest that the calcium content of feed given to pullets should be increased about two weeks prior to the attainment of sexual maturity. Alternatively, a separate source of calcium in the form of limestone grit could be made available during this period, which would enable the pullets to regulate their intake of calcium without having to under - or over - consume other nutrients in an attempt to obtain the desired amount of calcium prior to sexual maturity. In retrospect, the trial design could have been modified to take into account subsequent laying performance on the four dietary treatments, and shell strength and bone breaking strength could have been measured at different stages after sexual maturity. This would have made it easier to determine the best method of supplying calcium to pullets in the pre - laying period.

# CHAPTER 3

# CHANGES IN THE DAILY INTAKE OF CALCIUM DURING THE WEEKS PRIOR TO SEXUAL MATURITY IN PULLETS SUBJECTED TO DIFFERENT LIGHTING TREATMENTS DURING THE REARING PERIOD

## 3.1 Introduction

In the previous trial, all pullets were subjected to the same lighting programme, which resulted in the range in ages at sexual maturity being narrow. Under such circumstances it is difficult to separate out the effects of different dietary treatments on the response of pullets in the period just prior to sexual maturity. Consequently, in this trial three different lighting programmes were used in order to produce three different ages at sexual maturity. In this way, changes that take place in calcium intake prior to the onset of sexual maturity may be clearly distinguished from those that may occur chronologically.

The different lighting treatments were successful in altering age at sexual maturity. Pullets were photostimulated at 10, 14 and 18 weeks of age for the three treatments. Consequently, the changes that occurred in the variables of particular interest, namely food intake, body weight gain and limestone intake, may be expressed both chronologically (related to the age of the pullets) and relative to the age at sexual maturity. Both of these methods were used to determine the calcium requirements of pullets prior to the onset of lay.

## 3.2 Material and methods

### 3.2.1 Birds and management

Hy-line Brown and Silver pullets were raised from day old at Ukulinga Research Farm. At 10 weeks of age, 72 Silver and 72 Brown pullets were randomly selected from the main group. These pullets were weighed individually and randomly assigned to five light-tight rooms. Each room housed 24 (12 Brown and 12 Silver) individually caged pullets, with the exception of one room that housed 48 birds (24 Brown and 24 Silver). The growing cages measured 50, 45, and 55 cm in length, height and depth respectively (0.275 $m^2$ floor space). Each cage was equipped with a nipple drinker and two feeders measuring 25 cm long, 10 cm wide for choice feeding purposes.

3.2.2 Feeds

Pullets were fed *ad libitum* a commercial pullet starter mash from day old to six weeks and pullet grower mash from seven to 12 weeks of age. From 12 weeks till sexual maturity they were fed a commercial developer mash. The nutrient contents of the commercial grower and developer feeds are shown in Table 3.1. From 10 weeks of age all pullets were offered limestone grit in addition to the commercial feed supplied. This was supplied in a separate trough measuring 25 cm long, 10 cm wide.

**Table 3.1** Analysed nutrient content (as fed) (g/kg) of the two commercial feeds used from 10 weeks of age

| | Feed | |
|---|---|---|
| Nutrients | Grower | Developer |
| AME (MJ/kg) | 11.50 | 11.20 |
| Protein | 166.10 | 135.80 |
| Total phosphorous | 7.80 | 7.00 |
| Calcium | 13.80 | 11.90 |

3.2.3 Chemical Composition (Refer to Chapter 2, section 2.2.3)

3.2.4 Feeding Procedure

From 10 weeks until sexual maturity, one bucket that could hold up to 2 kg of feed was assigned to each cage and was filled with the feed and weighed. Feed was transferred from the buckets to the troughs when necessary. At the end of each week feed remaining in the trough was transferred back to the bucket, and each bucket with feed was weighed before adding more feed for the following week after which the bucket was weighed again. Feed intake was calculated by subtracting the amount remaining from the amount allocated and dividing by the number of birds and days between. The same procedure was used with the troughs containing limestone grit.

3.2.5 Lighting programmes (Treatments)

All birds were subjected to a lighting programme of 24 L: 0 D at day old. Day length was reduced during the first week to 8 L: 16 D. This day length was maintained until

the age at photostimulation. At 10 weeks of age, 48 pullets of the original 144 (housed in one room) were photostimulated by altering the lighting pattern to 14 L: 8D (LT 1). At 14 weeks of age another 48 pullets (housed in two rooms) were subjected to 14 L: 8D (LT 2). At 18 weeks of age the last 48 pullets (housed in two rooms) were photostimulated and the day length was increased to 14 L: 8D (LT 3).

### 3.2.6 Measurements

Body weights were recorded at the beginning of the trial and at the end of each subsequent week. Feed and limestone intakes were measured once a week. Age and body weight at sexual maturity were recorded for each pullet on the day that the first egg was laid.

### 3.2.6 Statistical analysis

The design of the experiment was a 2 x 3 (strain and lighting treatments respectively) factorial completely randomised design, with two replications. The ANOVA procedure (using the statistical package GENSTAT, 1998) was used to analysis the interactive effect between feed intake, calcium intake, and body weight.

## 3.3 Results

### 3.3.1 Feed intake

Feed intakes of the two strains and three lighting programmes, from 10 weeks of age until all birds on that treatment achieved sexual maturity are given in Table 3.2 and Figure 3.1. Feed intake for all strains and treatments increased with age and as the day length increased. Table 3.5 indicates the feed intakes of two strains of pullets on three lighting programmes at different stages of growth. There was no significant effect of strain on feed intake throughout the experiment.

### 3.3.2 Limestone grit intake and total calcium intake

The mean daily intakes of limestone grit for each week are shown in Table 3.3 and Figure 3.3. Figure 3.2 indicates calcium intake as limestone grit of pullets on the experimental treatments. The amounts of calcium consumed through the commercial feed supplied were calculated from the mean daily feed intakes, and these mean intakes

are given in Table 3.3 together with the sum of calcium consumed from both sources. In general calcium intake increased from 10 to 13 weeks of age and then decreased in LT 2 and LT 3, but continued to increase on LT 1 pullets. Pullets on LT 2 increased their intakes of calcium slightly after 14 weeks of age but considerably at 17 weeks of age. Pullets on LT 3 increased their intakes at 18 weeks (Brown strain) and 19 (Silver strain) weeks of age.

### 3.3.3 Body weight gain, body weight and age at sexual maturity

The results of change in body weight are shown in Table 3.4. Though the feed intake for LT1 pullets increased with age, the body weight change in all the weeks was almost the same and there was also no significant difference between the strains. Bodyweight gain increased as feed intake increased for pullets that were on LT 2 and LT 3 with the Silver strain showing a significantly higher ($P < 0.01$) body weight gain than the Brown strain on LT 2, but the effect was not significant for LT 3 pullets (Table 3.4). The mean body weight achieved among the treatments at sexual maturity significantly ($P < 0.01$) differs due to different lighting programmes practised. The earlier the light stimulation the smaller the body weight at sexual maturity. There were no significant differences between the strains for body weight at sexual maturity within each lighting treatment, there was a highly significant ($P < 0.001$) difference between the lighting treatments. Age at sexual maturity between strains within each lighting treatment was not significantly different (Table 3.6).

**Table 3.2** Mean weekly feed intakes (g/bird d) from 10 weeks until the attainment of sexual maturity of two strains on three lighting programmes

| Age (week) | Lighting treatments | | | | | |
|---|---|---|---|---|---|---|
| | LT 1 | | LT 2 | | LT 3 | |
| | Brown | Silver | Brown | Silver | Brown | Silver |
| 11 | 74.7 | 72.3 | 65.9 | 66.5 | 64.7 | 68.1 |
| 12 | 86.1 | 79.5 | 72.3 | 73.1 | 76.0 | 74.2 |
| 13 | 96.9 | 93.0 | 82.2 | 81.0 | 83.3 | 83.4 |
| 14 | 101.1 | 105.2 | 81.5 | 84.1 | 80.3 | 79.5 |
| 15 | 110.3 | 103.7 | 87.1 | 95.1 | 77.4 | 74.8 |
| 16 | 115.0 | 98.2 | 113.7 | 116.1 | 82.1 | 81.2 |
| 17 | 110.9 | 105.5 | 122.5 | 122.6 | 84.2 | 82.6 |
| 18 | | | 112.4 | 118.8 | 81.2 | 81.8 |
| 19 | | | 122.1 | 118.3 | 105.7 | 112.6 |
| 20 | | | | | 117.3 | 123.7 |
| 21 | | | | | 112.2 | 108.4 |
| Mean | 99.3 | 93.9 | 95.5 | 97.3 | 87.7 | 88.2 |
| SED: Strain | $5.7^{n.s}$ | | $1.5^{n.s}$ | | $1.0^{n.s}$ | |

*P < 0.05 **P < 0.01 ***P > 0.001 $^{n.s}$P > 0.05, wks (weeks),

**Table 3.3** Mean weekly calcium intake (g/bird d) of two strains of pullets subjected to three lighting programmes from 10 weeks of age till sexual maturity.

| Response variable | Age (weeks) | Basal Diet Ca | | Limestone Ca | | Total Ca | |
|---|---|---|---|---|---|---|---|
| | | Brown | Silver | Brown | Silver | Brown | Silver |
| | 11 | 1.03 | 1.00 | 0.57 | 0.25 | 1.61 | 1.25 |
| | 12 | 1.19 | 1.10 | 0.24 | 0.08 | 1.42 | 1.18 |
| | 13 | 1.15 | 1.11 | 0.39 | 0.30 | 1.55 | 1.41 |
| | 14 | 1.20 | 1.25 | 0.52 | 0.67 | 1.72 | 1.93 |
| LT 1 | 15 | 1.31 | 1.23 | 0.56 | 0.34 | 1.87 | 1.57 |
| | 16 | 1.37 | 1.17 | 0.47 | 0.26 | 1.84 | 1.42 |
| | 17 | 1.32 | 1.26 | 0.94 | 0.61 | 2.26 | 1.87 |
| | Mean | 1.22 | 1.16 | 0.53 | 0.37 | 1.75 | 1.52 |
| | SED: Strain = 0.06$^{ns}$ | | | | | | |
| | 11 | 0.91 | 0.92 | 0.24 | 0.11 | 1.15 | 1.03 |
| | 12 | 1.00 | 1.01 | 0.45 | 0.27 | 1.44 | 1.28 |
| | 13 | 0.98 | 0.96 | 0.63 | 0.39 | 1.61 | 1.35 |
| | 14 | 0.97 | 1.00 | 0.19 | 0.15 | 1.16 | 1.15 |
| LT 2 | 15 | 1.04 | 1.13 | 0.61 | 0.36 | 1.65 | 1.49 |
| | 16 | 1.35 | 1.38 | 0.26 | 0.29 | 1.61 | 1.67 |
| | 17 | 1.46 | 1.46 | 0.47 | 0.15 | 1.93 | 1.61 |
| | 18 | 1.34 | 1.41 | 1.18 | 1.50 | 2.52 | 2.91 |
| | 19 | 1.45 | 1.41 | 0.25 | 2.07 | 1.70 | 3.48 |
| | Mean | 1.17 | 1.19 | 0.48 | 0.59 | 1.64 | 1.77 |
| | SED: Strain = 0.071 $^{ns}$ | | | | | | |
| | 11 | 0.89 | 0.94 | 1.13 | 0.52 | 2.02 | 1.46 |
| | 12 | 1.05 | 1.03 | 1.11 | 0.72 | 2.16 | 1.74 |
| | 13 | 0.99 | 0.99 | 1.34 | 0.84 | 2.33 | 1.83 |
| | 14 | 0.96 | 0.95 | 0.80 | 0.43 | 1.75 | 1.38 |
| | 15 | 0.92 | 0.89 | 0.66 | 0.28 | 1.58 | 1.17 |
| | 16 | 0.98 | 0.97 | 0.45 | 0.13 | 1.43 | 1.09 |
| LT 3 | 17 | 1.00 | 0.98 | 0.67 | 0.26 | 1.67 | 1.25 |
| | 18 | 0.97 | 0.97 | 0.49 | 0.17 | 1.46 | 1.15 |
| | 19 | 1.26 | 1.34 | 1.05 | 0.09 | 2.30 | 1.43 |
| | 20 | 1.40 | 1.47 | 1.07 | 0.55 | 2.46 | 2.02 |
| | 21 | 1.34 | 1.29 | 0.69 | 1.10 | 2.03 | 2.39 |
| | Mean | 1.07 | 1.07 | 0.86 | 0.46 | 1.93 | 1.54 |
| | SED: Strain = 0.106*** | | | | | | |

*P < 0.05 **P < 0.01 ***P > 0.001 $^{n.s}$P > 0.05, S = Strain, W = Weeks, Limestone Ca = limestone grit calcium. Amount of calcium in limestone was assumed to be 38 %. SED for limestone grit intake.

**Table 3.4** Mean body weight gain (g/bird d) of two strains of pullets subjected to three lighting programmes from 10 weeks of age till sexual maturity

| Age weeks | Change in body weight (g/bird d) | | | | | |
|---|---|---|---|---|---|---|
| | LT 1 | | LT 2 | | LT 3 | |
| | Brown | Silver | Brown | Silver | Brown | Silver |
| 11 | 9.5 | 10.4 | 8.7 | 9.0 | 10.8 | 10.1 |
| 12 | 8.9 | 10.4 | 8.4 | 9.3 | 7.9 | 8.4 |
| 13 | 11.1 | 11.5 | 8.8 | 9.3 | 10.7 | 10.4 |
| 14 | 9.6 | 12.4 | 6.8 | 7.2 | 6.0 | 6.6 |
| 15 | 11.5 | 9.5 | 8.6 | 10.0 | 6.6 | 6.4 |
| 16 | 12.1 | 10.3 | 13.0 | 12.0 | 6.8 | 6.0 |
| 17 | 9.1 | 6.9 | 14.2 | 13.0 | 8.5 | 8.7 |
| 18 | | | 6.0 | 10.2 | 5.2 | 4.4 |
| 19 | | | 6.5 | 4.9 | 15.3 | 17.8 |
| 20 | | | | | 12.5 | 13.8 |
| 21 | | | | | 6.2 | 7.9 |
| Means | 10.3 | 10.2 | 8.9 | 9.4 | 8.7 | 9.1 |
| SED: Strain | $0.64^{n.s}$ | | 0.36** | | $0.34^{n.s}$ | |

*P < 0.05 **P < 0.01 ***P > 0.001 $^{n.s}$P > 0.05 wks (weeks), Initial mean weight of pullets at 10 weeks were: LT 1 Brown = 820 g/b and LT 1 Silver = 802 g/b, LT 2 Brown = 800 g/b and LT3 Silver = 810 g/b, and LT 3 Brown = 780 g/b and LT 3 Silver = 800 g/b

**Table 3.5** Mean feed intakes, body weight gain, intakes of limestone grit and total calcium (g/bird d) of two strains of pullets on three lighting programmes at different stages of growth

| Age | Mean feed intakes at different stages of growth (g/bird d) | | | | | | SED | |
|---|---|---|---|---|---|---|---|---|
| | LT 1 | | LT 2 | | LT 3 | | | |
| | Brown | Silver | Brown | Silver | Brown | Silver | Strain | LT |
| 16 to 18 weeks | 108.1 | 94.7 | 116.2 | 119.5 | 82.5 | 81.8 | $3.24^{ns}$ | 3.97*** |
| 3 weeks after P.S | 85.9 | 81.6 | 94.1 | 98.4 | 101.4 | 106.1 | $7.71^{ns}$ | $9.44^{ns}$ |
| 3 weeks before S.M | 108.1 | 94.7 | 119.0 | 120.0 | 111.7 | 114.7 | $3.73^{ns}$ | 4.56** |
| | Mean body weight gain at different stages of growth (g/bird d) | | | | | | | |
| 16 to 18 weeks | 8.8 | 10.4 | 11.1 | 11.7 | 6.8 | 6.4 | $1.42^{ns}$ | 1.74* |
| 3 weeks after P.S | 9.8 | 10.7 | 9.5 | 9.7 | 11.0 | 12.0 | $1.84^{ns}$ | $2.26^{ns}$ |
| 3 weeks before S.M | 8.8 | 10.4 | 8.9 | 9.4 | 11.3 | 13.2 | $2.02^{ns}$ | $2.47^{ns}$ |
| | Mean limestone grit intakes at different stages of growth (g/bird d) | | | | | | | |
| 16 to 18 weeks | 0.6 | 0.6 | 0.6 | 0.6 | 0.5 | 0.2 | $0.19^{ns}$ | $0.23^{ns}$ |
| 3 weeks after P.S | 0.4 | 0.2 | 0.4 | 0.3 | 1.2 | 0.7 | 0.15** | 0.19*** |
| 3 weeks before S.M | 0.6 | 0.6 | 0.6 | 1.2 | 0.9 | 0.6 | $0.25^{ns}$ | $0.30^{ns}$ |
| | Mean total calcium intakes at different stages of growth (g/bird d) | | | | | | | |
| 16 to 18 weeks | 1.9 | 1.7 | 2.1 | 2.1 | 1.5 | 1.2 | $0.22^{ns}$ | $0.18^{*}$ |
| 3 weeks after P.S | 1.5 | 1.3 | 1.5 | 1.4 | 2.1 | 1.5 | $0.19^{ns}$ | $0.16^{ns}$ |
| 3 weeks before S.M | 1.9 | 1.7 | 2.1 | 2.7 | 2.2 | 1.9 | $0.64^{ns}$ | $0.55^{ns}$ |

*P < 0.05 **P < 0.01 ***P > 0.001 $^{n.s}$P > 0.05, P.S = Photostimulation, S.M = Sexual maturity and LT = Lighting treatment

**Table 3.6** Mean body weight (g/bird), and age, at sexual maturity of two strains on three lighting programmes

| Responses variables | Treatment | Brown | Silver | Mean |
|---|---|---|---|---|
| Body weight at sexual maturity (g/bird) | LT 1 | 1457 | 1457 | 1457 |
| | LT 2 | 1573 | 1670 | 1621 |
| | LT 3 | 1704 | 1756 | 1730 |
| | Mean | 1578 | 1628 | |
| | SED: Treatment = 44.9** Strain = 114 $^{ns}$ | | | |
| Age at sexual maturity (days) | LT 1 | 113 | 111 | 112 |
| | LT 2 | 125 | 126 | 126 |
| | LT 3 | 146 | 145 | 146 |
| | Mean | 128 | 127 | |
| | SED: Treatment = 1.0*** Strain = 13.78$^{ns}$ | | | |

*P < 0.05 **P < 0.01 ***P > 0.001 $^{n.s}$P > 0.05

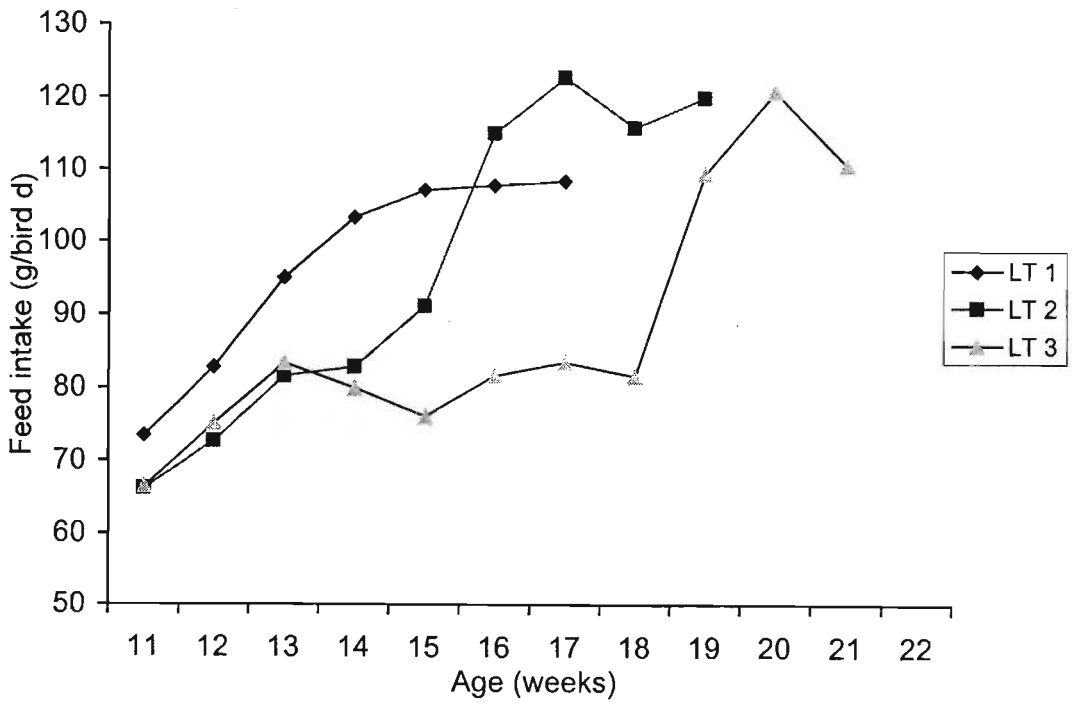


**Figure 3.1**: Mean daily feed intake of pullets on the experimental treatments. LT (Lighting treatment).

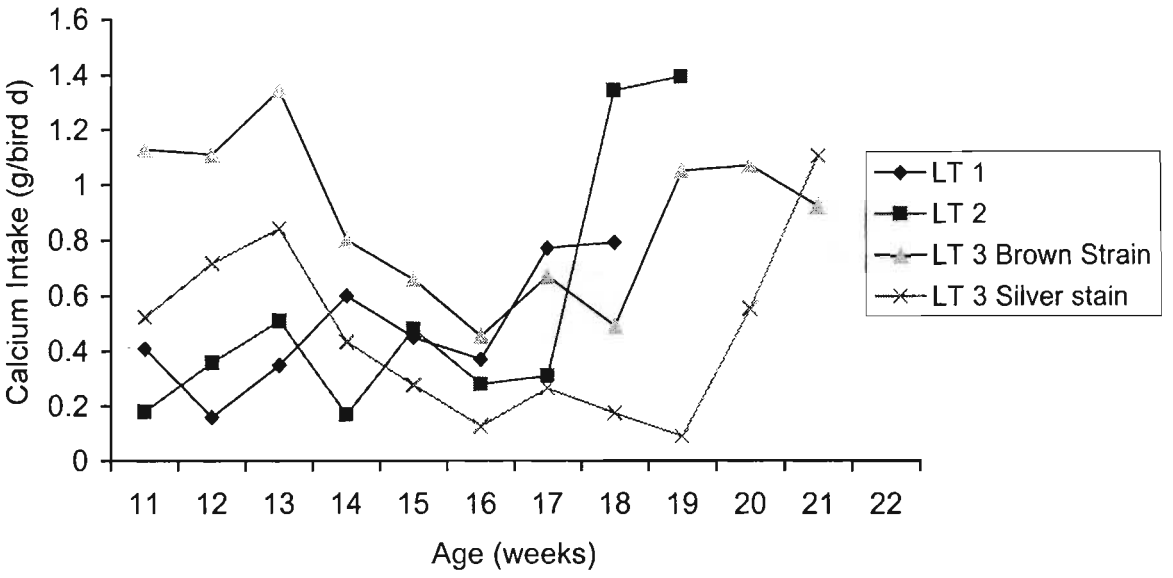


**Figure 3.2**: Calcium intake as limestone grit (380 g Ca/kg) of pullets on the experimental treatments. For LT 1 and LT 2 the mean for Brown and Silver strains are combined because there were no significant differences between strains

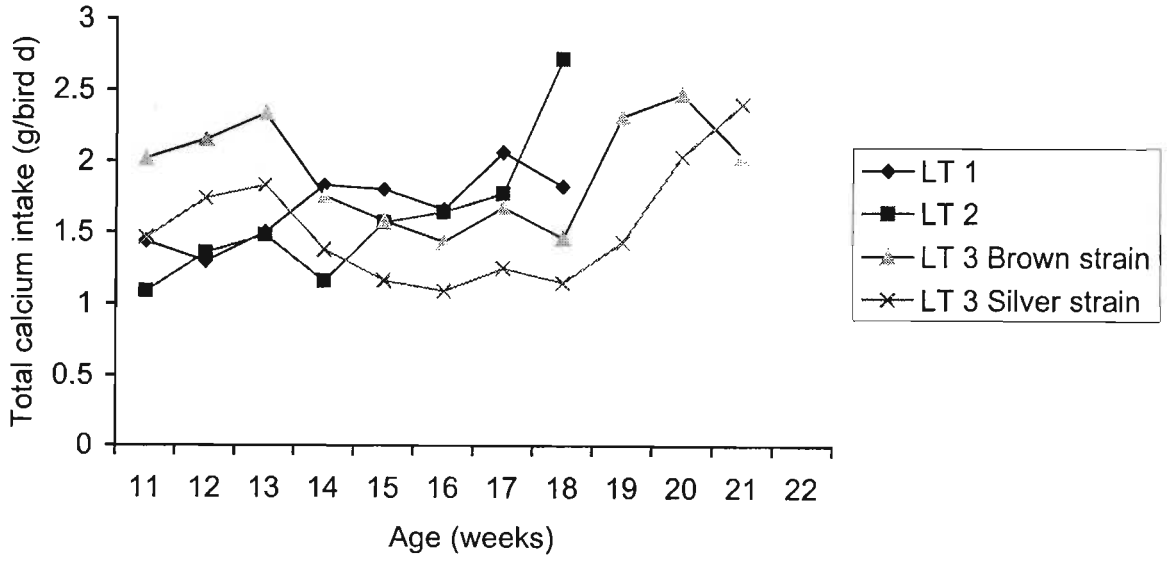


**Figure 3.3**: Total calcium intake of two strains of pullets on the three lighting treatments.

## 3.3 Discussion

In this trial the results show the responses of two strains of pullets to limestone grit supplied as choice when different ages at sexual maturity are attained with the use of different lighting programmes. From Figure 3.1, the similarity in the trends for LT 2 and LT 3 from 10 – 14 weeks of age indicates that pullets that were on the same lighting treatment pattern had the same nutrient intakes. Once the LT 2 pullets were photostimulated at 14 weeks of age, the two graphs diverged. The similarities to 14 weeks were because pullets had a similar environment, feed, they were of the same breed, and age. Roland *et al.* (1973) found that lighting has a major influence on growth, body composition of growing pullets and on the development of reproductive organs and these results in different calcium intakes and other nutrients. It can be seen from Figure 3.1 that pullets that were on LT 1 had higher feed intakes at an earlier stage than LT 2 and LT 3. It has been demonstrated that food intake by pullets increases proportionally with day length (Lewis, 1996). Because these pullets had a longer day length, they had more time to access the feed than pullets on the other treatments. Early photostimulation resulted in earlier sexual maturity than in the other treatments. But because they reached sexual maturity at an early age, most of their nutrients were directed to preparation of the reproductive organs, which explains why these pullets showed lower body weight gain and body weight at sexual maturity than the other treatments. The mean feed intakes (Table 3.5) from 16 to 18 weeks of age and three weeks before sexual maturity for all the treatments confirm that different ages of photostimulation resulted in different nutrients (e.g. calcium) requirements and as a result different body weight gains (Table 3.5) regardless of the fact that they were the same age. The calcium requirement should therefore be expressed in terms of particular stage of development rather than according to the age of the bird.

Generally, for all the treatments (Figure 3.1) feed intake increased considerably as the pullets approached sexual maturity. Additional nutrients are required at this stage for the development of the ovary and oviduct. And pullets that were photostimulated at 18 weeks of age had higher feed intakes (Table 3.5) three weeks before sexual maturity than the ealier-maturing pullets since heavier body requires more nutrients for maintenance. Classen and Scott (1981) found that feed intake by pullets offered calcium separately increased gradually and then dropped on the day of the first egg. Similar

trends were observed in this trial. However, the food intake was lowest a week before the first egg for LT 2 pullets, but it dropped on the week that the first egg was laid in LT 1 and LT 3 (Figure 3.1). In this trial it was not possible to determine feed intake at first egg since intake was measured only once a week.

There are no nutrients required specifically for the onset of sexual maturity, although this event is associated with substantial increases in consumptions of energy, protein and calcium to support shell formation. Mongin and Sauveur (1974) reported that calcium consumption was higher on egg formation days than on non-egg forming days. However, our results indicate that limestone intake could also be high two weeks prior to sexual maturity (Table 3.3) and that the amount of calcium required is related to the lighting pattern used; LT 1 pullets required less calcium than LT 3 pullets at this stage (Table 3.5). It can be seen from Figure 3.2 that limestone intake increased substantially two weeks prior to the onset of sexual maturity in each of the lighting treatment. Limestone intake was high for most of the treatments at the beginning of the trial and then decreased gradually. This might be because birds were still adjusting to limestone grit, since it was a new ingredient to them. If the birds are given a choice they sample the feeds to determine their composition, and when they have learned which are nutritious they eat predominantly from them, while sampling other feed occasionally to take advantage of any nutritious need which may become newly available as the birds grow (Forbes, 1995). This seems to be true for this trial, given that after a learning period limestone intake increased in a fluctuating manner. The fluctuating trends point to the fact that the pullets were predominantly sampling the limestone grit until the stage of medullary development.

Hurwitz (1976) recommended that at least two weeks before the onset of sexual maturity a diet containing approximately 20 g calcium/kg should be fed, noting that 10 g calcium /kg was not enough. The results of this trial are in accord with his finding. Figure 3.2 shows that limestone intake depends on the stage of medullary bone development, pullets increased limestone intake two weeks prior to sexual maturity on all treatments, even though they did not reach sexual maturity at the same age. It appears that the length of time between light stimulation and onset of lay is not constant, but decreases, as the birds get older and more somatically mature. So, the time between photostimulation and sexual maturity for those birds stimulated at 10

weeks of age was 42 days, for the 14 weeks birds was 28 days, and for the 18 weeks birds was 20 days, the results of this trial are in accord with the findings by Gous *et al.* (2000). The increase in calcium intake observed was therefore not directly related to the age at photostimulation but to age at sexual maturity and this proves the ability of pullets to determine how much and when calcium intake should be increased with respect to their physiological stages of development. To confirm the ability of pullets to regulate calcium, that has been reported by Holcombe *et al.* (1975), Joshua and Mueller (1979), and Irving *et al.* (1981), though LT 3 pullets were given a choice of limestone grit at an earlier age their limestone intake was lower than the rest up until two weeks prior to sexual maturity. So between 14 to 18 weeks of age the pullets did not see the need to increase limestone intake, since they reached sexual maturity at a later stage than the rest. From Figure 3.2 LT 3 , it can be seen that higher limestone intake was observed on Brown pullets than on Silver pullets but, on the other hand, the Silver strain had higher mean feed intakes than the Brown strain. This indicates that Silver strain pullets reached their calcium requirement by consuming more feed, consequently exhibiting higher bodyweight gain to and body weight at sexual maturity than the Brown strain. However, there was no difference in age at sexual maturity between strains (Table 2.9). Had the Brown strain reached sexual maturity a long way before the Silver strain, this could have explained the increased need for calcium. So it can be concluded that the significant difference that existed in limestone grit intake between strains is unlikely to be related to calcium requirement.

In conclusion, the present study indicates that pullets are able to regulate their own calcium intake depending on their physiological state; birds that reached sexual maturity earlier due to the lighting programme applied consumed more calcium at an earlier age than those that reached sexual maturity at a later stage. It appears that when pullets are given a choice they increase their intakes of calcium for medullary bone retention by either increasing feed intake or the intake of limestone grit two weeks before sexual maturity. Taking advantage of calcium appetite that seems to develop at the stage of medullary bone development, and the ability of pullets to regulate limestone intake, the introduction of limestone grit in a self-selection diet in the early stages of medullary bone development solves the problem of an inadequate or an over-supply of calcium by the basal diet and it overcomes the problem of variations that exist in different flocks brought about by different management practices. The principle of offering pullets a

calcium source in addition to a low calcium pullet developer feed, as used in this trial, should assist pullet producers to minimize eggshell and weak bone problems in laying pullets.

Trials of this nature would be improved by monitoring the calcium status of pullets through the laying period, using responses such as eggshell thickness, egg size, and production rate and bone breaking strength, as the information collected in this trial does not provide evidence that the changing intake of calcium by the pullets just prior to sexual maturity would be the most appropriate in the long term.

# CHAPTER 4

## GENERAL DISCUSSION

The composition of a feed for growing or laying pullets is based on the nutrients required by the bird at the stage of growth or production and the predicted food intake under the given environmental conditions. The daily requirement for each nutrient is often based on the assumed potential growth rate or rate of production of the bird at that time. In defining the calcium requirement of a growing pullet prior to sexual maturity, the difficulties faced are in predicting the age of sexual maturity, and the daily amount of food that will be consumed, as well as in knowing what are the consequences of feeding high or low calcium diets during this period. The aim of this research was to evaluate a system in which pullets were given the opportunity of adjusting their calcium intake according to demand, independently of the intake of other essential nutrients.

Because the measurements were taken only to the point where pullets started laying, the number of measurements that could be used for this evaluation was limited to weight gain, food and calcium intake, age at sexual maturity and bone strength. The latter measure proved to be unsuccessful in evaluating the different treatments, probably because too little time elapsed between the start and end of the trial to have any impact on bone strength. This measure may be worthwhile if birds are maintained on different calcium intakes for a long period.

In the first experiment, weight gains and food intakes provided useful information when evaluating the three calcium levels used. Assuming that the birds offered the low calcium feed and supplemental calcium as a choice were consuming the desired amount of calcium (which was found to be 80.1g/ bird d) during the period to sexual maturity, the higher (81.4g/bird d) intake of the low calcium feed was obtained, whereas the lower intake of 77.9g/ bird d and 77.8 g/ bird d were obtained for medium and high calcium food respectively. The average daily gain of 11.6, 11.2 and 10.0 g/bird d corresponded to the food intakes observed. In this trial all birds were on the same lighting programme, so resulting in differences in age at sexual maturity to confound the effect of dietary treatments.

In the second trial, differences in sexual development were created with the use of different lighting programmes. This enabled the separation of the chronological changes that take place in calcium intake prior to the onset of lay from those that might occur by different lighting programmes. As with the first trial, it appeared that the need for calcium increased around two weeks before sexual maturity, as in all cases calcium intake increased at that time. Before this increase, pullets preferred to keep calcium intake low.

The recommendation from these trials is therefore that pullets should be maintained on low calcium feeds until two weeks before sexual maturity, at which time they could either be offered a complete feed containing about 20 g calcium / kg, or, preferably, be given limestone grit in addition to a low calcium feed, as a source of calcium. Using the latter treatment enables birds at different stages of sexual development to consume the appropriate amount of supplementary calcium without having to overconsume other nutrients that may be unnecessary at that time. Because all the measurements were taken up to the age of sexual maturity it was not possible to follow the effects of the different rearing treatments through into the laying period. It is therefore not possible to resolve the dispute between researchers regarding the harmful effects, or otherwise, of feeding high levels of calcium to pullets during the rearing period. Nor is it possible to determine whether the choices made by pullets, when offered a separate source of calcium, would have resulted in the best possible laying performance. Such a follow up study would be important.

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