THE INFLUENCE OF CALCIUM INTAKE BY BROILER BREEDERS ON BONE DEVELOPMENT AND EGG CHARACTERISTICS

by

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31 July 2005

DECLARATION

I declare that the thesis hereby submitted by me for the **PHILOSOPHIAE DOCTOR** degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of the thesis in favour of the University of the Free State.

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ACRONYMS AND ABBREVIATIONS

ALP	alkaline phosphatase
AMP	adenosine monophosphate
ANOVA	analysis of variance
avP	available phosphorus
ASA	American Soybean Association
BBS	beta backscatter
BMU	basic multicellular unit
BS	bone strength
BW	body weight
BWG	body weight gain
Ca	calcium
Ca^{2+}	calcium ions
CaBp	calcium binding protein
$CaCO_3$	calcium carbonate
$Ca_3(PO4)_2$	tricalcium phosphate
cm^2	centimetre squared
CP	crude protein
CT	calcitonin
CV	coefficient of variation
DM	dry matter
EODES	erratic oviposition and defective egg syndrome
ESG	egg specific gravity
g	gram
GH	growth hormone
GLM	General Linear Models
hr.	hour(s)
ICU	international chick units
IGF	insulin-like growth factor
kcal	kilocalories
kg	kilogram
l	litre
ME	metabolisable energy
$Mg_3(PO4)_2$	magnesium phosphate

MJ	megajoules
mg	milligram
MSU	Mississippi State University
mm	millimetre
mRNA	messenger RNA
N	Newton
NaCl	sodium chloride
nm	nanometer
NPP	nonphytate phosphorus
NRC	National Research Council
P	phosphorus
PGE	prostaglandins of the E-series
pH	hydrogen ion concentration
Pi	inorganic phosphorus
PO_4^{2-}	phosphate ions
PRL	prolactin
PTH	parathyroid hormone
RNA	ribonucleic acid
SAS	Statistics Analysis Systems
SWUSA	shell weight per unit surface area
TCA	true cortical area
TGF	transforming growth factor
TRAP	tartrate-resistant acid phosphatase
vs.	versus
µg	microgram
µm	micron
^oC	degrees celsius
$1,25(OH)_2D_3$	dihydroxycholecalciferol

CHAPTER 1

GENERAL INTRODUCTION

Minerals constitute about 4% of vertebrate animals of which calcium and phosphorus make up more than half of this amount (Cromwell, 1991). These two minerals comprise more than 70% of the mineral content of the animal body (Todd, 1976; Maynard *et al.*, 1979; Singh & Panda, 1996). It is stated (Klasing, 1998) that calcium is the most prevalent mineral in the body and is required in the diet in larger quantities than any other mineral. The high calcium requirement of growing chicks is driven by the need for skeletal mineralisation.

Calcium is one of the key elements required for maintenance and egg production. It plays a major role in a wide variety of biological functions (Elaroussi *et al.*, 1994). According to Todd (1976) and Cromwell (1991), more than 99% of calcium and about 80% of phosphorus is found in the skeleton, where these elements are deposited as the hydroxyapatite. About 1% (or 5-10 mg) of calcium, which is found in the soft tissues, is of great importance, as it is the portion responsible for most body functions (Langemann, 1984; Perry, 1984). Similarly, 20% of phosphorus found outside the skeleton is responsible for many functions (Todd, 1976).

Calcium and phosphorus are essential for the formation and maintenance of the skeleton. In laying hens, calcium is utilised for eggshell formation [National Research Council (NRC), 1994; Ross Breeders, 1996]. According to Simons (1986), a laying hen will deposit in eggshells during one year of production 30 to 40 times the calcium present in its own skeleton. Phosphorus is also an important element in shell formation, not because eggshell contains much phosphorus (there is about 100 times as much calcium as phosphorus in eggshell), but because of the special relationship that exists between calcium and phosphorus in bone formation (Boorman *et al.*, 1985). The two minerals occur in the body in combination with each other most of the time, and an inadequate supply of either of the two in the diet limits the utilisation of both (Maynard *et al.*, 1979). Because of many interactions of calcium and phosphorus, the two elements are usually considered together (Singh & Panda, 1996;

Touchburn *et al.*, 1999). In broilers, the body calcium and phosphorus increase more than 60 times during 6 weeks of life (Simons, 1986).

Several factors affect the bone calcification process including age, hormones, dietary calcium and vitamin D. Parathyroid hormone that is produced in the parathyroid glands plays a major role in bone calcification. If this hormone is lacking, the serum level of calcium is halved (Perry, 1984). Sex hormones also play a role in calcification. The appearance of sex hormones at puberty hastens bone calcification and the hardening of the area of bone growth called epiphyseal junction. The level of dietary calcium will also affect bone formation; the presence or absence of vitamin D that is required for the absorption and utilisation of calcium, will affect this process, as well (Perry, 1984).

The rates of genetic change for production parameters such as growth, feed efficiency and yield have changed the physiology of the birds. Live weights of broilers at 42 days have more than doubled in the last 23 years from 1050 to 2600 g and are projected to reach 3 kg by the year 2007 at current rates of progress (McKay *et al.*, 2000). Therefore, the impact of rapid growth on two aspects of physiology such as skeletal development, heart and lung function has attracted great attention (McKay *et al.*, 2000). It is contended (Williams *et al.*, 1998, 2000) that the growth performance of broilers has changed considerably over recent years because of genetic selection for meat production but poultry diets have changed little in terms of mineral content. Williams *et al.* (1998) postulated that perhaps the porosity observed could be ascribed to the occurrence of more rapid bone modelling and remodelling in modern birds, together with inadequate supply of mineral (primarily calcium and phosphorus) in the commercial diets.

It is generally recognised that genetic selection for muscle growth in the broilers has resulted in an imbalance between the development of various body systems, which include increased demands being placed on the skeletal integrity. This process, however, is made difficult by the numerous specific causes of leg problems (Williams *et al.*, 2000). This is consistent with Turner (2000), who reported that the intense selection for fast growth and efficient feed conversion results in very serious welfare problems for broiler breeders. Bone breakages in live animals pose a significant

welfare problem that needs to be constantly monitored and addressed (Stewart, 1996). In broiler breeders, lameness is a worldwide problem, which causes the chickens a lot of discomfort and results in economic losses to the poultry industry (Hybro Breeders, 2001). According to Turner (2000), broilers suffer from three main types of lameness, which include lameness due to abnormal bone development, bone and joint diseases associated with infections, and lameness associated with degenerative diseases (*e.g.* osteoarthritis). Hybro Breeders (2001) estimated rearing mortality and culling due to lameness to be over 30% of the total losses.

Bone problems are a major health concern in meat-type and breeder poultry. Rapid growth results in skeletal deformities including problems caused by leg weakness that result in lameness and consequently poor animal welfare (Sanotra *et al.*, 2001). Skeletal problems are common in both young and old poultry and are often related to bone weakness. They affect mortality on the farm and condemnations within processing plant and thus raise both welfare and economic concerns (Rath *et al.*, 1999). Leg weakness is multifactorial in origin and can be influenced by management, genetics, environment and nutrition (Sanotra *et al.*, 2001). According to Julian (1998), skeletal deformities can also be mechanically or toxin induced. Mechanically induced problems are also more frequent in fast growing broilers. Genetic faults as well as toxins in feed or water can cause skeletal deformities, as rapidly growing birds would consume more of the offending product. However, many skeletal defects in broiler and roaster chickens are rare or absent in slower growing strains. Nutritional deficiencies can result in skeletal disease in all birds. Havenstein *et al.* (1994) reported that rapidly growing birds have a higher requirement for specific nutrients.

Broiler breeder hens are routinely reared to sexual maturity by applying feed restriction programme, which decreases costs and maximises reproductive fitness and health (Robinson *et al.*, 1993). Costa (1981) states that most primary breeders particularly those breeding the heavier strains would recommend feed restriction as early as 3 weeks of age in order to counteract the rapid growth, excess feed consumption and resulting over-fatness which would otherwise impair their reproductive performance. Feed restriction in broiler breeders results in changes in the relative growth of different body components, but there is little information on the

effect of this on bone structure and composition (McCormack *et al.,* 2001). Hocking *et al.* (2001) assessed the welfare of modified rearing programmes for broiler breeders and reported that the food-restricted birds consumed more water at all ages than birds on *ad libitum* feeding. In that study, water intake was high for restricted birds fed high protein diet compared with low protein diet. It is believed that excessive drinking is directly related to stress, which suggests that low protein diets may also improve welfare directly.

As mentioned earlier, calcium plays an important role in bone formation and development. Excessive dietary calcium levels can increase the pH in the gut resulting in decreased absorption of phosphorus from the intestines as well as that of magnesium, manganese and zinc (Ensminger, 1992; NRC, 1994; Van der Klis & Versteegh, 1996). Excess calcium may also lead to phosphorus deficiency by the formation of insoluble calcium phosphates in the digestive tract (Arthur *et al.*, 1983; Kaplan, 1995), impaired metabolic functions (Kaplan, 1995), a decline in feed intake and visceral calcification (Hubbard Feeds Inc., 2000), and reduced growth in chickens (Summers *et al.*, 1976; Shafey, 1993). On the other hand, low dietary calcium contributes to birds increasing feed and water intake in comparison to those getting adequate dietary calcium (Damron & Flunker, 1995). Feeding high dietary level of phosphorus leads to cessation of egg production. High dietary level of phosphorus also has adverse effects on shell quality as it contributes to the alteration of the acid-base balance (Keshavarz, 1994).

The calcium level in the starting and growing pullet diets must stimulate the development of the glands or organs that control the calcium feed back mechanism, which is essential for calcium mobilisation in egg production (Patrick & Schaible, 1980). Therefore, providing the starting chick with adequate calcium ranging from 0.8 to 1%, will initiate the development and functioning of the calcium feedback control mechanism so that when the need for calcium mobilisation for eggs arises, the laying hen's body will respond. In laying diets, the calcium level should start at about 2% two weeks before onset of egg production, slowly increasing to 3 to 3.6% (Patrick & Schaible, 1980).

In broiler breeder production, four types of breeder diets are used including chick starter, grower, pre-breeder and breeder all mash (ScanBrid Int., 2000; Ross Breeders, 2001). These breeding companies recommend that chick starter diet should contain 1% calcium and 0.45% available phosphorus. Ross Breeders' dietary calcium recommendation for the grower diet is the same as in the starter diet, while ScanBrid recommends a slightly higher level (1.1% calcium). Similarly, the suggested level of available phosphorus in the grower diet is 0.35% and 0.33% for Ross and ScanBro breeders, respectively. A calcium level of 1.5% is recommended for the pre-breeder diet (ScanBrid Int., 2000; Ross Breeders, 2001). Regarding the level of phosphorus in the grower diet, Ross Breeders (2001) recommends 0.40% and ScanBrid Int. (2000) 0.33%. While Ross Breeders (2001) recommends that breeder diets should contain 2.8% calcium and 0.35% available phosphorus, ScanBrid Int. (2000) recommends 3% calcium and 0.33% available phosphorus. These levels indicate that the nutrient requirements of broiler breeders are similar across the strains. For commercial layers, a dietary calcium level of 3.8% is recommended during lay (Dekalb Breeders, 1998). The NRC (1994) estimated the calcium and phosphorus (nonphytate phosphorus) requirements of broiler breeder hens to be 4% and 0.35%, respectively.

It is generally considered that modern poultry has been bred for superior meat production, possibly overlooking the consequences on bone quality (Rath *et al.*, 2000). For several years, there have been concerns about welfare of broiler chickens because of the unacceptably high incidence of bone disorders that result in lameness. The intense selection for rapid growth has produced problems not seen in slower growing birds such as skeletal and cardiovascular metabolic diseases (Classen, 2000). The objectives of this thesis were (1) to investigate the effects of feed restriction and *ad libitum* feeding on bone development in broiler breeder pullets during the first 18 weeks of age; and (2) to determine the effects of different levels of dietary calcium during rearing and laying periods on the reproductive performance and bone characteristics of broiler breeder hens.

This thesis is presented in the form of five separate articles, augmented by a general introduction, literature review and conclusions in an effort to create a single unit. Although care has been taken to avoid unnecessary repetition, some repetition has been inevitable.

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CHAPTER 2

LITERATURE REVIEW

The aim of this chapter is to extensively review literature on calcium metabolism of growing and laying hens. This entails examining previous and recent work on the effect of dietary calcium level on bone development and egg characteristics.

2.1 Functions of Calcium

2.1.1 Structural functions

Calcium is the most prevalent mineral in the body and is required in the diet in larger quantities than any other mineral (Siebrits, 1993; Elaroussi *et al.*, 1994). It is one of the key elements required for maintenance and egg production (Elaroussi *et al.*, 1994). Calcium plays a major role in a wide variety of biological functions in the body, of which the structuring of bones is the most important (Calnek *et al.*, 1991; Siebrits, 1993). It is therefore the most abundant inorganic component of the skeleton (Elaroussi *et al.*, 1994; Mateos & de Blas, 1998). The high calcium requirement of growing chickens is driven by the need for skeletal development. In laying hens, most calcium is used for shell formation (Klasing, 1998). Calcium constitutes more than a third of the total mineral content of an adult bird (Klasing, 1998) and comprises about 1.5% of the bird's weight (Underwood, 1981; Larbier & Leclercq, 1994). For instance, the skeleton contains 98-99% of a bird's calcium, most of which is in the form of hydroxyapatite, $(Ca_{10}(PO_4)_6(OH)_2$, with small amounts of noncrystalline calcium phosphate and calcium carbonate (Siebrits, 1993; Dudek, 1997; Klasing, 1998). The remaining 1% of calcium in the body is found in plasma and other body fluids.

Although calcium is only present within the extra and intra-cellular fluids in low concentrations, it plays an essential role in the control of a number of cellular functions. Living bone contains as much as 40% calcium and as little as 15% water depending upon its age and location in the body (Perry, 1984). The fat and water free portion of bone contains about 40% dry matter (DM) while the remainder is ash or minerals. The ash portion of bone consists of about 85% to 88% tricalcium phosphate ($Ca_3(PO4)_2$), 10% calcium carbonate ($CaCO_3$), and 1.5% to 2% trimagnesium phosphate ($Mg_3(PO4)_2$) (Perry, 1984; Seres, 1992). Therefore, the diet must supply significant amounts of phosphorus and calcium and reasonable quantities of

magnesium (Seres, 1992). In mammals, bone ash contains about 36% calcium, 17% phosphorus, and 0.8% magnesium (Maynard *et al.*, 1979). Bone ash also contains considerable amounts of citrate and small quantities of sodium, potassium, chlorine, fluorine, and traces of other elements.

The mineral component of bone consists mainly of hydroxyapatite crystals that contain large amounts of calcium and phosphorus (Weaver, 2001). In poultry, the major portion of dietary calcium is used for bone formation in growing chicks or poults and for eggshell formation in mature hens (Calnek *et al.*, 1991; Klasing, 1998). About 60-65% of the calcium in the eggshell is derived from dietary sources and the remaining 25-40% from medullary bone (skeletal stores) (Sugiyama & Kasuhara, 2001). According to Hopkins *et al.* (1987), the eggshell contains about 2.2 g calcium, in the form of 5.5 g $CaCO_3$ and 20 mg phosphorus.

2.1.2 Physiological functions

Calcium plays key roles in signal transduction and catalytic protein activation at the most fundamental levels of cell biology (Heaney, 1997). It plays a major role in mediating the constriction and relaxation of blood vessels (vasoconstriction and vasodilation), blood clotting, nerve transmission, the activation of enzymes including those necessary for the transmission of nerve impulses and for the contractile properties of muscle (Langemann, 1984; McDonald *et al.*, 1995; Dudek, 1997; Weaver, 2001). Calcium also plays a role in cell membrane permeability by facilitating the passage of nutrients in and out of the cell walls (Dudek, 1997; McWatters, 1997). It is usually associated with plasma proteins such as calcium binding protein (CaBp) or intracellular proteins (calmodulin) and in equilibrium with ionised state, the concentration of which is very precisely controlled both inside and outside the cells (Larbier & Leclercq, 1994). A more likely role of CaBp is buffering the calcium ions (Ca^{2+}) that enter the cell, so that the concentration of these ions in the cytoplasm is kept within physiological limits despite the large changes in calcium flow across the intestinal cells (Taylor & Dacke, 1984). According to Riggs & Bishop (2000), the rate of calcium absorption is directly proportional to the quantity of the CaBp in the intestinal cells.

Calcium plays a role in the control of nerve and muscle excitability (Cullinson, 1975; Perry, 1984). According to Weaver (2001), excitable cells, such as skeletal muscle and nerve cells, contain voltage-dependent calcium channels in their cell membranes, which allow for rapid changes in calcium concentrations. For example, as a muscle fibre receives a nerve impulse that stimulates it to contract, calcium channels in the cell membrane open to allow a few calcium ions into the muscle cell. These calcium ions bind to the activator proteins within the cell that release a large amount of calcium ions from storage vesicles inside the cell. The binding of calcium to the protein, troponin-c, initiates a series of steps that result in muscle contraction. The binding of calcium to protein, calmodulin, activates enzymes that break down muscle glycogen to provide energy for muscle contraction (Weaver, 2001). A decline in calcium levels results in increased excitability, whereas high levels result in a pseudotranquilizing effect. Reduction in the concentration of Ca^{2+} results in reduced nerve electrical across the membrane of the axon (Singh & Panda, 1996). Extremely low levels of calcium can result in tetany, which is followed by death (Perry, 1984). On the other hand, surplus calcium will not prevent the downward remodelling of excessively massive bone, but calcium deficiency will prevent the strengthening of bone (Heaney, 1997).

Calcium plays a vital role in acid-base balance by maintaining a pH of 7.4 to 7.6 (Cullinson, 1975; Perry, 1984; Singh & Panda, 1996; Mateos & de Blas, 1998). A lack of basic elements in the bloodstream causes acidosis, a condition often observed in lactating animals that are drawing too much calcium from the blood for milk formation resulting in acid-base balance being upset. Calcium is also responsible for initiation of neuromuscular functions, egg formation, immune system (Banerjee, 1992; Gillespie, 1987; 1998; McWatters, 1997), and contributes to more efficient weight gain and feed utilisation. Calcium can act as an activator or stabiliser of enzymes and plays a role in their secretion (Coon *et al.* 2001). It must also be present for the enzyme prothrombin to form thrombin, which reacts with fibrinogen to form the blood clot (Underwood, 1981). Blood will not clot in the absence of calcium. Additionally, calcium plays an important role in the secretion of hormones (Coon *et al*., 2001). Along with sodium and potassium, calcium is also needed for regulation of heartbeat (Banerjee, 1992; Singh & Panda, 1996). It also plays a role in fat and carbohydrate metabolism (Singh & Panda, 1996). Although most of the calcium in the

body is found in the skeleton where it makes up about one third of dry bone, calcium can also be found in body fluids.

In blood, calcium is found in the serum but not in the cellular portion. Approximately half of the blood calcium appears to be bound to protein, another half is in the ionic state (Perry, 1984). The plasma of mammals usually contains 80 to 120 mg calcium per litre, but that of laying hens contains 300 to 400 mg/l (McDonald *et al.*, 1995). With a layer diet of 3.56% calcium or higher, most shell calcium is derived by intestinal absorption, while for layers on a 2% calcium diet, 30 to 40% of the shell calcium is derived from bone. According to Fischer (1983), the completed shell weighs approximately 5 g and is 300 to 340 µm. Excessive levels of vitamin D as well as long periods of low calcium ingestion will result in a decline in serum calcium (Perry, 1984).

Calcium is one of the most metabolically active minerals and its metabolism is tightly regulated (Klasing, 1998). There is close hormonal control over concentration of calcium in the blood, and this is responsible for the absence of a correlation between the calcium intake and its concentration in blood. The two hormones that closely control the level of calcium in the blood are PTH and calcitonin (CT) (Fraser, 1988). PTH prevents hypocalcaemia by mobilising calcium from bone, while CT prevents hypocalcaemia by inhibiting bone resorption.

2.2 Factors Affecting Calcium Absorption

Several factors affect calcium absorption including site of absorption, vitamin D_3, Ca/P ratio, fats, pH, dietary Ca level, dietary P level, dietary level of salt, mycotoxins, phytates and oxalates, fibres, magnesium and iron. These factors are described briefly in the sections below.

2.2.1 Site

Calcium salts are more soluble in an acid solution; hence, absorption occurs mainly in the upper small intestine (duodenum) where feed contents are still somewhat acidic following digestion in the stomach (Ensminger *et al.*, 1990). Some absorption also occurs in the lower intestine (Perry, 1984; Underwood & Suttle, 1999). Calcium is

absorbed by active transport when dietary calcium levels are low. Passive absorption in the jejunum and ileum is the major absorptive process when calcium intake is adequate or high (Klasing, 1998; Bronner & Pansu, 1999). Bronner & Pansu (1999) stated that calcium that reaches the large intestine undergoes absorption there by both active process that requires energy and passive process. Active calcium transport has four primary steps: (i) energy-dependent uptake of Ca^{2+} across the enterocyte membrane; (ii) binding of Ca^{2+} to calbindin within endocytic vesicles; (iii) fusion of vesicles with lysosomes; and (iv) movement of lysosomes along microtubules and exocytosis of the contents at the basal lateral membrane (Klasing, 1998).

2.2.2 Vitamin D_3

Vitamin D that is perceived to be a precursor of vitamin D_3 is a crucial companion nutrient to calcium and phosphorus and it plays an essential role in the utilisation of the dietary calcium to maintain or regulate calcium homeostasis and to sustain metabolic functions of the birds' body (Fraser, 1988; Lopez, 2000). In the normal young chicken, about 70% of calcium absorption is vitamin D dependent (Hurwitz, 1992). Vitamin D promotes the intestinal absorption of calcium that mainly occurs in the duodenum (Roche, 2000). In nature, vitamin D exists in two major forms: ergocalciferol (D_2), and cholecalciferol (D_3). Vitamin D_3 ($1,25(OH)_2D_3$) enhances the intestinal absorption of both calcium and phosphate, and acts on the kidneys to stimulate absorption of calcium and phosphorus from the kidney and the bone.

The levels of PTH and vitamin D_3 control the efficiency of absorption. High levels of PTH and vitamin D_3 occur when levels of blood Ca^{2+} are low. They induce the synthesis of calbindin, which binds calcium and facilitates transport across the intestinal epithelial cells. As a result, a sufficient level of vitamin D_3 in breeding diets is necessary to ensure absorption of calcium by the hen (Klasing, 1998). Garlich & Wyatt (1971) reported that the minimum requirements for vitamin D_3 by the laying hen are 500 international chick units (ICU) per kilogram (kg) of feed.

In the opinion of Thompson & Fowler (1990), the absorption of calcium is regulated largely by animal requirements and is inversely related to intake. Calcium absorption is a closely regulated process that involves the action of vitamin D_3, PTH and CT (Thompson & Fowler, 1990). According to Fischer (1983), the average rate of

absorption of calcium from the digestive tract is 83 mg per hour and the short-term demand for calcification is met partly from labile stores in medullary bone.

2.2.3 Calcium/ phosphorus ratio

In growing diets, it is extremely important to have calcium to available phosphorus in the ratio of 2:1 (Schwartz, 1996), with 2.5 to 3.5 being too great and generally producing rickets. To avoid hyperparathyroid bone disease, Fraser (1988) suggested that for most animal species the dietary Ca-P ratio should be between 1.1 to 2:1. Singh & Panda (1996) reported that optimum results are achieved when Ca-P ratio is 1:1 to 2.2:1. These authors also claim that chickens may tolerate dietary Ca-P ratio of 2.5:1 without showing adverse effects, while a ratio of 3.3:1 is injurious. Roche (2000) recommends a Ca-P ratio of 1.4:1 for growing birds. A high molar ratio of calcium to phytate in the diet can lead to the formation of an extremely insoluble calcium-phytate molecule inaccessible to phytase. Low available phosphorus and/or improper ratios will also affect hatching quality and ultimately hatchability (Kornegay, 2000).

Excess calcium is excreted as a calcium-phosphorus (Ca-P) complex, which can result in a phosphorus deficiency if too much of the complex leaves the bird (Korver, 1999). Excretion of high levels of Ca-P complex may impair formation of both medullary (calcium reserve) and cortical (structural) bones. Excess dietary calcium will also contribute to decreased feed intake, visceral calcification, and other problems, as well as, a deficiency of other important nutrients (Hubbard Feeds Inc., 2000). Similarly, excess phosphorus in the diet forms insoluble calcium phosphate, which renders calcium unusable; as the body continues to absorb the phosphorus resulting in hypocalcaemia and metabolic bone disease (Kaplan, 1995; Henry, 1999).

According to Avian Farms (1996), feeding growing birds diets containing the calcium level in excess of 1.4% lead to development of gout and even crooked toes in male chickens aged less than 21 weeks. It is claimed (Singh & Panda, 1996) that nephrosis and visceral gout develop when dietary levels of calcium exceed 2.5% in growing birds. The digestibility of both calcium and phosphorus is depressed by an excess of either one but usually occurs because of excess calcium, the least expensive ingredient in feed (Touchburn *et al.,* 1999). The ratio of calcium to available

phosphorus of the diet is the main factor for achieving optimum weight gain during the starter period in this case of broilers (Hulan *et al.*, 1985, 1986). It is claimed (Hulan *et al.*, 1986) that as the total Ca + P and calcium to phosphorus ratios increase in the diets, biological performance [body weight gain (BWG), final live body weight and feed conversion] decreases. The authors also reported decreased tibia strength of normal broiler genotype due to increased ratio of calcium to available phosphorus in the starter and finisher diets. For dwarf genotypes, only the highest levels of calcium and available phosphorus in finisher diets decreased tibia strength.

A great excess of either calcium or phosphorus interferes with the absorption of the other, a fact that helps to explain why a certain ratio between these minerals in the diet is desirable for their absorption (Maynard *et al.*, 1979).

2.2.4 Fats

Moderate amounts of fat increase the transit time through the digestive tract, which makes for more time for mineral absorption. Acidity of the gastric juices, especially hydrochloric acid which is released by the stomach, lowers the pH of the contents of the digestive tract in the small intestine and favours dissociation and hence absorption. On the other hand, high fat diets may produce fatty acids in the intestines, which can also reduce the availability of calcium by forming insoluble calcium soaps that are assimilated with difficulty (Kaplan, 1995). In the opinion of Maynard *et al.* (1979) a certain amount of fat appears to favour the absorption of calcium. Faulty fat metabolism can also adversely affect the metabolism of vitamin D (Kaplan, 1995; Dudek, 1997) leading to a reduced calcium utilisation.

2.2.5 Intestinal pH

The chick's digestive system from 1 day to 3 weeks of age is extremely sensitive to dietary levels of calcium, which would increase the pH of the intestinal tract to 6.5 or above. When this pH is reached, manganese forms an insoluble complex and therefore is not absorbed. Zinc also forms an insoluble complex with phytic acid at this pH, which renders it and phosphorus unavailable to the young chick (Patrick & Schaible, 1980). Phosphorus absorption is optimal at pH 6 and when the pH is higher than 6.5, absorption is markedly decreased which results the absorption of calcium. A high calcium or phosphorus level in the intestine reduces the absorption of both calcium

and phosphorus (Anon, 2001). Recent results (Keshavarz, 2001) showed that increased dietary levels of phosphorus result in increased water and feed intakes, and total daily excreta output. Increased dietary level of calcium also decreased feed intake but did not have an effect on moisture content of the excreta, and daily water output.

2.2.6 Dietary level of calcium

The level of dietary calcium influences calcium absorption as high dietary calcium levels depress efficiency of absorption (Maynard *et al.*, 1979). Calcium levels greater than 4%, especially when supplied as $CaCO_3$ can reduce palatability, hence lower feed consumption, and can decrease the absorption of zinc and manganese resulting in the increased requirements of these minerals. Excessive calcium levels can also increase the pH in the gut resulting in decreased absorption of phosphorus from the intestines as well as that of magnesium, manganese and zinc (Ensminger, 1992; NRC, 1994; Van der Klis & Versteegh, 1996). Excess calcium may also lead to phosphorus deficiency by the formation of insoluble calcium phosphates in the digestive tract (Arthur *et al.*, 1983), impaired metabolic functions (Kaplan, 1995), a decline in feed intake and visceral calcification (Hubbard Feeds Inc., 2000). On the other hand, low dietary calcium contributes to birds increasing feed and water intake in comparison to those getting adequate dietary calcium (Damron & Flunker, 1995). Perry (1984) contends that the level of calcium available in the diet is inversely proportional to the absorbability of calcium across the wall of the small intestine.

A low calcium diet stimulates PTH secretion while a low phosphorus diet leads to an increase in concentration of ionised calcium in the plasma, which depresses PTH secretion. The effects of this depression are to conserve phosphorus (by reducing the PTH inhibition of tubular reabsorption of phosphate) and to allow the urinary excretion of the additional calcium absorbed from the gut during the low phosphate regimen (Taylor & Dacke, 1984).

2.2.7 High level of dietary phosphates

High levels of phosphates form calcium phosphates of low absorbability (Perry, 1984). In contrast, high plasma phosphorus decreases calcium absorption from the gut and calcium from the bone (Keshavarz, 1994). Feeding high dietary level of

phosphorus leads to cessation of egg production. High dietary level of phosphorus also has adverse effects on shell quality as it contributes to the alteration of the acid-base balance. On the other hand, feeding low phosphorus stimulates an increase in plasma $1,25(OH)_2D_3$ as well as ionised calcium/ total calcium (Frost *et al.*, 1991), while aflatoxins reduce plasma concentrations of 1,25 dihydroxyvitamin D_3 (Annison, 1995).

2.2.8 Dietary level of salt

Large amounts of salt (NaCl) in the diet may also interfere with calcium metabolism. The sodium ion interferes with the supply of bicarbonate ions to the shell gland that are essential for the formation of eggshell, which is mainly $CaCO_3$ (Korver, 1999). High sodium diet also increases urinary calcium excretion and causes loss of bone calcium (Chan & Swaminathan, 1998).

2.2.9 Mycotoxins in the diet

Some mycotoxins, particularly aflatoxin and ochratoxin appear to interfere either with vitamin D absorption, vitamin D metabolite production or vitamin D induced calcium absorption leading to development of rickets in spite of correct calcium/phosphorus ratio and adequate vitamin D (Swick, 1995; Roche, 2000). A higher level of feed contamination from aflatoxin, common in tropical regions is likely to result in higher incidence of leg disorders (Annison, 1995). Consequently, it is important that the balance between calcium and phosphorus is seriously considered when formulating poultry feeds (Keshavarz, 1994).

2.2.10 Phytates and oxalates

Several factors interfere with calcium absorption including compounds such as phytates (salts of phytic acid, a phosphoric derivative) in cereal grains and oxalates (oxalic acids). For example, sodium phytate, the hexaphosphate of the vitamin inositol, results in decreased absorption of calcium, probably by forming a complex with calcium, thus making it unavailable to the bird. An insoluble compound is also formed when calcium is combined with dietary oxalic acid (Perry, 1984; Dudek, 1997; Klasing, 1998). Compared with phytic acid, oxalic acid is the most potent inhibitor of calcium absorption, and is found in high concentrations in spinach and rhubarb and in lower concentrations in sweet potato and dried beans (Weaver, 2001).

The body cannot absorb this compound through the intestinal walls, and it passes through the digestive system and is excreted.

2.2.11 Fibres, magnesium and iron

According to Dudek (1997), excessive intake of certain fibres may also impair calcium absorption, as can excessive intakes of magnesium and iron.

2.3 Bone Metabolism and Function

Bone consists of living cells and an intercellular matrix that is impregnated with mineral salts. Bone is approximately 70% mineral, 20% organic matter, and 10% water. Collagen is the major organic matrix that confers tensile strength to the bone, whereas hydroxyapatite provides compressional strength (Rath *et al.*, 2000). Calcium phosphate makes up about 80% of the mineral matter, with the remainder composed largely of $CaCO_3$ and magnesium phosphate (Frandson, 1981). This mineral mass is attached to a living structure, the osein, which is soft and elastic and makes up the remaining third of the weight of fresh bone (Charray *et al.*, 1992). Bone is a vascularised, supporting skeletal tissue (although it may arise ectopically outside the skeleton), which is deposited by osteoblasts and by osteocytes, and removed, and hence remodelled, by osteoclasts and osteocytes (Villee *et al.*, 1989).

Bone is a dynamic tissue influenced by physiological, nutritional and physical factors such as mechanical and physical activities (Rath *et al.*, 2000). It is formed and destroyed continually under the control of hormones and physical factors. This constant activity allows the modelling process, i.e., modification of the bone architecture to meet physical stresses (Stevens & Lowe, 1992). Most bones have a large marrow cavity in the centre; this may contain yellow marrow, which is mostly fat, or red marrow, the connective tissue in which red and some white blood cells are made (Villee *et al.*, 1989).

2.3.1 Functions of bone

Bone serves important structural and metabolic functions. It supports the musculature and thus its growth and development are intimately associated with overall body growth. The hydroxyapatite form of calcium phosphate provides the structural rigidity of bone, and yet it is readily solubilised to supply its minerals to other tissues when

needed (Fraser, 1988; Rose, 1997; Klasing, 1998). Metabolically, bone provides a labile pool of calcium and phosphate that can be accessed during disturbances in mineral homeostasis (Norman, 1979; Loveridge *et al.*, 1992; Stevens & Lowe, 1992; Miller & Harley, 1999; Young & Heath, 2000). This is particularly important in poultry, as each egg shell contains about 2 g calcium that is equivalent to around 10% of the total body calcium of the hen (Loveridge *et al.*, 1992). Other functions of the bone are to provide protection for the soft tissues and vital organs and to permit locomotion (*e.g.* long bones) (Romanoff, 1960; Fraser, 1988; Banerjee, 1992; Stevens & Lowe, 1992; Bouxsein & Augat, 1999; Miller & Harley, 1999).

2.3.2 Bone formation and mineralisation

Bone formation and mineralisation is a complex process that is regulated by hormones, systemic growth factors and local factors. Information on the important role that systemic factors play in bone formation and resorption is relatively new. Growth factors such as insulin-like growth factor I (IGF-1), and transforming growth factor ß (TGF-ß) may increase bone matrix synthesis either directly or simply by increasing the number of collagen-synthesising cells (Ali, 1992). The prostaglandins of the E-series (PGE) have the greatest activity in bone tissues. PGE2 is a potent activator of bone resorption; however, both PGE2 and PGE1 stimulate collagen synthesis, bone formation, proliferation and differentiation of osteoprogenitor cells (Watkins, 1992). Vitamin D and its metabolites, as already mentioned, play an important role in the absorption and retention of calcium and are closely involved in bone formation and indirectly in its resorption. As in the cartilage cells, 1,25-$(OH)_2D_3$ enhances differentiation of bone cells (Hurwitz, 1992). PTH and CT determine the amount of calcium and phosphorus built into, retained and released from the bone (Ali, 1992). Calcitonin stimulates the proliferation of chondroblasts and increases chondrogenesis, while PTH enhances bone remodelling and promotes cartilage maturation during osteogenesis (Fraser, 1988).

Bones grow in length at flat pieces of cartilage, called growth plates. At the growth plates are cells, called osteoblasts, which synthesise and secrete osteoid, a collagen-rich protein. Osteoid forms a matrix on to which calcium and phosphate ions are adsorbed followed by crystallisation. Deficiencies of vitamins D and A can seriously reduce bone growth (Rose, 1997).

2.3.2.1 Bone formation

There are two primary types of bone formation: (a) intramembranous ossification and (b) endochondral ossification. The more metabolically important bone ossification is by endochondral ossification, which is relevant to most of the bone in the body (Ali, 1992). According to Stevens & Lowe (1992), endochondral ossification is the method whereby the foetus forms long and short bones. Endochondral ossification can also be defined as cartilage (which becomes calcified in mammals) that is subsequently eroded by vascular and marrow elements (Caplan, 1988). In this process, hyaline cartilage is deposited in the shape of the required bone and is subsequently transformed into bone by mineralisation. All the long bones are formed by endochondral ossification (Ali, 1992). On the other hand, intramembranous ossification results in the formation of flat bones (*e.g.* skull), and contributes to cortical bone shafts of long bones (Stevens & Lowe, 1992).

The formation of functional bone tissue can be categorised into (a) that concerned with production and secretion of the extracellular collagen bone matrix and (b) the deposition of mineral calcium hydroxyapatite crystals in the matrix (Norman, 1979). According to Ali (1992), the mineral, which gives bone its characteristic properties, has to be of a specific type (hydroxyapatite), and it has to be in a form that can be easily laid down and removed in response to the body's physiological requirements.

Bone development is a multi-step process that includes patterning of skeletal elements, commitment of haematopoietic and/or mesenchymental cells to chondrogenic and osteogenic lineages, and further differentiation into three specialised cell types: chondrocytes in cartilage and osteoblasts and osteoclasts in bone (Clement-Lacroix *et al.*, 1999). These workers established that osteoblasts express prolactin (PRL) receptors, suggesting that an effect of PRL on osteoblasts could be required for normal bone formation and the maintenance of bone mass. New bone forms on a core of mineralised cartilage by the action of lining cells or osteoblasts that have a mesenchymal origin. Bone forming surfaces can be categorised into three types: (1) bone forming surfaces; (2) quiescent or dormant surfaces where there is no new bone formation; and (3) resorptive surfaces where osteoclasts are seen dissolving the bone mineral (Ali, 1992). According to Owen (1971), there are two main types of cells in the body that are capable of osteogenesis: (1) undifferentiated

mesenchymal cells that are widely distributed throughout a variety of tissues and which produce bone only in the presence of an inducing agent, and (2) a population cells in marrow tissues which are predetermined in an osteogenic direction.

2.3.2.2 Bone mineralisation

Mineralisation is restricted to the extracellular matrix surrounding the hypertrophic chondrocytes. This matrix is extensively modified from the matrix of the other growth plate chondrocytes but the exact role of the matrix components during mineralisation is not clear (Loveridge *et al.*, 1992). According to Stevens & Lowe (1992), for mineralisation to take place the combined local concentrations of Ca^{2+} ions and phosphate (PO_4^{2-}) ions must be above the threshold value. A number of factors operate to bring about this. For instance, a glycoprotein (osteocalcin) in osteoid binds extracellular membrane Ca^{2+} ions, resulting in high local concentration. In addition, the enzyme alkaline phosphatase (ALP), which is abundant in osteoblasts, increases local Ca^{2+} and $PO4^{2-}$ ion concentrations.

Osteoblasts produce matrix vesicles that can accumulate Ca^{2+} and $PO4^{2-}$ ions, and are rich in enzymes such as ALP and pyrophosphatase, which can cleave $PO4^{2-}$ from larger molecules (Ali, 1992; Stevens & Lowe, 1992). According to Watkins (1992), a major proportion of matrix vesicles is phosphatidylserine, which is complexed with Ca^{2+} and inorganic phosphate (Pi). Matrix vesicles contain ion-transport proteins for Pi and Ca^{2+} and possess several active phosphatases, especially ALP. Osteoblast-derived matrix vesicles are believed to be the most important factor controlling the initial site of mineral deposition in osteoid of rapidly growing bone (Ali, 1992; Stevens & Lowe, 1992). It is also hypothesised that once the first few crystals of hydroxyapatite have precipitated out, they grow rapidly by accretion until they join foci growing from other matrix vesicles. Other cells that produce matrix vesicles are the ameloblasts and odontoblasts of the developing teeth, and chondrocytes hence the frequent mineralisation of cartilage (Stevens & Lowe, 1992). Although the growth hormone (GH) is considered the main regulator of postnatal bone growth, there is controversy regarding its action (Loveridge *et al.*, 1992).

2.3.3 Types of bone cells

Bone is a complex tissue made up of cells as well as extracellular material and is composed of both organic and mineral components such as calcium phosphate (Norman, 1979; Ali, 1992; Rose, 1997). The organic part of the matrix determines the orientation and structural organisation of bone, while the mineral gives bone its strength and rigidity (Rose, 1997). The organic phase is composed of organic fibres that are more able to withstand tension and torsion (Rose, 1997). Ninety percent of the organic matrix is type I collagen, the remainder being non-collagenous proteins such as osteocalcin, osteonectin, bone sialoprotein and osteopontein, which seem to have roles in bone mineralisation or resorption (Roach, 1994). Cells that are responsible for production, maintenance and modelling of the osteoid include osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts (Stevens & Lowe, 1992). Osteoblasts synthesise osteoid and mediate its mineralisation and osteoclasts are responsible for bone resorption (Stevens & Lowe, 1992; Young & Heath, 2000). These cells are briefly discussed in the sections below.

Osteoprogenitor cells

Osteoprogenitor cells are derived from the primitive mesenchymal cells and form a population of stem cells, which can differentiate into the more specialised bone-forming cells (i.e. osteoblasts and osteocytes) (Stevens & Lowe, 1992). The osteoprogenitor cells are usually a clearly defined layer between the cells on the surface of the bone and the fibroblastic layer (Owen, 1971). In trabecular bone in very young animals, the majority of the cells in the spaces between the trabeculae are osteoprogenitor cells. A few osteoprogenitor cells may also be present in Harvesian canals, in the space between the endothelial cells and the cells on the surface of the bone. The layer of osteogenic cells is always much thinner in old animals than in young animals and osteoprogenitor cells are often difficult to distinguish. Osteoprogenitor cells are recognised by their position in the tissue near to the surfaces of bone and their spindle-shaped mesenchymal appearance (Owen, 1971).

When osteoprogenitor cells change into osteoblasts, they undergo mitosis and may be identified by their uptake of tritiated thymidine, as demonstrated by autoradiography. They are also recognised by their changes in morphology, as they become osteoblasts, osteocytes, or osteoclasts. The transformation of these cells from one type to another,

which occurs spontaneously more frequently in developing bone, can be demonstrated in adult bone under certain conditions (McLean & Urist, 1968).

Osteoblasts

Osteoblasts are the skeletal cells responsible for synthesis, deposition, and mineralisation of the extracellular matrix of bone (Aubin, 1998). Osteoblasts are mononucleated cells found on the surface of bones, and are responsible for bone formation (Klasing, 1998). Osteoblasts are cuboidal in shape, with a breadth of 15-20 µm. The cytoplasm is intensely basophilic when stained, owing to its content of ribonucleic acid (RNA) (McLean & Urist, 1968). The most striking and abundant component of the osteoblast cytoplasm is the rough endoplasmic reticulum. The vesicular nucleus is spherical or ovoid and normally lies towards one of the cell. Only one of the nuclei, normally centrally placed, is a fairly well defined pale staining area, the juxta-nuclear vacuole, which can be demonstrated to contain a Golgi apparatus (Hodges, 1974). Osteoblasts are derived from osteoprogenitor cells and synthesise the organic component of the bone matrix (osteoid). These cells consist of many diverse materials (collagen, water, and small quantities of mucopolysaccharides) proportions of which depend on the species, age and the site of bone and often the position in a single long bone (Jowsey & Gordan, 1971; Ali, 1992; Stevens & Lowe, 1992). Osteoid is a collagenous support tissue of type 1 collagen embedded in a glycosaminoglycan gel containing specific glycoproteins (*e.g.* osteocalcin), which strongly bind calcium. The osteoblasts divide readily, but only a portion of the new cells actually secretes osteoid substance and forms bone. The rest of the osteoblasts is held in reserve as the osteogenic layer of the periosteum and of the endosteum within the marrow cavity and Harvesian canals (Frandson, 1981).

The osteoblast is a secretory cell with a highly structured and controlled directionality to its biosynthesis. Osteoblasts are connected as a continuous monolayer of cells held together in close-packed conformation with their side faces completely in contact with their neighbouring capillaries. This cobblestone arrangement allows continuous sheets of osteoid to be fabricated. As a result, it is predicted that this arrangement may play a role in the production of usually large diameter collagen fibrils indigenous and specific to osteoid (Caplan, 1988).

When fully active, osteoblasts are cuboidal or polygonal cells, with basophilic cytoplasm reflecting the abundance of rough endoplasmic reticulum in their cytoplasm from their role as active protein synthesising and secreting cells (Stevens & Lowe, 1992). The active osteoblast tends to be columnar in shape with the nucleus at the end farthest from the bone surface. It is orientated perpendicular to the bone surface (Vaughan, 1970, 1975). These cells are responsible for the deposition of calcium phosphate on to a collagen matrix located within the template previously created by cartilage, thus increasing the width of the bone (Klasing, 1998; Karsenty, 1999). Osteoblasts also produce prostaglandins, which strongly inhibit osteoclastic resorption (Chambers, 1988). When active bone formation ceases at a particular site, osteoblasts flatten out and become bone lining cells, which cover all endosteal bone surfaces from resorption. Although relatively inactive, these cells have an important function in protecting bone surfaces from resorption (Roach, 2000). Another function of osteoblasts, which is sometimes forgotten, is that they are required for longitudinal bone growth (Karsenty, 1999).

Compared to osteoclasts and chondrocytes, little is known about the molecular control of osteoblast differentiation and function. This limited knowledge is partly ascribed to the fact that the osteoblast could be viewed, from a genetic standpoint, as a sophisticated fibroblast. The genes that have been demonstrated to control osteoblast differentiation directly or indirectly are *Cbfa1* and *Indian hedge-hog (Ihh)* (Karsenty, 1999).

Osteocytes

The osteocyte is an osteoblast that has been surrounded by calcified or mineralising bone matrix (McLean & Urist, 1968; Hodges, 1974; Loveridge *et al.*, 1992; Stevens & Lowe, 1992). The true osteocyte is fully enclosed within a lacuna and its cytoplasmic processes extend through apertures of the lacuna into canaliculi in the bone. Because it is important that no bone cell is located very far from the nearest blood vessel, the osteocytes are arranged central to capillaries in concentric layers called lamellae, which form spindle-shaped units known as osteons (Villee *et al.*, 1989). The osteocyte is rich in glycogen and the cytoplasm is faintly basophilic (McLean & Urist, 1968). The osteocyte closely resembles an osteoblast with respect to cytoplasmic organelles but it has fewer of them. Its long cytoplasmic processes

branch very little, some of them extending toward the osteoid surface and others extending toward or into the mineralised zone (Hodges, 1974). They have less Golgi apparatus, endoplasmic reticulum and mitochondria than active osteoblasts. Osteocytes are regularly spaced and aligned along bone lamellae. Osteocytes have processes that extend into canaliculi in the surrounding matrix for some distance and these processes are surrounded by nonmineralised intralacunar matrix (McLean & Urist, 1968). The relatively constant number of osteocytes per cubic millimetre (mm) suggests that entombment is a controlled event (Loveridge *et al.,* 1992).

The function of the osteocytes is unknown, but each osteocyte in its lacuna maintains a narrow zone of osteoid around it and retains the prominent Golgi apparatus and a fraction of the rough endoplasmic reticulum of its parent osteoblast. This suggests that it may be able to maintain the organic matrix. Although the evidence is limited, osteocytes may also resorb bone matrix to release calcium through osteocytic osteolysis process (Stevens & Lowe, 1992). After resorption, the osteocytes disappear (probably self-destruct), and this probably attracts osteoblasts (Ott, 1998). McLean & Urist (1968) suggested that osteocytes might be involved in the transport mechanism by which transfer of materials from blood to bone is accomplished. It is believed that osteocytes play a role in the release of mineral from bone to blood, and hence in the homeostatic regulation of the concentration of calcium in the body fluids. Osteoblasts are also believed to play a central role in the response to mechanical stimuli, sensing mechanical strains and initiating an appropriate modelling or remodelling response via a number of chemical messengers including glucose-6-phosphate dehydrogenase, nitric oxide, and insulin-like growth factors (Compston, 2001). It is hypothesised that osteocytes are the major mechanosensory bone cells that detect the mechanical signals (Roach, 2000; Compston, 2001).

Osteoclasts

Osteoclasts are large, multinucleated bone-resorbing cells (1-50 nuclei per cell depending on the species) of highly variable size, which are derived from haematopoietic precursors of the monocyte/ macrophage (Hodges, 1974; Villee *et al.,* 1989; Karsenty, 1999; Compston, 2001). The multiple nuclei of the cell are even in shape and size and in many ways resemble those of osteocytes and osteoblasts (Hodges, 1974). Osteoclasts are formed by the fusion of mononuclear cells and are

characterised by a ruffled border, which consists of a complex infolding of plasma membrane, and a distinct cytoskeleton (Jowsey & Gordan, 1971; Miller, 1992; Rose, 1997; Karsenty, 1999; Compston, 2001). Osteoclasts are found in contact with calcified bone surfaces (Karsenty, 1999). Active osteoclasts are normally found lying in a cavity or groove of the underlying bone (Hodges, 1974). Osteoclasts are rich in lysosomal enzymes, including tartrate-resistant acid phosphatase (TRAP). During the bone resorption process, hydrogen ions generated by carbonic anhydrase II are transported across the plasma membrane by a proton pump to dissolve bone mineral. Subsequently, lysosomal enzymes including collagenase and cathepsins are released and degrade bone matrix (Compston, 2001).

According to Rose (1997), bone resorption is needed to increase the size of the internal bone spaces and provides a store of metabolically active calcium in the body. During the lifespan of the osteoclast, the cells continue to incorporate and perhaps expel nuclei (Miller, 1992). The nuclei resemble those of osteoblasts and osteocytes. The cytoplasm is often foamy and the cell has extensive processes. Jowsey & Gordan (1971) suggested that increased resorptive activity is associated with osteoclasts containing many nuclei. In the opinion of McLean & Urist (1968) osteocytes may originate from precursor cells in the stroma of the bone marrow, or they may represent fused osteoblasts and may also include fused osteocytes liberated from the bone being resorbed. Osteoclasts are found on the inner (endosteal) surface of the bone and function to absorb it (Klasing, 1998). These cells are also found attached to the bone surfaces at sites of active resorption, often in depressions, where they have eroded into the bone (McLean & Urist, 1968; Price & Russell, 1992; Stevens & Lowe, 1992). This according to McLean & Urist (1968) suggested that the lacunae were formed by an erosive action of the overlying osteoclasts.

Bone resorption consists of the removal of mineralised bone tissue (Jowsey & Gordan, 1971). Bone absorbing agents exert their effects by increasing the numbers and activity of cells mainly via the osteoblast lineage (Price & Russell, 1992). Osteoclasts are highly mobile and resorb bone; both the mineral and organic phases of bone as they move along the bone surface. Bone resorption occurs when the osteoclast is in contact with the bone (Stevens & Lowe, 1992). Contact with the bone is through a central 'ruffled border' where resorption occurs, and peripheral 'clear zone', which

acts as a seal (Loveridge *et al.,* 1992). According to Miller (1992), the "ruffled border" is the cell surface specialisation on the osteoclast that facilitates absorption. The ruffled border is comprised of cytoplasmic folds that interdigitate with the bone to provide a large surface area for the extrusion of lytic enzymes and uptake of degraded material from the resorption site. The cytoplasm adjacent to the ruffled border contains phagosomes, lysosomes and exocytolic vesicles. Osteoclasts can penetrate 50-70 µm into compact bone in 24 hours and destroy a volume of bone equivalent to that formed by 100-1000 osteoblasts. Osteoclasts possess an extensive Golgi apparatus and numerous mitochondria but a sparse endoplasmic reticulum and a few ribosomes (Loveridge *et al.,* 1992). The dissolution of bone mineral is followed by degradation of the exposed organic matrix components probably by lysosomal enzymes exocytosed across the ruffled border into the sub-osteoclastic space.

It is suggested that osteoclastic activity is directly controlled by intracellular signals produced by osteoblasts. Contraction of osteoblasts of the lining layer and/or their synthesis of collagenase may expose the mineralised matrix permitting osteoclastic resorption (Price & Russell, 1992). According to Chambers (1988), osteoclastic resorption of bone can be regulated at three distinct levels: induction of differentiation; initiation of bone resorption; and modulation of rate of bone resorption.

Although osteoclasts are generally considered to resorb old and fully mineralised bone and even to discriminate against new, incompletely mineralised tissue, they may be seen to resorb recently formed bone, as in the primary and secondary spongiosa beneath the epiphyseal plate in a growing animal (Jowsey & Gordan, 1971). Osteoclastic resorption contributes to bone remodelling in response to growth or changing mechanical stresses upon the skeleton (Young & Heath, 2000). Osteoclasts also participate in the long-term maintenance of blood calcium homeostasis by their response to PTH and CT. PTH stimulates osteoclastic resorption and release of calcium ions from bone, while CT inhibits osteoclastic activity (Young & Heath, 2000).

Osteoblasts and osteocytes secrete and nourish the osteoid, into which inorganic mineral salts are deposited to make it rigid and hard whereas osteoclasts reshape the deposited bone (*i.e*. mineralised osteoid) (Stevens & Lowe, 1992).

2.3.4 Collagen (bone matrix)

Although various proteins, glycoproteins, peptides, carbohydrates and lipids are present in bone, over 90% of the organic component is made up of a single protein, collagen (Ali, 1992). Collagen is composed of three left-handed polypeptide helices, which are twisted around each other to form a right-handed superhelix. Twisting the polypeptide of the superhelix in opposite directions is principally responsible for collagen's strength (McKee & McKee, 1999). The organic component of the matrix is comprised of Type I collagen, glycosaminoglycans and proteoglycans (Stevens & Lowe, 1992). Type I collagen is widely distributed and is the major component of the bone (Ali, 1992). It may be characterised as a hybrid molecule since its polypeptide chains include two a1(I) chains plus an a2 chain. Fibres derived from Type I molecules are prevalent in virtually all of the major connective tissues (Miller, 1977). According to McKee & McKee (1999), Type I collagen molecules, found in the teeth, bone, skin, and tendons, are about 300 nm long and approximately 1.5 nm wide. About one third of the amino acid content of collagen is glycine, whereas proline and 4-hydroxyproline may account for as much as 30% of collagen's amino acid composition (McKee & McKee, 1999; Elliot & Elliot, 2001). Small quantities of 3-hydroxyproline and 5-hydroxylysine also occur (McKee & McKee, 1999). Bone is also known to include Types V and IX collagens. In contrast, cartilage does not have Type I collagen but is composed mainly of Type collagen II (Ali, 1992), which is composed of three apparently identical a1(II) chains (Miller, 1977). Ali (1992) contends that most of the rest of the organic matrix is composed of non-collagenous proteins.

2.4 Types of Bones

Structurally, three types of avian bone are found including compact bone, cancellous bone and medullary bone (Hodges, 1974). The hard compact cortical bone (woven bone) is found largely in the shafts of long bones, which surround the marrow cavities. On the other hand, cancellous or spongy bone (also known as lamellar bone) is made of a network of fine interlacing partitions, the trabeculae, enclosing cavities

that contain either red or fatty marrow (Vaughan, 1970; Stevens & Lowe, 1992). These two types of bones can be identified according to the pattern of collagen forming osteoid.

2.4.1 Cortical bone

Cortical bone is always a temporary bone that will either be resorbed or replaced with lamellar bone via the remodelling process (Roach, 2000). Cortical bone is characterised by haphazard organisation of collagen fibres and is mechanically weak. This type of bone is produced when osteoblasts produce osteoid rapidly, as in the foetal bone development and in adults when there is pathological rapid new bone formation, *e.g.* healing fracture (Stevens & Lowe, 1992). Cortical bone is laid down in all directions rather than as unidirectional lamellae (Roach, 2000). The collagen fibres are deposited in an irregular, loosely intertwined pattern in the osteoid (Stevens & Lowe, 1992; Young & Heath, 2000). This happens initially in the foetal bones, but remodelling and the deposition of more resilient lamellar bone gradually replaces the resulting woven bone (Stevens & Lowe, 1992). The rapidly formed cortical bone is eventually remodelled to form lamellar bone, which is physically stronger and more resilient (Young & Heath, 2000).

2.4.2 Cancellous bone

Cancellous or spongy bone can be found in many places, particularly in the centre of the bones of the cranium and forming the bulk of the bony substance in the ends of long bones such as the femur. It consists of a delicate scaffolding or interconnecting fine bony plates and trabeculae, which is surrounded by spaces containing marrow. The trabeculae are composed of fine bony lamellae interspersed with lacunae containing osteocytes (Hodges, 1974). Cancellous bone is characterised by a regular parallel alignment of collagen into sheets (lamellae), and is mechanically strong (Stevens & Lowe, 1992; Young & Heath, 2000). Cancellous bone is a type of structural bone that gives internal support (Whitehead *et al.*, 1998). Cancellous bone has a large surface area and a greater blood supply than cortical bone, thus making it more responsive to changes in circulating hormones such as oestrogen, PTH, CT and testosterone, which can affect bone metabolism than cortical bone (Kenney, 2000).

2.4.3 Medullary bone

At the hen's onset of sexual maturity, the function of osteoblasts changes from forming lamellar cortical bone to producing a woven bone called medullary bone, which is unique to birds and crocodilians. This bone is laid down on the surfaces of structural bone and in spicules within the medullary cavities, especially in the leg bones (Whitehead, 2004). The development of medullary bone deposits in female birds during the reproductive cycle is unique in the class Aves, as medullary bone deposits permit the animal to store a considerable amount of readily available calcium without involving the structural skeleton (Miller, 1992). Medullary bone is a specialised form of secondary bone or woven bone used primarily as a labile and dynamic reserve of calcium to supplement dietary calcium for eggshell formation in laying hens (Hodges, 1974; Sykes, 1992; Sugiyama *et al.*, 1999; Whitehead 2004). Medullary bone forms shortly before the onset of egg production and persists throughout the laying period, its formation being concomitant with the maturation of the ovarian follicles (Dacke *et al.*, 1999). According to Parkinson & Cransberg (1999), the peak calcium reserves occur at 30 weeks of age, while the skeletal calcium depletion occurs in mid-lay (40-45 weeks of age). Knott & Bailey (1999) stated that the substantial non-collagenous component of medullary bone is likely to be related to its role as a calcium store. The majority of non-collagenous proteins in bone are involved in the mineralisation process. Many have calcium binding properties that would enhance the sequestration of mineral in a tissue required to respond promptly to the demands of egg production.

Medullary bone is non-structural, has a spongy appearance, due to an interlaced pattern of thin spicules (Dacke *et al.*, 1999). As compared to cortical or cancellous bone, medullary bone is low in collagen but high in mineral, proteoglycans, and carbohydrates (Rath *et al.*, 1999). It has a crumbly texture, while the woven bone of fracture callus or immature bone retains its structural integrity, suggesting that the medullary bone is even less organised than woven bone (Knott & Bailey, 1999). According to Whitehead (2004), medullary bone is fundamentally weaker than structural bone because (a) it is a form of a woven bone based upon a very irregular arrangement of collagen fibrils and, (b) because much of it is present in isolated spicules.

Medullary bone is formed on the endosteal surface by osteoblasts, stimulated by androgens and estrogens released by the maturing ovarian follicles. The femur and tibiotarsus are the two bones that are rich in medullary bone. However, small deposits of medullary bone can be found in the ischia, ilia, pubis, and ribs. The large surface of medullary bone is richly populated by foci of osteoblasts and osteoclasts, which mediate high rates of bone turnover (Hodges, 1974; Klasing, 1998). Once egg production has commenced a pattern of rapid build-up and break down of medullary bone is established in relation to the shell formation cycle (Hodges, 1974). Medullary bone supplies calcium for eggshell formation at periods when dietary supply is not sufficient (Hodges, 1974; Klasing, 1998). In immature animals, the medullary cavities of most bones contain active (red) marrow, which is responsible for the production of the cellular elements of blood. In the adult, active marrow is restricted to a few sites; the medullary cavities of other bones are filled with inactive (yellow) marrow that is largely composed of adipose tissue (Young & Heath, 2000).

During the early stages of shell calcification, the trabeculae are covered with both osteoblasts and osteoclasts. As calcification progresses the osteoblast population declines rapidly, and is replaced by large numbers of osteoclasts. During the post-oviposition period, and during the descent of the subsequent egg passage down the oviduct as far as the shell gland, either cell form may be dominant or present in equal numbers (Hodges, 1974).

2.5 Role of Parathyroid Hormone and Calcitonin

2.5.1 Parathyroid hormone

Parathyroid hormone is a polypeptide (containing 84 amino acids and has a molecular weight of about 8000), which is synthesised by parathyroid glands situated in the lower neck region (Hurwitz, 1987; Prince & Russell, 1992). It is synthesised from a precursor molecule, pro-PTH, by removal of an NH_2-terminal sequence of six amino acids in the Golgi apparatus of the secretory cell (Taylor & Dacke, 1984; Price & Russell, 1992). According to Taylor & Dacke (1984), pro-PTH is itself derived from a precursor polypeptide with 115 residues, known as pre-pro-PTH, by the removal of 25 amino acids from the NH_2-terminal end of the molecule during its translation in the rough endoplasmic reticulum. The primary targets for PTH in birds, as in mammals,

are the kidneys and bone. The decrease in calcium intake stimulates the secretion of PTH.

The main functions of PTH are mobilising calcium from the skeleton, promoting absorption of calcium and phosphorus from the digestive tract, and causing the kidneys to excrete phosphorus, while containing Ca^{2+} by reabsorption. PTH enhances renal reabsorption of calcium (Capen & Rosol, 1989) and promotes the synthesis of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) from 25-hydroxycholecalciferol in the kidney (Allen & Sansom, 1985). It promotes resorption by acting on the osteoclasts, inciting them to engulf and destroy matrix structure so that the trapped calcium in the matrix can be released into the blood. Bone calcium resorption and intestinal calcium absorption increase as a result of stimulated 1,25-$(OH)_2D_3$ and PTH secretion (Horst, 1986, Horst *et al.*, 1994).

When the parathyroid gland detects that the level of calcium in the blood is getting too low, it secretes extra PTH. This increased level of PTH in the blood acts on cells in the kidney to stimulate formation of an active form of vitamin D, which in turn acts on cells in the intestine to increase calcium-binding capacity. In this manner, more of the ingested calcium is absorbed in the intestines leading to increased blood calcium levels (Postman, 1998). The action of PTH hormone is counteracted by CT, which is secreted by thyroid C cells. CT replenishes the body stores of bone calcium at the times of calcium adequacy. CT decreases the concentration of calcium in blood plasma by reducing the rate of bone resorption (Allen & Sansom, 1985).

The concentration of ionic calcium in the extracellular fluid is the most powerful regulator of PTH secretion but a number of other factors such as magnesium, catecholamines and prostaglandins, act as modulators of hormone secretion. Cyclic adenosine monophosphate (AMP) appears to be a secondary messenger in the control of PTH secretion by calcium. Intracellular concentrations of cyclic AMP increase in parallel with increases in the rate of PTH secretion under the influence of both hypocalcaemia and secretagogues, such as adrenaline, dopamine and prostaglandin (Taylor & Dacke, 1984).

2.5.2 Calcitonin

Calcitonin is a lipophilic single-chain polypeptide consisting of 32 amino acids, with seven-membered disulphide ring at the N terminus and prolinamide at the C terminus (Copp, 1972; Frandson, 1981; Emslie-Smith *et al.*, 1988; Gennari & Gonnelli, 1999; Ieda *et al.*, 2001). There is also an aromatic amino acid (tyrosine or phenylalanine) at position 22 and glycine at position 28 (Copp, 1972). Calcitonin is secreted from the ultimobranchial glands in birds, which are just distal to the parathyroid glands, or from the thyroid gland in mammals (Hurwitz, 1987; Ieda *et al.*, 2001) and has a molecular weight of 3000 (Hurwitz, 1987). According to Frandson (1981) and Emslie-Smith *et al.* (1988), CT is produced and released from the parafollicular "C" cells of the thyroid gland when the calcium concentration of extracellular fluid bathing these thyroid cells is increased. This increased level of CT acts on cells within the bones to increase absorption of calcium into bones (Postman, 1998). Calcitonin acts by means of specific receptors, on osteoclasts as a selective physiological anti-resorptive agent (Gennari & Gonnelli, 1999).

The roles played by CT are twofold, namely to combat hypercalcaemia and to protect the skeleton against excessive resorption (Taylor & Dacke, 1984). As calcium is elevated (hypercalcaemia), CT is secreted, which indirectly prevents calcium from being mobilised into serum and probably acts on kidneys and intestine to block utilisation of calcium (Taylor & Dacke, 1984). In laying hens, the only time during the egg cycle when hypercalcaemia is likely to occur is immediately before or after oviposition, when calcium is still at a high level without being removed from the blood by the shell gland (Taylor & Dacke, 1984). Calcitonin stimulates the proliferation of chondroblasts while PTH enhances bone remodelling and promotes cartilage maturation during osteogenesis (Fraser, 1988).

2.6 Bone Modelling and Remodelling

Bone tissue is a complex tissue, which is continuously undergoing changes throughout the life of an animal. This involves successive cycles of resorption and deposition and/or bone formation (Sissons, 1971; Price & Russell, 1992). Bone modelling involves both the growth and shaping of bones. It occurs during the first two decades in humans and in animal species, while growth plates remain open (Compston, 2001). In mature adults, skeleton modelling may occur in response to

altered biochemical stress, or as part of the fracture healing process. The bone modelling process involves both bone formation and resorption; the former exceeds the latter and is not coupled to it temporally or spatially as in bone remodelling (Compston, 2001).

Like bone modelling, bone remodelling is a surface phenomenon. In the young growing animals where the tissue mass is expanding, the amount of bone formed exceeds the amount removed and there is a net increase in the number of cells produced. In the adult, where, the ideal state exists, the tissue mass is maintained at a steady size, bone formation is balanced by bone removal and the cell production is considered to be equal to cell loss (Owens, 1971).

Bone remodelling comprises two phases: bone resorption of a quantum of bone by the osteoclasts, followed by bone formation by the osteoblasts within the cavity so created of osteoid, which is subsequently mineralised (Compston, 2001; Takeda & Karsenty, 2001). In normal adult bone, the processes of resorption and formation are coupled in both space and time; thus, bone resorption always precedes formation (coupling). Compston (2001) states that in young adult skeletons, the amounts of bone formed and resorbed are quantitatively similar or balance. The sites at which bone remodelling occurs are termed basic multicellular units (BMUs) or bone remodelling units (Compston, 2001). The regulation of bone remodelling involves a complex interplay between systemic hormones, mechanical factors, and locally produced cytokines, growth factors, and other mediators (Compston, 2001). According to Takeda & Karsenty (2001), the bone resorption arm of bone remodelling is under a tight endocrine control. During the process of remodelling, a hormone, leptin inhibits bone formation by osteoblasts.

2.7 Calcium Homeostasis

Calcium homeostasis occurs primarily in cancellous bone, the anatomy of which provides a large surface area, well suited to rapid mineral exchange (Mosley, 2000). According to Whitehead (1991), calcium homeostasis is maintained in the hen by mobilisation of bone calcium. Thus, the hen can still supply calcium for the formation of eggshells during periods of low calcium intake. Much of the shell is formed during the night when calcium intake from feed might be expected to be low (Whitehead,

1991). The hormones with the greatest involvement in Ca regulation in the bird are PTH, CT, 1,25$(OH)_2D_3$ and 17 ß-estradiol (Beck & Hansen, 2004).

Most calcium absorption occurs in the duodenum and jejunum (Larbier & Leclercq, 1994). According to Cousine & Deluca (1972) and Miller (1992), the capacity of the intestine to absorb calcium is regulated to a large extent by the vitamin D endocrine system. The two main targets of vitamin D are the small intestine and bone. The action of vitamin D is to promote active absorption of calcium and secondarily phosphorus by the intestine and mobilisation of these minerals from bone (Cousine & Deluca, 1972; McWatters, 1997). Vitamin D is also essential for the breakdown and assimilation of phosphorus for the normal depositing of these minerals into bones, for normal growth and development (McWatters, 1997). Both effects help in maintaining the calcium and phosphate concentration of plasma at supersaturated levels with regards to bone mineral, which in turn is necessary for normal bone calcification.

The compound 1,25$(OH)_2D_3$ acts in a similar manner to a steroid hormone, regulating DNA transcription in the intestinal microvilli. It also induces the synthesis of messenger RNA (mRNA) that is responsible for the production of CaBp (calbindin) (McDonald *et al.*, 1995). The 1,25-$(OH)_2D_3$ results in the formation of CaBp in the intestines thereby facilitating absorption of calcium (Sreenivasaiah, 1998). According to Taylor & Dacke (1984), the main role of the active metabolite of vitamin D [1,25-$(OH)_2D_3$] is to elevate the plasma concentrations of calcium and inorganic phosphorus (Pi) to levels that will support normal mineralisation of the skeleton. This is mainly achieved by absorption of calcium and phosphate ions from the small intestine. In addition to its well documented effect on mineral absorption and thus on the supply of mineral to bone, vitamin D affects bone development directly by controlling differentiation and proliferation of a large number of cellular elements notably immune system, skin, and cancer cells (Hurwitz, 1992). PTH together with 1,25-$(OH)_2D_3$ mobilises calcium and phosphorus from the skeleton when the concentration of Ca^{2+} ions in the plasma decreases. The amount of 1,25-$(OH)_2D_3$ produced by the kidney is controlled by PTH. Vitamin D is also required for the release of calcium from the kidney mitochondria under the influence of PTH (Sreenivasaiah, 1998).

Since vitamin D controls calcium absorption, excessive vitamin D results in hypercalcaemia. This extra calcium has to be removed, and may be deposited in the heart or blood vessels, in the bone joints, in the pericardium or on the walls of the intestines (Roche, 2000). This can give rise to heart failure, stiffness, or intestinal problems frequently accompanied by muscular weakness, anorexia and excessive urination (Roche, 2000). When serum ionised calcium is low (hypocalcaemia), PTH is secreted to induce the kidney to produce more 1,25-$(OH)_2D_3$, which in turn enhances the intestinal absorption of the calcium (Larbier & Leclercq, 1992; Kaplan, 1995; McDonald *et al.*, 1995). Heaney (1997) argues that at low calcium intakes vitamin D is essential for the induction of a calcium-binding transport protein in the intestinal mucosa that enhances calcium extraction from the digesta. In addition to increasing intestinal absorption of calcium, 1,25-$(OH)_2D_3$ increases the absorption of phosphorus from the intestine and also enhances the reabsorption of calcium and phosphorus from the kidney and bone. In hypocalcaemic conditions, induced by a dietary deficiency of vitamin D, production of 1,25$(OH)_2D_3$ is greatly increased. A number of other hormones believed to regulate the production of 1,25$(OH)_2D_3$ include prolactin, GH, insulin, glucocorticoids, CT, reproductive steroids and 1,25$(OH)_2D_3$ itself (Taylor & Dacke, 1984).

2.8 Calcium Requirements for Growing and Laying Chickens

The amount of dietary calcium required to maximise bone or eggshell mineralisation and strength is greater than that needed for other functions (Klasing, 1998). Requirement levels are based on the premise that all of the calcium consumed has a bioavailability similar to that of $CaCO_3$ (Klasing, 1998). Laying hens require higher dietary levels of calcium than non-layers, as calcium is required for eggshell formation. To ensure maximum shell quality, it is recommended that hens consume a minimum of 3.75 g calcium/hen/day (Roland, 1986a, 1986b). Grau & Roudybush (1987) reported that the calcium requirement of laying hens is 100 times greater than that of non-layers.

The normal contents of starter diets are 10 g calcium and 4.5 g available phosphorus/kg (in an approximate ratio of 2:1) (Whitehead, 2002). According to Avian Farms (1999), calcium levels in starter and grower diets should be 0.9-1% and 0.85-0.95%, respectively. At the same time available phosphorus levels of 0.47-0.50%

and 0.42-0.47% are recommended for chick starter and grower diets, respectively. In addition, calcium levels of 3 to 3.5% are recommended for breeder hens aged between 23 and 65 weeks. Woolford (1994) suggested that a daily supply of about 3.5 to 4 g calcium is required for shell formation. According to Ross Breeders (1998), breeder hens require 4-5 g calcium per day from the day they lay the first egg, to maintain the calcium balance. This requirement is satisfied by making the change from pre-breeder diet (1.5% calcium) to breeder (2.8% calcium) diet immediately prior to first egg.

Temperatures above the thermoneutral zone tend to reduce feed consumption and hence result in increased mineral requirements when expressed as a proportion of the diet (Underwood & Suttle, 1999). According to Fisher (1983), high levels of calcium can also depress feed intake and egg production. On the other hand, Clunies *et al*. (1992), obtained contradictory results after feeding laying hens 3 levels of dietary calcium (i.e. 2.5%, 3.5% and 4.5%). The workers reported that birds on the 2.5% calcium diet had the lowest feed intake, while those fed 3.5% calcium exhibited the highest level of intake. Hens fed the 4.5% calcium diet had feed intakes that were no different from those fed 3.5% calcium. Again, as calcium intake increased, calcium retention increased linearly, while shell weight increased quadratically. The investigators concluded that in order to improve shell quality calcium intake must be increased; however, there is a diminishing response of shell weight at higher calcium intakes.

According to Singh & Panda (1996), the hen absorbs about 100 mg calcium per hour for eggshell formation, which is a very high rate considering the size of the birds. Roland & Farmer (1984) suggested that the hen needs 125 mg dietary calcium every hour for 16 hrs to form an eggshell. According to Roland (1986b), the average calcium requirement for eggshell formation within a population of hens is greatest at approximately peak production. However, because the amount of calcium deposited on the shell can increase slightly with the age of hen and production might not be a factor in the individual hen's daily requirement, the calcium requirement for an individual hen for a particular egg on a particular day could increase with age. As hens age, the average quantity of calcium deposited on the eggshell per day (percent production x calcium content of eggshell) declines. Calcium deposition in eggshell

prior to peak production is at least 5% less compared to the quantity of calcium deposited in eggshell after peak production (Roland, 1986b).

The calcium requirement for maximum shell thickness is greater than that for maximum egg production and as shell thickness is related to strength, the requirement quoted is for maximum or near maximum thickness (Hill, 1988). The NRC (1994) recommends a ratio as high as 12 calcium to 1 nonphytate phosphorus (NPP) (weight/weight). Dietary calcium content of 3.25 to 3.75% is believed to be desirable for laying hens. Scott *et al*. (1971) reported that a calcium level of 5% caused a decline in feed consumption, while it did not improve eggshell quality above that obtained with 3.5% calcium. For egg formation alone, the hen needs 2 - 2.24 g Ca/ day [Mississippi State University (MSU), 2000]. According to Etches (1987), if a hen lays 250 eggs/year, she will secrete 580 g of calcium. The researcher suggests that if the hen retains 50% of the calcium she eats for egg and eggshell production, she will consume 1.16 kg of Ca^{2+} per annum.

Both calcium and phosphorus play a role in egg production and hatchability of fertile eggs. Manley *et al.* (1980) conducted two experiments in which diets containing two levels of dietary calcium (2.5 and 3.5%) and 3 levels of dietary phosphorus (0.3, 0.4 and 0.5%) were fed to turkey hens. In both experiments increasing calcium in the diet of turkey hens, resulted in a slight numerical increase in egg production. Hens that received 3.5% dietary calcium laid at a rate of 2.5% points higher than hens receiving 2.5% calcium. On the other hand, Ahmad *et al.* (2003) reported that increasing dietary calcium level from 2.5 to 5% in Bovanes hens increased egg production from 75.3 to 82.4% and egg specific gravity (ESG) from 1.078 to 1.083 units. They reported that calcium level had no effect on feed consumption or egg weight. It was also observed that birds fed diets containing the high level of calcium, 3.5%, produced eggs with a hatchability that was 9.2% higher than eggs from hens fed a diet with a 2.5% calcium dietary level. Hatchability also increased as dietary phosphorus levels were increased from 0.3 to 0.5%. Menge *et al*. (1977) reported similar results in turkey hens. Furthermore, Shafey & McDonald (1991) observed that increasing dietary calcium from 0.89 to 2.33% reduced BWG, feed intake , tibia calcium content, and plasma Pi, while it increased feed conversion ratio and plasma total calcium in broiler chickens. In their study, high dietary levels did not affect tibia ash and phosphorus contents.

High levels of phosphorus causes excretion of calcium at a time when calcium requirement is at a premium, resulting in poor shell quality (Broadbent, 1989). Poor shell quality will have a deleterious effect on hatchability. Additionally, high calcium intake leads to the formation of kidney stones (Maynard *et al.*, 1979). The skeletal system is intimately involved in calcium storage for eggshell formation. Calcium mobilisation and storage is also under the influence of oestrogen hormones. Proper build-up of calcium stores is essential for the maintenance of bone integrity and acceptable shell quality (Robinson, 1999).

Larsen *et al.* (2000) investigated the effects of feeding 3 levels of dietary calcium (0.35, 0.94 and 1.64%) in growing pigs and found that that medium (0.94%) or high (1.64%) dietary calcium level resulted in a higher retention of calcium, more bone mass as indicated by a higher trabecular bone volume and a lower bone resorption. The mineral appositional rate in the bones was also higher in these groups, indicating a fast growth rate. In contrast, a low dietary calcium supply resulted in lower overall calcium retention, lower bone mass, and a higher extent of resorption lacunae and osteoid extent and width. These workers concluded that the medium level of dietary calcium was sufficient to sustain sound bone development.

2.9 Deficiency Symptoms of Calcium

A calcium deficiency may occur because of a low dietary calcium level or excess dietary phosphorus. A low calcium level, which usually implies abnormal parathyroid function, is rarely due to low dietary calcium intake since the skeleton provides a large calcium reserve for maintaining normal blood levels (Weaver, 2001). Other causes of abnormally low blood calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels (Weaver, 2001). Insufficient vitamin D may cause a secondary calcium deficiency, by impairing calcium absorption and bone formation. Increasing dietary calcium above the normal required levels improves but does not completely correct this secondary deficiency. Heaney (1997) contends that increasing calcium intake beyond the amount that produces the optimal bone mass will not result in more bone.

The skeleton of an animal normally contains large reserves of both calcium and phosphorus that can be resorbed without permanent or serious damage. These homeostatic mechanisms enable the animal to accommodate temporary shortages (Todd, 1976). Previous reports (Bar & Hurwitz, 1984; Gillespie, 1987; Schwartz, 1996) suggested that dietary deficiency of either calcium or phosphorus, if sufficiently prolonged leads to skeletal deformities, retarded growth and decreased feed intake (depraved appetite). If acute, retarded growth can lead to death (NRC, 1977). Imbalances of calcium, phosphorus or vitamin D intakes at any rate can result in skeletal defects (Todd, 1976). In growing chicks, a calcium deficiency causes skeletal abnormalities, including rickets, dyschondroplasia, lameness, enlarged painful joints and misshapen bones as well as large and uncalcified epiphyseal plate of long bones (Klasing, 1998).

Most calcium related problems are related to excess calcium or insufficient levels of available phosphorus. As a result, most calcium and/or phosphorus problems are manifested as a phosphorus deficiency (Schwartz, 1996). In immature or young flocks a deficiency of either calcium or available phosphorus or an imbalance in these nutrients will result in rickets (ASA, 1997). According to Todd (1976), the term 'rickets' is often used to describe the pathological condition that occurs in young animals due to defective calcification of the growing bone. Rickets is a condition in which bones are decalcified and weakened causing bowing of legs and other problems (Swick, 1995). The growth plate is decreased in width and birds become sluggish and reluctant to walk. The bones and beaks become soft and rubbery, while the joints become enlarged, the bones become weak, soft, deformed and are easily broken (Swick, 1995; ASA, 1997). Weakness and morbidity are also deficiency symptoms of phosphorus (Schwartz, 1996).

Calcium deficiency is seldom observed in young birds unless there is a feed formulation problem (Schwartz, 1996). In laying hens and breeders, osteomalacia, which is characterised by decreased skeletal density, may occur (ASA 1997). Osteomalacia differs from rickets inasmuch as little new bone growth occurs (Robert & Deluca, 1972). Other symptoms of a calcium deficiency include morbidity, increased basal metabolic rate, and increased susceptibility to internal haemorrhage.

Vitamin D deficiency can also lead to distortion of the breastbone and vertebral column, and other conditions such as soft-shelled eggs (Roche, 2000).

Osteoporosis in laying hens is defined as a progressive decrease in the amount of mineralised structural bone and leads to bone increased fragility and susceptibility to fracture. The fractures occur during the lifetime of hens in cages or during depopulation when transporting to a processing factory or when hanging on shackles (Bishop *et al.,* 2000; Whitehead & Fleming, 2000). Osteoporosis is also defined as a reduction in skeletal mass with associated bone microarchitectural deterioration that results in an increased risk of fractures (Spangler *et al.*, 1997). As a result, osteoporosis is referred to as a disease of increased fragility (Mosley, 2000). It is caused by defective mineralisation of bone tissue and is characterised primarily by thick seams of unmineralised or a poorly mineralised organic matrix, mainly osteoid, on the surfaces of medullary bone trabeculae (Whitehead & Wilson, 1992). In the opinion of Fraser (1988), osteoporosis is caused by an imbalance between the formation of bone by osteoblasts and its resorption by osteoclasts during bone turnover and remodelling. The major abnormality in most cases of osteoporosis involves increased bone porosity in the trabecular (larger marrow surfaces) and thinning of cortical bone (Ott, 1998). A bone remodelling sequence modulates these increases. A study by Chan & Swaminathan (1998) found that the loss of calcium is proportional to the dietary intake of sodium and that even at moderate intakes of sodium significant loss of bone occurred.

2.10 Feeding Systems

2.10.1 Methods of restricted feeding

There are two nutrient restriction programmes practised in pullet rearing. These are qualitative and quantitative programmes. Qualitative restriction involves modifying the quality of the diet (*e.g.*, decreasing diet's crude protein content), while quantitative restriction entails manipulating the amounts of feed given to the birds. The common feed restriction method is the quantitative one (Leeson & Summers, 1997).

Restriction of feed intake to control BW in broiler breeders has been practised for many years because full-fed hens selected for growth become lethargic and produce abnormal eggs at a higher rate (McDaniel & Brake, 1981; Crouch *et al.*, 2001).

According to Fisher (1998), the main objectives during rearing (i.e. from day old to sexual maturity) are to achieve the breeding company's recommended target BW and to maintain good uniformity (coefficient of variation of BW 10% or less) with an even growth curve, according to the standard. It is claimed (Hybro Breeders, 2001a) that BW at 6 weeks dictates to a large extent the final size of the skeletal frame. Most (90%) of the frame size is developed early, and by 12-16 weeks of age, the size of the pullet is fixed (Leeson, 2001a). Ross Breeders (2001) states that skeletal size is fixed at 84 days (12 weeks) in broiler breeder pullets. According to Hybro Breeders (2001b), the hormonal development that occurs at 12 weeks depends largely on BW development. Consequently, to control BW development, birds should be weighed weekly.

Broiler breeder BW is routinely controlled from an early age to reduce reproductive problems associated with genetic selection for growth. Overfeeding growth-selected poultry results in abnormal development of the ovary, which may cause an increase in the proportion of hatching eggs that will not produce chicks (Renema *et al.*, 1999). Broiler breeder chickens require dedicated feed restriction programmes to maximise egg production and chick production (Robinson *et al.*, 1993).

In two experiments conducted by Fontana *et al.* (1992) to investigate the effect of early feed restriction on growth, feed conversion, and mortality in broiler chickens, it was found that early feed restriction programmes reduced the consumption of the starter diets by an average of 22% in restricted broilers when compared with controls (*ad libitum*). Mortality rate was also reduced for male birds under early feed restriction. Again, the mean BW was lower for restricted birds compared to controls. In that study, feed conversion ratios of feed restricted birds were reported to be lower at 28 days of age in one experiment and 49 days of age in both experiments. The results of another study by Renema *et al.* (1994) showed that feed intake to sexual maturity was lower in full-fed than in feed restricted turkeys. In a separate experiment, Renema *et al.* (1995) also observed that full-fed turkey hens had the highest incidence of double-yolked and thin-shelled eggs. Defective egg production also decreased in feed-restricted turkey hens and increased in full-fed hens.

The two most commonly used commercial feed restriction programmes during the growing period (i.e., up to 18 weeks of age) are skip-a-day, in which amounts of feed calculated to achieve desired BW are fed on alternate days; and limited every day feeding, in which half of the skip amount is fed daily (Mench, 2002). According to Mench (2002), the skip-a-day programme is preferred for males in the United States, since it provides greater BW uniformity than limited every day feeding. Hudson *et al*. (2001) observed that prolonged skip-a-day feeding resulted in significant increases in uniformity, perhaps because of its effect on eating habits. Because feed is available for longer periods of time in skip-a-day feeding programme, the larger and more aggressive birds may be consuming feed immediately after it is provided, allowing smaller and less aggressive birds to eat adequate amounts of feed afterward. Another study at Auburn University (United States) reported that a restricted rearing programme reduced BW and was associated with higher uniformity in males compared to everyday feeding (Anon, 2000).

In fast growing birds, restricted feeding programmes may be initiated on a daily basis as early as 7 to 10 days of age (Leeson & Summers, 2000). For strains with slow initial growth rates *ad libitum* feeding is continued until 3-4 weeks and thereafter followed by restricted feeding. Crouch *et al.* (2001) state that current recommendations for broiler breeders include skip-a-day feed restriction beginning at day 3 or 4 accompanied by light restriction to 8 hours per day.

Control of BW in broiler breeder hens during the prelay period is extremely difficult, but this is of utmost importance (Theimsiri, 1993). The purpose of feed restriction is to prevent the flock from becoming overweight and developing a large skeletal frame. Feed restriction prevents excessive BWG and fatness, which results in low production later on (Robinson *et al.*, 1993; Avian Farms, 1999). According to Robinson *et al*. (1993), fertility is reduced in overweight broiler breeders due to reduced mating success (which limits sperm transfer to the female), by a reduction in the duration of fertility, and possibly by impaired sperm transport to the site of fertilisation (because the normal passage of developing eggs is more random than in egg-type hens). In addition, embryonic mortality is high in the eggs of overweight hens, as such eggs are often poorly calcified, which results in increased shell porosity and egg weight loss (Robinson *et al.*, 1993). Overweight hens also exhibit short laying sequences and may

also have poor overall chick production due to an increased incidence of first-of sequence eggs, which have been demonstrated to exhibit an increased incidence of embryonic mortality (Robinson *et al.*, 1993). Renema *et al.* (1995) reported longer sequence length in feed-restricted turkey hens during early lay. These workers claim that sequence length increased with the severity of restriction programme. They also found that shell deposition as a percentage of egg weight was significantly better in restricted than in full-fed turkey hens. Improved hatchabilities of eggs of restricted were also reported, which was attributed to better shell calcification due to reduced erratic oviposition and improved shell density when egg size was reduced (Renema *et al.*, 195).

It is recommended that feed intake must be restricted at 3 weeks of age (ScanBrid Int., 2000), 4 weeks (Avian Farms, 1999) or 5 weeks (Leclercq *et al.*, 1987). The slight differences in commencement of feed restriction are probably due to the differences in the growth potential of the strains of broiler breeders over time. Theimsiri (1993) proposes that BW at 4 weeks should be 400 – 450 g. Crouch *et al.* (2001) demonstrated that feed restricted turkey hens consumed less feed per day and laid more eggs than non-restricted hens. In addition, turkey hens that were feed restricted earlier (*i.e.* from 3-16 or 3-24 weeks) had higher peak egg production than hens that were fed *ad libitum* or restricted late (restricted from 16-24 weeks) (Crouch *et al.*, 2002). From a nutritional point of view, daily restriction is preferable. If conditions do not properly allow this, twice the daily amount may be distributed every other day (skip-a-day method).

Mismanagement of the broiler breeders' BW (both male and female) can delay the onset of production past 25 weeks of age, resulting in lower total egg production, reduced hatchability and fertility. This causes the laying hen to be extremely sensitive to hot weather, resulting in high mortality and poor performance (Theimsiri, 1993). According to Robinson (1999), overweight breeder pullets have excessive follicle development, and this increased development can be detrimental in achieving high egg production. When birds have too many large follicles a problem known as "Erratic Oviposition and Defective Egg Syndrome" (EODES) occurs. This condition is typified by a high incidence of: multiple-yolked eggs that can cause a prolapse of the oviduct; the laying of more than one egg per day (with or without good quality

shells); and internal laying which often results in "egg yolk peritonitis". Erratic oviposition also occurs. Excessive BWG in the female may be a primary factor in low fertility in commercial practice (Brake & McDaniel, 1981). Poor fertility is believed to arise from overfeeding (Robinson, 1999). On the contrary, excessive feed restriction in broiler breeder cockerels during part or all of the rearing period could significantly decrease early fertility (Lilburn *et al.*, 1990). It was concluded that excessive feed restriction during rearing should not be used to control BWG later in the production cycle.

2.10.2 Diet composition and types

In broiler breeder production (rearing and laying phases), four types of diets may be used, including starter, grower, pre-breeder and breeder all mash. However, it is not uncommon to feed three types of diets (i.e. starter, grower and breeder all mash). According to Peterson Farms (2001), breeder chick starter diet [17-18% crude protein (CP) and 12.18 MJ/kg of metabolisable energy (ME)] should be fed during the first three weeks of life followed by restriction after the third week. Starting from 4 weeks of age through 20 weeks pullet grower diet (17% CP and 12.0 MJ/kg ME) is fed. A pre-breeder diet with 17.5% CP and 12.2 MJ/kg ME is recommended from 21 weeks through 5% production. Thereafter a breeder diet of 17% CP and 12.4 MJ/kg ME may be fed (Peterson Farms 2001). In contrast, Cobb Breeders (1997) and Ross Breeders (2001) recommend that a chick starter diet could be fed from day-old up to 42 days. For a grower's diet, Cobb Breeders (1997) and Ross Breeders (2001) recommend that it should be fed from 43 to 126 days and 43 to 105 days, respectively. According to Cobb Breeders (1997) and Ross Breeders (2001), pre-breeder diet should be from 105 to 133 days (15-19 weeks), 127 to 154 days and/or 105 to 154 days, respectively. The recommendation of Cobb Breeders (1997) and Ross Breeders (2001) is that breeder diet should be fed at 155 weeks (22 weeks) of age. This indicates that the requirements of broiler breeder chickens are similar across the strains.

2.10.2.1 Pre-breeder nutrition

There has been considerable interest in recent years in the use of pre-breeder (or pre-lay) diets, which are traditionally used as a way of introducing a transition phase in terms of calcium metabolism (Leeson & Summers, 1997). For Leeson (2001a, 2001b), pre-breeder diets are often used to manipulate body size or to bring about a

transitional change in the birds calcium metabolism prior to maturity. Leeson & Summers (1997) contended that using a pre-breeder diet is based on the assumption that the bird's nutrient requirements change in this critical period of its life, which is at about 19-23 weeks of age. There are major changes that occur in the bird's metabolism, which relate to ovary and oviduct development, thus making this time the basis for a specialised diet. With egg laying birds, pre-breeder diets must involve a change in the level of calcium, in order to establish the bird's calcium reserves required for rapid and sudden onset of eggshell production. According to Lesson & Summers (2000), most often pre-breeder diets are used to condition or correct growth and/or body composition problems, which have arisen during 14-18 week growing period. For instance, pre-breeder diets may be of use where rearing programmes have not been adequate and the birds are underweight at the time of transfer to the breeder house. Leeson & Summers (1997) argued that when the rearing programme has been ideal, and birds are at or above the target BW when approaching sexual maturity, then little response to the pre-breeder diet could be expected.

Although it can be generalised that pre-breeder diets may be useful where rearing programmes have been inadequate and birds are underweight when they are moved to the breeder house (Leeson & Summers, 1987), it is considered that the ideal time for feeding pre-breeder diets is between 19-23 weeks (Leeson & Summers, 2000). During this period, the pullet is expected to increase in weight by about 570 g. This is somewhat more than growth expectation of around 350 g for the previous 4 weeks (15-19 weeks) or growth of 450 g from 23 to 27 weeks of age. A significant proportion of this growth spurt is associated with ovary and oviduct, which are developing in response to light stimulation (Leeson & Summers, 2000). According to Anon (2000), calcium is important in the pre-lay period (2 weeks prior to egg production), because this is the time for the pullets to build up their medullary bone to enable them form eggshells.

Commercially, three approaches are used in pre-breeder calcium nutrition. Firstly, is the use of grower diets that contain only 0.9 - 1.0% calcium being fed up to 5.0% egg production (Leeson & Summers, 2000). Pullets can produce about 2-3 eggs with a diet containing 1.0% calcium and then shut down the ovary. The second system involves the classical breeder diet containing around 1.5% calcium, which is really a

compromise situation. It allows for medullary bone reserves to develop, without resorting to 3.0-3.5% calcium, used in a breeder diet. However, 1.5% calcium is still inadequate for sustained eggshell formation. With this diet, the breeder can produce 4-6 eggs, before the ovulation pattern is affected (Leeson & Summers, 2000). Leeson (2001b) states that a good rule of thumb is to change from pre-breeder to breeder diet when the very first egg is noticed, because this occurs usually around 10 days before 1% egg production. The third option is perhaps the simplest solution, and involves changing from grower to breeder diet at first egg (10 days before 1% production). Feeding the breeder diet before maturity ensures that even the earliest maturing birds have adequate calcium for sustained early egg production. However, the proponents of pre-breeder diets suggest that breeder diets introduced early provide too much calcium, and that this contributes to kidney disorders, as the extra ingested calcium must be excreted in the urine (Leeson, 2001b).

2.10.2.2 Nutrition of hens during lay

The BW of broiler breeder stocks is routinely limited by quantitative feed restriction to improve productivity and fertility. Feeding after rearing is more generous but the quantities are decreased after the peak of egg production (Hocking *et al.*, 1998). According to Robinson *et al.* (1999), peak egg production occurs at 30-32 weeks of age. The investigators reported that after peak production the ovary of most genetic strains gradually becomes more immune to the detrimental effects of overfeeding. Overfeeding breeder females can cause abnormal ovary development by as early as 14 weeks of age, when some early follicle development can occur. Because feed intake is restricted in broiler breeder hens, with most feed being consumed during early morning hours, these breeder hens may be susceptible to periods of calcium deficiency during shell formation than hens fed *ad libitum* (Farmer *et al.*, 1983a, 1983b). In the laying hen, Ca^{2+} levels are affected by the ovulatory cycle; Ca^{2+} is elevated when the shell gland is empty, declines following entry of an egg into the organ, and reaches a minimum level approximately 16 hours prior to a subsequent oviposition (Parsons & Combs, 1981). The investigators suggested that upon entry of an egg into the shell gland, bone metabolism reverses and rapid resorption occurs, releasing both calcium and phosphorus.

Calcium metabolism in laying hens is dominated by the uterus for shell formation. As a result, inadequate calcium in the diet leads to disruption of ovulation and cessation of lay until their meagre calcium reserves are replenished. Under normal conditions, *i.e.* when a high calcium diet is being fed, the medullary bone is resorbed whenever supplies of calcium from the gut are not sufficient to provide for the demands of the shell gland. This occurs in the early hours of the morning when most of the previous day's food has been absorbed (Common *et al.*, 1948 cited by Taylor & Dacke, 1984). It is suggested that resorption of medullary bone is induced by an increase in the circulating concentration of PTH, secretion of which is stimulated by the decline in Ca^{2+} concentration that occurs during shell formation (Taylor & Dacke, 1984).

In their study, Clunies & Leeson (1995) reported that dietary calcium level had a significant effect on hens' BW. For instance, as dietary calcium concentration increased from 2.5% to 3.5%, hens' BW increased. Thereafter no further increases in weight were observed. In that study, 5 levels of calcium (i.e. 2.5, 3.0, 3.5, 4.0, or 4.5%) were fed to groups of 16 hens. It was also observed that feed and calcium intake increased as egg formation proceeded, and calcium intake increased as dietary calcium concentration increased. In addition, plasma calcium, shell ash and shell calcium increased as dietary calcium increased. A reduction in absolute quantity of medullary bone calcium and the proportion of calcium in this bone was observed when low calcium diets were fed.

2.11 Influence of Time on Eggshell Formation

The ability of hens to produce quality shells depends on the availability of calcium from ingested feed and skeleton (Farmer *et al.*, 1983a, 1983b). Blood Ca^{2+} levels are at a maximum whenever the shell gland is empty. It was observed that approximately 5.4 hours following oviposition, an egg entered the shell gland and blood Ca^{2+} level started to decline, and continued to do so as the rate of eggshell calcification increased (Parsons & Combs, 1981).

Time of feeding is believed to have an effect on egg specific gravity (ESG). The study of Roland *et al.* (1973) showed that ESG from commercial laying hens varied according to time of oviposition. These workers observed that the later in the afternoon the egg was laid the better the eggshell. A similar study by Harms (1991a)

reported that although ESG is increased when broiler breeder hens were offered their daily feed allowance at 1600 hours (hr) as compared with 0800 hr, the improvement was very small. These results are inconsistent with earlier findings by Farmer *et al.* (1983b), who demonstrated that when commercial type breeder hens that are 9 months in lay were fed in the afternoon, the ESG was significantly improved. The differences in the results may be due to differences in bird strain. Harms (1991a) also reported that changing the time of feeding resulted in a significant reduction in egg production. It was therefore suggested that the reduction in egg production might have contributed to the improvement in ESG. Previous studies (McDaniel & Brake, 1981; Roque & Soares, 1994) showed that eggs with ESG of <1.080 had lower fertility and hatch and higher embryonic mortality than eggs with higher ESG (>1.080). Roque & Soares (1994) classified eggs with ESG =1.080 as thin-shelled and those with ESG >1.080 as thick-shelled. These workers also observed that eggs with a higher ESG (>1.080) had higher chick viability.

Previous study of Farmer *et al.* (1983b) compared the effects of morning (0800) and afternoon (1600) feeding of calcium and found that afternoon fed hens had significantly more calcium available during the stages of eggshell calcification. Whitehead (1991) contends that morning feeding might limit the supply of calcium during the period of shell formation. This led Roland & Farmer (1984) to conclude that time of calcium intake is important for shell formation and that the most important time for the hens to receive calcium was during the afternoon when shell calcification is initiated.

2.12 Egg Weight and Shell Thickness

2.12.1 Egg weight

Egg weight is a factor, which the hatching industry has always taken into consideration. In six avian species (i.e., chicken, turkey, duck, goose, pheasant and quail) studied, Shanaway (1987) reported that hatching weight depended upon a linear function of egg weight at setting. Generally, many workers agree that it is preferable to have eggs of average weight to achieve good hatchability as far as chickens, turkeys and ostriches are concerned (Gonzales *et al.*, 1999). Shatokhina (1975) investigated the curvilinear relationship between egg weight and hatchability and observed that the hatchability of eggs weighing between 46 and 50 g as well as those

weighing between 66 and 74 g was between 8 and 10% lower than for eggs of average weight (50-66 g). Similar results were reported by Nordskog & Hassan (1969) who found that hatchability was maximised when the egg weighed about 50 g. A 10 g increase in egg weight above this optimum value resulted in a 10.7% decline in hatchability, whilst a 10 g decrease lowered it by 3.9%. Petek *et al.* (2003) also found that hatchability increased with increased egg weight in quails.

It is believed that egg size has an effect on post-hatch mortality (Narushin & Romanov, 2002). McNaughton *et al.* (1978) and Wyatt *et al.* (1985) found that mortality is higher among chicks hatched from eggs of lower weight. According to Petek *et al.* (2003), the relatively low hatchability of small eggs can be explained by insufficient essential nutrients, which might lead to increased chick mortality. On the other hand, Proudfoot & Hulan (1981) reported no significant effect of egg size on the post-hatch mortality of broiler chicks. The differences in results on the effect of egg weight on post-hatch mortality may be attributable to species differences and to some extent the intensity of management.

The primary factors that influence egg weight and egg mass are BW (pullet weight at point of lay), feed allowance, age (Triyuwanta *et al.*, 1992), energy intake, protein intake and intake of linoleic acid (Tiller, 2003).

Underweight pullets attain their weight late while overweight pullets lay the preferred medium weight and large sized eggs earlier in the cycle (especially birds kept in cages). There is a danger of fatty livers and a further, undesirable substantial increase in egg weight after the 40th week of lay (Tiller, 2003). Excessive energy intake is conceivable in situations where egg weights increase beyond target level. Feeding management (lower feed levels in troughs, fewer meals) restricts energy consumption. A daily intake of 310 kcal for white hens and 318 kcal for brown hens is sufficient**.** Roberts & Leary (2000) reported that HyLine Brown produced larger eggs at high levels of linoleic acid and smaller eggs at lower levels. On the contrary, the ISA Brown hens did not respond to changes in dietary linoleic acid. The authors concluded that the effects of linoleic acid are strain dependent for egg weight.

2.12.2 Shell thickness

The eggshell performs a double function during embryo development. It has to be thick and strong enough to protect the embryo from external insults. Simultaneously, the shell should be sufficiently thin and fragile not to act as a strong barrier to the hatching process. Furthermore, although the shell has to have enough pores to supply the developing embryo with oxygen, the high pore concentration should not allow pathogenic microorganisms to enter into the egg (Narushin & Romanov, 2002). Tsarenko (1988) found that the hatchability of thick-shelled eggs was 30% higher compared to those with thin-shells. High ambient temperature is one known cause of eggshell quality problems. During heat stress, feed intake is depressed and egg weight declines (Roberts & Leary, 2000). According to Taylor & Dacke (1984), shell thickness increases markedly as the daily intake of calcium rises from 2 to 4 g in laying hens.

2.13 Nutrition and Bone Breaking Strength

There is a need for better understanding of bone strength (BS) in poultry because bone breakage and related infections lead to mortality, low productivity, and carcass condemnations (Rath *et al.*, 2000). According to Arthur *et al.* (1983), BS is the point on the deformation curve where maximum force causes fracture (failure) of the bone. Bone strength is related to its material properties such as density, matrix chemistry, geometry, and architecture. The collagen that constitutes ~90% of the organic matrix of the bone, contributes to its tensile strength and plastic property, while the minerals contribute to the stiffness and compressional strength of the bone (Rath *et al.*, 1999). Bone ash and BS are highly correlated and are therefore suggested to be of value in predicting and studying bone breakage of hens (Rowland *et al.*, 1968).

A 6-week study by McCormack *et al.* (2001) demonstrated that restricted feeding of Cobb broiler breeder pullets significantly decreased all bone characteristics measured (*i.e.*, cortical porosity, ash content, calcium and phosphorous, BS) with the exception of hydroxyproline content, which was unaltered. These workers concluded that severe feed restriction of broiler breeders resulted in an overall reduction in tibia BS, which was only marginally less severe than the reduction in BW induced by the restriction. In addition, cortical bone in restricted birds was thicker and less porous than in *ad libitum* fed birds but had much lower radiographic density, caused by a marked

reduction in mineralisation. In another study, Lilburn (1994) observed that relative tibia development (length and width) is slower in broilers compared with turkeys and ducks, suggesting a greater susceptibility to biomechanical problems, the end result of which is abnormal long bone development. Compared with tibia, it appears that mineralisation rates and other aspects of femur development occur more slowly than what is observed for tibia. Nutrient restriction during short periods of growout has proven to be an effective way of decreasing leg abnormalities (Lilburn, 1994). In another study, Cox & Balloun (1972) observed that femur mineral depletion was concomitant with the onset of egg production and progressed rapidly during the production of the first 30 eggs.

A study by Knowles & Wilkins (1998) demonstrated that end-of-lay hens from battery cages have especially fragile bones and that these break easily during the rough handling, which is received during depopulation. On the contrary, birds from extensive systems have stronger bones and suffer fewer breaks during depopulation but have a greater prevalence of old healed breaks. These workers argued that the old breaks occur because of collisions in poorly designed housing systems. Several studies have demonstrated that birds maintained in cages have lower tibiae or humeri BS and bone ash values than floor birds (Rowland *et al.*, 1968; Rowland & Harms, 1970; Rowland *et al.*, 1971; Wabeck & Littlefield, 1972; Meyer & Sunde, 1974). Similar results were reported by Moore *et al.* (1977) in radii of laying hens. Meyer & Sunde (1974) also reported that floor birds had less broken bones than caged birds.

It was found (Wabeck & Littlefield, 1972) that humeri from broilers reared in floor pens had approximately twice the BS of humeri from broilers reared in cages. Rowland *et al.* (1968) and Rowland & Harms (1970) attributed the differences in tibia ash and tibia BS of hens in cages to confinement and probably lack of exercise. Furthermore, Travis *et al.* (1983) found that males had stronger humeri than females and that right humeri were stronger than left humeri in both sexes. The authors could not explain the strength of right *vs.* left wing. The humerus stress of males was also significantly greater than that of females (Mandour *et al.*, 1989).

In pigs, Crenshaw *et al.* (1981a) reported that higher levels of calcium and phosphorus resulted in an increase in the mechanical properties (bending moment, ultimate stress, yield stress and modulus of elasticity), geometrical measurements (moment of inertia and wall thickness) and percentage of ash bones. Other researchers have also reported an increase in BS due to increased levels of dietary calcium and phosphorus fed (Nimmo *et al.*, 1980a; Shafey, 1993). Nimmo *et al.* (1981) reported greater bending moment values for bones from gilts fed higher calcium-phosphorus (.975% Ca, .75% P) levels than for bones from those fed lower calcium-phosphorus (.65% Ca, .50% P) diet. It was also observed that gilts on high calcium-phosphorus consumed more feed per day than gilts on low calcium-phosphorus diet. This is inconsistent with, Summers *et al.* (1976), Shafey & McDonald (1991), Shafey (1993) and Hubbard Feeds INC. (2000) who reported that excess dietary calcium leads to reduced growth and feed efficiency, and a decline in feed intake in chickens. The differences in results may be ascribable mainly to species differences. Summers *et al.* (1976) also reported lower egg production with 4.0 and 2.5% calcium diets. It was observed that egg size increased linearly with higher level of calcium throughout the test period. These investigators observed that although eggshell deformation increased (weaker shells) for the low calcium diet throughout the test most of this change was noted during the first week (12 days) of lay. In another study, Roland (1980) reported that increasing calcium level from 3.00 to 4.25% did not prevent the decline in shell weight as egg weight declined.

2.13.1 Factors affecting bone strength

Factors that can directly or indirectly affect BS include nutrition, growth and ageing (Crenshaw *et al.*, 1981b; Rath *et al.*, 2000), genetics, endocrine, disease, physical loading, gender, toxins and antinutrient (Rath *et al.*, 2000). These factors are discussed briefly in the sections below.

Growth, gender and age

A study by Rath *et al.* (2000) in which the changes in the physical, compositional and mechanical properties of tibiae from chickens between 5 and 55 weeks of age, were compared showed that weight, length, diameter, and pyridinium cross link content were maximised at 25 weeks of age while the mineral content, density, and the BS of bones reached maximum at 35 weeks of age. These workers observed that tibia from

5-week-old chicks were strong but brittle because of low collagen crosslinks and high mineral content.

In another study, Rath *et al*. (1999) reported that 72-week-old hens had the highest ash content and exhibited a similar trend with respect to bone density, although the increase was between 25 and 40% in this parameter. The increase in biomechanical strength was also substantially greater in older birds compared with younger birds. In a report by Al-Batshan *et al.* (1994) it was found that femur ash decreased from 50.8% at 37 weeks of age to 47.6% at 58 weeks of age. Similarly, percentage shell and shell thickness declined from 9.79% and .403 mm at 22 weeks of age to 8.88% and .373 mm at 57 weeks of age, respectively.

Gender is another factor that influences bone growth. Size differences together with hormonal differences, can account for the differences in the growth and BS between males and females (Rath *et al*, 2000). Previous study of Rath *et al*., (1999) demonstrated that male and female birds of the same age show different diaphyseal diameters, with the females showing a consistently lower value. On the contrary, a comparative study with 7 and 72 week old male and female chickens showed no significant differences in BS between young males and females (Rath *et al*., 2000). In that study, the bones from 72-week-old hens were stronger and more rigid and had a lower strain value, which is perhaps due to the presence of medullary bone.

Genetics

Genetics is another determinant of BS. Generally, it is considered that modern poultry have been bred for superior meat production, possibly overlooking the consequences on bone fragility (Rath *et al*, 2000). Although Newman & Leeson (1997) reported that heavy-bred birds do not show any weaker bones than their lighter-weight egg-type counterparts, the findings of Nestor & Emmerson (1990) suggested that heavier birds might have stronger bones. Previous report of Mandour *et al.* (1989) indicated that it is possible to increase humerus strength through selection.

Physical activity and mechanical stress

Rowland *et al.* (1972) conducted three experiments in which 7-12 strains of hens were utilised for measurement of tibia BS and they found that caged hens had lower tibia

BS than floor hens of the same strain. It was also observed that some of the pullets that were maintained in cages had a higher BS than other strains maintained on the floor. In one experiment, tibia ash was not related to BS but in another experiment caged hens, which had lower BS had significantly lower tibia ash values than the floor hens. This indicates existence of conflicting literature on bone BS aspect. Rowland *et al.* (1968) showed in an earlier study that bone ash and BS were highly correlated.

Nutrition and vitamins

The results of a study (Mraz, 1972) in which three levels of calcium (1.5, 2.25 and 3% calcium and 0.4, 0.6 and 0.8% phosphorus) were fed to 20 weeks old pullets for 12 months indicated that the dry and ash weights of tibiae from pullets fed diets containing 2.25% more calcium were higher than those from pullets fed 1.5% calcium diets. It was also observed that the ratios of ash weight to dry weight tended to increase when dietary calcium or phosphorus was increased. This is in disagreement with findings of Francis (1972) who reported higher ash percentage values in the femurs of chickens on medium (1.01%) and high (1.61%) calcium diets compared to those fed low calcium (0.41%) diet. Atteh & Leeson (1983) also reported increased bone ash and bone calcium when dietary calcium was increased from 3 to 4.2%. Plasma calcium also increased with an increase in dietary calcium level.

The results of an earlier study (Hulan *et al.*, 1986) showed that tibia strength of normal broiler genotype decreased as a result of increased ratio of calcium to phosphorus in the broiler starter and finisher diets. For dwarf genotypes, only the highest levels of calcium and available phosphorus in finisher diets decreased tibia strength. Nimmo *et al.* (1980b) found that peak force and stress required to break bones from pigs fed 1% calcium (low level) were less than those required to break bones from pigs fed 1.3% calcium (high level), respectively). The workers suggested that the degree of mineralisation of bones was greater for boars fed diets containing 1.3% calcium than it was in those fed the lower level of calcium (1%).

Toxins and antinutrients

Aflatoxin and ochratoxin are the major mycotoxins of current concern to animal and public health, as they inhibit growth, including skeletal growth and development (Huff *et al.,* 1980). According to Hamilton *et al.* (1974), aflatoxin interacts with vitamin D to produce deficiencies that result in occurrence of several natural outbreaks of rickets in chickens fed diets low in vitamin D_3 and containing aflatoxin. The results of a study by Huff *et al.* (1980) showed that tibia strength decreased at aflatoxin concentrations of 2.5, 5, and 10 µg/g and by ochratoxin at 2, 4, and 8 µg/g. The extent of the decrease in BS was greater with ochratoxin, which caused a loss at its highest dose to about one-eighth of the control value, as compared with aflatoxin, which caused a loss of only about one-half.

Infection and stress

Infection and stress can be risk factors for bone integrity leading to bone weakness (Rath *et al.* 2000). Reece (1992) stated that bone infections such as osteomyelitis and osteonecrosis cause focal bone loss resulting in bone weakness.

Hormones and cytokines

Hormones and cytokines have a profound effect on bone metabolism, growth, and remodelling, and therefore, are consequential to their strength (Rath *et al.,* 2000). The medullary osteogenesis in poultry is under the control of synergistic action of oestrogen and androgens (Dacke *et al.,* 1993). Studies on the effects of various sex steroids on adolescent chickens demonstrated that testosterone implants caused a significant increase in BS of young chickens (Rath *et al.,* 1996*).*

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CHAPTER 3

INFLUENCE OF DIETARY CALCIUM LEVELS ON BONE DEVELOPMENT IN BROILER BREEDER PULLETS UP TO 18 WEEKS OF AGE

3.1 Introduction

Broiler breeders are continuously selected for faster growth. Therefore, feed restriction during the growing and laying period is a common practice to prevent excess body weight (Ingram *et al.*, 2001). Williams *et al.* (2000*a*) suggested that bone characteristics might be changing in modern fast growing broilers, with increases in cortical bone porosity and changes in composition that could affect mechanical properties of bone. According to McCormack *et al.* (2001), feed restriction in broiler breeders results in changes in the relative growth of different body components, but there is little information on the impact of this on bone structure and composition.

Calcium (Ca) performs various functions in the body, of which the structuring of bones is the most important. About 99% of Ca is contained in the skeleton (Hurwitz *et al.*, 1987). Several researchers (Rowland *et al.*, 1968; Reichmann & Connor, 1977; Shafey, 1993; Roberson *et al.*, 2004) reported an increase in bone breaking strength (BS) because of increased levels of dietary Ca and phosphorus (P) in commercial growing and laying chickens as well as growing turkeys. Accordingly, higher bone ash percentages were recorded as the Ca level in over one year old laying hen diets increased. On the other hand, Hubbard Feeds INC. (2000) and Shafey (1993) reported reduced growth and a decline in feed intake (feed efficiency) in growing and laying chickens due to excess dietary Ca. Accordingly, Hulan *et al.* (1986) found that biological performance (body weight gain, final live weight and feed conversion) declines as the total Ca+avP and Ca:avP ratio increase in the diets.

It seems from the literature that feed restriction could influence the bone structure and composition of growing broiler breeders. Calcium plays a major role in the structuring of bone and further research on higher Ca levels in broiler breeder pullets' diets during rearing is of utmost importance. Most of the studies regarding dietary Ca levels on bone development were done with laying hens. The effect of Ca intake on bone

development of broiler breeders on restricted feeding during the rearing period needs urgent investigation. Therefore, a study was undertaken to investigate the effects of dietary Ca levels and feed restriction on bone development of broiler breeders up to 18 weeks of age.

3.2 Materials and Methods

Six hundred and forty one-day-old Ross broiler breeder pullets were obtained from a commercial hatchery and were randomly assigned to 4 treatment groups, each having 4 replicates. The 4 treatments were 1.0% Ca (0.45% Pi), 1.5% Ca (0.7% Pi), 2.0% Ca (0.9% Pi) and 1.0% Ca (0.45% Pi). The first 3 treatments were feed restricted according to Ross Breeders (2001) recommendations while the last treatment was *ad libitum* fed (served as control) throughout the rearing period. Feed restriction started at 2 weeks of age. Individual feeds were analysed for Ca and P to ensure accurate diet formulation. Water was provided *ad libitum* for all treatments. The initial weight of the birds was determined by weighing 5.0% of the birds prior to allocation to 4 dietary treatments. The rearing period lasted until 18 weeks of age.

Pullets were fed different diets during the 3 feeding phases of the experimental period namely, pre-starter (0 to 2 weeks); starter (2 to 4 weeks) and grower (4 to 18 weeks). The physical and nutrient compositions of diets are shown in Tables 3.1 and 3.2, respectively. The diets for each feeding phase were isocaloric and isonitrogenous. Calcium and feeding levels were the only differences during each specific phase. A diet with 1.5% Ca was obtained by mixing the 1.0% and 2.0% Ca diets.

Day-old chicks were reared in 16 pens with 40 birds per pen and 4 pens (replicates) per treatment in a closed house with windows for ventilation. Each replicate was housed in floor pens, measuring 4 m^2 with wood shavings and/or grass as litter material. The stocking density at 12 and 18 weeks of age was 0.11 and 0.13 per m^2, respectively as numbers decreased due to birds that were sacrificed for bone samples. Each pen was equipped with an electric brooder, 2 tube-type feeders and 2 automatic drinkers.

Table 3.1 Physical composition of experimental diets on air dry basis (%)

	Pre-starter diet		**Starter**		**Grower**	
	1.0% Ca	**2.0% Ca**	**1.0% Ca**	**2.0% Ca**	**1.0% Ca**	**2.0% Ca**
Maize	58.62	58.15	58.15	58.37	67.12	64.82
Maize glutten	1.85	-	-	-	-	-
Wheat bran	6.50	12.00	12.00	5.45	12.0	9.30
Full fat soya	-	-	-	1.30	-	-
Soybean oil cake	17.85	17.85	17.85	18.95	6.70	11.60
Sunflower oil cake	8.00	8.00	8.00	8.00	10.0	6.40
Fishmeal	1.00	-	-	-	-	-
Calcium carbonate	1.30	1.45	1.45	3.00	1.70	2.95
Calcium monophosphate	1.27	1.31	1.31	3.37	1.47	4.08
Salt	0.17	0.23	0.23	0.24	0.23	0.26
Sodium bicarbonate	0.30	0.28	0.28	0.25	0.26	0.14
Choline liquid	0.03	0.021	0.02	0.03	0.052	0.05
Lysine	0.33	0.18	0.18	0.15	0.91	0.01
Threonine	0.33	-	-	-	-	-
Methionine	0.24	0.18	0.18	0.18	0.32	0.03
Trace mineral/ vitamin premix	0.35	0.35	0.35	0.35	0.35	0.35

Table 3.2 Nutrient composition of experimental diets on air dry basis (%)

	Pre-starter diet		**Starter**		**Grower**	
	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca
Moisture	11.19	11.31	11.31	10.93	11.20	10.96
ME (MJ/Kg)	12.10	11.80	11.80	11.60	12.10	11.70
Protein	20.26	17.99	17.99	17.99	14.00	14.32
Crude fat	3.01	3.05	3.05	3.05	3.26	3.12
Crude fibre	5.53	6.13	6.13	6.13	6.45	5.41
Calcium	0.99	1.01	1.01	2.00	1.10	2.01
Phosphorus	0.79	0.81	0.81	1.28	0.82	1.36
Available phosphorus	0.45	0.90	0.45	0.90	0.45	0.90
Arginine	1.25	1.15	1.15	1.16	0.88	0.90
Isoleucine	0.84	0.74	0.74	0.76	0.55	0.58
Methionine	0.59	0.49	0.48	0.48	0.30	0.29
TSAA[1]	0.95	0.81	0.81	0.81	0.58	0.57
Threonine	0.78	0.66	0.66	0.67	0.51	0.53
Tryptophan	0.23	0.21	0.21	0.21	0.16	0.16
TA[2] arginine	1.16	1.07	1.06	1.07	0.81	0.83
TA[2] isoleucine	0.76	0.67	0.67	0.69	0.49	0.55
TA[2] lysine	1.05	0.85	0.85	0.85	0.55	0.55
TA[2] methionine	0.56	0.45	0.45	0.45	0.27	0.51
TA[2] TSAA	0.87	0.73	0.73	0.73	0.51	0.46
TA[2] Threonine	0.69	0.58	0.58	0.59	0.45	0.46
TA[2] Tryptophan	0.21	0.19	0.19	0.19	0.14	0.15
Linoleic acid	1.59	1.68	1.68	1.65	1.82	1.72
Salt	0.21	0.23	0.23	0.25	0.24	0.27
Choline (mg/kg)	1410.68	1288.83	1288.83	1308.81	1311.38	1307.09
Sodium	0.18	0.18	0.18	0.18	0.18	0.16
Chlorine	0.24	0.22	0.66	0.66	0.22	0.22
Potassium	0.71	0.70	0.70	0.70	0.57	0.59

[1]Total sulphur amino acids

TA[2] – Chemically determined

Pullets were reared at a time when day length was decreasing (May to July). Chicks received continuous light for the first 2 days of life and thereafter it was reduced to natural day length pattern of a decreasing and increasing photoperiod throughout the rearing period. Pullets were vaccinated in accordance with a vaccination programme obtained from a local parent stock company.

Feed consumption was measured by giving pre-weighed feed allocations to each replicate group throughout the week and then weighing back all of the unconsumed feed at the end of the week. Pen body weights (BW) were also recorded on weekly basis.

At 6, 12 and 18 weeks of age, 5 pullets were randomly selected from each replicate (i.e. 20 birds per treatment at each age) and killed by either cervical dislocation or stunning in the abattoir and carcasses stored in the refrigerator overnight and the bones removed the following day. The tibiae (left and right) and right humeri from each of the birds were excised and defleshed without boiling. The right tibiae and right humeri were then weighed and total length and bone shaft widths measured by means of a calliper with an accuracy of 0.001 cm (Zhang & Coon, 1997). The tibiae and humeri were individually sealed in plastic bags to minimise moisture loss, and stored in a freezer at -18 ^{0}C for later analysis (Nimmo *et al.*, 1980a; Zhang & Coon, 1997). The bones were then removed for bone ash and BS determinations. The right tibiae and right humeri were used for BS while left tibiae were used for bone ash determination and histomorphometric analysis. Breaking strength (N) was determined according to procedures described by Fleming *et al.* (1998). Bone stress (N/mm^2), which is force per unit area of bone, was calculated by dividing BS with true cortical area (mm^2). True cortical area (TCA) was calculated by multiplying cortical area with mean percent bone and dividing by 100. Percent bone, which is the reciprocal of porosity, was determined from microscopic observations.

Left tibiae were dissected and a 5 mm ring from midshaft taken for histological processing. Two additional samples were taken, 20 mm on either side of the ring, and combined for ash measurements according to the procedures described by Fleming *et al.* (1998) and Williams *et al.* (2000*a*, 2000*b*). The bone cross-section taken for histology was fixed in 10% neutral buffered formalin, decalcified and processed for

histomorphometric analysis according to procedures described by (Fleming *et al.*, 1994, 1998).

Bone data obtained at each sampling period was regressed on average Ca intake and/or levels per chicken during the particular period, namely from day-old to 6 weeks of age, from day-old to 12 weeks of age and for the entire period of day-old to 18 weeks of age. Calcium intakes were calculated from average feed intake values of the birds on a particular dietary Ca level. The Minitab Statistical Software package (Release 8.2) (Minitab Inc., 1991) was employed to analyse data sets.

In a second analysis of the data the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1996) was used to estimate differences between treatment means for the different levels of Ca intake within and between age periods. In this analysis, the data was regarded as a split plot design with 4 dietary treatments being the main plots and age as split plots. The differences between treatment means were separated using the Tukey test.

3.3 Results and Discussion

3.3.1 General performance characteristics

A short overview will be given on the general performance parameters (feed consumption, Ca intake, growth rate and mortality) of the broiler breeders during rearing in the present study as these could have had an important bearing on the findings regarding bone characteristics.

3.3.1.1 Feed intake

The influence of Ca levels, age and feed restriction on the feed intake of birds is illustrated in Figure 3.1. It is clear that feed restriction was successfully applied and that feed consumption was essentially the same for restricted groups. The dietary Ca levels applied in the present study did not appear to influence consumption albeit feed was allocated in accordance with breeders' recommendations. This is in line with the findings of Smith *et al.* (2003) who found that increasing dietary Ca level from 0.9 to 1.5% had no effect on feed consumption of broilers. Similar results were reported by Hocking *et al.* (2002) in growing turkeys fed 4 Ca levels (6, 10, 14 and 18 g/kg) and avP (3, 5, 7, and 9 g/kg) up to 13 weeks of age. This is also consistent with Ahmad *et*

al. (2003) who found that increasing dietary Ca level from 2.5 to 5.0% had no effect on feed consumption in Bovans hens. Shafey & McDonald (1991) found that high dietary Ca (2.43 *vs.* 0.89%) reduced feed intake in broiler chicks reared up to 17 days of age. It seems that the influence of Ca levels on feed intake differs between *ad libitum* and feed restricted birds. In the case of restricted birds, Ca levels within limits had no pronounced effect on feed intake.

As can be expected, feed intake increased significantly (P<0.05) with age and the *ad libitum* group consumed significantly (P<0.05) more feed than restricted groups. In a previous study, Yu *et al.* (1992a) reported that average feed intake of restricted birds was 37.2% of the full-fed (*ad libitum*) birds during rearing (4-18 weeks). Similarly, the average feed intake of restricted birds (1.0% Ca diet) in the current study was 34.2% of that of the *ad libitum* fed birds (0-18 weeks).

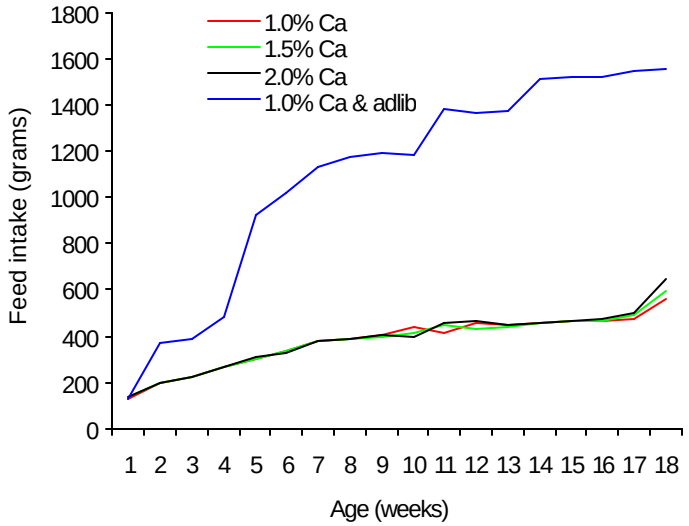


Figure 3.1 Effect of calcium level, age and feed restriction on feed intake of broiler breeder pullets

3.3.1.2 Calcium intake

From Figure 3.2, it seems that the daily Ca intake of the birds in general significantly (P<0.05) increased with increasing dietary Ca level. The daily Ca intake of the restricted pullets fed 1.0, 1.5 and 2.0% Ca diets, respectively was 0.66 g, 0.89 g and 0.91g at 6 weeks; 0.71 g, 0.93 g and 1.27 g at 12 weeks; and 0.72 g, 1.19 g and 1.85 g at 18 weeks. The average daily Ca intake per bird during the rearing period for birds

fed 1.0%, 1.5%, 2.0% Ca diets and *ad libitum*-fed birds was 0.66 g, 0.81 g, 1.0 g and 1.6 g, respectively. Accordingly, Yu *et al.* (1992b) reported daily average Ca intakes of 0.6 g and 1.67 g respectively, for feed restricted and *ad libitum* fed Indian River breeder hens (4 to 18 weeks). With the exception of weeks 14 to 18, the daily Ca intakes of birds in all treatments were higher compared to the recommended levels of Ross Breeders (2001). The rise in recommended levels of Ca intake from week 14 is attributable to a recommended increase in both diet concentration (1.0 to 1.5% Ca) and daily feed intake (Ross Breeders, 2001). A feed increase of 10-15% at 105 days (15 weeks) is recommended to ensure a significant increase in growth rate.

In accordance with feed intake, Ca intake of the restricted birds was significantly ($P<0.05$) lower than that of the *ad libitum* group (Figure 3.2).

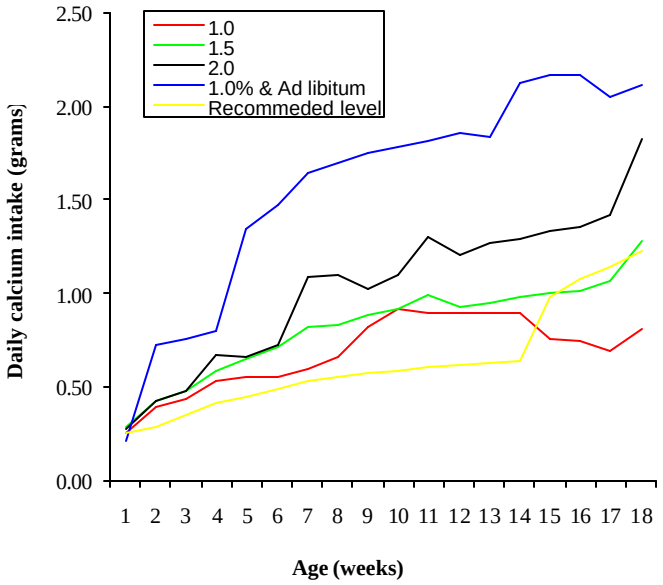


Figure 3.2 Effect of dietary calcium level, age and feed restriction on calcium intake of broiler breeder pullets

3.3.1.3 Body weight

From Figure 3.3, it is evident that Ca levels had no statistical significant ($P<0.05$) influence on the growth rate of birds fed a restricted isonitrogenous and isocaloric diet. Prince *et al.* (1984) reported that increasing dietary Ca and P levels from 0.65% Ca and 0.55% P to 1.20% Ca and 0.86% P resulted in increased ($P<0.01$) growth rate in pigs. In contrast, Shafey & McDonald (1991) found that increased dietary levels of

Ca alone or Ca (2.56%) and P (.49% available P) significantly (P<0.01) reduced body weight gain in broiler chickens. However, more recent research with 8 to 14 week poults fed 105 and 120% of NRC (1994) requirements of Ca resulted in linear (P<0.003) BW increase at 11 and 14 weeks of age (Roberson, 2004). It seems that literature on the effect of dietary Ca levels on BW is inconsistent probably due to differences in species, strain or breed, age, dietary Ca levels and stage of production. The results of the current study suggest that the NRC (1994) recommendations for broiler breeders (1.0% Ca) may be sufficient to support the required growth.

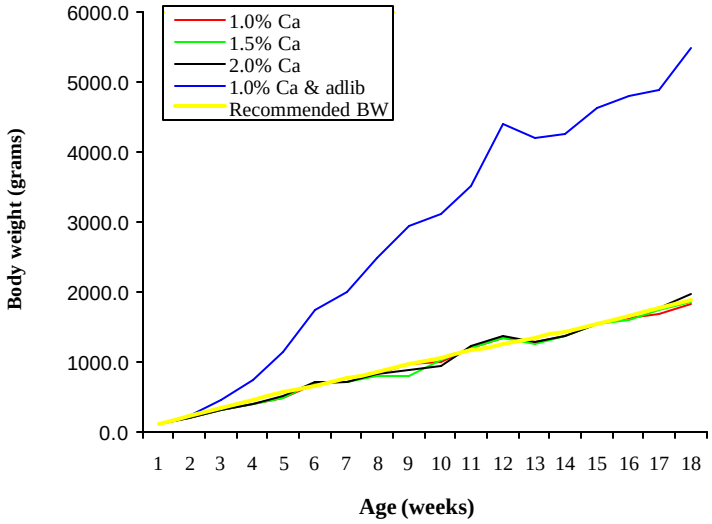


Figure 3.3 Effect of calcium levels, age and feed restriction on body weight of broiler breeder pullets

The *ad libitum* group as expected was significantly (P<0.05) heavier than restricted groups. Mean weight of restricted birds was 36% of the weight of *ad libitum* birds at 6 weeks of age, and decreased to 29% of the weight of *ad libitum* birds at 12 weeks of age and increased to 34% of *ad libitum* at 18 weeks of age. A previous study of Yu *et al.* (1992b) showed that restricted birds not only weighed less, but also had a significantly shorter length than *ad libitum*-fed birds, an indication of stunted growth. Similar results were reported by Fontana *et al.* (1992).

3.3.1.4 Mortality

Overall mortality for the entire rearing period was 7.2% (46 birds), which is higher than the Ross Breeders standard mortality of 5.1% at 18 weeks of age. The mortality rate calculated on individual treatment basis was 11 (6.9%), 13 (8.1%), 12 (7.5%) and

10 (6.3%) for birds on 1.0%, 1.5%, 2.0% Ca and *ad libitum* group, respectively. Restricted feeding tended to result in a higher percentage mortality compared to the *ad libitum* group. The high mortality observed in the restricted groups was mainly due to cannibalism as the birds were not debeaked. In early publications by Atkinson *et al*. (1967) and Wise & Ewins (1980) it was found that Ca levels *per se* had no significant influence on bird mortality during rearing.

3.3.2 Bone dimensions

3.3.2.1 Bone length

The mean values for tibia and humerus length are given in Table 3.3. It is evident that bone length increased non-significantly with increased dietary Ca intake.

A regression analysis of the tibia and humerus length data (Table 3.4) at all ages showed a highly significant response to Ca intake when data of the *ad libitum* group was included in the calculations. However, no significant response in tibia lengths due to Ca intake could be demonstrated for those groups that were reared on restricted feeding. The regression equations for this data are also shown in Table 3.4.

The graphical illustration of the response of tibia and humerus length to increasing dietary Ca levels is shown in Figures 3.4 and 3.5, respectively. It can clearly be seen that the response was mainly due to the much larger bone lengths of the birds on *ad libitum* intake and that the groups on restricted feeding did not show any response to increasing Ca intake.

The results of the current study have demonstrated that restricted feeding resulted in lower values for bone dimensions than when *ad libitum* feeding is applied. Restricted birds had shorter tibiae and humeri than *ad libitum* birds, an indication of "stunted growth", a term that was used by Yu *et al.* (1992b). These workers found that restricted birds had significantly shorter tibiae than *ad libitum* fed birds at 18 weeks of age.

Table 3.3 Effect of calcium levels on bone dimensions of broiler breeder pullets during rearing

		Age (weeks)				Significance of effect (P)			
	Treatment	**6**	**12**	**18**	**Means**	**Treatment**	**Age**	**Interaction**	**CV**
Right tibia									
Length (mm)	1% Ca	71.84 ± 5.43	100.00 ± 22.65	118.47 ± 11.31	96.76[b]	0.0064	0.0001	0.2692	70.71
	1.5% Ca	72.11 ± 7.01	109.54 ± 21.72	118.35 ± 8.09	98.50[b]				
	2% Ca	71.71 ± 5.58	105.54 ± 6.46	118.24 ± 9.00	100.00[ab]				
	1% Ca & *ad lib*	92.63 ± 5.54	190.14 ± 26.09	134.52 ± 4.69	139.09[a]				
	Means	77.07[a]	122.39[b]	126.30[b]					
Width (mm)	1% Ca	4.90 ± 0.66	6.81 ± 0.38	7.10 ± 0.67	6.27[b]	0.0001	0.0001	0.6769	9.40
	1.5% Ca	4.78 ± 0.48	6.62 ± 0.48	7.23 ± 0.67	6.21[b]				
	2% Ca	4.98 ± 0.71	6.62 ± 0.62	7.30 ± 0.82	6.30[b]				
	1% Ca & *ad lib*	7.03 ± 0.55	8.92 ± 0.64	9.63 ± 0.82	8.53[a]				
	Means	5.42[a]	7.24[b]	7.81[c]					
Weight (g)	1% Ca	4.05 ± 1.03[a]	11.08 ± 2.53[a]	15.22 ± 4.29[a]		0.0001	0.0001	0.0001	19.64
	1.5% Ca	4.35 ± 1.13[a]	11.19 ± 3.12[a]	14.57± 2.66[a]					
	2% Ca	4.07 ± 1.12[a]	11.00 ± 1.61[a]	14.70 ± 2.78[a]					
	1% Ca & *ad lib*	10.82±1.50[b]	20.48[b]	26.65[b]					
Right humerus									
Length (mm)	1% Ca	52.77 ± 3.80	70.44 ± 16	80.26 ± 5.60	67.82[b]	0.0001	0.0001	0.0644	8.59
	1.5% Ca	53.38 ± 3.80	74.32 ± 4.1	80.60 ± 5.90	69.44[a]				
	2% Ca	53.40 ± 4.80	73.60 ± 3.6	80.20 ± 3.80	69.07[b]				
	1% Ca & *ad lib*	65.80 ± 3.70	83.80 ± 4.4	86.30 ± 3.80	78.65[a]				
	Means	56.33[a]	75.46[b]	81.86[c]					
Width (mm)	1% Ca	4.40 ± 0.40	6.31 ± 0.40	6.61 ± 0.40	6.27[b]	0.0001	0.0001	0.4970	9.27
	1.5% Ca	4.36 ± 0.40	6.23 ± 0.50	6.49 ± 0.60	6.21[b]				
	2% Ca	4.45 ± 0.50	6.12 ± 0.30	6.59 ± 0.70	6.30[b]				
	1% Ca & *ad lib*	6.28 ± 0.40	7.91 ± 1.10	8.69 ± 0.60	8.53[a]				
	Means	4.88[a]	6.64[b]	7.09[c]					
Weight (g)	1% Ca	2.27 ± 0.77[b]	5.95 ± 1.81[a]	7.51 ± 2.86[a]		0.0001	0.0001	0.0001	27.19
	1.5% Ca	2.50 ± 0.81[b]	5.71 ± 1.82[a]	6.53 ± 1.70[a]					
	2% Ca	2.33 ± 0.64[b]	5.56 ± 1.47[a]	6.98 ± 1.83[a]					
	1% Ca & *ad lib*	5.69 ± 1.09[c]	11.46 ± 2.29[b]	15.09 ± 2.92[a]					

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred.
Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

Table 3.4 Multiple regression analysis of bone dimensions on calcium intake of pullets during rearing

		Data from restricted groups only			**Data of *ad lib* group included**		
Age	**Variable**	**Equations**	**Adj -R^2**	**P value**	**Equation**	**P value**	**Adj -R^2**
6 weeks	Tibia length	Tiblength6 = 72.50 - 0.83 Caint6		0.893	Tiblength6 = 52.2 + 26.2 Cain6	0.000	0.753
	Humerus length	Humlength6 = 52.10 + 1.33 Caint6	0.000	0.755	Humlength6 = 41.0 + 16.2 Caint6	0.000	0.583
	Tibia width	Tibwidth6 = 4.54 + 0.446 Caint6		0.486	Tibwidth6 = 2.79 + 2.77 Caint6	0.000	0.645
	Humerus width	Humwidth6 = 4.30 + 0.152 Caint6	0.000	0.732	Humwidth6= 2.62 + 2.38 Caint6	0.000	0.711
	Tibia weight, g	Tibweight6 = 4.23 - 0.01 Caint6		0.995	Tibweight6 = 1.89 + 8.14 Caint6	0.000	0.699
	Humerus weight	Humweight6 = 2.45 – 0.103 Caint6	0.000	0.892	Humweight6 = -0.789 + 4.21 Caint6	0.000	0.655
12 weeks	Tibia length, mm	Tiblength12 = 107.00 - 0.39 Caint12		0.956	Tiblength12 = 92.3 + 15.2 Caint12	0.000	0.639
	Humerus length	Humlength12 = 68.30 + 4.53 Caint12	0.000	0.355	Humlength12 = 65.5 + 7.41 Caint12	0.000	0.229
	Tibia width	Tibwidth12 = 6.96 - 0.278 Caint12		0.272	Tinwidth12 = 5.39 + 1.37 Caint12	0.000	0.639
	Humerus width	Humwidth12 = 6.52 – 0.297 Caint12	0.000	0.148	Humwidth12 = 5.26 + 1.02 Caint12	0.000	0.449
	Tibia weight	Tibweight12 = 10.80 + 0.30 Caint12		0.769	Tibweight12 = 5.40 + 5.94 Caint12	0.000	0.699
	Humerus weight	Humweight12 = 6.34 – 0.598 Caint12	0.000	0.482	Humweight12 = 2.41 + 3.51 Caint12	0.000	0.542
18 weeks	Tibia length	Tiblength18 = 119.00 - 0.24 Caint18		0.939	Tiblength18 = 107 + 9.91 Caint18	0.000	0.230
	Humerus length	Humlength18 = 80.50 - 0.07 Caint18	0.000	0.966	Humlength18 = 76.0 + 3.67 Caint18	0.000	0.127
	Tibia width	Tibwidth18 = 6.93 + 0.203 Caint18		0.389	Tibwidth18 = 5.30 + 1.58 Caint18	0.000	0.436
	Humerus width	Humwidth = 6.57 – 0.009 Caint18	0.000	0.962	Humwidth18 = 5.01 + 1.31 Caint18	0.000	0.424
	Tibia weight	Tibweight18 = 15.70 - 0.61 Caint18		0.583	Tibweight18 = 6.67 + 7.00 Caint18	0.000	0.376
	Humerus weight	Humweight18 = 7.68 – 0.502 Caint18	0.000	0.487	Humweight8 = 1.39 + 4.80 Caint18	0.000	0.368

Tib = tibia; Hum = humerus; Caint = calcium intake

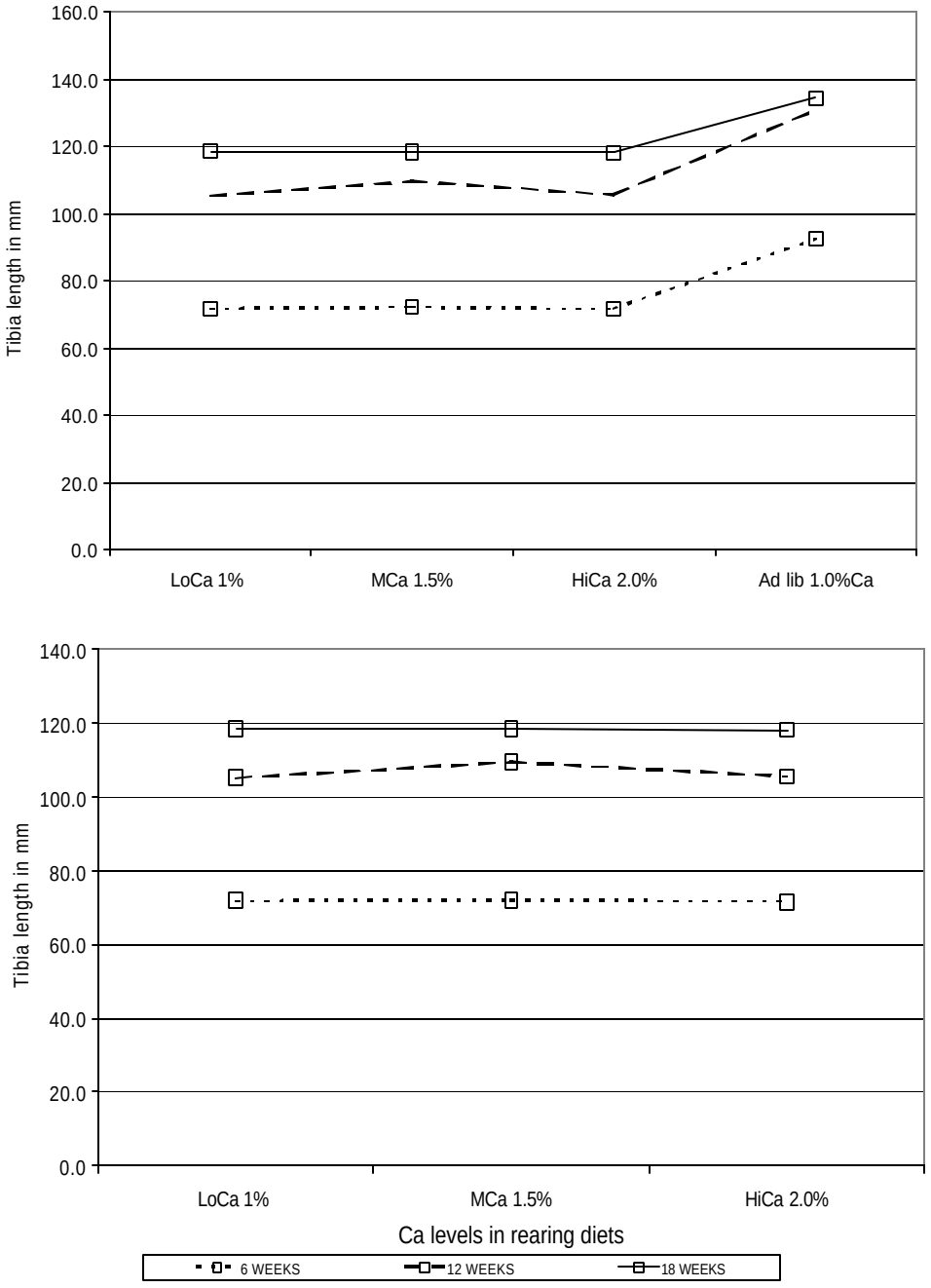


Figure 3.4 Right tibia length at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

As shown in Table 3.3 the length of tibia increased significantly (P<.0001) with age up to 12 weeks of age while that of humerus increased with age throughout rearing. This indicates differences in the developmental growth patterns of humerus and tibia of the growing broiler breeder pullets. For instance, tibia length increased by 46% and

13% between 6 and 12 weeks and 12 and 18 weeks, respectively. On the other hand, increases in humerus length of 37 and 10% were noted at 6 and 12 weeks and 12 and 18 weeks, respectively. These values show that bone development and growth in broiler breeder pullets is rapid during the first 12 weeks of age.

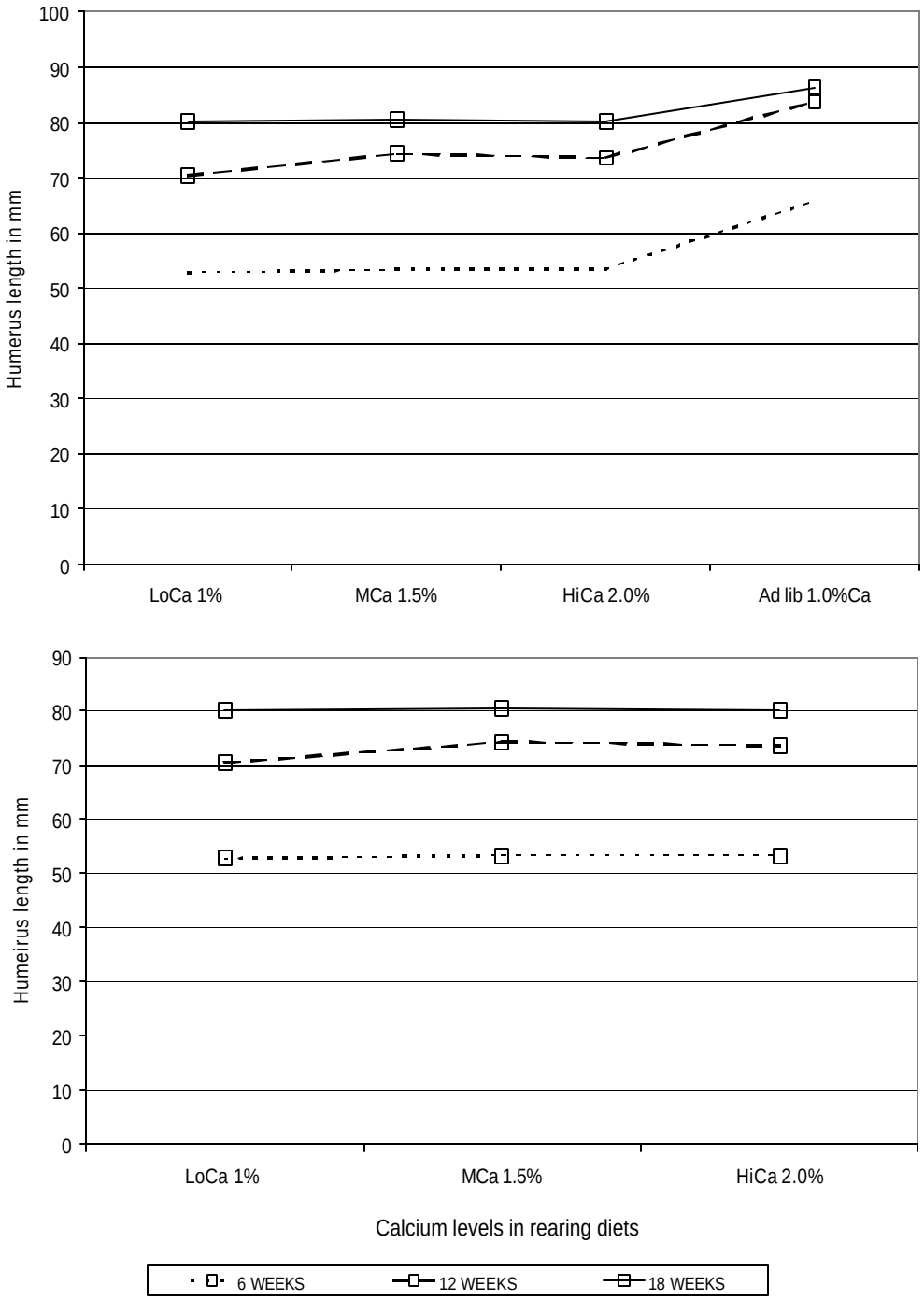
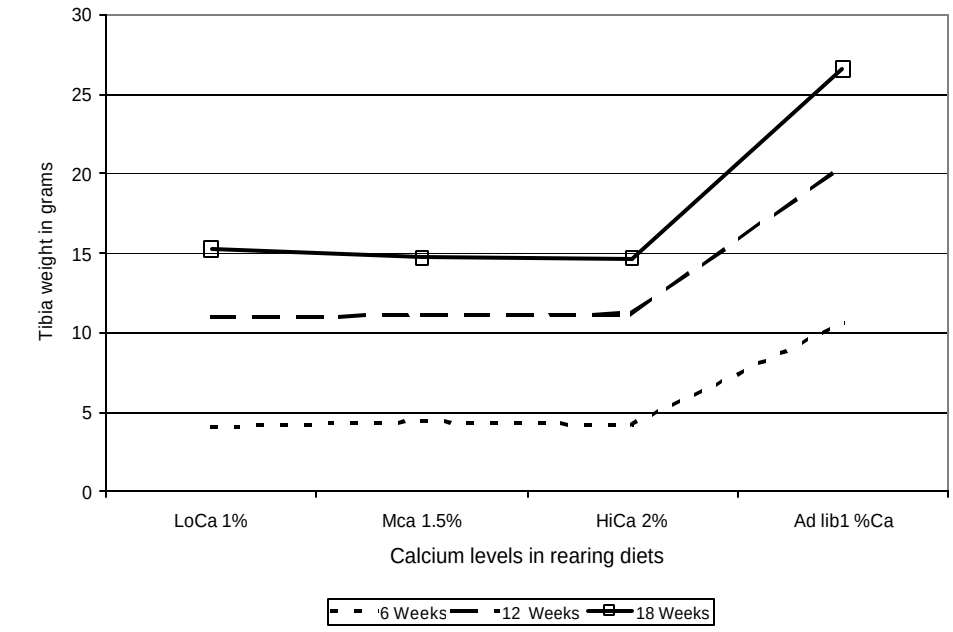


Figure 3.5 Right humerus length of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

3.3.3.2 Bone width and weight

The average values for the bone widths and weights are presented in Table 3.3 and a graphical display of the responses is given in Figures 3.6 to 3.9. No significant influence of dietary Ca intake on bone width of restricted birds could be detected (Table 3.3). Williams *et al* (2000*b*), however, reported that tibiotarsus width decreased linearly with increasing dietary Ca content. This is consistent with the trend reported for lower BW at high Ca content, since lighter birds generally have smaller bones. In the current study Ca levels in the restricted groups, however, had no significant influence (Figure 3.3) on BW, and therefore bone width. Bone width showed, however, a constant significant increase, as the birds got heavier because of age and *ad libitum* feeding. Accordingly Williams *et al.* (2000a) reported that heavier birds had longer and wider tibiotarsi.

The results of the regression analysis accordingly did not result in any response of bone width to increasing dietary Ca levels (Table 3.4). However, when the bones from the *ad libitum* group were included in the data set a significant response in bone dimensions to increasing Ca intake occurred (Table 3.4). The explanation for the significant responses in bone dimensions when data from the *ad libitum* group was included in the data set lies most probably in the larger bone mass that was formed due to *ad libitum* feeding. In the initial planning of the experiment the *ad libitum* group was included to compare Ca content of bones under *ad libitum* conditions to the Ca content of bones from chickens under conditions of feed restriction as practised in the commercial poultry industry. Research conducted by Yu *et al.* (1992b) on broiler breeders on *ad libitum* and feed restriction during rearing also found that feed restriction caused significant decreases in tibia lengths. These workers ascribed the differences in bone dimensions to the larger body mass of the *ad libitum* fed group (4.2 kg) as opposed to the restricted group (1.9 kg). Feed restriction in their study only started after 4 weeks of age whereas in the present work it was already implemented at 2 weeks of age.



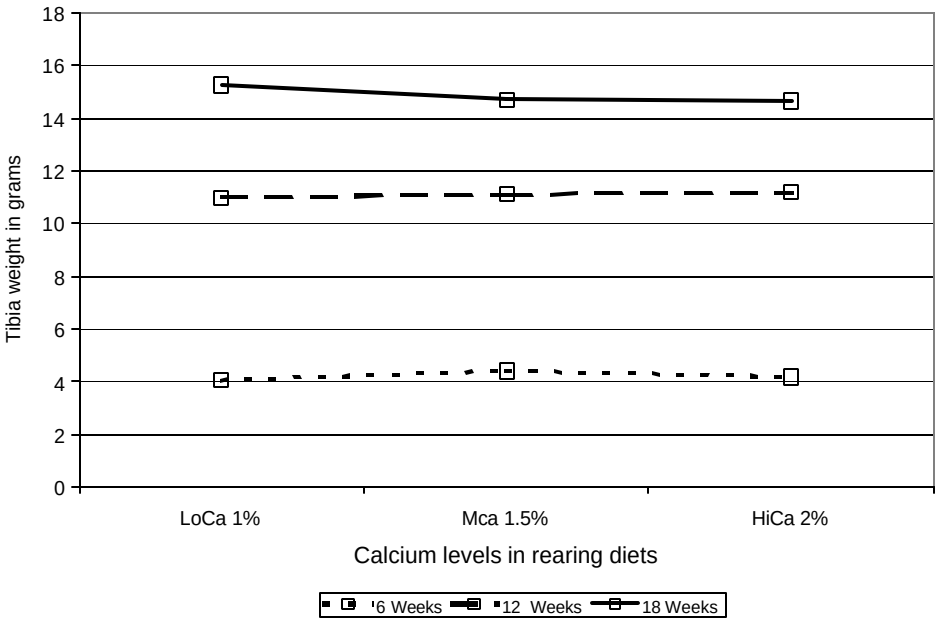


Figure 3.6 Tibia weight of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

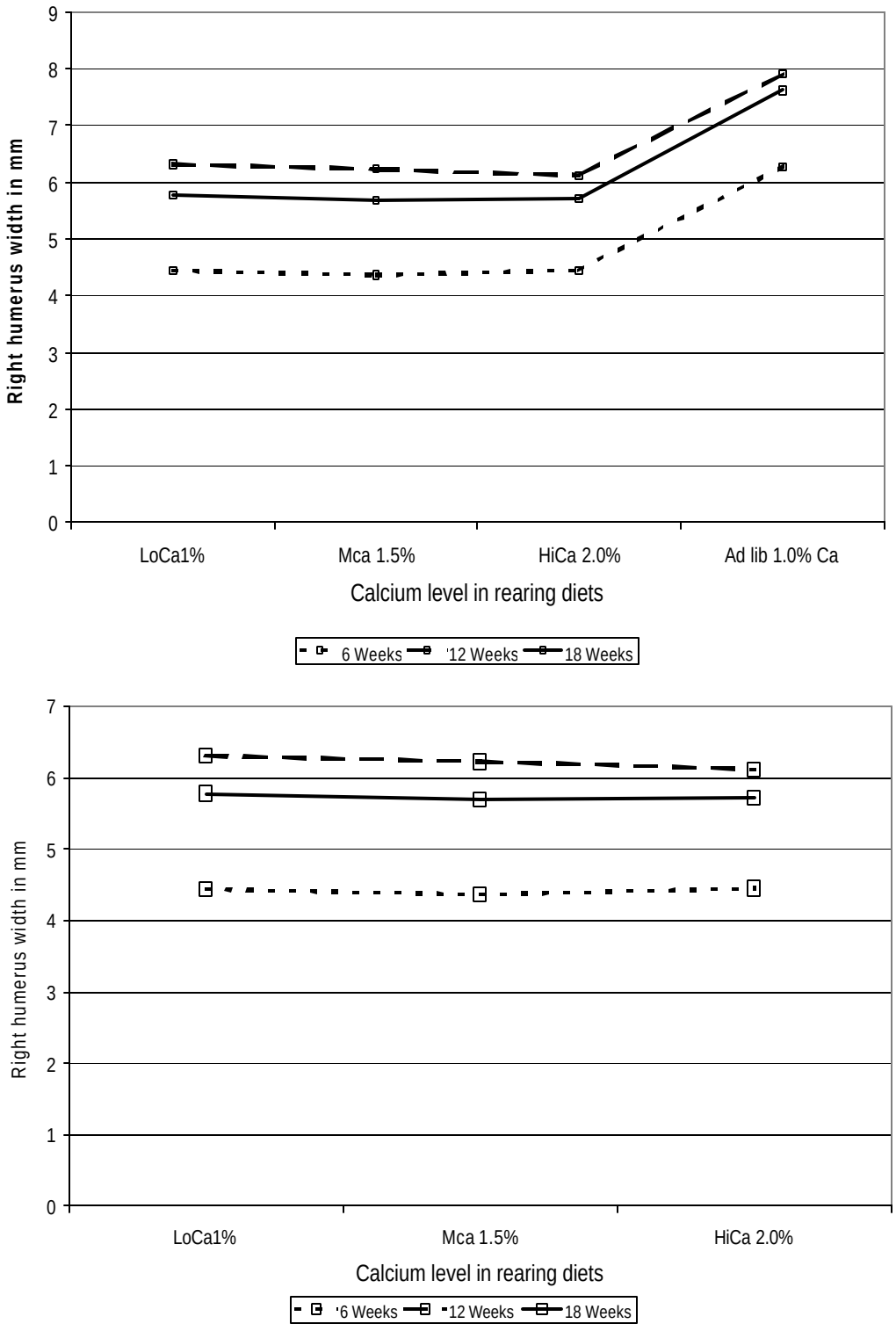


Figure 3.7 Humerus weight of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

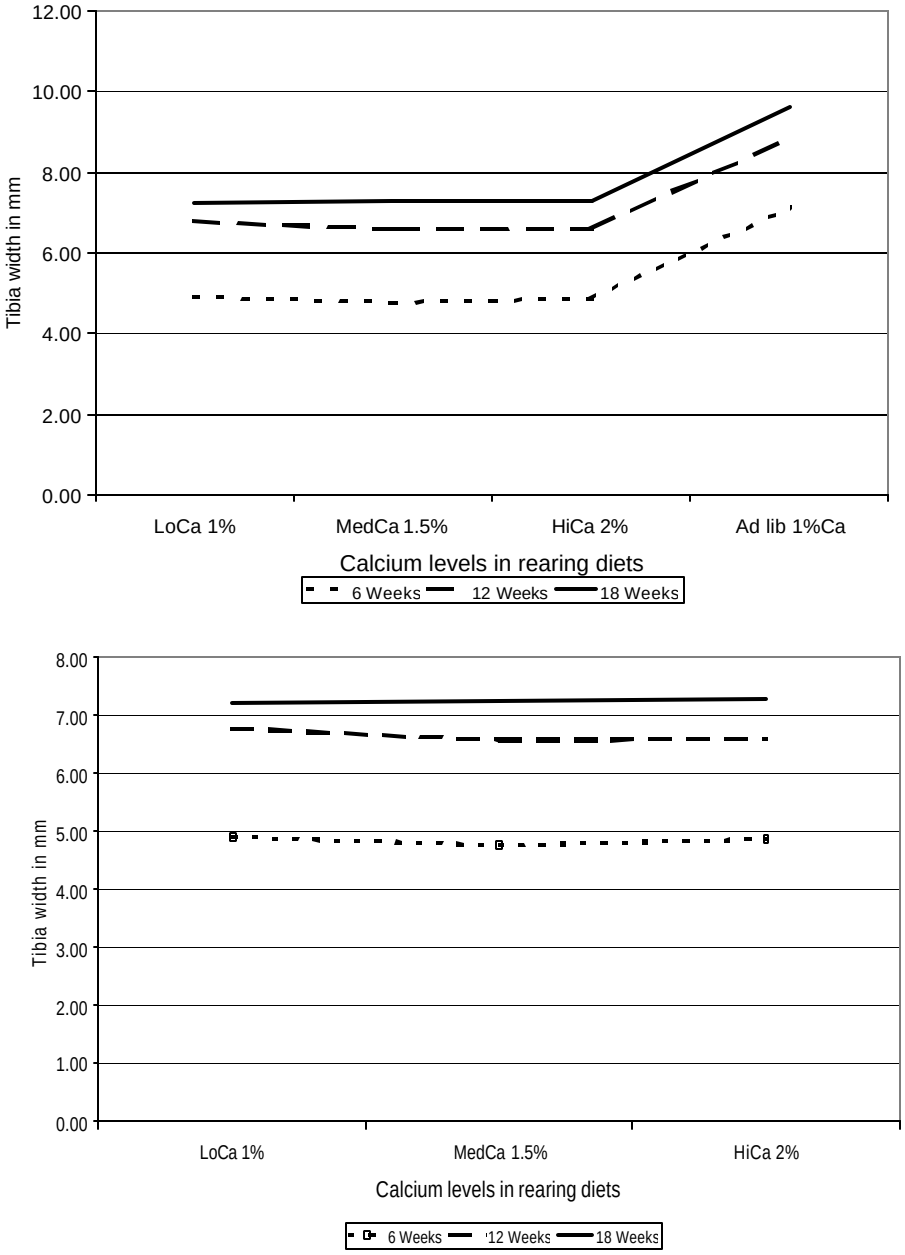


Figure 3.8 Tibia width of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

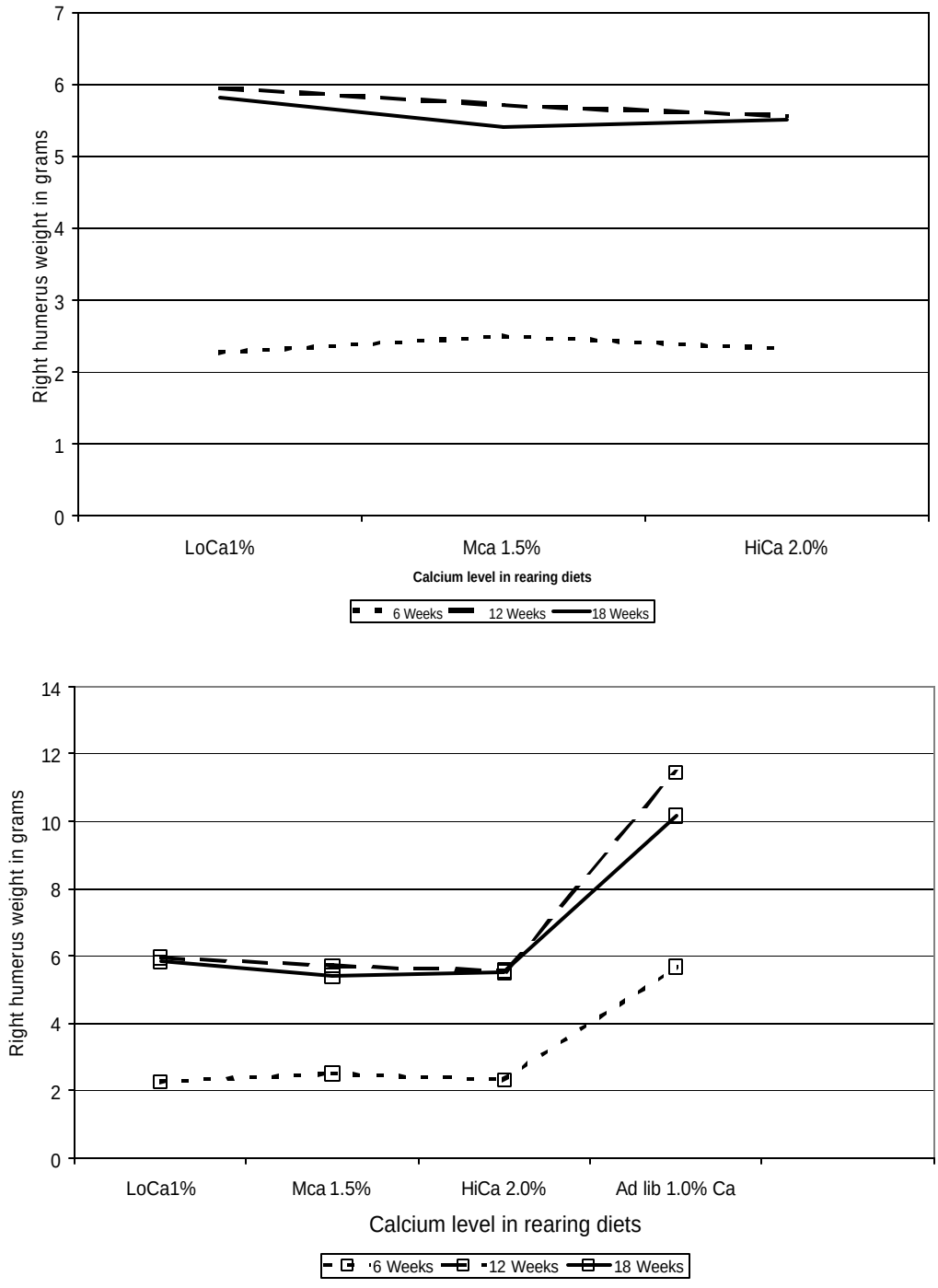


Figure 3.9 Humerus width of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

A significant (P<0.0001) Ca level x age interaction for bone weight did occur which indicated that the influence of dietary Ca on bone weight varied during different periods. Therefore, the effect of dietary Ca levels on bone weight was compared statistically within each age and the effect of age within Ca levels (Table 3.3). Dietary Ca levels did not significantly (P>.05) influence bone weight of the restricted birds (Table 3.4). This result is consistent with Williams *et al.* (2000*b*) who reported a tendency for bone weight to decrease with increasing dietary Ca concentration. Additionally, Summers *et al*. (1991) reported a significant reduction in weight as well as tibia length after feeding White Leghorn chicks diets containing 1.2% *vs.* 0.8% Ca for a 4 week period.

The *ad libitum* feeding of pullets resulted in a heavier bone weight at all ages in agreement with McCormack *et al.* (2001). It is also of interest that tibia weights of the restricted groups at 18 weeks in the study by Yu *et al.* (1992b) amounted to 85% of the *ad libitum* group. In the present study, tibia weight of the restricted birds was 88% of that of the *ad libitum* group at 18 weeks of age. The slight differences in the results on tibia weights in the present and previous study of Yu *et al.* (1992b) may be attributable to the differences in bird strain, age at which feed restriction commenced and housing system.

The weight of the right tibia increased significantly (P<0.0001) for each 6 weeks increment up to 18 weeks. On the other hand, the weight of the right humerus increased significantly (P<0.0001) up to 12 weeks and thereafter flattened off. This suggested differences in the developmental maturity of the tibia and humerus. This finding lends support to the view of Fisher (1998) and Ross Breeders (2001) who stated that skeletal size in broiler breeder pullets is fixed at 12 weeks.

3.3.3 Bone mechanical properties

Breaking strength (BS) data for humeri and tibiae are shown in Table 3.5 and Figure 3.10. A significant (P<0.0036) Ca level x age interaction for BS occurred. Although different Ca levels did not significantly (P>0.05) influence BS, birds fed 2.0% Ca diet tended to have greater BS than those fed 1.0% and 1.5% Ca diets. According to regression analyses (Table 3.6), however, tibia BS in the restricted groups responded significantly at 6 weeks, as well as at 12 weeks of age, to increasing intakes of Ca.

Table 3.5 Effect of calcium levels on bone mechanical properties of broiler breeder pullets during rearing

		Age				Significance of effect (P)			
	Treatment	**6**	**12**	**18**	**Means**	**Treatment**	**Age**	**Interaction**	**CV**
Right tibia									
Bone strength (N)	1% Ca	97.65 ± 22.23^{c}	213.90 ± 22.23^{b}	269.78 ± 22.23^{a}		0.0001	0.0001	0.0030	17.43
	1.5% Ca	93.30 ± 22.23^{b}	221.00 ± 22.23^{a}	249.28 ± 22.23^{a}					
	2% Ca	108.55 ± 22.23^{b}	253.50 ± 22.23^{a}	268.15 ± 22.23^{a}					
	1% Ca & *ad lib*	298.30±22.23^{c}	387.50±22.23^{b}	599.5± 22.23^{a}					
Bone stress (N/mm^{2})	1% Ca	23.52 ± 1.51	19.0 ± 1.51	23.09 ± 1.51	21.87^{b}	0.0001	0.0003	0.1951	14.80
	1.5% Ca	24.86 ± 1.51	20.25 ± 1.51	26.84 ± 1.51	23.98^{b}				
	2% Ca	21.16 ±1.51	19.23 ± 1.51	26.89 ± 1.51	22.43^{b}				
	1% Ca & *ad lib*	11.23 ± 1.51	12.89 ± 1.51	15.22 ± 1.51	13.11^{a}				
	Means	20.19^{ab}	17.84^{a}	23.01^{b}					
Right humerus									
Bone strength (N)	1% Ca	93.65 ± 29.38^{b}	236.00 ± 29.38^{a}	273.85 ± 29.38^{a}		0.0001	0.0001	0.0036	20.70
	1.5% Ca	90.20 ± 29.38^{b}	258.50 ± 29.38^{a}	262.63 ± 29.38^{a}					
	2% Ca	120.70 ± 29.38^{b}	276.50 ± 29.38^{a}	294.43 ± 29.38^{a}					
	1% Ca & *ad lib*	294.60 ± 29.38^{c}	501.00 ± 29.38^{b}	704.38 ± 29.38^{a}					

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred.

Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

Table 3.6 Multiple regression analysis of bone mechanical properties data on calcium intake of boiler breeder pullets during rearing

		Data from restricted groups only			***Ad libitum* group data included**		
Sampling age	**Variables**	**Equations**	**P value**	**Adj-R^2**	**Equations**	**P value**	**Adj-R^2**
6 weeks	Tibia breaking strength	RTib6 = 53.4 + 57.8 Caint6	0.029	0.0638	RTib6 = -98.70 + 261 Caint6	0.000	0.793
	Humerus breaking strength	RHum6 = 21.1 + 104 Caint6	0.021	0.0728	Rhum6 = -93.7 + 257 Caint6	0.000	0.740
	Bone stress	Stress6 = 27.2 – 6.6 Caint6	0.545	-0.0022	Stress6 = 30.5 – 10.9 Caint6	0.006	0.0532
12 weeks	Tibia breaking strength	Rtib12 = 165.0 + 64.7 Caint12	0.008	0.0986	RTib12 = 124 + 107 Caint12	0.000	0.608
	Humerus breaking strength	RHum12 = 201.0 + 55.8 Caint12	0.055	0.0458	Rhum12 = 102 + 160 Caint12	0.000	0.730
	Bone stress	Stress12 = 19.4 + 0.13 Caint12	0.960	0.0163	Stress12 = 23.2 – 3.92 Caint12	0.001	0.0073
18 weeks	Tibia breaking strength	Rtib18 = 252.0 + 4.4 Caint18	0.855	-0.0166	RTib18 = 4.3 + 213 Caint18	0.000	0.366
	Tibia breaking strength	Rhum18 = 251.0 + 21.2 Caint18	0.549	-0.0109	Rhum18 = -36.8 + 264 Caint18	0.000	0.415
	Bone stress	Stress18 = 20.5 + 3.79 Caint18	0.232	-.0222	Stress18 = 31.3 – 5.31 Caint18	0.061	0.0990

Rtib = right tibia; RH = right humerus; Caint = calcium intake

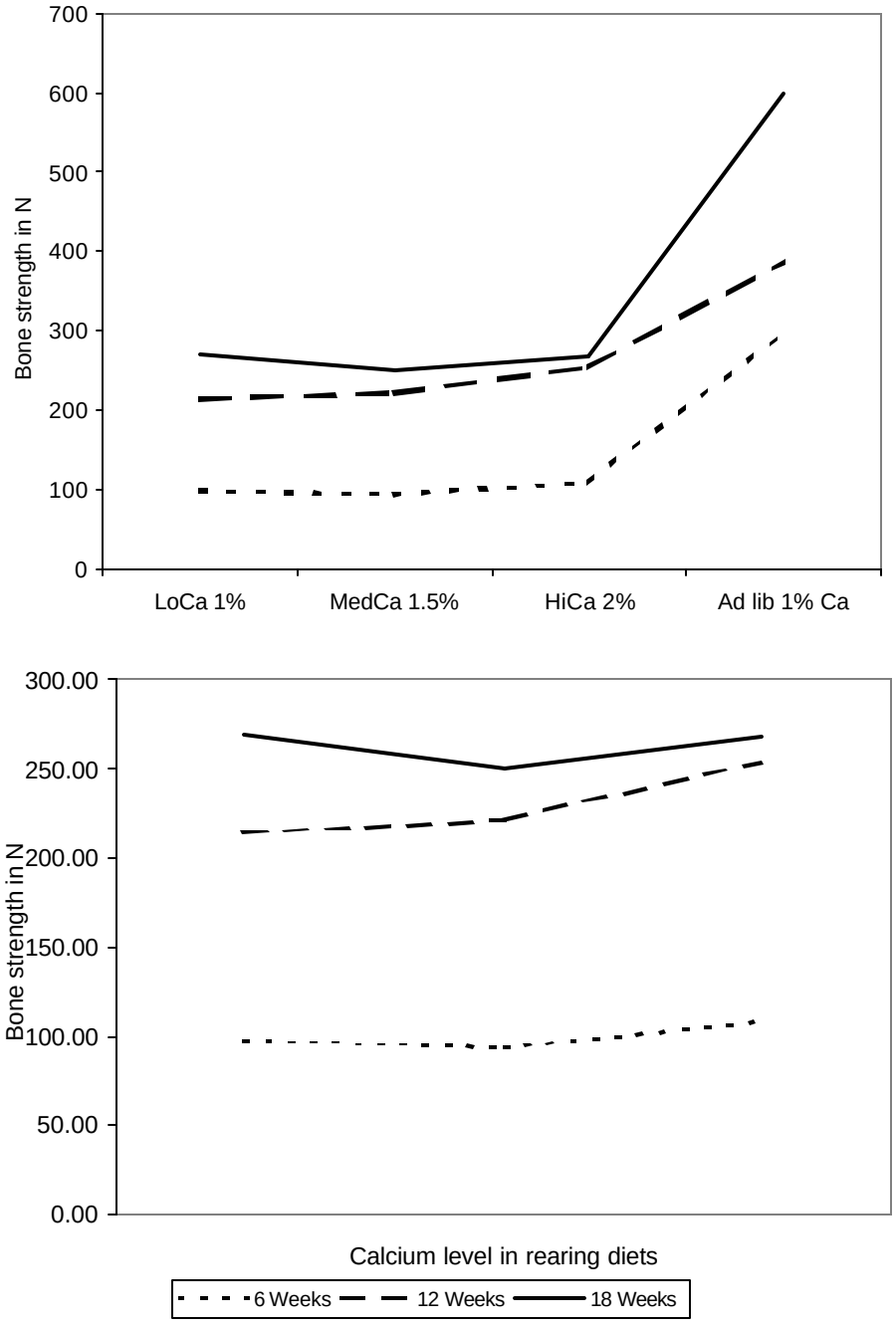


Figure 3.10 Combined breaking strength of humerus and tibia of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

The response in humerus BS was only significant for the data collected at 6 weeks of age (Table 3.6). These findings are different from what was found for other bone characteristics such as bone lengths, bone weights and bone widths for pullets on restricted feeding. For those characteristics no significant (P>.05) responses were found to increasing Ca intakes by birds on restricted feeding. Only when data of the *ad libitum* group was included in the data sets, significant responses in bone characteristics were noted (Table 3.5 and Figure 3.10).

The tibia BS significantly (P<.0001) increased with age. This finding confirms the results of several investigators (Gregory & Wilkins, 1992; McCoy *et al.*, 1996; Frost, 1997; Rath *et al.*, 1999, 2000).

From the results in Table 3.5, it seems that dietary Ca levels did not have a significant effect on tibia stress in the restricted groups. This is in agreement with McCormack *et al.* (2001) who found no significant influence of Ca levels on the bone stress of 6 weeks old Cobb broiler breeder pullets on restricted feeding. In contrast with these findings, Nimmo *et al.* (1980b) reported higher BS and stress required to break bones for bones from pigs fed higher Ca and P levels (1.3% Ca/ 1.0% P) than for those from pigs on low Ca-P diets (0.65% Ca/ 0.50% P). The differences in results are mainly attributable to differences in species and Ca levels.

As already discussed, stress required to break bones from *ad libitum* birds was significantly (P<0.0001) lower than that required to break bones from restricted group (Table 3.5 and Figure 3.11). Crenshaw *et al.* (1981a) state that as bone mineralisation increases, maximum stress of the bone increases. According to these results, the degree of bone mineralisation was greater for restricted groups than it was for the *ad libitum* group. Such finding is to be expected, as rapid growth does not allow enough time for the production of strong tissue, remodelling, and alignment of bone resulting in less mineralisation. These results suggest that less mineralisation had occurred in birds on *ad libitum* feeding, an observation that agrees with that of Nimmo *et al.* (1980b).

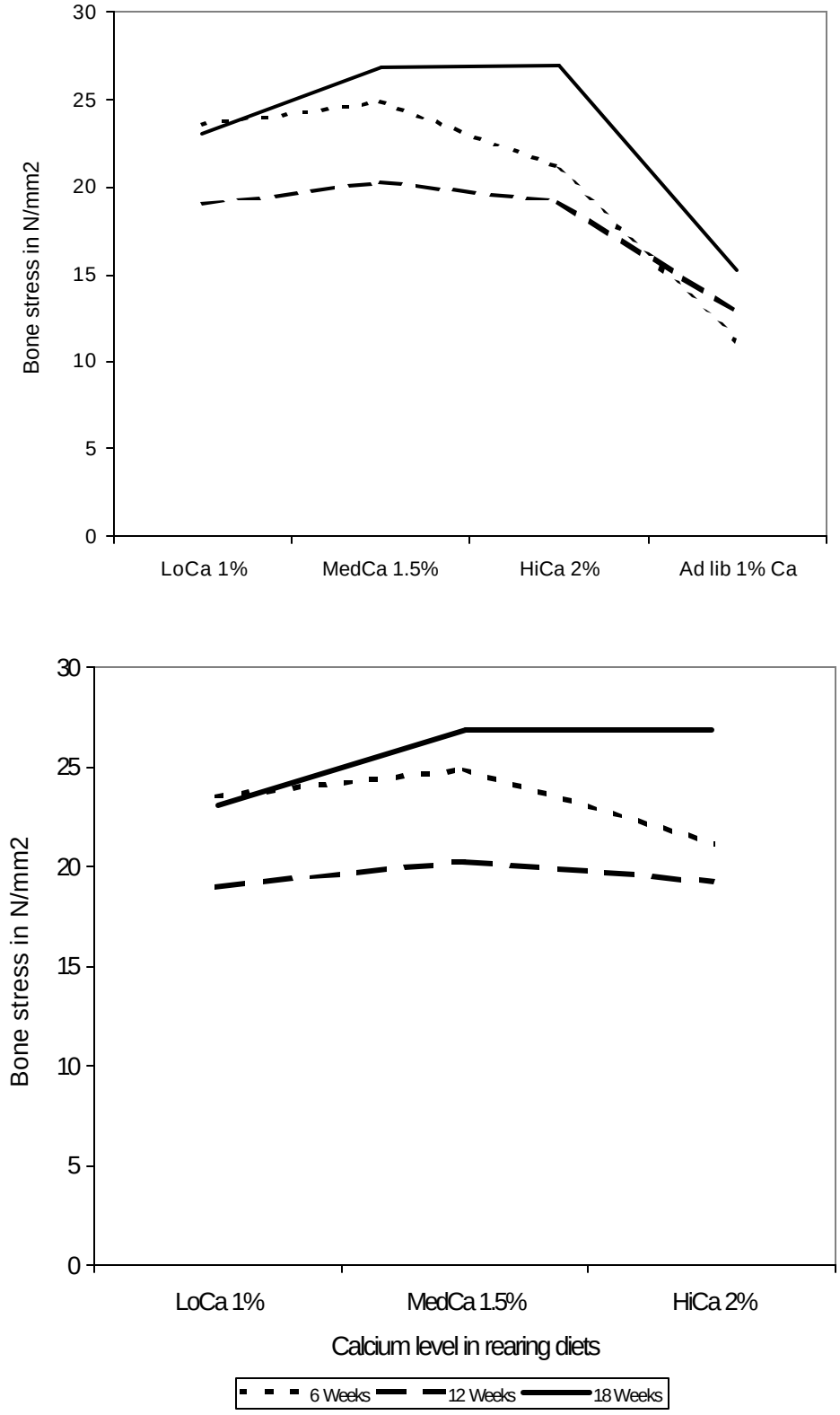


Figure 3.11 Bone stress of tibia of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

3.3.4 Bone chemical composition

It is evident from Table 3.7 and Figure 3.12 that different dietary Ca levels resulted in no significant differences in the ash percentage, Ca and P content of the restricted groups. The figures for the Ca and P contents of bone ash are not shown as these variables are influenced by dietary ash percentage. The result on bone Ca is consistent with Hocking *et al.* (2002) and Smith *et al.* (2003) who reported no benefits of feeding growing turkeys and broilers diets containing Ca levels ranging from 0.6 to 1.5%. The results of the current study are, however, inconsistent with those of Nimmo *et al.* (1980a), Crenshaw *et al.* (1981b) and Atteh & Leeson (1983) in pigs and poultry. Nimmo *et al.* (1980b) reported higher percentage ash values after feeding pigs diets containing 1.3% Ca and 1.0% P compared with 0.65% Ca and .50% P. Although 2.0% Ca level in the current study did not significantly influence Ca content of bone of restricted birds, this level showed a slightly lower Ca value. A tendency for bone ash to decline with increased Ca intake of the pullets was observed at 18 weeks of age for the restricted groups (Figure 3.12). Shafey & McDonald (1991) also reported a decline in tibia Ca and increased plasma Ca after feeding broiler chicks diets containing 2.53% *vs.* 1.07% Ca. It seems from the results of the current study that too high levels of Ca (2.0% and more) could influence the Ca content of the bone detrimentally. The mechanism of suppression of bone calcification by high dietary levels of Ca remains unclear. The feeding of high levels of Ca during the rearing period of broiler pullets could probably result in the body's mechanism for Ca mobilisation to malfunction, as well as, gear the body for high levels of excretion.

According to Table 3.7, *ad libitum* feeding did not have a significant ($P>.05$) effect on bone ash percentage, Ca and P contents.

There was a significant ($P<.0001$) increase in bone ash and Ca with age (Table 3.7). Bone P, however, declined ($P<.0007$) with age. The finding on bone ash in the current study is in agreement with Rath *et al.* (1999) who reported an increase in tibia bone ash of female broiler breeder chickens aged 7 and 72 weeks. These authors noted, however, a decrease in tibia bone ash of males with age. Hocking *et al.* (2002) reported a decline in Ca, P and bone ash due to age. Only the result on the P reported in the present study confirms the work of Hocking *et al.* (2002) who reported a decline in P in growing turkeys up to 13 weeks of age. The reason for the decline of P

Table 3.7 Bone chemical composition of broiler breeder pullets reared on different calcium levels in feed

		Age (weeks)				Significance of effect (P)			
	Treatment	**6**	**12**	**18**	**Means**	**Treatment**	**Age**	**Interaction**	**CV**
Left tibia									
Ash content (%)	1.0% Ca	34.43 ± 1.70	29.31 ± 1.70	58.40 ± 1.70	40.71± 0.91[a]	0.8606	0.0001	0.4952	8.32
	1.5% Ca	33.91 ± 1.70	29.53 ± 1.70	60.93 ± 1.70	41.46 ± 0.91[a]				
	2.0% Ca	33.57 ± 1.70	32.24 ± 1.70	56.37 ± 1.70	40.73 ± 0.91[a]				
	1.0% Ca & *ad lib*	33.00 ± 1.70	29.07 ± 1.70	58.76 ± 1.70	40.27 ± 0.91[a]				
		33.73 ± 0.85[a]	30.04 ± 0.85[b]	58.62 ± 0.84[c]					
Calcium, %	1.0% Ca	26.77 ± 3.62	29.19 ± 3.62	35.28 ± 3.62	30.41 ± 0.79[a]	0.8228	0.05	0.9199	24.62
	1.5% Ca	27.57 ± 3.62	30.74 ± 3.62	32.82 ± 3.62	30.37 ± 0.79[a]				
	2.0% Ca	26.70 ± 3.62	29.00 ± 3.62	29.21 ± 3.62	28.30 ± 0.79[a]				
	1.0% Ca & *ad lib*	24.13 ± 3.62	26.75 ± 3.62	34.64 ± 3.62	28.51 ± 0.79[a]				
		26.29 ± 1.81[a]	28.92 ± 1.81[a]	33.00 ± 1.81[b]					
Phosphorus, %	1.0% Ca	17.71 ± 0.87	16.63 ± 0.87	15.95 ± 0.87	16.76 ± 47[a]	0.3785	0.0007	0.7347	10.64
	1.5% Ca	18.26 ± 0.87	17.03 ± 0.87	14.86 ± 0.87	16.72 ± 47[a]				
	2.0% Ca	17.05 ± 0.87	17.67 ± 0.87	14.24 ± 0.87	16.32 ± 47[a]				
	1.0% Ca & *ad lib*	16.95 ± 0.87	15.68 ± 0.87	14.28 ± 0.87	15.64 ± 47[a]				
	Means	17.49 ± 0.43[a]	16.75 ± 0.43[a]	14.83 ± 0.43[b]					
TCA[1] (mm^2)	1.0% Ca	6.08 ± 0.71[b]	12.25 ± 0.71[a]	13.35 ± 0.71[a]		0.0001	0.0001	0.0458	10.66
	1.5% Ca	6.44 ± 0.71[b]	12.65 ± 0.71[a]	13.15 ± 0.71[a]					
	2.0% Ca	6.72 ± 0.71[b]	13.52 ± 0.71[a]	13.40 ± 0.71[a]					
	1.0% Ca & *ad lib*	17.70 ± 0.71[b]	20.25 ± 0.71[b]	24.60 ± 0.71[a]					
Percent bone	1.0% Ca	83.52 ± 0.81[b]	89.86 ± 0.81[a]	92.46 ± 0.81[a]		0.0017	0.0001	0.0047	1.85
	1.5% Ca	83.13 ± 0.81[b]	90.46 ± 0.81[a]	91.10 ± 0.81[a]					
	2.0% Ca	82.50 ± 0.81[b]	91.43 ± 0.81[a]	91.93 ± 0.81[a]					
	1.0% Ca & *ad lib*	77.08 ± 0.81[b]	90.32 ± 0.81[a]	90.79 ± 0.81[a]					

[1]TCA – true cortical area (cortical area multiplied by mean % bone divided by 100)
Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred.
Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

Table 3.8 Multiple regression analysis of bone chemical composition on calcium intake of pullets during rearing

		Data from restricted groups only			**Data of *ad lib* group included**		
Age	**Variable**	**Equations**	**P value**	**Adj -R^2**	**Equation**	**P value**	**Adj -R^2**
6 weeks	Ash, %	%Ash6 = 26.20 + 10.1 Cain6	0.090	0.032	%Ash6 = 35.4 – 2.2 Caint6	0.312	0.000
	Percent bone	%bone6 = 86.6 – 3.25 Cain6	0.204	0.000	%bone = 89.1 – 7.95 Caint6	0.000	0.780
	True cortical area, mm^2	TCA6 = 5.13 + 1.57 Cain6	0.360	0.000	TCA6 = -4.53 + 14.5 Caint6	0.000	0.873
12 weeks	Ash, %	%Ash12 = 27.7 + 1.77 Cain12	0.228	0.008	%Ash12 = 29.5 – 0.117 Caint12	0.823	0.000
	Percent bone	%bone12 = 88.1+ 2.48 Cain12	0.609	0.081	%bone = 90.3 + 0.133 Caint12	0.854	0.000
	True cortical area, mm^2	TCA12 = 10.7 + 2.00 Cain12	0.052	0.140	TCA12 = 8.03 + 4.90 Caint12	0.000	0.854
18 weeks	Ash, %	%Ash18 = 62.4 - 1.83 Cain18	0.326	0.000	%Ash18 = 66.3 – 5.14 Caint18	0.000	0.000
	Percent bone, %	%bone18 = 92.5- 0.45 Cain18	0.305	0.000	%bone318 = 92.9 – 0.837 Caint18	0.103	0.120
	True cortical area, mm^2	TCA18 = 13.3 + 0.013 Cain 18	0.988	0.000	TCA18 = 4.96 + 7.02 Caint18	0.001	0.516

TCA = true cortical area

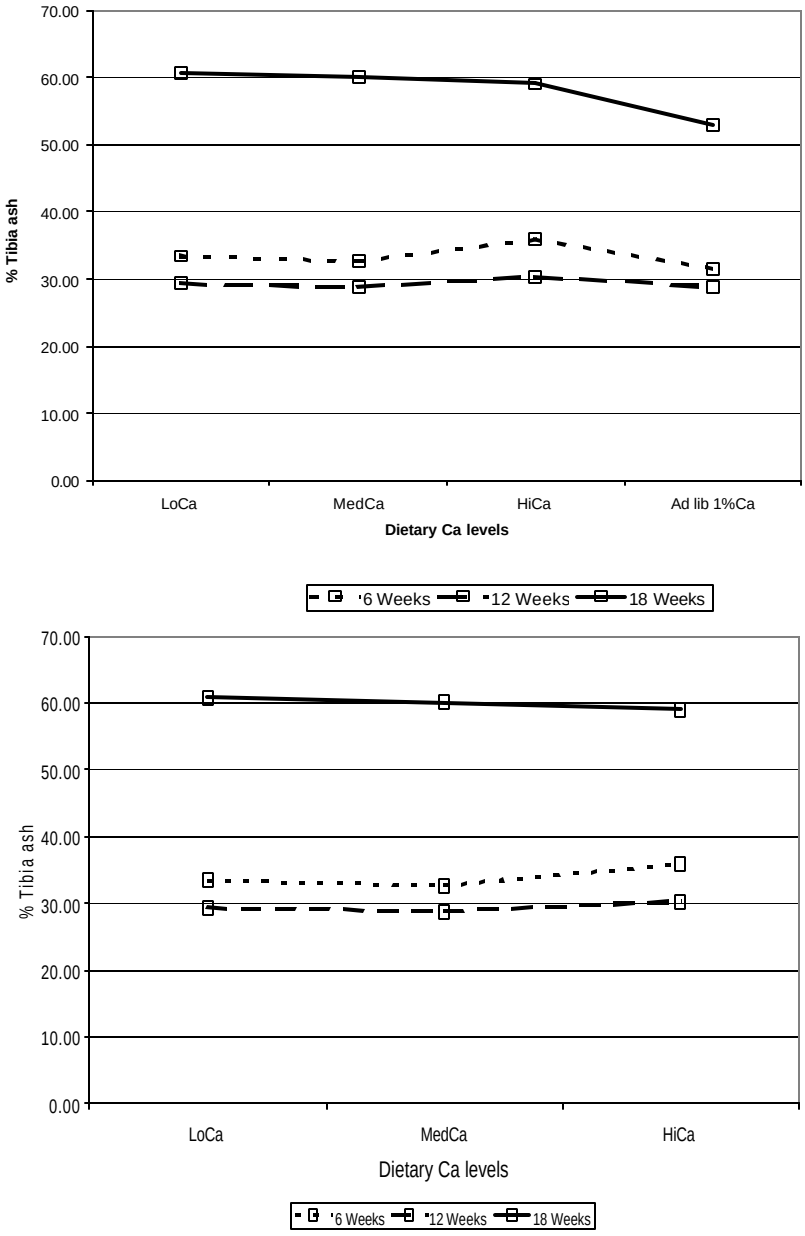


Figure 3.12 Percent tibia ash at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

with age is unclear. The differences in results with regards to bone ash and Ca may be attributable to length of experiment and differences in animal species but not Ca levels, as levels of Ca used in the present study and that of Hocking *et al.* (2002) were similar. The decline in bone ash values from 33.7 to 30% between 6 and 12 weeks of age could be associated with the birds increased demand for nutrients, notably Ca due to rapid growth rates. Accordingly, skeletal size is fixed at 12 weeks of age (Fisher,

1998). This pattern in bone development probably explains the high bone ash content at 18 weeks of age (Table 3.7 and Figure 3.12).

Bone ash values obtained at 6 weeks age for the restricted groups in this study are consistent with the results of McCormack *et al.* (2001) who reported ash value of 33.63% after feeding Cobb broiler breeder pullets diets containing 0.9 g Ca and 5.8 g total P during a 6-week period. In the current study the average bone ash value for the birds on restricted feeding was 33.97% at 6 weeks. However, McCormack *et al.* (2001)) found a higher ash value (40.65%) for *ad libitum* group compared to 33.0% reported in the current study. The differences in the results relating to bone ash values for the *ad libitum* group could be attributable to differences in experimental conditions (*e.g.*, open-sided *vs.* climate controlled housing systems) and strain of birds (Ross *vs*. Cobb).

According to Ruff & Hughes (1985), bone ash content is correlated with its BS. This does not appear to be supported by the results of the current study. In the present study, Ca and P contents did not increase significantly with increasing Ca level (Tables 3.7 and 3.8) while BS did according to regression analysis (Table 3.6).

The finding on bone ash in the current study is in agreement with Rath *et al*. (1999) who reported an increase in tibia bone ash of female broiler breeder chickens aged 7 and 72 weeks. These workers noted, however, a decrease in tibia bone ash of males with age.

3.3.5 True cortical area and percent bone

A significant (P<.05) Ca level x age interaction for true cortical area (TCA) and percent bone occurred, indicating that not all bones responded similarly at each age period or Ca level. The means for TCA and percent bone are presented in Table 3.7. The average TCA values for restricted birds fed 1.0, 1.5 and 2.0% Ca diets were 6.08, 6.44 and 6.72 mm^2 at 6 weeks, 12.25, 12.65 and 13.52 mm^2 at 12 weeks and 13.35, 13.15 and 13.40 mm^2 at 18 weeks of age. As for percent bone, the average values for birds fed 1.0, 1.5 and 2.0% Ca diets were 83.52, 83.13 and 82.5% at 6 weeks, 89.86, 90.46 and 91.43% at 12 weeks and 92.46, 91.1 and 91.93 at 18 weeks of age. A regression analysis of these parameters at all ages (Table 3.8) showed a significant

(P<.001) response to Ca intake when data of the *ad lib* group was included in the calculations. However, dietary Ca did not have significant (P>.05) influence on TCA and percent bone of the restricted birds (Table 3.8).

Figure 3.13 illustrates tendencies for percent bone of restricted birds to decline with increasing dietary Ca level at 6 weeks and to increase at 12 and 18 weeks of age. Percent bone of restricted birds was not significantly (P>.05) different at 12 and 18 weeks, perhaps indicating that the bone is fully developed at 12 weeks (Table 3.7), thus confirming the theory that bone in broiler breeder pullets is fixed at 12 weeks of age as suggested by Fisher (1998) and Ross Breeders (2001). The TCA of restricted birds was also similar at 12 and 18 weeks as illustrated in Figure 3.14.

Ad libitum birds had significantly (P<.05) higher TCA values than restricted birds (Table 3.7 and Figure 3.14).

At 6 weeks, percent bone for *ad libitum* group was lower than that of restricted treatments but appeared to be similar to that of restricted birds at 12 and 18 weeks of age as shown in Figure 3.13. The lower percent bone of the *ad libitum* birds may be attributable to rapid growth rate due to higher feed consumption rates. This probably indicates that the rate of mineralisation could not keep pace with rapid growth rate of the *ad libitum* group. It can be observed from Table 3.7 that though bone ash was not significantly (P>.05) different between restricted and *ad libitum* groups during rearing, *ad libitum* group compared to restricted groups tended to have lower bone ash values at 6 weeks of age, indicating less mineralisation. The *ad libitum* group had higher TCA vales compared to restricted treatments and this could be ascribed to higher bone dimension values (i.e, length, width and weight) due to faster growth rate.

The TCA and percent bone were significantly (P<.0001) influenced by age (Table 3.7). The two parameters increased significantly from 6 to 12 weeks and then significantly flattened off. Between 6 and 12 weeks TCA and percent bone increased by 59.3% and 9.9%, respectively. Smaller increases of 9.7% (TCA) and 1.2% (percent bone) were observed between 12 and 18 weeks of age.

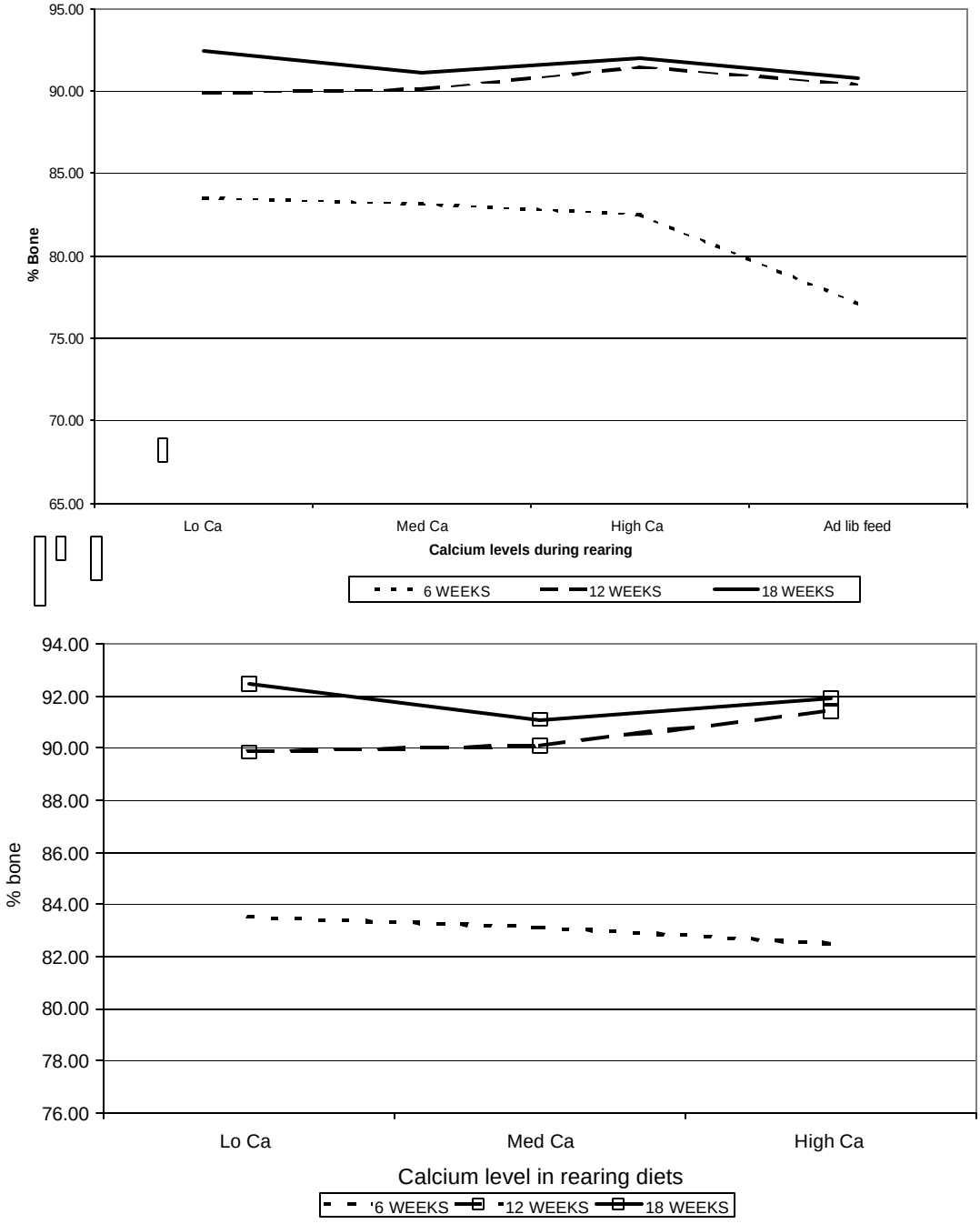
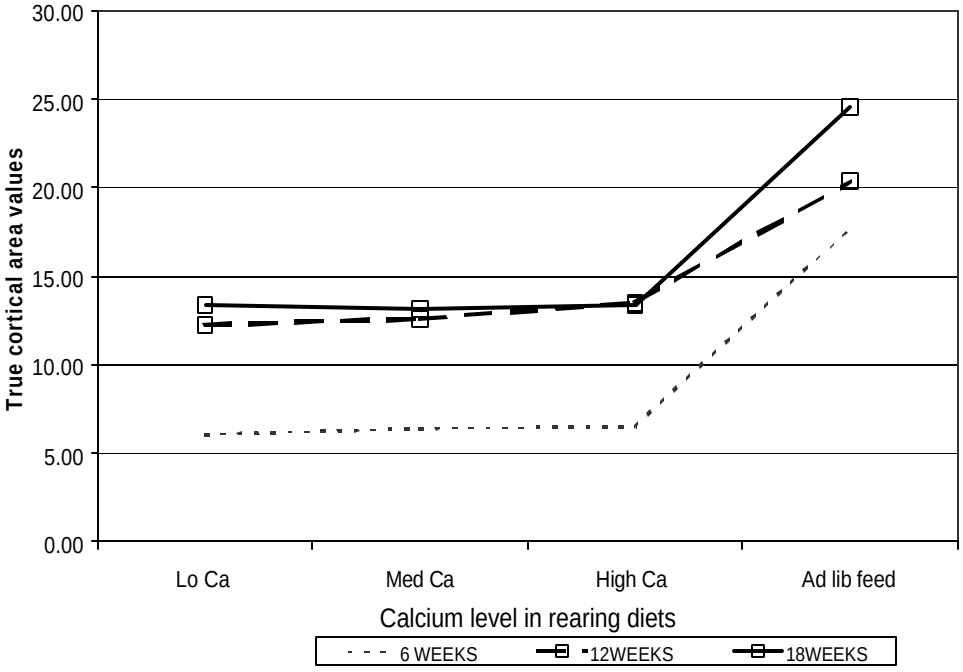


Figure 3.13 Percent bone of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded)



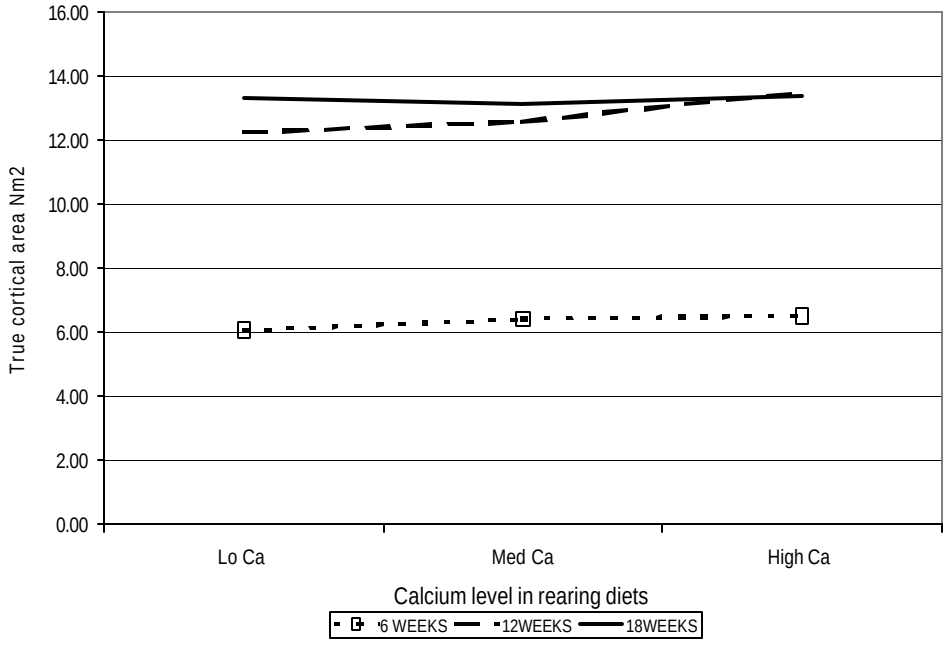


Figure 3.14 True cortical area of pullets at different ages and reared on different dietary Ca levels (*ad libitum* included and excluded).

3.4 Conclusions

The results of the present study showed that dietary Ca level had no significant effect on bone formation in broiler breeder pullets on restricted feeding up to 18 weeks of age, except bone breaking strength. The results found for breaking strength were, however, not supported by stress required to break bones, percent bone ash, Ca and P contents as well as percent bone. Thus, it seems that dietary Ca levels higher than 1.0% will not influence bone development advantageously in feed restricted breeder pullets during the rearing period. Therefore, it is concluded that increasing the levels of dietary Ca alone cannot optimise bone formation in restricted broiler breeders during rearing. The levels of other nutrients like protein in the diet should probably be increased as well.

Ad libitum feeding of broiler breeder pullets in the present study resulted in a significant increase in bone dimensions and breaking strength. Bone variables studied such as ash content (percentage), percent bone, true cortical area, Ca and P contents were, however, not significantly influenced by *ad libitum* feeding of broiler breeder pullets. In fact stress required to break bones from *ad libitum* birds was significantly lower than that of restricted groups. This means that *ad libitum* feeding resulted in less bone mineralisation. The detrimental effect of a rapid growth rate and resulting overfatness on reproductive performance and health should also be borne in mind.

According to bone length and breaking strength values, bone development of restricted broiler breeder pullets is fixed at 12 weeks of age.

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CHAPTER 4

INFLUENCE OF DIETARY CALCIUM LEVELS ON BONE CHARACTERISTICS OF BROILER BREEDER HENS

4.1 Introduction

There are several different types of bones in laying hens. The main types that provide structural integrity are cortical and cancellous (or trabecular) bones, both of which are forms of lamellar bone. These bones are formed during growth, but when a hen reaches sexual maturity, a third type of nonstructural bone, medullary bone, is formed (Whitehead & Fleming, 2000). Medullary bone persists throughout the laying period and its formation is concomitant with maturation of the ovarian follicles (Dacke *et al.*, 1999). Young pullets deposit medullary bone in the marrow cavity of long bones and it builds up during the laying period (Whitehead *et al.*, 1998). It is argued (Whitehead & Fleming, 2000) that the conventional view that medullary bone contributes little to overall bone strength (BS) may not be totally correct. Fleming *et al.* (1998a) demonstrated that the presence of large amounts of medullary bone in the humerus of hens during the laying period improves BS. Medullary bone supplies calcium (Ca) for eggshell formation at periods when dietary supply is not sufficient (Hodges, 1974; Klasing, 1998). The femur and tibiotarsus are the two bones that are rich in medullary bone.

According to Parkinson & Cransberg (1999), the peak Ca reserves occur at 30 weeks of age, while the most skeletal Ca depletion occurs in mid-lay (40-45 weeks of age). Once egg production has commenced a pattern of rapid build-up and break down of medullary bone is established in relation to the shell formation cycle.

Modern laying hens have a high susceptibility to bone fracture. The high incidence of fractures in live birds, which can occur both during the egg production period and in the course of depopulation and subsequent transport and handling, represents a severe welfare problem (Fleming *et al.*, 1994). Williams *et al.* (2000a) state that although the growth performance of the modern broiler has changed considerably over recent years, their diets have changed little. It has been postulated that probably the porosity observed arises from the occurrence of more rapid bone modelling and remodelling in modern birds, together with an inadequate dietary supply of Ca and phosphorus (P). Therefore, Ca supply during the rearing and laying periods is of utmost importance to

ensure adequate bone development and Ca reserves for eggshell formation and BS. The objective of this experiment was to determine the effects of three different levels of Ca during the rearing period on the bone characteristics of broiler breeder hens during the laying period.

4.2 Materials and Methods

One hundred and ninety eight Ross broiler breeder pullets were reared up to 22 weeks according to body mass guidelines on diets containing 1.0, 1.5 and 2.0% Ca (Chapter 3). The pullets in each experimental diet were randomly divided into 3 treatment groups with 1.5, 2.5 and 3.5% dietary Ca (66 birds per treatment) fed from 23 to 60 weeks of age. A constant Ca:P ratio was maintained in all the diets. The pullets were placed in individual cages, which were equipped with individual feed troughs, water nipples and perches. All data were collected on an individual bird basis and each bird was considered as an experimental unit.

Birds were first photostimulated at 154 days (22 weeks) in accordance with Ross Breeders' recommendation (Ross Breeders, 2001). The photoperiod was extended with artificial light by 2 to 3 hours at 22 weeks and thereafter by 1 hour per week from 24 to 26 weeks of age when the birds received 16 hours of light. This was held constant until birds were depopulated at 60 weeks of age.

The composition of experimental diets is given in Tables 4.1 and 4.2. Pullets were fed pre-breeder diets containing 1.0, 1.5 and 2.0% Ca from 19 to 22 weeks of age. Three types of breeder diets containing 1.5, 2.5 and 3.5% Ca were fed from 23 to 60 weeks of age (laying period) and these include: breeder phase 1 (23 to 34 weeks), breeder phase 2 (35 to 46 weeks) and breeder phase 3 (47 to 60 weeks). A diet with 2.5% Ca was obtained by mixing the 1.5% and 3.5% Ca diets. Each dietary treatment of the layer phase was fed to 66 replicates (22 birds per subgroup). Experimental diets were isocaloric and isonitrogenous. Feed was provided in restricted amounts in accordance with the breeders' recommendations, while water was provided *ad libitum*. Feed intake by individual birds was recorded on a weekly basis and body weight (BW) was determined by weighing each bird at 3-weekly intervals. Mortality was recorded during the course of the experiment.

Table 4.1 Physical composition of laying diets on air dry basis (%)

	Pre-breeder diet		**Breeder Phase 1**		**Breeder Phase 2**		**Breeder Phase 3**	
	1.5% Ca	**3.5% Ca**	**1.5% Ca**	**3.5% Ca**	**1.5% Ca**	**3.5% Ca**	**1.5% Ca**	**3.5% Ca**
Maize	63.54	63.51	61.92	59.66	63.11	60.81	56.43	62.23
Pollard Glutten	-	-	4.45	2.3	1.8	1.0	-	-
Wheat bran	12.65	6.65	5.15	-	6.55	-	14.90	1.00
Full fat soya	-	-	-	10.0	-	9.95	-	1.70
Soybean oil cake	7.75	11.4	8.6	10.3	8.4	7.55	8.75	9.50
Sunflower oil cake	12.45	11.1	15.0	7.75	15.0	10.00	15.00	15.0
Calcium carbonate (grit)	-	-	2.0	6.15	2.3	6.75	2.25	6.60
Calcium carbonate (fine)	1.15	2.2	0.5	1.5	0.6	1.65	0.6	1.65
Mono calcium phosphate	1.49	4.25	1.29	1.36	1.40	1.50	1.28	1.53
Salt	0.24	0.26	0.41	0.40	0.43	0.44	0.44	0.44
Bicarbonate	0.20	0.15	-	-	-	-	-	-
Choline liquid	0.04	0.04	0.03	0.03	-	0.03	-	-
Lysine	0.10	0.04	0.15	-	0.10		0.03	0.03
Methionine	0.05	0.05	0.005	0.06	0.01	0.05	0.01	0.02
Trace mineral/vitamin premix	0.35	0.35	0.50	0.50	0.30	0.30	0.30	0.30

Table 4.2 Nutrient composition of laying diets on air dry basis (%)

	Pre-breeder diet		**Breeder phase 1**		**Breeder phase 2**		**Breeder phase 3**	
	1.0% Ca	2.0% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca
Moisture	11.07	10.37	10.58	9.96	9.77	9.10	9.85	9.19
Metabolisable Energy (MJ/kg)	11.96	11.70	12.09	12.00.	11.94	11.87	11.46	11.43
Protein	15.22	15.50	18.33	17.72	17.03	16.77	16.68	16.06
Crude fat	3.30	3.06	3.00	4.20	2.97	4.07	3.09	2.98
Crude fibre	7.01	5.99	0.00	0.00	6.65	5.08	8.28	6.64
Ash			6.21	11.23	6.74	12.05	6.90	11.98
Calcium	1.00	2.01	1.51	3.50	1.52	3.50	1.59	3.46
Phosphorus	0.84	1.37	0.78	0.71	0.80	0.74	0.84	0.78
Available phosphorus	0.45	0.90	0.41	0.40	0.43	0.43	0.43	0.54
Arginine	0.98	1.01	1.11	1.12	1.08	1.09	1.10	1.07
Isoleucine	0.60	0.64	0.74	0.76	0.69	0.71	0.67	0.67
Lysine			0.81	0.83	0.76	0.78	0.73	0.72
Methionine	0.35	0.34	0.38	0.38	0.35	0.36	0.33	0.33
T SAA[1]	0.06	0.64	0.73	0.70	0.68	0.67	0.66	0.64
Threonine	0.55	0.57	0.66	0.66	0.62	0.63	0.61	0.60
Tryptophan	0.17	0.18	0.19	0.20	0.18	0.19	0.19	0.18
TA[2] Arginine	0.91	0.93	1.04	1.04	0.99	1.01	1.01	0.99
TA[2] Isoleucine	0.54	0.57	0.67	0.69	0.62	0.65	0.59	0.60
TA[2] Lysine	0.60	0.60	0.70	0.71	0.64	0.67	0.61	0.61
TA[2] Methionine	0.31	0.31	0.34	0.35	0.31	0.33	0.29	0.30
TA[2] TSAA	0.57	0.57	0.64	0.63	0.59	0.60	0.57	0.56
TA[2] Threonine	0.48	0.50	0.59	0.59	0.55	0.56	0.26	0.53
TA[2] Tryptophan	0.15	0.16	0.17	0.18	0.17	0.17	0.17	0.17
Linoleic acid	1.83	1.68	1.65	2.32	1.65	2.26	1.71	1.64
Xanthophylls			23.51	17.68	17.12	14.66	11.29	12.45
Salt	0.24	0.27	0.42	0.41	0.44	0.44	0.45	0.45
Choline	1300.01	1309.56	1205.18	1204.08	1008.79	1003.18	1087.10	993.06
Sodium	0.16	0.16	0.18	0.18	0.19	0.20	0.20	0.20
Chlorine	0.22	0.57	0.33	0.29	0.33	0.31	0.32	0.32
Potassium	0.60	0.60	0.60	0.63	0.63	0.63	0.71	0.61
Magnesium			0.22	0.20	0.23	0.21	0.25	0.23
Manganese			46.82	63.94	50.82	68.71	61.84	71.60

[1]Total sulphur amino acids
[2]Chemically determined

At 35 and 60 weeks of age, 12 birds were randomly selected from each of the 9 treatments and killed by cervical dislocation. The carcasses were stored overnight in a refrigerator at 5 ^{o}C until the following day when the tibiae (left and right) and right humeri from each of the birds were excised and defleshed without boiling. The right tibiae and right humeri were then weighed and total length and bone shaft widths measured by means of a calliper with an accuracy of 0.001 cm (Zhang & Coon, 1997). The tibiae (both left and right) and right humeri were individually sealed in plastic bags to minimise moisture loss, and stored in a freezer at -18 ^{0}C for later analysis (Nimmo *et al.*, 1980; Zhang & Coon, 1997). The bones were then removed from refrigerator for bone ash and BS determinations. The right tibiae and right humeri were used for BS determination, whereas left tibiae were used for bone ash determination and histomorphometric analysis. Breaking strength (N) was determined according to procedures described by Fleming *et al.* (1998b). Bone stress (N/mm^2), which is force per unit area of bone, was calculated by dividing BS with true cortical area (mm^2). True cortical area (TCA) and percent bone were calculated as in Chapter 3. The histological processing of left tibiae was done according to the procedures of Fleming *at al.* (1994) and Williams *et al.* (2000a, 2000b), which have been described in Chapter 3 (Section 3.2).

4.2.1 Statistical analysis

Bone data obtained at 35 and 60 weeks were regressed on average Ca intake during the rearing and laying periods. Calcium intakes were calculated from average feed intake values of the birds on a particular dietary Ca level. Data were analysed using the Minitab Statistical Software package (Release 8.2) (Minitab Inc., 1991). Regression equations were referred to where applicable in the discussion of the data. One-way analysis was employed to determine significant differences in response to parameters for Ca levels within a sampling period.

Calcium level during the rearing period (1-day-old to 22 weeks) had no significant effect on bone measurements. Therefore, the data during the laying period (23 to 60 weeks) were also analysed as a 2 x 3 factorial block design (effect of 2 ages and 3 Ca levels) in which data from individual birds served as replicates. Data were subjected to ANOVA using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1996) to assess the effect of dietary treatment on response variables relating to mechanical properties (bone strength and stress), bone dimensions (length, width

and weight), bone chemical composition (ash percentage, Ca and P contents) and Ca intake. The differences between treatment means were separated using the Tukey test.

4.3 Results and Discussion

4.3.1 Feed intake

The effect of Ca levels and age on feed intake of hens is illustrated in Figure 4.1. In spite of restricted feeding the hens' feed intake was significantly (P<.0001) different among dietary treatments. Feed intake increased with increasing dietary Ca level with 1.5 and 3.5% Ca diets giving the lowest (989.62±4.72 g) and highest (1059.60±4.80 g) average feed intake values per hen for the total period, respectively. Such findings are to be expected in view of the important role that Ca plays in the formation of the skeletal system and body metabolism. These results are in agreement with those of Clunies *et al*. (1992a) who, fed three levels of Ca (2.5, 3.5 and 4.5%) to white Leghorn hens and found that birds fed 2.5% Ca diet had the lowest feed intake while those fed 3.5% Ca showed the highest. Summers *et al.* (1976) also reported similar differences by feeding laying hens on 1.5 and 2.96% Ca diets, respectively. In contrast to these results, Menge *et al.* (1977), Reichmann & Connor (1977), Chandramoni *et al.* (1998) and Ahmad *et al*. (2003) reported no significant effect of increasing dietary Ca level on feed consumption of commercial laying hens fed diets containing Ca levels ranging from 2.5 to 5.0%. The disagreements with findings of some previous workers might be attributable to differences between strains or breeds, climatic variation, sources and levels of Ca.

Figure 4.1 shows that feed intake of hens fluctuated during the laying period. It is clear from Figure 4.1 that an increased feed intake occurred at 28, 35, 45, 53 and 56 weeks of age. Fluctuations in feed intake could be attributable to ambient temperature as this study was carried out during periods when extremely high and low temperatures prevailed and birds were kept in a housing system that was not climate controlled.

4.3.2 Calcium intake

From Figure 4.2, it is evident that different intakes of Ca by the hens in each treatment were achieved by feeding the various Ca levels. Keshavarz & Nakajima (1993) and Clunies & Leeson (1995) reported a significant increase in Ca intake by feeding dietary Ca levels ranging from 2.5 to 5.5%. Similar results were reported by

Fernández (1995) and Larsen *et al.* (2000) in pigs. Ross Breeders (1998) and Kemp & Kenny (2004) suggested that breeders need 4-5 g of Ca per day from the first egg throughout the laying period. This requirement is satisfied by making the change from pre-breeder (1.5% Ca) to breeder (2.8% Ca) diets immediately prior to the first egg (Ross Breeders, 1998). The 2.5% dietary Ca level in the current study appeared to provide the recommended requirements (4-5 g). On the other hand, the Ca intake by hens fed 3.5% dietary Ca exceeds the proposed intake.

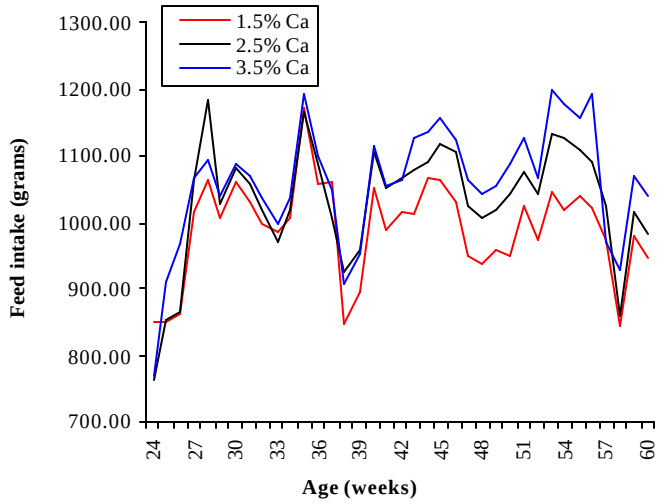

Figure 4.1 Effect of calcium level and age on feed intake of broiler breeder hens.

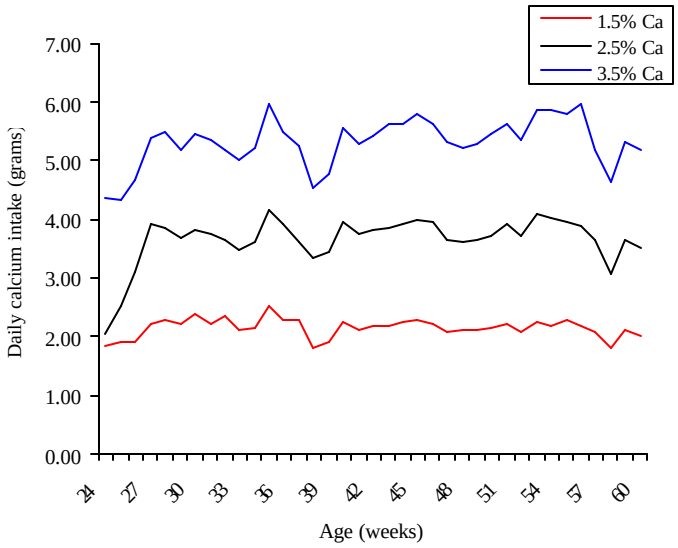

Figure 4.2 Effect of Ca level and age on Ca intake of broiler breeder hens.

It is evident from Figure 4.2 that daily Ca intake of hens was lower at weeks 38 and 58. The variation in Ca intake at especially weeks 38 and 58 could be attributable to high and low temperatures. The maximum and minimum temperatures at week 38 were 35.6 ^{o}C and 17 ^{o}C, respectively. At week 58, maximum and minimum temperatures of 19.4 ^{o}C and 4.0 ^{o}C were recorded. These temperatures are regarded as extreme. It is suggested that the thermoneutral zone for layers lies between 20 and 30 ^{o}C (Australian Poultry Convention Report, 1985). The lower Ca intake at week 38 could be attributable mainly to high temperature, which was outside the comfort zone of 20-30 ^{o}C.

4.3.3 Body weight

As dietary Ca concentration increased from 1.5 to 3.5% (Figure 4.3), hen BW increased ($P<.05$). Such findings would be expected in view of the higher feed intake and important role Ca plays in the formation of the skeletal system and body metabolism. Birds fed 1.5% Ca diet had significantly ($P<.05$) lower BW than those fed 2.5 and 3.5% Ca diets, respectively. However, BW for birds fed 2.5% and 3.5% Ca diets was not significantly different, indicating that either of these two levels is sufficient to support growth. The results of this study support those of Menge *et al.* (1977) and Clunies & Leeson (1995) who reported improved BW in laying Single Comb White Leghorn hens and turkey hens fed increasing dietary Ca levels ranging from 1.16 to 5.5%. A significant ($P<.0001$) Ca level x age interaction occurred indicating that the influence of dietary Ca on BW varied during different periods.

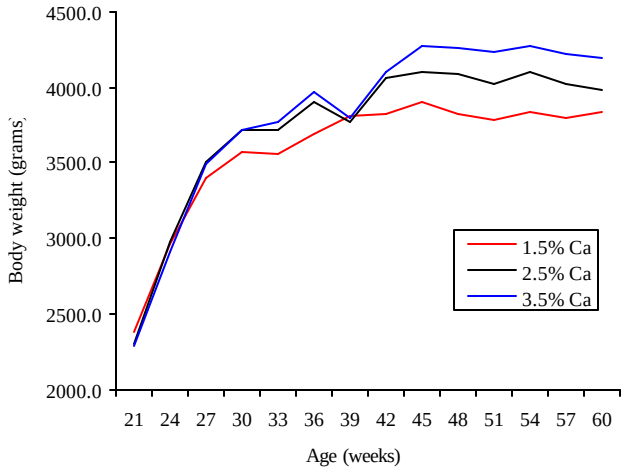

Figure 4.3 Effect of Ca level and age on body weight of broiler breeder hens.

As illustrated in Figure 4.3, the BW of hens increased with age up to 45 weeks.

4.3.4 Mortality

Twelve (4 from each treatment) birds died during the laying period representing a cumulative mortality rate of 6.0%. This indicates that treatment did not influence mortality in agreement with Atkinson *et al*. (1967). Two birds from treatment groups on 2.5 and 3.5% Ca diets were culled due to lameness and broken humeri.

4.3.5 Bone dimensions

According to Table 4.3 Ca level during the rearing period had no influence on bone dimensions of laying broiler breeders. This is in agreement with the results observed during the rearing period (Chapter 3).

As illustrated in Table 4.4, bone length, width and weight were not significantly (P>.05) influenced by dietary Ca level during the laying period. With the exception of tibia weight, regression analysis (Table 4.3) showed the same results. Tolon & Yalcin (1997), however, reported a decrease in the weight of the humerus of caged broilers. These workers attributed the decrease in humerus weight to the birds' high sensitivity to cage rearing. The results of the current study (Table 4.4) are also inconsistent with Williams *et al.* (2000b), who reported that the tibiotarsus width of broilers fed higher levels of Ca decreased linearly with increasing dietary Ca content. These workers suggested that the small dietary Ca effects on BW and bone ash could have combined to give a stronger, but indirect, effect on bone width. In agreement with the current results, Koutoulis *et al.* (1997) reported no effect of dietary Ca supply on bone length of hens up 72 weeks of age.

In contrast with the humerus, the length and width of tibia significantly (P<.001) increased and decreased with age, respectively (Table 4.4). Although no clear explanation could be given for the decline in bone width, it is possible that the resorption of bone for eggshell calcification could have been a contributory factor.

From Table 4.4 it is apparent that bone weight did not change significantly (P>.05) with age. The gradual resorption of medullary bone for eggshell formation during the laying period could have contributed to this non-significant bone weight result.

Table 4.3 Multiple regression analysis of bone characteristics on calcium intake of broiler breeder hens during lay

Sampling age	Variables	Equations	Adj R-sq	Rear P value	Lay P value
35 weeks	Tibia length, cm	Avtiblen35 = 129 – 0.212 LayCain – 3.83 RearCain	0.012	0.148	0.648
	Tibia width, mm	AvtibWidt35 = 7.07 + 0.100 LayCain + 0.461 RearCain	0.029	0.281	0.190
	Tibia weight, g	AvtibWt35 = 17.6 + 0.505 LayCain – 0.84 Rearcain	0.090	0.508	0.031
	Humerus length, cm	RHLENG35 = 86.9 – 3.44 RearCain + 0.122 LayCain	0.040	0.157	0.794
	Humerus width, mm	RHWIDTH35 = 5.50 + 1.29 RearCain – 0.031 Lay Cain	0.000	0.169	0.853
	Humerus weight, g	RHUMWT35 = 9.27 + 0.96 RearCain – 0.180 LayCain	0.000	0.607	0.590
	Breaking strength[1], N	STRENGTH35 = 112 – 78.3 RearCain + 45.1 LayCain	0.273	0.179	0.000
	Bone stress, N/m^2	STRESS35 = 13.1 – 0.12 RearCain + 3.16 LayCain	0.039	0.223	0.045
	Bone ash, %	%Ash35 = 53.1 + 2.77 RearCain + 0.198 LayCain	0.000	0.715	0.363
	True cortical area, mm^2	TCA35 = 13.0 0.32 RearCain+ 0.940 LayCain	0.028	0.777	0.000
	Percent bone	%Bone = 0.87 + 0.01 RearCain – 0.04 LayCain	0.0309	0.4904	0.1418
60 weeks	Tibia length, cm	Avtiblen60 = 126 + 2.83 RearCain – 0.479 LayCain	0.005	0.309	0.314
	Tibia width, mm	AvtibWidt60 = 7.38 – 0.022 RearCain – 0.0104 LayCain	0.000	0.956	0.879
	Tibia weight, g	AvtibWt60 = 17.7 + 0.61 RearCain + 0.140 LayCain	0.000	0.626	0.513
	Humerus length, cm	RHLENGTH60 = 85.9 – 5.84 Rearcain + 0.908 LayCain	0.011	0.269	0.314
	Humerus width, mm	RHWIDTH60 = 6.22 + 0.14 Rearcain + 0.0199 LayCain	0.000	0.568	0.644
	Humerus weight, g	RHWT60 = 6.22 + 2.28 Rearcain + 0.388 LayCain	0.01	0.281	0.281
	Breaking strength[1], N	STRENGTH60 = 258 – 68.5 RearCain + 29.6 LayCain	0.1244	0.4968	0.011
	Bone stress, N/m^2	STRESS60 = 11.329 + 0.487 RearCain + 0.827 LayCain	-0.0350	0.4500	0.4420
	Bone ash, %	%Ash60 = 48.1 + 2.06 RearCain + 1.24 LayCain	0.006	0.685	0.160
	True cortical area, mm^2	TCA60 = 12.84 – 1.11 RearCain + 1.29 Lay Cain	0.4840	0.4893	0.0001
	Percent bone	%BONE60= 0.908 – 0.003 RearCain + 0.004 LayCain	0.0199	0.8143	0.1362

RearCain = rearing calcium intake; Lay Cain = rearing calcium intake; TCA = true cortical area; Avtibleng = average tibia length; Avtibwidt = average tibia width; Avtibwt = average tibia weight; RH = right humerus; RHwt = right humerus weight

Table 4.4 Effect of calcium level and age on bone dimensions of broiler breeder hens

Variable	Treatment	Age (weeks)			Significance of effect (P)			
		35	60	Means	Treatment	Age	Interaction	CV
Right tibia								
Length (mm)	1.5% Ca	124.36 ± 1.11	128.87 ± 1.16	126.62 ± 0.80[a]	0.3220	0.0004	0.5903	3.1
	2.5% Ca	124.23 ± 1.11	126.44 ± 1.11	125.33 ± 0.79[a]				
	3.5% Ca	123.19 ± 1.11	126.79 ± 1.11	124.99 ± 0.79[a]				
	Means	123.93 ± 0.64[a]	127.37 ± 0.65[b]					
Width (mm)	1.5% Ca	7.87 ± 1.89	7.24 ± 0.20	7.55 ± 0.14[a]	0.8833	0.0001	0.4287	8.6
	2.5% Ca	7.90 ± 1.89	7.39 ± 0.19	7.65 ± 0.13[a]				
	3.5% Ca	8.10 ± 1.89	7.12 ± 0.19	7.61 ± 0.13[a]				
	Means	7.96 ± 0.11[a]	7.25 ± 0.11[b]					
Weight (g)	1.5% Ca	17.81 ± 0.54	18.60 ± 0.56	18.21 ± 0.39[a]	0.2059	0.9566	0.3813	10.0
	2.5% Ca	18.75 ± 0.54	18.77 ± 0.54	18.76 ± 0.38[a]				
	3.5% Ca	19.55 ± 0.54	18.82 ± 0.54	19.19 ± 0.38[a]				
	Means	18.71 ± 0.31[a]	18.73 ± 0.32[a]					
Right humerus								
Length (mm)	1.5% Ca	84.09 ± 1.55	81.99 ± 1.62	83.04 ± 1.22[a]	0.7338	0.7061	0.6245	6.4
	2.5% Ca	82.94 ± 1.55	83.88 ± 1.55	83.41 ± 1.10[a]				
	3.5% Ca	84.39 ± 1.55	84.10 ± 1.55	84.25 ± 1.10[a]				
	Means	83.81 ± 0.90[a]	83.32 ± 0.91[a]					
Width (mm)	1.5% Ca	6.53 ± 0.11	6.50 ± 0.12	6.51 ± 0.80[a]	0.8781	0.4454	0.7765	6.1
	2.5% Ca	6.56 ± 0.11	6.39 ± 0.11	6.48 ± 0.80[a]				
	3.5% Ca	6.47 ± 0.11	6.44 ± 0.11	6.45 ± 0.80[a]				
	Means	6.52 ± 0.07[a]	6.44 ± 0.07[a]					
Weight (g)	1.5% Ca	10.28 ± 0.74	9.83 ± 0.77	10.05 ± 0.53[a]	0.0927	0.5169	0.4503	26.0
	2.5% Ca	8.77 ± 0.74	9.00 ± 0.74	8.89 ± 0.52[a]				
	3.5% Ca	9.76 ± 0.74	11.16 ± 0.74	10.46 ± 0.52[a]				
	Means	9.60 ± 0.42[a]	10.00 ± 0.43[a]					

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable.

Although it is generally thought that medullary bone has non-structural properties, Fleming *et al.* (1996) have shown that it contributes to overall BS. Resorption of medullary bone could result in weaker bones.

4.3.6 Bone mechanical properties

From Table 4.3 it is evident that Ca levels during the rearing period did not influence BS and bone stress of laying hens.

The GLM and regression analyses indicated that BS was significantly influenced by dietary Ca level at 35 and 60 weeks of age (Tables 4.3 and 4.5). As illustrated in Table 4.5, BS significantly ($P<.02$) increased as dietary Ca concentration increased from 1.5 to 2.5%. Thereafter there were no further increases in BS, indicating that the addition of more than 2.5% Ca did not seem to have a beneficial effect. Although the BS for birds fed 2.5 and 3.5% Ca diets was not statistically different, birds on 3.5% Ca diet tended to have numerically higher BS values. These results are in agreement with those of Rowland *et al.* (1968) who reported significantly ($P<.05$) higher BS for birds fed 6.8% Ca diets compared to 1.0%. The finding that increasing Ca levels above 2.5% may not be beneficial supports a previous report of Moore *et al.* (1977) who observed no statistical differences in the BS of radii of 4 months old commercial layer hens fed 3.78% Ca and 1.0% P and 3.22% Ca and 0.65% P diets.

Stress, which is a force per unit area, allows comparisons to be made between strengths of bones that differ in length, size and shape (Crenshaw *et al.*, 1981a). A regression analysis of bone stress data on Ca intake showed that bone stress was significantly ($P<.045$) influenced by dietary treatment at 35 weeks but not at 60 weeks of age (Table 4.3). When means were compared between Ca levels by means of GLM analysis it was observed that dietary Ca levels had no significant ($P>.05$) effect on bone stress (Tables 4.5). The fact that stress values were not significantly different (Table 4.5) indicates that mineralisation was similar across treatment groups. Although it was apparent from Table 4.5 that dietary Ca did not influence bone stress, birds fed 3.5% Ca tended to have higher stress values compared to those fed 1.5% and 2.5% Ca diets. The high coefficient of variation could have contributed to the non-significant results as shown in Table 4.5.

Table 4.5 Effect of calcium level and age on bone mechanical properties of broiler breeder hens during lay

	Treatment	Age (weeks) 35	60	Means	Treatment	Age	Interaction	CV
Right tibia								
Bone strength (N)	1.5% Ca	235.00 ± 68.74	252.00 ± 93.43	242.20 ±15.56[a]	0.0001	0.9809	0.7308	23.3
	2.5% Ca	320.42 ± 56.18	315.00 ± 58.54	317.71 ± 14.44[b]				
	3.5% Ca	350.00 ± 54.10	340.00 ± 105.45	343.50 ± 14.79[b]				
	Means	300.93 ± 12.00[a]	301.34 ± 12.38[a]					
Bone stress (N/mm^2)	1.5% Ca	27.90 ± 5.47	19.33 ± 6.86	23.61 ± 4.39[a]	0.5893	0.0081	0.7790	68.5
	2.5% Ca	32.63 ± 5.47	16.50 ± 6.42	24.57 ± 4.22[a]				
	3.5% Ca	37.39 ± 6.05	21.38 ± 5.74	29.38 ± 4.17[a]				
	Means	32.33 ± 3.27[a]	19.07 ± 3.67[b]					
Right humerus								
Bone strength (N)	1.5% Ca	235.00 ± 22.38	252.00 ± 23.47	243.50 ± 16.22[b]	0.0001	0.9768	0.8149	24.4
	2.5% Ca	320.42 ± 21.43	315.00 ± 21.43	317.71 ± 15.15[a]				
	3.5% Ca	350.00 ± 21.43	340.00 ± 22.38	345.00 ± 15.49[a]				
	Means	301.81 ± 12.56[a]	302.33 ± 1.96[a]					

Previous studies (Whitehead & Wilson, 1992) have shown that there is a constant decline in structural bone content of hens throughout the laying period. Whitehead (2004) suggested that the general net effect of the replacement of structural bone is to weaken the overall strength of the hen's skeleton and thus increase fracture. In the present study, BS was not influenced (P=0.98) by age (Table 4.5), in disagreement with McCoy *et al.* (1996) who reported an increase in BS with age in caged commercial layers. The apparent contradiction may be due in part to the fact that data from the two studies were obtained from different sample populations, clinically normal birds in one and mortalities in the other, as well as differences in strains or breeds of birds.

It is evident from Table 4.5 that bone stress significantly (P<.0081) decreased with age. This is in disagreement with Rath *et al.* (1999) who reported an increase in failure stress with age for 7 and 72 weeks old broiler breeder chickens. In the work of Rath *et al.* (1999) the content of protein, Ca, and P in the feed were, however, different for the young and breeder birds. This could probably have influenced the results and contributed to the various results obtained.

Bone stress values were significantly (P<.0001) greater at 35 weeks compared to 60 weeks. Bone stress decreased by approximately 46% between 35 and 60 weeks of age. This indicates progressive loss of structural bone over the life of the laying hen and its subsequent replacement with medullary bone. Crenshaw *et al* (1981b) stated that as bone mineralisation increases, maximum stress and bending moment of the bone increase. At a point of optimum mineralisation, stress reaches a maximum. Conversely, the lower stress indicates that the hens had bones that were less mineralised, which could be a result of resorption for eggshell calcification that occurred during the laying cycle.

4.3.7 Bone chemical composition

In accordance with bone dimensions and mechanical properties, the chemical composition of broiler breeder laying hens was not influenced by various Ca levels during the rearing period (Table 4.3).

The results of regression analysis and GLM for bone ash, Ca and P contents are presented in Tables 4.3 and 4.6. No significant (P>.05) differences in tibia ash due to dietary Ca levels during the laying period occurred. These results are in disagreement with those of Atteh & Leeson (1983) who reported significantly higher bone ash content in layers with increasing dietary Ca from 3.0 to 4.2%. The differences in results may be ascribed to level of feeding (*ad libitum vs.* restricted), strain of birds (commercial layers *vs.* broiler breeders), Ca levels and possibly housing system.

In the present study, BS did not appear to be positively related to percent tibia ash. Breaking strength increased with dietary Ca levels in contrast with tibia ash. This is in disagreement with Rowland *et al.* (1968, 1972). These workers reported that caged hens that had low BS had significantly lower tibia ash values than floor hens. Because bone ash did not show any significant increase due to dietary treatment in the current study, it is likely that other factors other than increased mineral content may have contributed to the increase in the strength of bones. One possibility is the intermolecular crosslinks of collagens, which are responsible for its tensile strength and stress properties (Frost, 1994) and may provide fibrillar reinforcement causing an increase in the BS (Rath *et al*, 1996).

No significant (P<.05) differences were observed between the two age periods with respect to tibia ash content. In the current study, average tibia ash content at 35 and 60 weeks of age was 55.2 and 54.8%, respectively (Table 4.6). Newman & Leeson (1999) suggested that low ash values probably indicate that medullary bone was being resorbed at a faster rate in order to supply sufficient Ca to maintain shell formation. Whitehead (2004) suggested that the considerable rise in circulating oestrogen at the onset of hen's sexual maturity has a stimulatory effect on osteoblasts, causing them to produce medullary bone instead of structural bone. In the absence of structural bone formation, continued osteoclastic resorption would be expected to give rise to a net depletion of structural bone, leading ultimately to osteoporosis (Fleming *et al.* 1998b). The decline (P<.0001) in Ca content observed from 35 to 60 weeks of age in the current study support the view that the medullary bone was being resorbed to support shell formation resulting in bones with lower bone stress values. These results were, however, not supported by the bone ash values though a tendency for ash to decline with age was observed (Table 4.6).

Table 4.6 Effect of calcium level and age on bone chemical composition (left tibia) of broiler breeder hens

		Age (weeks)			Significance of effect (P)			
	Treatment	**35**	**60**	**Means**	**Treatment**	**Age**	**Interaction**	CV
Left tibia								
Ash content (%)	1.5% Ca	56.82 ± 3.24	52.39 ± 7.33	54.61 ± 1.04[a]	0.1080	0.7504	0.0661	9.3
	2.5% Ca	52.74 ± 3.26	54.66 ± 7.11	53.70 ± 1.04[a]				
	3.5% Ca	56.10 ± 4.65	57.47 ± 3.66	56.78 ± 1.04[a]				
	Means	55.22[a]	54.84[a]					
Calcium, %	1.5% Ca	38.22 ± 0.96	14.33 ± 0.92	26.28 ± 0.67[a]	0.7197	0.0001	0.2439	12.2
	2.5% Ca	39.63 ± 0.92	13.73 ± 0.92	26.68± 0.65[a]				
	3.5% Ca	37.33 ± 0.92	14.52 ± 0.92	25.93 ± 0.65[a]				
	Means	38.89 ± 0.54[a]	14.19 ± 0.53[b]					
Phosphorus, %	1.0% Ca	16.87 ± 0.34	6.61 ± 0.33	11.74 ± 0.24[b]	0.0415	0.0001	0.9244	10.2
	1.5% Ca	16.29 ± 0.33	5.92 ± 0.33	11.11 ± 0.23[ab]				
	2.0% Ca	15.98 ± 0.33	5.89 ± 0.33	10.92 ± 0.23[a]				
	Means	16.38 ± 0.19[a]	6.13 ± 0.19[b]					
TCA[1] (mm^2)	1.0% Ca	13.64 ± 1.45	14.14 ± 1.45	13.89 ± 0.99[a]	0.0856	0.0987	0.5925	24.5
	1.5% Ca	15.24 ± 0.99	16.84 ± 1.36	16.04 ± 0.84[a]				
	2.0% Ca	15.25 ± 1.28	18.43 ± 1.21	16.84 ± 0.88[a]				
	Means	14.71 ± 0.72[a]	16.47 ± 0.76[a]					
Percent bone	1.5% Ca	0.82 ± 0.07	0.91 ± 0.06	0.86 ± 0.05[a]	0.9116	0.0209	0.8706	20.6
	2.5% Ca	0.82 ± 0.04	0.92 ± 0.06	0.87 ± 0.04[a]				
	3.5% Ca	0.77 ± 0.06	0.92 ± 0.06	0.85 ± 0.04[a]				
	Means	0.81 ± 0.03[a]	0.92 ± 0.03[a]					

[1]TCA – true cortical area (cortical area multiplied by mean % bone divided by 100)
Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable.

The percentage of Ca present in the mineral component of the tibia was not significantly (P=0.72) different between Ca levels (Table 4.6). The mean values for 1.5, 2.5 and 3.5% Ca levels were 26.28, 26.68 and 25.93%, respectively. Clunies *et al.* (1992b) suggested that perhaps with higher levels of dietary Ca there is a decreased dependence upon medullary bone mineral to supply Ca for shell formation. According to Hurwitz & Bar (1966), medullary bone Ca increases with increasing dietary Ca levels. The results of the present study are, however, consistent with those of Clunies & Leeson (1995) and Keshavarz & Nakajima (1993), who found no beneficial effects of feeding increased dietary Ca levels (2.5 to 5.5% Ca) on bone Ca levels of laying hens.

During the test period (35 to 60 weeks) both Ca and P content of bone ash significantly (P<.0001) declined by 63.5 and 62.6%, respectively. This represents a monthly decline of 2.1 % for both parameters. The decline in Ca content of bone is expected, as the hen requires Ca for eggshell formation during the laying period. It is well documented that egg weight increases with age, indicating that the heavier egg requires more Ca to be deposited as shell than a lighter egg. Most of the Ca required for eggshell formation is obtained from the medullary bone, which is continuously resorbed during the laying period resulting in low bone Ca.

4.3.8 True cortical area and percent bone

The results of regression analysis for true cortical area (TCA) and percent bone on average Ca intake per hen are presented in Table 4.3. A regression analysis indicated that only TCA was significantly affected by dietary Ca treatment at 35 and 60 weeks. The means for TCA and percent bone are given in Table 4.6. In contrast with regression, GLM analysis revealed that dietary Ca levels did not statistically influence both parameters (Table 4.6). No relevant literature on these parameters could be found in existing literature.

According to Table 4.6 percent bone significantly (P<.02) increased with age and TCA did not.

4.4 Conclusions

According to the results of the present study, various dietary Ca levels during the rearing period of broiler breeder pullets did not influence bone dimensions, bone mechanical properties and bone chemical composition during the laying period. It seems therefore that Ca levels higher than 1.0% in feed restricted rearing diets for breeder pullets (up to 22 weeks) will not influence the bone characteristics during the laying period.

The present results demonstrated no beneficial effects of feeding Ca levels higher than 1.5% during the laying period on all bone parameters except BS. Regarding BS, an increase of dietary Ca above 2.5% during the laying period resulted in no further increase. The feed intake and BW of broiler breeder hens were accordingly lower when 1.5% Ca was included in the diet. Therefore, it seems that 2.5% Ca (4 g Ca/hen/day) is required to stimulate feed intake and support growth of broiler breeder hens on restricted feeding. This level will also supply the Ca requirements for bone development of feed restricted broiler breeder hens.

According to the tibia width and bone stress values as well as Ca and P contents, the bones of broiler breeder hens were less mineralised at the end of the laying period. This is probably an indication of bone mineral resorption for eggshell calcification during the laying period.

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CHAPTER 5

EFFECT OF DIETARY CALCIUM LEVEL ON EGG PRODUCTION AND EGGSHELL QUALITY IN BROILER BREEDER HENS AT PEAK PRODUCTION

5.1 Introduction

The ability of hens to produce quality shells depends largely on the availability of calcium (Ca) from ingested food and skeleton (Farmer *et al.* 1983). Boorman *et al.* (1985) stated that Ca could be derived directly from the diet in the light whereas it must be mobilised from the skeleton in the dark when the birds do not feed. The skeletal system is intimately involved in Ca storage for eggshell formation. According to Klasing (1998), the amount of dietary Ca required to maximise bone or eggshell mineralisation and strength is greater than that needed for other functions. Consequently, proper build-up of Ca stores is essential for the maintenance of bone integrity and acceptable shell quality (Robinson, 1999). Although the shell represents no more than 10% of the egg's weight, it is a vital component to protect and contain food contents of the egg until the consumer uses these (Hunton, 1982). According to Roland (1986), the average Ca requirement for eggshell formation within a population of hens is greatest at approximately peak production. The significance of Ca requirements in layers can be determined by the fact that eggshell contains about 94% calcium carbonate, which is equivalent to 2-2.2 grams (g) of Ca (Simons, 1986; Hopkins *et al.*, 1987; Roland, 1988).

According to Hamilton (1982), the term eggshell quality is often used as a synonym for "shell strength" and denotes the ability of eggshells to withstand applied forces without cracking or breaking. The quality of eggshells is most commonly defined in terms of the amount of shell present and is assessed by measuring shell specific gravity, shell weight and shell thickness (Messens *et al.*, 2005), shell weight per unit surface area (SWUSA) and percent shell of total egg weight (Hunton, 1982). Factors that affect eggshell quality include: the strain of bird, the age of the hen, environmental temperature (>21 ^{o}C usually leads to deterioration in shell quality), dietary factors, dietary electrolytes, stress, disease and other chemical compounds (Roberts & Brackpool, 1994). To ensure maximum shell quality, the hens should

consume a minimum of 3.75 g Ca/hen/day (Roland, 1986). According to Mississippi State University (2000), the hen needs 2 - 2.24 g Ca/ day for egg formation alone.

Broiler breeders require an average of 0.7 g Ca (per bird) per day during the growing period (Chapter 3) and 4-5 g Ca per day from first egg throughout the laying period (Ross Breeders, 1998, 2001; Kemp & Kenny, 2004). It is suggested (Ross Breeders, 2001) that this requirement could be satisfied by making the change from pre-breeder (1.5% Ca) to breeder (2.8% Ca) diets immediately prior to first egg. However, Summers *et al.* (1976) stated that although absolute intake of Ca and phosphorus (P) will depend on strain of bird, energy level of the diet and environmental temperature, it is not uncommon to see intakes of Ca in excess of 4.5 g and available phosphorus (avP) of 0.6 g per day.

Calcium intake is recognised to be a major factor in the regulation of reproductive activity in the hen. A decline from the usual dietary concentration of between 30 and 35 g Ca/ kg diet can interfere with egg and shell formation (Luck & Scanes, 1979). As feed intake is restricted in broiler breeder hens and most feed is consumed during the early hours of morning, these hens are likely to be more susceptible to periods of Ca deficiency during shell formation than *ad libitum* fed birds (Farmer *et al.*, 1983). Accordingly the Ca requirements of hens are greatest at peak production (Roland, 1986). Therefore, the present study was undertaken to gain additional information on the effects of dietary levels of Ca in the rearing and laying periods on shell quality and egg production up to 35 weeks of age of broiler breeder hens selected for faster growth rate and heavier body weight (BW).

5.2 Materials and Methods

5.2.1 Birds and husbandry

One hundred and ninety eight Ross broiler breeder pullets were reared up to 22 weeks of age on restricted diets with 1.0, 1.5 and 2.0% Ca (Chapter 3). The pullets on each experimental diet were further randomly divided into three treatments with 1.5, 2.5 and 3.5% dietary Ca (22 birds per treatment). The hens were placed in individual cages within a common room for all treatments. The cages were fitted with feed troughs, water nipples and perches. Following 3-week adjustment period the hens were fed test diets containing 1.5, 2.5 and 3.5% Ca, respectively.

The composition of experimental diets is given in Tables 5.1 and 5.2. Pullets were fed pre-breeder diet containing 1.0, 1.5 and 2.0% Ca from 19 to 22 weeks of age. Thereafter, two types of breeder diets containing 1.5, 2.5 and 3.5% Ca were fed from 23 to 35 weeks of age and these include breeder phase 1 (23 to 34 weeks) and breeder phase 2, which was fed at 35 weeks. The feeds were isocaloric and isonitrogenous but differed only in the Ca and P contents. The feed intake was administered in accordance with Ross Breeders (2001) recommendations. Individual BW measurements were taken on 3-weekly intervals for the duration of the experiment. The hens were photostimulated at 22 weeks of age and received 16 hours of light by week 26. This photoschedule was continued to 60 weeks of age.

5.2.2 Experimental parameters measured

Egg parameters

Egg numbers were recorded daily and summarised on a weekly basis throughout the experimental period (i.e., 25 to 35 weeks). Abnormal eggs (large and misshapen), shell-less and those with defective shells were recorded for production calculations. Cumulative egg production was calculated on a per bird basis throughout the experimental period. First egg laid was considered as age at point of lay while flock attaining maximum percent lay on any day and/or week was considered as peak percent lay and the day was regarded as age at peak of lay (Ali *et al.*, 2003). Percent lay on daily basis was calculated using the formula given by North & Bell (1990).

Individual egg weights were recorded for all the eggs produced by each hen on daily basis. All eggs except shell-less were included in the weight data. Average egg weight per hen was recorded on a weekly basis. After the mean egg weight had been determined in grams each, daily egg mass was computed by multiplying percent hen day production by mean egg weight (North & Bell, 1990; Ross Breeders, 2001). The surface area (cm^2) of each egg was calculated using the formula of Carter (1975a), $3.9782W^{.7056}$, where W is the egg weight in grams.

Eggshell quality

Three eggs from each hen were used to determine eggshell thickness at 3-weekly intervals (i.e., 27, 30 and 33 weeks of age). The eggs were weighed individually and stored in a cool room at 5 ^{o}C. Following the measurement of egg weight, each egg

Table 5.1 Physical composition of laying diets on air dry basis (%)

	Pre-breeder diet		**Breeder Phase 1**		**Breeder Phase 2**		**Breeder Phase 3**	
	1.5% Ca	**3.5% Ca**	**1.5% Ca**	**3.5% Ca**	**1.5% Ca**	**3.5% Ca**	**1.5% Ca**	**3.5% Ca**
Maize	63.54	63.51	61.92	59.66	63.11	60.81	56.43	62.23
Pollard Glutten	-	-	4.45	2.3	1.8	1.0	-	-
Wheat bran	12.65	6.65	5.15	-	6.55	-	14.90	1.00
Full fat soya	-	-	-	10.0	-	9.95	-	1.70
Soybean oil cake	7.75	11.4	8.6	10.3	8.4	7.55	8.75	9.50
Sunflower oil cake	12.45	11.1	15.0	7.75	15.0	10.00	15.00	15.0
Calcium carbonate (grit)	-	-	2.0	6.15	2.3	6.75	2.25	6.60
Calcium carbonate (fine)	1.15	2.2	0.5	1.5	0.6	1.65	0.6	1.65
Mono calcium phosphate	1.49	4.25	1.29	1.36	1.40	1.50	1.28	1.53
Salt	0.24	0.26	0.41	0.40	0.43	0.44	0.44	0.44
Bicarbonate	0.20	0.15	-	-	-	-	-	-
Choline liquid	0.04	0.04	0.03	0.03	-	0.03	-	-
Lysine	0.10	0.04	0.15	-	0.10		0.03	0.03
Methionine	0.05	0.05	0.005	0.06	0.01	0.05	0.01	0.02
Trace mineral/vitamin premix	0.35	0.35	0.50	0.50	0.30	0.30	0.30	0.30

Table 5.2 Nutrient composition of laying diets on air dry basis (%)

	Pre-breeder diet		**Breeder phase 1**		**Breeder phase 2**		**Breeder phase 3**	
	1.0% Ca	2.0% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca
Moisture	11.07	10.37	10.58	9.96	9.77	9.10	9.85	9.19
Metabolisable Energy (MJ/kg)	11.96	11.70	12.09	12.00.	11.94	11.87	11.46	11.43
Protein	15.22	15.50	18.33	17.72	17.03	16.77	16.68	16.06
Crude fat	3.30	3.06	3.00	4.20	2.97	4.07	3.09	2.98
Crude fibre	7.01	5.99	0.00	0.00	6.65	5.08	8.28	6.64
Ash			6.21	11.23	6.74	12.05	6.90	11.98
Calcium	1.00	2.01	1.51	3.50	1.52	3.50	1.59	3.46
Phosphorus	0.84	1.37	0.78	0.71	0.80	0.74	0.84	0.78
Available phosphorus	0.45	0.90	0.41	0.40	0.43	0.43	0.43	0.54
Arginine	0.98	1.01	1.11	1.12	1.08	1.09	1.10	1.07
Isoleucine	0.60	0.64	0.74	0.76	0.69	0.71	0.67	0.67
Lysine			0.81	0.83	0.76	0.78	0.73	0.72
Methionine	0.35	0.34	0.38	0.38	0.35	0.36	0.33	0.33
T SAA[1]	0.06	0.64	0.73	0.70	0.68	0.67	0.66	0.64
Threonine	0.55	0.57	0.66	0.66	0.62	0.63	0.61	0.60
Tryptophan	0.17	0.18	0.19	0.20	0.18	0.19	0.19	0.18
TA[2] Arginine	0.91	0.93	1.04	1.04	0.99	1.01	1.01	0.99
TA[2] Isoleucine	0.54	0.57	0.67	0.69	0.62	0.65	0.59	0.60
TA[2] Lysine	0.60	0.60	0.70	0.71	0.64	0.67	0.61	0.61
TA[2] Methionine	0.31	0.31	0.34	0.35	0.31	0.33	0.29	0.30
TA[2] TSAA	0.57	0.57	0.64	0.63	0.59	0.60	0.57	0.56
TA[2] Threonine	0.48	0.50	0.59	0.59	0.55	0.56	0.26	0.53
TA[2] Tryptophan	0.15	0.16	0.17	0.18	0.17	0.17	0.17	0.17
Linoleic acid	1.83	1.68	1.65	2.32	1.65	2.26	1.71	1.64
Xanthophylls			23.51	17.68	17.12	14.66	11.29	12.45
Salt	0.24	0.27	0.42	0.41	0.44	0.44	0.45	0.45
Choline	1300.01	1309.56	1205.18	1204.08	1008.79	1003.18	1087.10	993.06
Sodium	0.16	0.16	0.18	0.18	0.19	0.20	0.20	0.20
Chlorine	0.22	0.57	0.33	0.29	0.33	0.31	0.32	0.32
Potassium	0.60	0.60	0.60	0.63	0.63	0.63	0.71	0.61
Magnesium			0.22	0.20	0.23	0.21	0.25	0.23
Manganese			46.82	63.94	50.82	68.71	61.84	71.60

[1]Total sulphur amino acids
[2]Chemically determined

was broken and shell thickness and shell weight (including membranes) determined. The shells were washed under gentle running water to remove adhering albumen (Nordstrom & Ousterhout, 1982; Strong, 1989; Kul & Seker, 2004) and wiped with a paper towel to remove excessive moisture. Eggshell thickness was measured using thickness meter sensitive to 0.01 mm. Two measurements were made on the broad end, the equator (waist region) and the sharp end of each egg and the average of each of the two measurements calculated (Ikeme *et al.*, 1983; Ehtesham & Chowdhury, 2002). The shells from individual eggs were then placed in crucibles, dried at 60 ^{o}C overnight, and cooled in the dessicator for approximately 30 minutes, thereafter shell weight was recorded. Percentage shell was calculated by dividing dry shell weight by egg weight and multiplying by 100 (Buss & Stout, 1981; Chowdhury & Smith, 2001). The SWUSA (mg/cm^2) was calculated using the formula of Carter (1975b) while the weight of egg contents was obtained by subtracting eggshell weight from egg weight.

5.2.3 Statistical analysis

The effects of Ca level and age on the egg characteristics data during laying period (25 to 35 weeks) were analysed as a 3 x 3 factorial block design in which data from individual birds served as replicates. Data were subjected to ANOVA using the General Linear Models (GLM) procedure (SAS Institute, 1996) to assess the effect of dietary Ca level and age on response variables relating to egg production (as well as egg mass, egg weight, egg surface area and egg contents) and eggshell quality (shell thickness, shell weight, shell percentage and SWUSA). The differences between treatment means were separated using the Tukey test.

As the results of individual hens at different ages were dependent, average egg production and eggshell quality data up to 33 weeks were also regressed on Ca intake (levels) during the rearing and lay periods using Minitab Statistical Software package (Release 8.2) (Minitab Inc., 1991).

5.3 Results and Discussion.

5.3.1 Calcium intake

The hens Ca intake during lay (25 to 60 weeks) has been discussed in detail in Chapter 4. Hence, this chapter will only discuss the Ca intake for the three specific age periods (i.e., 27, 30 and 33 weeks). A significant ($P<.0178$) Ca level x age

interaction was noted indicating that the influence of dietary Ca on Ca intake varied from period to period. Dietary Ca level had a significant (P<.0001) effect on hen's daily Ca intake (Table 5.3). The Ca intake of the hens significantly (P<.0001) increased as dietary Ca concentration increased from 1.5 to 3.5%. These results are consistent with those of Clunies *et al*. (1992) who reported that Ca intake of Single Comb White Leghorn hens significantly increased as dietary Ca concentration increased from 2.5 to 3.5%. Average Ca intakes (g/hen/day) of 2.2, 3.7 and 5.3 for the 1.5, 2.5 and 3.5% Ca levels, respectively were recorded during the experimental period. It is suggested that broiler breeders require 4-5 g Ca/hen/day throughout the laying period (Ross Breeders 1998, 2001; Kemp & Kenny 2004). The inclusion of approximately 3.0% Ca in the diet of broiler breeders used in the current study would probably supply this requirement (i.e., 4-5 g Ca/hen/day).

The hens Ca intake declined (P<.0001) significantly with age. At 33 weeks of age the Ca intake was significantly (P<.05) lower compared to 27 and 30 weeks. Webster (2002) contends that older hens may have reduced ability to absorb Ca that cannot be compensated by increased Ca percentage in the ration.

Table 5.3 Effect of dietary calcium intake (g) and age on hen's daily calcium intake

Age (weeks)	**Dietary level of calcium**		
	1.5%	2.5%	3.5%
27	2.21±0.06[a]	3.91±0.06[b]	5.38±0.06[c]
30	2.29±0.06[a]	3.82±0.06[b]	5.45±0.06[c]
33	2.11±0.06[a]	3.47±0.06[b]	4.99±0.06[c]
CV%	12.8		
Significance level (P)			
Treatment	0.0001		
Age	0.0001		
Interaction	0.0178		

[a,b,c]Means within rows with no common superscripts differ significantly (P<.05).

5.3.2 Egg production

From Table 5.4 as well as Figure 5.1, it can be observed that egg mass was the only variable affected by rearing treatment. This finding is, however, difficult to explain.

Table 5 4 Multiple regression analysis of egg production, egg contents and eggshell characteristics on Ca intake of broiler breeder hens (27 to 33 weeks of age)

Variables	Regression equations	Adjusted R-sq	Rear P value	Lay P value
Hen production (%)	AVGHD = 65.36 + 1.92 RearCa + 1.90 LayCa	0.0251	0.0635	0.0640
Egg mass (g)	AVGM = 37.896 + 1.307 RearCa + 1.456 Lay Ca	0.0347	0.0486	0.0270
Egg weight (g)	EW = 60.948 – 0.504 RearCa + 0.140 Lay Ca	-0.0003	0.1827	0.7094
Shell weight (g)	SW = 5.098 – 0.051 RearCa + 0.187 LayCa	0.0867	0.2432	0.0001
Shell thickness (mm)	SHTHK = 37.214 – 0.100 RearCa + 1.148 LayCa	0.1071	0.6684	0.0001
Shell percentage	SHPER = 8.362 – 0.007 RearCa + 0.301 LayCa	0.1195	0.8995	0.0001
Egg surface area (cm^2)	ESA = 72.280 – 0.418 RearCa + 0.097 LayCa	-0.0008	0.1873	0.7573
Shell weight per unit surface area (mg/cm^2)	SWUSA = 66.350 + 0.406 RearCa + 0.032 LayCa	-0.0007	0.9151	0.1739
Egg contents (g)	EGGCONT = 55.294 – 0.445 RearCa + 0.302 LayCa	0.0030	0.1881	0.3797

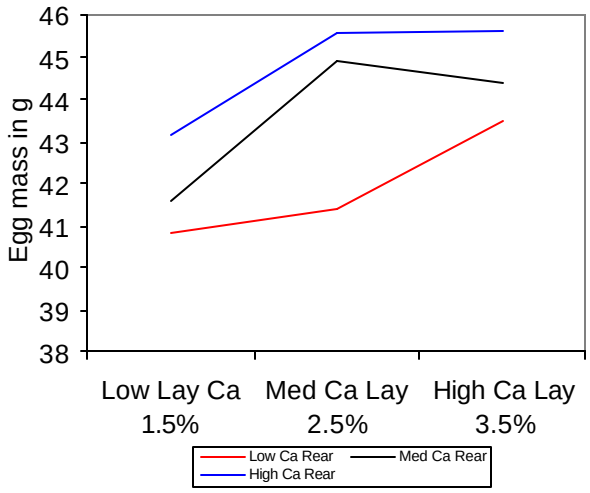

Figure 5.1 Effect of Ca levels on egg mass during rearing and laying periods

The influence of Ca levels and age during early lay on egg production and egg mass is shown in Figures 5.2 and 5.3 respectively as well as Table 5.4. The regression analysis showed that egg production was not significantly (P>.05) influenced by experimental diets and that egg mass increased significantly (P<.05) with increased dietary Ca level during the laying period (Table 5.4).

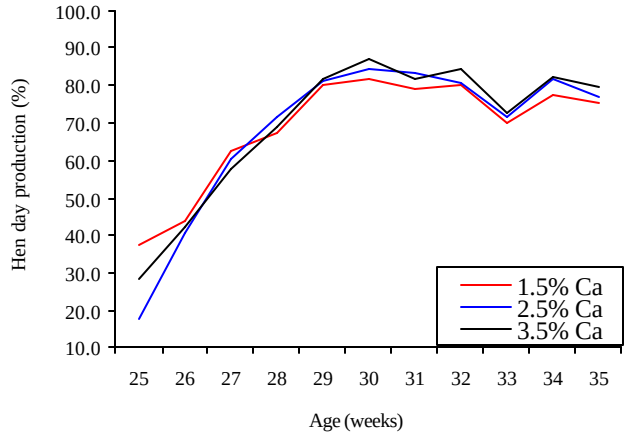

Figure 5.2 Effect of dietary Ca levels and age on egg production

There was a 1.6% average egg production difference (68.63 *vs.* 69.74%) between 1.5% and 3.5% dietary Ca levels. Davidson & Boyne (1970) reported that feeding commercial laying hens diets containing Ca level above 2.6% resulted in no significant improvement in egg production up to 26 weeks of age. The finding on egg

production is in agreement with published reports (Reichmann & Connor, 1977; Manley *et al.*, 1980; Ousterhout, 1980; Keshavarz & Nakajima, 1993). In contrast to these findings Pepper *et al.* (1968), Reddy *et al.* (1968), Quisenberry & Walker (1970), Helence *et al.* (1973), Menge *et al.* (1977), Roland *et al.* (1996) and Ahmad *et al.* (2003) reported increased egg production with increasing dietary Ca level. The type of bird (broiler breeders *vs.* commercial layers), as well as, bird strain differences (Ross *vs*. Bovanes) and Ca levels may have contributed to the various results reported in the literature.

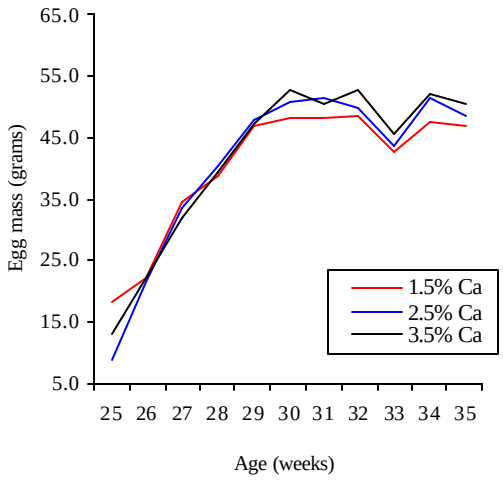

Figure 5.3 Effect of dietary lay Ca levels and age on egg mass during lay period

The egg mass finding is consistent with the results of Scott *et al.* (2000) though Ca levels used in that study were higher than those used in the current study. The fact that egg mass was affected ($P<.05$) by treatment while egg production and egg weight were not is surprising given that egg mass is a function of these two variables.

In the current study, age at point of lay (first time egg was laid) and age at peak of lay were 25 weeks (175 days) and 30 weeks (210 days), respectively. The age at peak of lay in the current study is in accordance with Ross Breeders (2001). North & Bell (1990) stated that even though today's breeder flocks are best brought into 5% hen-day production at 24 weeks of age, factors such as hatching date, season, strain, temperature, ration and feeding programme may lead to flocks varying 2 or 3 weeks from this age. Ali *et al.* (2003) reported average age at point of lay and age at peak of

lay of broiler breeder hens to be about 24 weeks (164.67 days) and 33 weeks (232.83 days), respectively.

Egg production and egg mass significantly (P<.0001) increased with age up to 30 weeks and thereafter a tendency for these variables to decline was noted (Figures 5.2 and 5.3). A sharp decline in egg production and egg mass was noted at 33 weeks of age and this decline could probably be ascribed to among other factors a high ambient temperature. North & Bell (1990) state that at 38 ^{o}C egg production drastically declines, and many birds may die from heat exhaustion. The house temperature of 36.7 ^{o}C was recorded at 33 weeks, which was 0.4 ^{o}C higher compared to week 32. Although literature seems to be conflicting regarding the thermoneutral zone, the accepted general rule of thumb is 20-30 ^{o}C for layers (Australian Poultry Convention Report, 1985). Additionally, North & Bell (1990) state that normally egg production does not decline until average house temperature reaches 27 ^{o}C. According to Barlett (1984), for each 1 ^{o}C rise in temperature above the thermoneutral point of around 30 ^{o}C, there is a reduction of 1.5 g in feed intake. During the hot weather, feed consumption declines and the daily intake of critical minerals such as Ca and protein may be less than optimum resulting in low production.

5.3.3 Mortality

The birds that died or were culled (i.e., birds with broken bones or sick) during production period (25 to 35 weeks) were considered as mortality. Consequently, mortality calculated and presented here also includes culls. Mortality rate during this period as calculated per dietary treatments were 4.5, 3.0 and 0% for birds on the 1.5, 2.5 and 3.5% Ca diets, respectively. The fact that mortality declined with increased dietary Ca level suggests that Ca level had an effect on mortality. Accordingly Quisenberry & Walker (1970) reported that dietary Ca level is positively correlated to survival rate. These results are in disagreement with those of Atkinson *et al.* (1967) and Scott *et al.* (1999, 2000) who reported no differences in mortality in turkey and commercial laying hens fed diets containing Ca levels ranging from 1.24 to 4.5%. As already mentioned the Ca levels, length of lay, age, bird strain and species could have contributed to the differences in the results.

5.3.4 Egg weight and egg contents

According to Zinpro Cooperation (2002), the growth of the broiler chick after hatching is directly related to egg weight. As a general rule, a 1 g change in egg weight results in a 7 to 10 g change in the weight of the 42-day-old finished broiler. In the current study, egg weight was not significantly (P>.05) different among dietary Ca levels (Tables 5.4 and 5.5). From the results in Tables 5.4 and 5.5, it is clear that in accordance with egg weight, egg contents was not significantly (P>.05) influenced by dietary Ca levels. The lack of response in egg weight due to increasing Ca intake levels is consistent with the reports of Atkinson *et al.* (1967), Helence *et al.* (1973), Atteh & Leeson (1983), Zapata & Gernat (1995) and Ahmad *et al.* (2003) who fed commercial layers and turkey breeder hens diets containing Ca levels ranging from 1.24 to 5.0%. The current results are also in agreement with the findings of Roland *et al.* (1996) who reported no adverse effect of dietary Ca on egg weight of commercial Leghorn hens fed diets containing six Ca levels (2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%) from 22 to 32 weeks of age.

Age and BW are the primary factors that influence egg weight. In Chapter 4 (Section 4.3.3) it was found that BW increased with dietary Ca level up to 2.5% as well as with age. Regardless of Ca level in the diet, the egg weight significantly (P<.05) increased from week 27 to 33 (Table 5.5). The present results confirm previous observations that egg weight is lowest at the beginning of the production cycle but increases as age and BW increase throughout the laying period (Leeson & Summers, 1982; McDaniel, 1983). According to Table 5.5, the greatest increases in egg weight were observed during the first six weeks of egg production (i.e., from 25 to 30 weeks). According to Reddy *et al.* (1968) who fed commercial laying hens the effect of age on egg weight was of quadratic response.

The mean egg weights for 1.5, 2.5 and 3.5% Ca diets in the current study were 57.76, 58.47 and 58.35 g, respectively. These average weights were slightly higher than the mean egg weight for meat-type birds suggested by North & Bell (1990). According to these workers, the minimum egg weight for meat-type birds is between 49.6 and 52.0 g during the first 12 weeks of egg production and after 12 weeks of egg production, respectively. According to these workers, the minimum size is determined by the needs of the hatchery using the eggs and the size of the bird laying the eggs.

Table 5.5 Effect of calcium level and age on egg weight, egg contents and eggshell characteristics

Variable	Treatment	Age (weeks) 27	30	33	Means	Significance of effect (P) Treatment	Age	Interaction	CV
Egg weight (g)	1.5% Ca	56.16 ± 0.74	60.07 ± 0.60	61.49 ± 0.61	59.24 ± 0.38[a]	0.5480	0.0001	0.2274	7.3
	2.5% Ca	56.35 ± 0.75	60.07 ± 0.57	62.97 ± 0.62	59.80 ± 0.38[a]				
	3.5% Ca	55.10 ± 0.73	60.51 ± 0.57	63.39 ± 0.60	59.67 ± 0.37[a]				
	Means	55.87 ± 0.43[a]	60.22 ± 0.33[b]	62.62 ± 0.35[c]					
Egg surface are (cm^2)	1.5% Ca	68.20 ± 0.62	71.54 ± 0.50	72.73 ± 0.51	70.83 ± 0.32[a]	0.5686	0.0001	0.2167	5.2
	2.5% Ca	68.38 ± 0.63	71.54 ± 0.48	73.95 ± 0.52	71.29 ± 0.32[a]				
	3.5% Ca	67.26 ± 0.62	71.88 ± 0.48	74.29 ± 0.50	71.14 ± 0.31[a]				
	Means	67.95 ± 0.36[a]	71.65 ± 0.28[b]	73.66 ± 0.29[c]					
SWUSA[1] (mg/cm^2)	1.5% Ca	73.56 ± 1.02	72.20 ± 0.82	69.48 ± 0.84	71.75 ± 0.52[a]	0.0001	0.0015	0.1731	8.0
	2.5% Ca	76.82 ± 1.03	78.13 ± 0.78	75.11 ± 0.84	76.69 ± 0.51[b]				
	3.5% Ca	75.99 ± 1.00	77.53 ± 0.78	76.27 ± 0.82	76.60 ± 0.50[b]				
	Means	75.46 ± 0.59[b]	75.95 ± 0.46[b]	73.62 ± 0.48[a]					
Egg contents (g)	1.5% Ca	51.14 ± 0.70	54.90 ± 0.56	56.44 ± 0.57	54.16 ± 0.35[a]	0.9399	0.0001	0.3113	7.5
	2.5% Ca	51.10 ± 0.71	54.48 ± 0.53	57.41 ± 0.58	54.33 ± 0.35[a]				
	3.5% Ca	49.98 ± 0.69	54.93 ± 0.54	57.72 ± 0.56	54.24 ± 0.35[a]				
	Means	50.74 ± 0.40[a]	54.77 ± 0.31[b]	57.19 ± 0.33[c]					
Shell weight (g)	1.5% Ca	5.02 ± 0.09[a]	5.16 ± 0.07[a]	5.05 ± 0.07[a]		0.0001	0.0001	0.0365	9.8
	2.5% Ca	5.26 ± 0.09[a]	5.59 ± 0.07[b]	5.55 ± 0.07[b]					
	3.5% Ca	5.12 ± 0.09[a]	5.57 ± 0.07[b]	5.67 ± 0.07[b]					
Shell percentage (%)	1.5% Ca	8.95 ± 0.12	8.61 ± 0.10	8.23 ± 0.10	8.59 ± 0.06[a]	0.0001	0.0001	0.3962	8.1
	2.5% Ca	9.33 ± 0.13	9.32 ± 0.09	8.83 ± 0.10	9.16 ± 0.06[b]				
	3.5% Ca	9.30 ± 0.12	9.23 ± 0.09	8.95 ± 0.10	9.16 ± 0.06[b]				
	Means	9.19 ± 0.07[bc]	9.05 ± 0.06[c]	8.67 ± 0.06[a]					

Table 5.5 continues on the next page

Table 5.5 Effect of calcium level and age on egg weight and egg characteristics (continued from previous page)

Variable	Treatment	Age (weeks)				Significance of effect (P)			
		27	30	33	Means	Treatment	Age	Interaction	CV
Shell thickness									
Sharp end (mm x 10^{-2})	1.5% Ca	39.32 ± 0.48^{a}	38.60 ± 0.39^{a}	37.00 ± 0.39^{a}		0.0001	0.0007	0.01337	7.3
	2.5% Ca	40.59 ± 0.50^{a}	41.32 ± 0.38^{b}	39.86 ± 0.41^{b}					
	3.5% Ca	39.66 ± 0.49^{a}	41.09 ± 0.38^{b}	40.46 ± 0.39^{b}					
Equator (mm x 10^{-2})	1.5% Ca	38.63 ± 0.49	37.71 ± 0.40	36.46 ± 0.40	37.60 ± 0.25^{a}	0.0001	0.0023	0.0707	7.5
	2.5% Ca	39.59 ± 0.50	40.58 ± 0.38	39.17 ± 0.41	39.78 ± 0.25^{b}				
	3.5% Ca	39.43 ± 0.49	40.23 ± 0.38	39.56 ± 0.40	38.74 ± 0.25^{b}				
	Means	39.22 ± 0.29^{ab}	$39.51 \pm 0.0.22^{b}$	38.40 ± 0.23^{a}					
Broad end (mm x 10^{-2})	1.5% Ca	38.71 ± 0.48	37.97 ± 0.39	36.10 ± 0.40	37.59 ± 0.25^{a}	0.0001	0.0001	0.0842	7.4
	2.5% Ca	40.02 ± 0.50	40.83 ± 0.37	39.03 ± 0.40	39.96 ± 0.25^{b}				
	3.5% Ca	39.70 ± 0.49	40.49 ± 0.38	39.46 ± 0.39	39.88 ± 0.25^{b}				
	Means	39.47 ± 0.28^{b}	39.76 ± 0.22^{b}	38.20 ± 0.23^{a}					

[1] SWUSA – shell weight per unit surface area.

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant ($P>0.05$) interaction occurred.

Means with the same letter within a row (age) are not significantly different for the same variable, where a significant ($P<0.05$) interaction occurred.

In accordance with egg weight, egg contents significantly (P<.001) increased with age (Table 5.5). The increase in egg contents could be attributed mainly to the increase in egg yolk. O'Sullivan *et al.* (1991) reported disproportional deposition of yolk albumen into the egg with advancing age in laying chickens. Reidy *et al.* (1994) reported that the weight of eggs in commercial turkey hens increased approximately by 11.0% between the onset of lay and 24 weeks of production. During this period, yolk weight increased by 21.0% compared to 7.0% of albumen weight.

5.3.5 Eggshell quality

Table 5.5 presents data on egg surface area, SWUSA, shell weight, shell percentage and eggshell thickness (sharp end, equator and broad end). Regression of these variables on Ca intake during the rearing and laying periods resulted in the equations presented in Table 5.4. It is clear that the rearing period had no influence on eggshell quality.

According to regression analysis (Table 5.4) the shell quality variables significantly (P<.05) influenced by dietary lay treatment were shell thickness, shell weight and shell percentage. From the results in Tables 5.4 and 5.5, it is clear that in accordance with egg weight, egg surface area was not significantly (P>.05) influenced by dietary Ca levels. On the contrary, SWUSA, shell weight, shell percentage and shell thickness improved significantly (P<.0001) when dietary Ca increased from 1.5 to 2.5% (Table 5.5). A further increase in dietary Ca resulted in no (P>.05) further improvement in these characteristics indicating that addition of 2.5% Ca in the diet did not seem to have beneficial effect. Although Table 5.5 indicates that SWUSA was significantly influenced by dietary treatment the opposite result was obtained when data for SWUSA was regressed against Ca intake (Table 5.4). According to Richards & Staley (1967), shell thickness, shell weight, shell percentage and SWUSA are significantly correlated with one another. Correlations will be discussed later in Chapter 7 of this thesis. Results suggest that for maximum shell weight, SWUSA, shell thickness and percentage shell the dietary Ca concentration for broiler breeder hens should be 2.5%. In contrast to these results, Ousterhout (1980) reported highly significant influence of dietary Ca on shell weight after feeding Hubbard White Leghorn hens diets containing Ca levels ranging from 2.75 to 4.75%. The results of the current study for shell weight are also inconsistent with those of Clunies *et al.* (1992) and Chandramoni *et al.* (1998)

who fed Single Comb White Leghorn hens diets containing Ca levels ranging from 2.5 to 4.5% and reported that increasing dietary Ca resulted in significantly increased shell weight. In the study of Chandramoni *et al.* (1998), optimum shell quality was achieved by feeding 3.6% Ca level compared to 2.5% Ca level in the current study. Waldroup *et al.* (1974) fed caged turkey breeder hens two levels of Ca (2.5 and 3.5%) and 4 levels of P (0.1, 0.2, 0.3 and 0.4%) and found no significant effect of dietary Ca and P on eggshell thickness. Similar results were reported by Kuhl *et al.* (1977) after feeding Hyline hens three dietary Ca levels (2.5, 3.0 or 3.5%) in a 48-week study. On the other hand, Damron & Flunker (1995) reported significantly higher specific gravity (indicating good shell thickness) as Ca level increased from 2.5 to 3.5% in diets of laying hens. The finding of shell percentage in the current study is in partial agreement with the results of Reddy *et al.* (1968) who reported greater response of percent shell by increasing Ca levels from 2.25 to 3.05%. A different response in eggshell quality due to increasing Ca levels among breeds is to be expected as breeds differ in weight, frame size, egg production potential and composition.

In accordance with egg weight, egg surface area significantly ($P<.001$) increased with age (Table 5.5), indicating that egg surface area is positively correlated to egg weight. The equation ($3.9782W^{.7056}$) of Carter (1975a) indicates that the determination of egg surface is dependent on egg weight. As a consequence, it is logical that an increase in egg weight due to age would result in a concomitant increase in egg surface area.

Eggshell thickness, shell percentage and SWUSA significantly ($P<.0001$) declined with age (Table 5.5). The SWUSA was significantly ($P<.05$) lower at 33 weeks of age compared to 27 and 30 weeks. The decline in SWUSA could be associated with increased egg weight, which is negatively correlated with shell weight. It is evident from Table 5.5 that shell thickness was in general significantly ($P<.05$) lower at 33 weeks compared to other age periods. These results confirmed previous reports that eggshell thickness declines with age. Roland (1980) contended that shell thickness decreases with hen age because total shell deposition after the first three months of lay remains fairly constant while eggs continue to increase in size. This causes the shell to be spread thinner, forcing shell quality to decline. A significant ($P<.0001$) Ca level x age interaction for eggshell weight occurred. This indicates that the influence of dietary Ca on egg weight varied during different periods. With the exception of 1.5%

dietary Ca level, eggshell weight increased significantly (P<.0001) from 27 to 30 weeks of age. A further increase in age (30 to 33 weeks) did not influence eggshell weight significantly (P>.05). The lower eggshell weight at 27 weeks of age could be attributable to the fact that smaller eggs are produced at the beginning of laying period compared to the later stages of the laying cycle. Eggshell percentage tended to decline by 1.6% between 27 and 30 weeks, and significantly (P<.05) between 30 and 33 weeks, respectively. The 4.1% decline in eggshell percentage from 30 to 33 weeks could be associated with peak production and the gradual increase in egg size with age. North & Bell (1990) contended that as eggs get larger during the laying period the shells become thinner to cover the larger egg contents. Another possible contributory factor to thinner shells is the decline of Ca from the medullary bone with age due to resorption for shell calcification. This is consistent with recent findings of Kul & Seker (2004) who reported that shell percentage decreases with increased egg weight. The results of the present study therefore confirmed the view that eggshell percentage is negatively correlated with egg weight.

5.4 Conclusions

No influence of Ca during rearing could be detected except for egg mass. The results of the present study suggest that increasing Ca level from 1.5 to 2.5% can improve eggshell quality as determined by eggshell weight, percentage shell, shell thickness and SWUSA. Ross Breeders (2001) recommended a slightly higher level of 2.8% Ca in the diet. The results of the present study to some extent support the current recommended dietary level of 2.8% Ca (4-5 g) to ensure good eggshell quality throughout the laying period. A Ca level of 2.5% and Ca intakes (g/hen/day) of 3.9, 3.8 and 3.5 g at weeks 27, 30 and 33 resulted in a good eggshell quality.

Shell weight, percentage shell, shell thickness and SWUSA declined with age while egg weight, egg surface area, egg contents increased with age. The decline in the first four factors is probably ascribable to a concomitant decline in the hens' Ca intake, as they grow older.

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CHAPTER 6

EFFECT OF DIETARY CALCIUM LEVEL ON EGG PRODUCTION AND EGGSHELL QUALITY IN BROILER BREEDER HENS FROM 36 TO 60 WEEKS OF AGE

6.1 Introduction

The ability of hens to produce quality shells depends largely on the availability of calcium (Ca) from ingested food and skeleton (Farmer *et al.* (1983). It is claimed (Klasing, 1998) that the amount of dietary Ca required to maximise bone or eggshell mineralisation and strength is greater than that needed for other functions. Therefore, proper build-up of Ca stores is essential for the maintenance of bone integrity and acceptable shell quality (Robinson, 1999).

Eggshell is a bioceramic composite comprising a mineral part (calcite aggregates, 95%) pervaded with an organic matrix (1 to 3.5%), resulting in a structure that has excellent mechanical properties (Hunton, 1995). The function of the eggshell is to protect the contents of the egg from mechanical impacts and microbial invasions and to control the exchange of water and gases through the pores during the extrauterine development of the chick embryo (Nys *et al.*, 1999). Rodriguez-Navarro *et al.* (2002) state that in food market, the eggshell function as a packaging material and its good quality is of utmost importance to the consumer selection and safety. As a result, great care is required to preserve it intact.

The term “shell quality” is frequently used as a synonym for “shell strength”, and denotes the ability of eggshells to withstand externally applied forces without cracking or breaking (Hamilton, 1982). Eggshell quality can be defined by variables such as egg specific gravity through its relationship to shell porosity as shown by positive correlation with pore concentration (Peebles & Brake, 1987). Factors that affect the strength of eggshells are heredity, clutch position, rate of production (Hammerle, 1969) age, health status (disease), season, temperature, nutrition (Hammerle, 1969; Wolford & Tanaka, 1970), strain of hen, time of the day eggs are laid, eggshell ultrastructure (Hamilton *et al*. 1979a, 1979b), housing system, length of lay and neuro-humoral reproductive control mechanisms (Wolford & Tanaka, 1970).

The most common physical properties associated with eggshell strength are shell thickness and shell egg specific gravities. Richards & Staley (1967) suggested that shell thickness, shell weight, shell percentage and shell weight per unit surface area (SWUSA), may be classified as shell quality measurements, as these variables are significantly correlated with each other.

In Chapter 5 the influence of dietary Ca levels in the rearing and laying periods on eggshell quality and egg production up to 35 weeks of age of broiler breeder hens was investigated. It was found that increasing Ca level above 1.0% during rearing had beneficial effect only on egg mass. On the other hand, increasing Ca level from 1.5 to 2.5% during the laying period led to improvements in eggshell quality as determined by shell weight, percentage shell and shell thickness as well as egg mass (27 to 33 weeks). Ousterhout (1980) suggested that as the hen gets older, minimisation of handling loss by maintenance of eggshell quality becomes more important than egg weight. Shell quality is governed by the quantity of the SWUSA of the egg. Shell deposition increases for several months and then plateaus, as the hen gets older. Since egg weight increases throughout the laying period, once shell deposition plateaus, shell quality declines. In this regard, dietary Ca content is important as shell deposition and quality are directly related to the level of Ca in the diet (Ousterhout, 1980).

Because feed intake is restricted in broiler breeder hens, with most feed being consumed during the early morning hours, these and especially older breeder hens may be more susceptible to periods of Ca deficiency during shell formation than hens that are fed *ad libitum* (Farmer *et al.*, 1983). Accordingly Webster (2002) stated that older hens have reduced ability to absorb Ca. The present study was undertaken to gain additional information on the effects of dietary Ca levels during rearing and laying periods on shell quality and egg production of broiler breeder hens from 36 to 60 weeks of age.

6.2 Materials and Methods

6.2.1 Birds and husbandry

The experimental birds described in Chapter 5 were used to investigate the effects of dietary Ca levels on shell quality from 36 to 60 weeks. As already described in the

previous chapter, 198 Ross broiler breeder pullets were reared on restricted diets with 1.0, 1.5 and 2.0% Ca treatment (Chapter 3). The pullets on each experimental diet were further randomly divided into three treatments with 1.5, 2.5 and 3.5% dietary Ca (66 birds per treatment) and fed from 23 to 60 weeks the diets given in Chapter 4. The hens were placed in individual cages within a common room for all treatments. The cages were fitted with feed troughs, water nipples and perches. The hens were photostimulated at 22 weeks of age and received 16 hours of light by week 26. This photoschedule was continued to 60 weeks of age.

Birds were fed pre-breeder diet from 19 to 22 weeks of age, breeder diet phase 1 (23 to 34 weeks), breeder diet phase 2 (35 to 46 weeks) and breeder diet phase 3 (47 to 60 weeks). The chemical and nutrient composition of the experimental diets are presented in Chapter 5 (Tables 5.1 and 5.2) of this thesis. The feed intake was administered in accordance with Ross Breeders' recommendations. Individual body weight (BW) measurements were taken at 3-weekly intervals for the duration of the experiment.

6.2.2 Experimental parameters measured

Egg parameters

Egg numbers were recorded daily and summarised on a weekly basis throughout experimental period. Abnormal eggs (large and misshapen), shell-less and those with defective shells were recorded. Shell-less eggs were, however, not included in the weight data. Cumulative egg production was calculated on a per bird basis throughout the experimental period. Percent lay on daily basis was calculated using a formula given by North & Bell (1990).

Individual egg weights were recorded for all the eggs produced by each hen on daily basis throughout the test period. After the mean egg weight had been determined individually, daily egg mass was computed by multiplying percent hen day production by mean egg weight (North & Bell, 1990).

Eggshell quality

Eggshell thickness was determined according to the procedures of Nordstrom & Ousterhout (1982), Ikeme *et al.* (1983), Ehtesham & Chowdhury (2002) and Kul &

Seker (2004). These procedures have been described in detail in Chapter 5. Following determination of shell thickness, eggshells from individual eggs were then placed in crucibles and dried in the oven at 60 ^{o}C overnight and cooled in the dessicator for approximately 30 minutes, and weight was recorded. The weight of egg contents was calculated by subtracting shell weight from egg weight. Shell percentage was calculated by dividing dry shell weight by egg weight and multiplying by 100 (Chowdhury & Smith, 2001). The surface area (cm^2) of each egg was calculated using the formula of Carter (1975), $3.9782W^{.7056}$, where W is the egg weight in grams. Shell weight was divided by egg surface area to give the SWUSA, expressed as mg/cm^2 (Wells, 1967).

6.2.3 Statistical analysis

The effects of dietary Ca level and age on the egg characteristics data during laying period (36 to 60 weeks) were analysed as a 3 x 9 factorial block design in which data from individual birds (22 birds per treatment) served as replicates. Data were subjected to ANOVA using the General Linear Models (GLM) procedure of SAS (SAS Institute, 1996) to assess the effect of dietary Ca level and age on response variables relating to egg production, egg weight, egg mass, shell thickness, shell weight, shell percentage, egg surface area and SWUSA. The differences between treatment means were separated using the Tukey test. Average egg production and shell quality data (36 to 60 weeks) were also regressed on average Ca intake during the rearing and lay periods.

6.3 Results and Discussion

6.3.1 Calcium intake

The Ca intakes of laying hens up to 35 weeks have been discussed in Chapter 5. Hence, this Chapter will only discuss the Ca intake of hens from 36 to 60 weeks of age. A significant ($P<.0001$) Ca level x age interaction occurred for Ca intake indicating that the influence of dietary Ca levels on Ca intake varied during different periods. As illustrated in Table 6.1, dietary Ca levels had a significant ($P<.0001$) effect on Ca intake of the hens throughout the laying period. An average Ca intake (g/hen/day) of 2.14, 3.76 and 5.39 for the 1.5, 2.5 and 3.5% Ca levels respectively occurred during the experimental period (36 to 60 weeks).

Low Ca intake values of hens were noted at 39 weeks compared to other age periods (Table 6.1) and this could be ascribable to high ambient temperature above the comfort zone, which occurred during this period. The average maximum temperatures at 36, 39 and 42 weeks were 32.6, 35.6 and 30.9 ^{o}C, respectively. In general, the hens' Ca intake did not appear to decline with age until 54 weeks when it started to decline.

Table 6.1 Effect of dietary Ca levels and age on Ca intake (g) of broiler breeder hens

	Dietary level of calcium		
Age (weeks)	1.5%	2.5%	3.5%
36	2.27±0.06[a]	3.90±0.06[b]	5.50±0.06[c]
39	1.91±0.06[a]	3.42±0.06[b]	4.77±0.06[c]
42	2.18±0.06[a]	3.81±0.06[b]	5.82±0.06[c]
45	2.28±0.06[a]	3.99±0.06[b]	5.78±0.06[c]
48	2.12±0.06[a]	3.59±0.06[b]	5.21±0.06[c]
51	2.19±0.06[a]	3.92±0.06[b]	5.62±0.06[c]
54	2.18±0.06[a]	4.02±0.06[b]	5.88±0.06[c]
57	2.09±0.06[a]	3.65±0.06[b]	5.18±0.06[c]
60	2.03±0.06[a]	3.51±0.06[b]	5.18±0.06[c]
CV%	10.9		
Significance level (P)			
Treatment	0.0001		
Age	0.0001		
Interaction	0.0001		

[a,b,c]Means within a row with no common superscripts differ significantly (P<.05).

6.3.2 Egg production

Dietary Ca level during rearing had no effect on egg production and egg mass (Table 6.2). According to Table 6.2, these two variables were significantly (P<0.05) influenced by dietary Ca treatment during lay. From Figure 6.1 it is evident that hen day egg production from week 36 for birds fed 1.5% Ca diets was in general significantly (P<.05) lower than those fed the 2.5 and 3.5% Ca diets. Accordingly Davidson & Boyne (1970) fed commercial laying hens three levels of Ca (1.7, 2.8 and 3.9%) and reported that 1.7% level gave significantly lower egg production than the 2.8 and 3.9% levels. These results are consistent with reports of Clunies *et al.* (1992a) and Ahmad *et al.* (2003) in commercial laying hens. Reichmann & Connor (1977),

Table 6.2 Multiple regression analysis of egg production and egggshell characteristics on calcium intake of broiler breeder hens during lay (36 to 60 weeks)

Variables	**Regression equations**	**Adjusted R-sq**	**Rear P value**	**Lay P value**
Hen day production, %	HD60 = 59.1 + 1.15 RearCa+ 1.40 LayCa	0.039	0.692	0.005
Egg mass, g	EM60 = 38.5 + 1.78 RearCa+ 1.41 LayCa	0.079	0.411	0.000
Egg weight, g	EW60 = 55.4 + 1.95 RearCa + 2.70 LayCa	0.005	0.216	0.004
Shell thickness, mm	SHTHIK60= 34.6 + 1.27 RearCa + 1 .19 LayCa	0.055	0.080	0.006
Shell weight, g	SW60 = 3.86 + 0.432 RearCa + 0.399 RearCa	0.113	0.013	0.000
Shell percentage, %	%Shell = 7.23 + 0.389 RearCa + 0.254 LayCa	0.042	0.048	0.030
Egg contents, g	EGGCONT = 51.6 + 1.51 RearCa + 2.30 LayCa	0.038	0.302	0.009
Shell surface area, cm^2	ESA = 67.9 + 1.60 RearCa + 2.17 LayCa	0.049	0.209	0.004
Shell weight per unit surface area, mg/ cm^2	SWUSA = 58.9 + 4.10 RearCa + 3.04 LayCa	0.079	0.017	0.003

EGGCONT = egg contents; HD = hen day production; EM = egg mass ; EW = egg weight; ESA = egg surface area; SHTHIK = shell thickness;
SWUSA = shell weight per unit surface area

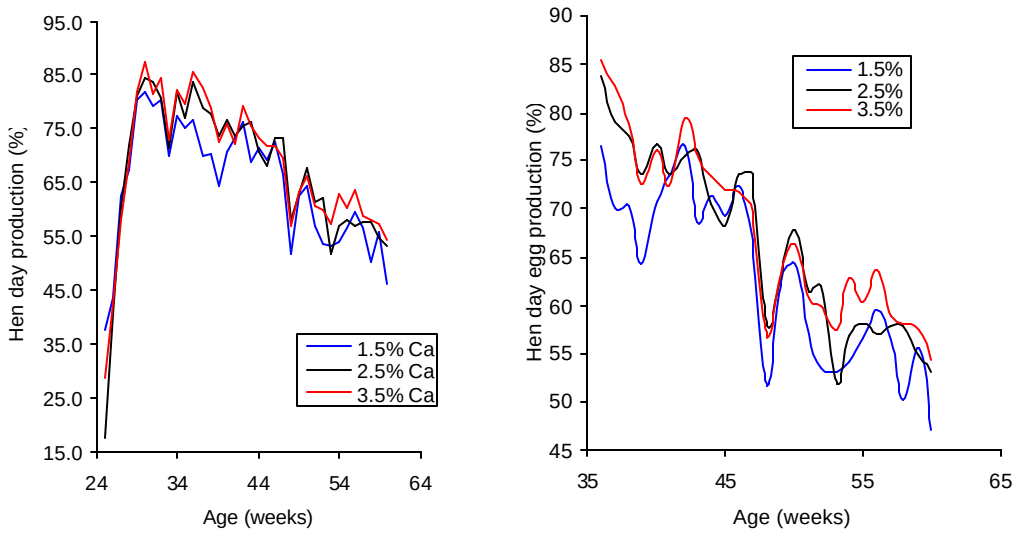

Figure 6.1 Effect of dietary calcium levels and age on egg production.

Manley *et al*. (1980) and Atteh & Leeson (1983) reported no effect of feeding Ca levels ranging from 2.4 to 5.69% on egg production in commercial laying hens and turkey breeder hens.

In the present study, the mean percent hen day production values during the entire laying period for the 1.5, 2.5 and 3.5% dietary Ca levels were 62.84±0.49, 66±0.47 and 67.46±0.46, respectively. The average number of eggs produced by each hen from 25 to 60 weeks of age (245 days) for the 1.5, 2.5 and 3.5 Ca levels was 150.21±3.24, 161±3.14 and 165±3.11, respectively. Results indicated that 2.5% Ca level during the laying period resulted in the highest ($P<.05$) egg production. The yearly egg production from birds fed the 2.5 and 3.5% Ca diets was in agreement with Ciacciariello & Gous (2002) who reported that a broiler breeder hen produces about 165 eggs in the 60 weeks of hen's production life. No significant ($P>.05$) influence of dietary Ca on egg production for weeks 25 to 35 was found in Chapter 5 indicating that effects of Ca on this variable occur in later stages of lay.

From Figures 6.1, it is clear that egg production significantly ($P<.0001$) declined after peak production (week 30) with age. Rose (1997) attributed the decline in egg production immediately after peak production to a lengthening of egg formation time.

A slow and continuous reduction in the rate of egg yolk deposition as the birds' age also contributes to a decline in egg production.

The effect of Ca level and age on egg mass is shown in Figures 6.2. In accordance with egg production egg mass was significantly (P<.05) lower for birds fed 1.5% Ca diets compared to 2.5 and 3.5% Ca diets. No statistical (P>.05) differences were observed between the 2.5 and 3.5% dietary Ca levels.

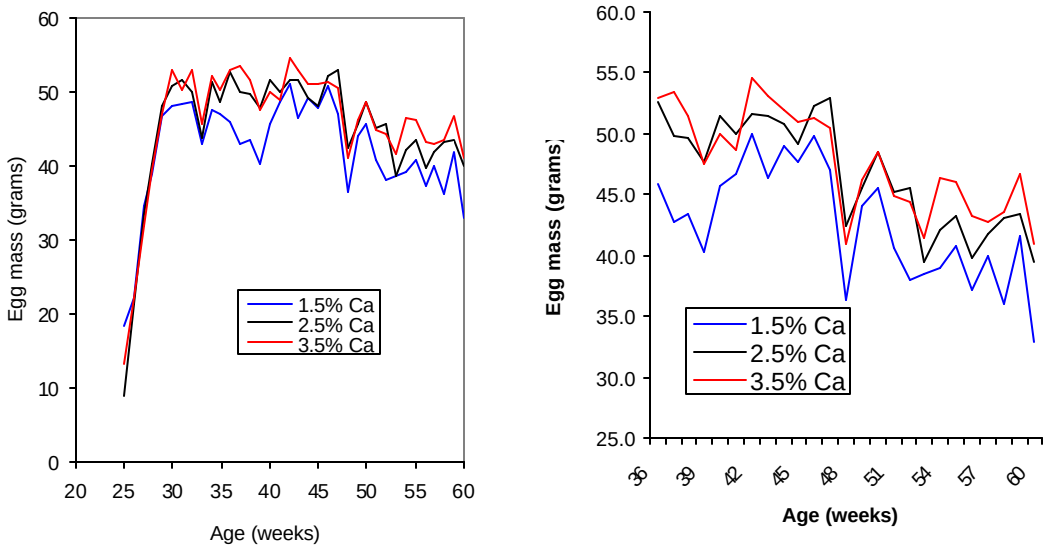

Figure 6.2 Effect of dietary calcium levels and age on egg mass

As shown in Figure 6.2, egg mass in accordance with egg production significantly (P<.0001) declined with age. This is in agreement with Rose (1997) who stated that egg mass output rises to a peak shortly after a flock has reached peak egg production and thereafter declines steadily until egg production ceases.

6.3.3 Mortality

Ali *et al.* (2003) stated that mortality plays a major role in determining performance of the broiler breeder enterprise, as it is a function of the dead and culled birds over the growth and production period. High mortality has been associated with poor laying performance of broiler breeders. In the present study, mortality included birds that died or were culled from the flock during the second laying cycle (ie., 36 to 60 weeks). Nine hens (3 from each treatment) died during this phase indicating that

treatment had no effect on mortality. In contrast with Chapter 5, it seems that Ca level had no effect on mortality from 36 to 60 weeks of age. Atkinson *et al.* (1967), Pepper *et al.* (1968), Reichmann & Connor (1977) and Scott *et al.* (1999, 2000) observed similar results when they fed turkey hens and Shaver Starcross hens diets containing Ca levels ranging from 1.24 to 6.0%.

6.3.4 Egg weight and egg contents

Table 6.3 and Figures 6.3 and 6.4 present the mean values for egg weight and egg contents. As illustrated in Tables 6.2 and 6.3, egg weight and egg contents were significantly (P<.0001) influenced by lay treatment but not rearing treatment. Table 6.3 and Figures 6.3 and 6.4 indicate that the feeding of 2.5 and 3.5% Ca resulted in greater egg weight and egg contents than the lower level (1.5% Ca). No significant (P>.05) differences in these variables were found between the 2.5 and 3.5% Ca levels.

The present results (36 to 60 weeks) are in disagreement with the results of the first phase of the laying cycle (25 to 35 weeks), where no significant (P<.05) differences in egg weight was observed due to increases in dietary Ca level. According to these results, the effect of different dietary Ca levels on egg weight appears during the later stages of the laying period. The results of the present study (36 to 60 weeks) agree with the findings of Summers *et al.* (1976) who reported linear increase in egg weight with higher level of Ca (2.96 *vs*. 1.50%). These results are also in accordance with Atteh & Leeson (1983), Zapata & Gernat (1995) and Ahmad *et al.* (2003) who reported that increasing dietary Ca level from 2.5 to 5.0, 3.0 to 4.2 and 3.0 to 3.5%, respectively had no effect on egg weight in commercial laying hens. Reddy *et al.* (1968) fed commercial layers diets containing Ca levels ranging from 2.25 to 5.05% and reported that feeding up to 2.25% Ca level resulted in greater egg weight. They found that Ca levels above 3.05% caused small but significant decreases in egg weight.

Table 6.3 Effect of dietary calcium level and age on egg weight, egg contents and eggshell characteristics of broiler breeder hens

Variable	Treatment	Age (weeks) 36	39	42	45	48	51	54	57	60	Means	Significance of effect (P) Treatment	Age	Interaction	CV
Egg weight (g)	1.5% Ca	62.75±0.66	62.86±0.77	66.95±0.65	69.72±0.69	71.09±0.79	71.59±0.82	72.25±0.87	72.62±0.87	73.41±1.16	69.25±0.27[a]	0.0001	0.0001	0.9830	6.2
	2.5% Ca	64.15±0.61	65.21±0.65	68.12±0.63	70.86±0.69	71.74±0.69	72.70±0.75	74.55±0.78	74.09±0.87	73.73±0.95	70.57±0.25[b]				
	3.5% Ca	64.99±0.62	65.70±0.69	68.89±0.65	71.14±0.68	72.24±0.66	73.85±0.72	73.76±0.73	73.74±0.79	75.96±0.92	71.14±0.24[a]				
	Means	63.96±0.36[a]	64.59±0.41[a]	68.00±0.37[b]	70.57±0.40[c]	71.69±0.41[c]	72.71±0.44[cd]	73.52±0.46[cd]	73.48±0.49[cd]	74.36±0.59[d]					
Shell weight (g)	1.5% Ca	5.12±0.07[a]	5.00±0.09[a]	6.02±0.07[a]	6.14±0.08[a]	6.11±0.09[a]	6.29±0.09[a]	6.41±0.10[a]	6.16±0.10[a]	6.31±0.13[a]		0.0001	0.0001	0.0001	8.0
	2.5% Ca	5.70±0.07[b]	5.71±0.07[b]	6.02±0.07[a]	6.30±0.08[a]	6.32±0.08[ab]	6.49±0.09[a]	6.63±0.09[a]	6.54±0.10[b]	6.46±0.11[a]					
	3.5% Ca	5.81±0.07[b]	5.79±0.08[b]	6.07±0.07[a]	6.26±0.07[a]	6.40±0.07[b]	6.43±0.08[a]	6.56±0.08[a]	6.54±0.09[b]	6.69±0.10[a]					
Egg contents (g)	1.5% Ca	57.63±0.62	57.87±0.72	60.93±0.61	63.58±0.65	64.98±0.75	65.30±0.77	65.84±0.82	66.45±0.82	67.10±1.09	63.30±0.26[a]	0.0001	0.0001	0.9900	6.4
	2.5% Ca	58.45±0.57	59.51±0.62	62.10±0.60	64.56±0.65	65.42±0.65	66.21±0.71	67.92±0.73	67.55±0.82	67.27±0.89	64.33±0.23[b]				
	3.5% Ca	59.18±0.58	59.91±0.65	62.82±0.62	64.88±0.64	65.83±0.62	67.43±0.68	67.20±0.69	67.19±0.75	69.26±0.87	64.86±0.23[b]				
	Means	58.42±0.34[a]	59.10±0.38[a]	61.95±0.35[b]	64.34±0.37c	65.41±0.39[cd]	66.31±0.42[de]	66.99±0.43[de]	67.07±0.46[de]	67.87±0.55[e]					
Egg surface area (cm^2)	1.5% Ca	73.78±0.53	73.87±0.62	77.24±0.52	79.48±0.56	80.58±0.64	80.98±0.66	81.51±0.70	81.79±0.70	82.42±0.93	79.07±0.22[a]	0.0001	0.0001	0.9823	4.4
	2.5% Ca	74.93±0.49	75.79±0.53	78.17±0.51	80.37±0.56	81.09±0.55	81.85±0.61	83.32±0.63	82.94±0.70	82.66±0.76	80.12±0.20[b]				
	3.5% Ca	75.62±0.50	76.20±0.56	78.79±0.53	80.60±0.55	81.48±0.53	82.76±0.58	82.69±0.59	82.67±0.64	84.40±0.74	80.58±0.19[b]				
	Means	74.77±0.29[a]	75.29±0.33[a]	78.07±0.30[b]	80.15±0.32[c]	81.05±0.33[cd]	81.86±0.36[de]	82.51±0.37[de]	82.47±0.39[de]	83.16±0.47[e]					
Shell percentage (%)	1.5% Ca	8.17±0.09[a]	7.95±0.11[a]	9.01±0.09[a]	8.82±0.10[a]	8.60±0.11[a]	8.78±0.12[a]	8.91±0.12[a]	8.50±0.12[a]	8.62±0.16[a]		0.0001	0.0001	0.0001	7.0
	2.5% Ca	8.90±0.09[a]	8.77±0.09[b]	8.85±0.09[a]	8.90±0.10[a]	8.82±0.10[a]	8.94±0.11[a]	8.94±0.11[a]	8.85±0.12[a]	8.80±0.13[a]					
	3.5% Ca	8.96±0.09[a]	8.83±0.10[a]	8.86±0.09[a]	8.81±0.10[a]	8.87±0.09[a]	8.74±0.10[a]	8.91±0.10[a]	8.89±0.11[a]	8.82±0.13[a]					
SWUSA[1] (mg/cm^2)	1.5% Ca	69.39±0.79[a]	67.55±0.91[a]	77.97±0.77[a]	77.30±0.83[a]	75.82±0.94[a]	77.59±0.98[a]	78.58±1.03[a]	75.36±1.03[a]	76.67±1.38[a]		0.0001	0.0001	0.0001	6.7
	2.5% Ca	76.07±0.72[b]	75.33±0.78[b]	77.04±0.75[a]	78.37±0.83[a]	77.90±082[a]	79.29±0.90[a]	79.53±0.93[a]	78.84±1.03[ab]	78.29±1.13[a]					
	3.5% Ca	76.87±0.74[b]	76.01±0.83[b]	77.02±0.78[a]	77.65±0.81[a]	78.52±0.79[a]	77.63±0.86[a]	79.34±0.87[a]	79.19±0.94[b]	79.25±1.10[a]					
Shell thickness															
Sharp end (mm x 10^{-2}))	1.5% Ca	37.04±0.41[a]	37.57±0.47[a]	41.67±0.40[a]	42.08±0.43[a]	39.87±0.49[a]	39.89±0.51[a]	40.12±0.54[a]	39.13±0.54[a]	39.43±0.72[a]		0.0001	0.0001	0.0001	6.6
	2.5% Ca	40.19±0.37[b]	40.95±0.40[b]	41.77±0.39[a]	42.50±0.43[a]	40.70±0.42[a]	40.44±0.47[a]	41.24±0.48[a]	40.95±0.54[a]	40.40±0.58[a]					
	3.5% Ca	40.73±0.38[b]	41.57±0.43[b]	41.48±0.40[a]	42.41±0.42[a]	40.73±0.41[a]	39.95±0.45[a]	40.65±0.45[a]	40.82±0.49[a]	40.73±0.57[a]					
Equator (mm x 10^{-2})	1.5% Ca	36.29±0.39[a]	36.72±0.45[a]	40.68±0.38[a]	41.24±0.41[a]	38.99±0.46[a]	39.26±0.48[a]	40.26±0.51[a]	38.95±0.51[a]	39.44±0.68[a]		0.0001	0.0001	0.0001	6.3
	2.5% Ca	39.51±0.35[b]	40.39±0.38[b]	40.95±0.37[a]	41.94±0.41[a]	40.38±0.40[b]	39.79±0.44[a]	40.65±0.45[a]	40.73±0.51[b]	40.29±0.55[a]					
	3.5% Ca	40.00±0.36[b]	40.82±0.41[b]	40.84±0.38[a]	41.75±0.40[a]	40.09±0.39[ab]	39.40±0.42[a]	40.71±0.43[a]	40.53±0.46[ab]	40.55±0.54[a]					
Broad end (mm x 10^{-2})	1.5% Ca	36.03±0.39[a]	36.50±0.46[a]	40.77±0.39[a]	41.54±0.41[a]	39.10±0.47[a]	39.29±0.49[a]	40.53±0.52[a]	38.75±0.52[a]	39.24±9.69[a]		0.0001	0.0001	0.0001	6.4
	2.5% Ca	39.27±0.36[b]	40.35±0.39[b]	41.10±0.38[a]	42.16±0.41[a]	40.38±0.41[a]	40.25±0.45[a]	40.78±0.46[a]	41.51±0.52[b]	40.93±0.56[a]					
	3.5% Ca	39.82±0.37[b]	40.77±0.41[b]	40.94±0.39[a]	41.92±0.40[a]	39.99±0.39[a]	39.47±0.43[a]	40.97±0.44[a]	40.63±0.47[b]	40.38±0.55[a]					

[1]SWUSA – shell weight per unit surface area.

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred.

Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

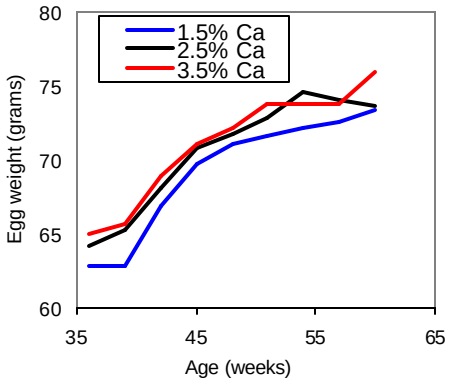

Figure 6.3 Effect of dietary Ca and age on egg weight

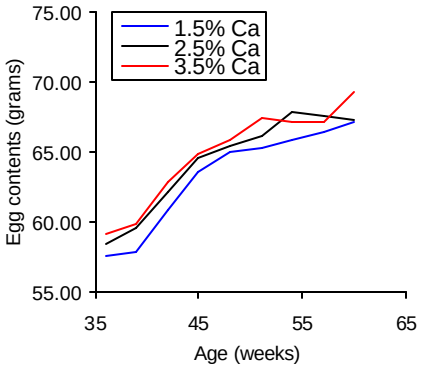

Figure 6.4 Effect of dietary Ca and age on egg contents

As already mentioned, egg contents significantly (P<.00001) increased as dietary Ca concentration increased from 1.5 to 2.5%. Thereafter further increases resulted in non-significant (P>.05) increase in egg contents indicating that the addition of more than 2.5% Ca in the diet seems not to have beneficial effect (Table 6.3). In contrast to these results (36 to 60 weeks), egg contents was not significantly (P>.05) affected by treatment in Chapter 5. The differences in results obtained in the present study and Chapter 5 could be ascribed mainly to an increase in egg weight (size), which was smaller from 25 to 35 weeks compared to the later stages of egg production. An increase in egg contents could be attributable to increases in egg yolk size with age. North & Bell (1990) argued that although the size of the yolk has a greater relationship with egg size than the amount of albumen, the amount of the latter is also related to the size of the egg. Reidy *et al.* (1994) reported that egg weight from commercial turkey breeders increased by about 11.0% between onset of lay and 24 weeks, whereas yolk weight and albumen weight increased by 21 and 7.0%, respectively during this period.

As illustrated in Figures 6.3 and 6.4 as well as Table 6.3, egg weight and egg contents increased over time from 36 to 60 weeks of age. Similar observations were made in Chapter 5. Egg weight increased by an average of 1.9% and 1.7% per month from 36 to 60 weeks (present study) and 25 to 35 weeks (Chapter 5), respectively.

6.3.5 Eggshell quality

The mean values for shell weight, egg surface area, percentage shell, SWUSA and eggshell thickness (sharp end, equator and broad end) are presented in Table 6.3 and Figures 6.5 to 6.9. Table 6.2 gives the regression analysis of eggshell quality variables on Ca intake. According to Table 6.2 and Figures 6.10 to 6.12, dietary Ca during the rearing period had a significant (P<.05) influence on shell weight, shell percentage and SWUSA. In spite of the conclusions in Chapter 3 that dietary Ca level above 1.0% had no influence on bone characteristics (bone development), it seems from the present results that Ca levels higher than 1.0% during the rearing period influenced eggshell strength (quality) beneficially. Shell thickness tended (P<.08) to be influenced by rearing treatment (Table 6.2 and Figure 6.13). The influence of these variables by rearing treatment during lay is unexpected as up to 33 weeks of age, egg mass was the only variable influenced (P<.05) by rearing treatment. This influence of dietary Ca level during the rearing period on eggshell quality was also not supported by a 3x3 (effect of 3 Ca levels during rearing and laying periods, respectively) GLM analysis.

The results of the regression analysis (Table 6.2) showed that nearly all eggshell quality variables were significantly influenced by lay treatment, thus confirming GLM analysis shown in Table 6.3. As illustrated in Table 6.3 and Figures 6.5 to 6.9, the feeding of 2.5 and 3.5% Ca in general resulted in greater shell weight, egg surface area, SWUSA, shell percentage and shell thickness than the lower level (1.5% Ca). No significant (P>.05) differences in these variables were found between the 2.5 and 3.5% Ca levels.

A significant (P<0.0001) Ca level x age interaction for shell weight occurred indicating that the influence of dietary Ca during lay on eggshell weight varied during different periods (Figure 6.5 and Table 6.3). The results of the current study disagree with those of Clunies *et al.* (1992b) and Chandramoni *et al.* (1998) who reported that feeding commercial Leghorn laying hens diets containing Ca levels ranging from 2.5 to 4.5% significantly increased shell weight. In the study of Chandramoni *et al.* (1998) optimum shell quality in terms of shell weight was obtained with 3.6% Ca level, whereas the poorest shell weight was obtained with 2.6% Ca. In the present study, lower shell weight values were obtained when 1.5% Ca level was fed. The

different results between layer and broiler breeder hens are to be expected in light of their relative egg production potential. Higher Ca levels used in previous studies as well as differences in bird strain or breed may have contributed to differences in the results.

As indicated in Table 6.3 and Figure 6.6 egg surface area was significantly (P<.05) influenced by dietary Ca level up to 2.5% during lay but thereafter there were no further statistical significant (P>.05) increases. Egg surface area significantly (P<.0001) increased over time (Figure 6.6). The increase in egg surface area is related to an increase in egg weight with age. Egg weight is required in the calculation of egg surface area, hence an increase in egg weight affects egg surface area. Previous findings (Leeson & Summers, 1982; McDaniel, 1983) indicated that egg weight increased with age throughout the laying period.

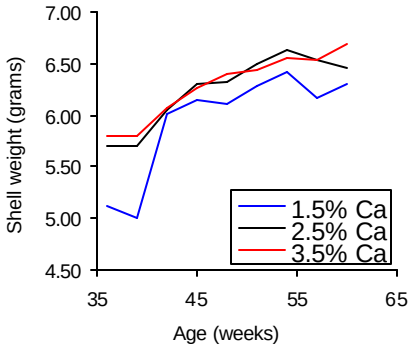

Figure 6.5 Effect of dietary Ca and age on shell weight

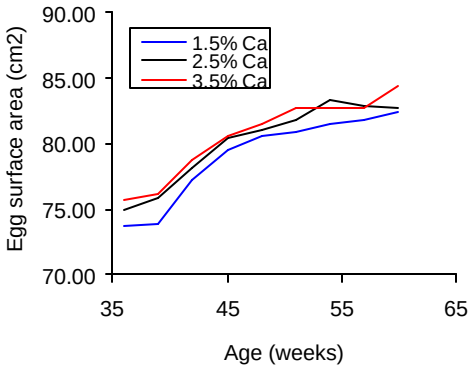

Figure 6.6 Effect of dietary Ca and age on egg surface area

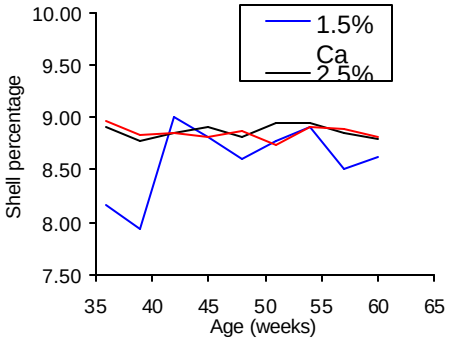

Figure 6.7 Effect of dietary Ca and age on shell percentage

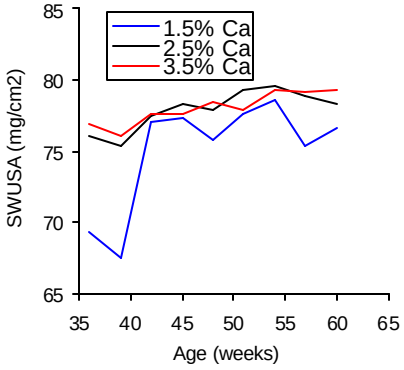

Figure 6.8 Effect of dietary Ca and age on SWUSA

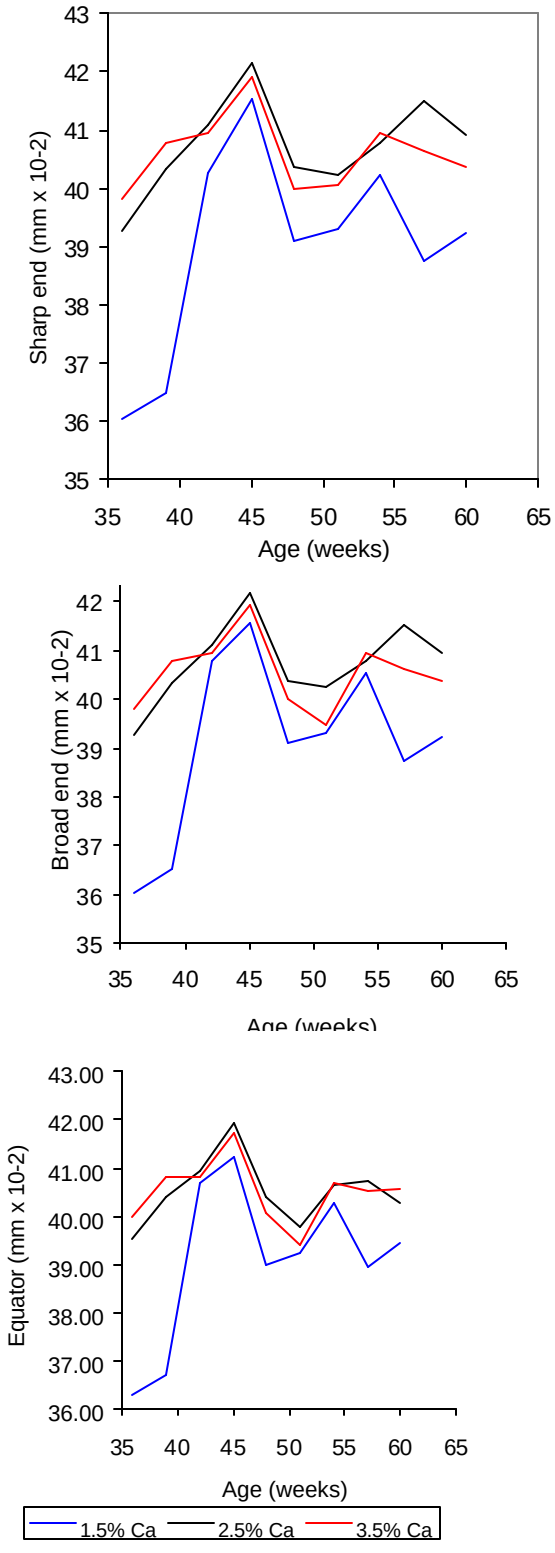

Figure 69 Effect of dietary Ca level and age on shell thickness (sharp end, equator and broad end).

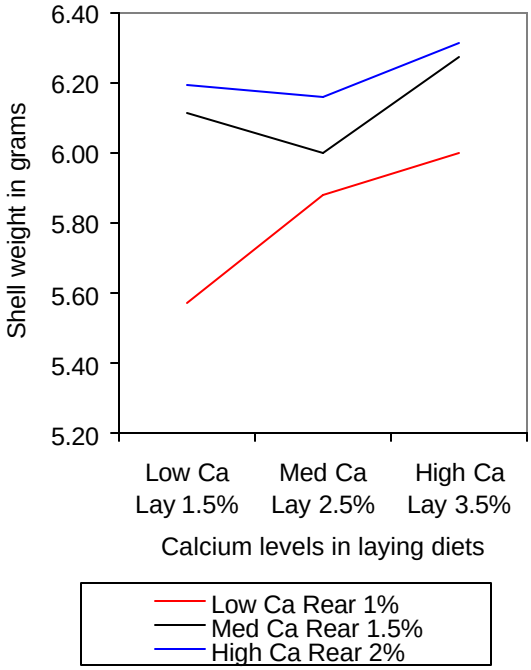

Figure 6.10 Effect of rearing dietary Ca on shell weight.

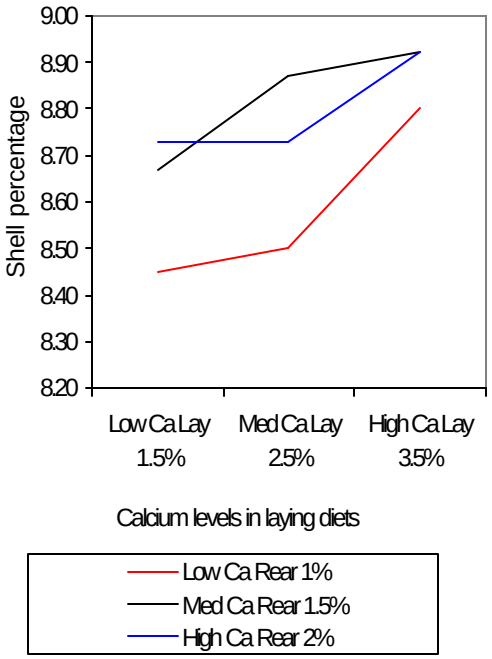

Figure 6.11 Effect of rearing dietary Ca on shell percentage.

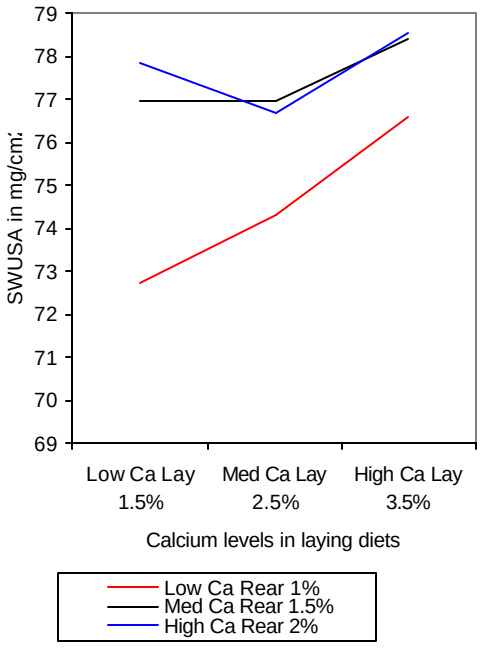

Figure 6.12 Effect of rearing dietary Ca on SWUSA

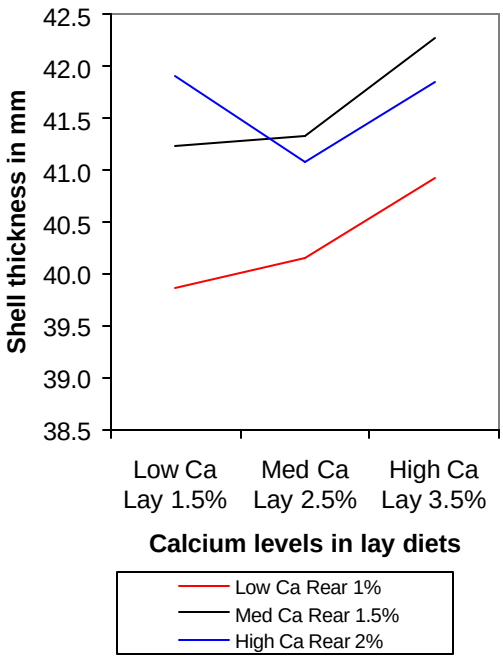

Figure 6.13 Effect of rearing dietary Ca on shell thickness

It was observed that at 36 and 39 weeks shell percentage was not higher (P>.05) when dietary Ca level during lay increased from 2.5 to 3.5% (Figure 6.7). Thereafter shell percentage was not significantly (P>.05) different among Ca levels (Table 6.3). In the first laying phase (25 to 35 weeks) reported in Chapter 5, shell percentage also increased significantly (P<.05) with increased Ca level up to 2.5%. These results are in disagreement with those of Reddy *et al.* (1968) who reported that feeding commercial layers diets containing 3.85 and 3.05% Ca resulted in significantly (P<.05) greater shell percentage than lower levels (i.e., 2.25% and 2.65% Ca). Differences in results are probably mainly attributable to higher Ca levels and differences in breeds or strains. A more recent study of Kul & Seker (2004) in quails demonstrated that shell percentage decreased with increased egg weight. Similar observations were made in the current study (Figures 6.3 and 6.7).

Table 6.3 and Figure 6.8 illustrated that dietary Ca level during lay had a significant (P<.05) effect on SWUSA only at 36 and 39 weeks of age. No significant (P>.05) influence of dietary Ca levels on SWUSA was observed at 42, 45, 48, 51, 54 and 60 weeks of age. In the present study SWUSA was not significantly different for 2.5 and 3.5% Ca levels at 36 and 39 weeks of age indicating that additions of Ca level above 2.5% in diets do not have beneficial effect. These results appear to be in disagreement with the findings of Chandramoni *et al.* (1998) who reported that SWUSA increased with increasing dietary Ca level from 2.6 to 3.6% in a 120 days experimental study. The disagreement with the findings of some previous workers might be attributable to differences in strain or breeds (Rani Shaver White Leghorn layers *vs.* Ross breeders), climatic variation, duration of experiment, managemental factors, Ca levels, variation between sources of Ca and age. The experiments in the current study and previous study (Chandramoni *et al.*, 1998) were conducted in uncontrolled environments.

Increasing Ca levels resulted in thicker shells (Table 6.3 and Figure 6.9). However, it was noted that increasing Ca above 2.5% resulted in no significant (P <.05) thicker eggshells. A significant (P<.0001) Ca level x age interaction for shell thickness occurred, indicating that the influence of dietary Ca on shell thickness varied during different periods. Menge *et al.* (1977) observed similar results as dietary Ca level increased from 1.16 to 2.25%. They reported significantly increased beta-backscatter (BBS) counts; indicating increased eggshell thickness and density, which are the

principal elements of shell quality. Waldroup *et al.* (1974) and Zapata & Gernat (1995) also reported no beneficial effects of increased Ca level on eggshell thickness after feeding caged turkey breeder hens and commercial layers diets containing Ca levels ranging from 2.5 to 3.5%. Nordstrom & Ousterhout (1982) stated that in order for shell thickness to increase, shell weight must increase, egg surface area must increase, or a combination of these two changes must occur. In the present study, a combination of these two changes occurred.

Table 6.3 indicates that shell percentage and SWUSA were significantly (P<.0001) affected by age. It is evident from Figures 6.7 and 6.8 that in general shell percentage and SWUSA declined with age. Al-Batshan *et al.* (1994) reported a decline in shell percentage with age in commercial layers. The decline in SWUSA and shell weight with age may be attributable to increased egg weight, which influences shell weight. Ousterhout (1980) contends that shell quality is governed by the amount of SWUSA of the egg.

Regardless of the Ca level in the diet, the shell weight and egg surface area increased (P<.05) over time from 36 to 60 weeks of age (Figures 6.5 and 6.6). Similar observations were made in Chapter 5 (27 to 35 weeks) with the exception of shell weight, which increased significantly (P<.0001) from week 27 to 30. Thereafter a further increase in age (30 to 33 weeks) did not significantly (P>.05) influence shell weight. In the current study (36 to 60 weeks), shell weight increased by an average of 2.2% per month compared to 1.5% in Chapter 5 (25 to 35 weeks). The greatest (P<.0001) increase in shell weight was noted from 39 to 45 weeks (Table 6.3). The finding that shell weight and egg surface area increased with age is in agreement with previous report of Nordstrom & Ousterhout (1982) who noted that shell weight is influenced by egg weight. As a consequence, the increase of shell weight and egg surface area with age is related to increased egg weight. The findings of Kul & Seker (2004) showed a positive correlation (P<.01, r=0.60) between shell weight and egg weight in Japanese quail.

In accordance with the results of Sparks (1998), shell thickness tended to decline with age (Figure 6.9). Roland (1980) contended that shell thickness decreases with hen age because total shell deposition after the first three months of lay remains fairly constant

while eggs continue to increase in size. This causes the shell to be spread thinner, forcing shell quality to decline. These results confirmed previously reported findings (North & Bell, 1990) that eggshell thickness declines with age.

6.4 Conclusions

According to the results of the present study eggshell characteristics (shell weight, shell percent, SWUSA and shell thickness) seemed to be affected by rearing treatment. This indicates that feeding Ca levels above 1.0% during the rearing period (up to 18 weeks) may be beneficial for eggshell quality. These results were, however, not supported by bone characteristics during the rearing and laying periods. The GLM statistical analysis also showed no beneficial effect of dietary Ca levels higher than 1.0% during the rearing period on eggshell quality. The influence of dietary Ca level during the rearing on eggshell quality of laying broiler breeder hens needs further investigation.

The results of the present study suggest that for maximum egg production and eggshell quality the dietary Ca concentration for caged broiler breeder hens reared from 36 to 60 weeks of age should be 2.5% (11.9 and 11.45 MJ ME/kg diet). There were no beneficial effects of increasing Ca level from 2.5 to 3.5%. The Ca level of 2.5% during lay is close to Ross Breeders (2001) recommended level of 2.8% (4.5 Ca g/bird/day). These results suggest that the 2.5% Ca (3.8 g Ca/hen/day) during lay is adequate to support egg production and to maintain eggshell quality in broiler breeder hens.

Egg production, egg mass, shell percentage and shell thickness declined with age while other parameters such as egg weight, egg contents, egg surface area, shell weight and SWUSA increased.

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CHAPTER 7

EFFECT OF DIETARY CALCIUM INTAKE ON ITS RETENTION BY CAGED BROILER BREEDER HENS

7.1 Introduction

Calcium (Ca) together with phosphorus (P) is necessary for the formation and maintenance of the skeleton. In layers, Ca has an additional function in eggshell formation (Simons, 1986). The formation of eggshells depends on both dietary Ca and medullary bone reserves. The shell enables the egg to withstand considerable biological and mechanical abuse while still maintaining its structural integrity and the wholesomeness of its contents (Parsons, 1982). According to Narushin & Romanov (2002), the eggshell performs a double function during embryo development. It has to be thick and strong enough to protect the embryo from external insults. At the same time, the shell should be sufficiently thin and fragile not to act as a strong barrier to the hatching process.

According to Roland & Farmer (1984), the hen needs 125 mg dietary Ca every hour (hr) for 16 hr to form an eggshell. Farmer & Roland (1986) reported that there is a decline in skeletal Ca contribution to the shell as the dietary level of Ca increases. Furthermore, Roland & Farmer (1984) reported an inverse relationship between skeletal Ca utilisation and the availability of dietary Ca. The greater the percentage of skeletal Ca is used in shell formation the poorer the shell quality. The beneficial effect of increasing the dietary Ca level from 1.5 to 2.5% on egg production and shell quality of broiler breeder hens was illustrated in Chapters 5 and 6. This was probably an indication of a more efficient utilisation and retention of Ca by the broiler breeder hens.

Calcium metabolism in laying hens is most intensive due to high Ca content of eggs (Simons, 1986). For example, eggshells contain 2-0-2.2 g Ca; hence, with increasing egg production, layers need more Ca. The Ca content of the eggshell has been taken as 373 mg/g. Simkiss (1961) stated that there is an increase in Ca retention approximately two weeks before the onset of egg production. This increase is associated with the appearance of the medullary bone in the bone marrow cavities of certain bones. The increased Ca retention is assumed to occur as the hen prepares for

the marked output of Ca in the form of eggshell, which occurs during a normal laying cycle (Summers *et al.*, 1976).

The study of Clunies *et al.* (1992a) demonstrated that Ca retention increased linearly with increasing Ca intake of laying hens. In laying hens, Summers *et al.* (1976) reported, however, much higher Ca retention for the low Ca (1.5%) as compared to the high Ca (2.96%) diet; however, absolute retention was significantly higher on the high Ca diet. These workers found that levels of P significantly influenced Ca retention and that more Ca was retained on the low Ca diet as compared to the high P diet. In addition, Keshavarz & Nakajima (1993) reported significantly increased Ca intake with increasing dietary level of Ca and reduced percentage retention of Ca with increases in the Ca intake of laying hens aged 20 to 64 weeks. Similar results were reported by Leeson & Summers (1997). This would suggest that the results in the available literature on Ca intake and retention seems to be inconsistent and conflicting.

The present study was undertaken to further examine the effect of dietary Ca intake on Ca retention of broiler breeder hens and to investigate the relationship between Ca retention and egg characteristics.

7.2 Materials and Methods

7.2.1 Animal experiments

Management of the birds and care of the eggs was similar to that described in Chapters 4, 5 and 6. The test diets used in this trial have already been described in Chapters 4 and 5 of this thesis. The diets were isocaloric and isonitrogenous. The only nutrients varied were Ca and P. The experiment was conducted for a period of 15 weeks (27 to 42 weeks of age). Birds were housed in individual cages with separate feeders, with metal trays placed below cages for collection of excreta. Ten replicate birds were used per treatment. Hens were subjected to 16 hr of light throughout the test period. Feed was provided in restricted amounts in accordance with Ross Breeders Manual (Ross Breeders, 2001) while water was provided *ad libitum* with feed weighed back on weekly basis. Egg production was recorded daily and egg weight was determined and recorded on daily basis as well.

7.2.2 Chemical analyses

The excreta samples from 90 birds (30 birds per treatment) were collected during a 7-day period at 3-weekly intervals (27, 33, 36 weeks) and at 42 weeks of age. Excreta samples were collected on metal trays, and transferred into paper bags and dried in the oven at 100 ^{o}C for 24 to 48 hr and air-cooled. Thereafter, dried excreta were weighed and pulverized using a hammer mill with a 1 mm sieve. The pulverized samples were homogenised before taking a representative sample for determination of Ca. The Ca content of the excreta and feeds were analysed after a preceding destruction of organic matter content of samples. Approximately 1-gram samples of excreta were ashed in a muffle furnace at 550^{o} C for 16 hr and digested in 10 mL nitric acid and 20 mL distilled water added before analysis. Calcium was determined by atomic absorption spectrophotometry (Hocking *et al.*, 2002). Calcium retention was calculated by subtracting shell Ca, egg contents Ca and faecal Ca from the hens Ca intake. In order to cancel differences in Ca intake as a factor, Ca retention was also expressed as a percentage of Ca intake. The Ca content of the egg contents (g Ca/bird/day) was calculated by multiplying eggs weighing 50 or 60 g by 0.025 and 0.030, respectively (Simons, 1986). Shell Ca (mg/g) was obtained by multiplying average egg weight (g) by 373 mg/g.

7.2.3 Statistical analysis

The data during the laying period (25 to 60 weeks) were analysed as a 3 x 4 factorial (3 Ca levels x 4 age periods) in which data from individual birds served as replicates. Both tables and figures were used to facilitate the interpretation of results especially where interactions occurred. Data were subjected to ANOVA using the General Linear Models (GLM) procedures of SAS® (SAS Institute, 1996) to assess the effect of dietary Ca level on Ca retention of the broiler breeder hens. The differences between treatment means were separated using the Tukey test. Correlation analyses were used to determine the relationship between egg production, egg weight, shell weight, egg contents, shell percentage, egg surface area, SWUSA, average shell thickness, age, shell Ca, Ca intake and Ca retention according to the Pearson correlation procedures of SAS® (SAS Institute, 1996).

7.3 Results and Discussion

7.3.1 Calcium intake

The results of daily Ca intake and daily excretion of Ca are summarily illustrated in Table 7.1. Dietary Ca level had a significant (P<.0001) effect on Ca intake and Ca retention of hens. As dietary Ca concentration increased from 1.5 to 3.5%, the Ca intake of hens significantly (P<.05) increased accordingly (Table 7.1). These results are consistent with Atkinson *et al.* (1967), Clunies *et al.* (1992a) and Clunies & Leeson (1995) who reported increased Ca intake with increased dietary Ca concentration in breeder turkeys and Single Comb White Leghorn pullets, respectively.

7.3.2 Egg and shell weight

Data on egg weight were presented and discussed in detail in Chapters 5 and 6 of this thesis. Hence, this chapter will briefly discuss egg weights for the four specific age periods (27, 33, 36 and 42 weeks). Egg weight was not significantly (P>0.05) different among dietary Ca levels (Table 7.1). Similar results were reported in Chapter 5 while opposite results were obtained in Chapter 6. This lack of response in egg weight due to dietary Ca levels is in agreement with previous reports (Atkinson *et al.*, 1967; Gleaves *et al.*, 1977; Clunies *et al.*, 1992a; Keshavarz & Nakajima, 1993; Scott *et al.* 2000; Zapata & Gernat, 1995; Ahmad *et al.*, 2003). It seems from the current results that the influence of dietary Ca level on egg weight differs between the early and later stages of the egg production period. In contrast with early lay (25 to 35 weeks), where Ca level had no significant influence on egg production, significant differences in egg weight due to dietary Ca level were observed from week 36 to 60. It is worth noting that none of the previous studies except that of Clunies *et al.* (1992b) attempted to separate shell-forming from non shell-forming days. On the non shell-forming days, Clunies *et al.* (1992b) observed no significant effect of dietary Ca content on absolute Ca and P retention. The present results are, however, inconsistent with those of Scott *et al.* (1999) who reported increased egg weight with higher Ca. The differences in results are probably due to differences in experimental conditions, bird strains and dietary Ca levels. Higher Ca levels (3.7 and 4%) were fed in the previous study of Scott *et al.* (1999) than in the present study.

Table 7.1 Daily calcium intake, egg weight, shell weight, shell calcium and faecal excretions of hens fed diets varying in calcium concentration

Variable	Treatment	Age (weeks)					Significance of effect (P)			
		27	33	36	42	Means	Treatment	Age	Interaction	CV
Daily Ca intake (g)	1.5% Ca	2.21 ± 0.08	2.12 ± 0.08	2.26 ± 0.08	2.19 ±0.09	2.19 ± 0.04[a]	0.0001	0.0001	0.1120	11.6
	2.5% Ca	3.91 ± 0.08	3.54 ± 0.08	3.89 ± 0.08	3.85 ± 0.08	3.80 ± 0.04[b]				
	3.5% Ca	5.40 ± 0.08	4.91 ± 0.08	5.52 ± 0.08	5.32 ± 0.09	5.29 ± 0.04[c]				
		3.84 ± 0.05[a]	3.52 ±0.05[b]	3.89 ± 0.05[ac]	3.79 ± 0.05[ac]					
Egg weight (g)	1.5% Ca	55.17 ± 0.63	61.33 ± 0.57	62.66 ± 0.61	67.19 ± 0.64	61.59 ± 0.31[a]	0.0628	0.0001	0.2280	7.2
	2.5% Ca	54.65 ± 0.65	62.62 ± 0.56	63.89 ± 0.62	68.22 ± 0.63	62.34 ± 0.31[a]				
	3.5% Ca	54.15 ± 0.61	62.50 ± 0.56	64.82 ± 0.61	68.75 ± 0.64	62.55 ± 0.30[a]				
	Means	54.66 ± 0.36[a]	62.15 ± 0.33[b]	63.79 ± 0.35[c]	68.05 ± 0.37[d]					
Shell weight (g)	1.5% Ca	5.02 ± 0.09[a]	5.05 ± 0.07[a]	5.12 ± 0.08[a]	6.02 ± 0.08[b]		0.0001	0.0001	0.0001	9.2
	2.5% Ca	5.26 ± 0.09[a]	5.55 ± 0.07[a]	5.70 ± 0.07[ab]	6.02 ± 0.07[b]					
	3.5% Ca	5.12 ± 0.09[a]	5.67 ± 0.07[b]	5.81 ± 0.07[bc]	6.07 ± 0.08[c]					
Shell Ca excretion (g)	1.5% Ca	1.87 ± 0.03[a]	1.89 ± 0.03[a]	1.91 ± 0.03[a]	2.24 ± 0.03[b]		0.0001	0.0001	0.0001	9.2
	2.5% Ca	1.96 ± 0.03[a]	2.07 ± 0.03[a]	2.13 ± 0.03[ab]	2.25 ± 0.03[b]					
	3.5% Ca	1.91 ± 0.03[a]	2.11 ± 0.03[b]	2.17 ± 0.03[bc]	2.26 ± 0.03[c]					
Faecal Ca excretion (g)	1.5% Ca	0.34±0.10[a]	0.10±0.10[a]	0.13 ±0.10[a]	0.26±0.11[a]		0.0001	0.000`	0.0001	43.0
	2.5% Ca	1.30±0.10[b]	0.75±0.10[a]	0.70 ±0.10[a]	0.67±0.09[a]					
	3.5% Ca	3.28±0.10[b]	3.03±0.10[b]	1.94 ±0.10[a]	1.55±0.10[a]					
Total Ca excretion (g)	1.5% Ca	2.83±0.09[ab]	2.42±0.08[a]	2.52 ±0.08[a]	3.21±0.08[b]		0.0001	0.0001	0.0001	9.7
	2.5% Ca	3.99±0.09[a]	3.56±0.08[b]	3.50 ±0.08[b]	3.78±0.07[ab]					
	3.5% Ca	4.90±0.08[a]	5.04±0.07[a]	4.48 ±0.07[b]	4.36±0.08[b]					
Calcium retention (g)	1.5% Ca	0.11 ± 0.14[ab]	0.16 ± 0.11[ab]	0.31 ± 0.11[b]	-0.27 ± 0.12[a]		0.0001	0.0001	0.0001	83.3
	2.5% Ca	0.89 ± 0.13[a]	0.83 ± 0.12[a]	1.14 ± 0.11[a]	0.93 ± 0.11[a]					
	3.5% Ca	0.57 ± 0.12[a]	0.02 ± 0.11[b]	1.47 ± 0.11[c]	1.58 ± 0.12[c]					
Faecal Ca as % of Ca intake	1.5% Ca	15.23±2. 00[ac]	4.76±1.94[b]	5.52±2.05[b]	12.01±2.13[bc]		0.0001	0.0001	0.0001	35.8
	2.5% Ca	33.28±1.94[a]	21.71±1.97[b]	18.11±1.97[b]	17.47±1.94[b]					
	3.5% Ca	60.88±1.91[a]	61.18±1.94[a]	35.44±2.01[b]	29.20±2.05[b]					
Shell Ca as % of Ca intake	1.5% Ca	83.22±2.29[a]	88.91±1.81[a]	82.75±1.85[a]	96.75±1.89[b]		0.0001	0.0001	0.0007	14.6
	2.5% Ca	50.23±2.15[a]	57.85±1.89[a]	53.98±1.74[a]	58.76±1.71[a]					
	3.5% Ca	34.30±1.98[a]	41.99±1.78[a]	39.77±1.74[a]	43.21±1.99[a]					

Table 7.1 continues on the next page

Table 7.1 (continued)

Variable	Treatment	Age (weeks) 27	33	36	42	Means	Treatment	Age	Interaction	CV
Total excreted Ca as % of Ca intake	1.5% Ca	95.96±3.26[a]	93.49±2.58[a]	88.32±2.63[a]	113.38±2.69[b]		0.0001	0.0001	0.0001	14.8
	2.5% Ca	77.83±3.06[a]	77.14±2.69[a]	71.73±2.48[a]	76.09±2.43[a]					
	3.5% Ca	90.15±2.82a[b]	99.71±2.52[b]	74.27±2.48[a]	70.83±2.82[a]					
Ca retained as % of Ca intake	1.5% Ca	4.04±3.26[a]	6.51±2.58[a]	11.68±2.63[a]	-13.38±2.29[b]		0.0001	0.0001	0.0001	86.2
	2.5% Ca	22.17±3.06[a]	22.86±2.69[a]	28.27±2.48[a]	23.91±2. 43[a]					
	3.5% Ca	9.85±2.82[b]	0.29±2.52[a]	25.73±2.48[b]	29.17±2.82[b]					

[1]Percent shell Ca as a percentage of Ca intake (shell Ca/Ca intake x 100).

Means with the same letter within a column (treatment) or row (age) are not significantly different (P>.05).

A previous study (Clunies *et al.*, 1992a) reported that shell weight increased significantly due to increasing dietary Ca from 3.5 to 4.5% in a short-term experiment. In the current study, increasing dietary Ca from 1.5 to 2.5% significantly (P<.05) improved shell weight only at 33 and 36 weeks of age (Table 7.1 and Figure 7.1). No differences (P>.05) in shell weight were observed as Ca level increased from 2.5 to 3.5%. This is in agreement with Clunies *et al.* (1992a). Shell weight was, however, not significantly (P>.05) influenced by Ca intake at 27 and 42 weeks of age. As expected, egg weight significantly (P<.0001) increased over time.

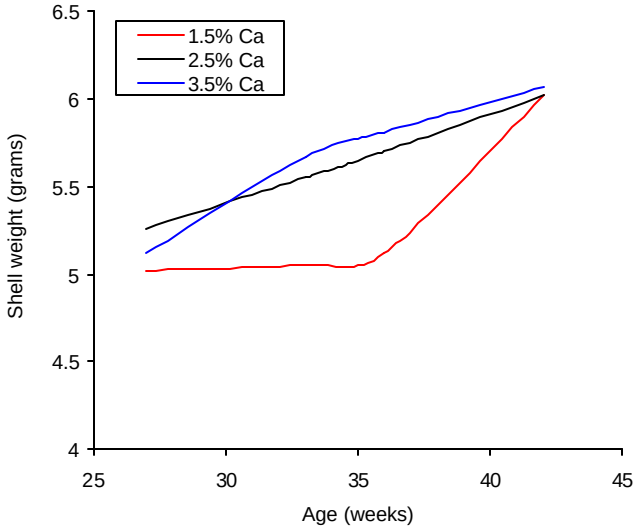

Figure 7.1 Effect of dietary Ca levels on shell weight

7.3.3 Calcium excretion

Dietary Ca had a significant (P<0.0001) effect on shell Ca excretion (Table 7.1 and Figure 7.2). As dietary Ca concentration increased from 1.5 to 3.5% there was a significant (P<.05) increase in shell Ca excretion. This was in accordance with the significant increase in shell weight. There were no differences (P>.05) in shell weight and shell Ca excretion of hens fed diets containing 2.5 and 3.5% Ca. As dietary Ca concentration increased from 1.5 to 2.5%, shell Ca excretion increased by 6.1 %. Smaller increases of less than 1% were noted when dietary Ca increased from 2.5 to 3.5%. Previous results of Hurwitz & Bar (1966) demonstrated that shell Ca excretion increased as dietary Ca increased from 1.7 to 3.7%. Atteh & Leeson (1983) reported that increasing dietary Ca content from 3 to 4.2% resulted in a non-significant increase in shell Ca excretion. This was confirmed by the present results.

The quantity of Ca deposited in the eggs increases slightly as the hen ages. For this reason, it is suggested that the hen's Ca requirement increases with age (Roland, 1986). Calcium excretion through the shell significantly (P<.0001) increased with age (Table 7.1 and Figure 7.2). The results of the present study are therefore in agreement with Roland (1986).

From Table 7.1 and Figures 7.3 and 7.4, it is clear that dietary Ca had a significant (P<.0001) effect on faecal Ca and total Ca excretions. Increasing the level of Ca in the diet from 1.5 to 3.5% resulted in increased faecal and total Ca excretions (Figures 7.3 and 7.4). The faecal Ca excretion significantly (P<.0001) declined at the 2.5 and 3.5% Ca levels over time. No clear trend could be detected for total Ca excretion over time.

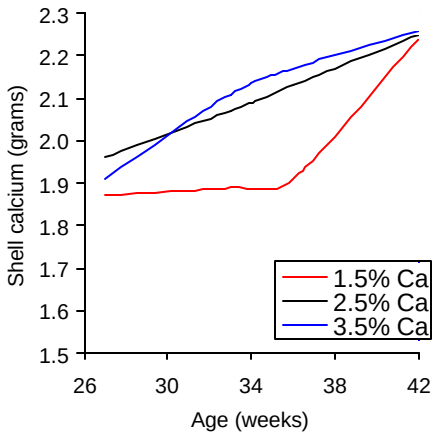

Figure 7.2 Effect of dietary Ca level on shell Ca excretion at different ages

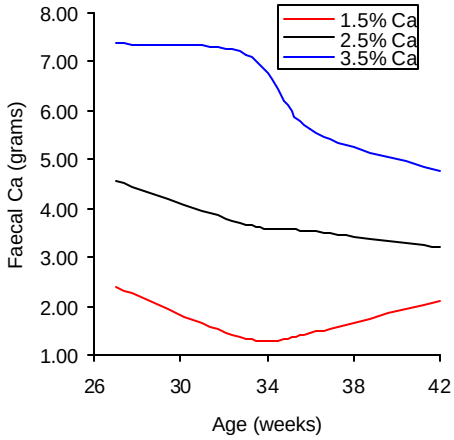

Figure 7.3 Effect of Ca level on faecal Ca excretion at different ages

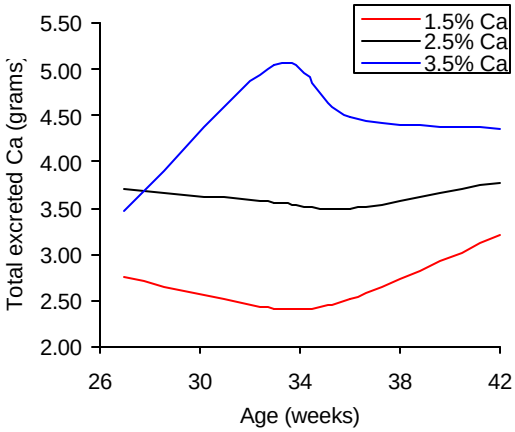

Figure 7.4 Effect of Ca level on total Ca excretion at different ages

In an effort to eliminate differences in Ca intake as a factor, Ca excretion and retention were expressed as a percentage of Ca intake. As indicated in Table 7.1 and Figures 7.5 to 7.8, statistically significant (P<.05) differences were observed when faecal Ca excretion, shell Ca excretion, total Ca excretion and Ca retention were expressed as percentage of Ca intake of hens. A significant Ca level x age interactions occurred for all the parameters investigated indicating that the influence of dietary Ca on these parameters varied during different periods. It was observed that faecal Ca excretion in relation to intake increased significantly (P<.05) with increasing dietary Ca concentration up to 3.5%. Shell Ca excretion as a percentage of intake showed the opposite results. This is probably an indication that Ca in the diet was less efficiently utilised for eggshell formation at the higher Ca levels. Total Ca excretion as a percentage of intake was at weeks 27 and 33 significantly (P<.0001) lower when 2.5% Ca level was included in the diet. At weeks 36 and 42, less total Ca as a proportion of intake was excreted when the higher Ca levels (2.5 and 3.5%) were included in the diet.

A non-significant (P>.05) influence of age on percentage faecal Ca excretion for birds on 1.5% Ca diets was noted throughout the test. At the 2.5 and 3.5% Ca levels, the percentage faecal Ca excretion for birds significantly (P<.05) declined with age.

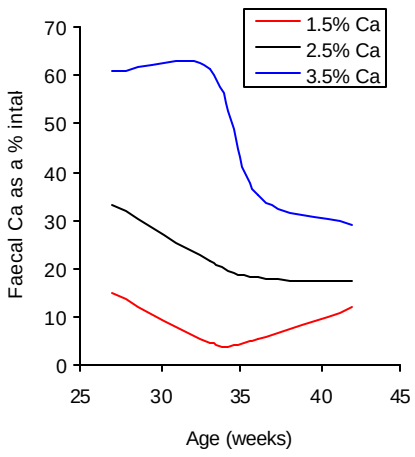

Figure 7.5 Effect of Ca level on faecal excretion as as a percentage of Ca

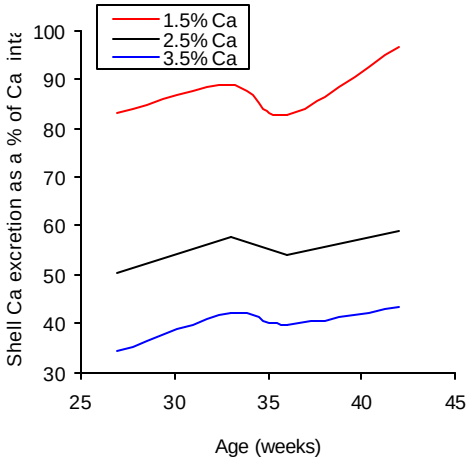

Figure 7.6 Effect of Ca level on shell Ca as percentage of Ca intake

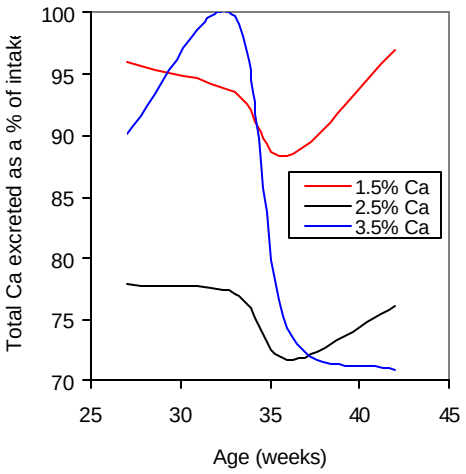

Figure 7.7 Effect of Ca level on total Ca excretion as as a percentage of Ca intake

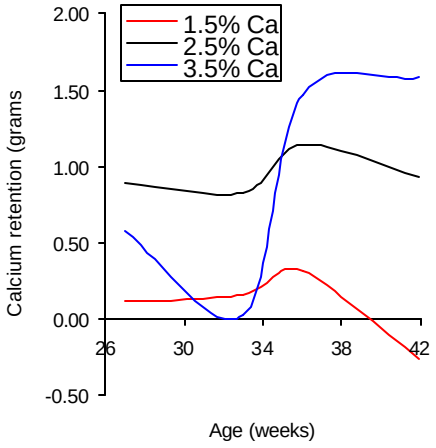

Figure 7.8 Effect of Ca level on Ca retention of broiler breeder hens

Percentage shell Ca excretion for birds on 2.5 and 3.5% Ca diets remained constant over time, while significantly (P<.05) increasing with age for birds on 1.5% Ca diets. Accordingly, total Ca excreted as a percentage of intake at the 1.5% Ca level at week 42 increased (P<.0001). The opposite results occurred at the 3.5% Ca level.

Calcium retention increased (P<.0001) with increasing dietary Ca levels at weeks 36 and 42. (Table 7.1). From Table 7.1 and Figures 7.8 and 7.9, it is, however, evident, that percentage Ca retention increased and/or was significantly (P<.05) higher at the 2.5% Ca level. The findings of Leeson & Summers (1997) showed that percentage Ca retention increased as dietary Ca level increased from 1.5 to 2.96%. These results demonstrated that feeding the 2.5% Ca diet resulted in greater percentage of Ca retained by the hens. This suggests that this level supplied enough Ca to support egg production and good shell quality as well as bone formation. In disagreement with these results, Summers *et al.* (1976) and Qin & Klandorf (1991) reported much higher Ca retention for the low Ca diet (1.5%) compared to the high Ca diet (2.96 to 3.5%) in broiler breeder hens and White Leghorn females, respectively. The differences in results may be ascribable to differences in type of birds (broiler breeders *vs*. commercial layers), dietary Ca levels, duration of the experiments and age of birds. Birds in the study of Qin & Klandorf were older (>60 weeks) compared to those used by Summers *et al.* (1976). In this regard, Atteh & Leeson (1983) reported a tendency for Ca retention to decline with increasing dietary Ca level from 3% to 4.2% for

laying hens over a 7-week period. These workers concluded that a high retention of Ca is probably associated with a high level of egg production and, hence a high Ca requirement. This argument seems to be supported by the egg production results reported in Chapter 6. In Chapter 5, it was observed that dietary Ca level had no significant effect on egg production. The age of birds and possibly bird strain or breed could have contributed to the differences in the results of the current and previous studies.

A significant (P<.0001) age effect for percentage total Ca excretion and percentage Ca retention was noted. No clear pattern could, however, be detected with increasing age.

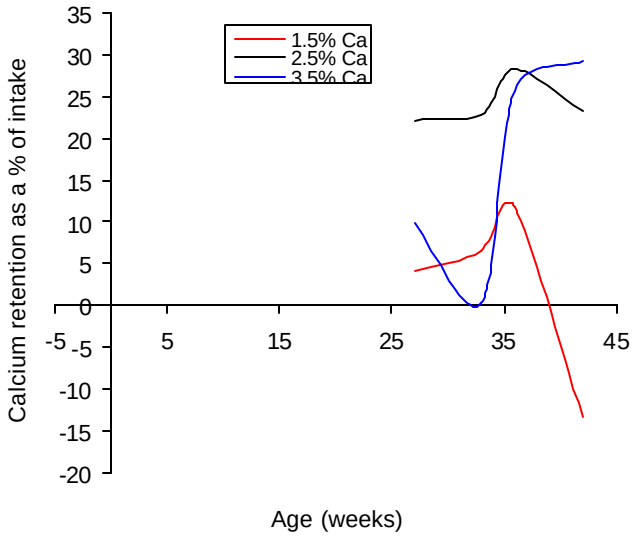

Figure 7.9 Effect of Ca level on Ca retention as a percentage of Ca intake

7.3.4 Correlation coefficients

The Ca retention of hens was correlated with age, Ca intake and eggshell characteristics as shown in Table 7.2. Data presented in Table 7.2 indicate that age was significantly (P<.01) correlated with all traits except daily Ca intake, Ca retention and shell percentage. The correlation coefficients were, however, low to moderate (r^2 less than 0.42). These results confirm previous results (North & Bell, 1990) that age is correlated to egg weight and shell thickness. In the current study, age was moderately correlated to egg weight (r^2=0.42) and lowly correlated to shell thickness (r^2=0.08) (Table 7.2).

Table 7.2 The correlations between age, calcium retention and egg characteristics of broiler breeder hens

Traits	Daily Ca intake	Daily Ca retention	Hen day production	Egg mass	Egg weight	Shell weight	Shell percent	ESA	SWUSA	Shell Ca excretion	Faecal Ca excretion	Average shell thickness
Age	0.01	0.04	0.18**	0.46***	0.65***	0.52***	-0.07	0.66***	0.17**	0.0.53***	-0.26***	0.27***
Daily Ca intake		0.78***	0.07	0.08	0.01	0.10	0.13*	0.002	0.13*	0.27***	0.50***	0.12
Daily Ca retention			0.13*	0.11	-0.01	-0.04	-0.04	-0.01	-0.05	-0.04	0.38***	-0.06*
Hen day production				0.89***	0.11	0.06	-0.05	0.11	-0.01	0.09	-0.001	0.01
Egg mass					0.52***	0.37***	-0.10	0.52***	0.08	0.35***	-0.06	0.15*
Egg weight						0.71***	-0.18**	1.00***	0.16*	0.58***	-0.11	0.27***
Shell weight							0.55***	0.71***	0.80***	0.74***	0.08	0.83***
Shell percent								-0.19**	0.94***	0.33***	0.25***	0.83***
ESA [1]									0.16**	0.58***	-0.11	0.27***
SWUSA [2]										0.55***	0.21**	0.94***
Shell Ca excretion											-0.05	0.60***
Faecal Ca excretion												0.16**

*P<05; **P<.01; ***P<.0001

[1]Egg surface area (cm^2)

[2]Shell weight per unit surface area (mg/cm^2)

Correlation analysis showed that daily Ca intake was positively significantly (P<.05) correlated to all traits studied except hen day production, egg mass, egg weight, egg surface area, and average shell thickness. In contrast with these results, Ousterhout (1980) reported that egg weight was highly significant and inversely related to dietary Ca level in commercial layers. The differences in results may be mainly attributable to differences in experimental conditions as well as dietary Ca levels and bird strains. In the study of Ousterhout (1980), Leghorn hens were fed three constant Ca levels (2.75, 3.75 and 4.75%) and two variable levels, decreasing from 3.75 to 2.75% and increasing from 3.75 to 4.75%, each in 0.25 steps every 8 weeks from 28 weeks to 68 weeks of age. Constant Ca levels were fed in the present study. A moderate correlation (r^2=0.61) was observed between daily Ca intake and Ca retention. This is in agreement with the significant (P<.0001) increase in Ca retention with increasing Ca levels as already discussed (Table 7.1).

As statistically significant (P<.01) but a low positive correlation was found between Ca retention and hen day production (r^2=0.04). Table 7.2 indicates that hen day production was significantly (r=0.89) correlated with egg mass. The highly significant (P<.0001) correlation (r^2=0.79) between hen day production and egg mass is to be expected as one is a function of the other. This also applies for the low (P<.05) correlation between egg mass (r^2 less than 0.27) and egg weight, shell weight, egg surface area, shell Ca excretion and shell thickness, respectively. Accordingly egg weight was moderate to highly significantly (P<.0001) correlated with shell weight (r^2=0.50), egg surface area (r^2=1.00) and shell Ca excretion (r^2=0.50). A recent study (Kul & Seker, 2004) also showed statistically significant correlation between egg weight, shell weight and average shell thickness. The results of the present study and those of Kul & Seker (2004) support a previous report of Stadelman (1989), which showed that shell thickness, is directly correlated to egg weight as well as shell weight. The low significant correlation (r^2=0.07) observed in the current study between egg weight and shell thickness is in disagreement with Hunt *et al.* (1977) who reported a negative correlation (r=-0.037) between these parameters. The differences between current and previous results could be due to species difference, age, strain of birds, diet (Ca content) as well as housing systems. According to Baumgartner (1994), genetic correlations between total egg weight and the weights of its component parts are well above 0.5. In the present study, these correlations vary

from low to high (0.10 to 1.0). The non-significant correlation between egg weight and egg production observed in the current study confirmed the earlier work of McDaniel *et al.* (1981) in broiler breeders aged 40 and 53 weeks. However, these workers found a significant negative correlation in force-moulted breeder hens.

Shell weight is significantly influenced by egg weight (Nordstrom & Ousterhout, 1982). These workers found that 47% of variation in shell weight was accounted for by egg weight while a slightly higher value (50%) was obtained in the present study. Roland (1979) explained that this indicated that as a hen lays larger eggs, the amount of the eggshell produced also increases, but not at a rate sufficient to maintain shell thickness.

The moderate to high (r^2=0.50 to 1.0) relationships between shell weight and egg surface area, SWUSA, shell Ca excretion and shell thickness, respectively is to be expected, as one is a function of the other. The same applies for the high correlation (r^2=1.0) between egg contents and egg surface area, shell percentage and SWUSA (r^2=0.88), shell percentage and shell thickness (r^2=0.69), as well as shell Ca excretion and shell thickness (r^2=0.69).

In the current study, shell percentage was negatively and lowly correlated (r^2=0.30) to egg weight, indicating that as egg weight increased, shell percentage tended to decline. This result confirmed to some extent the recent findings of Kul & Seker (2004) who studied the external and internal quality traits of eggs in Japanese quail. The results of the current study also support the view of Roland (1979) who stated that as a hen lays larger eggs, the amount of the shell produced also increases but not at a sufficient rate to maintain shell thickness. These workers suggested that the increase in shell percentage is less than the increase of other components that form the egg.

Richards & Staley (1967) suggested that shell thickness, shell weight, shell percentage and SWUSA, may be classified as shell quality measurements, as these variables are significantly correlated with each other. Accordingly, Richards & Staley (1967) obtained strong relationships among these quality measurements. With the exception of shell weight and shell percentage (r^2=0.30) a higher level of significance

(P<.0001) of these variables was observed in the current study than by Richards & Staley (1967). On the other hand, Pepper *et al.* (1968) found no significant differences in egg weight, shell weight and SWUSA for eggs studied in the last month of the experiment in which Shaver Starcross hens were fed diets containing Ca levels ranging from 3 to 6%. These workers concluded that failure to show a difference in shell weight or SWUSA would suggest that there is little or no correlation between these measurements of eggshell quality and that of specific gravity (an indicator of shell thickness). In disagreement with findings of Pepper *et al*. (1968), the results of the current study demonstrated that these variables are correlated (Table 7.2). The differences in results may be attributable to differences in strain of birds, dietary Ca levels (1.5 to 3.5% *vs.* 3 to 6% Ca in current and previous studies, respectively) as well as differences in experimental conditions.

Generally, eggshell thickness depends on the amount or weight of shell present in relation to the egg surface area (Carter, 1975). Nordstrom & Ousterhout (1982) stated that for shell thickness to increase, shell weight must increase, egg surface area must decrease, or a combination of these must occur. The results of the current study are in partial agreement with the findings of Nordstrom & Ousterhout (1982) because of positive and significant correlations obtained. Egg surface area in the present study was not negatively correlated to shell thickness, in disagreement with the findings of Nordstrom & Ousterhout (1982).

7.4 Conclusions

Shell (r=0.27) and faecal (r=0.50) Ca excretions were positively related to Ca intake of broiler breeder hens. Although not calculated, the same relationship was found for Ca intake and faecal Ca excretion as a percentage of intake. The opposite relationship was, however, observed between Ca intake and eggshell Ca excretion as a percentage of intake. Therefore, proportionally less of the Ca intake was used for eggshell formation as the intake of broiler breeder hens increased. The rest of the Ca intake could be utilised for bone formation and/or excreted through the faeces. According to Chapter 4, bone mineralisation was similar at different Ca intakes of layers. Therefore, it seems that a higher Ca intake was mainly accompanied by a higher Ca excretion through the faeces.

The net effect of Ca intake and total Ca excretion was that the 2.5% Ca level (3.8 g Ca/hen/day) exhibited a significantly ($P<.05$) higher Ca retention compared to 1.5% Ca level. In accordance with the results in previous chapters, this Ca level and/or intake will support egg production, good shell quality and sufficient bone formation in broiler breeder hens.

It further seems from the results that daily Ca retention was moderately ($r^2=0.61$) correlated with daily Ca intake. Daily Ca retention was, however, not indicative of any egg characteristic.

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CHAPTER 8

GENERAL CONCLUSIONS

Calcium is one of the key elements required for the formation and maintenance of bone as well as eggshell formation. This especially applies for broiler breeders where intense selection for fast growth and efficient feed conversion occur. Therefore, knowledge regarding the calcium requirements of broiler breeder pullets/hens during rearing (up to 22 weeks), earlier (23 to 35 weeks) and later (36 to 60 weeks) stages of the laying period is of utmost importance.

From the results of the present study, it seems that 1.0% Ca (12.1 MJ ME/kg) is adequate for normal bone development in broiler breeder pullets on restricted feed up to lay (22 weeks). This Ca level (average intake of 0.7 g/bird/day during rearing period) supported the desired body weight gain. Accordingly, bone dimensions (length, width and weight), bone stress and bone chemical composition (content of ash, calcium and phosphorus) were not detrimentally influenced by this dietary Ca level. The same applies for true cortical area and percent bone. According to regression analysis dietary Ca during the rearing period could influence shell weight, shell percentage and shell weight per unit surface area during the later stages of the laying period. These results were, however, not supported by bone characteristics results during the rearing and laying periods as well as General Linear Model statistical analysis procedure. This aspect needs further investigations by using a different experimental design and slaughtering more birds during the experimental period. Altering other nutrients in the diet like protein to influence bone development should also be investigated.

As expected, the *ad lib* feeding of pullets resulted in heavier body and bone weights. Although bones from the *ad libitum* group were stronger, the degree of bone mineralisation and percent bone was greater for the restricted groups.

It further seems that 2.5% Ca (3.5 – 4.0 Ca g/hen/day) is needed for growth, bone development and good egg quality of broiler breeder hens during the laying phase. Although 1.5% Ca (±2.2 g/hen/day) in the breeder diet has been shown to be adequate

to support bone development and bone mechanical properties (with the exception of bone strength), the feed intake and body weight of broiler breeders were lower. Therefore, 2.5% Ca (3.5 – 4.0 g/hen/day) in the diet is needed to ensure that the recommended feed intake and subsequent desired body weight are achieved.

In accordance with feed intake and body weight, 2.5% Ca (3.5 – 4.0 g/hen/day) is needed for egg production and good eggshell quality. During the earlier stages of laying period (23 to 35 weeks), 1.5% dietary Ca was sufficient to achieve maximum hen day production, egg weight and egg mass. More Ca, namely 2.5% in the diet was, however, needed during the earlier stages of the laying period to ensure the highest eggshell quality as determined by eggshell weight, shell weight per unit surface area (SWUSA), percentage shell and shell thickness. In the later stages of the laying period (36 to 60 weeks), 2.5% Ca in the breeder diet is required for maximum hen day production, eggshell weight and egg mass as well as eggshell quality. These findings were supported by a higher Ca retention in broiler breeders consuming the diet with 2.5% Ca compared with 1.5% Ca.

It therefore seems that 1.0% dietary Ca (average 0.7 g/pullet/day) is needed during the rearing period up to 22 weeks. Thereafter 2.5% Ca (3.5 – 4.0 g/hen/day) should be included in the broiler breeder diets up to 60 weeks.

During the rearing period bone weight, length and width increased up to 18 weeks as can be expected. Accordingly, mechanical properties, true cortical area and percent bone increased. The same increase was found from 35 to 60 weeks for the tibia length and opposite for the tibia width. The other bone dimensions, mechanical properties, true cortical area and percent bone showed no changes over time.

Egg production and egg mass increased from week 25 to 30 and thereafter declined. A clear increase in egg weight, egg contents, egg surface area and SWUSA occurred as the lay period progressed.

ABSTRACT

1. A study was conducted to investigate the effects of dietary Ca levels and feed restriction on the bone development of broiler breeder pullets up to 18 weeks of age. Six hundred and forty one-day-old Ross breeder pullets were randomly assigned to 4 treatment groups; namely 1.0% Ca (0.45% Pi), 1.5% Ca (0.7% Pi), 2.0% Ca (0.9% Pi) and 1.0% Ca (0.45% Pi). The first three treatments were feed restricted while the last treatment was fed *ad lib.* At 6, 12 and 18 weeks of age 20 pullets were randomly selected from each treatment and killed. Treatment effects on bone dimensions (length, width and weight), bone ash, Ca and P contents of tibia, mechanical properties (bone strength and stress) were investigated. Increasing dietary Ca levels had no significant ($P>0.05$) effect on bone measurements. As expected, all the bone measurements significantly ($P<0.05$) increased with age. Feed restriction significantly ($P<0.05$) reduced all the bone characteristics. The results suggested that 1.0% Ca (average 0.7 g/hen/day) is sufficient to support bone development and growth for feed restricted broiler breeder pullets up to 18 weeks of age.

2. One hundred and ninety eight Ross broiler breeder pullets were reared on restricted diets with 1.0, 1.5 and 2.0% Ca up to 22 weeks of age. The pullets in each experimental diet were further randomly divided into three treatments with 1.5, 2.5 and 3.5% dietary Ca (66 birds per treatment) fed from 23 to 60 weeks. At 35 and 60 weeks of age, 12 pullets per treatment were randomly selected and killed to obtain tibiae and humeri. Treatment effects on bone dimensions, bone ash, Ca and P content of tibia, mechanical properties, true cortical area (TCA) and percent bone were investigated. The 1.5% Ca diet resulted in lower ($P<.05$) feed intake and body weight. Calcium level had no effect on bone dimensions, bone stress, ash content, Ca content of ash, TCA and percent bone. These results suggest that the 2.5% dietary Ca (4 g/hen/day) may be adequate to support bone development and growth of feed restricted broiler breeder pullets.

3. The broiler breeder pullets described in paragraph 2 were used to investigate the effects of three levels of dietary Ca (1.5, 2.5 and 3.5%) during the early lay period (23 to 35 weeks) on egg production and eggshell quality. Increasing dietary Ca level from 1.5 to 3.5% had no significant effect ($P>.05$) on egg production, egg weight, egg mass, egg surface area and egg contents. Increasing the level of Ca from 1.5 to 2.5%

increased shell weight, shell weight per unit surface area, shell percentage and shell thickness. As anticipated, egg weight increased with age. The converse was true for egg production and eggshell parameters. It was concluded that a dietary Ca level of 2.5% and Ca intakes of 3.8, 3.9, and 3.5 g at weeks 27, 30 and 33, respectively were sufficient to sustain good eggshell quality of feed restricted broiler breeder hens.

4. The mentioned broiler breeder hens in paragraph 3 were fed three dietary Ca levels, 1.5, 2.5 and 3.5% from week 36 to 60. Regression analysis suggested that Ca level during the rearing period could positively influence eggshell quality during the later stages of lay (36 to 60 weeks). Increasing dietary Ca level from 1.5 to 2.5% during the laying period significantly ($P<.05$) increased egg production, egg weight, egg mass, shell weight, egg contents, egg surface area, shell percentage, shell weight per unit surface area and shell thickness. As anticipated, egg weight, shell weight, egg contents and egg surface area increased with age while egg production, egg mass, shell percentage and shell thickness declined. These results suggest that the 2.5% Ca (3.8 g/hen/day) is adequate to support egg production and to improve eggshell quality in feed restricted broiler breeder hens.

5. The effect of dietary Ca intake (1.5, 2.5 and 3.5% dietary Ca) on Ca retention of broiler breeder hens (30 per treatment) and the relationship between Ca retention and egg characteristics was investigated. Shell ($r=0.27$) and faecal ($r=0.50$) Ca excretions were significantly ($P<.05$) positively related to Ca intake of broiler breeders. Proportionally less of the Ca intake was used for eggshell formation as the intake of broiler breeders increased. It seems that a higher Ca intake was mainly accompanied by a higher Ca excretion through the faeces. The net effect of Ca intake and total Ca excretion was that the 2.5% Ca level (3.8 g Ca /hen/day) exhibit a significant ($P<.05$) higher Ca retention compared to 1.5% Ca.

It was concluded from the results that 1.0% dietary Ca (0.7 g /pullet/day) is needed during the rearing period up to 22 weeks. Thereafter 2.5% Ca (3.5 to 4.0 g /hen/day) should be included in broiler breeder diets up to 60 weeks. The possible effect of Ca levels during the rearing period on eggshell quality needs further investigation.

OPSOMMING

1. ’n Studie is uitgevoer om die effek van kalsiumpeile en voerbeperking op beenontwikkeling van jong braaikuikenhenne tot 18 weke na te gaan. Seshonderd een- en veertig dagoud Ross braaikuikenhenne is ewekansig aan vier behandelings toegeken naamlik: 1.0% Ca (0.45% P), 1.5% Ca (0.7% P), 2.0% Ca (0.9% P) en 1.0% Ca (0.45% P). Die eerste drie behandelings is beperk gevoer en die laaste groep *ad lib*. Op 6, 12 en 18 weke is 20 henne ewekansig per behandeling geselekteer en geslag. Behandelingseffekte op beenafmetings (lengte, wydte en gewig), beenas, Ca- en P-inhoud van tibia, beenbreeksterkte, beenspanning en porositeit is ondersoek. ’n Verhoogde dieet Ca-peil het beenafmetings nie betekenisvol ($P>0.05$) beïnvloed nie. Soos verwag het alle beenmetings betekenisvol ($P<0.05$) met ouderdom toegeneem. Volgens die resultate is 1.0% dieet Ca (gemiddeld 0.7 g/hen/dag) voldoende vir beenontwikkeling en groei van voerbeperkte braaikuikenhenne tot op 18 weke ouderdom.

2. Eenhonderd agt-en negentig Ross braaikuikenhenne is op innamebeperkte diete met 1.0, 1.5 en 2.0% Ca tot op 22 weke grootgemaak. Die jonghenne van elke eksperimentele dieet is verder ewekansig in drie behandelings met 1.5, 2.5 en 3.5% dieet Ca (66 henne per behandeling) ingedeel en vanaf 23 tot 60 weke gevoer. Op 35 en 60 weke is 12 jonghenne ewekansig per behandeling geslag om die tibia en humerus te verkry. Behandelingseffekte op beenafmetings (lengte, wydte en gewig), beenas, Ca en P-inhoud van tibia, meganiese-eienskappe (beensterkte en-spanning), ware skorsoppervlakte en persentasie been is ondersoek. Die 1.5% Ca-dieet het voerinname en liggaamsgewig verlaag ($P<0.05$). Ca-peil het geen invloed op beenafmetings, been sterkte, asinhoud, Ca-inhoud van as, skorsoppervlakte en persentasie been uitgeoefen nie. Volgens die resultate is 2.5% dieet Ca (4 g/hen/dag) nodig om beenontwikkeling en groei van voerbeperkte braaikuikenhenne te ondersteun.

3. Die jong braaikuikenhenne soos beskryf in paragraaf 2 is gebruik om die effek van dieet Ca (1.5, 2.5 en 3.5%) gedurende die lêperiode (week 23 tot 35) op eierproduksie en eierdopkwaliteit te ondersoek. ’n Toenemende Ca-peil vanaf 1.5 tot 3.5% het geen betekenisvolle ($P>0.05$) invloed op eierproduksie, eiergewig, eiermassa, eieroppervlakte, eiervolume en eierinhoud uitgeoefen nie. ’n Verhoging

van Ca-peil vanaf 1.5 tot 2.5% het eierdopgewig, dopgewig per eenheidsoppervlakte, persentasie dop en eierdopdikte verhoog. Soos verwag het eiergewig met toenemende ouderdom verhoog. Die teenoorgestelde het gegeld vir eierproduksie en eierdop parameters. Daar is tot die slotsom gekom dat 'n dieet Ca-peil van 2.5% en Ca-inname van 3.8, 3.9 en 3.5 g gedurende onderskeidelik weke 27, 30 en 33 voldoende sal wees vir goeie eierdopkwaliteit van voerbeperkte braaikuikenhenne.

4. Die genoemde braaikuikenhenne in paragraaf 3 is aan drie dieet Ca-peile, naamlik 1.5, 2.5 en 3.5% vanaf weke 36 tot 60 onderwerp. Regressie ontledings dui daarop dat Ca-peile gedurende die grootmaakperiode, eierdopkwaliteit gedurende die latere stadiums van die lêperiode (36 tot 60 weke) positief kan beinvloed. 'n Verhoging van Ca-peil vanaf 1.5 tot 2.5% gedurende die lêperiode het eierproduksie, eiergewig, eiermassa, dopgewig, eierinhoud, eieroppervlakte, persentasie dop, dopgewig per eenheids oppervlakte en dopdikte betekenisvol ($P<0.05$) verhoog. Soos verwag het eiergewig, dopgewig, eierinhoud en eieroppervlakte met ouderdom toegeneem terwyl eierproduksie, eiermassa, persentasie dop- en dopdikte verlaag het. Die resultate dui daarop dat 2.5% Ca (3.8 g/hen/dag) benodig word om eierproduksie te ondersteun en eierdopkwalitiet van braaikuikenhenne te verhoog.

5. Die effek van dieet Ca-inname (1.5, 2.5 en 3.5% dieet Ca) op Ca-retensie van braaikuikenhenne (30 per behandeling) en die verwantskap tussen Ca-retensie en eiereienskappe is ondersoek. Dop- ($r = 0.27$) en mis ($r = 0.50$) Ca-ekskresie was betekenisvol ($P<0.05$) positief gekorreleerd met Ca-inname van braaikuikenhenne. Proporsioneel is minder van die Ca-inname vir eierdopvorming gebruik soos Ca-inname van henne verhoog het. Dit blyk dat 'n hoër Ca-inname hoofsaaklik met 'n groter Ca-uitskeiding in die mis gepaard gegaan het. Die netto effek van Ca-inname en totale Ca-ekskresie was dat die 2.5% Ca-peil (3.8 g Ca/hen/dag) 'n betekenisvol ($P<0.05$) hoër Ca-retensie in vergelyking met 1.5% Ca getoon het.

Daar is tot die slotsom gekom dat 1.0% dieet Ca (gemiddeld 0.7 g/hen/dag) benodig word gedurende die grootmaakperiode tot 22 weke. Daarna moet 2.5% Ca (3.5 tot 4.0 g/hen/dag) in diete vir braaikuikenhenne tot 60 weke ingesluit word. Die moontlike invloed van Ca-peile gedurende die grootmaakperiode op eierdopkwaliteit vereis verdere ondersoek.