Chapter 1

Literature Review

1.1 General Introduction

Over the past few decades, production performance of modern commercial laying hens has improved considerably. The egg industry makes a large contribution to the Australian economy about $785.6 m in the 2013/2014 financial year (AECL, 2014). This industry strives to improve production performance such as egg production and egg quality through breeding, nutrition, and management. Various factors, such as genetics, housing, vaccination, lighting, nutrition, induced moult, ambient temperature and processing, may affect the productivity in egg production.

Body weight of birds is an important criterion for optimizing on-farm performance in the poultry industry. When nutrient requirements for individual birds are more homogeneous, there is less variability and a smaller safety margin is needed to meet the requirements of all birds (Madsen and Pedersen, 2010). All nutrients should be present in sufficient amounts to cover requirements for optimal growth, so that feed costs can be reduced and bird performance improved (Madsen and Pedersen, 2010). Larger body weight and increased fat accumulation have led to leg problems, early onset of sexual maturity, accelerated ovarian follicular development, and the incidence of multiple hierarchies and multiple ovulations (De Beer and Coon, 2007).

Flock uniformity is a major goal for achieving maximum performance for broiler breeders and egg producers. The aim is to have 80% of pullets within plus or minus 10 per cent of the average flock body weight. Parkinson *et al*. (2007) studied the influence of flock uniformity in several layer commercial farms and found that flocks recorded had an average body weight 100-300 g above the breed standards, which indicated obesity. These obese birds produced excessively large eggs which resulted in lower eggshell quality (Parkinson *et al*., 2007). Obese birds started laying earlier, produced fewer eggs and a greater percentage of double-yolked eggs (Abbas, *et al*., 2010). Poor uniformity is associated with variation in the degree of sexual maturity of hens, where underweight pullets have delayed onset of egg weights and egg production (Gilbert, 1983). On the other hand, a good uniform flock results in lower mortality and good feed conversion (Boerjan, 2004). Body weight uniformity and average body weight are related to one another.

Eggshell quality is an important factor for consumer appeal and for ensuring that each egg remains intact throughout the production chain. In addition, the economic success of a producer depends on the total number of eggs sold. Egg internal quality is increasingly important to the egg industry, since supermarkets set minimum standards for albumen quality (Roberts and Ball, 2003).

A number of factors influence eggshell quality and egg internal quality. These include strain of hens (Tůmová, *et al.*, 2009), the housing system in which the hens are kept (Đukić-Stojčić, *et al*., 2009; Tůmová *et al*., 2009), the age of the laying hens (Roberts and Ball, 2003; Silversides *et al*., 2006), nutrition, disease, environmental conditions and stress (Roberts, 2004).

1.2 Body weight and laying performance

Body weight is known to be correlated with egg size. Pullets are grown to attain a certain body weight at a particular age, in line with the recommendations of the particular breeder company. There is a direct relationship between the pullet's development during rearing and subsequent performance during the laying cycle. Body weight has been demonstrated to influence egg production, particularly egg weight and feed intake (Harms, *et al*., 1982; Bish *et al*., 1985).

Problems occurring during the early part of lay can be traced back to insufficient or improper body weight attained during the various stages of the growing period (Miles and Jacob, 2011). Leeson and Summers (1987) observed a correlation between body weight and age at maturity. They concluded that immature body weight at 15-19 weeks of age can influence egg weight, with each 100 g increase in body weight being associated with 3.5 g increase in feed intake and 1.2 g increase in egg weight. Variation exists throughout the growing period with respect to nutritional demands for the various tissues and organs (Miles and Jacob, 2011). Underweight pullets approaching peak egg production simply cannot consume enough energy each day to maintain or even attain peak production. According to Miles and Jacob (2011), pullets at this stage will use their body stores of fat or protein to furnish the energy in an attempt to produce eggs at their full genetic potential. This explains why immature body weight at point of lay is a major factor influencing subsequent egg size. Blokhuis and Van der Haar (1989) reported that egg-type chickens must reach a minimum age and body weight before they can commence egg production. Lacin, *et al*. (2008) reported that hen body weight significantly affected some parameters of laying performance and egg quality such as shape index, yolk colour, albumen index and Haugh

Unit, but was not a significant influence on shell strength, shell thickness and yolk index. In their study, the light body weight group had higher egg production, HU and better feed conversion ratio.

1.3 Flock uniformity

Uniformity is a measurement of the extent of body weight variation in a flock. Flock uniformity is the percentage of birds which are within ±10% or ±15% of the average flock body weight recommended for a particular age (Abbas *et al*., 2010). The main reasons for flock variation are related to the breed and to farm management (Madsen and Pedersen, 2010). Uniform flocks with the correct body weight give several benefits: birds are managed in a large group and are more efficiently affected by management changes (lighting, feeding and housing). Haider and Chowdhury (2010) found the uniformity of commercial brown layer chicks (Shaver 579) at 8-17 weeks of age achieved an average of 84% which was higher than minimum standards (80%) provided by the Shaver 570 Management guide. Flocks with high uniformity have been reported to reach peak egg production earlier and have higher peak production than flocks of low uniformity (Kosba *et al*., 2009). A more uniform flock exhibited significantly higher egg production than the less uniform flock (Petitte, *et al*., 1982). On the other hand, within non-uniform flocks, heavy hens will cause economic loss because of their decreased production, over-consumption of feed and poorer shell quality (McDaniel, *et al*., 1981). Uniformity based on chick body weight can be used to predict mortality in the first week of age (Boerjan, 2004).

Control of mature body weight during rearing is likely to have a major influence on egg size, especially if management and feed programs are designed to bring pullets into production at an early age (Leeson and Summer 1987). Dunnington and Siegel (1984) and Summers, *et al*. (1987) also showed that pullets must achieve a minimum age and body weight before commencing egg production. A similar situation occurred for meat-type chickens and Japanese quail (Dunnington and Siegel, 1984). Petitte *et al*. (1982) reported significantly larger eggs from heavier birds in cages. Miles and Jacob (2011) noted that the two most important criteria of pullet quality are uniformity within the flock and correct body weight at a specific age. Almost anything that adversely affects a pullet will usually be reflected in lower body weights and poorer flock uniformity.

1.4 Factors affecting uniformity

The main reason for poor uniformity is management, particularly feed, lighting, hatching egg size, diseases and parasites, and environmental factors such as house temperature and ventilation (Abbas, *et al*., 2010). Feed is recognized as the major cost of production, which accounts for more than 70% of the total cost. Any savings in feed consumption will usually increase the profit margin. Therefore, reducing feed consumption is an obvious objective in order to reduce production costs, especially when pullets are bought from outside suppliers. All nutrients should be present in sufficient quantities to meet requirements for optimal growth. Lordelo *et al*. (2004) reported that feeding a diet with cotton-seed meal as the major protein source during the rearing period of broiler breeder pullets improved body weight uniformity without adversely affecting future reproductive fitness. Pullets with similar body weight commence egg production at the same time and lay eggs of uniform weight. A skip-a-day feed restricted program has also been reported to improve flock uniformity in broiler breeder hens (De Beer and Coon, 2009). Segregating pullets based on their body weight can be used to control body weight and flock uniformity (Petitte *et al.*, 1981). Moreover, feed restriction could also be used without an associated decrease in body weight uniformity.

For optimal growth, poultry also require intensive light during rearing and production. Artificial lighting in the laying house should provide a 16-hour day once birds are in production (Scanes *et al*., 2004). Leeson *et al*. (2005) investigated the general responses to various step-down lighting regimes during the rearing period and subsequent rates egg production of Shaver White pullets. The lighting regimes were either an 8 hour day length or a step-down lighting regimen from 23 hours to 8 hour over periods of between 1 and 15 weeks. Other pullets, which were initially maintained on 8 hours of light, were given an abrupt increase in day length prior to transfer to step down lighting at various ages between 1 and 13 weeks. All birds were changed to 14 hour day lengths at 18 weeks of age and 16 hour at 20 weeks of age and throughout the experiment. These authors pointed out that body weight uniformity of pullets at 18 weeks of age can be improved by step-down lighting. Poultry producers should keep records of the body weight, flock uniformity, vaccination schedule, feeding program, lighting, environmental conditions during grow-out, and the general management of their pullets (Miles and Jacob, 2011).

1.5 Measurement of body conformation of laying hens

A range of techniques is available to gain information about an animal's body mass and body composition. Some of these techniques use simple, inexpensive equipment, and others require sophisticated, expensive equipment.

Body composition analysis measures the total mass of each component. It is a dynamic variable that often shows a high level of variation (Reynolds and Kunz, 2001). However, because there is variation between individuals in total body mass, differences in these mass-dependent measures may not reflect differences in relative body composition. Mass-independent variables are often used to control the effects of variation in body mass between individuals (Reynolds and Kunz, 2001).

Body weight is a function of skeletal size, fleshing (muscle) and condition (fat) (Tierce and Nordskog, 1985). Fat represents the major energy storage compartment of animals, and is therefore often the focus of body composition studies. However, because fat mass is the most variable aspect of an individual's body composition, it is the most difficult component to estimate (Reynolds and Kunz, 2001). Fat is the critical controlling factor for onset of puberty and lay in pullet development (Kwakkel, *et al.*, 1995). Body composition is a dynamic variable that often shows a high level of variation. When it has been studied, variation in body composition has been found at the level of the individual, population, and species. Limiting body weight may actually involve a specific body composition such as a minimum amount of body fat or body lean tissue to permit sexual maturity (Blokhuis and Van der Haar, 1989).

1.6 Application of computed tomography

The use of computerized tomography to predict body composition in poultry has been reported by Bentsen and Sehested (1989), Svihus and Katie (1993), and Andrassy-Baka *et al*. (2003). Computerized tomography (CT) is a method of describing the density in a cross-section of an object. Young *et al*. (2001) reported that CT scans generate uniform images in high resolution, conjugated with very good distinction between fat, lean and bone.

Computerized tomography works on the principle of acquiring information based on the X-ray radiation being transmitted in many directions through the cross-sectional plane of the object (Svihus and Katie, 1993). These transmitted radiations account for the linear attenuation

coefficient which is transformed to a matrix element which gives the X-ray absorption in CT values (Bentsen and Sehested, 1989). The CT values can range from -1023 (no absorption) to 1024 (total absorption) (Bentsen and Sehested,1989), with zero for water and -1024 for air; -10 to -200 for fat; 20 to 90 for lean tissue and 1000 for bone density (Thompson and Kinghorn, 1992).

A compatible standard software is required to display the images and measure area and specific density of each "slice" produced by the CT scanner. OsiriX (Rosset *et al*., 2004), Image J (Abramoff *et al*., 2004; McEvoy, 2007) and Catman (Thompson and Kinghorn, 1992) are programs used to analyse the CT values.

OsiriX is an open-source software that is more compatible for navigating through a large set of image data, which is used to display digital images from the CT scanner in DICOM format (Rosset *et al*., 2004). However, OsiriX also recognises many file formats include TIFF, JPEG, PDF, AVI, MPEG and Quicktime (Limberg, 2008)

In a study by Purushothaman *et al.* (2013), a closed polygon region of interest (ROI) was drawn to remove extraneous objects such as the fiberglass cradle from each of the CT images. The area outside the ROI was set to −1024 (air). This new setting deleted the area outside the ROI and allowed ROI to be exported and saved in 16-bit black and white image in DICOM format. The saved images were then processed using ImageJ.

ImageJ is also an open-source software base on Java Image (Abramoff *et al*., 2004). It is used to convert 16-bit CT images to 8-bit binary images. This modification was a prerequisite for the next image analysis program used (Abramoff *et al*., 2004).

Catman is software which is able to display an image and allows the specific area of tissue and organ to be calculated and stores the results in an ASCII file (Thompson and Kinghorn, 1992). The program partitions the CT images into fat, lean, and bone, based on the Hounsfield units range for each tissue and measures their area, mean pixel value, and variance. Data from the scanner have to be converted from the 16-bit CT format to an 18-bit binary image format and the CT values will be rescaled to a 256 grey scale.

1.7 Bone types

Bone is made up of hydroxyapatite crystals of calcium phosphate deposited on an organic collagen matrix (Whitehead and Fleming, 2000). There are several different bone types in laying hens. The main types providing structural integrity are cortical and cancellous (or trabecular) bone, both of which are forms of lamellar bone. These bone types are formed during growth but, when a hen reaches sexual maturity, a third type of non-structural bone, medullary bone, is formed (Whitehead and Fleming, 2000). After formation, bone undergoes a constant process of remodelling, in which osteoclast cells resorb areas of bone and are then replaced by osteoblasts that deposit new bone. Osteoporosis arises where there is an imbalance between these processes, resulting in a net resorption of structural bone (Whitehead, 2004a).

Medullary bone is described as a secondary bone tissue that develops within marrow cavities of long bones. It is a woven bone whose purpose is to provide calcium for the eggshell during formation, and has minimal biomechanical function (Kim *et al*., 2005; Kim *et al*., 2012; Dacke *et al*., 2015). It is characterized by the haphazard organization of collagen fibres in its matrix and is mechanically weaker than structural bone types. The highest content of medullary bone is usually found in the leg bones (Whitehead, 2002). The amount of medullary bone varies from a partial filling around the periphery of the cortical cavity to complete filling of the cavity. Measurements of humeral bone three-point breaking strength have been shown to be highly correlated with the amount of humeral medullary bone present (Fleming *et al*., 1998a). Medullary bone may thus make some contribution to the overall fracture resistance of bone, although not to the same degree as structural bone.

Skeletal problems in laying hens are important issues of welfare, health, and economic issue for the poultry industry. In the mid-20th century, cage layer fatigue was first noticed shortly after laying hens were housed in cages. This condition was associated with osteoporosis and bone brittleness (Webster, 2004). Structural bone loss related to osteoporosis is the major skeletal problem in laying hens (Gregory and Wilkins, 1989). Osteoporotic hens show evidence of widespread loss of structural bone throughout the skeleton (Whitehead and Fleming, 2000). Osteoporosis in laying hens is defined as a decrease in the amount of fully mineralized structural bone, leading to increased fragility and susceptibility to fracture (Whitehead and Fleming, 2000) and it arises where there is an imbalance between these processes, resulting in a net resorption of structural bone. A number of predisposing causes of bone breakage in laying hens has been

investigated (Knowles and Wilkins, 1998; Webster, 2004; Whitehead, 2004b; Koutoulis *et al*., 2009).

Bone quality is closely related to egg production and eggshell quality. High egg-producing hens are susceptible to osteoporosis (Fleming *et al.,* 2006). Estrogen activity is related to the onset of osteoporosis, which stimulates medullary bone formation for eggshell formation, contributing to weakened skeleton strength (Fleming *et al*., 1998b). This is in contrast to another cause of bone mineral loss, osteomalacia, in which defective mineralization of bone tissue occurs, with thick seams of poorly mineralized organic matrix. Both conditions will lead to poor quality bone, but osteomalacia is primarily associated with nutritional deficiencies of calcium, phosphorus, or vitamin D, whereas osteoporosis is a more complex problem.

Strain differences in susceptibility to bone breakage have been described (Whitehead, 2002, 2004 a,b), possibly associated with differences in body size or egg production, and genetic selection for skeletal characteristics has been shown to be effective (Fleming *et al*., 2004).

1.8 Calcium metabolism

Calcium and phosphorus are the most important mineral nutrients affecting eggshell quality. Bouvarel, *et al*. (2011) asserted that hens must consume about 4 g calcium per day which takes into account the mean calcium retention and an average of 2.2 g calcium per egg. The limited supply of calcium will affect the shell strength, egg production, and will increase mortality (Sherwood *et al*., 2013). During shell formation, an amount of calcium equivalent to 8-10 % of the total calcium in hen's body is secreted into the shell (Sherwood *et al*., 2013). Furthermore, PTH (parathyroid hormone) can rapidly mobilize calcium from the medullary bone whenever the rate at which calcium deposition on the shell exceeds the rate at which it is absorbed from the intestinal tract (Sherwood *et al*.,2013)

Active shell formation occurs during the night, therefore the further act of eating towards the end of the day is to ensure an adequate supply of calcium in the gut contents while shell formation is actually taking place (Fleming *et al*., 2006).

1.9 Structure of the ovary and oviduct

The female reproductive system of the chicken is divided into two separate parts: the ovary and the oviduct. Only the left ovary and oviduct of chickens are functional (Scanes, *et al*., 2004). The left ovary develops within the abdominal region ventral to the caudal vena cava and adjacent to the left kidney and adrenal gland. The developing ovary is eventually suspended from the body wall, primarily by the mesovarium (Johnson, 2015).

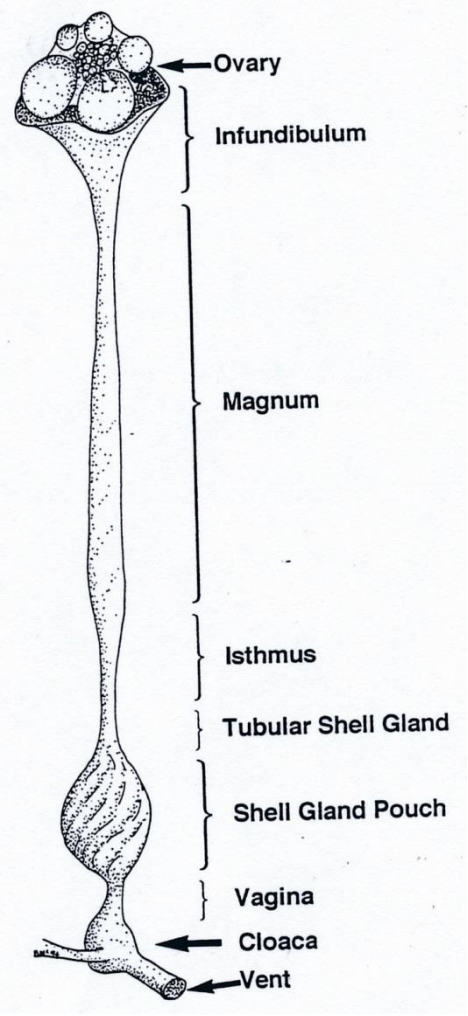

Figure 1.1. Structure of the oviduct (source: Roberts and Brackpool (1995)

The right ovary and oviduct typically regress during development and are non-functional in the adult bird. The ovary of immature birds consists of a mass of small ova, of which as many as 2000

are visible to the naked eye. At the time of hatch, avian oocytes are mostly in meiotic prophase I. Only a relatively few of these (250-500) will reach maturity and ovulate within the life span of most domesticated species (Johnson, 2015). When mature, the yolk is released from the follicle by rupture of the follicle wall along a line called stigma (Scanes *et al*., 2004).

The left oviduct of the hen develops rapidly after 16 weeks of age and becomes fully functional just prior to the onset of egg production (at approximately 20 weeks). The oviduct is suspended within the peritoneal cavity by dorsal and ventral ligaments and consists of five distinguishable regions: infundibulum, magnum, isthmus, tubular shell gland, and the vagina, (Figure 1.1). Each region is functionally and morphologically distinct in the formation of the whole egg. The ovum is engulfed by the infundibulum and resides for approximately 18 minutes (range 15-30 minutes), then travels to the magnum, the largest portion of the oviduct, which generates the albumen. The ovum remains in the magnum for approximately 3 hours. The developing egg then passes from the magnum to the isthmus, which produces the fibres that make up the inner and outer shell membranes that enclose the egg albumen, over about one hour. Next, the egg enters the tubular shell gland where water and electrolytes are added to the albumen (a process called 'plumping') over approximately 5 hours (Roberts, 2004). It is here that the first calcite crystals nucleate on specific sites (mammillary knobs) on the outer shell membrane composed of organic aggregates (Gautron and Nys, 2006; Dacke, *et al.*, 2015). Then, the incomplete egg moves to the shell gland pouch where it remains for at least 15 hours to complete the process of shell formation (Roberts, 2004). In the shell gland pouch, calcite crystal growth continues outward to give rise to the mammillary and palisade layers (Gautron and Nys, 2006). The deposition of calcium carbonate on the outer membrane fibres occurs in the space between the dilated shell membranes that envelop the hydrated albumen and the mucosa of the uterine wall (Dacke *et al*., 2015). The egg rotates during the linear deposition of calcium carbonate as the mammillary and palisade layers are sequentially formed (Nys, *et al*., 2004). One and half hours before oviposition, mineralization stops and finally, a thin-non calcified layer, the cuticle, coats the eggshell and the egg is laid via the vagina and cloaca (Nys *et al*., 2004; Roberts, 2004; Dacke *et al*., 2015). Within 30 minutes after the egg is laid, another ovum/yolk is released from the ovary to be laid on the following day (Scanes *et al*., 2004).

1.10 Egg defence

The egg possesses both physical and chemical defences to resist bacterial contamination as well as temperature variations and harsh physical condition (Rose-Martel and Hincke, 2013). The first one is the eggshell, together with the cuticle and membranes. For the hatching egg, the main role of the eggshell is to shield the embryo from external aggression, and must be compatible with easy breakage from inside to allow hatching of the embryo. Moreover, the eggshell structure must permit water and gas exchange, and provide essential compounds such as a source of calcium for the developing embryo (Nys *et al*., 2004; Gautron *et al*., 2007). In the case of the table egg, the shell function as a food packaging material must remain intact from point of lay, along the production process, to the consumer (Fraser *et al*., 1999).

The second natural defence of the egg is a chemical barrier consisting of proteins which exhibit anti-microbial activity found in the albumen and, to a lesser extent, in the other compartments of the egg (yolk and shell) (Gautron and Nys, 2006).

1.11 Egg structure

The egg is composed of a central yolk surrounded by the perivitelline membrane, albumen, eggshell membranes, calcified eggshell and cuticle (Roberts, 2004; Mikšík *et al*., 2010). The components of an avian egg and their proportionate parts of the total weight are yolk, 32%; albumen, 57%; and shell, 11% (Johnson, 2000). The shell is separated from the albumen by the shell membranes, and the yolk is separated from the albumen by the yolk membrane (vitelline membrane).

1.11.1 The yolk

The yolk is formed in the ovary during the ten to twelve days prior to the laying of the egg. It consists of the latebra, germinal disk, and concentric layers of light and dark surrounded by the vitelline membrane (Figure 1.2) (Stadelman, *et al*.,1995).

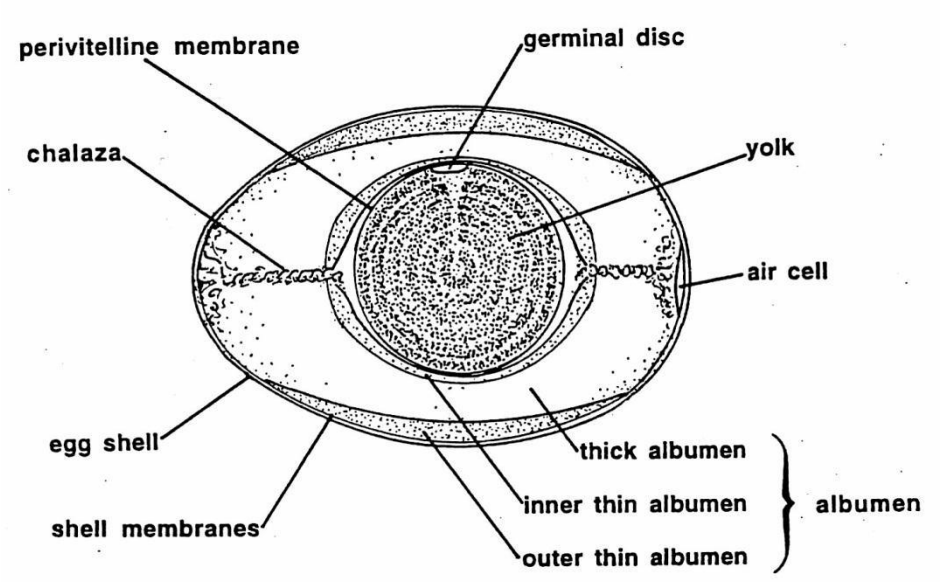

Figure 1.2. Schematic diagram of egg structure (source: Roberts and Brackpool, 1995)

The germinal disc is a small white spot about 2 mm in diameter on the surface of the yolk (Jacob *et al*., 2000). The yolk serves as a food for embryonic development and makes up 31 % of the total egg weight, which is consists of 33% lipid, 17% protein and approximately 1% free carbohydrates and inorganic elements (Johnson, 2000). The yolk is held together inside a fine elastic cover called the vitelline membrane. At the time of ovulation, the yolk sac, called the follicular membrane, releases the fully developed yolk into the open upper end of the oviduct (Stadelman, *et al*.,1995).

1.11.2 The albumen

Surrounding the yolk is the albumen, which is commonly called the egg white. It is a clear jelly-like substance which makes up approximately 60% of the total egg weight (Stadelman, *et al*.,1995) and consists of 88% of water, about 9%-11% of protein, 0.4-0.9% of carbohydrates, 0.5-0.6% minerals and a small amount (0.03%) of lipids.

There are four discrete layers of albumen in the egg: chalaziferous layer (2.7%) attached to the yolk; the inner thin layer (6.8%); the outer thick layer (57.3%) and the outer thin layer (23.2%) (Figure 1.2.) (Johnson, 2000).

The major proteins in the egg white are ovalbumin, 54%; ovotransferrin, 13% (binds iron, zinc, and copper); ovomucoid, 11% (inhibits protease); ovoglobulins, 8% (antibodies); lysozyme, 3.5%

(an enzyme that lyses or breaks down bacteria); and ovomucin, 2% (antimicrobial) (Scanes *et al*., 2004). An additional 16 proteins were identified; among them, two have never previously been detected in hen egg white: Tenp, a protein with strong homology with bacterial permeability-increasing protein (BPI), and VMO-1, an outer layer vitelline membrane (Guérin-Dubiard *et al*., 2006). Studies by Mann (2007) identified about 78 specific gene products. One year later, D'Ambrosio *et al*., (2008) identified 148 proteins in egg white, including the complete ovomucin β sub-unit and the N-terminal sequence of the ovalbumin gene X product.

1.11.3 Shell membrane

The next layers of the egg are the inner and the outer shell membranes which are deposited during a 1 to 2 hour period as a highly cross-linked fibrous meshwork (Rose and Hincke, 2009). These fibres consist of a core of type X collagen surrounded by a fuzzy material referred to as the mantle (Arias and Fernandez, 2003). The inhibitory effect of Type X collagen explains why the shell membranes do not become mineralized. The shell membranes act as a substrate for deposition of the mammillary knobs, which are the nucleation sites for calcite crystals (Arias *et al.* 1997).

1.11.4 The shell

The eggshell has been described as a bioceramic structure, in which the inorganic fraction is complexed with protein (Solomon, 1999) (Figure 1.3). It consists of organic (organic matrix, cuticle) (5%) and inorganic (calcite) components (95%) which are produced by the shell gland (uterus) of the oviduct, and is composed of a two-layered membrane and calcified extra-cellular matrix (Jacob *et al.,* 2000; Arias and Fernandez, 2003). The eggshell mineral component is associated with an organic matrix composed of proteins, glycoproteins and proteoglycans, termed "eggshell matrix proteins", which are progressively incorporated from precursors in the uterine fluid during calcification (Rose and Hincke, 2009). The mineralized shell consists primarily of calcite, the most stable polymorph of calcium carbonate, and extends from the inner mammillary cone layer, through the central palisade and outer vertical crystal layers (Rose and Hincke, 2009).

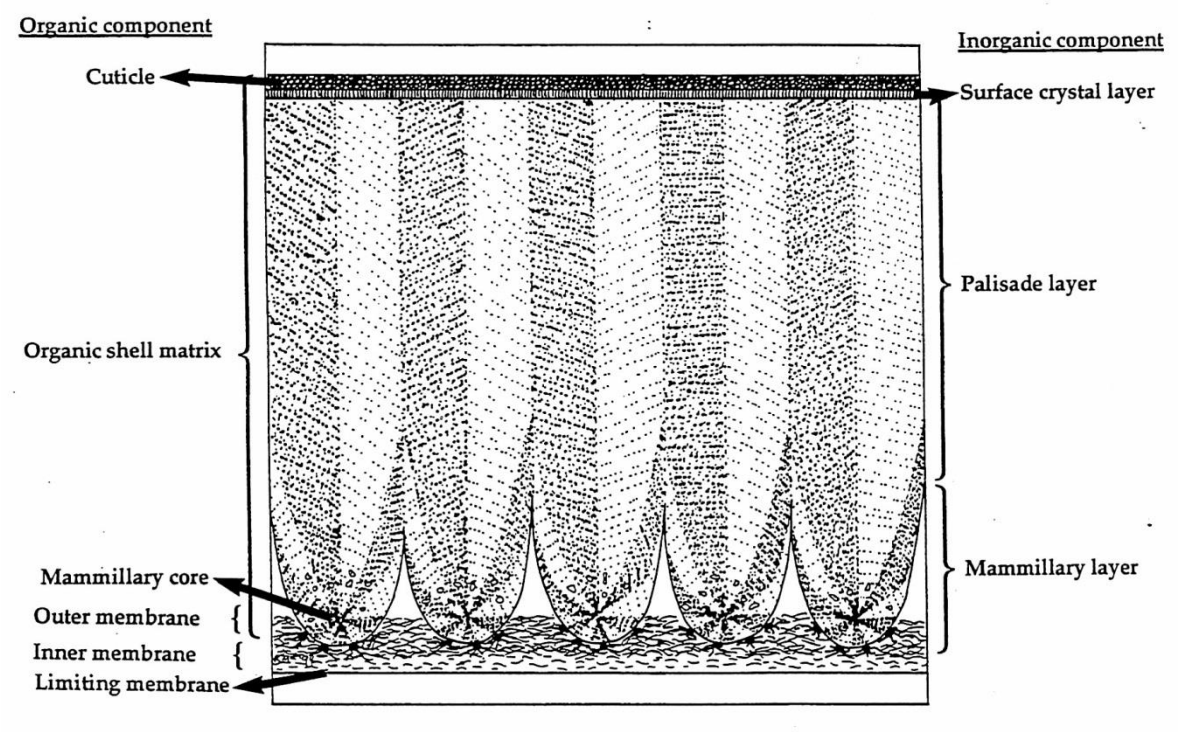

Figure 1. 3. Eggshell ultrastructure in transverse section (source: Roberts and Brackpool, 1995)

In the case of the table egg, the chamber must remain intact from point of lay, along the production process, until it reaches the consumer. As the first barrier against bacterial trans-shell penetration, shell integrity is important. Leleu *et al*. (2011) found that the presence of micro-cracks did not significantly affect the occurrence of bacterial ingress. However, a trend was observed towards enhanced penetration in the presence of micro-cracks. These authors came to the conclusion that micro-cracks most probably do not present a major risk of bacterial ingress, although this can depend on the eggs' origin.

The eggshell also functions as a chamber for housing the developing embryo (Fraser *et al*., 1999; Liao *et al*., 2013). In this capacity it provides physical protection, regulates gas, water and ionic exchange as well as providing a source of calcium for bone development in the embryo (Fraser *et al*., 1999; Scanes *et al*., 2004).

Mammillary knob layer

The mammillary layer is the innermost of the calcified portions of the eggshell. It is composed of a regular array of cones or knobs, with highly organic cores, into which is embedded the outer

eggshell membrane with numerous membrane fibres penetrating the calcified structure (Parson, 1982; Nys *et al*., 2004). Within the mammillary cone layer, microcrystals of calcite are arranged with spherulitic texture, which facilitates the propagation of cracks during pipping as well as the mobilization of calcium to nourish the embryo by dissolution of highly reactive calcite microcrystals (Nys *et al*., 2004).

The mammillary layer is the major source of calcium for the developing embryo (Liao *et al*., 2013); any variation in its thickness may affect the amount of calcium available (Abdel-Salam, *et al*., 2006) and reduce shell strength (Bain, 1992). The mammillary layer contains anchor points to the inner and outer shell membranes (Burley and Vadehra, 1989; Nys *et al*., 1999). The mammillary layer consists of the calcium reserve assembly and crown region, each with a unique substructure (Dennis *et al*., 1996) and comprises about one third to one fifth of the total thickness of the shell (Hodges, 1974). Liao *et al*. (2013) found that eggs with a thicker mammillary layer have a higher ability to hatch successfully. On the surface of the outer membrane, mammillary knobs appear to be the centre where calcification starts and form as the first stage of shell formation (Hunton, 2005).

The eggshell matrix is a series of layers of protein and acid mucopolysaccharide, on which calcification takes place (Johnson, 2000; Nys, *et al*., 2001). It has a function in the fabrication of the eggshell and participates in antimicrobial defence (Hincke, *et al*. 2011). The study of the distribution of proteins in each layer, conducted by Mikšík *et al*. (2007), indicated that ovalbumin was found only in the mammillary layer. Mikšík *et al*. (2003) concluded that proteins of the eggshell matrix interact with calcite during crystallization and participate on the formation of the eggshell. The quality of the mammillary layer ultrastructure is directly correlated with the eggshell's physical and material properties (Bain, 1992).

During embryonic development, calcium from the eggshell is mobilized exclusively from the mammillary cones; thus each mammillary cone can be viewed as a functional unit that provides the embryo with a limited amount of calcium (Liao *et al*., 2013). This layer loses about 50% of its original thickness by the time of hatching, owing to the consumption of the inner mammillary layer by the embryo during incubation (Abdel-Salam *et al*., 2006).

Palisade layer

The palisade layer grows from each mammillary knob and, as the calcification mechanism proceeds, adjacent columns fuse (Hunton, 2005; Solomon, 2010). It is a thick calcified layer

(200–300 µm thick) (Mikšík, *et al.*, 2003; Solomon, 2010; Dacke *et al*., 2015) and composed mainly of crystalline calcium carbonate in calcite form which arises from the nuclei of mammillary knobs (Johnson, 2000; Rodriguez-Navarro *et al*., 2002; Rose and Hincke, 2009). Burley and Vadehra (1989) reported that this layer is composed of about 97% calcium carbonate. In the palisade layer the crystals increase their size progressively and elongate along the calcite c-axis towards the eggshell surface (Dacke *et al*., 2015).

The dietary ingredient normally used to provide calcium to the laying hen is calcium carbonate, the same chemical as the shell. However, the chemical must be broken down in the digestive system and then re-synthesized in the shell gland to form the shell (Hunton, 2005). According to Itoh and Hatano (1964), apart from the calcium carbonate, which makes up the bulk of the shell, the remainder is made up of a small amount of magnesium carbonate, tri-calcium phosphate and other trace minerals. The total shell magnesium content is 5 times as much as the phosphorus and both elements are secreted during several hours prior to oviposition. The palisade layer ends at the vertical crystal layer which has a crystalline structure of higher density than that of the palisade region (Hincke, *et al*., 2010). The palisade layer constitutes the major calcified component of the eggshell and is considered that portion of the shell most closely associated with shell strength (Carnarius, *et al*.,1996).

Vertical crystal layer

The thin vertical crystal layer is deposited on the surface of the palisade layer; it is located between the cuticle and the palisade layer in varying thicknesses, betweent 3 and 8 µm thick (Simons, 1971; Johnson, 2000). The calcite crystals assume a vertical orientation, which overlies the polycrystalline columns of the palisade which form the bulk of the true shell (Solomon, *et al*.,1994). This layer may be an extension of the palisade layer (Roberts and Brackpool, 1995; Mikšík, *et al.,* 2007). In the vertical crystal layer, the matrix undergoes a transition from the vesicle-rich palisade region with an orientation of matrix parallel to the eggshell surface to a region devoid of microspheres and containing matrix oriented perpendicular to the eggshell surface (Mikšík *et al*., 2007). The vertical deposition may result from the orientation of the matrix perpendicularly to the surface (Dennis *et al*., 1996). A thin layer of hydroxyapatite crystals is located at the inner surface of the cuticle (Dennis *et al*., 1996).

Cuticle

The cuticle is the outermost layer of the egg and consists of organic matter and eggshell pigments (Gautron and Nys, 2006). It is a protective coating which prevents bacterial penetration through the gas exchange pores in the eggshell. The cuticle is an organic layer consisting of 90% proteins and 10% lipids which is deposited on to the surface of the egg during the final 1-1.5 hours prior to oviposition (Baker and Balch, 1962; Roberts and Brackpool, 1995; Nys *et al*., 2004).

The cuticle is composed of two layers, a mineralized inner layer and an outer layer consisting only of an organic matrix (Dennis *et al*., 1996). The inner cuticular layer is composed of a matrix-like material containing a core and a mantle. The core material is electron lucent, while the mantle is electron dense, therefore being referred to as the vesicular layer. The outer cuticle layer is much more compact and homogenous and does not appear to contain any matrix vesicles, hence it is referred to as the non-vesicular cuticle (Fraser *et al*., 1999).

The cuticle also contains glycoproteins, polysaccharides, lipids and inorganic phosphorus including hydroxyapatite crystals (Dennis *et al*., 1996). The cuticle is largely organic with protein content as high as 90% and with a high content of cystine, glycine, glutamic acid, lysine and tyrosine (Du, 2013). At least 47 proteins have been identified in the outer cuticle layer of the chicken eggshell and two proteins, similar to Kunitz-like protease inhibitor and ovocalyxin-32 (a carboxypeptidase A inhibitor), are the most abundant of the cuticle proteins (Rose-Martel, *et al*., 2012).

The thickness of the cuticle varies around the shell surface between 0.5 and 12.8 µm (Simons, 1971), and becomes greater near the pores, where the cuticle fills in and spans the upper pore space (Dennis *et al*., 1996; Kusuda, *et al*., 2011). The amount of the cuticle present on the eggshell also affects shell thickness, which is directly linked to shell strength and the absence of cuticle may decrease shell thickness (Belyavin and Boorman, 1980). Sparks and Board (1984) stated that cuticle thickness decreases significantly with the increasing age of the hen. However, Roberts, *et al*. (2013) found that there was no significant effect of flock age in a conventional cage production system on the extent of the cuticle cover. Ruiz and Lunam (2000) reported a thick cuticle layer in peak production compared to the beginning and end lay periods in broiler breeder hens, with a lesser amount of cuticle deposition at the end of the lay period (Sparks and Board, 1984; Messens, *et al*., 2005).

The cuticle is thought to play a role in controlling water exchange by repelling water or preventing its loss, and may function in limiting microbial colonization of the eggshell surface (Hincke *et al*., 2008). Together with the mineralized shell and shell membranes, the cuticle constitutes a physical barrier against microorganism invasion and contamination of the egg content. (De Reu *et al*., 2008).

1.12 Amorphous calcium carbonate

Amorphous calcium carbonate (ACC) is an unstable mineral which is easy to dissolve (Raz *et al*., 2002). There are eight known polymorphs of calcium carbonate, seven are crystalline and one is amorphous. Three of the polymorphs (calcite, aragonite and vaterite) are pure calcium carbonate, while two (monohydrocalcite and the stable forms of amorphous calcium carbonate) contain one water molecule per calcium carbonate molecule (Addadi *et al*., 2003). These authors further explained that amorphous calcium carbonate is the only form of calcium carbonate which is isotropic in polarized light and does not diffract X-rays.

Different groups of proteins and the equilibrium of uterine fluid solution chemistry, which control the formation and dissolution of ACC, play an important role during eggshell mineralization (Lakshminarayanan *et al*., 2006; Rodriguez-Navarro *et al*., 2015). One of the major challenges in the field of bio-mineralization is to understand the mechanism(s) by which biological systems determine which polymorph will precipitate. Rodriguez-Navarro *et al*., (2015) reported that the ACC mineral deposited around mammillary core sites progressively transforms directly into calcite crystals without the occurrence of any intermediate phase.

1.13 Ultrastructural characteristics in the mammillary layer

The ultrastructure of the chicken eggshell is relatively regular. It is a polycrystalline calcium carbonate ceramic consisting of only one polymorph, calcite. The mammillary cones are composed of calcite crystals which are small in size and are deposited without a specific orientation. The palisade layer is arranged as groups of columns extending for 200 µm (Nys *et al*., 1999; Rodriguez-Navarro *et al*., 2002) outwards from the mammillary knobs perpendicular to the surface (Silyn-Roberts and Sharp, 1986; Hincke *et al*., 2010) and at high magnification presents a faceted appearance (Nys *et al*., 1999).

The mammillary cores are the initial templates on which the rest of the calcified shell is built. The attachment of the mammillary layer to the shell membranes and the quality of construction of the mammillary layer play an important role in determining the strength of the entire eggshell (Roberts and Brackpool, 1995). The relationship of eggshell mammillary structure to eggshell strength has been studied. A higher density of mammillary knobs results in weaker eggshells (Van Toledo, *et al.,* 1982). Therefore, it seems reasonable that any changes in the morphology or composition of mammillae will affect shell structure and strength. A comprehensive description of these ultrastructural variations has been described in (Solomon, 1991; Roberts and Brackpool, 1995).

Twelve structural variations in mammillary layer have been described to illustrate the range of abnormalities found in weak or poor quality eggshells (Solomon, 1991). Bain (1992) categorized the structural variations described in Solomon (1991), which increase the resistance of eggshell to unstable fracture as early fusion, cuffing, confluent mammillae and a low mammillary density. Late fusion, Type-B bodies, aragonite, pitting, depression, erosion, pin holes, alignment, and a high mammillary density are the ultrastructural variations which decrease the resistance of the eggshell. Microporosity of the palisade layer was the most common defect detected. This is incomplete calcification leading to porosity throughout the eggshell, which is the most common factor in low quality eggshells.

A scoring system was developed by Bain (1992) to quantify the incidence of the structural variation throughout the mammillary layer.

1.13.1 Mammillary caps

The part of the mammillary layer embedded in the outer shell membrane is termed the basal, or mammillary cap. These caps have a very irregular surface (Simons, 1971).

1.13.2 Confluence

Confluence refers to the characteristic appearance of mammillary caps when they join with one another (Roberts and Brackpool, 1995). It alters pore distribution and influences the palisade layer formation (Solomon, 1991).

1.13.3 Early and late fusion

Early and late fusion refers to how early or how late the mammillae fuse during eggshell formation. The earlier the mammillae fuse, the greater the effective thickness of the shell and presumably, the shell strength will tend to be (Roberts and Brackpool, 1995).

1.13.4 Alignment of the mammillae

Alignment of mammillae is the condition where mammillae appear to "line up", resulting in a long continuous groove between the cones (Roberts and Brackpool, 1995). Alignment increases the ease with which a crack can propagate.

1.13.5 Type-A body

Type A bodies are mammillary cones that have minimal or no contact with shell membranes (Solomon, 1991), that is, the cones lack mammillary caps. Although the isolated appearance of Type A bodies may not affect the shell strength, and large numbers of this feature result in a weaker shell (Roberts and Brackpool, 1995).

1.13.6 Type-B body

Type B bodies are small spherical calcified bodies located within the mammillary layer, with or without attachment to the membrane layer (Solomon, 1991; Roberts and Brackpool, 1995). These features make no contribution to the shell thickness and reduce the extent of attachment between the shell membranes and mammillary caps. They are often found in shells laid by young birds and in those of birds exposed to stress. (Solomon, 1991)

1.13.7 Aragonite

Aragonite is another crystalline form of calcium carbonate most commonly found in mammillary layer. The presence of aragonite indicates changed conditions in the shell gland pouch during shell deposition (Roberts and Brackpool, 1995).

1.13.8 Cubics

Cubics are calcium carbonate crystals with a cubic morphology and are free-growing calcite crystals found in the spaces between the mammillae (Roberts and Brackpool, 1995). The presence of cubics could be a sign of any changed conditions in the shell gland pouch during shell deposition.

1.13.9 Cubic cone formation

Cubic cone formations refer to the presence of cubic shape crystals attached to the side of the mammillary cones (Roberts and Brackpool, 1995). The presence of cubic cone formations also a signal of any circumtances in the shell gland pouch during shell deposition.

1.13.10 Cuffing

Cuffing refers to the condition where extra crystalline cuffs are laid down at the junction of the cone and palisade layers (Solomon, 1991; Roberts and Brackpool, 1995). Cuffing is thought to contribute to shell strength.

1.13.11 Changed membrane

Changed membrane is the membrane that remains attached to the mammillary cones and cannot be completely removed by the plasma etcher. It comprises a range of shell membrane defects. Changed membrane may be indicative of abnormal conditions within the oviduct while the eggshell was being laid down (Roberts and Brackpool, 1995).

1.13.12 Pitting

Pitting refers to the presence of depression or erosion. Pitting creates areas of weakness within the eggshell (Roberts and Brackpool, 1995).

1.14 Factors affecting egg quality

Monitoring egg quality characteristic is important mainly in terms of production economy. Attention is devoted especially to eggshell quality, because cracked eggshells represent higher losses for market-egg producers (Zita, *et al*., 2009). The frequency of the occurrence of defective eggs may increase from 7% to 11% during the laying, collecting and packing stages of egg production (Rayan *et al*., 2010). Maintaining quality of eggshells throughout the production cycle is essential for the economics of egg production. Thus, understanding the various egg quality defects and causes will assist in minimizing their occurrence, and in the reduction of losses in the egg value chain.

Egg quality encompasses several factors related to the shell (external quality) and to the albumen and yolk (internal quality) and is influenced by the age of laying hens (Leeson and Summers, 2001; Roberts, 2004; Mertens *et al*., 2006; Silversides, *et al*., 2006; Sarica, *et al*., 2008; Roberts and Chousalkar, 2014;). Problems with egg quality are rarely related to just a single factor. Factors affecting egg quality include nutrition (Leek, 2015), stress (Banga-Mboko, *et al*., 2010), hen age (Rodriguez-Navarro, *et al*., 2002; Zita *et al*., 2009), flock density (Benyi, *et al*., 2006; Hegelund, *et al.,* 2006), housing systems (Clerici, *et al*., 2006; Singh, *et al*., 2009; Sekeroglu *et al*., 2010), genetic strain (Silversides *et al*., 2006; Zita *et al*., 2009) and disease (De Reu *et al*., 2008).

A decrease in eggshell quality of older hens has been reported. Egg production rate decreases and egg weight increases as birds increase in age. Shell quality declines as the hens get older as a result of the decreased ability to absorb calcium (Cordts, *et al.,* 2002; Roberts, *et al*., 2013). Rodriguez-Navarro *et al*. (2002) reported a lower breaking strength and greater variability in structural properties such as thickness, grain morphology and crystallographic texture from older hens. The cuticle, as the first barrier of the eggshell against microorganism penetration, is highly dependent on hen age and egg freshness (Rodríguez-Navarro, *et al*., 2013). Furthermore, these authors asserted that the thickness and degree of glycosylation of the cuticle decreases with hen age and is significantly depleted in lipids at the end of the laying cycle.

Ultrastructure studies have demonstrated the relationship between eggshell strength and the mechanical properties of the eggshell ultrastructure (Bain, 1992; Dennis *et al*., 1996; Fraser *et al*., 1999). Kemps *et al*. (2006) separated eggshell strength into material strength which depends on the association of the mineral and the organic components of the shell, and structural strength

which depends on several variables, such as size, shape, thickness, and distribution of the shell components. Van Toledo *et al*. (1982) reported that the density of mammillary knobs in the shell is inversely related to the breaking strengths of shells of a similar thickness. Rayan, *et al*. (2010) showed the effects of strain, age of hens and their interaction on total palisade and cap thickness of eggshells.

1.15 Housing system

Many authors have investigated the effect of housing systems on egg quality. Different housing systems for laying hens have considerable effects on performance and production traits, such as egg weight, feed efficiency, and mortality (Sherwin and Nicol, 1992; Tauson, *et al*., 1999; Van den Brand, *et al.*, 2004; Vits, *et al*., 2005; Singh *et al*., 2009; Sekeroglu *et al*., 2010; Tůmová, *et al*., 2011;). Guesdon and Faure (2004) concluded that egg production could be similar in furnished and standard cages if most of the eggs were laid in the nest in furnished cages. Tůmová *et al*. (2011) have investigated the interaction between housing system and genotype in relation to internal and external egg quality. They noted that genotype affected eggshell quality more than housing system and differences in egg quality occur not only between strains but also within strains. Thicker shells were observed for a barn system and a tendency to bigger eggs in organic production (Clerici *et al*., 2006). In addition, eggs from a barn system showed a higher deformability than free-range and organic eggs, and a lower hardness than free-range eggs. However, these authors concluded that shell characteristics are not clearly influenced by the housing system, but seem to be more affected by producer management and other factors, such as hen age and strains.

Housing systems significantly influenced body weight and mortality, but not feed consumption or feed efficiency (Singh *et al*., 2009). In a tropical climate, hens in battery cages consumed significantly more food than hens in floor pens (Banga-Mboko *et al*., 2010). In terms of stocking density, Benyi *et al*. (2006) found that housing 2 or 3 birds per cage resulted in greater body weight gain, higher egg production, heavier eggs, better feed conversion ratio and lower mortality than housing 4 birds per cage. Hens reared on litter showed more aggressive pecking than organic hens. This is probably due to differences both in light intensity and in stocking density (Ferrante, *et al*., 2009).

Rearing animals in a system which allows the development of all aspects of behaviour is regarded as important by many people. Housing standards are intended to provide a suitable environment for chickens in which the stress to the birds can be minimized, because high stress levels are likely to have a negative effect on both production capacity and health of the birds. Rodenburg and Koene (2007) suggest providing a complex environment which ample behavioural opportunities and separate functional areas to reduce damaging behaviour, fear and stress.

The majority of commercial layers in the world are kept in confined housing systems with light control, power ventilation and mechanical feeding. The space per hen in conventional cages is very limited, making it difficult or impossible to express natural behaviours like sand bathing and wing flapping. In general, today's egg producer has the choice of three main housing systems:

- Laying cages; conventional ones are small enclosures with welded wire mesh sloping floor; enriched ones are larger and also equipped with perches, nest boxes and litter.
- Barn systems; larger enclosures (barns) with litter on the floor and freedom of movement for the birds within the poultry house.
- Free-range systems; similar to barn systems, but with access to an outdoor run (Van Horne and Achterbosch, 2008).

The raising of poultry receives a lot of attention whether or not the birds are raised without the use of antibiotics for growth, without animal by-products, and in the case of organic, without synthetic chemicals. Some consumers are also interested in birds raised with access to the outdoors (free-range). Many consumers buy these products because they believe that the products have superior sensory qualities and report that they taste better. Some countries (European Union, United States) have very specific definitions for free-range and other specialty production systems. Housing systems for laying hens have changed a lot in the last decade. The reasons for this are mainly ~~on~~ animal welfare-focused issues (Fiks-van Niekerk, 2005). There is an increasing awareness in many countries that hens in conventional cages are restricted to some extent in their freedom and ability fully to express the full range of their normal behavioural patterns (Elson, 2004). Australia has a number of different systems of production, but mainly conventional cage, barn and free range ones. Furnished cages and aviary systems are rare in Australia. Each production system has advantages and disadvantages in terms of efficiency, production costs, animal welfare, food safety and environmental impact.

The increased use of alternative housing systems for hen egg production represents clear evidence of the animal housing and husbandry trend towards extensive rearing methods. Consumer demand is oriented towards healthy ethically produced foods, viewed from a safety point of view but also including a welfare assessment of the animals' living conditions (Ferrante *et al*., 2009). The conditions under which layer hens are housed remain a major animal welfare issue for some consumers, the egg production industry and legislators. Recently, a growing demand for organic and naturally grown agricultural products is a small but significant trend in agriculture. There are advantages and disadvantages of each production system, but consumers in many countries want to be able to choose eggs from a particular production system.

1.15.1 Cage systems

The conventional cage is a housing system found worldwide in modern poultry egg production (Pavlovski, *et al*., 2005). Cage housing systems were developed for layers to permit better environmental control, including control of the amount of light necessary to stimulate higher levels of egg production. Cages are widely used for laying hens because egg production is cheaper in cages than in alternative husbandry systems (Appleby *et al*., 2003). The conventional laying cage is usually a small enclosure with a sloping wire mesh floor and ancillary equipment for feeding, drinking and egg collection mounted on the front (Elson, 2004). Cages are usually arranged in rows and tiers, with a gently sloping floor that allows eggs to roll to the front collection belt (Craig and Swanson, 1994; Fraser *et al*., 2001; Awoniyi, 2003).

Cages are made of welded wire and can accommodate a number of hens depending on the dimension of the cage and the cage density regulations in a particular country. The floors of cages are made of wire and this permits the passage of the faecal droppings to a collection pit or onto a manure belt, thus reducing the incidence of worms and coccidiosis (Awoniyi, 2003). Cage systems represent about 53% of egg production in Australia, as this is currently the most cost-effective system and most consumers currently purchase their eggs based on price (AECL, 2014). Cage production systems are commonly used by commercial egg producers because of their efficiency (space savings, reduced labour, equipment costs). Commercial cages exist in a variety of sizes and shapes with different arrangements of feeding and watering systems designed to minimise the risk of entrapment and feather loss because of abrasion, particularly at the feeder trough (Craig and Swanson, 1994). Conventional laying cages in Australia must now comply with the following criteria: at least 550 cm2 cage area per hen, 10 cm feed trough length per hen, 10 cm drinker trough length per hen or at least two nipple or cup drinkers within reach of each cage,

a floor with a maximum slope of 8 degrees which adequately supports the forward-facing claws of each foot (Elson, 2004).

Modern cage production facilities consist of cages inside sheds or houses. Feeding, watering, egg and manure collection are all automated (Fraser, 2001; Fraser *et al*, 2001). Hens are housed in these cages from prior to the start of lay at around sixteen to eighteen weeks of age, through one or more laying cycles. There is evidence that hens prefer to have personal space and, where stocking densities are high, will maximize this by spacing themselves out evenly, both in cage (Albentosa and Cooper, 2004) and in colony systems (Lindberg and Nicol, 1996). In the European Union, conventional cage production systems were prohibited as of 2012, which means that alternative housing systems, including free-range or outdoor systems, have been developed.

1.15.2 Free range systems

Free-range systems provide hens with access to the outdoors, with pens for ranging and foraging (Elson, 2004). The principle is to allow the animals, as much as possible, for the best positive welfare outcomes, to live in a reasonably natural way, enabling them to express their instinctive behaviour. These behaviours include natural foraging behaviour, having access to large areas of open ground with weather-proof sheds to return to for roosting, laying, feeding, drinking and protection (Nicol *et al*., 2006; Lay *et al*., 2011). However, from a negative perspective, birds may also have access to the herbicide and insecticide applied to pastures and/or crops (Miao, *et al*., 2005).

Free-range accommodation is the system under which about 38% of eggs are produced in Australia (AECL, 2014). The average flock size is much smaller than in the other systems; typically being 1,000 – 7,000 birds, with a few of the larger farms having as many as 20,000 to 100,000 birds. The sheds protect the birds from the elements and predators while the free-range area allows them access to open space and vegetation. Increasingly, free range systems have automated nesting, feeding and watering systems (McGahan *et al*., 2008).

In hot weather, hens should be provided with cool water. This can be achieved by regularly flushing the drinker lines, keeping incoming water lines out of direct sunlight, insulating water lines and ensuring water storage tanks are shaded (Miao *et al*., 2005). If the water is too hot, birds will drink less, which will result in reduced feed intake, egg production and poorer shell quality

(Glatz, 2001). In very hot or heat wave conditions, hens can die or stop laying within 4 hours, if water is not available (David, 2010).

As outdoor systems or free-range systems become more commonly used in commercial production, their effects on egg characteristics need to be determined (Van der Brand *et al*., 2004). Savory, *et al*. (2006) suggested any space allowance of less than about 5000 cm2 per hen imposes at least some constraint on free expression of behaviour. However, it would be uneconomic to provide this amount of space (two hens per m2) in commercial housing systems. Nevertheless, the results suggest that laying hens would benefit from any increase above the current minimum 1111 cm2 usable area (nine hens per m2) in alternative housing, especially as their behaviour appears to change most markedly at around this density. Miao, *et al*. (2005) state that the average commercial free-range flock in Australia consists of 1,000-2,000 hens. In Australia, it is recommended that the range area should have a stocking density not in excess of 1500 birds/ha (Primary Industries Standing Committee, 2002; McGahan *et al*., 2008)

Generally, a free range poultry production system is characterized by lower productivity and lower input although this is changing as free range production becomes more commercially oriented. Egg production may fluctuate with the seasons under the free range system (Miao, *et al*., 2005). Consumers are the main driving force for free range poultry production worldwide.

In free range systems, improving the welfare of hens is important and this includes how hens utilize the outdoor areas (Bubier, 1998). A standard requirement in free range production systems is that birds must have easy access to an area on which to range during daylight hours. Range access allows the birds to perform spatial behaviours and decreases the average stocking density inside the house (Lay *et al*., 2011). However, not all birds use the range frequently. Range use is affected by many factors such as vegetation, trees and shade structures, climatic conditions, flock size, and time of day (Singh and Cowieson, 2013), the number of pop holes (Keeling *et al*., 1988) and the strain of bird (Kjaer and Sørensen, 1997). Shade, shelters and palatable vegetation should be provided in the range areas. Some birds never go outside the house, whereas some others spent more than 75 % of their time on the range (Singh and Cowieson, 2013). Grigor *et al*. (1995) suggested that the familiarity with the range affected the willingness of birds to access it. Keeling *et al*., (1988) show that, at any given time, 15–22% of birds were in the range area, but the frequency with which particular birds left the house was variable. Dawkins *et al*. (2003) and Zeltner and Hirt (2008) indicate that addition of shelters on the range increases the confidence of broilers, as they stay for longer in outdoor areas compared with unsheltered plots. Smaller flock

sizes kept at lower densities may encourage a much larger percentage of hens to go outdoors (Bubier, 1998).

1.16 Welfare issues of housing system

Animal welfare is becoming more and more important as consumers consider food production and how animals are treated. There is a high profile ethical concern for the animals that are under our care and management (Lawrence and Stott, 2009). The debate over animal welfare is complex and needs to be understood when making rational decisions about the future. The debate in many countries has stimulated many developments to enhance cage design, such as furnished laying cages, alternative systems such as aviaries, perches, and deep litter, and free-range production in fixed mobile houses. Welfare can be satisfactory in each of these systems, if they are well designed and managed (Elson, 2004).

Generally, hens in non-cage systems have more space and greater freedom than hens in laying cages. This is often considered a welfare benefit. However, various risks which may reduce welfare, lower or absent in cages, do occur in alternative systems (Elson, 2004). In the UK, the Farm Animal Welfare Council (FAWC) believes that animal welfare should be considered with reference to its 'Five Freedoms'. This can be taken as a general guideline of animal welfare. The Five Freedoms are:

- Freedom from hunger and thirst - ready access to fresh water and a diet to maintain full health and vigour;
- Freedom from discomfort-providing an appropriate environment including shelter and a comfortable resting area;
- Freedom from pain, injury or disease -prevention or rapid diagnosis and treatment;
- Freedom to express normal behaviour provision of sufficient space, proper facilities and company of the animals' own kind and;
- Freedom from fear and distress by ensuring conditions and treatment to avoid mental suffering (Sastry, 2006).

The World Organisation for Animal Health (OIE) definition of animal welfare refers to how an animal copes with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express

innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress (Appleby, 2014).

For some consumers, egg labelled free-range eggs are preferable to other non-free-range eggs (Pavlovski *et al*., 2005). Some consumers prefer to buy free-range eggs because they feel they are doing something good and of value (Blokhuis and Van der Haar, 1989). However, consumers do not necessarily really understand if the welfare of the animals is what they expected. These authors found that 51% of respondents to their survey could rarely identify whether the production system was animal welfare friendly or not by merely reading the label. One-third of consumers could not do this at all.

There are many concerns about the correlation between housing systems where animals are kept and welfare issues. Rodenburg and Koene (2007) reported that larger group size increases the risk of fear and stress, causing damaging behaviours such as feather pecking in laying hens. Stocking densities did not affect the eggshell quality traits, such as egg weight, specific gravity, and shell breaking resistance, shell weight, percentage shell, and shell thickness in the study of Sarica *et al*. (2008). However, these authors found that limiting the space allowance tended to decrease yolk and albumen quality, with a higher incidence of meat and blood spots in cages with a space allowance of 500 cm2 and 667 cm2 per hen. Feeding management has also been shown to influence feather pecking in laying hens (Van Krimpen *et al*., 2005).

Another consideration is bone strength. Michel and Huonnic, (2003) compared hens in aviaries and cages and found that the strengths of both tibia and humerus were higher in birds from aviaries compared to cages. The results suggested that the aviary system allows more vertical movements (flying, jumping) and that this led to better bone strength, compared to animals reared on the floor, even with perching enrichments. This result is in agreement with Meyer and Sunde (1974), who found that exercising reduced bone breakage substantially. Higher tibia breaking strength was found in birds from cages with perches than in those without perches (Duncan, *et al.*, 1992). Floor birds had stronger tibia than any of the caged birds (Meyer and Sunde, 1974).

1.17 Egg quality and housing systems

A number of authors have investigated the effects of housing systems on egg quality. Rossi and De Reu (2011) reported in their review that first quality eggs from conventional cages are better than those produced by furnished cages or non-cage systems.

Different housing systems for laying hens have considerable effects on performance and production traits, such as egg weight, feed efficiency, and mortality (Sherwin and Nicol, 1992; Tauson *et al*., 1999; Van den Brand *et al*., 2004; Vits *et al*., 2005; Hidalgo *et al*., 2008; Singh *et al*., 2009; Sekeroglu *et al*., 2010; Tůmová *et al*., 2011). Van ben Brand (2004) compared egg quality in two housing systems (outdoor versus cages system) and found that hens in cages produced heavier eggs than hens in a free-range system at the start of the experiment (25 weeks of age), but laid lighter eggs after 40 weeks of age. Ferrante *et al* (2009) reported lighter eggs in a barn system than in an organic system at the beginning of the experiment, but heavier eggs at the age of 35 weeks. Tůmová *et al*., (2011) reported that genotype had a greater effect on egg quality than the housing systems, with significant interactions between genotype and housing system for eggshell weight and strength, but not for shell thickness.

Thicker shells were observed for a barn system and a tendency to bigger eggs in organic production than in conventional cages (Clerici *et al*., 2006). Küçükyılmaz *et al*., (2012) reported thicker shells from an organic housing system than from conventional cages. However, these authors concluded that housing systems do not clearly influence the shell characteristics, but producer management and other factors such as hen age and strains seem to have more effect on shell characteristics. Guesdon and Faure (2004) concluded that egg production would be similar in furnished and standard cages if most of the eggs are laid in the nest in furnished cages.

Rossi and De Reu (2011) summarized the various studies on eggshell strength and shell thickness from cage and non-cage production systems and noted that there was no significant difference between eggs from conventional cages and non-cage systems, including barn and free-range systems, in shell breaking strength and shell thickness. The absence of any significant effect from the housing system on shell thickness was also reported by Guesdon and Faure (2004); Đukić-Stojčić, *et al*. (2009) and Tůmová *et al*., (2011).

In terms of stocking density, Benyi *et al*. (2006) found that housing 2 or 3 birds per cage resulted in greater body weight gain, higher egg production, heavier eggs, a better feed conversion ratio and lower mortality than housing 4 birds per cage. Hens reared on litter showed more aggressive pecking than organic hens. This is probably due to differences both in light intensity and in density (Ferrante *et al*., 2009).

1.18 Introduction to the current study

The main objective of the current study was to evaluate the importance of body weight at point of lay and body weight uniformity at different stages of lay, to egg production, egg size and egg quality. The study tested the hypothesis that correct body weight and good uniformity through the lay will result in good egg production and egg quality. The current study also initiated some preliminary analysis of the most efficient birds, to define the ideal proportions of bone and body fat for high sustained production of high quality eggs. Flocks from two different production systems (cage and free-range) were studied over time, for body weight, body weight uniformity, overall egg quality, extent of cuticle cover, and the incidence of mammillary layer shell ultrastructural variations. Bone breaking strength and body conformation were also determined late in lay for the cage flock.

A laboratory experiment was established more systematically to model some of the important variables identified in studies on commercial farms. The laboratory model established different initial body weight groups, with maximum flock uniformities to examine the effect of hen age and body weight group on overall egg quality, cuticle cover, the incidence of mammillary layer shell ultrastructural variation, bone breaking strength and body conformation using computerized tomography scanning.

Chapter 2

General Materials and Methods

2.1 Birds housing and general management

Hy-Line Brown laying hens were used to investigate body weight, flock uniformity, eggshell quality, egg internal quality and eggshell ultrastructure. Birds were kept in different production systems: cages (Chapters 3, 5), and free-range (Chapter 4). Water and feed were provided *ad libitum* and birds were maintained on a 16:8 light:dark cycle. Feed was formulated by a consultant nutritionist. All samples were collected from the New South Wales region.

2.2 Body weight and flock uniformity

Body weight was measured at a range of ages, as outlined in Chapters 3, 4 and 5. Body weight uniformity was calculated as:

$$Uniformity\ (\%) = \frac{Number\ of\ birds\ within \pm 10\%\ of\ target\ body\ weight}{Total\ number\ of\ birds\ weighed}\ X\ 100\%$$

2.3 Egg production and quality

Egg production records of laying hens were kept daily throughout the experiments. Group records for production were converted to weekly egg production percentages (eggs/hen/week as a percentage).

$$\frac{Number\ of\ eggs\ produced\ on\ weekly\ basis}{Number\ of\ birds\ available\ in\ the\ flock\ on\ that\ week}\ \text{x}\ 100\%$$

Eggs were collected at the same as hen age when birds were weighed for experiments described in Chapters 3, 4 and 5. For the experiment described in Chapter 5, eggs were collected and analysed on a weekly basis.

2.3.1 Traditional eggshell and egg internal quality measurements

For each egg collection, ninety eggs were collected; sixty eggs were analysed for eggshell and egg internal quality, and thirty eggs were used for cuticle cover analysis. Eggshell translucency was scored by placing the intact egg over a light source in an egg candling box. The translucency scores were 1 to 5, representing the least to the highest incidence of translucency.

Traditional eggshell quality measurements were shell colour measured as reflectivity, egg weight, eggshell breaking strength, shell deformation, and shell weight. All measurements were made using specialized egg quality equipment (Technical Services and Supplies, TSS, Dunnington, York, UK). Egg weight was measured in grams on a weighing balance (Ohaus). Shell reflectivity (indicator of lightness of shell colour) was measured using a reflectivity meter (measuring the amount of light reflected from the shell surface). Eggshell breaking strength (Newtons) and deformation to breaking point (microns) were measured by quasi-static compression using the Q/C- SPA machine (TSS equipment). Shell thickness was measured in microns, using a custom-built gauge, based on a Mitutoyo Dial Comparator Gauge Model ID-F150E (Kawasaki, Japan) mounted on a metal frame. Shells were washed to remove the adherent albumen and were dried under a fan overnight. Dried eggshells were then weighed and three pieces of shell with intact shell membranes were taken from around the equator of the egg for shell thickness measurement. Percentage shell was calculated from shell weight and egg weight.

Egg internal quality was measured in the form of albumen height, Haugh Unit (HU) and yolk colour score (TSS equipment). The HU takes into account the albumen height and the size of the egg and albumen height and HU are used as an indicator of internal egg quality or freshness. The equation for calculation of HU is:

$$\text{H. U.} = 100 \text{ Log } (\text{H} - \frac{\sqrt{\text{G } (30\ W^{0.37} - 100)}}{100} + 1.9)$$

H. U. = Haugh Unit

H = albumen height in mm

G = 32. 2

W = weight of whole egg in grams

2.3.2 Estimation of the amount of cuticle

2.3.2.1 MST Cuticle blue stain preparation

MST cuticle blue dye (MS Technologies, Europe Ltd, Kettering, Northamptonshire, UK) was used for staining the cuticle of the eggshell. The solution was prepared according to the manufacturer's instructions.

2.3.2.2 Cuticle staining

Eggs were immersed in the MST cuticle blue dye stain for 1 minute. They were then rinsed in water three times to remove the excess stain, placed on a plastic egg filler and allowed to dry thoroughly.

2.3.2.3 Measurement of shell reflectivity and spectrophotometry prior to staining

For estimation of the amount of cuticle present on the shell, shell reflectivity (%) was measured using the TSS shell reflectivity meter. A hand-held Konica Minolta spectrophotometer (CM-2600d; Ramsey, NJ, USA) was used to measure the cuticle colour. The Konica Minolta hand-held spectrophotometer functions on the SCI (Specular Component Included) and SCE (Specular Component Excluded) L*a*b space system where L* represents the grading between white (100) and black (0). The SCI values were used in this study. The higher the value for L*, the lighter the shell colour, and vice versa. The value for a* represents the colour grading between green and red where green is towards the negative end of the scale and red towards the positive end. More negative values for a* mean the eggs acquired more stain and thus the amount of cuticle is greater and vice versa. The b* component of the L*a*b space system is the grading between yellow and

blue. For b* value, blue towards the negative end and yellow towards the positive end of the scale. Among all the three components of the L*a*b colour space system, a* is the most important value that shows the amount of cuticle present on the stained eggs. The reading was taken 3 times per egg at three locations around the equator and an average recorded.

2.3.2.4 Spectrophotometry measurement following after staining

Shell colour (L*a*b) was measured following staining with cuticle blue dye, using the same procedures described earlier.

Based on the study described by Leleu *et al*. (2011), a single score value, $\Delta E^*_{ab,}$ was calculated as:

$$\Delta E^*_{ab} = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

2.3.2.5 Verification of cuticle staining using light microscopy and scanning electron microscopy

For confirmation of the reliability of the MST cuticle blue stain as an indicator of the amount of cuticle present, egg internal contents were emptied by making a small hole at the blunt end of the eggs using a Dremel High Speed rotary tool, 300 series (Robert Bosch Tool Corporation, Racine, WI, USA). The egg contents were removed and then internal walls were rinsed with water using a 10 mL syringe with attached tubing. Care was taken not to wash away stain from the cuticle surface. After drying thoroughly, small pieces were cut out from the different areas representing various amounts of stain, mounted on aluminium stubs using conductive silver paint (I005 aqueous conductive silver liquid-SEM adhesive, ProSciTech) and photographed under a dissecting microscope with attached camera at a magnification of 12. The same specimens were then gold sputter-coated for 5 minutes in a Neocoater (MP-19020NCTR) and viewed under a JEOL JCM-5000 NeoScope benchtop scanning electron microscope. A scoring method for the SEM of the cuticle, modified from Leleu *et al.* (2011), was used for the quantification of the amount of the cuticle present as shown in Table 2.1. The cuticle verification under the scanning electron microscope was applied in Chapters 3 and 4.

Table 2.1. Scoring sheet for cuticle quantification by Scanning electron microscope

Good intact cuticle (91-100%)	Less patchy cuticle (61-90%)	More patchy cuticle (11-60%)	Negligible or no cuticle (1-10%)
Score = 1	Score = 2	Score = 3	Score = 4

2.4 Scanning electron microscopy

Eggshell preparation

The ultrastructural features of the mammillary layer were scored using a scanning electron microscope (JCM-5000 NeoScope). Pieces of shell approximately 1 cm square were cut out from around the equator of the eggshell using a Dremel tool and soaked overnight in small containers of tap water. Shell membranes were manually peeled away to remove as much membrane as possible and the shell pieces were allowed to dry. The dried pieces were then plasma etched in a BioRAD RF Plasma Barrel Etcher (PT 7150, Hertfordshire, UK) for 4 hours to remove any remaining membrane following the method of Reid (1983). In the plasma etcher, oxygen gas is ionized by the application of radio frequency power under carefully controlled pressure conditions to form a highly reactive plasma at relatively low temperature. The reaction between the plasma and the carbon in the sample removes the organic component while leaving the crystalline material intact. After plasma etching, each shell was air dusted to remove the ash particles and mounted, inner surface uppermost, on a 9 mm diameter aluminium stub, using conductive silver liquid SEM adhesive. The stubs and samples were then placed in a Neocoater gold sputter-coater for 5 minutes; the thin coating of gold particles improving image quality. The samples were imaged using a Neoscope JM-5000 SEM, at 10kV; the machine was focused and photographs were taken. Images were scored for ultrastructural features of the mammillary layer using the score sheet developed by Solomon (1991).

2.5 Ultrastructural scoring of the shell mammillary layer

Mammillary cap size was scored as 1 (similar), 2 (variable) or 3 (highly variable). Mammillary cap quality was assessed as both the size of the cap in relation to its cone and the degree of membrane attachment from 1 (best) to 5 (worst). Confluence, early fusion, late fusion, alignment, Type-A bodies, Type-B bodies, aragonite, cubic, cubic cone formations, changed membrane (membrane not removed by plasma ashing) and cuffing were each ranked for incidence from 1 (none) to 4 (extensive). The same was done for the incidence of depressions, erosion and holes although these were rarely observed.

2.6 Bone breaking strength

Bone breaking strengths were determined on whole humerus and femur bones. At euthanasia, the bones were removed from the left side, carefully cleaned of tissue, wrapped individually in cling wrap to exclude air, labelled, sealed in a double plastic bag and stored at -20^0C until further bone quality measurements were conducted. Bones were slowly thawed in a refrigerator (at 4^0C) for approximately 7 hours then placed at room temperature overnight, prior to bone quality analysis.

Bone breaking strength was measured using a Lloyd LRX Material Testing Machine fitted with a standard 500 Newton load cell. A 30 mm distance between the 2 fixed points supporting the bone and a crosshead speed of 10 mm/min were held constant throughout all measurements. The surface of each supporting bar was covered with a layer of rubber 2 mm thick. The force was applied to the midpoint of the same facial plane of each bone, and the breaking strength was recorded from the failure point (peak) of each loading curve. The details of numbers of bones are outlined in Chapters 3 and 5.

2.7 Statistical analysis

Data were analyzed using Statview Software (SAS Institute Inc., Version 5.0.1.0). Level of significance was indicated by probability of less than 5%. The Fishers LSD test was used to differentiate levels of significance between mean values.

Chapter 3

Body weight uniformity of hens in a commercial cage production system and the effects on egg quality

3.1 Introduction

In the commercial egg industry, egg quality is important not only for producers but also for consumers. The natural role of the hen's egg is as an incubation chamber for a developing chick. The egg protects its contents by the presence of the cuticle and the architectural organization of the palisade layer, mammillary layer, shell membranes and albumen. The evaluation of egg quality is important to both the layer and broiler industries. Good eggshell quality ensures the supply of pathogen-free nutritious food to the consumer. Good eggshell quality also provides for the hatching of defect-free new chickens if fertile eggs are brooded properly. When measuring eggshell quality, it is traditional to assess using shell thickness and shell percentage, but more recently it has become possible to make an evaluation of shell ultrastructure. Ultrastructural studies have demonstrated that the eggshell is comprised of morphologically distinct calcified layers with the mammillary layer being the "foundation" of the eggshell. Studies have identified ultrastructural variations in the mammillary layer that can be used as indicators of eggshell quality (Roberts and Brackpool, 1995).

Poor shell and internal egg quality is still a big concern in the commercial egg industry. There are many factors that affect the overall quality of the egg. Hen age has been reported to influence egg weight, egg quality and internal egg characteristics (Silversides and Scott, 2001; Van den Brand *et al*., 2004). Apart from hen age, the next most important variable influencing egg quality is probably the flock body weight, growth and body weight uniformity. In Australia, Parkinson *et al*. (2007), studied the influence of flock uniformity in several commercial layer farms and found that the flocks studied had an average body weight 100-300 g above the breed standard, probably indicating obesity beyond the expectations of the commercial breeders. These obese birds produced excessively large eggs with associated lower eggshell (Parkinson *et al.*, 2007), and probably internal egg quality. The Breeder standards for flock uniformity aim to have 80 per cent of pullets within plus or minus 10 per cent of the average flock body weight. Flocks with high uniformity have been reported to reach peak egg production earlier and have higher peak

production than flocks of low uniformity (Hudson *et al*., 2001; Kosba *et al.*, 2009). On the other hand, poor uniformity is associated with variation in the degree of sexual maturity of hens, where underweight pullets have delayed onset of egg production (Yuan *et al*., 1994). Productive and profitable layers begin with good quality pullets. Having the correct body weight at the start of egg production will enable pullets to achieve their genetic potential. Problems which develop during the growing period cannot be corrected after egg production begins.

It has long been speculated that frame size and skeletal calcium reserves in pullets have important implications for egg production and eggshell quality, particularly in the later part of the egg production cycle. Flock body weights, and growth of flocks in the transition between onset of lay and peak production, together with flock uniformity, may influence these skeletal reserves and therefore influence long term eggshell quality.

In the present chapter, a study was conducted on caged production flocks to evaluate the influence of divergent flock uniformities on the eggshell and egg internal quality parameters, amount of cuticle on the shell, mammillary ultrastructural variables, and skeletal size and bone breaking strength. This on-farm study was a preliminary investigation on a commercial farm to evaluate flock body weights and compliance with breeder recommendations, to determine flock uniformity patterns during egg production, and to develop more detailed scientific methodologies for subsequent studies of egg quality.

3.2 Materials and methods

3.2.1 Bird management

This was an on-farm study on cage production system. Two flocks of Hy-Line Brown commercial layers were studied throughout their production cycles. They were sourced at the same age from the same hatchery but reared in different rearing houses before being transferred at 15 weeks of age into conventional cages in the same environmentally-controlled system in the region of Tamworth, NSW. Shed A had a total of 10500 chicks and shed B had a total of 5500 chicks, with the stocking density being slightly higher in Shed A. The rearing sheds were located at slightly different altitudes and were about 2 km distant from each other.

The flock was provided with feed formulated to recommended commercial standards for HyLine Brown laying hens throughout the production life of the flock.

A total of 100 birds from each flock was randomly selected and weighed at the ages of 6, 15, 19, 26, 37, 50 and 60 weeks. The birds weighed were not the same at each age. Body weight averages and uniformity were calculated at each age. Feed formulation and composition were not made available by the producers because those details are considered commercial in confidence.

3.2.2 Egg quality measurements

Egg production was recorded at the farm from both flocks combined. It was not possible to separate production from the two original rearing flocks.

A total of 90 eggs was collected directly from the cage fronts of each of the original flocks at 19, 26, 37, 50 and 60 weeks of age, prior to analysis for egg internal and eggshell quality, as described in Chapter 2. Eggs were collected randomly from cages over the study period for each flock. The amount of cuticle present and shell ultrastructural features were also analysed under the SEM and scored, as described in Chapter 2.

3.2.3 Bone breaking strength

A total of twelve birds was randomly selected at 86 weeks of age from both flocks (A) and (B); twelve bones (six left femur and left humerus), were used to measure the bone breaking strength, length and width. Details of bone measurements are described in Chapter 2. Body weights of these 12 individual birds were also recorded and related to the skeletal weight, strength, length and width.

3.2.4 Statistical analysis of data

Data were analyzed using Statview Software (SAS Institute Inc., Version 5.0.1.0). A two-way analysis of variance was conducted taking flock age and shed/flock as the independent variables, and body weight, egg quality measurements, spectrophotometry (L*a*b), single score measurements for cuticle cover, and ultrastructural features as dependent variables. Level of significance was indicated by probability of less than 5%. The Fishers PLSD test was used to differentiate between mean values.

3.3 Results

3.3.1 Body weight and flock uniformity

Table 3.1. shows the body weight with the lowest and the highest body weight from 6 weeks to 60 weeks of age.

Table 3.1. Body weight (BW) of hens in a cage production system

Flock age	BW (kg/bird)		BW > 2.3 kg (%)		Lowest BW (kg)		Highest BW (kg)	
(weeks)	A	B	A	B	A	B	A	B
6	0.6 ± 0.004[f]	0.6 ± 0.005[g]	0	0	0.5	0.5	0.6	0.7
15	1.3 ± 0.009[e]	1.4 ± 0.010[f]	0	0	1.1	1.1	1.5	1.6
19	1.7 ± 0.014[d]	1.8 ± 0.011[e]	0	0	1.3	1.5	2.0	2.0
26	1.9 ± 0.014[c]	1.9 ± 0.014[d]	1	1	1.6	1.6	2.5	2.4
37	2.1 ± 0.017[b]	2.0 ± 0.016[c]	13	5	1.7	1.7	2.6	2.5
50	2.1 ± 0.023[b]	2.0 ± 0.019[b]	14	6	1.2	1.0	2.6	2.5
60	2.1 ± 0.019[a]	2.1 ± 0.021[a]	17	19	1.8	1.6	2.6	2.8
P Value								
Age (A)	<0.0001							
Shed (S)	NS							
A*S	<0.0001							

[a,b,c,d,e,f,g] Within a column, values with different superscripts are significantly different from each other. Values are mean ± SE

Body weight was significantly affected (P<0.0001) by flock age and there was a significant interaction between flock age and shed. Body weight increased with increasing flock age in both sheds. Figure 3.1 also shows that body weight of most birds exceeded the breed standard from the beginning of the study.

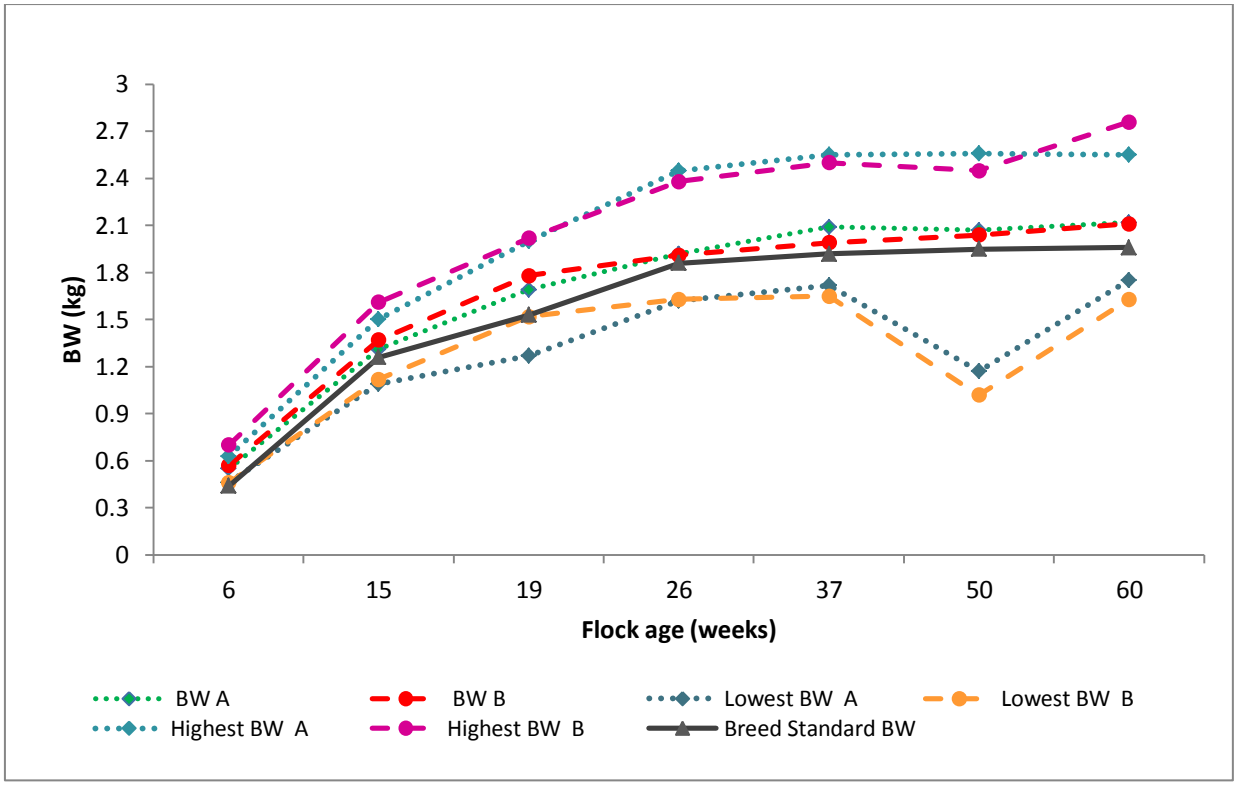

Figure 3.1. Body weight of flocks with the lowest and highest BW weight compared to breed standards

Between the two sheds, body weight and body weight uniformity were not significantly different for all ages combined although body weight was lower for Shed A until 26 weeks of age when pairwise comparisons were made between sheds at each flock age.

Pullet weight at 19 weeks of age exceeded the breed standard by between 100 to 200 g for Sheds A and B. Figure 3.1 also shows the body weights of the birds from the two original sheds with the lowest and the highest body weights.

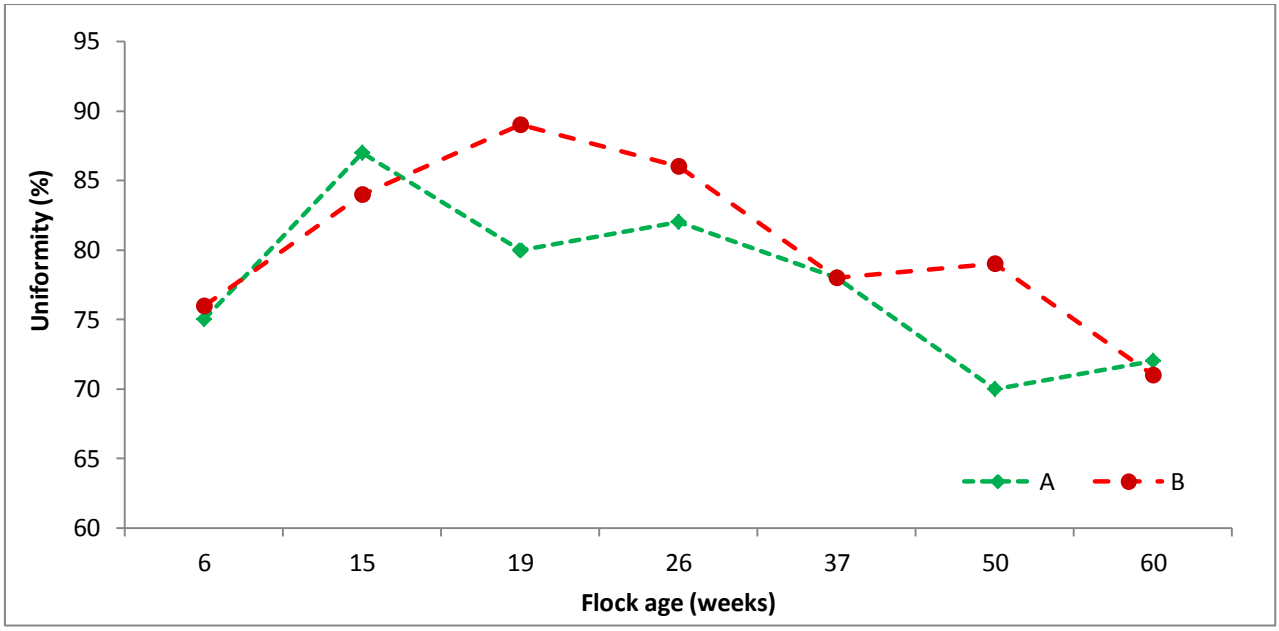

Figure 3.2. Flock uniformity between two sheds

Body weight uniformity ranged from 70% to 87 % for shed A (Figure 3.2). The highest uniformity in Shed A was at age 15 weeks (87%) with the lowest body weight uniformity was at age 50 weeks (70%). For shed B, body weight uniformity was highest (89%) at 19 weeks and lowest (71%) at 60 weeks of age. Shed B had consistently superior uniformities between 19 and 50 weeks of age, compared to Shed A.

3.3.2 Egg production and quality

Figure 3.3 presents the hen-day egg production for both sheds in comparison to breeder recommendations. The highest egg production was attained at 36 weeks of age, then generally decreased as hen age increased. Peak production was therefore delayed by approximately 10 weeks and this may have been influenced by the lower uniformity standards of the larger shed flock (Shed A) at 19 and 26 weeks of age.

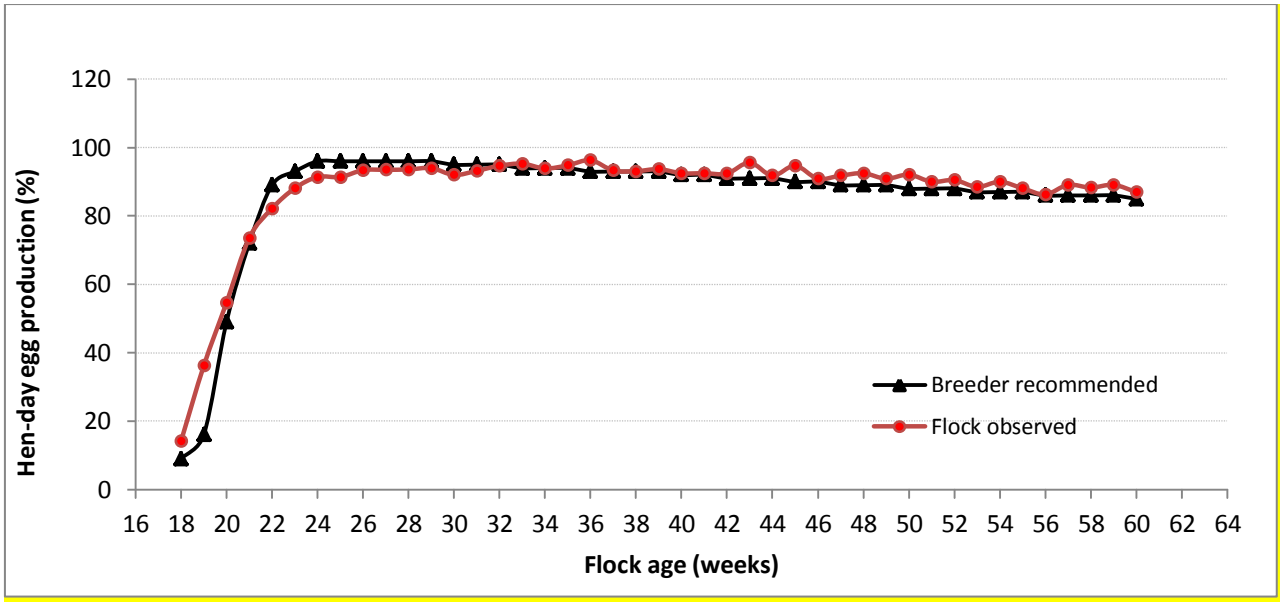

Figure 3.3. Hen-day egg production (%)

There was a significant main effect (P<0.0001) of flock age for all eggshell quality measurements (Table 3.2) and the interaction between flock age and sheds for shell reflectivity, egg weight and shell weight (Table 3.3).

Table 3.2. Main effect of flock age on egg quality measurements

Measurement	Flock age (weeks)					P Value
	19	26	37	50	60	
Translucency score	2.6±0.08[b]	2.6±0.06[b]	2.8±0.07[a]	2.1±0.07[c]	2.7±0.86[ab]	<0.0001
Shell reflectivity (%)	28.4±0.33[b]	26.9±0.27[c]	28.9±0.40[ab]	29.4±0.35[a]	28.3±0.31[b]	<0.0001
Egg weight (g)	50.5±0.35[e]	58.6±0.31[d]	61.2±.039[c]	63.0±0.32[b]	64.9±0.44[a]	<0.0001
Breaking strength (N)	45.5±0.57[a]	43.9±0.51[b]	40.9±0.59[c]	40.3±0.58[c]	41.2±0.70[c]	<0.0001
Deformation (µm)	311.2±2.09[a]	284.8±2.45[b]	283.9±3.31[b]	253.9±3.05[c]	256.7±2.92[c]	<0.0001
Shell weight (g)	5.0±0.04[d]	5.7±0.04[c]	5.9±0.05[b]	6.1±0.04[a]	6.1±0.05[a]	<0.0001
Percentage shell (%)	9.8±0.07[a]	9.7±0.05[ab]	9.6±0.05[b]	9.6±0.05[b]	9.4±0.09[c]	<0.0001
Shell thickness (µm)	395.5±2.19[b]	409.6±2.00[a]	389.7±2.25[b]	409.5±2.32[a]	407.1±2.87[a]	<0.0001
Albumen Ht (mm)	10.8±0.07[a]	9.4±0.09[b]	8.9±0.12[c]	9.0±0.11[c]	8.3±0.10[b]	<0.0001
HU	104.6±0.3[a]	96.8±0.41[b]	93.9±0.61[c]	94.0±0.58[c]	95.0±0.54[c]	<0.0001
Yolk colour score	10.3±0.08[c]	11.1±0.05[b]	11.3±0.07[b]	11.7±0.06[a]	11.7±0.07[a]	<0.0001

[a,b,c,d] Across a row, values with different superscripts are significantly different from each other.

Values are mean ± SE

Table 3. 3. Interaction of flock age and sheds on the eggshell quality measurements at 19, 26, 37, 50 and 60 weeks of age

Measurement		Flock age (weeks)					P Value		
		19	26	37	50	60	A	S	A*S
Shell reflectivity (%)	A	29.1 ± 0.5	26.5 ± 0.3	27.1 ± 0.4	29.0 ±0.5	27.9 ± 0.4	<0.0001	0.0025	<0.0001
	B	27.7 ± 0.4	27.3 ± 0.4	30.8 ± 0.6	29.7 ± 0.5	28.6 ± 0.5			
Egg wt (g)	A	49.5 ±0.5	59.5 ± 0.4	62.9 ±0.5	63.4 ±0.4	65.9 ±0.5	<0.0001	0.0001	<.0001
	B	51.4 ±0.5	57.8 ±0.5	59.4 ± 0.5	62.7 ± 0.5	63.8 ± 0.7			
Shell wt (g)	A	4.9±0.05	5.8±0.04	6.1±0.06	6.1±0.06	6.2±0.08	<0.0001	<0.0001	0.0050
	B	5.0±0.06	5.6±0.06	5.6±0.06	6.0±0.06	6.0±0.07			

There was significant effect of rearing shed on eggshell quality measurements, except for translucency score, shell breaking strength, shell deformation and percentage shell (Table 3.4).

Table 3.4. Main effect of sheds on egg quality measurements

Measurement	Sheds		P Value
	A	B	
Translucency score	2.5±0.05	2.6±0.05	ns
Shell reflectivity (%)	27.9±0.19[b]	28.8±0.23[a]	0.0025
Egg weight (g)	60.3±0.39[a]	59.0±0.35[b]	0.0001
Breaking strength (N)	42.7±0.39	42.0±0.39	ns
Deformation (µm)	279.3±0.21	276.8±0.20	ns
Shell weight (g)	5.8±0.04[a]	5.6±0.04[b]	<0.0001
Percentage shell (%)	9.7±0.04	9.7±0.04	ns
Shell thickness (µm)	405.7±1.46[a]	398.9±1.62[b]	0.0010
Albumen Ht (mm)	9.7±0.08[a]	9.3±0.07[b]	0.0001
HU	97.5±0.41[a]	96.3±0.38[b]	0.0059
Yolk colour score	11.3±0.05[a]	11.1±0.05[b]	0.0033

[a,b] Across a row, values with different superscripts are significantly different from each other. Values are mean ± SE

Translucency score was significantly higher at age 37 weeks than for all other ages except 60 weeks. There was no significant difference between the sheds for translucency score. Shell reflectivity varied significantly among the age groups although there was no consistent trend and shell reflectivity was higher in Shed B than in Shed A (Figure 3.4).

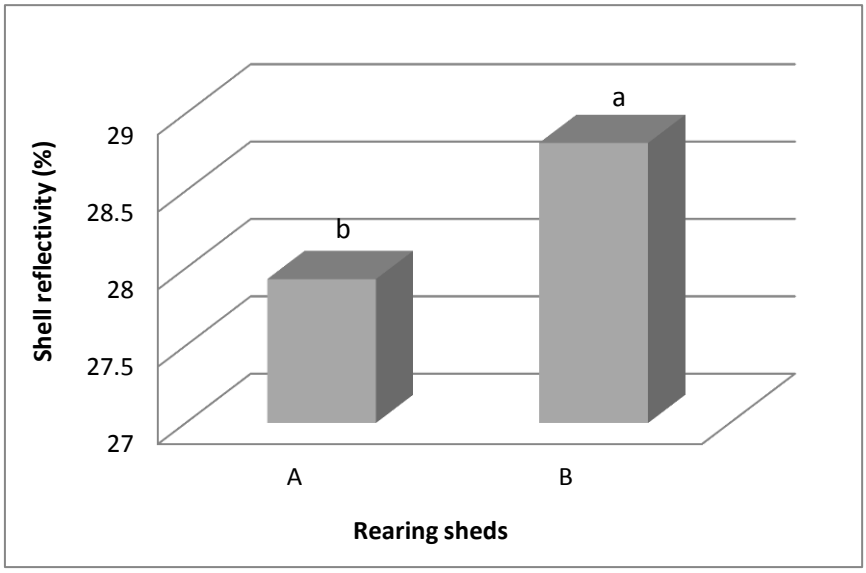

Figure 3.4. Shell reflectivity between the two sheds

Egg weight increased significantly with increasing hen age and body weight; egg weight was higher in Shed A than in Shed B (Figure 3.5 and 3.6).

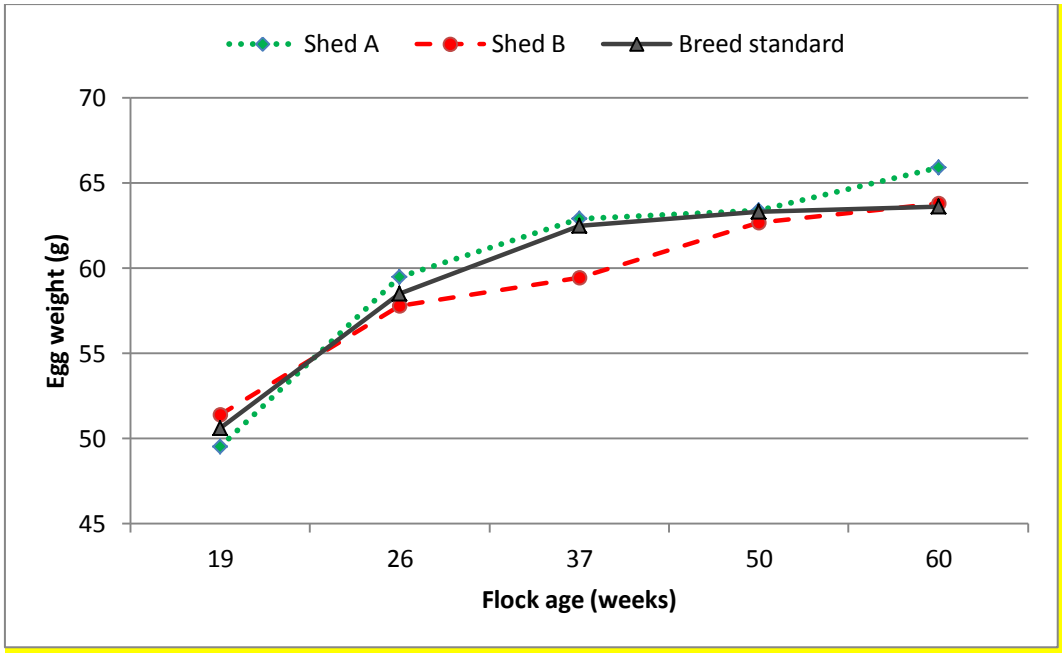

Figure 3.5. Egg weight comparison between the two rearing sheds

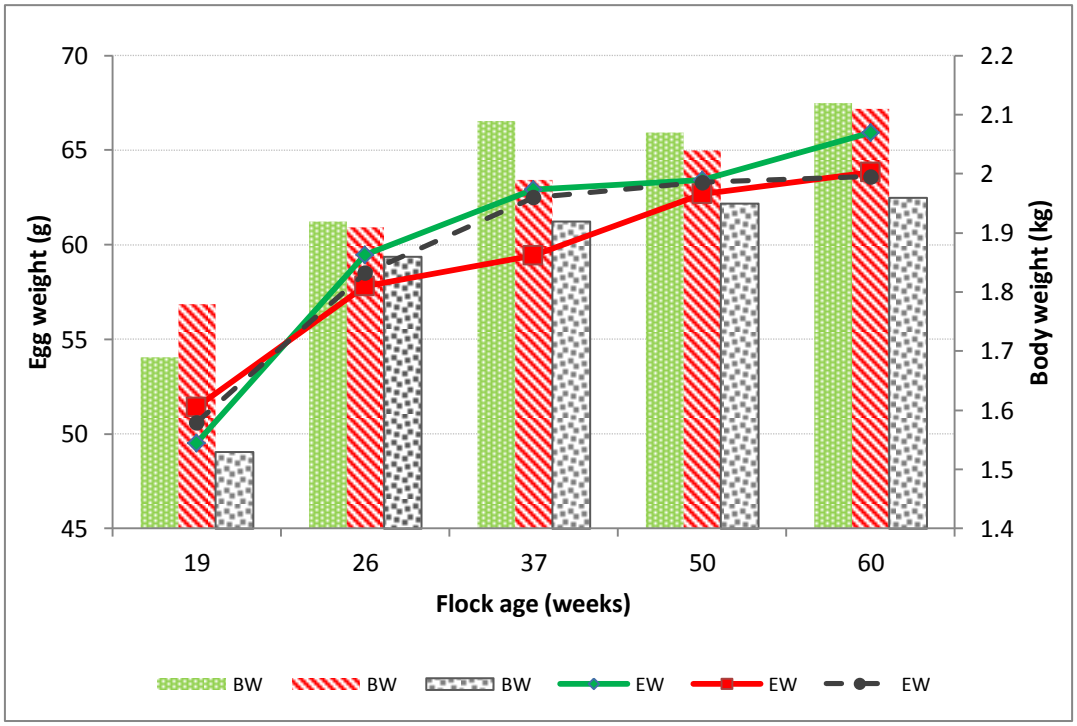

Figure 3.6. Egg weights and body weight between the rearing sheds, measured against the breed standard

Shell breaking strength decreased significantly (P<0.0001) (Table 3.2) with increasing flock age although there was no significantly difference from age 37 to 60 weeks. There was also no significant difference between the sheds for shell breaking strength.

Shell deformation to breaking point decreased from age 19 weeks to 50 weeks then remained relatively constant to age 60 weeks. There was no significant difference between the sheds for shell deformation.

Shell weight increased with increasing bird age with no significant difference from 50 to 60 weeks of age. Shell weight was significantly higher (P<0.0001) in Shed A than in Shed B (Figure 3.7). Clearly Shed A produced bigger eggs and bigger shells with thicker shells.

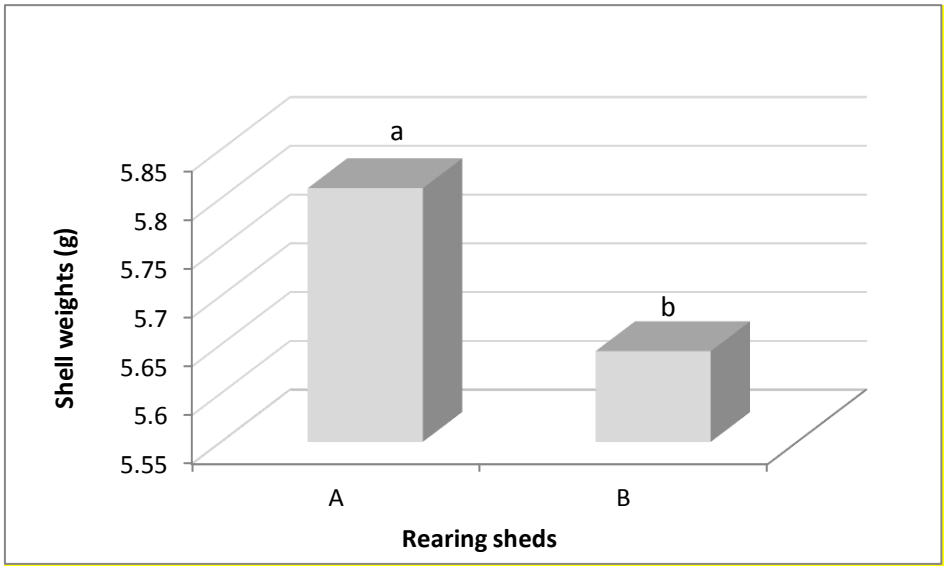

Figure 3.7. Shell weights between the rearing sheds

Percentage shell decreased with increasing bird age with no significant difference between age 37 and 50 weeks. There was no significant difference between sheds for percentage shell.

Shell thickness varied among the age categories, being highest at the ages of 26, 50 and 60 weeks. Shell thickness was higher in Shed A than in Shed B (Figure 3.8).

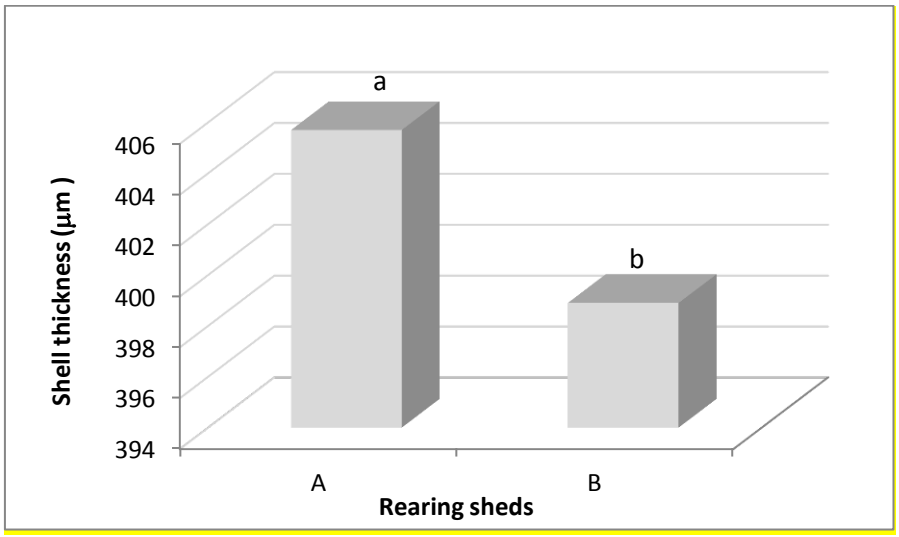

Figure 3.8. Shell thickness between the rearing sheds

For egg internal quality measures, albumen height varied among the ages, but generally decreased with increasing hen age. Albumen height was significantly higher in Shed A than in Shed B. Hus decreased to 37 weeks of age and then remained relatively constant. HU was higher in Shed A than in Shed B. Yolk colour significantly increased with increasing bird age but was not significantly different between the sheds.

3.3.3 Estimation of the amount of cuticle

Shell reflectivity (%) and spectrophotometry (L*a*b) measurements for cuticle cover

Table 3.5 summarizes the results for shell reflectivity and the spectrophotometry of eggshells before and after staining. There was a significant main effect of flock age for shell reflectivity before staining and values of the L*a*b* colour space system before and after staining. Shell reflectivity fluctuated with flock age, with the highest reflectivity being at age 50 weeks. There was also a significant effect of flock age on the difference in a* and b* before and after staining, but not on the difference in L* before and after staining.

Table 3.5 Main effect of flock age on shell reflectivity before staining and spectrophotometric measurements (L*a*b*) of stained cuticle

Measure-ment	Flock age (weeks)					P Value
	19	26	37	50	60	
Before staining						
Shell reflectivity (%)	28.1±0.59^{b}	28.0±0.42^{b}	28.2±0.49^{b}	31.0±0.60^{a}	28.9±0.47^{b}	0.0001
L	59.4±0.52^{c}	59.1±0.38^{c}	59.7±0.40^{c}	62.8±0.52^{a}	61.0±0.41^{b}	<0.0001
a	19.0±0.32^{a}	19.0±0.19^{a}	19.0±0.19^{a}	16.8±0.29^{c}	17.9±0.22^{b}	<0.0001
b	27.5±0.47^{c}	30.3±0.19^{a}	29.9±0.22^{a}	28.8±0.26^{b}	28.9±0.22^{b}	<0.0001
After staining						
L*	53.9±0.66^{b}	53.0±0.51^{b}	53.1±0.49^{b}	56.3±0.68^{a}	54.4±0.53^{b}	0.0003
a*	3.8±0.79^{a}	0.3±0.67^{b}	-1.5±0.60^{bc}	-2.3±0.81^{c}	-1.1±0.61^{bc}	<0.0001
b*	30.1±0.32^{c}	30.8±0.17^{b}	29.7±0.16^{c}	31.2±0.24^{ab}	31.6±0.18^{a}	<0.0001
ΔL*	5.6±0.35	6.0±0.25	6.6±0.44	6.6±0.31	6.5±0.24	ns
Δa*	15.1±0.75^{b}	18.7±0.70^{a}	20.5±0.70^{a}	19.3±0.82^{a}	19.0±0.63^{a}	<0.0001
Δb*	-2.6±0.54^{b}	-0.5±0.24^{a}	0.3±0.24^{a}	-2.4±0.32^{b}	-2.7±0.29^{b}	<0.0001
Single score	16.7±0.85^{c}	19.7±0.74^{b}	21.9±0.68^{a}	20.5±0.88^{ab}	20.4±0.68^{ab}	<0.0001

^{a,b,c} Across a row, values with different superscripts are significantly different from each other. Values are mean ± SE

The spectrophotometric measurements of shells with stained cuticle indicated that the value for L* showed the same pattern as for shell reflectivity, with a strong correlation between the two measurements (R^2= 0.951) (Figure 3.9).

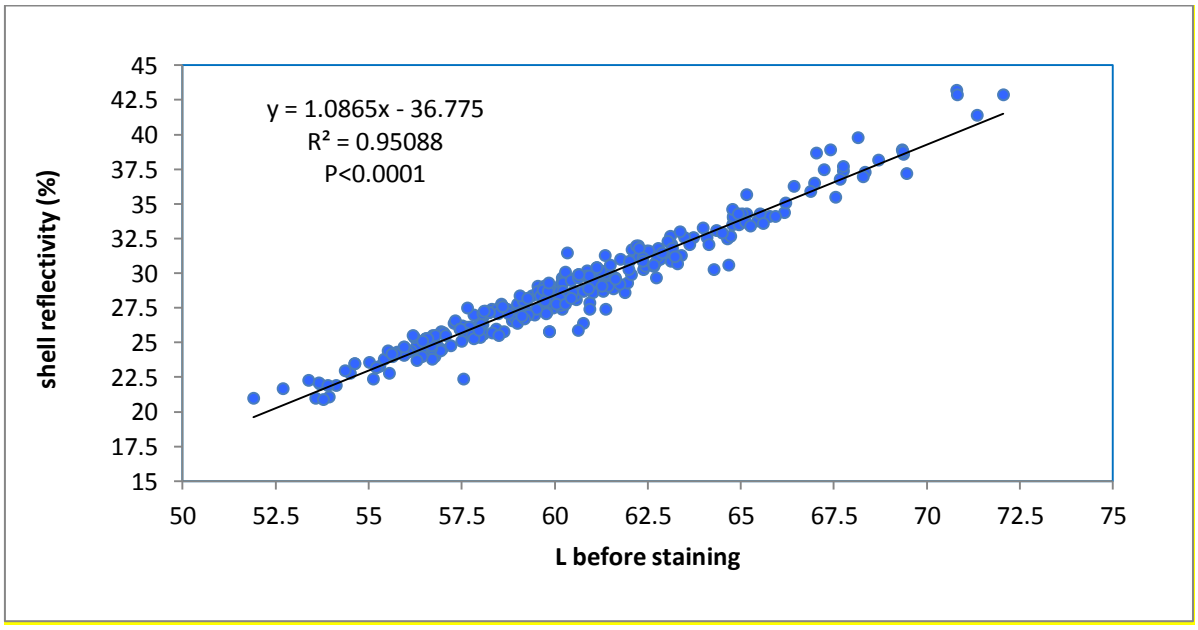

Figure 3.9. The correlation between shell reflectivity and L value before staining

The value for a* was significantly different among age categories, with the highest a* value at 19 weeks, indicating the lowest cuticle cover, and the lowest a* value which indicated more cuticle cover was at age 50 week.

The value for b* was highest at 50-60 weeks of age. The differences in L* values before and after staining were not significantly different among the age categories. The differences in a* values before and after staining were lowest at 19 weeks of age. The differences in b* values before and after staining were highest at 26-37 weeks of age. The single score value, calculated after the method of Leleu *et al*. (2011), was significantly higher at 37-60 weeks of age than at 19 and 26 weeks of age. In addition, there was a strong correlation between single score value and a* value after staining (Figure 3.10).

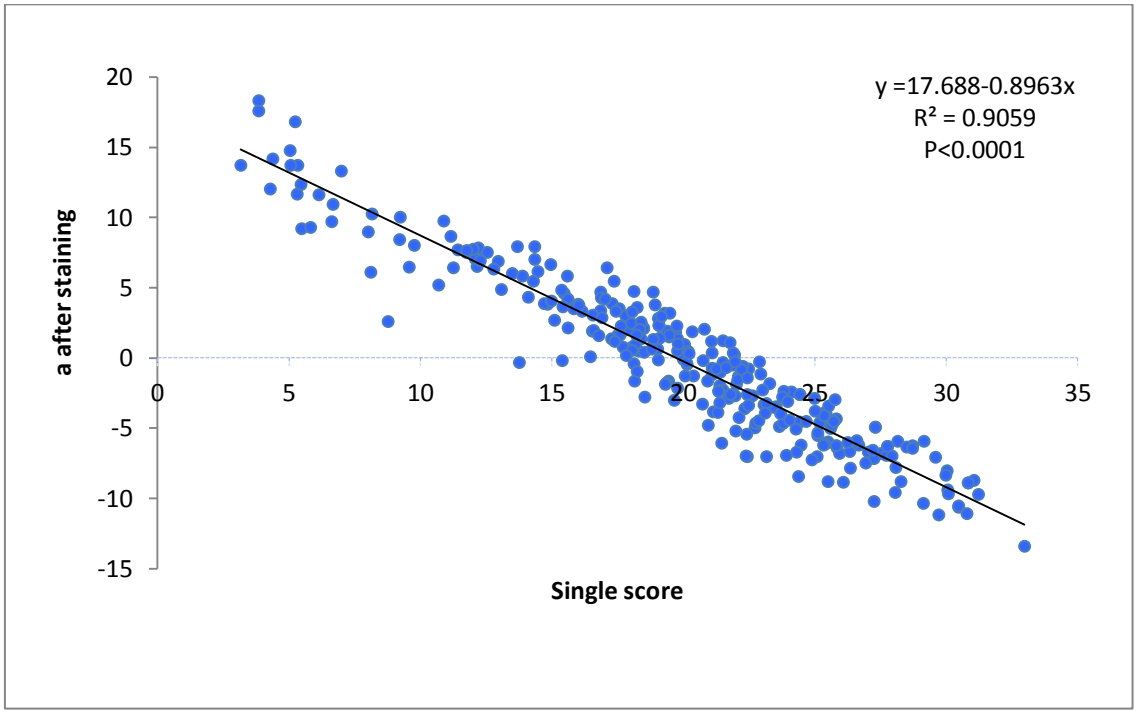

Figure 3.10. The correlation between a* value after staining and the single score

There was no significant difference between the rearing sheds for shell reflectivity before staining and the L*a*b* values before and after staining, except for the difference in L and the single score value were higher in Shed A than in Shed B (Figure 3.11).

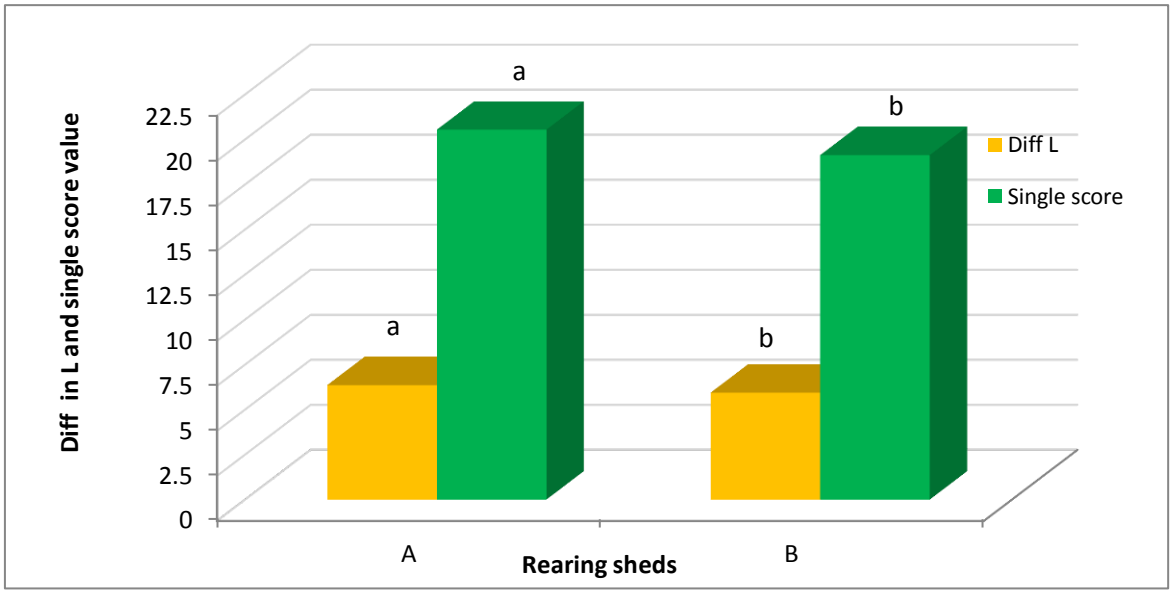

Figure 3.11. The different L and single score value between rearing sheds

Verification of the cuticle presence on eggshells by observation under the scanning electron microscope

The extent of cuticle cover, as observed under the scanning electron microscope, was scored on the scale described in Chapter 2, where 1 represents a good intact cuticle and 4 represents little or no cuticle. There was a significant effect of flock age on the amount of cuticle, as shown in Table 3.6, with the cuticle score being lower (indicating good intact cuticle) at age 37 weeks than for all other ages. This finding is consistent with the single score value and the difference in a* before and after staining, both of which indicated that the most complete cuticle cover was present in the flock at 37 weeks of age. However, the a* value after staining indicated that the highest level of cuticle cover was at 50 weeks of age.

Table 3.6. Cuticle cover scores under scanning electron microscopy (SEM)

Measurement	Flock age (weeks)					P Value
	19	26	37	50	60	
Cuticle cover	-	2.0±0.13[a]	1.6±0.13[b]	2.2±0.13[a]	2.2±0.13[a]	0.0052

[a, b,] Values with different superscripts are significantly different from each other. Values are mean ± SE

3.3.4 Eggshell ultrastructural variations of the mammillary layer

There were significant main effects of flock age on all ultrastructural scores except for the incidence of cubics, cubic cone formation and cuffing (Table 3.7). Mammillary cap size variability was lower at 26-37 weeks of age than for 50-60 weeks. The incidence of confluence tended to decrease with increasing flock age. Cap quality score generally increased with increasing hen age. The incidence of early fusion decreased, and the incidence of late fusion increased with increasing flock age. Alignment was higher in age 60 weeks and 50 weeks than at other ages. The incidence of Type-A bodies, Type-B bodies and aragonite increased with increasing flock age. The incidence of changed membrane decreased with increasing flock age. The incidence of depression and erosion were higher at age 60 weeks than other ages.

Table 3.7. The main effect of flock age on the mammillary ultrastructure scores of the eggshell

Ultrastructure features	Flock age (weeks)				P value
	26	37	50	60	
Mammillary cap size variability	1.6±0.08^{b}	1.8±0.09^{b}	2.2±0.10^{a}	2.3±0.09^{a}	<0.0001
Confluence	2.5±0.12^{a}	2.6±0.13^{a}	2.0±0.10^{b}	2.3±0.13^{ab}	0.0069
Cap quality	2.3±0.10^{b}	2.3±0.09^{b}	2.5±0.08^{ab}	2.7±0.10^{a}	0.0102
Early fusion	3.5±0.09^{a}	3.6±0.11^{a}	3.4±0.09^{a}	3.0±0.12^{b}	0.0004
Late fusion	2.9±0.13^{b}	2.5±0.14^{c}	3.1±0.12^{ab}	3.3±0.12^{a}	<0.0001
Alignment	2.0±0.06^{b}	2.2±0.10^{b}	2.6±0.11^{a}	2.8±0.12^{a}	<0.0001
Type A	1.4±0.08^{b}	1.6±0.07^{b}	1.6±0.08^{b}	1.8±0.09^{a}	0.0119
Type B	1.9±0.08^{c}	2.0±0.04^{bc}	2.2±0.08^{b}	2.7±0.11^{a}	<0.0001
Aragonite	1.1±0.07^{b}	1.2±0.06^{b}	1.2±0.08^{b}	1.5±0.11^{a}	0.0222
Cubics	1.1±0.03	1.1±0.03	1.1±0.03	1.1±0.03	NS
Cubic cone formation	1.0±0.03	1.0±0.02	1.0±0.00	1.0±0.00	NS
Cuffing	1.0±0.03	1.1±0.04	1.0±0.02	1.0±0.02	NS
Changed membrane	1.4±0.10^{a}	1.2±0.08^{b}	1.0±0.02^{b}	1.0±0.00^{b}	<0.0001
Depression	1.0±0.02^{b}	1.0±0.03^{b}	1.0±0.02^{b}	1.2±0.06^{a}	0.0004
Erosion	1.1±0.05^{b}	1.2±0.06^{b}	1.1±0.06^{b}	1.5±0.08^{a}	0.0002

[a, b,] Within a column, values with different superscripts are significantly different from each other. Values are mean ± SE

There was no significant effect of rearing sheds on shell mammillary layer ultrastructure, except for changed membrane, which had a higher incidence for rearing Shed B (Figure 3.12).

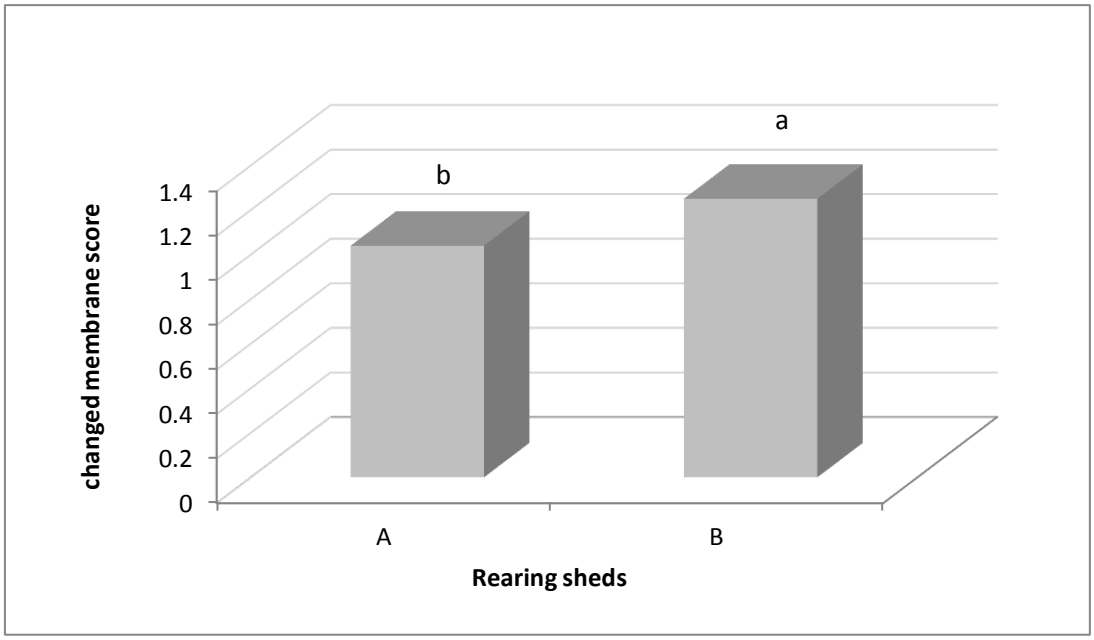

Figure 3.12. Changed membrane score between the rearing sheds

In this experiment, a new feature was found (Figures 3.13 and 3.14) in both rearing sheds at 26 weeks of age. Further analysis to identify this feature is still underway.

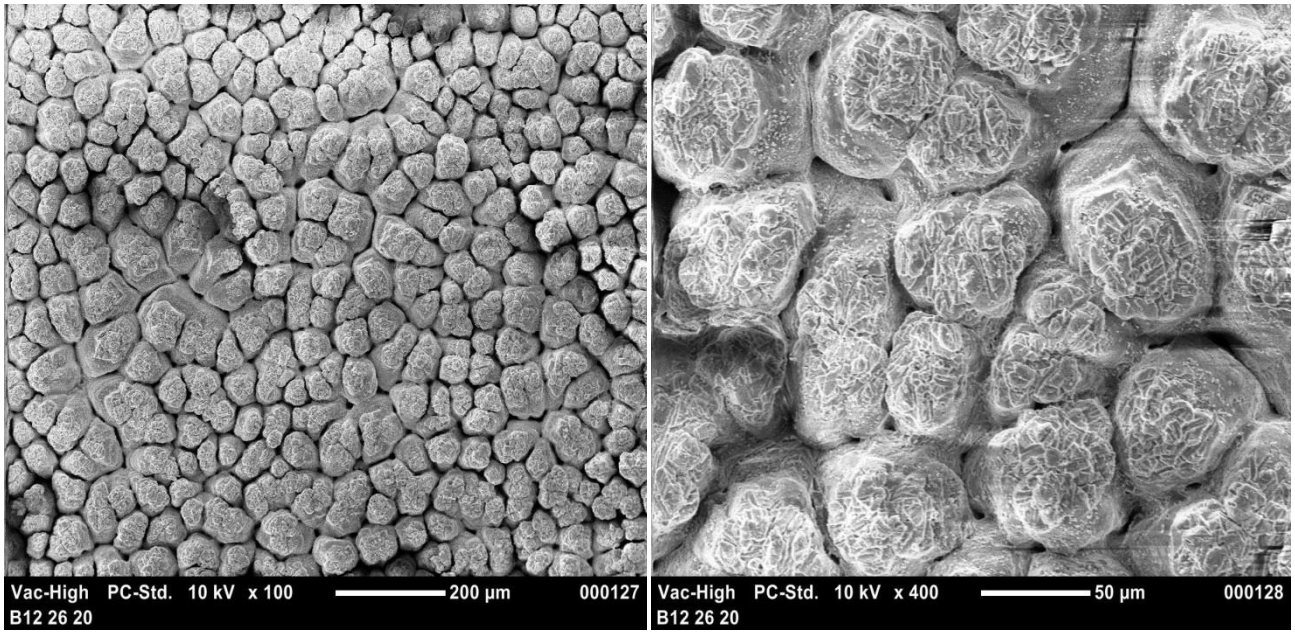

Figure 3.13. The SEM appearance of the new features in rearing Shed A at 26 weeks of age low (left) and high (right) magnification

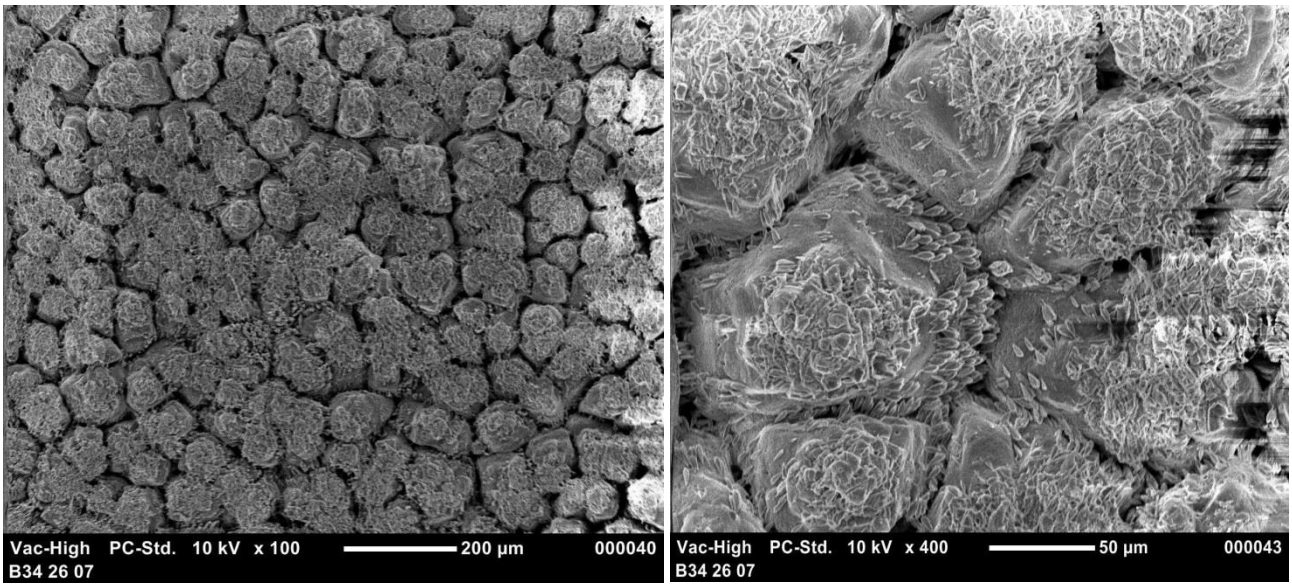

Figure 3.14. The SEM appearance of the new features in rearing Shed B at 26 weeks of age low (left) and high magnification (right)

3.3.5 Bone size and strength

Table 3.8 shows the breaking strength, length and width of both humerus and femur. The bone was taken from the hens at 86 weeks of age. There were no significant differences between the rearing sheds for bone breaking strength, length and width. Also, there was no significant correlation between body weight any of the bone measurements for either Shed A or Shed B (Figure 3.15)

Table 3.8. The length, width and breaking strength of humerus and femur bones for different rearing sheds

Sheds	BW (kg)	Humerus			Femur		
		Strength (kg)	Length (mm)	Width (mm)	Strength (kg)	Length (mm)	Width (mm)
A	2.2	21.9±0.81	74.6±0.84	8.0±0.10	41.2± 2.82	78.6±0.53	8.5±0.11
B	2.2	20.0±1.22	74.3±1.52	8.4±0.10	37.7±3.42	80.8±1.64	8.6±0.13

There were no significant differences for bones breaking strength, length and width.

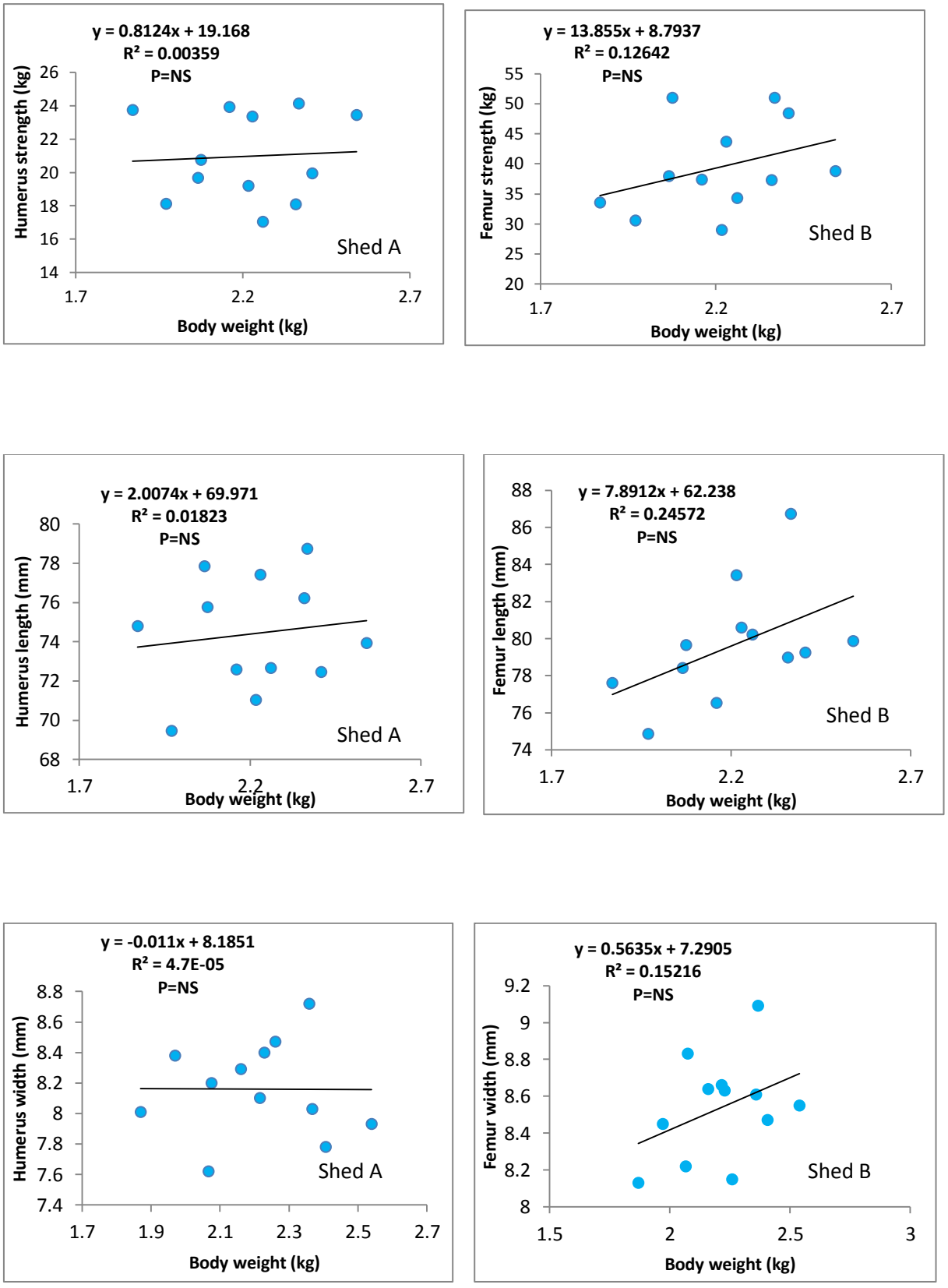

Figure 3.15. The correlation between body weight, bone strength, length and width in Sheds A and B

3.4 Discussion

3.4.1 Body weight and body weight uniformity

The body weight of hens in this study was higher than the Hy-Line Brown breeder performance standards, from 6 weeks of age until the end of the experiment at 60 weeks of age. On average, body weight was approximately 121 g above the breed standards between 6 and 60 weeks. This validates observations by Parkinson *et al*, (2007) who are of the opinion that the quality of a flock is indicated by its uniformity. Although the flock uniformity in this experiment was below 80% at six weeks of age, at point of lay it exceeded 80 % for both rearing sheds, until 26 weeks of age, and declined significantly thereafter. Contemporary information available in Australia indicates that many commercial flocks are achieving uniformities of approximately 90% at point of lay (18-19 weeks of age).

The flock in Shed B maintained a higher uniformity than that from Shed A between 19-50 weeks of age and had an estimated uniformity of 89% at 19 weeks of age, which is in line with the new potential standards for uniformity described by recent research (Parkinson, 2015, personal communication). Flocks with high uniformity have been reported to reach peak egg production earlier and have higher peak production than flocks of low uniformity (Hudson, *et al*., 2001; Kosba *et al*., 2009). On the other hand, poor uniformity is associated with variation in the degree of sexual maturity of hens, where underweight pullets have delayed onset of egg production (Yuan *et al*., 1994). Having the correct body weight at the start of egg production will enable pullets to achieve their genetic potential. Uniform flocks with the correct body weight mean that management changes (lighting, feeding and housing) can be more easily instituted (Kosba *et al*., 2009). The optimisation of flock body weight will provide producers with the maximum opportunity to push birds to their genetic potential.

Egg production was recorded as single-house egg production; it was not possible to compare the egg production of the two original flocks under commercial conditions. The egg production at point of lay was higher than the breeder recommendation, then fell slightly below the breeder standards to 33 weeks of age, after which time production slightly exceeded breeder standards. Peak egg production was delayed by some 10 weeks from 26 to 36 weeks of age, and this may be a consequence of the poorer uniformity standards of the larger flock density in Shed A. Overall

egg production met industry standards, and it seems likely that the data on egg quality derived from this flock would in all probability reflect industry norms.

In this on-farm study, the egg mass could not be counted, due to the absence of egg production records from the original rearing sheds. Although there was a decrease in body weight in Shed A at 50 weeks of age, the overall results indicate that both body weight and egg weight increased with increasing hen age. The reason for this is unknown, as there was no report from the owner at this stage. The pattern showed by Shed B was consistent: as the body weight increased, the egg weight increased.

3.4.2 Eggshell and egg internal quality measurements

Translucency score varied among age categories within a mean range of 2.11 to 2.83. The significance of this finding is not clear. In this experiment, eggs were analysed within 24 hours after collection. Ray (2012) evaluated the development of translucency of eggs, with daily observations over a period of 22 days after eggs were collected and stored, refrigerated, in cardboard cartons. He found that most freshly laid eggs showed relatively few translucent spots. Translucency developed rapidly within the first 24 hours after the egg was laid and continued to increase for about one week. The translucency score is a relatively subjective measurement of the incidence and extent of light patches in the eggshell, when the egg is placed on the candling light source. Translucency develops when moisture escapes from the egg albumen through the shell membranes into the ultrastructure of the mammillary layer (Solomon, 1991).

Shell reflectivity increase with increasing hen age has been reported by several authors (Zita *et al.*, 2009; Roberts *et al.*, 2013,). However, in this experiment, shell reflectivity varied among age categories, with the highest values at age 50 weeks, then decreased at age 60 weeks. The small but statistically significant decrease in shell reflectivity between 50 and 60 weeks of age may be explained by the slightly rougher surface of the 60 week eggs. However, this seems unlikely to be the case, as the pattern for L* of shells before staining was also significantly lower at 60 weeks than at 50 weeks. Shell reflectivity was higher in Shed B than in Shed A, indicating paler shell colour in Shed B.

Egg weight increased with increasing hen age, being highest at the age of 60 weeks and lowest at the 19 weeks of age. Increased egg weight with increasing hen age has been reported by several authors (Van Den Brand *et al.*, 2004; Silversides *et al.*, 2006; Ferrante *et al.*, 2009; Tůmová and Ledvinka, 2009; Ledvinka *et al.*, 2011; Roberts *et al.*, 2013,).

Shell breaking strength is the force in Newtons that causes the breakage of an intact shell when the egg is subjected to quasi-static compression. High breaking strength is indicative of a good quality eggshell. Shell breaking strength decreased from 19 to 37 weeks, then remained relatively constant to 60 weeks of age, with no significant difference from age 37 to age 60 weeks.

Shell deformation to breaking point is an indicator of the degree of elasticity of the eggshell. Shell deformation was highest at the age of 19 weeks then decreased with increasing hen age. A higher shell deformation in younger flocks has also been described by Roberts *et al.*(2013) and has been attributed to changes in shell structure resulting in a less elastic shell as flock ages.

Shell weight increased with increasing flock age although not in proportion to the increasing egg weight. This resulted in a percentage shell which decreased as hen age increased. This reduction in shell percentage results in an increased risk of fracture and this will be accentuated in very large eggs. However, between the sheds, shell weight was higher in Shed A than in Shed B, although the percentage shell was not significantly different between the two sheds.

The shell thickness varied among the age groups, being lower at 19 and 37 weeks than at 26, 50 and 60 weeks. Most studies report a steady decrease in shell thickness with increasing hen age, as has been described by other authors (Roberts *et al.*, 2013, Silversides, 1994).

In terms of internal quality, Shed A showed the highest value for albumen height, HU and yolk colour. The yolk colour increased with increasing hen age with no significant different for 50 and 60 weeks of age. Yolk colour mostly depends on the amount of xanthophyll in the feed. In this experiment, locally formulated feed was used.

3.4.3 Estimation of the amount of cuticle

The L* component of the L*a*b colour space system measures the grading between white (100) and black (0) colour. The use of shell colour, as measured by the spectrophotometer, is confounded by the underlying colour of the eggshell. L* values increased with flock age to 50 week of age, indicating that the eggs were lighter in colour with increased flock age. However, L* was significantly lower at 60 weeks of age, in parallel with the findings for shell reflectivity. The reason for this improvement in shell colour at 60 weeks of age is now clear.

The results from the a* value following staining with cuticle blue dye, indicated that the mean cuticle cover on the shell was highest at 50 weeks of age although this was not significantly

different from the results at 37 and 60 weeks. Roberts *et al.* (2013) also reported highest cuticle cover in mid (40-55 weeks) and late lay (55-65 weeks). However, Spark and Board (1984) reported that cuticle cover decreased with the increasing age of hens. Leleu *et al*. (2011) and Roberts *et al*. (2013) showed that a higher single score value denotes a higher staining affinity and hence more cuticle coverage, but the single score value does not always show the same pattern as the a* value after staining. In this experiment, the highest mean single score was at age 37 weeks (although not significantly different between age 37 and 50 weeks), which indicated more cuticle deposited on the shell. The a* value after staining was lowest at age 50 weeks (although not significantly different from the 37 and 60 week values). However, the single score value showed a strong correlation with the a* value ($R^2 = 0.906$) (Figure 3.10).

The appearance of the shell cuticle under the scanning electron microscope also showed that the thickest cuticle was at age 37 weeks and that the extent of staining with cuticle blue dye was a good indicator of the amount of cuticle present on an eggshell, as measured by the a* value after staining and the single score value. When verifying the cuticle appearance under the scanning electron microscope, only a single small piece of shell was taken from the equatorial region of the eggshell, whereas the spectrophotometric measurements of shell colour were conducted at three points around the equator of the egg. The single score value, as described by Leleu *et al*. (2011), measures the L*,a*, and b* values, before and after staining, then calculates a single value based on the differences in the values before and after staining. The higher the single score value and the more negative the a* value after staining, the higher the extent of cuticle cover. The single score was higher for eggs from birds reared in Shed A than for those from birds raised in Shed B, although no differences were found between the two sheds for the a* value after staining. The significance of the amount of the cuticle cover is still uncertain although there is good evidence that sufficient cuticle cover is important for the microbiological safety of eggs. The results from the b* value shows that the shells of the eggs from the older hens were less yellow than for the other group of ages, which may correlate with the paler brown-coloured shell of this group. However, there is no clear indication that the b* value correlates to the amount of cuticle cover.

3.4.4 Shell ultrastructure

Ultrastructural studies have demonstrated that the eggshell is comprised of morphologically distinct calcified layers with the mammillary layer being the “foundation” of the eggshell. Studies

have identified ultrastructural variations in the mammillary layer that can be used as indicators of eggshell quality (Roberts and Brackpool, 1995). In this experiment, the mammillary cap size was least variable at the youngest age (26 weeks) and deteriorated as the hens aged. The incidence of confluence, which is thought to increase shell strength, was more extensive at age 37 weeks and decreased as hens aged. Although no significant difference was found between the two sheds, Shed B tended to have a higher incidence of confluence than Shed A. The attachment of the mammillary layer to the shell membranes and the quality of construction of the mammillary layer plays an important role in determining the strength of the entire eggshell (Roberts and Brackpool, 1995). The incidence of changed membrane was significantly decreased ($P<0.0001$) as hens aged, and was most extensive in Shed B at 26 weeks of age. Changed membrane may reflect abnormal conditions within the oviduct while the eggshell was being laid down (Roberts and Brackpool, 1995). As hen age increased, the incidence of ultrastructural features known to be associated with poorer shell quality increased: alignment of mammillae, Type-A bodies, Type-B bodies, aragonite, late fusion and pitting. The incidence of ultrastructural features shown to be associated with good shell quality, such as early fusion and confluence, decreased as the flocks aged.

Understanding the ultrastructure of eggshells has reinforced the view that the mechanical properties of the eggshell cannot be defined by a simple thickness measurement (Bain, 2005). Therefore it seems reasonable that any changes in morphology or composition will affect shell structure. A comprehensive description of these variations has been described previously (Solomon, 1991; Roberts and Brackpool, 1995).

In this experiment, a new characteristic was found in the mammillary layer (Figure 3.13-3.14.). that has not yet been described in any references This feature was found at 26 weeks of age in both sheds. We assume that this feature is another form of calcite. Personal communication with Professor Joel Gautron of INRA, France, suggests that this is a feature of amorphous calcium carbonate, as described by Rodriguez-Navarro *et al*. (2015). Amorphous calcium carbonate (ACC) is an unstable mineral which is easily dissolved (Raz *et al*., 2002). There are eight known polymorphs of calcium carbonate; seven crystalline and one amorphous. Three of the polymorphs (calcite, aragonite and vaterite) are pure calcium carbonate, while two (mono-hydrocalcite and the stable forms of amorphous calcium carbonate) contain one water molecule per calcium carbonate (Addadi *et al*., 2003). Different proteins and the equilibrium of uterine fluid solution chemistry, which is controlled by the formation, and dissolution of ACC, might play an important role during eggshell mineralization (Lakshminarayanan *et al*., 2006; Rodriguez-Navarro *et al*., 2015). One of

the major challenges in the field of bio-mineralization is to understand the mechanism(s) by which biological systems determine which polymorph will precipitate. However, an analysis is underway to examine eggshells using X-ray diffraction and IR systems, in an attempt to further explain the phenomenon of this feature (Bain, M and Gautron, J; personal communication).

3.4.5 Bone size and strength

The result from bone measurements in the current study indicated that birds from the two original rearing sheds were not significantly different.

3.5 Conclusions

It can be concluded from this experiment that a comprehensive set of methods have been developed for the determination of egg quality traits on farm sampling. The heavy body weight in Shed A resulted in low uniformity and large egg size. The higher percentage of larger birds in Shed A than in Shed B could explained the variation in body weight uniformity. By maximising both body weight uniformity and the appropriate body weight category it may be possible to investigate maximum physiological potential for egg production and to then evaluate the egg quality consequences of such high egg- mass outputs.

The magnitude of the differences in commercial flock body weight with the Breeder Standards has been validated and the total body weight ranges identified.

Considering the importance of flock body weight uniformity in production performance, a model will be established attempting to maximise uniformity and then study the impacts of the range of weight categories likely to exist in commercial flocks,

In the next experiment these body weight categories will be modelled in a more systematic way to examine closely the interaction between body weight and production performance, egg size and egg quality.

Chapter 4

Effect of body weight uniformity on eggshell quality of hens in a free-range production system over the laying period

4.1 Introduction

Free-range poultry production has been developing rapidly in many countries. The demand for free-range products increases with the pressure on improving poultry welfare in the poultry industry (Miao, *et al.*, 2005). The definition of the term "free-range" varies greatly between countries and even within a single country. There are no legal guidelines for the definition of free-range in Australian legislation; as long as birds can access pasture each day, the eggs can be labelled "free range" (Primary Industries Standing Committee, 2002). In the European Union (EU), this definition is more stringent and free-range chickens must have continuous daytime access to open air runs, which must be covered with vegetation (Shimmura *et al*., 2008). The USA, Australia and European countries have developed Codes of Practice for free-range poultry farming which detail the minimum standards of husbandry and welfare for birds (Miao *et al*., 2005). The common characteristic of this production system is that flocks are not in cages and have daily access to an outdoor run with a minimum area per hen of 4 m^2 (Hegelund, *et al*., 2006). Rodenburg *et al*. (2012) reported that a maximum of 9 birds/m^2 can be kept, equal to 1111 cm^2 of floor space per bird in barn and free-range systems.

Australia uses several production systems for egg production, including conventional cage (53%), free-range (38%), aviary and barn systems (8%) (AECL, 2014). Each production system has advantages and disadvantages in terms of efficiency, production costs, animal welfare, food safety and environmental impact.

Alternative housing systems for egg production represent clear evidence of the animal housing and husbandry trend towards extensive rearing methods, mainly in developed countries. Consumers are interested in specialty eggs from free range poultry for two main reasons; welfare and safety issues. Appleby (2012) indicates that welfare considerations have played a greater role in egg sales than in any other sector of poultry production. In egg production, costs are generally higher in systems perceived to have higher welfare, but the demand for eggs is relatively constant

and sales of eggs from systems such as free-range have led the way for welfare improvements in all livestock production (Appleby, 2012).

Housing systems have been thought to influence eggshell quality characteristics (Silversides and Scott, 2001; Van den Brand *et al*., 2004). Clerici *et al*. (2006) reported a significant difference between the housing systems for egg weight, surface area, and shell thickness. However, they came to the conclusion that producer management and other factors such as hen age and strain are more likely to influence the shell characteristics than the housing system. Some of the early problems associated with egg quality in alternative housing systems have been solved by improvements in housing design.

Apart from strain, nutrition and disease, body weight uniformity is thought to be another factor which may influence overall egg quality. Maintaining high body weight uniformity is a major objective during the rearing period, and provides an estimate of the variability in a given flock at a given age. The more uniform the flock, the better the performance of that flock, and the more consistent the nutritional responses. The conventional goal for flock uniformity is to have 80 per cent of the pullets within plus or minus 10 per cent of the average flock body weight. Flocks with high uniformity have been reported to reach peak egg production earlier and have higher peak production than flocks of low uniformity (Hudson *et al*., 2001; Kosba *et al*., 2009). On the other hand, poor uniformity is associated with variation in the degree of sexual maturity of hens, and underweight pullets have delayed onset of egg production (Yuan *et al*., 1994).

The presence of obese birds on several commercial layer farms in Australia, reported by Parkinson *et al*. (2007), resulted in lower eggshell quality. Productive and profitable layers begin with good quality pullets achieving the correct average body weight at the start of egg production within the contemporary uniformity standards. This ensures a flock has a high probability of achieving their genetic potential. Problems which develop during the growing period cannot easily be corrected after egg production begins. It has been generally assumed that flock uniformity is more difficult to achieve in free range production than in cage production (Parkinson, personal communication), and these differences between conventional cage performance and free-range seem likely to respond to additional research defining the mechanisms for the performance differences

In this Chapter, a study was conducted on commercial free-range flocks to evaluate the effects of body weight uniformity on production, egg size, and both external and internal egg quality. A

systematic analysis of the flock characteristics could then be compared to similar data accumulated for cage production, in both commercial and laboratory settings

4.2 Materials and methods

Seven flocks of Hy-Line Brown commercial layers in free-range production systems in the Kemps Creed region of New South Wales (NSW) were followed throughout the production cycle. Five flocks were randomly sampled for body weight uniformity at 6 weeks of age, six flocks were studied for body weight at 16 and 19 weeks of age, and all seven flocks were recorded for body weight and eggshell and internal quality from 26-60 weeks of age. The total number of birds in each flock was 15814, 15314, 18476, 16300, 15614, 15663 and 16412, and flocks reached 26 weeks of age on May 11 2012, July 13 2012, September 21 2012, November 23 2012, January 25 2013, July 23 2013 and August 22 2013 for flocks 1, 2, 3, 4, 5, 6 and 7, respectively.

At least 100 birds were weighed from each flock at the different ages: 6, 16, 19, 26, 37, 50 and 60 weeks of age, and body weight uniformity was calculated.

A total of 90 eggs was collected randomly from each flock at 26, 37, 50 and 60 weeks of age. Eggs were collected, stored in a cool room at prior to transport (within 1-7 days), then analysed for egg internal and eggshell quality soon after, as described in Chapter 2. The extent of cuticle cover and ultrastructural features of the mammillary layer were also analysed.

4.3. Statistical analysis of data

Data were analyzed using Statview Software (SAS Institute Inc., Version 5.0.1.0). A two way analysis of variance was conducted taking flock age and shed/flock as the independent variables and body weight, egg quality measurements, spectrophotometry (L*a*b) measurements, single score measurements for cuticle cover, and ultrastructural features as dependent variables. Level of significance was indicated by probability of less than 5%. The Fishers PLSD test was used to differentiate between mean values.

4.4 Results

4.4.1 Body weight and flock uniformity and egg production

Seven flocks were studied in this experiment, however, only five flocks had body weight recorded at six weeks of age. Figure 4.1 shows the mean body weight at 6 weeks of age of these five flocks. There were significant differences in body weight among the flocks, with body weight being highest in Flock 4 and lowest in Flock 1. Flock 3 was clearly above the breed standard and Flock 1 below the standard, at 6 weeks of age.

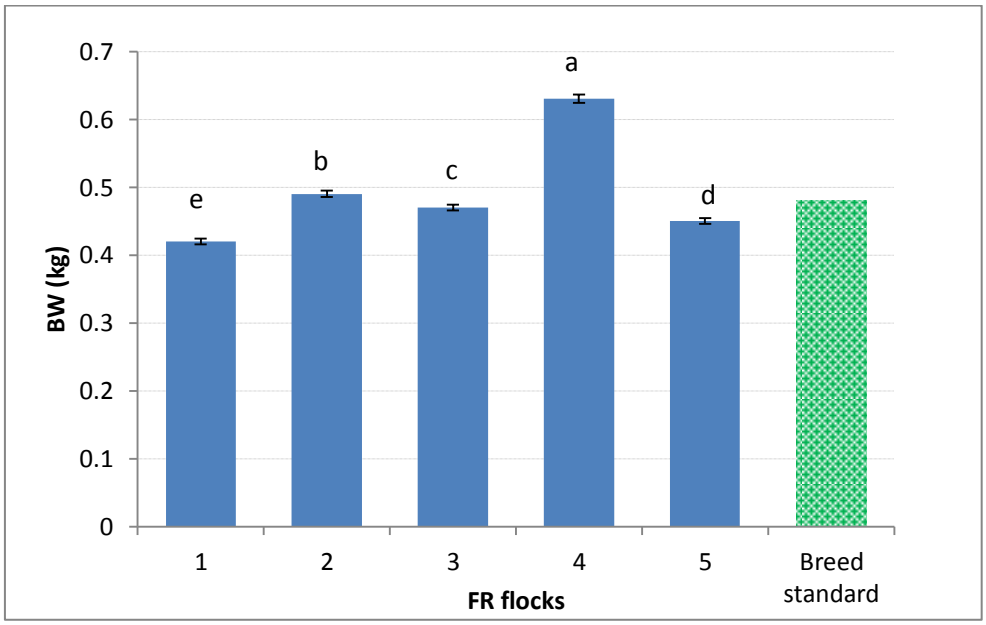

Figure 4.1. Body weights at 6 weeks of age in free range systems, compared to the breed standard

At 16 weeks of age, body weight was recorded for six of the flocks, as shown in Figure 4.2. Flocks 3 and 4 had the highest body weight compare to the breed standard and Flocks 1 and 2 had the lowest body weight. Flocks 5 and 7 were of similar body weight to the breed standard.

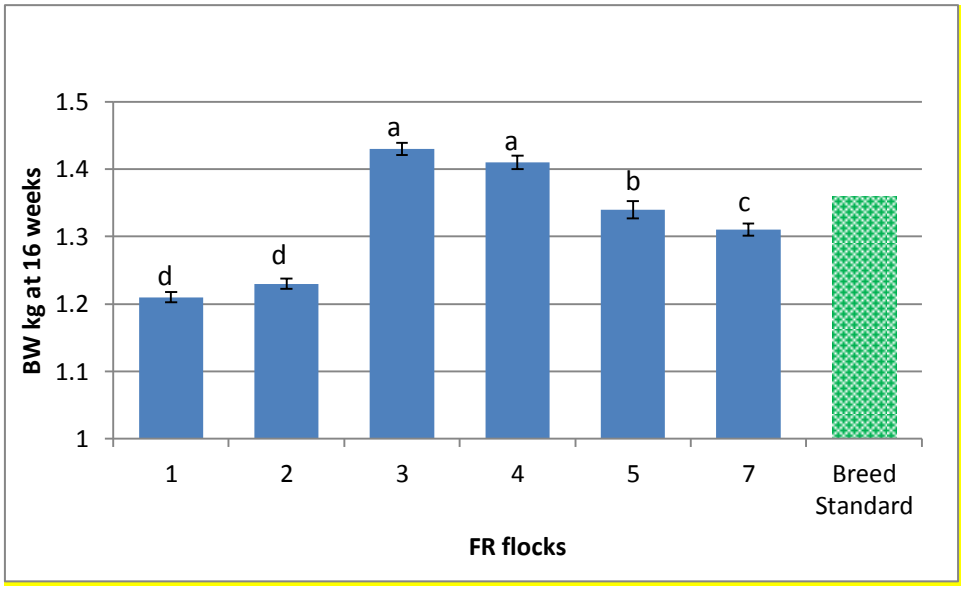

Figure 4.2. Body weights at 16 weeks of age in free range systems, compared to the breed standard

At 19 weeks of age, body weight was recorded for six flocks, as shown in Table 4.1. Body weight was highest for Flocks 3 and 6, and lowest for Flocks 1, 2 and 5, with Flock 7 being intermediate. From age 26 to 60 weeks of age, body weight was recorded for all seven flocks, as shown in Table 4.1.

Table 4.1. Flock body weight in free-range systems from age 19 to 60 weeks

Flocks	Flock age (weeks)				
	19	26	37	50	60
FR 1	1.4±0.01^{c}	1.9±0.02^{a}	2.0±0.02^{a}	1.9±0.02^{ab}	1.9±0.02^{bc}
FR 2	1.5±0.01^{c}	1.8±0.02^{b}	2.0±0.01^{a}	1.9±0.02^{ab}	2.0±0.02^{b}
FR 3	1.7±0.01^{a}	1.9±0.01^{a}	1.9±0.02^{b}	2.0±0.02^{a}	2.0±0.02^{a}
FR 4		1.8±0.02 ^{b}	1.9±0.01 ^{b}	1.9±0.01^{ab}	2.0±0.02 ^{ab}
FR 5	1.5±0.01^{c}	1.7±0.01^{c}	1.8±0.01^{d}	2.0±0.02^{ab}	2.0±0.02^{b}
FR 6	1.7±0.01^{a}	1.9±0.01^{a}	1.9±0.01^{c}	1.9±0.02^{b}	1.9±0.02^{cd}
FR 7	1.6±0.01^{b}	1.9±0.02^{a}	1.8±0.02^{c}	1.9±0.01^{c}	1.9±0.01^{d}
Breed	1.6	1.9	1.9	2.0	2.0
P Value	<0.0001	<0.0001	<0.0001	0.0001	<0.0001

^{a,b,c,d} within a column, values with different superscripts are significantly different from each other. Values are mean ± SE

There were significant differences between flocks for body weight at all ages recorded. At 26 weeks of age, body weight was highest for Flocks 1, 3, 6 and 7 and lowest for Flock 5, with flocks Flocks 2 and 4 intermediate. At 37 weeks of age, body weight was highest for Flocks 1 and 2, followed by Flocks 3 and 4, then Flocks 6 and 7, with Flock 5 being the lowest. At 50 weeks of age, body weight was relatively similar among the flocks, with Flock 3 highest and Flock 7 lowest. There was a similar pattern at 60 weeks of age. Three flocks experienced slow or below average growth rates between 26 to 37 weeks of age, relative to breed standards. Flock 1 had an average growth rate between 19-37 weeks of age which was aligned very closely with the breed standard, despite a lower pullet weight at 19 weeks of age.

Body weight uniformity of the flocks studied ranged from 62% to 89% (Table 4.2). There was no clear pattern of body weight uniformity in relation to flock age. Flocks 1 and 6 maintained high uniformity standards between 19 and 50 weeks of age, relative to the other 5 flocks. There was a marked reduction in uniformity in most flocks between 16 and 19 weeks of age, as illustrated in Figure 4.3).

Table 4.2. Flock uniformity in seven free range flocks at different ages

Flocks	Flock age (weeks)						
	6	**16**	**19**	**26**	**37**	**50**	**60**
FR 1	72.6	84.7	80.9	83.7	85.5	81.3	75
FR 2	71.4	85.4	80	79.1	72.2	78	76
FR 3	73.9	89.2	77.5	84.9	76.7	78.9	79.4
FR 4	68.8	88.9	-	74	74.4	81.8	73.8
FR 5	72.3	62.0	68.2	83.1	77.4	80.4	77.3
FR 6	-	-	83.5	84.2	80	81	80.8
FR 7	-	84.1	79.4	76.3	73.3	81	84.3

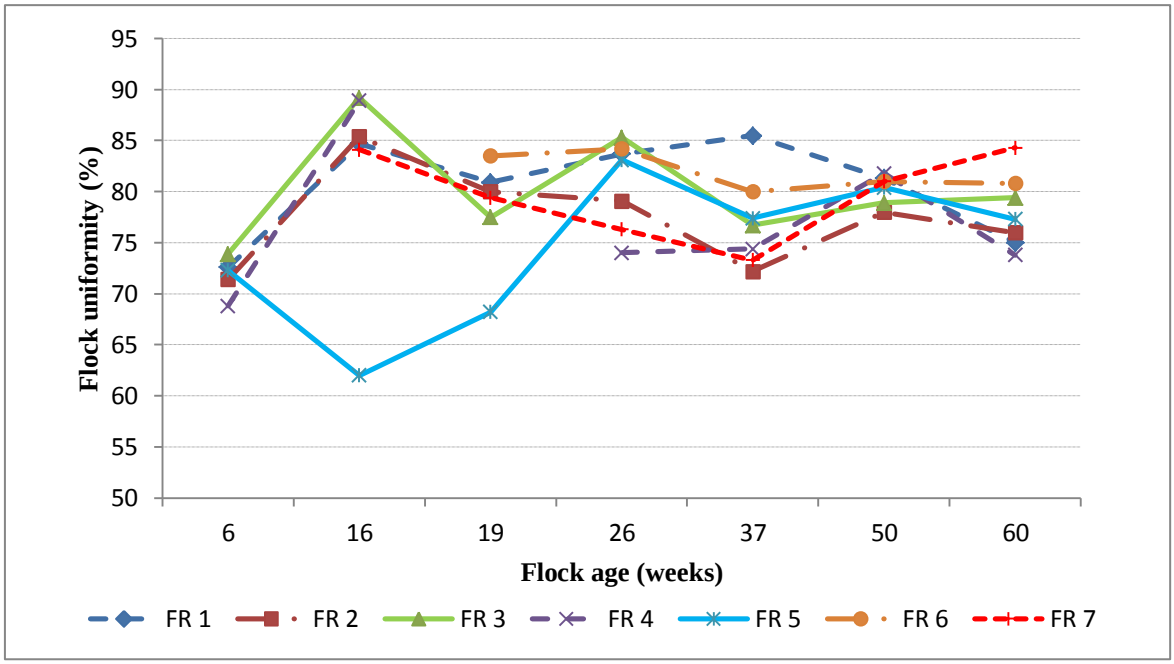

Figure 4. 3. Flock uniformity of seven flocks from age 6 to 60 weeks

It can be seen from Figure 4.3 that the timing of onset of lay varied among the seven flocks with Flocks 3, 5, 6 and 7 reaching point of lay at a younger age than Flocks 1, 2 and 4.

4.4.2 Egg quality

Figure 4.4 shows the hen-day egg production of the commercial free range flocks. Egg production varied among the flocks (Figure 4.4). There was considerable variation in the age at which birds came into lay and the extent of persistency of lay. The sudden decrease in production in Flock 5 at 30-31 weeks was not explained by the producer.

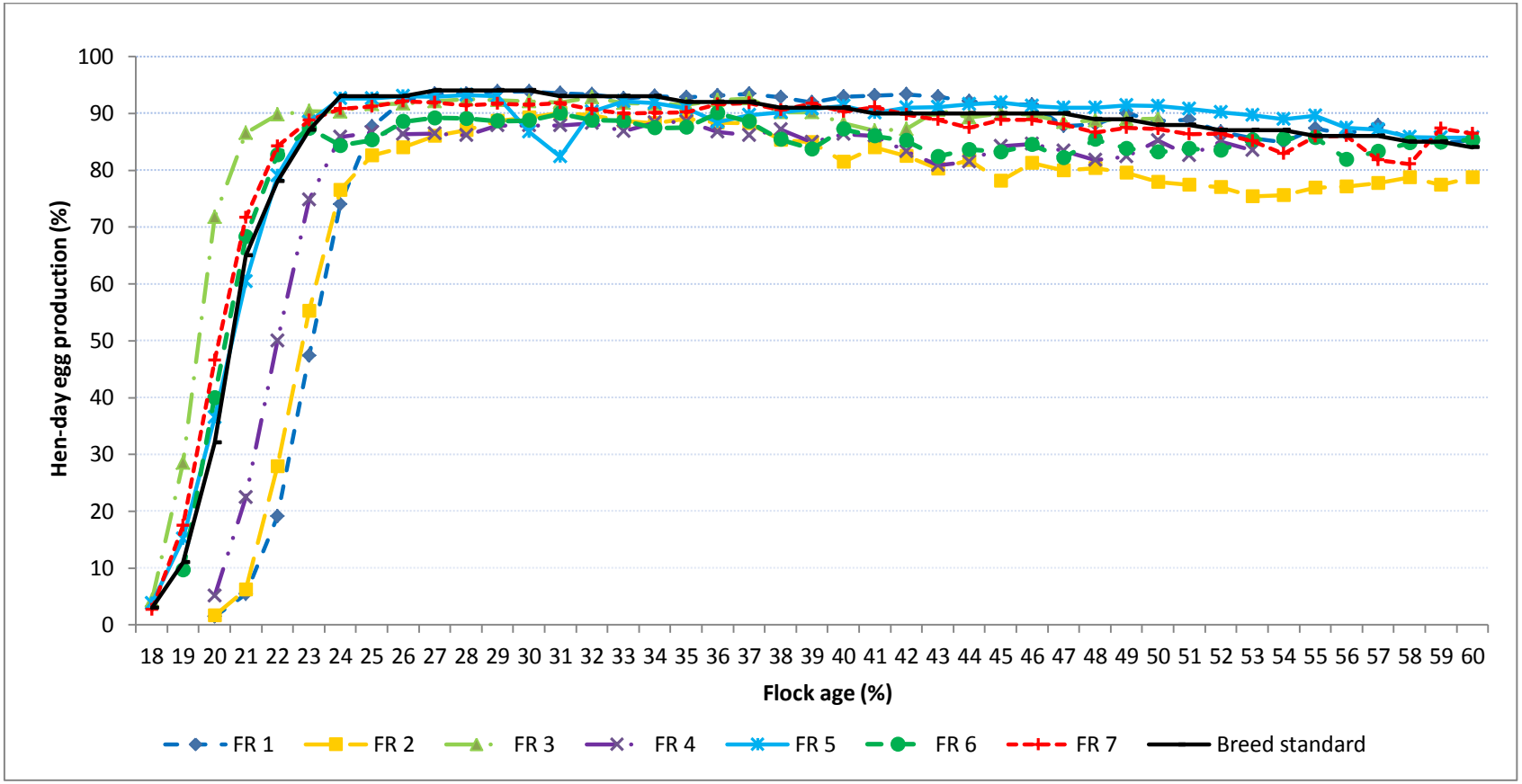

Figure 4.4. Hen-day egg production of the seven free range flocks, as compared to the breed standard

Table 4.3. Hen-housed egg production (%) of the seven free range flocks

Flocks	Flock age (weeks)			
	26	37	50	60
FR 1	92.2	93.4	88.7	85.2
FR 2	83.8	87.7	76.9	76.3
FR 3	91.3	83.4	79.1	NA
FR 4	90.6	89.6	75.6	NA
FR 5	92.6	89.0	90.2	83.3
FR 6	87.7	86.6	76.2	72.7
FR 7	91.8	91.1	86.0	84.6
Breed standard	90	90	84	78

Egg Production in Flock 1 was maintained at or above breed standards from 26 to 60 weeks of age (Table 4.3). The average egg production was very low in three flocks (Flocks 2, 4, 6) during time observed.

There was no significant correlation between average body weight and average egg production from 26 to 60 weeks of age (Figure 4.5) and the correlation between flock uniformity and egg production was low (R^2= 0.0629) (Figure 4.6).and was not statistically significance.

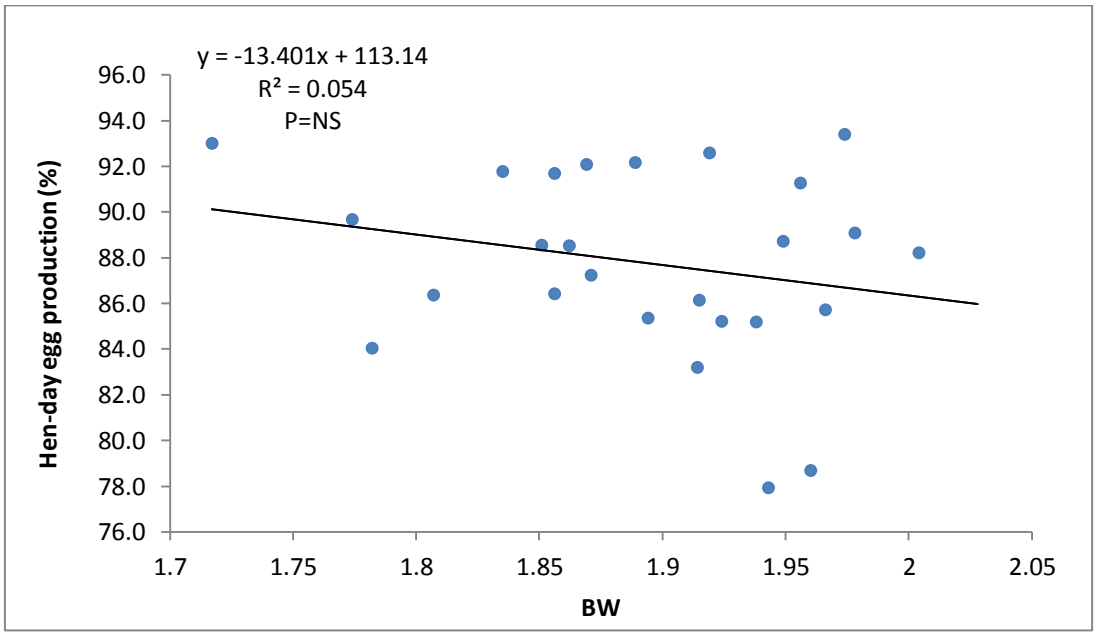

Figure 4.5. The correlation between the average body weight and egg production from 26 to 60 week

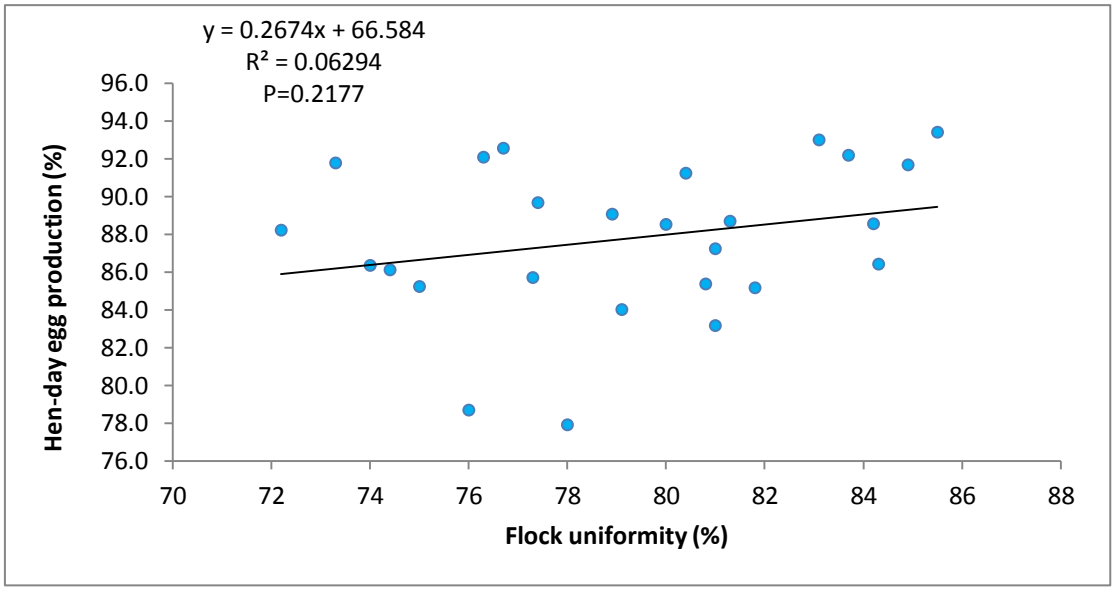

Figure 4.6. The correlation between flock uniformity from 26 to 50 weeks, and egg production 26-60

Table 4.4 shows the main effect of hen age on eggshell quality measurements and Table 4.5 shows the main effect of flock. There were significant interactions between hen age and flock for all eggshell quality measurements (see Appendix d). Translucency score was significantly higher at age 26 weeks than for all other ages. Shell reflectivity varied significantly among the age groups, being lowest at 26 weeks of age. Egg weight increased significantly with increasing flock age, although there was no significant difference between 50 and 60 weeks of age.

Table 4.4. Effects of hen age on the traditional measures of eggshell quality corrected

Measurements	Flock age (weeks)				P Value
	26	37	50	60	
Eggshell quality					
Translucency score	3.8±0.05^{a}	3.5±0.05^{b}	3.3±0.05^{c}	3.6±0.05^{b}	<0.0001
Shell reflectivity (%)	28.0±0.20^{c}	30.5±0.22^{a}	29.9±0.27^{b}	31.0±0.24^{a}	<0.0001
Egg weight (g)	58.7±0.21^{c}	61.7±0.22^{b}	63.5±0.24^{a}	63.3±0.25^{a}	<0.0001
Breaking strength (N)	44.1±0.41^{a}	43.5±0.39^{a}	42.0±0.35^{b}	40.3±0.39^{c}	<0.0001
Deformation (µm)	293.0±3.93^{a}	287.2±2.31^{a}	269.4±1.73^{b}	260.7±2.05^{c}	<0.0001
Shell weight (g)	5.6±0.03^{d}	5.8±0.03^{c}	6.1±0.03^{a}	6.0±0.03^{b}	<0.0001
Percentage shell (%)	9.5±0.04^{a}	9.4±0.04^{b}	9.5±0.04^{a}	9.5±0.04^{ab}	0.0018
Shell thickness (µm)	401.1±1.44^{b}	404.2±3.73^{b}	415.8±3.73^{a}	407.9±1.32^{b}	0.0001
Internal quality					
Albumen height (mm)	9.0±0.06^{a}	7.1±0.06^{b}	6.9±0.07^{c}	6.3±0.07^{d}	<0.0001
HU	94.5±0.28^{a}	83.1±0.41^{b}	80.9±0.45^{c}	77.0±0.52^{d}	<0.0001
Yolk colour score	10.3±0.05^{b}	10.0±0.06^{c}	10.4±0.05^{ab}	10.5±0.05^{a}	<0.0001

Shell breaking strength decreased significantly with increasing flock age, although there was no significant difference from age 26 to 37 weeks. Shell breaking strength showed a significant positive correlation R^2= 0.3236 with shell thickness (Figure 4.7), but showed a slight negative correlation with egg weight (Figure 4.8).

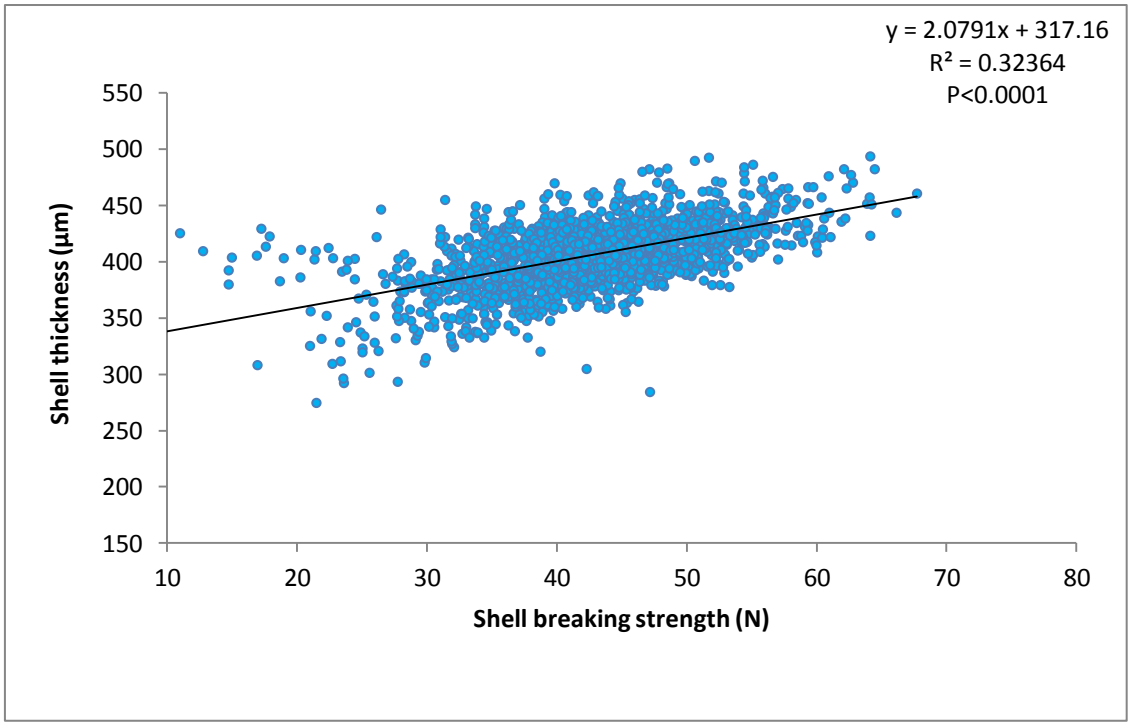

Figure 4.7. The correlation between shell thickness and shell breaking strength

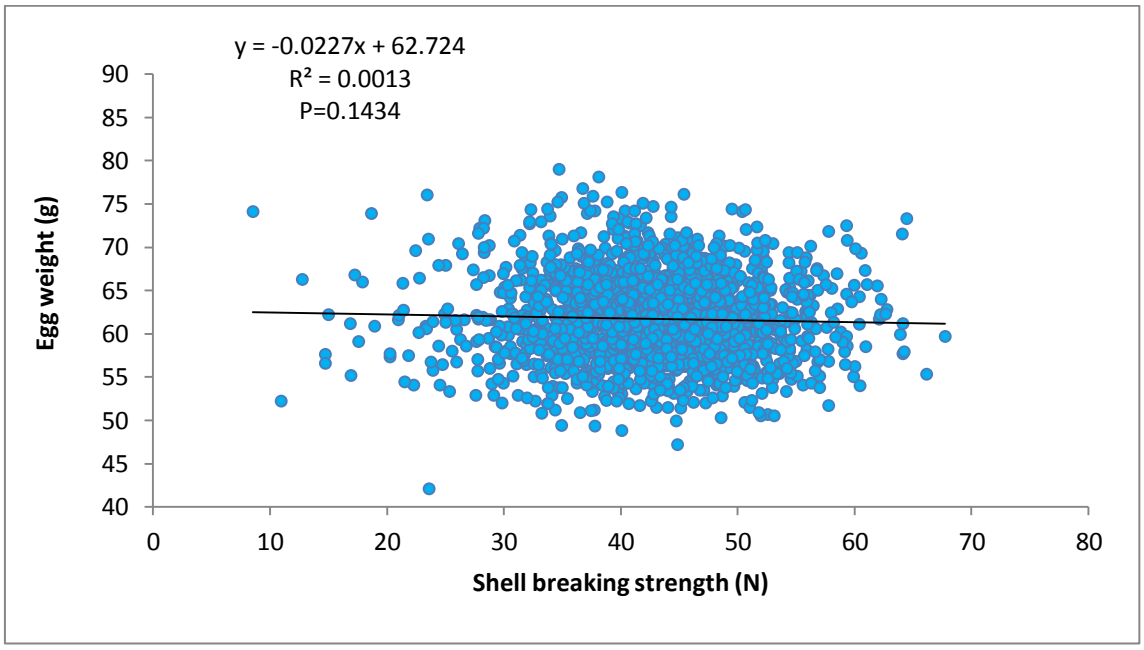

Figure 4.8. The correlation between egg weight and shell breaking strength

Shell deformation to breaking point decreased significantly with increasing flock age with no significant difference between age 26 and 37 weeks. Shell weight was highest at 50 weeks of age and lowest at 26 weeks. Percentage shell was lowest at 37 weeks of age. Shell thickness was highest at 50 weeks of age. Shell deformation and breaking strengths were most strongly correlated with age than with shell percentage and shell thickness.

For egg internal quality measures, albumen height and HU generally decreased with increasing flock age and was highest in age 26 weeks. Yolk colour was highest at 60 weeks and lowest at 37 weeks.

Table 4.5. Traditional measures of eggshell quality among the flocks

Measurements	Flocks							P Value
	FR 1	FR 2	FR 3	FR 4	FR 5	FR 6	FR 7	
Eggshell quality								
Translucency score	3.7±0.06^{a}	3.7±0.06^{a}	3.4±0.07^{c}	3.4±0.06^{bc}	3.5±0.07^{bc}	3.5±0.07^{bc}	3.6±0.07^{ab}	<0.0001
Shell reflectivity (%)	30.7±0.36^{ab}	30.9±0.32^{a}	29.9±0.31^{b}	30.9±0.29^{a}	31.3±0.30^{a}	28.2±0.27^{c}	27.0±0.27^{d}	<0.0001
Egg weight (g)	63.2±0.29^{ab}	62.5±0.37^{bc}	59.8±0.27^{e}	63.8±0.34^{a}	59.9±0.31^{e}	62.2±0.30^{c}	61.0±0.31^{d}	<0.0001
Breaking strength	43.1±0.52^{b}	42.6±0.50^{b}	40.3±0.51^{c}	39.5±0.49^{c}	43.7±0.45^{b}	45.6±0.56^{a}	42.6±0.49^{b}	<0.0001
Deformation (µm)	285.2±6.86^{a}	283.7±2.45^{ab}	277.4±2.51^{ab}	274.8±3.54^{bc}	275.3±2.59^{bc}	274.3±2.53^{bc}	272.3±2.29^{c}	0.0545
Shell weight (g)	6.0±0.03^{ab}	5.9±0.04^{b}	5.6±0.04^{d}	5.8±0.04^{c}	5.8±0.04^{c}	6.0±0.04^{a}	5.8±0.03^{c}	<0.0001
Percentage shell (%)	9.5±0.04^{b}	9.5±0.05^{b}	9.3±0.06^{c}	9.0±0.05^{c}	9.7±0.05^{a}	9.7±0.05^{a}	9.5±0.04^{b}	<0.0001
Shell thickness (µm)	409.6±1.52^{a}	406.8±1.97^{a}	395.4±2.23^{b}	393.1±1.84^{b}	416.5±6.35^{a}	413.4±1.69^{a}	415.9±6.16^{a}	<0.0001
Internal quality								
Albumen height	8.0±0.10^{a}	7.0±0.11^{d}	7.3±0.1^{c}	7.6±0.11^{b}	7.5±0.1^{bc}	6.3±0.09^{e}	7.5±0.11^{bc}	<0.0001
HU	87.7±0.65^{a}	81.0±0.80^{c}	84.6±0.62^{b}	85.0±0.69^{b}	85.8±0.61^{b}	77.7±0.65^{d}	85.4±0.68^{b}	<0.0001
Yolk colour score	10.9±0.06^{a}	10.2±0.08^{c}	9.7±0.05^{e}	10.0±0.09^{d}	10.4±0.06^{b}	10.2±0.07^{c}	10.7±0.06^{a}	<0.0001

Table 4.5 shows the main effect of flocks on eggshell and internal quality measurements. Translucency scores varied among the flocks within the mean range from 3.40 to 3.73. Shell reflectivity was highest in Flocks 1, 2, 4 and 5 and lowest in Flock 7. Egg weight ranged from an average of 59.84 in Flock 3 to 63.75 in Flock 4. Among the flocks, shell breaking strength was highest in Flock 6 and lowest in Flocks 3 and 4. Shell deformation to breaking point ranged from 272.29 in Flock 7 to 285.19 in Flock 1. Shell weight was significantly higher in Flocks 1 and 6 than other flocks, with Flock 3 having the lowest shell weight. Percentage shell varied between means of 9.04 for Flock 4 to 9.73 for Flock 6. Shell thickness was lower in Flocks 3 and 4 than for all other flocks. There was a significant positive correlation between shell thickness and egg weight (Figure 4.9).

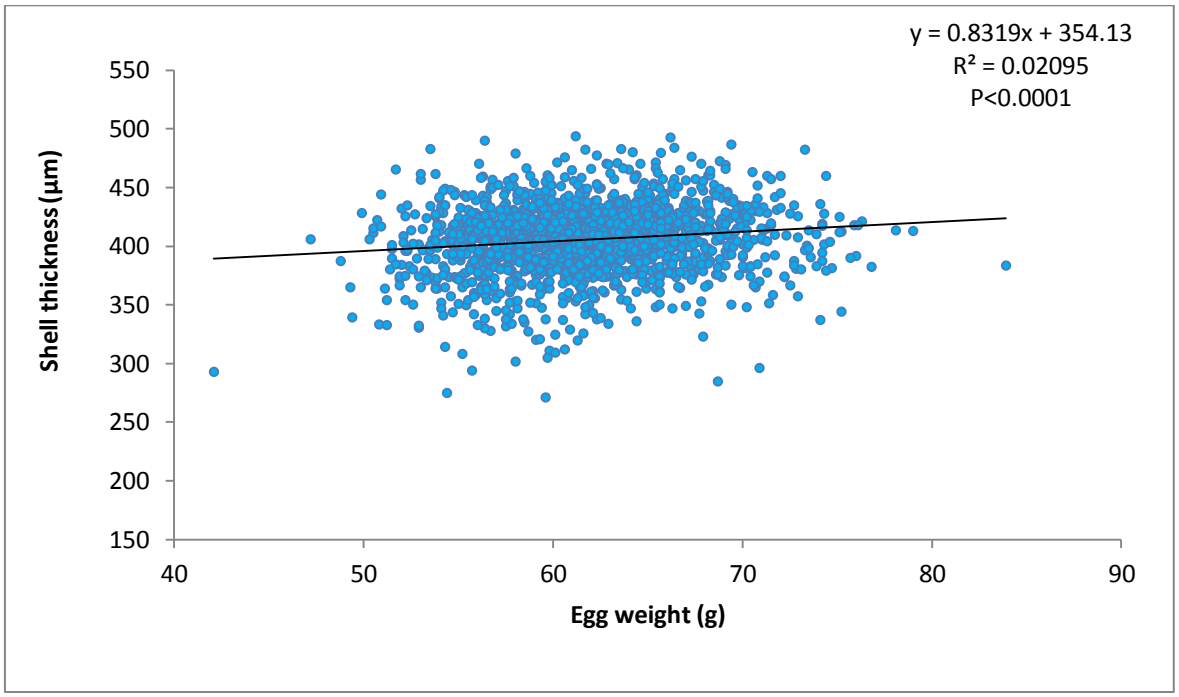

Figure 4.9. The correlation between egg weight and shell thickness

There was a significant difference among the flocks for egg internal quality measurements, with variation in albumen height, HU and yolk colour score.

4.3.3 Estimation of the amount of cuticle

Shell reflectivity (%) and Spectrophotometry (L*a*b) measurements for cuticle cover

Table 4.6 summarizes the results for shell reflectivity and spectrophotometry of eggshells before and after staining with cuticle blue dye, for the different age categories. There was a significant main effect of hen age for shell reflectivity before staining, and values of the L*a*b* colour space system before and after staining. Shell reflectivity fluctuated with flock age, the lowest reflectivity being at 26 weeks of age and the highest at the ages of 37 and 60 weeks.

Table 4.6. Spectrophotometric measurements

Measurement	Flock age (weeks)				P Value
	26	37	50	60	
Before staining					
Shell Reflectivity (%)	29.0±0.28^{c}	31.0±0.31^{a}	29.9±0.29^{b}	30.9±0.36^{a}	<0.0001
L*	59.5±0.37^{c}	63.0±0.29^{a}	62.1±0.23^{b}	63.4±0.29^{a}	<0.0001
a*	18.1±0.13^{a}	17.6±0.14^{b}	17.2±0.14^{c}	16.1±0.16^{d}	<0.0001
b*	29.1±0.21^{a}	29.2±0.16^{a}	28.8±0.16^{a}	28.2±0.16^{b}	<0.0001
After staining					
L*	54.2±0.37^{c}	56.3±0.31^{b}	56.2±0.29^{b}	58.0±0.35^{a}	<0.0001
a*	1.5±0.34^{a}	-0.7±0.36^{b}	-0.1±0.42^{b}	0.1±0.39^{b}	<0.0001
b*	32.9±0.18^{a}	31.6±0.16^{b}	31.3±0.16^{bc}	31.1±0.19^{c}	<0.0001
ΔEab	18.5±0.43^{b}	19.9±0.39^{a}	18.8±0.45^{b}	17.4±0.44^{c}	<0.0001
Cuticle cover	1.9±0.07^{c}	1.9±0.07^{c}	2.2±0.08^{b}	2.4±0.08^{a}	<0.0001

$^{a, b, c}$ Across a column, values with different superscripts are significantly different from each other. Values are mean ± SE

The pattern was very similar for the L* value before staining. There was a high correlation (R2= 0.8962) between shell reflectivity and L value before staining (Figure 4.10). The L value after staining generally increased with increasing flock age, with the highest value at 60 weeks and the lowest value was at 26 weeks of age.

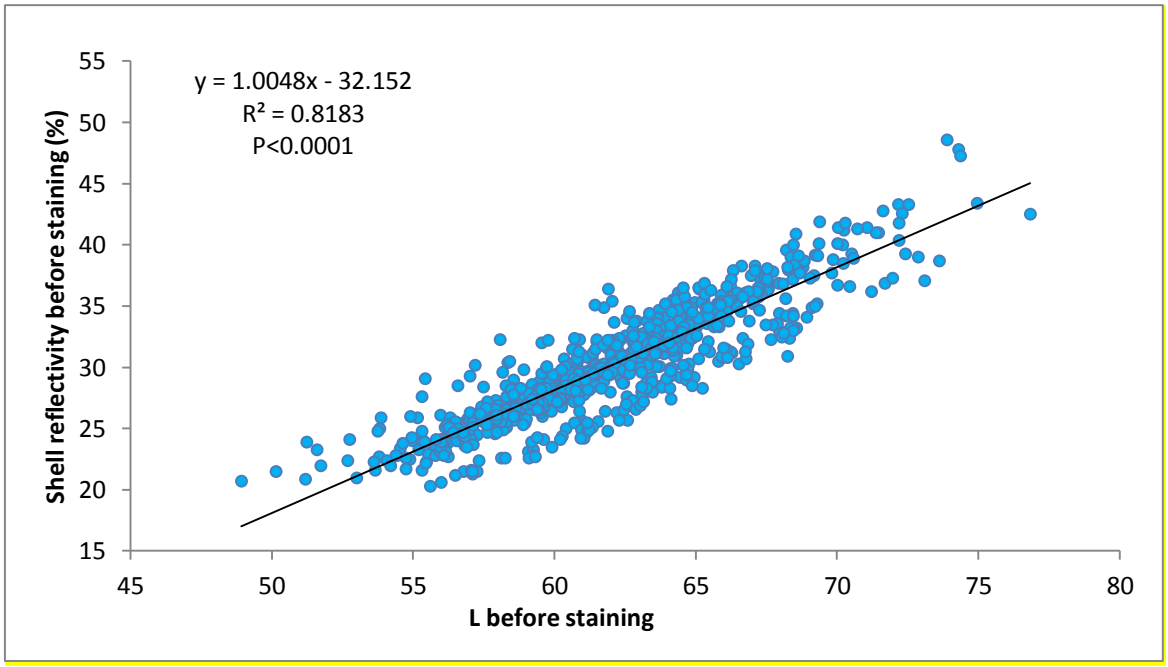

Figure 4.10. Correlation between shell reflectivity and L value before staining

The a* value before staining decreased with increasing flock age, while values after staining were higher at 26 weeks than for all other ages. The b* value before and after staining remained relatively constant before decreasing at 60 weeks of age. The b* value after staining decreased with increasing flock age.

There was also significant difference among flocks for shell reflectivity and the spectrophotometry of eggshells before and after staining (Table 4.7). Shell reflectivity varied among the flocks, being highest in Flocks 1 and 5 and lowest in Flock 7. L value before staining was highest in Flocks 4 and 5 and lowest in Flocks 1, 2 and 3 with no significant difference between Flocks 6 and 7. L* value after staining was highest in Flock 5 and lowest in Flock 6. The a* value before staining was highest in Flock 6 and lowest in Flock 5. The a* value after staining was highest in Flocks 1 and 4 and lowest in Flock 6. A lower value indicates more cuticle cover on the shell. The b* value before staining was highest in Flock 6 and lowest in Flocks 1 and 2. The b* value after staining was highest in Flock 6 and lowest in Flocks 1 and 7. The single score value was highest for Flock 6 and lowest for Flock 1. However, the cuticle score as viewed under the SEM (Table 4.7), had the highest score in Flock 1 and the lowest score in Flock 6. The single score value showed a strong correlation with the a* value (R^2 = 0.8644) (Figure 4.11).

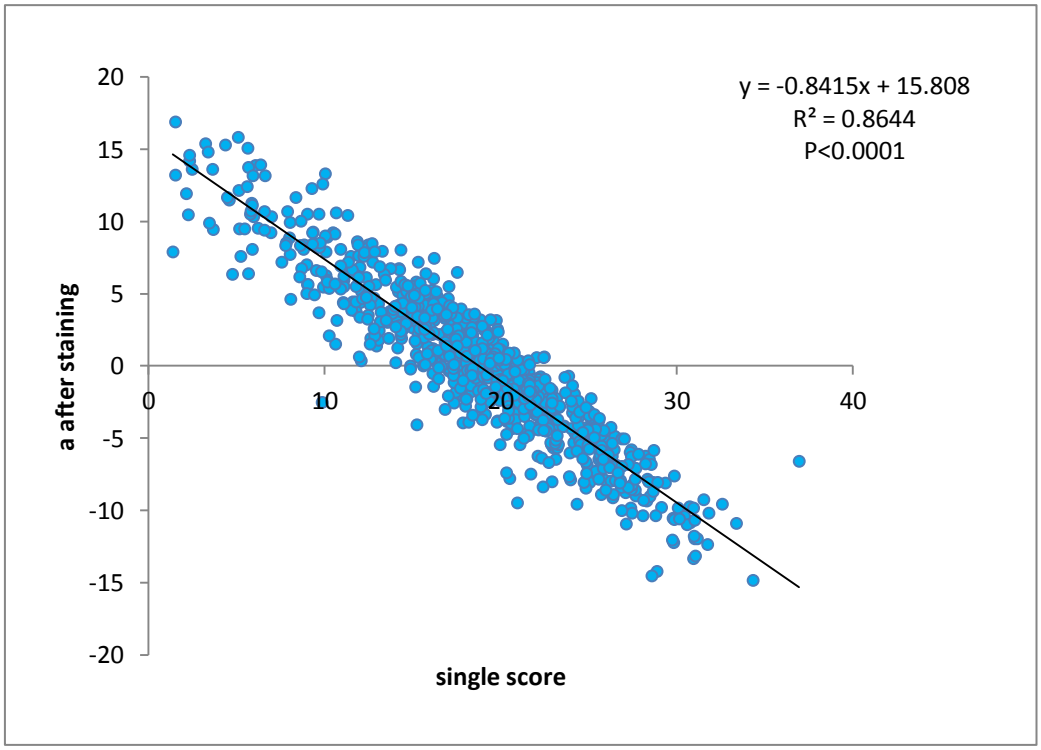

Figure 4. 11. The correlation between a* value after staining and the single score

Table 4.7. Spectrophotometric measurements of stained cuticle among the flocks

Measurements	Flocks							P Value
	FR 1	FR 2	FR 3	FR 4	FR 5	FR 6	FR 7	
Before staining								
Shell reflectivity (%)	31.5±0.50^{a}	30.2±0.38^{bc}	29.9±0.36^{c}	31.0±0.39^{ab}	31.4±0.43^{a}	29.1±0.36^{cd}	28.2±0.39^{d}	<0.0001
L*	61.1±0.46^{c}	61.4±0.34^{c}	61.4±0.32^{c}	63.4±0.41^{a}	62.6±0.59^{ab}	62.0±0.31^{bc}	62.0±0.36^{bc}	0.0001
a*	17.3±0.22^{ab}	17.3±0.19^{ab}	17.2±0.19^{bc}	17.1±0.19^{bc}	16.7±0.21^{c}	17.7±0.18^{a}	17.4±0.22^{ab}	0.0136
b*	28.4±0.25^{c}	28.4±0.23^{c}	28.6±0.24^{bc}	29.1±0.21^{b}	28.7±0.19^{bc}	29.8±0.19^{a}	28.8±0.28^{bc}	<0.0001
After staining								
L*	56.3±0.49^{b}	56.0±0.41^{b}	56.1±0.38^{b}	56.7±0.58^{ab}	57.5±0.44^{a}	54.6±0.39^{c}	56.1±0.44^{b}	0.0003
a*	2.5±0.51^{a}	0.4±0.46^{b}	0.1±0.43^{b}	1.9±0.49^{a}	0.5±0.54^{b}	-4.1±0.41^{c}	0.1±0.45^{b}	<0.0001
b*	31.0±0.24^{c}	32.0±0.25^{ab}	32.0±0.18^{ab}	31.7±0.20^{b}	31.8±0.24^{b}	32.4±0.27^{a}	31.1±0.26^{c}	<0.0001
ΔE^*_{ab}	16.1±0.49^{c}	18.4±0.52^{b}	18.5±0.49^{b}	17.3±0.63^{bc}	18.0±0.64^{b}	23.5±0.46^{a}	18.7±0.53^{b}	<0.0001
Cuticle cover SEM	2.5±0.09^{a}	1.9±0.10^{c}	2.1±0.09^{bc}	2.3±0.09^{ab}	2.1±0.09^{bc}	1.6±0.08^{d}	2.5±0.09^{bc}	<0.0001

$^{a, b, c}$ Across a column, values with different superscripts are significantly different from each other. Values are mean ± SE

4.4.4 Eggshell ultrastructural variations of the mammillary layer

Table 4.8 shows the effect of flock age and Table 4.9 shows the variation among flocks for mammillary layer ultrastructural variations of the eggshell. Flock age had the most significant effect on shell ultrastructural variations, although there was no effect on the incidence of Type A bodies, cubics, cuffing, and changed membrane.

Table 4.8. The main effect of flock age on the mammillary ultrastructure scores of the eggshell in a free range system

Ultrastructure features	Flock age (weeks)				P value
	26	37	50	60	
Mammillary cap	2.0±0.05^{c}	2.0±0.05^{c}	2.3±0.04^{b}	2.5±0.4^{a}	<0.0001
Confluence	2.5±0.07^{ab}	2.6±0.07^{a}	2.3±0.07^{bc}	2.1±0.07^{c}	<0.0001
Caps quality	2.4±0.05^{b}	2.2±0.05^{c}	2.6±0.06^{a}	2.6±0.06^{a}	<0.0001
Early fusion	3.5±0.06^{b}	3.7±0.05^{a}	3.3±0.06^{b}	2.9±0.07^{c}	<0.0001
Late fusion	2.8±0.07^{c}	2.5±0.07^{d}	3.1±0.07^{b}	3.4±0.06^{a}	<0.0001
Alignment	2.2±0.05^{b}	2.2±0.05^{b}	2.6±0.06^{a}	2.7±0.06^{a}	<0.0001
Type A	1.7±0.05	1.7±0.05	1.8±0.05	1.8±0.05	NS
Type B	2.1±0.04^{c}	2.2±0.04^{c}	2.5±0.05^{b}	2.8±0.06^{a}	<0.0001
Aragonite	1.2±0.05^{c}	1.3±0.05^{c}	1.7±0.06^{b}	2.1±0.07^{a}	<0.0001
Cubics	1.2±0.03	1.2±0.03	1.2±0.04	1.3±0.04	NS
Cubic cone	1.5±0.05^{a}	1.3±0.04^{b}	1.2±0.03^{c}	1.2±0.03^{c}	<0.0001
Cuffing	1.1±0.02	1.1±0.02	1.1±0.02	1.1±0.02	NS
Changed membrane	1.1±0.03	1.1±0.02	1.1±0.03	1.2±0.03	NS
Depression	1.0±0.01^{b}	1.0±0.01^{b}	1.1±0.02^{b}	1.2±0.03^{a}	<0.0001
Erosion	1.2±0.03^{c}	1.2±0.03^{c}	1.3±0.04^{b}	1.6±0.05^{a}	<0.0001

$^{a, b, c}$ Across a column, values with different superscripts are significantly different from each other. Values are mean ± SE

In general, the incidence of mammillary layer ultrastructural features associated with good quality eggshells decreased with increasing flock age. Conversely, the incidence of mammillary layer ultrastructural features associated with poor quality eggshells increased with increasing flock age. As flock age increased, mammillary cap size became more variable and the quality of mammillary caps deteriorated. The incidence of early confluence and early fusion decreased with increasing flock age. At the same time, the incidence of negative features such as late fusion, alignment, Type B bodies, aragonite, depression and erosion increased. The incidence of cubic cone formation, which has been associated with poor shell quality, actually decreased with increasing hen age.

Table 4.9. Effect of flock on the mammillary ultrastructure scores of the eggshell

Flocks	Align ment	Con-fluence	Mam cap size	Cap quality	Early fusion	Late fusion	Type A body	Type B body	Aragoni te	Cubics	Cubic cones	Cuffing	Changed mem-brane	Depre ssion	Erosion
FR 1	2.2± 0.06^{c}	2.3± 0.08^{ab}	2.3± 0.05^{bc}	2.5± 0.06^{ab}	3.3± 0.08	3.0± 0.09	1.5± 0.05^{c}	2.3± 0.05^{b}	1.4± 0.06^{cd}	1.2± 0.04	1.3±0. 05^{ab}	1.1± 0.03^{a}	1.3± 0.05^{a}	1.0± 0.00^{b}	1.1± 0.03^{b}
FR 2	2.4± 0.07^{bc}	2.4± 0.11^{ab}	2.1± 0.06^{c}	2.6± 0.07^{ab}	3.5± 0.07	2.8± 0.08	1.7± 0.07^{bc}	2.4± 0.07^{b}	1.5± 0.08^{bcd}	1.3± 0.05	1.4± 0.05^{ab}	1.1± 0.03^{a}	1.1± 0.03^{bc}	1.0± 0.01^{b}	1.3± 0.05^{a}
FR 3	2.5± 0.07^{ab}	2.5± 0.10^{a}	2.5± 0.05^{a}	2.4± 0.07^{c}	3.4± 0.09	2.9± 0.09	1.9± 0.06^{a}	2.2± 0.06^{b}	1.7± 0.08^{ab}	1.2± 0.04	1.1± 0.04^{cd}	1.0± 0.01^{ab}	1.1± 0.03^{bc}	1.1± 0.03^{a}	1.4± 0.06^{a}
FR 4	2.4± 0.07^{b}	2.2± 0.10^{b}	2.3± 0.05^{ab}	2.6± 0.08^{a}	3.3± 0.09	2.8± 0.10	1.8± 0.07^{ab}	2.4± 0.07^{b}	1.6± 0.08^{bc}	1.2± 0.04	1.4± 0.06^{ab}	1.0± 0.00^{b}	1.2± 0.05^{a}	1.1± 0.03^{a}	1.4± 0.06^{a}
FR 5	2.4± 0.07^{bc}	2.3± 0.09^{ab}	2.1± 0.07^{c}	2.5± 0.08^{ab}	3.3± 0.08	3.0± 0.09	1.8± 0.06^{ab}	2.6± 0.08^{a}	1.6± 0.09^{bc}	1.2± 0.04	1.3± 0.05^{bc}	1.1± 0.04^{a}	1.1± 0.03^{b}	1.1± 0.03^{a}	1.3± 0.05^{a}
FR 6	2.5± 0.08^{ab}	2.6± 0.09^{a}	2.1± 0.07^{c}	2.1± 0.06^{d}	3.3± 0.09	3.1± 0.09	1.8± 0.06^{ab}	2.3± 0.06^{b}	1.3± 0.07^{d}	1.3± 0.05	1.1± 0.04^{d}	1.1± 0.03^{a}	1.1± 0.03^{bc}	1.1± 0.03^{a}	1.3± 0.05^{a}
FR 7	2.7± 0.08^{a}	2.9± 0.09^{ab}	2.1± 0.07^{c}	2.4± 0.07^{bc}	3.3± 0.09	3.0± 0.09	1.8± 0.08^{a}	2.6± 0.09^{a}	1.9± 0.11^{a}	1.3± 0.06	1.4± 0.06^{a}	1.1± 0.03^{a}	1.0± 0.02^{c}	1.1± 0.03^{a}	1.4± 0.06^{a}
P value	0.0001	0.0414	<0.0001	<0.0001	NS	NS	<0.0001	<0.0001	<0.0001	NS	0.0001	0.0074	<0.0001	0.0010	0.0031

$^{a, b, c,d}$ Within a column, values with different superscripts are significantly different from each other. Values are mean ± SE

4.5. Discussion

4.5.1. Body weight, body weight uniformity and egg production

A significant proportion of the free range flocks had pullet weights above the breed standards and achieved inappropriately low average flock growth rates between 19 to 37 weeks of age, despite the heavier pullet weight. In contrast, Flock 1 had a lower pullet weight, and the average growth rate between 19-37 weeks of age was aligned closely with the breed standards. Flock 1 also maintained high uniformity standards between 19 and 50 weeks of age and achieved egg production standards slightly above the breed standard until 60 weeks of age.

This outlying flock provides evidence for the true potential of free range production and many of the causal variables seem consistent with knowledge accumulated for cage production. The poor performance of many of the other flocks also illustrates likely variation at a commercial level; poor compliance with average growth rates patterns and low uniformity standards.

There also appears to be a trend for the heavy pullets to achieve poorer growth rates between 19 and 37 weeks of age, but more data on the relationship between initial pullet weight, production performance and growth is required to establish this relationship.

Flock uniformity varied from 62% to 89.2% and uniformity declined significantly between 16 weeks to 19 weeks for the majority of flocks, but was maintained at higher levels in Flocks 1 and 6 from 19 to 50 weeks of age. This loss of uniformity in flocks between 16 to 19 weeks of age may reflect the transition of the pullets into the free range housing systems. However, Flock 1 achieved consistently higher production with good average growth rates, and maintained uniformity, but Flock 6 had a poor average growth pattern between 26 to 37 weeks of age, which may have impacted on the production capacity of the flock.

Also, uniformity was not consistent within individual flocks across the ages sampled. The importance of body weight and flock uniformity has been noted by Miles and Jacob (2011). These authors pointed out that, when the proper pullet body weight and condition at the desired age of sexual maturity has been achieved, many problems associated with lower profits from a commercial layer flock can be eliminated. A lack of uniformity can be due to genetic variation or environmental influences, including social dominance. Chickens establish a social order which

involves competition for feeder and water space (Petite *et al.*, 1981). Use of the range area is a benefit to animal welfare, because usage will lower the density of hens in the house during the daytime, increase access to resources and opportunity to perform spatial behaviours, and provide the hens on the range with an enriched environment. It is also considered by many as the most acceptable housing system for poultry (Miao *et al.,* 2005). However, many factors are associated with on-farm performance, such as management.

In this experiment, egg production was recorded from age 26 to 60 weeks in most flocks. However, egg production in Flocks 3 and 4 was recorded only up to 50 weeks due to management changes adopted on the farms. Egg production in Flock 5 decreased at 31 weeks. The reason for this is unknown but may possibly be due to on-farm technical errors in record keeping. However, production showed a good recovery with higher production and extended peak production up to 51 weeks. Flock 2 had the lowest egg production, while Flock 1 had the highest production, with an extended peak in production. It can be seen from Figure 4.4 that the timing of onset of lay varied among the seven flocks with Flocks 3, 5, 6 and 7 reaching point of lay at a younger age than Flocks 1, 2 and 4.

Akbas and Takma (2005) used canonical correlation analysis to estimate the relationships between age at sexual maturity and egg production, body weight and egg weight in layers at three different stages (28, 36 and 40 weeks of age). They found negative correlations between egg numbers and both age at sexual maturity and body weight, with a higher correlation with age at sexual maturity than for body weight. They came to the conclusion that age at sexual maturity has a greater effect than body weight or egg weight on variation in egg production during the three age periods studied.

4.5.2. Eggshell and egg internal quality measurements

Translucency score was higher in age 26 weeks than other age category. The translucency score is a relatively subjective measurement of the incidence and extent of light patches in the eggshell when the egg is placed over a light source. Translucency develops when moisture escapes from the egg albumen through the shell membranes into the ultrastructure of the mammillary layer (Solomon, 1991). Ray (2012) reported that translucency develops rapidly within the first 24 hours after the egg is laid and continues to increase for about one week. In this experiment, the eggs

were collected from various free-range farms and analysed within 3-7 days following collection, which explains the high translucency score. However, the significance of this finding is not clear as to why 26 weeks of age had higher translucency than other ages.

Shell reflectivity as a measure of shell colour was only slightly higher in the present experiment than in the studies of cage flocks reported in Chapters 3 and 5. Shell reflectivity generally increased with increasing hen age, as reported previously by Zita *et al*. (2009) and Roberts *et al*. (2013). Shell reflectivity was higher in Flocks 1, 2, 4 and 5 than in other flocks, indicating slightly paler shell colour in these flocks. However, it appears that none of the seven flocks had a problem with pale shell colour, as compared with cage flocks of the same age.

Several authors (Silversides, 1994; Van Den Brand *et al*., 2004; Ferrante *et al*., 2009; Roberts *et al*. 2013) have reported the increase in egg weight with increasing hen age found in the present study. Egg weight was highest in Flocks 1 and 4 and lowest in Flocks 3 and 5.

Shell breaking strength decreased with advancing flock age indicating a gradual reduction in shell strength. However, there is little correlation between egg weight and shell breaking strength (Figure 4.8). However, Clerici *et al*., (2006) found a highly significant inverse correlation (r= -0.691) between egg weight and breaking strength. Butcher and Miles (2003) reported that smaller eggs have stronger shells than larger ones, as hens have a finite capacity to deposit calcium in the shell and, as a result, the same amount of calcium is spread over a larger area.

Clerici *et al*. (2006) who also found that 'poultry farm' factors seemed to affect eggshell characteristic much more than the 'housing system', recorded a highly significant correlation between shell thickness and displacement and breaking strength.

Shell deformation to breaking point is an indicator of the degree of elasticity of the eggshell. Shell deformation decreased with increasing hen age in the present study, which is in agreement with previous studies (Roberts *et al*., 2013). In this experiment, differences between the flocks for shell deformation approached statistical significance (P=0.0545). Shell weight increased with increasing flock age almost in proportion to the increasing egg weight, resulting in only small differences in percentage shell. However, between the flocks, shell weight was higher in Flocks 1 and 6 than other flocks. A number of studies have shown that shell weight was heavier in older hens than in younger hens (Singh *et al*., 2009; Rayan *et al*., 2010; Roberts *et al*., 2013). The reason for this is that older hens produce larger eggs, which also have larger shells. The

percentage shell was also higher in Flock 5 and 6 than other flocks. In the present study shell thickness was highest at 50 weeks of age, although most studies report a steady decrease in shell thickness with increasing hen age (Silversides, 1994; Roberts *et al.*, 2013). Figure 4.9 illustrates that there was a very low correlation between egg weight and shell thickness (R^2= 0.0209) in the present study. Correlation between egg size and shell thickness may be influenced by the numbers of eggs in the 70-80 category. Perhaps the lighter free range flocks do not have as many really large eggs.

In terms of internal quality, albumen height and HU decreased with increasing flock age, as has been demonstrated in previous studies (Van Den Brand *et al*., 2004; Ferrante *et al*., 2009) Yolk colour mostly depends on the amount of xanthophyll in the feed. In this experiment, locally formulated feed was used. Yolk colour has been reported to be darker in free-range eggs than in eggs from cages (Van Den Brand *et al*., 2004).

The results of this experiment have demonstrated a significant interaction between flock and hen age for all egg quality parameters. Egg quality of non-cage eggs is very variable (Fiks-Van Niekerk (2005), possibly due to higher environmental variation which leads to more factors contributing to egg quality and the higher influence of bird behaviour, influencing the percentage of floor eggs. It is suggested that this may be caused by stress as a result of inter-bird conflicts. This author concluded that non-cage systems are confronted with a variety of different factors influencing egg quality, so that a good egg quality is not easy to obtain. In recent years, however, non-cage systems have been improved to a large degree and egg quality may be equivalent to that in cage systems (Fiks-Van Niekerk., 2005). In comparison, the study of conventional cage and free-range production by Van den Brand *et al.* (2004) reported that, with increasing hen age, shell quality remained constant or increased, in free-range eggs, whereas shell quality decreased in caged birds. However, Mertens *et al*., (2006) compared egg quality from birds in conventional cages, furnished cages, aviaries, and free-range systems, and reported shell strength to be greatest in aviary eggs and weakest in free-range eggs.

4.5.3. Estimation of the amount of cuticle

In the current study, the L* values showed the same pattern as shell reflectivity, with a strong correlation between the two measurements (R^2= 0.8183) (Figure 4.10). Prior to staining with cuticle blue dye, shell colour became less red and less yellow as hen age increased.

The results from a* after staining with cuticle blue dye was lower at 37, 50 and 60 weeks of age as compared with at 26 weeks, indicating that the mean cuticle cover on the shell was lowest at 26 weeks. However, the results of the single score and scoring of the amount of cuticle present under the scanning electron microscope show a different pattern, indicating that cuticle cover is lowest at 60 weeks of age. Sparks and Board (1984) stated that cuticle thickness decreases significantly with the increasing age of the hen. However, Roberts, *et al.,* (2013) found that there was no significant effect of flock age in a conventional cage production system, on the extent of the cuticle cover. Ruiz and Lunam (2000) reported a thick cuticle layer in peak production, compared to the beginning and end lay periods in broiler breeder hens. Other researchers also report a lesser amount of cuticle deposition at the end of the lay period (Sparks and Board, 1984; Messens, *et al*., 2005). The cuticle is thought to play a role in controlling water exchange by repelling water or preventing its loss, and may function in limiting microbial colonization of the eggshell surface (Hincke *et al*., 2008). Together with the mineralized shell and shell membranes, the cuticle constitutes a physical barrier against microorganism invasion and contamination of the egg content. (De Reu *et al*., 2008).

4.5.4. Eggshell ultrastructural variations of the mammillary layer

The incidence of ultrastructural variations associated with good shell quality, such as confluence and early fusion, decreased with increasing flock age, whereas the incidence of late fusion, alignment, Type B bodies, aragonite, depression and erosion increased with increasing flock age. Bain (1992) categorized the structural variations described in Solomon (1991), which increase the resistance of eggshell to unstable fracture, as early fusion, cuffing, confluent mammillae and a low mammillary density. Late fusion, Type-B bodies, aragonite, pitting, depression, erosion, pin holes, alignment and a high mammillary density are the ultrastructural variations which decrease the resistance of the eggshell.

4.6. Conclusions.

Free-range flocks appear to have lighter mature body weights than equivalent cage flocks. Many free-range flocks experience compromised growth patterns between 19-37 weeks of age. Compromised growth in free-range flocks is not an inevitable consequence of the housing and social competition. Elite uniformity standards do not seem possible at this stage, but average and consistent uniformities can be maintained in free-range flocks. Elite free range production will be achieved only if nutrient intake patterns, growth patterns and flocks uniformities can mimic those of cage production, and Flock 1 is close to cage patterns and breed standards. The major variables identified are uniform pullets, heavy pullets, age at photo stimulation, early growth patterns, and maintenance of flock uniformity throughout egg production.

It seems likely that the lower average weight in free-range flocks will result in lower average egg weights, and this may confer some advantages for shell quality. More information is required on the impact of heavy pullet weights on growth and production in free-range flocks. Preliminary data suggested that heavier pullets may alter growth patterns. Additional larger epidemiological studies of growth and uniformity patterns will be required to attribute impacts of these traits on production and egg quality.

Chapter 5

Effect of body weights at point of lay on egg production performance and egg quality of Hy-Line Brown hens

5.1 Introduction

The poultry industry has undergone major changes since the late 1960's with genetic selection and highly developed management practices greatly improving the efficiency of meat and egg production. Genetic selection has substantially improved the performance of laying hens. The combined effects of reduced body weight, lower maintenance requirements and better egg production have resulted in improved feed conversion ratios (Parkinson and Stanhope, 2011). Thiele (2012) recommended that changing feed from starter to grower should be conducted based on target body weight, not just bird age. Bell (2011) and Thiele (2012) reported that incorrect rearing management can lead to body weight problems and poor laying performance of a flock. The correct weight at point of lay will optimize the flock's performance during the laying period.

To ensure that the maximum genetic potential is expressed in a commercial environment, one of the most important management strategies for poultry producers is to maintain body weight uniformity within the flocks. Maintaining uniform body weight is a major objective, particularly during the rearing period, as is reinforced in all Commercial Breeder manuals.

Uniformity provides an estimate of the variability in a given flock at a given age. A flock with high uniformity will reach peak egg production earlier and will peak higher than a non-uniform flock (Hudson *et al*.. 2001, Kosba *et al.*, 2009). The more uniform the flock, the better the performance of that flock, and the more consistent the responses to nutrition. On the other hand, poor uniformity is associated with variation in the degree of sexual maturity of hens, where underweight pullets have delayed onset of egg production (Yuan *et al*., 1994). The historical goal for flock uniformity is to have 80 per cent of the pullets within plus or minus 10 per cent of the average flock body-weight.

Although uniformity is mentioned repeatedly in the Production Manuals for all the major breeds of layer, there has been surprisingly little scientific research conducted into ways of ensuring

pullet flock uniformity, and then maintaining uniformity throughout lay. Parkinson *et al.* (2007) reported that the average body weight of hens were above breed standard in their study, indicating obesity and most probably lower feed conversion ratios. These larger birds produced excessively large eggs, which resulted in lower eggshell thickness and shell percentages (Leeson and Summers, 1987; Parkinson *et al*., 2007).

Body weight at point of lay is a major factor influencing subsequent growth, production, and egg size (Leeson and Summers, 1987) and may influence flock uniformities during egg production (Balnave, 1984). A closer examination of these relationships is important to the Australian Egg Industry, because there is good evidence that both pullet weights and subsequent mature weights of commercial layers deviate substantially from the body weight patterns described by the commercial breeders, and there is a dearth of knowledge about uniformity patterns during egg production in a commercial environment.

This experiment is part of a project which examines these issues in more depth, and follows on from studies undertaken on both a commercial conventional cage farm (Chapter 3) and seven free-range farms (Chapter 4), focusing on how the body weight at point of lay, average growth rate and flock uniformity impact on egg production, feed efficiency and both external and internal egg quality. The study described in this chapter established an experimental model with different point of lay body weights, but with extreme high uniformities in all treatments. The study attempts to model the interrelationships between the important commercial traits and to study uniformity patterns, in a more systematic way than is possible on a commercial farm.

5.2 Materials and methods

5.2.1 Body weight and flock uniformity

In this research station experiment, body weight groups were deliberately established using information derived from earlier studies (Chapter 3 and 4). A total of one hundred fifty birds was weighed and sorted according to body weight from the lowest to the highest. Three groups representing light, medium and heavy weights, relative to the mean, were selected with mean weights of 1.170, 1.337, and 1.507 kg at 16 weeks of age, respectively. Each weight grouping was represented by 20 birds with a total of sixty Hy-Line Brown birds being housed individually in cages and randomised through the shed. Body weights (BW) were recorded at the ages of 16, 19, 26, 37, 50, 60, 70 and 80 weeks. Body weight uniformity was calculated as mentioned in Chapter 2.

Feed intake was recorded and calculated weekly. The diet was formulated by a consultant nutritionist and mixed by Riverina Stockfeeds, Warwick, Queensland. Detailed feed ingredients and composition are presented in Table 5.1.

5.2.2 Egg production and ultrastructures parameters

Egg production records of the flock were monitored daily throughout the experiment. Egg records for production were converted to hen-day egg production (eggs/hen/day as a percentage) as described in Chapter 2.

Sixty eggs were collected weekly, then analysed for egg internal and eggshell quality and shell ultrastructure measurements. These eggs were first measured for cuticle cover, then the same eggs analysed for egg internal and eggshell quality and shell ultrastructure. Ultrastructure measurements were analysed at ten week intervals from age 20 to 80 weeks.

5.2.3 Bone breaking strength

Six birds from each group were euthanized at 80 weeks of age for bone breaking strength measurements. The left humerus and femur were used. Details are contained in Chapter 2.

Table 5.1. Feed composition and formulation of the experimental diets

Description	Kgs	%	Nutrient	Units	LP_ min	actual	LP_ max
Sorghum 9.5 Hammer	600	30	[Volume]	%	100	100	100
Wheat 11.5 hammermill	632.6	31.63	Protein	%	17.5	17.83798	
millrun 16.0	80	4	C- fibre	%		3.51863	
Cottonseed meal 43%	80	4	AME - A - MJ	MJ/Kg	11.1	10.85963	
Canola meal 37%	120	6	AME_A_MC	Mcal/	2.65	2.595518	
Soybean meal 47.5%	175	8.75	Arginine	%		1.110406	
Meat meal 51%	90	4.5	Leucine	%		1.384434	
Recyc veg oil -mix	10	0.5	Isoleucine	%	0.65	0.670346	
T-Limestone/B	165	8.25	Lysine	%	0.868	0.871522	
Sodium Bicarbonate M	1	0.05	Methionine	%	0.54	0.489327	
Salt - micro mix	4.8	0.24	Threonine	%	0.582	0.666896	
Lysine HCl - micro	2.8	0.14	Tryptophan	%	0.22	0.214087	
MHA Calcium Methionine	5.4	0.27	M+C	%	0.883	0.801539	
Threonine - micro	1.2	0.06	Arg TidPou	%		0.965991	
Tryptophan	0.2	0.01	ISO TidPou	%		0.580204	
Sodium Bentonite	20	1	Leu TidPou	%		1.16073	
‘Elite Hens’ Choice’ Mi	12	0.6	Lys TidPou	%		0.737911	
	2000	100	Met TidPou	%		0.455269	
			Thr TidPou	%		0.530588	
			Try TidPou	%		0.177163	
			M+C TidPou	%		0.707513	
			Calcium	%	3.8	3.738953	4.2
			Phosphorus	%		0.663366	
			Av -phos	%	0.48	0.388818	
			#cal/pho			5.636335	
			Sodium	%	0.18	0.174311	
			Potassium	%	0.56	0.601041	
			Chloride	%		0.24256	0.23
			Fat/EE	%		2.76358	
			C18:2W6lin	%	1.6	1.022815	

5.3 Results

At 43 weeks of age, the water supply to the light body weight group of birds was accidentally disrupted and some birds were left without water for differing periods of time. This caused a set-back to these birds which may have affected the remainder of the trial for this group. For the light body weight group, subjected to the water deprivation, the growth and production performance returned to high levels by 55 weeks of age and there was no apparent impact on these traits after this period.

Several of the light body weight birds were severely impacted by the water deprivation (fifteen birds) with two birds (L3 and L7) dying and egg production for the duration of the experiment was compromised in some of thesurviving birds. The remaining thirteen birds recovered full production two weeks after the incident, hence the comparison for egg production performance was restricted to 19- 43 weeks of age for all three body weight groups. A comparison for the two unaffected body weight groups (Medium and Heavy) was able to be undertaken from 19-72 weeks of age. One of the birds from the heavy BW group was excluded from the analysis as this bird had not laid since data recording commenced, was euthanized at 44 weeks of age and found to have a tumour in the oviduct.

5.3.1 Body weight, body weight uniformity, feed intake and egg production

The average body weight of the one hundred and fifty birds from which the body weight groups were selected was 1.338 kg at 16 weeks of age and average uniformity percentage was 98 percent. The average body weight of birds in the three body weight groups from age 16 weeks to 80 weeks is presented in Figure 5.1. There were significant effects of BW groups and age on the body weight. The body weight increased as the flock aged with the final body weight at 80 weeks of age being 2.0 kg, 2.2 kg and 2.3 kg for the light, medium and heavy BW group respectively. The highest body weight was reached at 60 weeks of age; 2.1 kg, 2.2 kg and 2.4 kg for the light, the medium and the heavy BW groups, respectively.

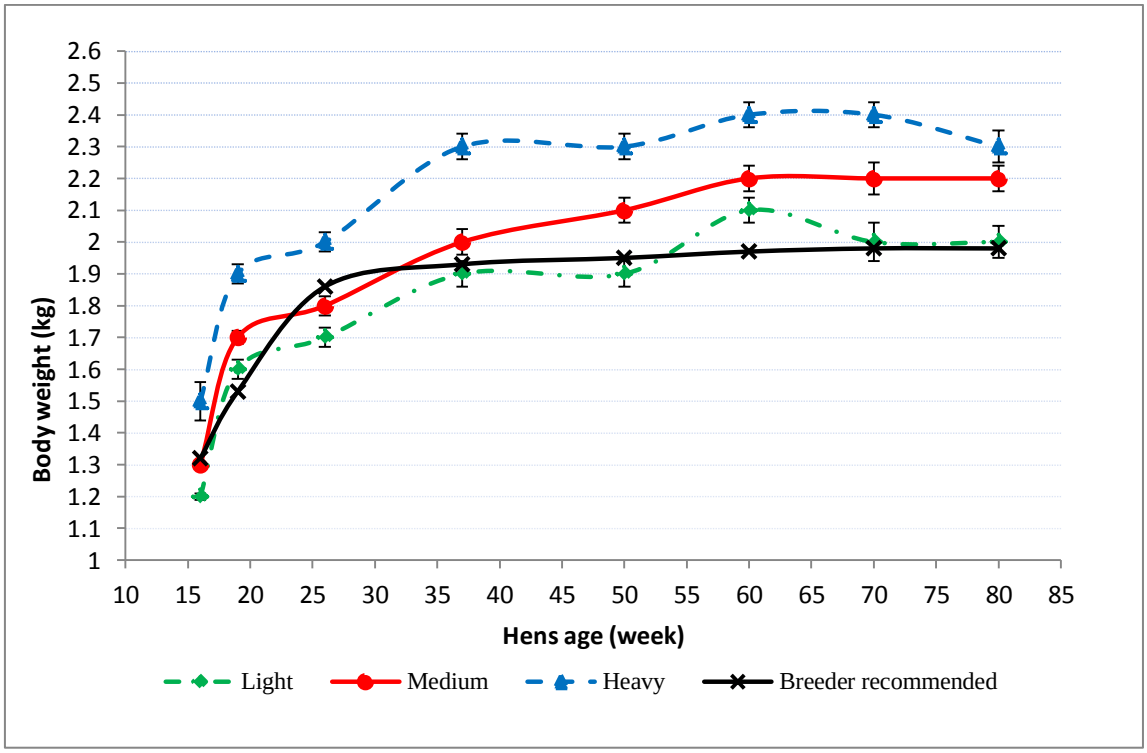

Figure 5.1. Average body weights of the birds from 16 weeks to 80 weeks of age

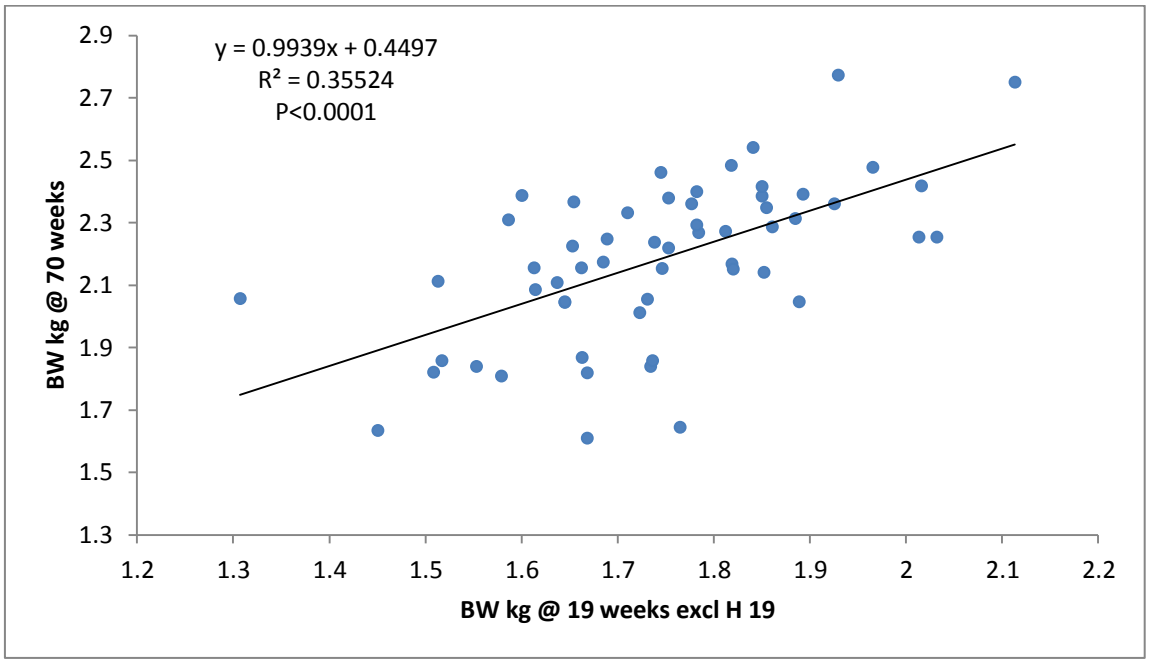

Figure 5.2. The correlation between BW at 19 weeks and BW at 70 weeks

There was a significance correlation between the BW at point of lay and BW at 70 weeks of age (Figure 5.2) The body weight at point of lay was a good predictor of mature body weight at 70 weeks of age, despite the impact of the acute water deprivation in the light body weight group (43-45 weeks of age).

Body weight uniformity as indicated by percentage for each group is presented in Table 5.2. Uniformity ranged from 61% to 100%. The highest uniformity, 100%, was recorded at 16 weeks (the age at which the body weight groups were selected) for the light and the medium BW groups and remained constant until 19 weeks of age in the medium group.

Table 5.2. Body weight uniformity of body weight groups from age 16 weeks to 80 weeks in percentages

BW groups	Flock age (weeks)							
	16	**19**	**26**	**37**	**50**	**60**	**70**	**80**
Light	100	90	80	85	83	75	61	67
Medium	100	100	80	75	80	75	70	80
Heavy	95	89	89	89	89	84	84	84

As the age of flock increased, the body weight uniformity decreased. Although the uniformity of body weight declined to 67% at 80 weeks in the light BW group, and 70% at 70 weeks in the medium BW group, the body weight uniformity was 84 % and 80% at 80 weeks of age in the heavy and medium groups, respectively.

Figures 5.3, 5.4, 5.5, 5.6, 5.7, and 5.8 show the correlation between body weight at point of lay (19 weeks) and the body weight at each of 50 and 70 weeks for the different BW groups. There was no significant correlation for the light BW group between body weight at point of lay and body weight at 50 and 70 weeks, nor for the heavy BW group between body weight at point of lay and body weight at 70 weeks. However, there was a positive correlation between body weight in the heavy BW group at point of lay and the BW at 50 weeks.

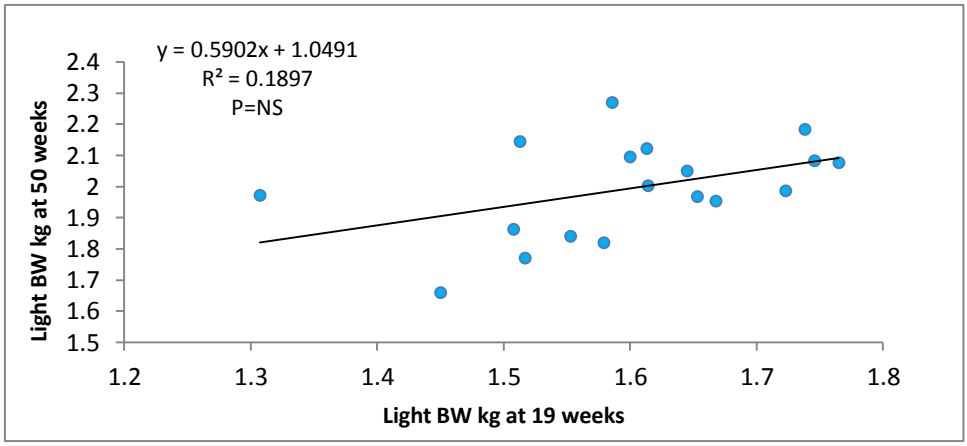

Figure 5.3. The correlation between point of lay of Light BW and body weight at 50 weeks

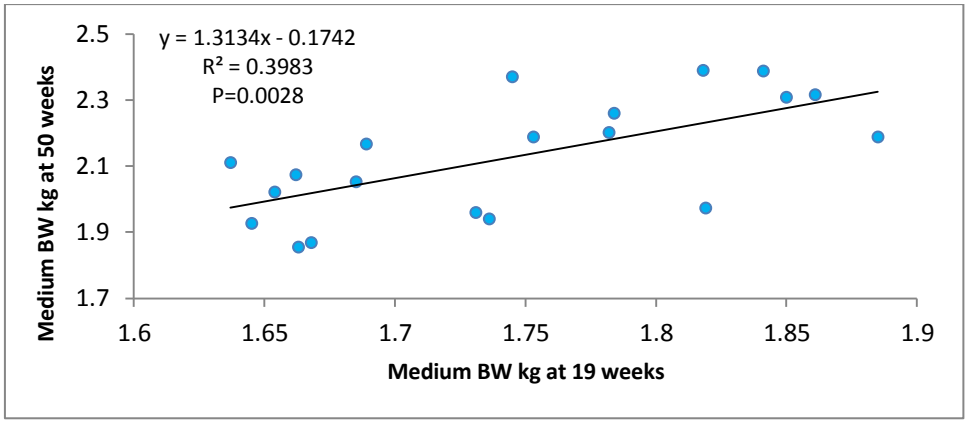

Figure 5.4. The correlation between point of lay of Medium BW and body weight at 50 weeks

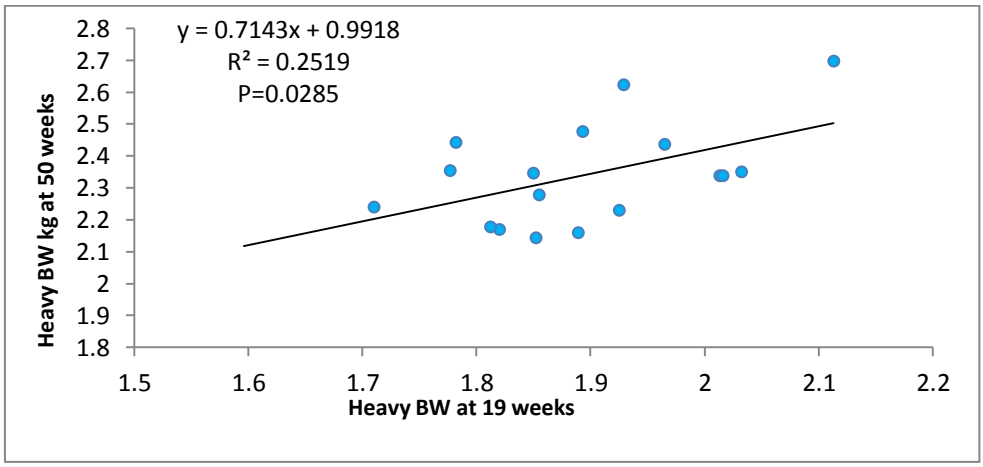

Figure 5.5. The correlation between point of lay of Heavy BW and body weight at 50 weeks

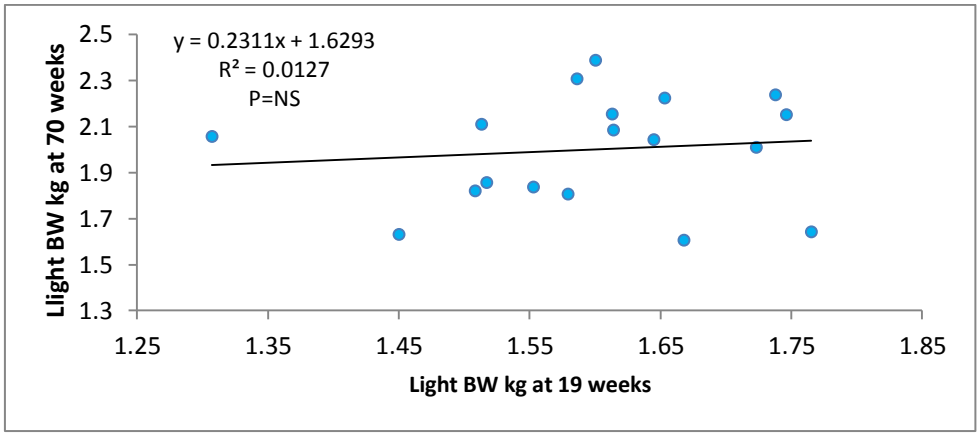

Figure 5.6. The correlation between point of lay of Light BW and body weight at 70 weeks

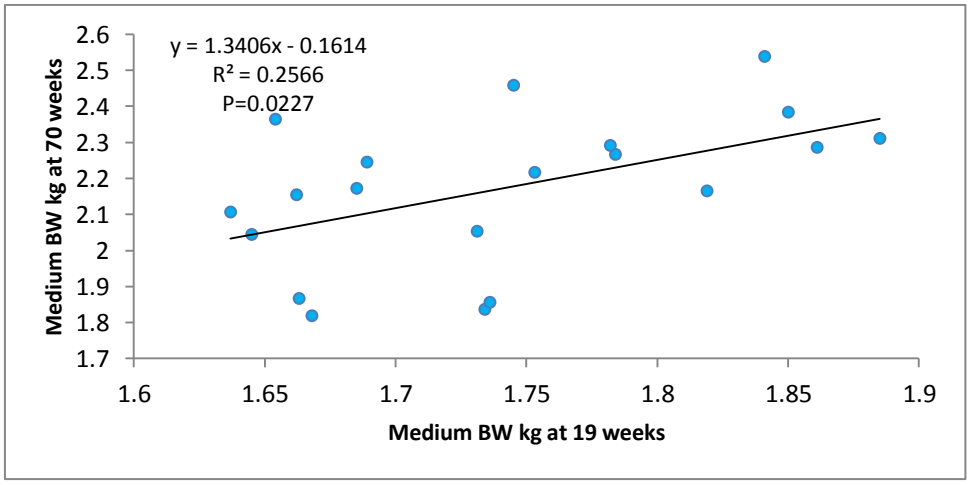

Figure 5.7. The correlation between point of lay of Medium BW and body weight at 70 weeks

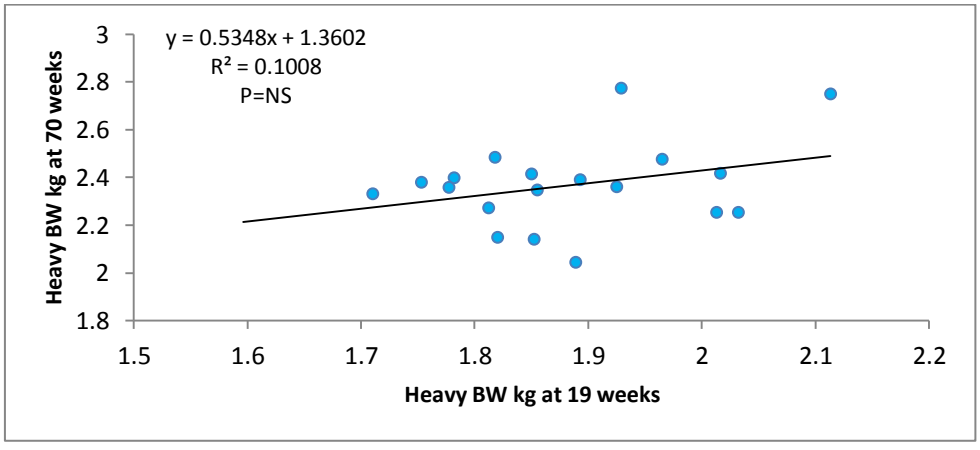

Figure 5.8. The correlation between point of lay of Heavy BW and body weight at 70 weeks

5.3.2 Egg production

Egg production records commenced at 20 weeks of age, when the flock reached 5% production, and continued until 80 weeks of age. Table 5.3 and Figure 5.9 illustrate the hen-day egg production from age 20 weeks to 80 weeks. The egg production results do not include one bird from the heavy BW group, as this bird laid early at 17 -18 weeks of age, then stopped laying and was euthanized at 44 weeks of age.

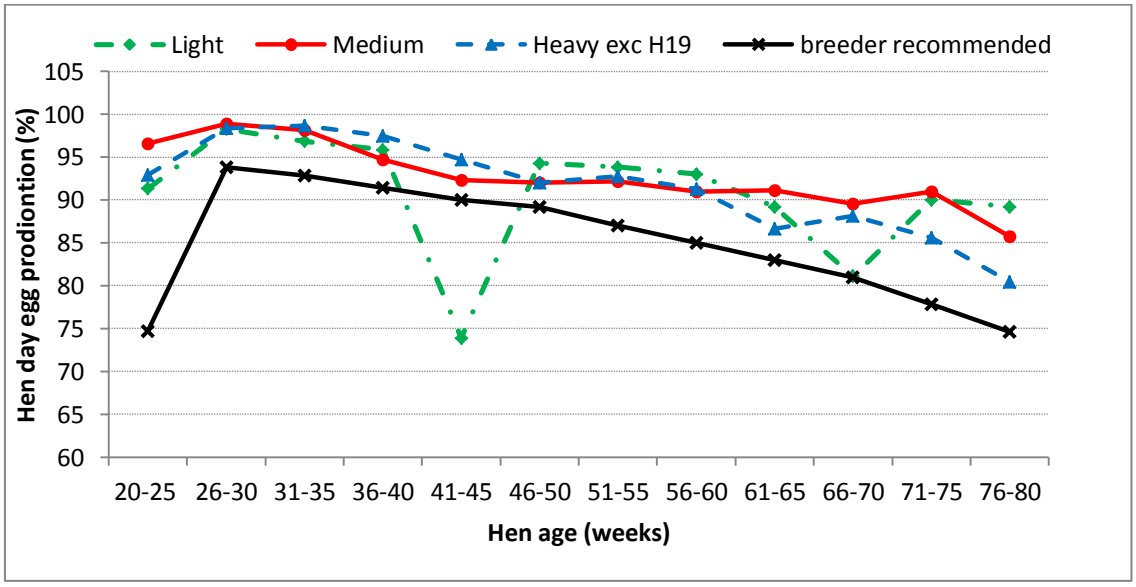

Figure 5.9. Figure hen day production at different body weights from 20 to 80 weeks of age.

All BW groups reached production above the breed standard at about 98-100% and maintained high production between 26 and 34 weeks of age (Figure 5.9). The average body weight during this period of high production was between 1.7 and 1.88 kg; 1.8 and 2.0; and 2.0 and 2.2 kg for light, medium and heavy BW groups, respectively. Figure 5.10, 5.11, 5.12 show the correlation between BW at 19 weeks of age and total egg produce during the time before water deprivation and the time during laying period from 20 to 72 weeks of age.

Table 5.3. Average Hen-day egg production from 20 weeks to 80 weeks of age

Flock age (weeks)	Light	Medium	Heavy	Breed recom mendation	Flock age (weeks)	Light	Medium	Heavy	Breed recom mendation
20	67.9	82.9	73.7	32	51	95.2	91.4	96.2	88
21	90.7	98.6	90.2	65	52	92.9	90.0	90.2	87
22	94.3	100.0	96.2	78	53	94.4	89.3	94.0	87
23	100.7	100.0	99.2	87	54	90.5	97.1	94.0	87
24	97.1	98.6	101.5	93	55	96.0	92.9	89.5	86
25	97.1	99.3	97.7	93	56	91.3	87.9	91.0	86
26	97.9	99.3	100.0	93	57	93.7	94.3	91.0	85
27	100.0	99.3	97.7	94	58	93.7	86.4	89.5	85
28	98.6	100.7	97.7	94	59	92.9	92.1	91.7	85
29	97.9	97.1	97.7	94	60	93.7	94.3	93.2	84
30	97.1	97.9	98.5	94	61	88.1	85.7	85.0	84
31	99.3	98.6	100.0	93	62	87.3	91.4	81.2	83
32	97.1	98.6	99.2	93	63	86.5	95.0	87.2	83
33	97.1	97.9	98.5	93	64	92.1	92.9	91.0	83
34	98.6	97.1	98.5	93	65	92.1	90.7	88.7	82
35	92.1	98.6	97.0	92	66	78.6	88.6	92.5	82
36	95.0	99.3	99.2	92	67	80.2	91.4	91.0	81
37	94.3	92.9	92.9	92	68	80.2	89.3	89.5	81
38	98.6	96.4	95.5	91	69	80.2	89.3	84.2	81
39	97.1	92.1	96.2	91	70	86.5	89.3	83.5	80
40	94.3	92.9	98.5	91	71	88.9	90.0	89.5	79
41	95.0	90.0	93.2	90	72	92.9	94.3	85.0	79
42	96.4	92.9	97.0	90	73	91.3	88.6	87.2	78
43	90.7	92.1	95.5	90	74	89.7	94.3	82.7	77
44	51.6	93.6	94.0	90	75	87.3	87.9	83.5	76
45	35.7	92.9	94.0	90	76	88.9	89.3	89.5	76
46	89.7	92.1	92.5	90	77	90.5	85.7	82.7	75
47	97.6	91.4	94.7	90	78	90.5	90.7	84.2	74
48	95.2	93.6	90.2	89	79	90.5	82.1	76.7	74
49	93.7	93.6	91.7	89	80	85.7	80.7	69.0	74
50	95.2	89.3	91.0	88					

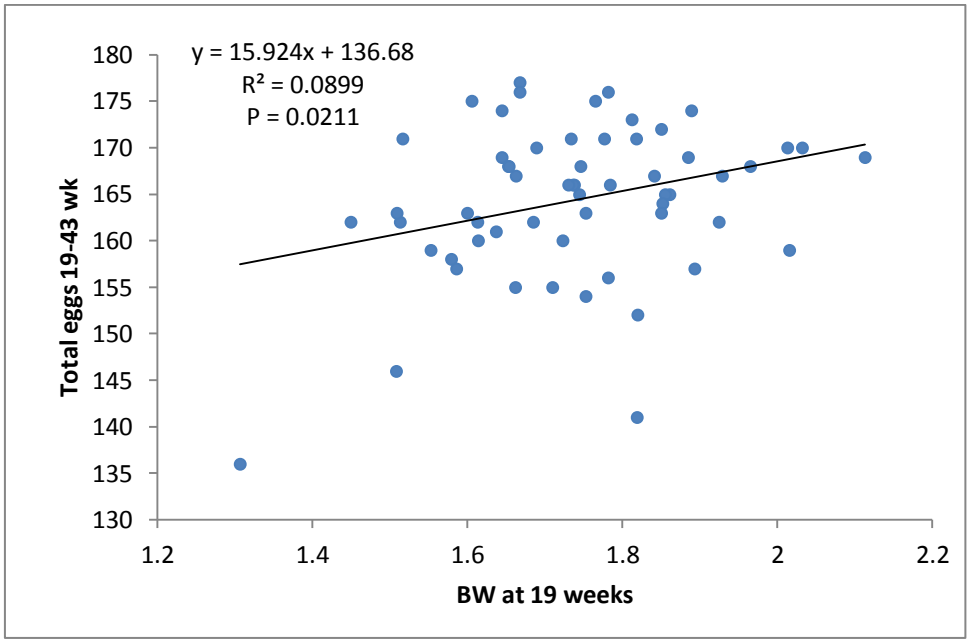

Figure 5.10. Correlation between BW at 19 weeks and total egg produced to 43 weeks

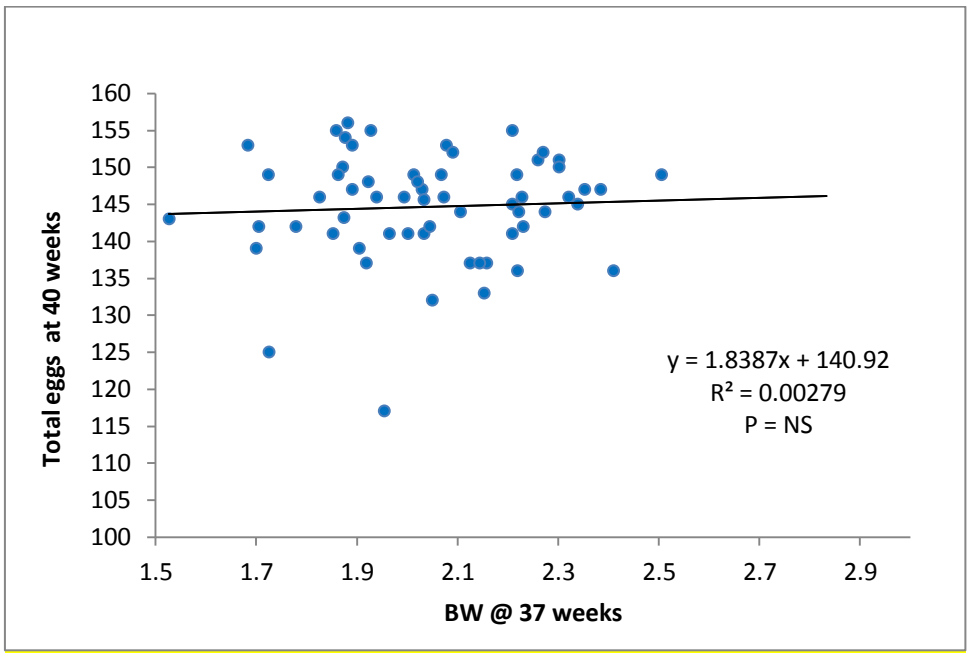

Figure 5.11. Correlation between body weight at 37 weeks and egg produced to 40 weeks

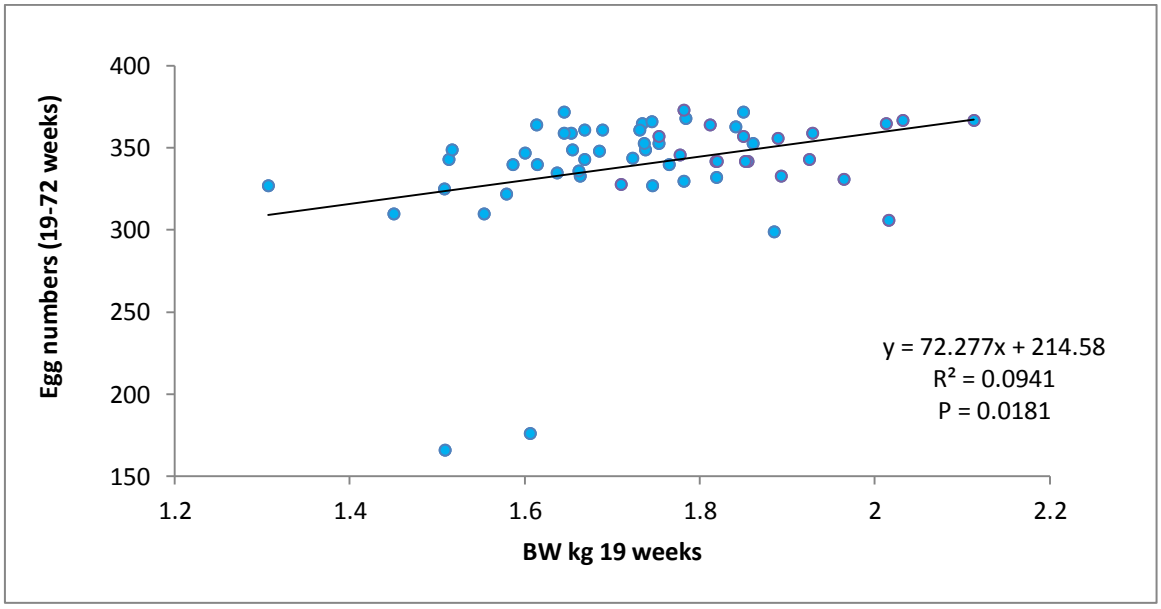

Figure 5.12. Correlation between BW @19 week and total eggs produced 19-72 weeks

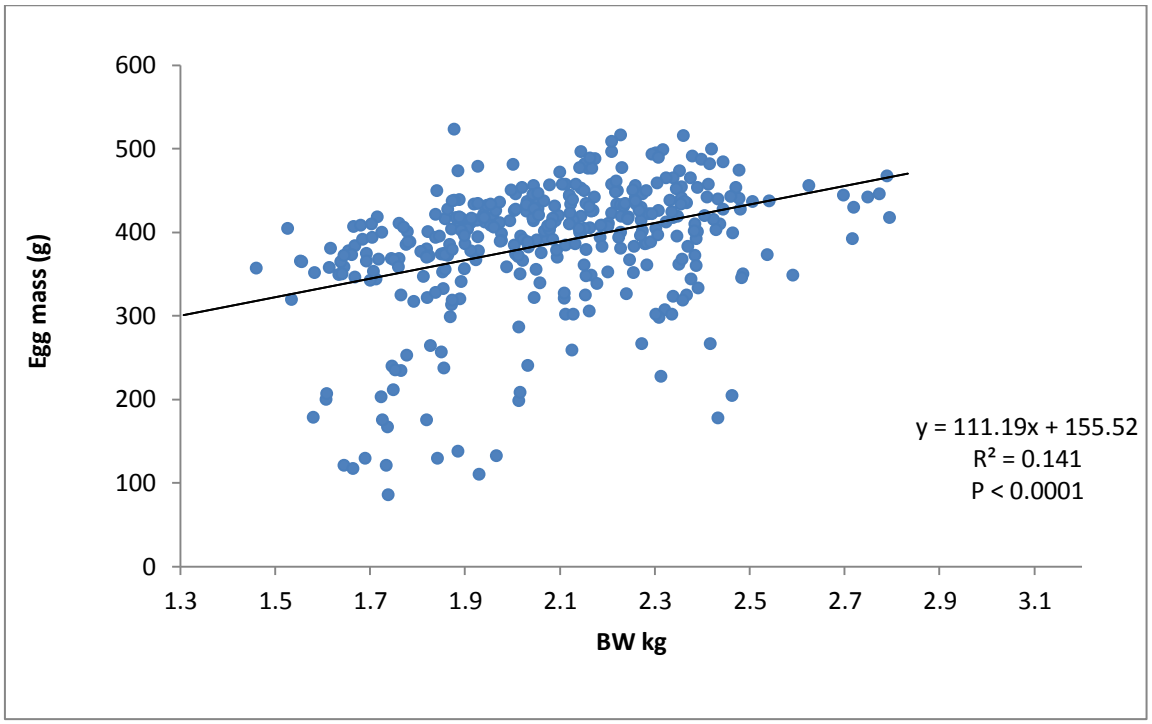

Figure 5. 13. Correlation between body weight and the egg mass

There was also strong correlation between body weight and the egg mass (Figure 5.13).

The results of feed intake from 19 weeks to 80 weeks of age are presented in Figure 5.14. Feed intake was significantly affected (P<.0001) by BW group and hen age, and there was a significant interaction between the two. The light BW group consumed less feed than the other BW groups

throughout the experiment. Feed intake was related to body size as the heavy BW group consumed more feed, followed by the medium and the light BW group, respectively, as expected. Interestingly, by the age of 46-50 weeks, the medium group consumed slightly more feed than the heavy BW group and this continued until the end of the experiment, while the light BW group remained the lowest consumers of feed. There was a significant positive correlation between body weight and feed intake (Figure 5.15) and a significant negative correlation between body weight and FCR (Figure 5.16)

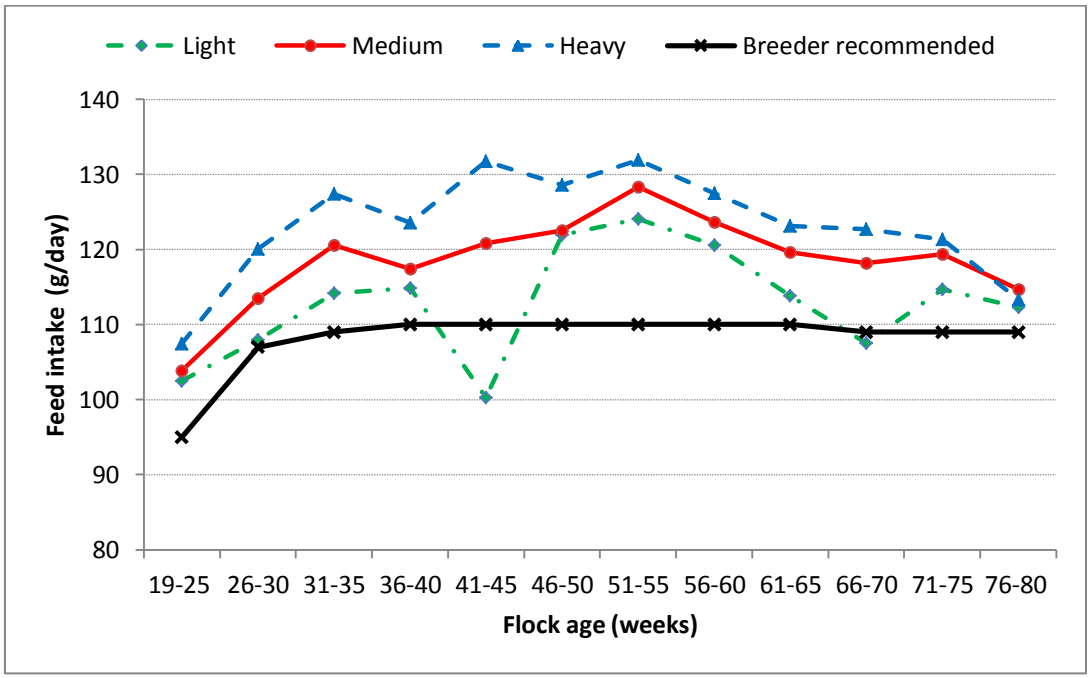

Figure 5.14. Feed intake of flock on the different BW groups from 19 to 80 weeks of age

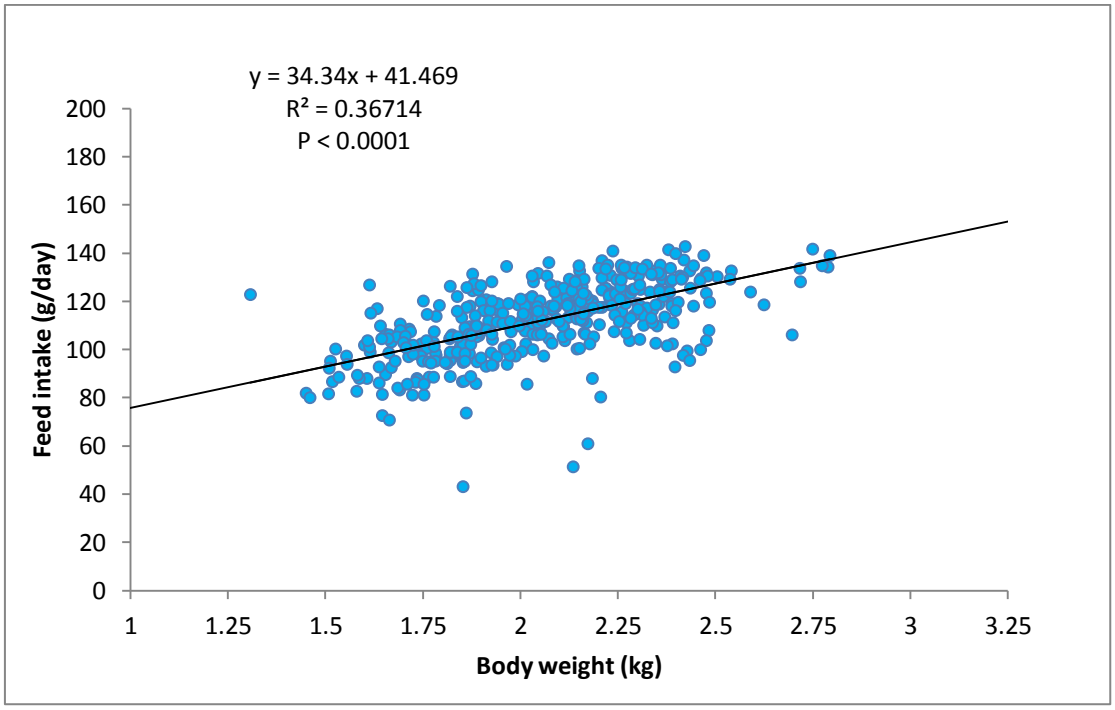

Figure 5.15. Correlation between body weight and feed intake

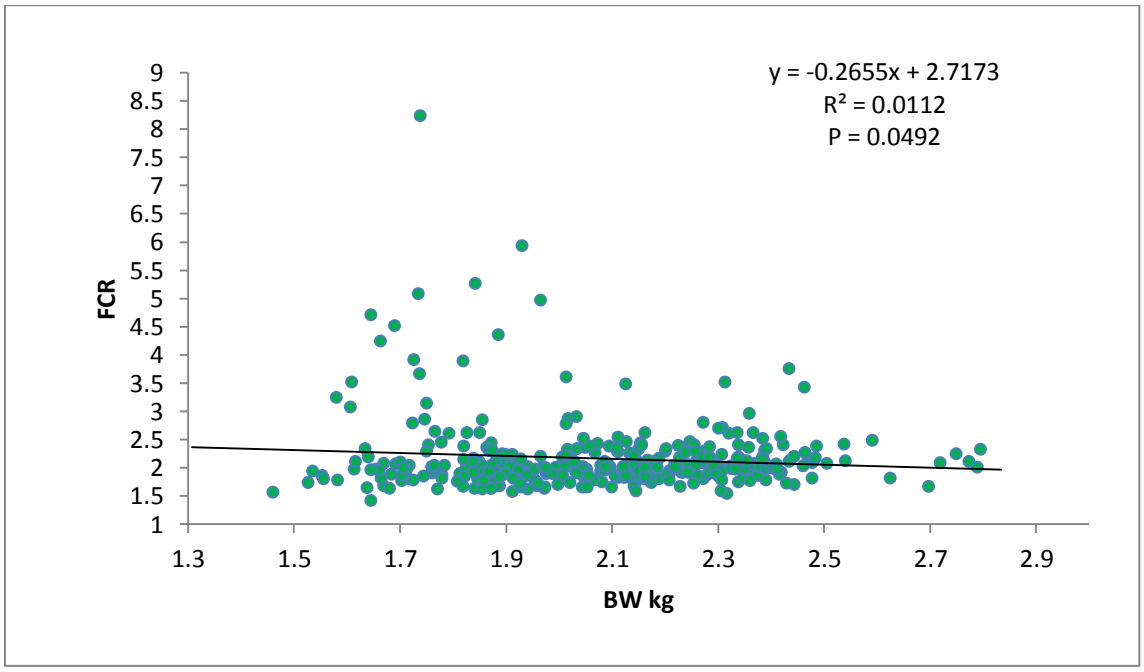

Figure 5.16. Correlation between body weight and FCR

5.3.3 Egg quality measurements

There were significant effects of flock age and body weight ($P<0.0001$) for most of the eggshell and internal quality variables (Tables 5.4 and 5.5). There was a significant interaction between flock age and BW group for both shell reflectivity and egg weight (Table 5.6). Flock age significantly affected the translucency score, which was highest at 26-30 weeks. Translucency score was also significantly higher in the light BW group (2.6) followed by the heavy BW group (2.5) and the medium BW group (2.4) with all groups being significantly different from one another. Shell reflectivity increased as the flock aged but decreased after 70 weeks of age, and was higher in the medium BW group than the other BW groups with all groups being significantly different from one another.

Egg weight was correlated with body weight, with all groups being significantly different from one another. Egg weight increased from early lay (19 weeks) to the end of the experiment.

Shell breaking strength varied with age, with the highest breaking strength being at 46-50 weeks, before decreasing as the flock aged. Shell breaking strength was higher in the light BW group than the medium and the heavy groups, which were not significantly different from each other. Shell deformation to breaking point was highest at 19-30 weeks of flock age, after which it decreased to age 80 weeks. Deformation was higher in the light BW group than in the medium and heavy groups, which were not significantly different from one another. Shell weight varied among the ages during the experiment, being highest at age 46-50 weeks then decreasing with increasing flock age. Shell weight was significantly different among the body weight groups, being highest in the heavy BW group and lowest in the light body weight group. Percentage shell varied significantly with hen age, generally increasing from 19 to 46-50 weeks of age, then decreasing as the flock aged. Percentage shell was significantly different among the BW groups, being highest in the light BW group, lowest in the heavy BW group, with that of the medium BW group intermediate.

Shell thickness increased from age 19 weeks to age 46-50 weeks, then decreased to 80 weeks (Figure 5-17). Shell thickness was significantly higher in the light and medium BW groups than in the heavy BW group (Table 5-5).

Table 5.4. Main effect of flock age on egg quality measurements

Flock age (weeks)	Shell quality								Internal quality		
	Translu-cency score	Shell reflectivity (%)	Egg weight (g)	Breaking strength (N)	Deformation (µm)	Shell weight (g)	Percentage shell (%)	Shell thickness (µm)	Albumen height (mm)	HU	Yolk colour score
19-25	2.4±0.06^{de}	24.1±0.16^{g}	52.8±0.3^{h}	41.9±0.4^{ab}	299.4±1.9^{a}	4.9±0.03^{h}	9.4±0.06^{b}	384.4±1.8^{ef}	9.9±0.09^{a}	99.9±0.2^{a}	8.0±0.06^{h}
26-30	3.3±0.05^{a}	24.4±0.17^{f}	57.8±0.3^{g}	41.5±0.4^{ab}	301.1±1.7^{a}	5.2±0.03^{g}	9.1±0.05^{de}	388.7±1.8^{de}	9.5±0.06^{d}	97.3±0.3^{bc}	7.9±0.07^{i}
31-35	2.9±0.04^{b}	25.5±0.16^{e}	60.5±0.2^{f}	41.8±0.4^{ab}	289.7±1.9^{b}	5.6±0.03^{d}	9.3±0.04^{bc}	390.6±1.6^{d}	9.6±0.06^{cd}	97.1±0.3^{cd}	9.7±0.07^{f}
36-40	2.8±0.04^{c}	25.8±0.17^{de}	62.1±0.3^{e}	42.3±0.4^{ab}	281.6±1.9^{c}	5.7±0.03^{c}	9.3±0.04^{cd}	397.2±1.6^{c}	9.5±0.06^{d}	96.6±0.3^{cd}	10.2±0.04^{d}
41-45	2.5±0.04^{d}	26.4±0.21^{c}	62.5±0.3^{de}	41.4±0.4^{b}	269.6±2.0^{d}	5.8±0.04^{bc}	9.3±0.05^{bc}	400.8±1.9^{bc}	9.8±0.06^{ab}	98.2±0.3^{b}	10.2±0.07^{d}
46-50	2.3±0.05^{fh}	25.7±0.21^{de}	63.2±0.3^{cd}	42.6±0.4^{a}	269.8±2.1^{d}	6.0±0.03^{a}	9.6±0.04^{a}	411.1±1.5^{a}	9.4±0.06^{d}	96.3±0.3^{d}	12.1±0.04^{a}
51-55	2.4±0.4^{ef}	26.5±0.21^{c}	63.8±0.3^{abc}	39.9±0.4^{c}	259.9±1.9^{e}	5.9±0.03^{b}	9.2±0.05^{cd}	403.4±1.8^{b}	9.7±0.05^{bc}	97.5±0.2^{bc}	11.2±0.06^{b}
56-60	2.2±0.03^{fh}	27.4±0.23^{b}	64.1±0.3^{ab}	39.3±0.4^{c}	260.6±1.9^{e}	5.8±0.03^{bc}	9.0±0.05^{e}	399.3±1.8^{bc}	9.2±0.07^{e}	94.5±0.4^{e}	10.6±0.04^{c}
61-65	2.4±0.04^{de}	29.2±0.27^{a}	64.2±0.3^{ab}	36.1±0.4^{d}	253.8±2.1^{ef}	5.6±0.04^{de}	8.7±0.05^{f}	386.8±1.9^{def}	8.1±0.07^{f}	89.2±0.4^{f}	10.1±0.05^{de}
66-70	2.2±0.04^{gh}	29.4±0.29^{a}	64.1±0.3^{ab}	36.5±0.5^{d}	258.6±2.6^{efg}	5.6±0.04^{d}	8.8±0.06^{f}	389.1±1.9^{de}	7.6±0.07^{g}	85.9±0.5^{g}	9.9±0.06^{e}
71-75	2.0±0.03^{h}	28.9±0.31^{a}	63.7±0.3^{bc}	33.5±0.5^{e}	251.9±5.3^{g}	5.5±0.04^{ef}	8.6±0.06^{f}	383.3±2.2^{f}	7.4±0.06^{h}	84.7±0.4^{h}	10.2±0.05^{d}
76-80	2.2±0.03^{g}	26.1±0.27^{cd}	64.5±0.4^{a}	33.3±0.5^{e}	249.1±3.7^{f}	5.4±0.05^{f}	8.4±0.06^{g}	374.7±2.3^{g}	7.0±0.06^{i}	82.1±0.5^{i}	8.9±0.05^{g}
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d,e,f,g,h,i} Within a column, values with different superscripts are significantly different from each other. Values are Mean ± SE

Table 5.5. Main effect of BW groups on egg quality parameters

Measurement	Light	Medium	Heavy	P value
Shell Quality				
Translucency score (after Stain)	2.6±0.02[a]	2.4±0.02[c]	2.5±0.02[b]	<0.0001
Shell reflectivity (%)	25.2±0.11[c]	27.9±0.13[a]	26.4±0.12[b]	<0.0001
Egg weight (g)	59.5±0.2[c]	61.8±0.2[b]	63.7±0.2[a]	<0.0001
Breaking Strength (N)	40.6±0.2[a]	38.6±0.2[b]	38.8±0.0 [b]	<0.0001
Deformation (µm)	282.3 ±1.7[a]	265.5±1.3[b]	267.1±1.2[b]	<0.0001
Shell Weight (g)	5.5±0.02[c]	5.6±0.02[b]	5.7±0.02[a]	<0.0001
Percentage Shell (%)	9.2±0.03[a]	9.1±0.03[b]	8.9±0.03[c]	<0.0001
Shell Thickness (µm)	394.6±1.0[a]	392.4±0.9[a]	389.8±1.0[b]	=0.0005
Internal Quality				
Albumen Ht (mm)	8.8±0.04[b]	8.9±0.05[b]	9.1±0.04[a]	<0.0001
HU	93.7±0.2[a]	93.2±0.2[b]	93.7±0.3[a]	ns
Yolk Colour Score	9.7±0.05[c]	9.9±0.04[b]	10.0±0.04[a]	<0.0001

[a,b,c] Across a row, values with different superscripts are significantly different from each other. Values are Mean ± SE

Table 5.6. Interaction between body weight group and flock age for egg quality measurements

Measurements	BW groups	Flock age (weeks)												P Value		
		19-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	BW	A	BW*A
Shell reflectivity (%)	L	18.5± 0.3	19.8± 0.4	20.8± 0.3	20.9± 0.3	21.9± 0.4	21.3± 0.3	21.1± 0.4	21.4± 0.4	21.8± 0.5	22± 0.5	22.4± 0.6	20.5± 0.6	<0.0001	<0.0001	<0.0001
	M	19.9± 0.3	21.7± 0.3	23.3± 0.3	24.3± 0.4	25.1± 0.4	25.3± 0.4	26.1± 0.4	26.4± 0.5	27± 0.5	27.2± 0.5	27.5± 0.6	25.2± 0.6			
	H	19.7± 0.3	19.8± 0.3	21.8± 0.3	22.7± 0.3	23.5± 0.4	23.2± 0.4	23.8± 0.4	24.2± 0.4	24.9± 0.6	25.5± 0.6	25.8± 0.6	22.2± 0.5			
Egg weight (g)	L	51.8± 0.5	56.3± 0.3	59.2± 0.3	59.8± 0.4	60.2± 0.4	61.2± 0.4	61.4± 0.4	61.6± 0.4	61.7± 0.4	61.2± 0.5	61.3± 0.5	61.9± 0.6	<0.0001	<0.0001	0.05
	M	52.5± 0.5	58.1± 0.5	60.4± 0.4	62.5± 0.4	62.5± 0.5	63± 0.5	64.3± 0.5	64.5± 0.5	63.9± 0.5	64± 0.5	63.8± 0.4	64.6± 0.5			
	H	54.3± 0.5	59.1± 0.4	62.0± 0.4	64.2± 0.4	64.5± 0.4	62.2± 0.5	65.5± 0.5	65.9± 0.5	66.8± 0.6	66.7± 0.5	65.9± 0.5	67.3± 0.7			

Continued…

Measurements	BW groups	Flock age (weeks)												P Value		
		19-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	BW	A	BW*A
Albumen Ht (mm)	L	9.6± 0.1	9.2± 0.09	9.3± 0.09	9.0± 0.1	9.5± 0.1	9.3± 0.1	9.5± 0.09	9.2± 0.1	8.2± 0.1	8± 0.1	7.6± 0.1	7.2± 0.1	<0.0001	<0.0001	<0.0001
	M	10.1± 0.2	9.5± 0.1	9.6± 0.1	9.6± 0.1	9.8± 0.1	9.3± 0.1	9.8± 0.09	9.0± 0.1	8.1± 0.1	7.6± 0.1	7.2± 0.1	6.8± 0.1			
	H	10.1± 0.08	9.8± 0.1	9.9± 0.1	9.8± 0.09	10± 0.08	9.7± 0.1	9.9± 0.1	9.3± 0.1	8.2± 0.1	7.5± 0.1	7.4± 0.1	7± 0.1			
HU	L	98.9± 0.5	96.3± 0.4	95.7± 0.5	95.0± 0.5	97.4± 0.5	96.2± 0.5	96.8± 0.4	95.3± 0.5	90.1± 0.5	88.7± 0.6	86.7± 0.6	84.4± 0.7	NS	<0.0001	<0.0001
	M	100.1± 0.4	97.3± 0.5	97.3± 0.4	97.0± 0.4	98.2± 0.5	95.7± 0.6	97.7± 0.4	93.8± 0.7	88.8± 0.7	85.6± 0.8	83.5± 0.7	80.7± 0.9			
	H	100.6± 0.4	98.4± 0.5	98.1± 0.4	97.9± 0.4	98.8± 0.4	96.9± 0.5	97.9± 0.5	94.5± 0.7	88.8± 0.7	83.9± 1.2	83.9± 0.8	81.1± 0.8			
Yolk colour score	L	7.8± 0.1	7.8± 0.1	9.4± 0.1	10.0± 0.1	9.8± 0.1	12.0± 0.1	11.2± 0.1	10.5± 0.1	9.9± 0.1	10.1± 0.1	10.2± 0.1	9.1± 0.1	<0.0001	<0.0001	0.005
	M	8.1± 0.1	7.9± 0.1	9.8± 0.1	10.2± 0.1	10.3± 0.1	12.0± 0.1	11.2± 0.1	10.7± 0.1	10.1± 0.1	9.9± 0.1	10.1± 0.1	8.9± 0.1			
	H	8.2± 0.09	8.1± 0.1	9.9± 0.1	10.2± 0.1	10.4± 0.1	12.2± 0.1	11.3± 0.1	10.7± 0.1	10.3± 0.1	10.0± 0.1	10.2± 0.1	8.9± 0.1			

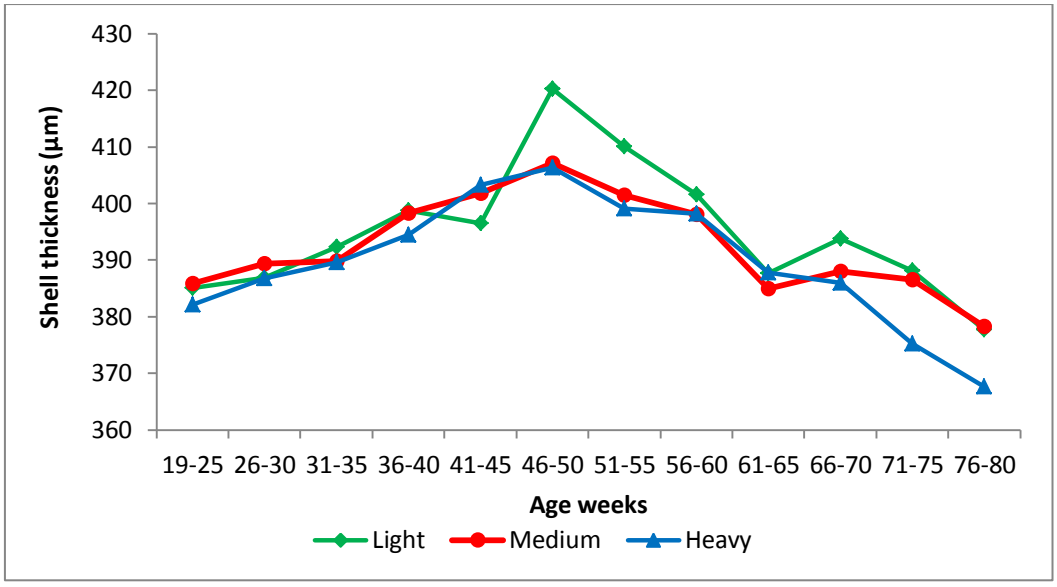

Figure 5.17. Shell thickness in different BW groups

Egg internal quality, measured by albumen height and HU, decreased consistently with increasing flock age (Table 5.4). Albumen height was significantly higher for the heavy BW group than for the light and medium BW groups. HU also generally declined with increasing hen age. However, there was no statistically significant effect of BW group on HU. The yolk colour varied over the course of the experiment due to the different batches of feed. Yolk colour was significantly different among the BW groups, being highest in the heavy BW group and lowest in the light BW group with the medium BW group having intermediate yolk score. There was a statistically significant interaction between flock age and BW group for albumen height, HU and yolk colour score.

5.3.4 Estimation of the amount of cuticle

Shell reflectivity (%) and Spectrophotometry (L*a*b) measurements

Table 5.7. Summarizes the results for shell reflectivity and the spectrophotometric measurements of eggshells before and after staining with MST cuticle blue dye. There was a significant main effect of both flock age (Table 5.7) and BW group (Table 5.8) on shell reflectivity (%) and all values of the L*a*b colour space system before and after staining.

Table 5.7. Main effect of flock age on shell reflectivity and spectrophotometric measurements (L*a*b*) before and after staining

Flock age (weeks)	Shell reflectivity (%)	L*	a*	b*
Before staining				
19-25	24.1±0.16^{g}	55.9±0.16^{i}	19.0±0.11^{a}	27.6±0.18^{bc}
26-30	24.4±0.17^{f}	56.5±0.17^{h}	18.7±0.11^{b}	28.7±0.12^{a}
31-35	25.5±0.16^{e}	57.7±0.15^{g}	18.2±0.08^{c}	28.8±0.12^{a}
36-40	25.8±0.17^{de}	58.1±0.17^{fg}	17.7±0.09^{d}	27.8±0.13^{b}
41-45	26.4±0.21^{c}	58.9±0.2^{e}	17.3±0.11^{e}	27.8±0.11^{b}
46-50	25.7±0.21^{de}	58.2±0.19^{f}	17.4±0.1^{de}	27.6±0.13^{bc}
51-55	26.5±0.21^{c}	59.2±0.2^{de}	16.9±0.11^{f}	27.4±0.13^{bcd}
56-60	27.4±0.23^{b}	59.7±0.21^{d}	16.7±0.12^{f}	27.6±0.14^{bc}
61-65	29.2±0.27^{a}	61.1±0.23^{c}	16.0±0.13^{g}	27.3±0.14^{cd}
66-70	29.4±0.29^{a}	61.9±0.24^{b}	15.6±0.15^{h}	26.9±0.16^{ef}
71-75	28.9±0.31^{a}	62.7±0.26^{a}	14.8±0.16^{i}	26.5±0.18^{f}
76-80	26.1±0.27^{cd}	61.6±0.25^{b}	15.4±0.15^{h}	27.1±0.15^{de}
P Value	<0.0001	<0.0001	<0.0001	<0.0001
After staining				
19-25	19.4±0.2^{i}	50.0±0.2^{i}	1.2±0.33^{de}	28.6±0.11^{d}
26-30	20.4±0.2^{h}	51.2±0.24^{h}	1.4±0.29^{d}	29.7±0.10^{c}
31-35	22.0±0.2^{g}	52.7±0.21^{g}	2.2±0.29^{bcd}	30.2±0.09^{a}
36-40	22.6±0.2^{fg}	53.5±0.22^{f}	1.7±0.32^{cd}	30.3±0.08^{a}
41-45	23.6±0.3^{cd}	54.5±0.27^{de}	2.6±0.35^{ab}	29.8±0.09^{c}
46-50	23.3±0.2^{de}	54.1±0.28^{ef}	3.4±0.33^{a}	30.1±0.09^{ab}
51-55	23.8±0.3^{cd}	55.0±0.26^{d}	2.7±0.33^{ab}	30.1±0.10^{ab}
56-60	24.1±0.3^{bc}	55.2±0.28^{d}	1.7±0.35^{cd}	29.9±0.10^{bc}
61-65	24.7±0.3^{ab}	55.9±0.31^{c}	-0.3±0.38^{f}	28.4±0.1^{d}
66-70	25.1±0.3^{a}	56.5±0.32^{bc}	-0.5±0.39^{f}	28.3±0.13^{d}
71-75	25.3±0.4^{a}	58.2±0.34^{a}	0.4±0.38^{ef}	28.0±0.13^{e}
76-80	22.7±0.3^{ef}	57.1±0.34^{b}	0.9±0.41^{de}	28.3±0.11^{d}
P Value	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d,e,f,g,h,i} Within a column, values with different superscripts are significantly different from each other. Values are mean ± SE

Table 5.8. Main effect of body weight groups on shell reflectivity and spectrophotometric L*, a*,b* before and after staining and the single score

BW Group	Light	Medium	Heavy	P value
Before staining				
Shell Reflectivity (%)	25.2±0.11[c]	27.9±0.13[a]	26.4±0.12[b]	<0.0001
L*	57.9±0.1[c]	60.5±0.12[a]	59.1±0.11[b]	<0.0001
a*	17.8±0.06[a]	16.3±0.07[c]	17.1±0.07[b]	<0.0001
b*	27.7±0.07[b]	27.2±0.08[c]	27.9±0.07[a]	<0.0001
After staining				
Shell Reflectivity (%)	20.9±0.13[c]	24.8±0.15[a]	23.0±0.13[b]	<0.0001
L*	52.1±0.14[c]	56.2±0.15[a]	54.5±0.14[b]	<0.0001
a*	-0.5±0.17[b]	2.4±0.17[a]	2.4±0.17[a]	<0.0001
b*	29.1±0.05[b]	29.4±0.06[a]	29.4±0.06[a]	0.0002
Single score	19.4±0.18[a]	15.0±0.19[c]	15.7±0.18[b]	<0.0001

[a,b,c] Across a row, values with different superscripts are significantly different from each other. Values are mean ± SE

Table 5.9. The differences between shell reflectivity, L*, a*, b* value before and after staining and the single score

Flock age (week)	Δ Reflectivity	Δ L	Δ a	Δ b	Single score
19-25	4.7±0.13[a]	6.0±0.13[a]	17.8±0.30[a]	-1.0±0.16[a]	19.0±0.33[a]
26-30	4.0±0.10[cd]	5.3±0.16[b]	17.2±0.29[a]	-0.9±0.13[a]	18.3±0.32[ab]
31-35	3.5±0.1[ef]	5.0±0.11[c]	16.0±0.29[b]	-1.6±0.14[c]	17.0±0.31[c]
36-40	3.2±0.12[fg]	4.6±0.13[cd]	16.0±0.32[bc]	-2.5±0.15[ef]	17.0±0.34[c]
41-45	2.8±0.13[g]	4.4±0.14[de]	14.7±0.36[de]	-2.0±0.12[d]	15.6±0.38[d]
46-50	2.3±0.13[h]	4.2±0.13[e]	14.0±0.33[e]	-2.5±0.13[ef]	15.0±0.36[e]
51-55	2.8±0.13[i]	4.2±0.13[e]	14.2±0.34[de]	-2.9±0.16[f]	15.3±0.37[de]
56-60	3.3±0.15[ef]	4.6±0.14[de]	15.1±0.36[cd]	-2.3±0.15[de]	16.1±0.38[cd]
61-65	4.6±0.17[ab]	5.2±0.16[b]	16.2±0.40[b]	-1.0±0.13[ab]	17.3±0.42[bc]
66-70	4.3±0.18[bc]	5.4±0.15[b]	16.1±0.41[b]	-1.5±0.15[c]	17.2±0.44[c]
71-75	3.6±0.17[de]	4.6±0.16[cde]	14.4±0.41[de]	-1.5±0.15[c]	15.3±0.44[de]
76-80	3.4±0.18[e]	4.7±0.16[cd]	14.5±0.43[de]	-1.2±0.13[bc]	15.5±0.46[de]
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a,b,c,d,e,f,g,h,i] Within a column, values with different superscripts are significantly different from each other. Values are mean ± SE

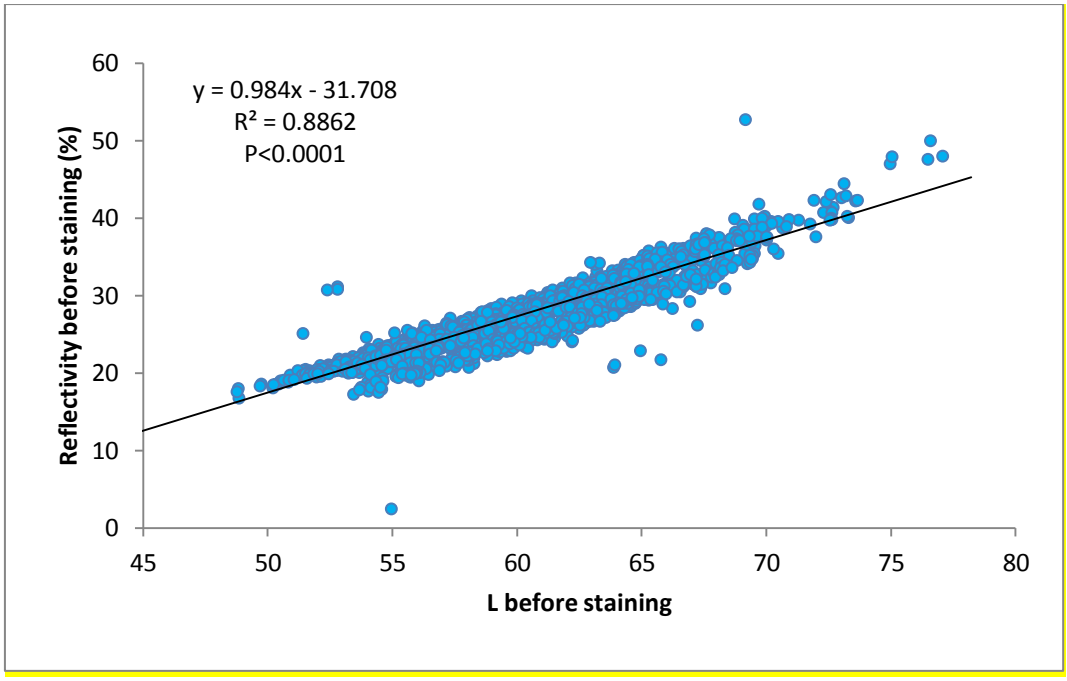

Figure 5.18. Correlation between shell reflectivity and L* value before staining

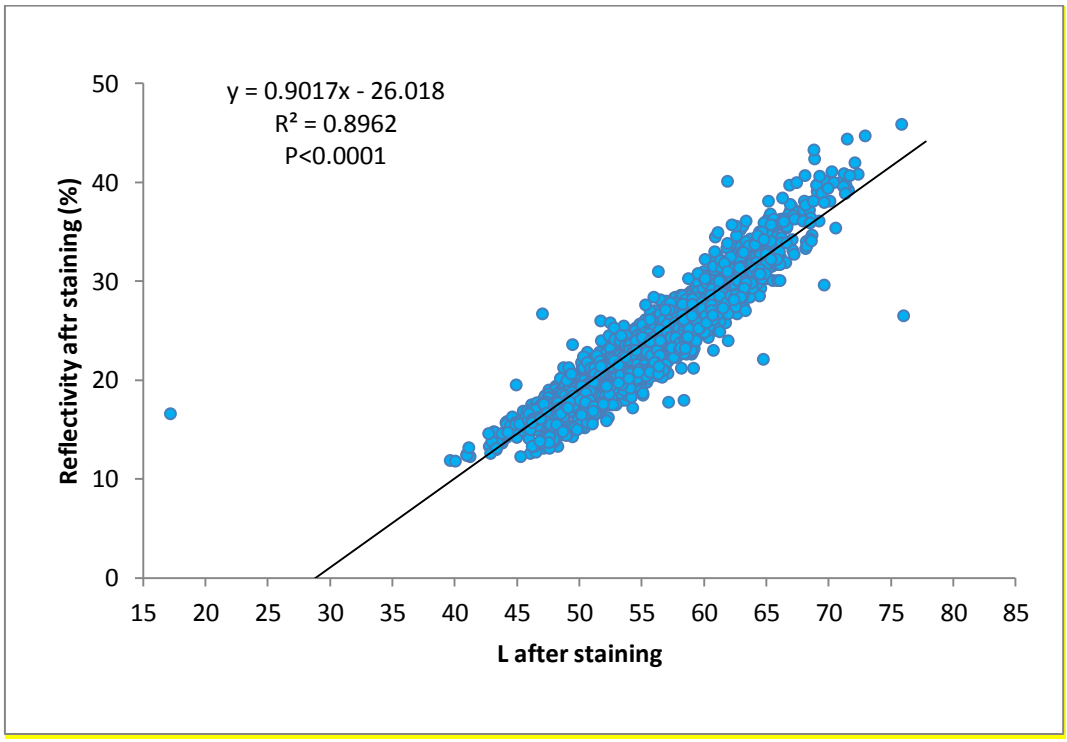

Figure 5.19. Correlation between shell reflectivity and L value after staining

With respect to flock age (Table 5.7), shell reflectivity before staining increased to age 70 weeks then decreased slightly. However, after staining, the shell reflectivity increased to age 71-75

weeks then decreased at 76-80 weeks. The pattern was very similar for the L* values (Table 5.8). There was a significant difference of hen age for the difference value of L*,a*,b* before and after staining (Table 5.9). There was high correlation between shell reflectivity and L* value before and after staining (Figure 5.18 and Figure 5.19). The a* values before staining decreased with increasing flock age, whereas values after staining were varied among the ages, being lowest in the age period 61to 75 weeks. The b* values, before and after staining, varied with flock age being highest at 26-35 weeks and 31-36 weeks of age, respectively. Single score values generally decreased over the course of the experiment (Table 5.8). Differences in reflectivity, L*, a* and b* are shown in Table 5.8. These values are used to calculate the single score values.

Both before and after staining, shell reflectivity was highest in the medium and lowest in the light BW group, with the heavy BW group intermediate. The L* values before and after staining followed the same pattern. The a* values before staining were highest in the light BW group followed by the heavy and medium BW groups, respectively. After staining, a* values were lowest and negative in the light BW group and positive in the heavy and the medium groups, which were not significantly different from each other. The single score values were highest for the light BW group, followed by the heavy and the medium BW groups. The b* value before staining was highest in the heavy BW group, and lowest in the medium BW group with the light BW group intermediate (Table 5.8). After staining, the b* value was higher in the heavy and medium BW groups than in the light BW group. The single score values were highest in the light BW group and lowest in the medium BW group, with the heavy BW group intermediate.

5.3.5 Ultrastructure variations of the eggshell

For shell mammillary layer ultrastructural variations, there was no significant main effect of BW group on mammillary layer ultrastructure except for alignment, changed membrane and cap quality (Table 5.10). The incidence of alignment was highest for the medium BW group, lowest for the heavy BW group with the light BW group intermediate. The incidence of changed membrane was higher for the light and medium BW groups than for the heavy BW group. The cap quality was higher in the medium and heavy BW groups than in the light BW group.

Table 5.10. The main effects of BW group on the ultrastructural properties

BW groups	Alignment	Changed membrane	Cap quality
Light	3.2±0.09[ab]	1.2±0.07[a]	2.4±0.08[b]
Medium	3.2±0.09[a]	1.2±0.05[a]	2.6±0.07[a]
Heavy	3.0±0.09[b]	1.0±0.02[b]	2.7±0.08[a]
P Value	=0.092	0.009	0.021

[a,b,c] Within a column, values with different superscripts are significantly different from each other. Values are mean ± SE

There were statistically significant effects of flock age on most of the ultrastructural scores, with the exception of the incidence of cubics, cuffing, changed membrane and depression (Table 5.11). As the flock aged, there was an increase in the incidence of ultrastructural features associated with decreased shell quality, such as increased mammillary cap size variability, decreased mammillary cap quality and an increased incidence of late fusion, alignment, Type A bodies, Type B bodies, aragonite, erosion. There was also a decreased incidence of ultrastructural features associated with good shell quality, such as confluence and early fusion.

Table 5.11. The main effect of flock age on the mammillary ultrastructure scores of the eggshell

Ultrastructure properties	Flock age (week)							P Value
	20	30	40	50	60	70	80	
Mammillary cap size variability	2.4±0.1^{bc}	2.5±0.1^{b}	2.7±0.1^{ab}	2.7±0.1^{ab}	2.9±0.1^{a}	2.8±0.1^{a}	2.8±0.1^{a}	0.0008
Confluence	3.3±0.1^{a}	2.4±0.1^{b}	2.3±0.1^{bc}	2±0.2^{bcd}	2.1 ±0.2^{bcd}	1.7 ±0.1^{d}	2±0.2^{cd}	<0.0001
Caps quality	2.2	2.7	2.7	2.6	2.4	2.5	2.8	0.0042
Early fusion	3.4±0.1^{a}	3.3±0.2 ^{ab}	3.1±0.2^{abc}	3.1±0.2^{abc}	3±0.2^{bc}	2.8±0.2^{c}	2.7±0.2^{c}	0.03
Late fusion	2.8±0.1^{c}	3±0.2^{bc}	3±0.1^{bc}	3.2±0.1^{abc}	3.3±0.1^{ab}	3.4±0.1^{ab}	3.5±0.1^{a}	0.018
Alignment	2.5±0.1^{d}	2.8±0.1^{cd}	3.1±0.1^{bc}	3.3±0.1^{ab}	3.2±0.1^{ab}	3.5±0.1^{a}	3.3±0.1^{ab}	<0.0001
Type A	1.5±0.1^{d}	1.6±0.1^{cd}	1.8±0.1^{bc}	1.9±0.1^{ab}	1.9±0.1^{ab}	2.2±0.1^{a}	2.2±0.1^{a}	<0.0001
Type B	2.2±0.1^{c}	2.5±0.1^{bc}	2.5±0.1^{bc}	2.6±0.1^{ab}	2.5±0.1^{bc}	2.9±0.1^{a}	3.1±0.1^{a}	0.0001
Aragonite	1.6±0.2^{cd}	1.8±0.1^{bcd}	1.4±0.1^{d}	1.5±0.2^{cd}	1.9±0.1^{abc}	2.2±0.2^{ab}	2.3±0.2^{a}	0.0001
Cubics	1.2±0.1	1.4±0.1	1.4±0.1	1.4±0.1	1.4±0.1	1.3±0.1	1.4±0.1	NS
Cubic cone formation	2. 2±0.1^{a}	2±0.1^{ab}	1.8±0.1^{bc}	1.9±0.1^{bcd}	1.6±0.1^{cd}	1.6±0.1^{d}	1.4±0.1^{d}	<0.0001
Cuffing	1±0.0	1±0.0	1±0.0	1±0.0	1.1±0.05	1.0±0.03	1.0±0.0	NS
Changed membrane	1.1±0.1	1.2±0.1	1.1±0.1	1.1±0.1	1.3±0.1	1.3±0.1	1.1±0.1	NS
Depression	1.1±0.1	1.1±0.1	1.0±0.0	1.0±0	1.0±0.0	1.0±0	1.1±0.1	NS
Erosion	1.1±0.1^{cd}	1.4±0.1^{b}	1.1±0.1^{cd}	1.0±0.03^{d}	1.3±0.1^{bc}	1.5±0.1^{b}	1.9±0.1^{a}	<0.0001

^{a,b,c,d} Across a row, values with different superscripts are significantly different from each other. Values are mean ± SE

5.3.6 Bone strength

Table 5.12 shows the strength, length and width of both humerus and femur. There were no significant differences among the body weight groups for any of the bone measurements. However, the breaking strength of both humerus and femur of the medium BW group tended to be greater than in the other groups.

Table 5.12. The length, width and breaking strength of humerus and femur bones for different BW groups

BW group	BW (kg)	Humerus			Femur		
		Strength (kg)	Length (mm)	Width (mm)	Strength (kg)	Length (mm)	Width (mm)
Light	2.2	16.9±1.34	74.3±1.01	8.3±0.09	31.2±2.89	80.7±0.79	8.4±0.45
Medium	2.1	21.3±1.29	75.2±0.74	8.5±0.12	31.5±1.41	80.3±0.91	8.6±0.17
Heavy	2.3	18.4±1.84	76.4±0.72	8.5±0.17	29.1±1.69	81.6±0.92	8.5±0.36

There was no significant different in any parameters among the BW groups (P>0.05) n=6 birds.

5.4 Discussion

5.4.1 Body weight and body weight uniformity

Body weight at point of lay is important as it can influence egg production and egg quality over the entire laying period. The results of the present study show that average body weight at 19 weeks of age was 1.595 kg, 1.741 kg, and 1.870 kg for the light, medium and heavy BW groups, respectively, and this is similar to what is seen in many commercial flocks. The light BW group achieved the minimum weight at that age, as recommended by the breeder manual (1.53kg - Hy-Line Brown, Performance Standards Manual). The light BW group continued to have body weights similar to that of the breeder recommendation, although body weight was lower at 26 weeks and higher at 60 weeks than the recommended standard. At 26 weeks of age, weight in the medium BW group was lower than the breeder standard by 60 g, whereas the light BW group was below the breeder standard from 26 to 50 weeks of age. The body weight in both medium and heavy BW groups continued to exceed the breeder standard by an average of 170-370 g during the experiment. Although the body weight exceeded the recommended weight at point of lay, the medium group reached 5% production at age 20 weeks whereas both the heavy and the light groups reached 5% production at 21 weeks of age. All groups attained significantly higher egg production than the breeder recommendation, with the exception of the light BW group at 41-45 weeks, where the birds inadvertently experienced water restriction. However, the light BW group showed good recovery after the incident, reaching their highest production at 46-50 weeks of age. Dunnington and Siegel (1984) studied the age at onset of sexual maturity and egg production in relation to body weight from high (HA) and light (LA) lines of antibody production to sheep erythrocyte antigen, in single Comb White Leghorn chickens. These authors concluded that egg-type chickens must attain a minimum age and body weight before commencing egg production. Balnave (1984) also reported that age at sexual maturity was significantly influenced by body weight at 21 weeks, with the heavier birds maturing earlier and producing significantly heavier eggs, compared with those in the lightest body weight groups. Balnave (1984) reported no differences in egg production over a body weight range of 1.36, 1.45, 1.55, 1.64 and 1.73 in the White Leghorn **x** Australorp strain.

In this experiment, birds were allocated to the body weight groups at 16 weeks of age, so that all groups had extremely high body weight uniformity (90 – 100 %) at point of lay, in order to assess the maximum productive potential of different body weight categories at the onset of sexual

maturity. Body weight uniformity remained high during the trial, generally decreasing with increasing flock age. Commercial egg producers and pullet growers aim for a particular average body weight and uniformity of the flock at the age of sexual maturity. Producers aim to have at least 80% of the pullets within a range of ± 10% of the average weight of the flock (Akanbi and Goodman, 1982), in order to reach optimum egg production. Furthermore, Akanbi and Goodman (1982) found that body weight uniformity at 19 weeks of age was 80.7, 84.0, and 81.2% for pullets from the lowest, middle and heaviest groups, respectively, with restricted feeding for the middle and heavy groups to attain the recommended weight at 19 weeks of age. They assumed that separation of pullets based on weight at 9 weeks might be used as an indicator of their genetic potential for growth. Others, including Leeson, *et al*., (2005) found that body weight uniformity improved with step-down lighting. However, Frikha, *et al.*, (2009) found that body weight uniformity of pullets was not affected by dietary treatment when corn was substituted by wheat. Studies undertaken on commercial farms in Australia have indicated that uniformities as high as 90% are possible in brown egg layers at or near point of lay (16-20 weeks of age) (Parkinson personal communication, 2015). The model in this experiment therefore exceeds these industry standards and was able to be achieved in all three weight categories. It may be possible for uniformities above 90% at point of lay to be achieved, particularly using advanced housing technologies.

The most interesting finding, however, was the gradation of uniformities at 70 weeks of age for the different body weight groups, with the light and medium groups having low uniformities, but higher production overall. However, there was no significant correlation between flock uniformity and egg production. The medium body weight group produced 350 eggs to 72 weeks of age, with a body weight range of 1.86 to 2.37 kg, a uniformity at 70 weeks of 70% and a pullet weight of 1.7 kg at 19 weeks of age, compared with the light BW (322 eggs) and heavy BW (348 eggs) eggs at that age.

For the light body weight group, the production to 40-42 weeks of age was extremely high (93-94%) with a body weight range of 1.53 to 2.22 kg at 37 weeks of age, and a uniformity of 85%. One bird from the heavy BW group was excluded from the analysis. This pullet was 1.62 kg at 16 weeks of age and 2.83 kg at 37 weeks of age, was laying eggs early at 17-18 weeks of age, then stopped producing eggs from 19 weeks of age onwards. This excluded bird was euthanized at 44 weeks after appearing unwell. This bird illustrates the problems arising from heavy weight at an early age causing early maturity.

The light body weight group had the poorest correlation between body weight at 19 weeks of age and the 50 week body weight and this seems likely to reflect the greater variation in age at sexual maturity and potential for growth in individual birds. In the heavy body weight group, the lower correlation between 19 and 50 week body weights probably reflects the differences in the potential of the heavier birds to deposit body fat, compared with the medium sized birds that have the strongest correlation between 19 and 50 week body weights (Figures 5.3, 5.4, 5.5). The significant correlation between body weight at point of lay and egg production (Figures 5.10, 5.12) shows that maintaining target body weight at point of lay is very important for reaching the peak and then sustaining egg production. Abbas *et al*. (2010) also reported that the high uniformity group (75-80%) consistently had the highest hen-day and hen-house production over all ages, while the low uniformity group had the lowest hen-day production.

Feed intake was directly associated with body weight in the present study (R^2= 0.3671) (Figure 5.15). The current study found that the heavy birds consumed more feed in early lay than other groups; however, from 50 weeks of age onwards, the medium group consumed slightly more feed than the other groups. Birds in the medium and heavy BW groups consumed about 10.4 and 10.7 g feed per bird, respectively, above the feed intake recommended. Heavy birds consuming more feed has been reported by other studies (Jensen, *et al*., 1976; Harms, *et al*., 1982; Leeson *et al*., 2005).

The poultry house used in the present study was not environmentally controlled and it is assumed that this caused the birds to consume more feed, resulting in body weight exceeding the recommended levels. However, egg production for all groups was higher than recommended guidelines, with some variation among the groups. By controlling feed intake, producers will maintain the average target body weight and flock uniformity and, as a result, will reach maximum production. The regression analysis showed that there was a negative correlation between body weight and FCR (Figure 5.16). The high values for FCR are due to the very low egg production at 19 weeks in all BW groups. However, there was a positive correlation between body weight and egg mass (R^2= 0.141) as shown in Figure 5.13. The low values in this figure are due to the low egg production at 19 weeks in all BW groups. Management is key in controlling flock uniformity by controlling body weight and using of feed restriction to achieve optimum production (Abbas *et al.*, 2010).

5.4.2 Eggshell and egg internal quality measurements

Flock body weight at point of lay affected the internal and eggshell quality of the eggs. Shell reflectivity was significantly different among the BW groups. The light BW group had darker shell colour and lower egg weight than the other groups. It has been suggested that, regardless of egg size, the same amount of colour is distributed over the shell surface (Odabasi, *et al*., 2007). This may explain why small eggs have darker shell colour than larger eggs. The heavy BW group had significantly higher in egg weight and shell weight than the other BW groups. Summers and Leeson (1983) found a strong correlation between body weight and egg weight and other studies report that differences in egg weight generally reflected differences in body weight, with heavy birds producing larger eggs than smaller birds (Jensen *et al*., 1976; Kırıkçı, *et al.*, 2004; Lacin, *et al*., 2008).

Shell breaking strength (BS) measures the shell resistance to breakage when force is applied to the surface of the eggshell. In the current study, BS and shell deformation were higher in the light BW group, indicating stronger shells. This might be related to egg weight, as this BW group produced the smallest eggs (59.5 g). However, Lacin *et al*. (2008) did not find a significant effect of body weight on shell strength and shell thickness.

For egg internal quality, albumen height and HU were higher in the heavy BW group, which produced larger eggs, than the other BW groups. These results contradict the findings of (Lacin *et al*., 2008), who reported a higher albumen index and HU in light weight birds. Yolk colour was higher in the heavy BW group. The primary determinant of yolk colour is plant pigment (xanthophyll) content in the diet and is influenced by feed intake. This result most likely reflects the level of feed consumed by heavy BW groups. Fluctuations in yolk colour score with the age of the flock will reflect feed intake and the amount of yolk colour pigment in the different batches of feed.

Overall, BW groups and flock age had significant effects on all measures of eggshell and egg internal quality. As flock age increased, shell reflectivity, egg weight, shell weight and shell thickness increased, and translucency, BS, and shell deformation decreased. These results are comparable to the findings of several authors (Summers and Leeson, 1983; Rodriguez-Navarro, *et al*., 2002; Guesdon and Faure, 2004; Lacin *et al*., 2008). Rodriguez-Navarro *et al*. (2002) correlated BS with the orientation of calcite crystals in the eggshell and, in their studies, eggs from older flocks had lower BS values compared to younger flock eggs. Change in components of

the organic matrix with flock age may be a contributing factor to poor shell strength in older hens (Fraser *et al.*, 1999; Gautron and Nys, 2006; Panheleux *et al.*, 1999).

5.4.3 The amount of cuticle cover on eggshell

The SCI L* component of the L*a*b system measures the grading between white and black and gives results similar to shell reflectivity, which simply indicates the colour lightness of the shell. In the current study, the increasing L* value with increasing flock age indicated that the amount of colour deposited decreased with flock age and this was observed despite the staining with cuticle blue dye. There was a significant correlation between shell reflectivity and L* value. R^2 values for the shell reflectivity vs L* before staining and shell reflectivity vs L* value after staining were 0.888 and 0.885, respectively, and for shell reflectivity before and after staining, and L* value before and after staining were 0.725 and 0.76, respectively (data not shown). This finding indicated that shell colour became lighter as the flock aged, except for the decreased reflectivity and slightly decreased L* value at 76-80 weeks of age. A possible explanation is that, as the flocks get older, birds produced larger eggs, which had rougher surfaces, and some had speckled shells. The speckled shell surfaces were darker in colour, which affected the results recorded by the reflectivity meter and the spectrophotometer.

The a* value measures the grading between red and green, with green towards the negative end of the scale. A more negative value following staining with cuticle blue dye indicates the presence of more eggshell cuticle. Eggs with good quality intact cuticle stained well; eggs with patchy cuticle acquired patchy stain, whereas eggs did not stain at all in the absence of cuticle. Our results indicate that the cuticle cover was greater in the light BW group, which produced smaller eggs than the other groups. Cuticle cover was higher at the early stage of lay (20 weeks) and higher again in late lay (age 51-70 weeks). A possible explanation for our findings of greater cuticle cover in the light BW group is more protein distributed in the cuticle, because eggs are smaller than those of the other groups.

Mikšík *et al.* (2007) reported that 70% of protoporphyrin, the main pigment of the eggshell, was found in the cuticle layer. This finding was contradicted by Samiullah and Roberts (2013) who found only 13-20% of protoporphyrin was located in the cuticle.

The finding of better cuticle cover in late lay contradicts the findings of Rodríguez-Navarro, *et al.*, (2013), who found that thinner and more irregular cuticle was found on eggs laid by old flocks (70

weeks) than on eggs of young flocks, which had thicker and more regular cuticle. Roberts, *et al.* (2013) noted that, although the cuticle cover on eggshells was not significantly different based on age, the thickest cuticle was found at mid lay (40-55 weeks) and late lay (55-65 weeks). In the current experiment, the thickest cuticle cover was at 61-70 weeks of age and eggs from individual birds, from the beginning of lay to the age of 80 weeks, were analysed. In comparison, the study by Roberts *et al*. (2013) collected eggs randomly from the flock. The cause of thicker cuticle found in older flocks is still uncertain.

5.4.4 Scoring of ultrastructure variations of the shell

There was a tendency to higher incidence of confluence in the light BW group, as compared to the other groups. The reason for this result could possibly be the smaller egg size produced in this group. As hen age increased, the incidence of ultrastructural features shown to be associated with good shell quality, such as early fusion and confluence, decreased. At the same time, the incidence of ultrastructural features known to be associated with poorer shell quality increased: alignment of mammillae, Type-A bodies, Type-B bodies, aragonite, late fusion and pitting.

The significant effects of flock age on early fusion, late fusion, alignment, aragonite and erosion are evidence of deterioration in shell quality. Bain (2005) noted that late fusion and alignment in the mammillae predispose an egg to initiation and propagation of cracks. However, cuffing improved shell strength (Bain, 2005). The incidence of ultrastructural features shown to be associated with good shell quality, such as early fusion and confluence, have been reported by other workers to decrease as the flock increased in age (Roberts and Brackpool, 1995; Bain, 2005). As the flock age increased, a number of structural variations altered in the mammillary layer increased and reduced shell membrane bonding (Nascimento, *et al*., 1992; Roberts and Brackpool, 1995) thus weakening the shell strength (Van Toledo, *et al*., 1982; Bain, 1992). The tendency of higher incidence of Type-A bodies, Type-b bodies and aragonite in the heavy BW group showed that the eggshells were poorer in quality. The scoring of ultrastructural features under the SEM and the photographs taken revealed that weak eggshells can possess major ultrastructural abnormalities.

5.4.5 Bone strength

The result from bone measurements in the current study indicate that although the BW groups were not significantly different, the medium group tended to have the best bone strength. A

further analysis, such as bone ash, needs to be conducted, in order to obtain an explanation of how the body weight of flocks affects calcium deposition in bones. Despite the large differences in body weight, bone dimensions and breaking strength were not significantly different among the body weight groups. The skeletal dimensions and bone breaking strength appear not to be influenced by body weight. Although the body weights of the medium and heavy group birds in the trial were significantly higher than those of the light weight group at 80 weeks of age, the random sampling of birds for measurement of bone breaking strength resulted in the average body weight from light and medium groups being not significantly different (Table 5.12).

5.5 Conclusion

In this experiment, body weight exceeded the breed standard by an average of 170-370 g for the medium and heavy BW groups. Although uniformity was high in comparison with the breed standard from 16 to 60 weeks of age, uniformity decreased as hen age increased. In addition, egg production in this experiment exceeded the breed standards, with the exception of the light BW group at 41-45 weeks of age, as a result of accidental water deprivation. Despite the water deprivation episode, the light body weight group, which had high uniformity at point of lay and a body weight range at 50 weeks of 1.66 to 2.27 kg, produced at levels exceeding the breed standard. Maintaining flock uniformity at 80% will optimize the egg production during the laying period, concerning the body weight at point of lay.

The uniformity of the heavy BW group was steady at 89% from 19 to 50 weeks, which may be responsible for maintaining the peak production higher than the breed standard. The bird that was excluded from the heavy BW group had heavy BW at point of lay that may be as an example of early maturation, which produced large egg. Produce a large egg at the beginning of lay may indicate problems in the oviduct as this organ is not yet mature. Maintaining body weight at the level recommended will reduce the incidence of birds having problems of the reproductive tract.

Body weight at point of lay affects egg quality. Stronger shell was found in the light BW group which correlated to small eggs in this group. On the other hand, bigger egg and low shell breaking strength in the heavy BW group indicated low shell quality. There was no correlation between body weight uniformity and egg production. Hen age is the main factor affecting egg quality, with egg quality deteriorating as hens grow older. However, bone breaking strength and bone dimensions were not affected by body weight.

Chapter 6

Application of Computerized Tomography scanning for assessing body conformation of hens at the end of the laying period

6.1 Introduction

Genetic selection has substantially improved the performance of laying hens. When studying the composition of growing chickens, it can be informative to measure whole body composition. Body composition is a dynamic variable that often shows a high level of variation (Reynolds and Kunz, 2001). These authors noted that body composition analysis measures the total mass of each component such as water, fat, lean and inorganic constituents.

Heavy body weight of pullets can result in lower egg production and poorer egg quality (Leeson and Summers 1987; Parkinson *et al*., 2007). Although results from the previous chapter show high egg production in the heavy BW group, the peak production was not sustained, and dropped as the overall egg quality decreased with increased flock age. One of the birds in the heavy BW group from previous chapter commenced lay early but then stopped laying due to a tumour in the oviduct; this is one of the problems that can occur in heavy pullets. The concern over the high incidence of obese hens accentuates the need for an accurate method of measuring body composition. Several techniques have been evaluated in chickens. Among these techniques, computed tomography (Bentsen and Sehested, 1989, Svihus and Katie, 1993), magnetic resonance imaging (MRI) (Mitchell *et al*., 1991) and Dual Energy x-ray (DXA) (Mitchell *et al*., 1997) have been evaluated as being accurate techniques. Computerized tomography (CT) measurements of live animals can also accurately quantify the body proportions of lean mass, fat and bone, as reported in sheep (Young *et al.,* 2001; Haynes, *et al.,* 2010), cats (Buelund *et al*., 2011), pigs (Jopson *et al*., 1995), and beagle dogs (Ishioka *et al*., 2005).

Computerized tomography is a method of measuring the density in a cross-section of an object. This is achieved by the use of an X-ray tube that fires X-ray beams at many different positions around the cross-sectional plane of the object (Svihus and Katie, 1993). At the opposite side of the

object, the X-ray beams are measured by detectors, providing a measurement of the absorption of beams by the object. Information from the detectors is then used to calculate the absorption in small sectors of the cross-section, the so-called pixels. The X-ray absorption (CT-value) of each pixel has values between -1023 and 1024, the lowest value indicating that no beams have been absorbed and the highest value that all the beams have been absorbed. Water will give a CT-value of approximately zero (Svihus and Katie, 1993).

An image contains information concerning just one 'slice' through the body. Fuller *et al*(1994) reported that the three imaging methods (Ultra sound, X-Ray Computerized axial tomography (CAT) and MRI) share the problem of deriving an estimate of the composition of the whole body from these images.

The present study was conducted to provide a more accurate evaluation of body composition of laying hens, in relation to flock uniformity at the end of the laying period for hens from a commercial farm (Chapter 3), and from a research station experiment (Chapter 5).

6.2 Material and Methods

Birds

The birds came from two studies; on-farm and in a laboratory setting.

1) For the on-farm study, twelve hens weighing 1.870-2.540 kg at 87 weeks of age were studied. These hens came from the same original rearing sheds as described in Chapter 3, six hens per shed.

2) In the research station study, eighteen hens weighing 1.870-2.725 kg at 80 weeks of age as described in Chapter 5 were studied, six hens per body weight group (light, medium, and heavy).

Live weights were recorded immediately prior to scanning on an electronic weighing scale (VEIT electronics Poultry scale BAT 1) with maximum weight 30 kg, then euthanized using CO_2. Birds were scanned in groups of three. Following CT scanning, the abdominal fat depots were removed and weighed.

CT Scanning and images

A whole body scan with regular intervals was performed using a GE HiSpeed QXi 4 Slice CT scanner (manufactured June 2003). The acquisition parameters of the CT scanner were as follows: helical scanning120 kV; 140mA; 5mm thickness; 5 mm spacing and 1 s scanning time.

The resulting images were analysed using the software programs OsiriX, ImageJ and AutoCAT as described by Haynes and colleagues (Fuller *et al*. 1994; Rosset *et al*., 2004; McEvoy 2007; Haynes *et al*., 2010).

6.3 Statistical analysis of data

Data were analysed using Statview Software (SAS Institute Inc., Version 5.0.1.0). A two-way analysis of variance was conducted, taking rearing sheds and body weight groups as the independent variables, and fat measured, fat, bone predicted, CT entire and CT carcass as dependent variables. Level of significance was indicated by a probability of less than 5%. A linear regression was performed to investigate the correlation between the body weight of hens and fat, lean and bone, predicted by CT scan. Level of significance was indicated by a probability of less than 5%.

6.4 Results

1. On-farm study (from Chapter 3)

There was no significant difference between original rearing sheds for the body weight, fat pad weight, and all the variables derived from CT scanning (Table 6.1).

Table 6.1. Body weight, abdominal fat and the variables predicted from CT

	Shed A	Shed B
Measured BW kg	2.2±1.0	2.2±0.06
Fat pad g	147±0.03	141±0.01
Predicted CT entire weight (%)		
Fat	18.9±2.12	19.1±0.69
Lean	68.5±1.48	68.1±0.47
Bone	12.7±0.79	12.8±0.46
Predicted CT carcass weight (%)		
Fat	17.4±1.79	17.9±0.55
Lean	66.5±1.13	65.8±0.71
Bone	16.1±0.96	16.3±0.48

Figure 6.1 shows the positive correlation between measured body weight, abdominal fat and percentages of fatmeasured by CT scans, observed in the on-farm study. There was highly significant positive correlation between body weight and abdominal fat (R^2= 0.8057). A similar pattern existed for the CT predicted entire and carcass fat percentages, with R^2= 0.5524 and R^2= 0.5783, respectively

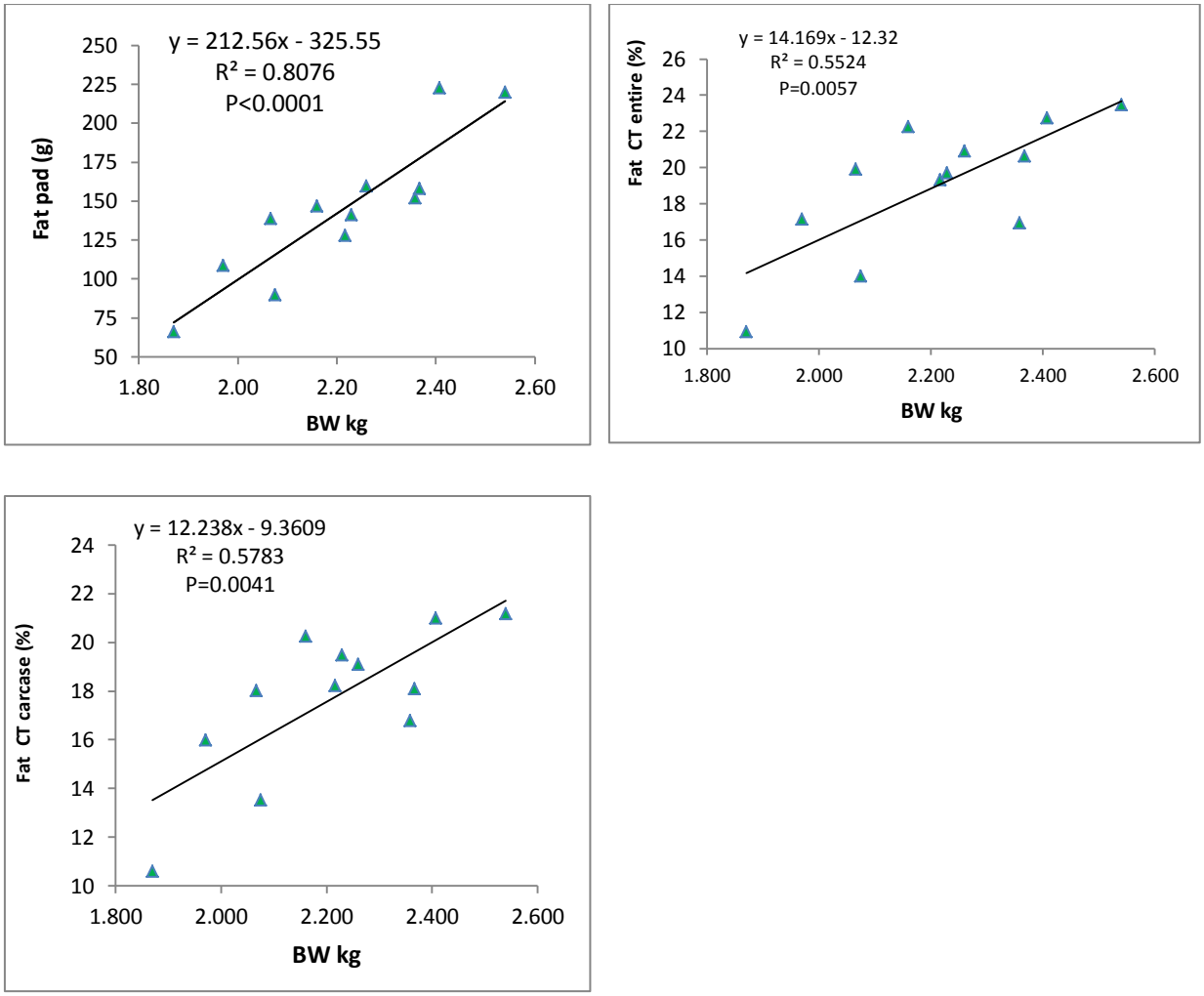

Figure 6.1. The correlation between body weight and measured abdominal fat, CT predicted entire percentage fat and CT predicted carcass predicted fat

There was also a significant negative correlation between body weight and lean composition, as shown in Figure 6.2.

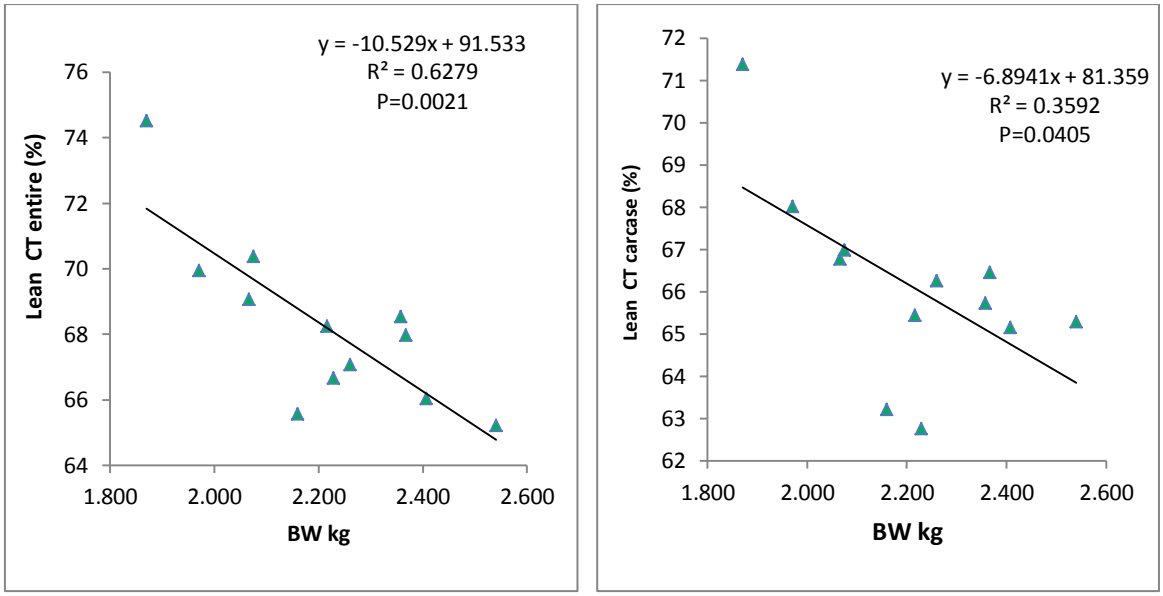

Figure 6.2. The correlation between body weight and percentage lean predicted CT entire and percentage CT carcass

The correlation between body weight and percentage bone predicted by CT was not significant. However, a positive correlation was found between body weight and CT predicted carcass bone percentage with R^2= 0.3371 (Figure 6.3).

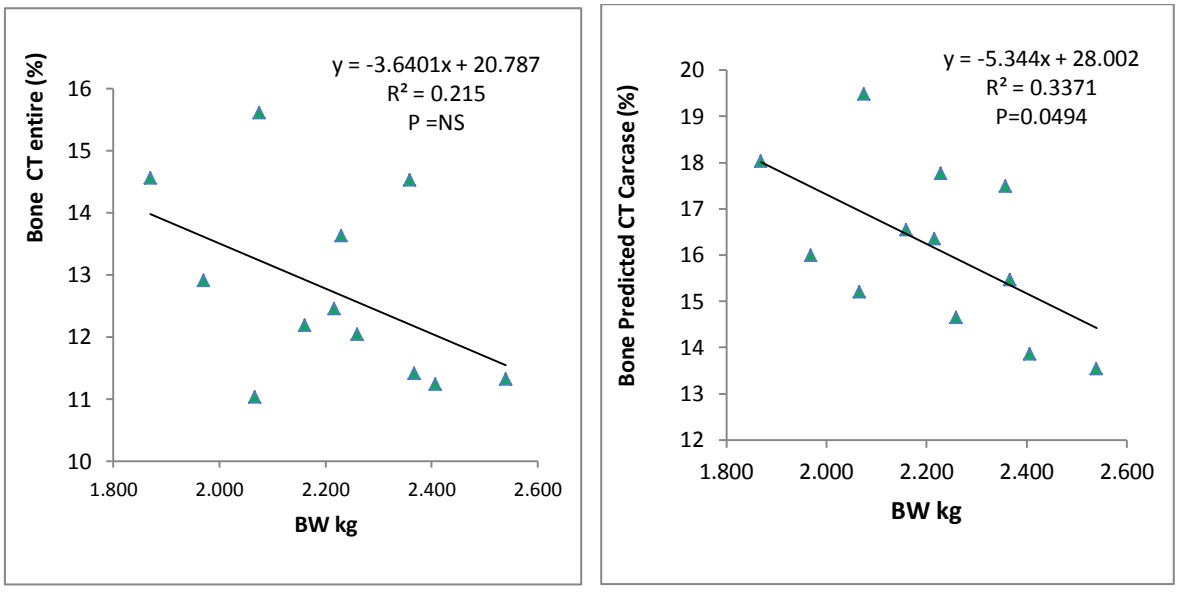

Figure 6.3. The correlation between body weight and bone predicted by CT

There was a significant correlation between measured abdominal fat and CT predicted entire and carcass fat (Figure 6.4)

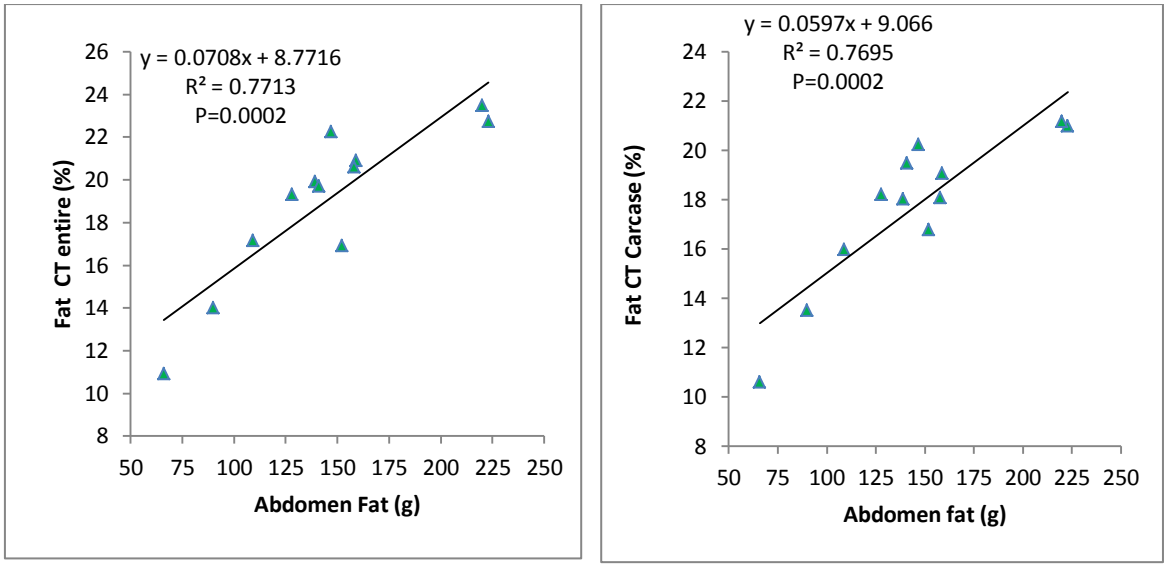

Figure 6.4. The correlation between abdomen fat measured and fat predicted by CT

2. Research station at UNE

There was no significant difference between body weight groups for body weight, fat pad measured and all the variables derived from CT scanning (Table 6.2).

Table 6.2. Body weight, abdominal fat and variables predicted from CT

	Light	Medium	Heavy
Measured BW (kg)	2.1±0.07	2.1±0.07	2.3±0.10
Fat pad (g)	120±0.01	90±0.02	98±0.02
Predicted CT entire weight (%)			
Fat	16.8±1.03	14.7±1.58	16.0±1.50
Lean	70.0±0.94	71.4±1.39	70.7±1.18
Bone	13.2±0.77	13.9±0.69	13.4±0.52
Predicted CT carcass weight (%)			
Fat	14.2±0.65	13.1±1.19	15.4±1.45
Lean	67.4±0.92	68.2±0.98	66.5±0.97
Bone	18.4±0.91	18.7±0.73	18.1±0.72

However, there was a significant correlation between measured body weight and measured abdominal fat pad weight and CT predicted entire percentage fat (Figure 6. 5).

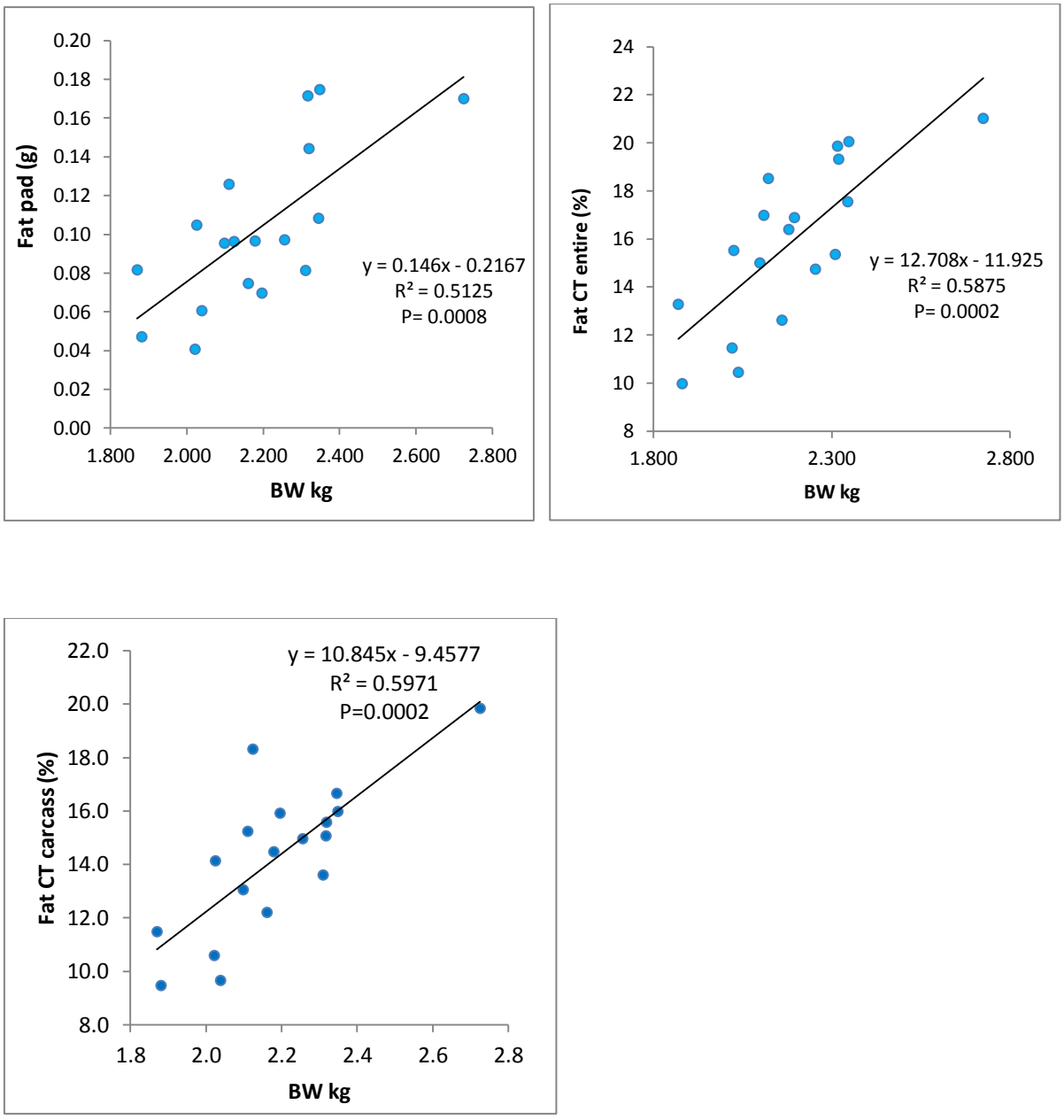

Figure 6.5. The correlation between body weight and abdominal fat measured in the laboratory setting experiment, and fat predicted by CT

There was a significant negative correlation between body weight and CT predicted entire lean percentage (Figure 6.6). However, the correlation between body weight and CT predicted carcass lean percentage was not significant.

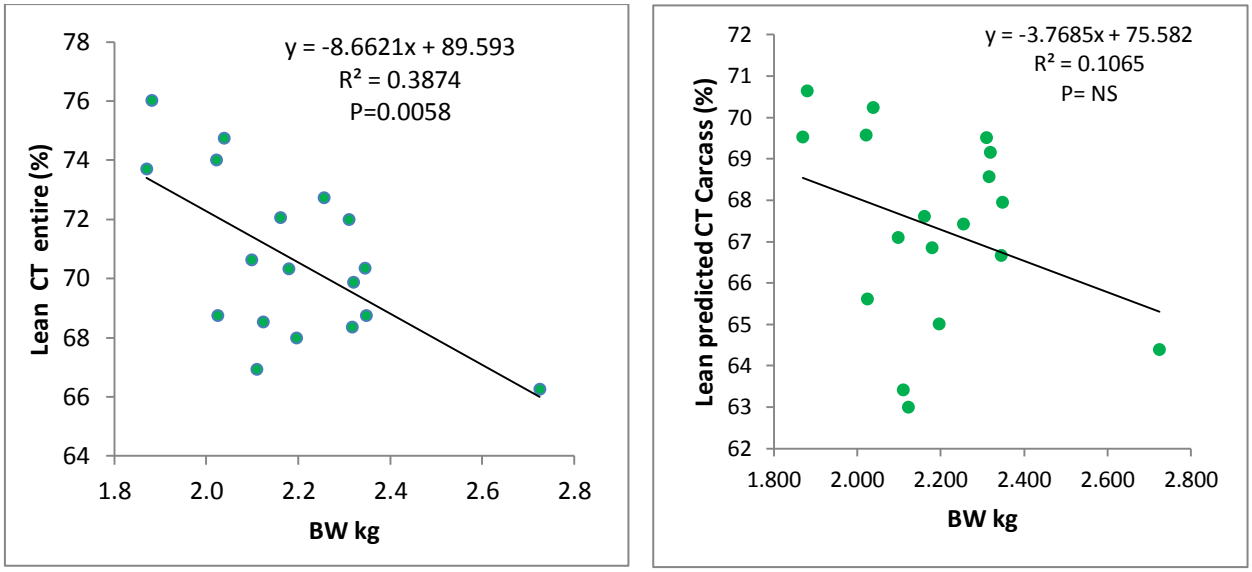

Figure 6.6. The correlation between body weight and lean percentage predicted by CT entire

There was a significant negative correlation between body weight and CT predicted entire bone percentage, and CT predicted carcass bone percentage (Figure 6.7).

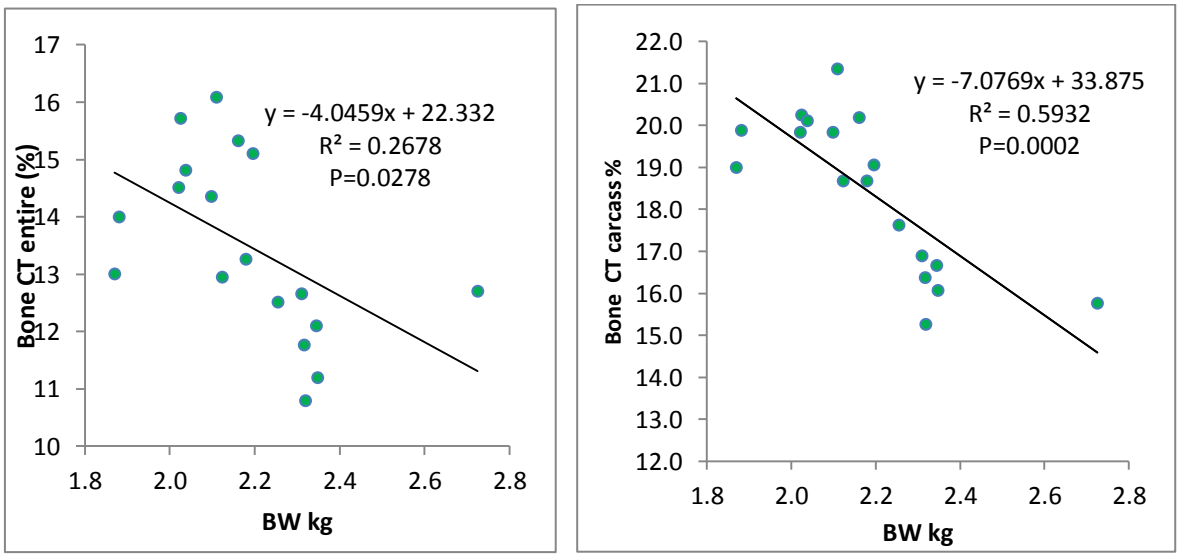

Figure 6.7. The correlation between body weight and bone percentage predicted by CT

There was a highly significant positive correlation between measured fat pad and CT predicted entire fat percentage, with R^2= 0.783 (Figure 6.8).

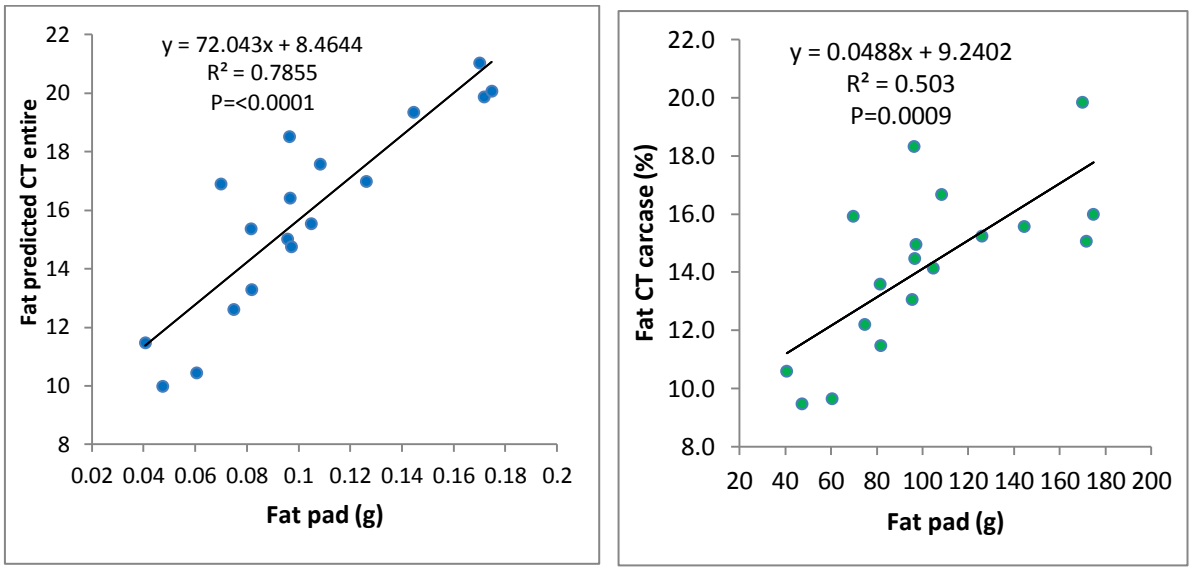

Figure 6.8. The correlation between abdominal fat and fat predicted by CT

There was a significant negative correlation between fat pad and CT predicted entire percentage lean, and carcass percentage lean (Figure 6.9).

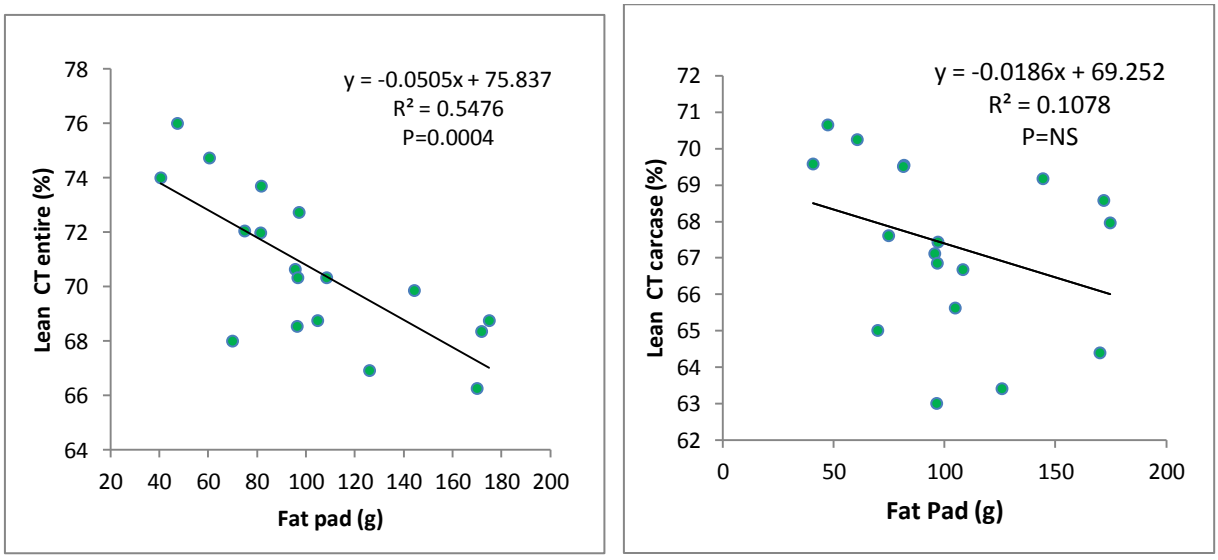

Figure 6.9. The correlation between fat pad weight and lean predicted by CT

Table 6.3 summarises the correlations between body weight and fat pad weight and the variables predicted by CT for data from the two experiments combined.

Table 6.3. The comparison of correlations between body weight and fat pad with the variables predicted by CT

Measurement correlations	On-farm study (Chapter3)	Research station experiment (Chapter 5)	Both studies
BW vs Fat pad	P<0.0001 R^2=0.8076	P=0.0008 R^2=0.5125	P0.0001 R^2=0.5371
BW vs Fat CT entire	P=0.0057 R^2=0.5524	P=0.0002 R^2=0.5875	P=0.0004 R^2=0.365
BW vs Lean CT entire	P=0.0021 R^2=0.6279	P= 0.0058 R^2=0.3874	P=0.0054 R^2=0.2465
BW vs Bone CT entire	P=NS R^2=0.215	P= 0.0278 R^2=0.268	P=0.0003 R^2=0.2749
BW vs Fat CT carcass	P=0.0041 R^2=0.5783	P= 0.0002 R^2=0.5971	P=0.0008 R^2=0.3356
BW vs Lean CT carcass	P=0.0405 R^2=0.3592	P=NS R^2=0.1065	P=NS R^2=0.0873
BW vs Bone CT carcass	P=0.0494 R^2=0.3371	P=0.0002 R^2=0.5932	P=0.0005 R^2=0.3586
Fat pad vs Fat CT entire	P=0.0002 R^2= 0.7711	P<0.0001 R^2=0.7855	P<0.0001 R^2=0.6608
Fat pad vs Lean CT entire	P=0.0005 R^2= 0.7171	P=0.0004 R^2=0.5476	P<0.0001 R^2=0.4873
Fat pad vs Bone CT entire	P=0.0102 R^2=0.4886	P=0.0151 R^2=0.3162	P=<0.0001 R^2=0.4217
Fat pad vs Fat CT carcass	P= 0.0002 R^2=0.7695	P=0.0009 R^2=0.503	P=<0.0001 R^2=0.5385
Fat pad vs Lean CT carcass	P=0.0496 R^2=0.337	P= NS R^2=0.1078	P=0.0337 R^2=0.1521
Fat pad vs Bone CT carcass	P=0.0016 R^2=0.6532	P= 0.0024 R^2=0.45	P<0.0001 R^2=0.5494

6.5 Discussion

High body mass in broilers is associated with excessive fat deposition, lameness and high mortality rates (often due to skeletal or cardiovascular disease, or both). To regulate weight gain, limit health risks, and maintain high fertility, husbandry practices for the parent stock of broiler chickens encompass a high degree of feed restriction (Renema and Robinson, 2004).

For the on-farm experiment, although there were no significant differences between the two original rearing sheds for any of the values measured or predicted, but there were significant positive correlations between body weight and measured abdominal fat, CT predicted entire fat percentage and CT predicted carcass fat percentage. Conversely, there were significant negative correlations between body weight and CT predicted entire lean percentage, CT predicted carcass lean percentage, CT predicted entire bone percentage and CT predicted carcass bone percentage. A similar pattern was found for the laboratory setting at research station. Birds from the three body weight groups were selected randomly for the CT scanning. Random sampling was used to minimize bias in the experiment. However, as it happened, the birds taken from the medium BW group were towards the high end of the range of the medium BW group, whereas the birds taken from the heavy BW group were towards the low end of the range for this group. By chance, although the body weights of the birds in the different body weight groups were significantly different for all birds at 80 weeks of age, the birds selected for CT scanning were not significantly different in body weight. This will, in part, explain why there were no significant differences between the body weight groups for the CT predicted measurements. Another factor influencing this result is the relatively small sample size used. High fat pad also found in light group is not relevant with the body weight as compared with other BW groups. It could be contained other than fat during sampling.

Taherkhani *et al.*, (2010) reported that obesity and associated high leptin levels may impair ovarian steroid biosynthesis, gonadotropin secretion, and also follicle rupture and ovulation. These authors suggested that increased feeding frequency could improve the reproductive performance of broiler breeder hens through preventing or delaying lipotoxicity development. Some of these effects may also occur in overweight commercial laying hens In these two experiments, there was significant correlation between fat pad measured and fat predicted entire using CT. The heavy hens had a heavier fat pad and higher percentages of CT predicted fat. Highly correlation between fad pad measured and fat predicted by CT also reported by Andrassy-

Baka *et al.*, 2003. This resulted in the heavier hens having proportionally lower percentages of lean and bone tissue.

CT is a reliable and convenient method for the measurement of abdominal fat, and has the advantages of distinguishing visceral and subcutaneous adipose tissue (Chang *et al.*, 2011).

6.6 Conclusion

It can be concluded that a significant correlation between body weight and variables measured for body conformation was found. Body weight correlated positively with fat pad measured and correlated negatively with lean and bone content. As body weight increased, fat content increased, while other content such as lean tissue and bone deceased. CT is an accurate method to evaluate body composition in laying hens.

Chapter 7

General discussion

Good performance in commercial poultry production is the result of good management. In laying hens, egg production and egg quality are the indicators of that performance. However, many factors can influence the ability of flocks to sustain high rates of production of first quality eggshells, including body weight and body weight uniformity amongst a flock. Body weight at point of lay is a major factor influencing subsequent egg size, and this applies to both immature and more mature ages (Leeson and Summers, 1987). It has been proposed that high standards of flock uniformity at genetically defined body weights may augment the capacity of high performing flocks to maintain shell quality throughout the laying life in a commercial setting. Conversely, flocks with lower uniformity are likely to experience an accelerated loss of shell quality. The heavy birds could be categorized as being obese, beyond the expectation of the commercial growers. The cost of feed consumed to attain body weight above breed standard represents extra returns to farmers, if the body weight can be maintained as per breeder recommendation (Parkinson *et al*., 2007).

These thoughts led to the studies reported in this thesis, which investigated the effects of hen body weight and flock uniformity on production performance, egg quality (internal and external) and body conformation, in laying hens. Emphasis was placed on body weight and flock uniformity at point of lay, which are very important for the overall performance and egg quality of a flock.

Data from both a commercial cage production farm and a number of commercial free-range farms demonstrated the performance and the quality of eggs typically produced in the commercial market. The laboratory experiment was designed as a model to evaluate the influence of uniform body weight in three different ranges (light, medium and high) at point of lay, on subsequent flock performance. The purpose of this study was to demonstrate any positive or negative effects of variation in body weight, from the recommended breed values, on egg production and quality, with a view to applying this information to commercial production.

In this thesis, on-farm data from both conventional cage and free-range production systems showed a variation in average body weight of birds and flock uniformity from the rearing period to the end of the laying cycle. The on-farm studies showed that hens exceeded the body weight

standards as recommended by the breeder company, at particular ages. In the conventional cage production system study, the hens exceeded the breed standards body weight by about 100-200 g, while, in free-range production systems, the body weight varied among the flocks.

A significant proportion of the free-range flocks had pullet weights above the breed standard and achieved inappropriately low average flock growth rates from 19 to 37 weeks of age, despite the heavier pullet weight. In contrast, one of the free-range flocks (F1) had a lower pullet weight, and average growth rate between 19-37 weeks of age aligned closely with the breed standard. This outlying flock provides evidence of the true potential for free-range production, and many of the causal variables seem consistent with knowledge accumulated for cage production.

Data from on-farm studies also varied, owing to random sampling, which would have contributed in part to the differences in results. Unfortunately, the eggs taken as samples were not the same from the same birds that were weighed. The birds and eggs sampled in Chapters 3 and 4 would have differed among age periods, as we could not track the same birds on each sampling occasion. From the laboratory experiment, birds were selected, from a larger group, into body weight groups of light, medium and heavy, with the average initial body weight at 19 weeks being 1.6, 1.7 and 1.9 kg, for light, medium and heavy BW groups, respectively. The light BW group attained the minimum weight recommended by breeder manuals at 19 weeks of age and continued to have an average body weight similar to breeder recommendations. On the other hand, the medium and heavy BW groups exceeded the recommended body weight by an average of 170 and 370 g, respectively, at 19 weeks of age and continued to exceed the breeder standard throughout the experiment. In relation to body weight uniformity, because birds were allocated to groups on the basis of body weight at 16 weeks of age, the groups showed high uniformity at 19 weeks of age (90-100%) in all BW groups. Although the body weight uniformity decreased with increasing hen age, the egg production in this experiment was greater than the breeder standards with the exception of the light BW group at 41-45 weeks of age, this being a result of accidental water deprivation. High body weight uniformity enhanced egg production, however it did not affect the shell ultrastructure and eggshell quality. Overall egg quality was affected mainly by hen age which is consistent with the findings of Roberts *et al*., (2013).

Egg production is a major variable for evaluating a flock's performance. Egg production was significantly affected by hen age, generally decreasing as hen age increased from peak production. Egg production from the commercial cage study was lower than the breed standard from point of lay to about 30 weeks. Peak production in this experiment was delayed by approximately 10

weeks. The explanation for this could be the lower uniformity for Shed A from 19 weeks of age to about 26 weeks of age, although Shed B had better body weight uniformity from age 15 weeks (above 80%) to 26 weeks, before decreasing to below 80% at 37 weeks. In addition, the egg production in this cage experiment (from 2 rearing flocks) was recorded as a single egg production. It is assumed that the low body weight uniformity in Shed A might has contributed to low egg production in the first ten weeks. This is one of the constraints of an on-farm study, which could not be avoided.

Egg production in the free-range flocks varied among the flocks. The poor performance of some of the other free-range flocks illustrates the likely variation, at a commercial level, resulting from poor alignment with average growth rates patterns and low uniformity standards (Parkinson *et al*., 2007). There also appears to be a trend for heavier pullets to achieve poorer growth rates between 19-37 weeks of age, but more data on the relationship between the initial pullet weights, production performance and growth will be required. The correct management of both average body weight and flock distribution of body weights in the rearing period has highly significant impacts on egg production. Having the correct body weight at the start of egg production will enable pullets to achieve their genetic potential. Uniform flocks with the correct body weight give several benefits, mainly that birds can be managed in a large group and management changes (lighting, feeding and housing) can be more easily instituted (Kosba *et al.*, 2009). The importance of body weight and flock uniformity has been noted by Miles and Jacob, (2011). These authors pointed out that, when the proper pullet body weight and condition at the desired age of sexual maturity have been achieved, many problems associated with lower profits from a commercial layer flock can be eliminated. Furthermore, management is a key factor controlling a flock's uniformity and maintaining the target body weight at each flock age.

As it has been hypothesised, body weight and body weight uniformity at point of lay affects eggshell and egg internal quality. The BW groups in this the laboratory experiment showed that the heavy birds produced larger eggs with low percentage shell and shell thickness. However, the findings from the commercial flocks for egg quality were varied. Both on-farm experiments (cage and free-range flocks) showed that good uniformity had a positive impact on overall egg quality. In addition, the average body weight, especially at 19 weeks of age should be maintained at the breeder standard to achieve good performance. Leeson and Summers (1987) and Parkinson *et al*. (2007) have reported that larger birds consume more feed while producing larger sized eggs with inferior eggshell quality. However, this finding is contradicted by the findings of Lacin *et al*.

(2008), who reported that body weight did not significantly affect shell strength and shell thickness.

The eggshell is a micro-environmental chamber for housing the developing embryo and protecting it from external aggression (Fraser, *et al*., 1999; Liao *et al*., 2013). In this capacity, it provides physical protection, regulates gas, water and ionic exchange as well as providing a source of calcium (Fraser *et al*., 1999). In the case of the table egg, the chamber must remain intact from point of lay, along the production process, to the consumer (Fraser *et al*., 1999). In the studies reported in this thesis, shell quality declined as the flock aged. Deterioration of shell quality as hens get older has been reported by many researchers (Leeson and Summers, 2001; Mertens *et al*., 2006; Silversides, *et al*., 2006; Roberts *et al*., 2013). Cordts *et al.* (2002) reported that the deterioration in eggshell quality is associated with changes in the shell matrix material, which affects the mechanical properties of the shell. These authors further explained that the ability to absorb calcium is lower in older hens, while the amount of calcium deposited on the shell is relatively constant.

The other eggshell quality parameter measured in this study was the amount of cuticle. The cuticle is the outermost layer of the egg and consists of organic matter and eggshell pigments (Gautron and Nys, 2006; Gautron *et al.*, 2007). It is a protective coating which prevents bacterial penetration through the gas exchange pores in the eggshell. The greatest influence on cuticle cover in the current study was the effect of hen age, with an overall trend for better cuticle cover later in lay. The contradictory findings for cuticle cover in Chapter 4, as indicated by a* after staining, as compared with the single score value and the SEM scores, require further clarification. The findings from the laboratory experiment showed that the cuticle cover was higher in the period of 61-75 weeks in the light BW group. Previous studies have reported that the amount of cuticle cover decreased as hen age increased (Sparks and Board, 1984; Ruiz and Lunam, 2000; Rodriguez-Navarro *et al*., 2013) with no significant difference reported by Roberts *et al*. (2013). The present study also showed that the light BW group had a greater amount of cuticle than the other BW groups. A possible explanation for greater cuticle cover in the light BW group is that more protein was distributed in the cuticle, because the eggs were smaller than those of the other groups. Du (2013) reported that the cuticle is largely organic with protein content as high as 90%, and with a high content of cystine, glycine, glutamic acid, lysine and tyrosine. However, the cause of thicker cuticle found in older flocks is uncertain. Together with the

mineralized shell and shell membranes, the cuticle constitutes a physical barrier against microorganism invasion and contamination of the egg content. (De Reu *et al*., 2008).

When considering eggshell quality, it is useful to have an understanding of the shell ultrastructure. Ultrastructural studies have demonstrated that the eggshell is comprised of morphologically distinct calcified layers with the mammillary layer being the "foundation" of the eggshell. Studies have identified ultrastructural variations in the mammillary layer that can be used as indicators of eggshell quality (Roberts and Brackpool, 1995). The attachment of the mammillary layer to the shell membranes and the quality of construction of the mammillary layer, play an important role in determining the strength of the entire eggshell (Roberts and Brackpool, 1995). Understanding the ultrastructure of eggshells has reinforced the view that the mechanical properties of the eggshell cannot be defined by a simple thickness measurement (Bain, 2005). Therefore it seems reasonable that any changes in morphology or composition will affect shell structure. A comprehensive description of these variations has been described previously (Roberts and Brackpool, 1995; Solomon, 1997).

The current studies investigated the relationship between hen body weight uniformity and changes in ultrastructural characteristics of the eggshells. However, the body weight of hens did not affect the ultrastructure characteristic. In these experiments, the mammillary ultrastructure was affected mainly by hen age. As hen age increased, the incidence of ultrastructural features, shown to be associated with good shell quality, such as early fusion and confluence, decreased. At the same time, the incidence of ultrastructural features known to be associated with poorer shell quality increased: alignment of mammillae, Type-A bodies, Type-B bodies, aragonite, late fusion and pitting. The significant effects of flock age on early fusion, late fusion, alignment, aragonite, and erosion are evidence of deterioration in shell quality. Although there was no significant effect of body weight in the laboratory experiment, cap quality was best in the lighter birds. The light BW group tended to have a higher incidence of confluence than other BW groups. Confluence refers to the characteristic appearance of mammillary caps when they join with one another. The attachment of the mammillary layer to the shell membranes, and the quality of construction of the mammillary layer plays an important role in determining the strength of the entire eggshell (Roberts and Brackpool, 1995). This ultrastructure results confirmed that lower egg size is associated with different ultrastructure of the eggshell. From the commercial cage study, a new feature was found in the mammillary layer at 26 weeks of age, which has not yet been formally identified. It is assumed that this new feature is amorphous calcium carbonate, as described by

Rodriguez-Navarro *et al*. (2015); Gautron J, 2015, (personal communication)). A further analysis of this new feature is under way.

The bone breaking strength measurements in Chapters 3 and 5 showed that there was no significant effect of body weight on bone breaking strength, length, and width from both studies. A further analysis, such as bone ash, needs to be conducted in order to obtain an explanation of how the body weight of flocks affects calcium deposition in bones.

For body conformation, it is reported by de Beer and Coon (2007) that larger body weight and fat accumulation causes many problems in laying hens, such as leg problems, early onset of sexual maturity, accelerated ovarian follicular development, and the incidence of multiple hierarchies and multiple ovulations. Although the results from the bone strength were not statistically significant, the experiment using computerised tomography confirmed that there is a high positive correlation between body weight and the total percentage of fat, and a negative correlation between body weight and total percentage of lean and bone tissue. Taherkhani *et al.*, (2010) reported that obesity can have negative effects on a hen's reproductive performance. This experiment confirmed that body weight increased linearly with the composition of body fat, while the composition of lean and bone decreased. The heavy birds deposited proportionally more fat than the light birds, as measured by computerised tomography. The accuracy of the CT method for the measurement of abdominal fat has been reported by Chang *et al.*, (2011); Bentsen and Sehested, (1989), and Svihus and Katie, (1993).

Conclusions

It can be concluded from these experiments that:

1. In the on-farm cage study, the heavier body weight in birds originating from rearing Shed A resulted in lower uniformity and larger egg size. The higher percentage of larger birds in Shed A compared to Shed B could explain the variation in body weight uniformity. By maximising both body weight uniformity and the appropriate body weight for age, it may be possible to investigate maximum physiological potential for egg production and to then evaluate the egg quality consequences of such high egg mass outputs. The magnitude of the differences in commercial flock body weight from the breeder standards has been validated, and the total body weight ranges identified.

2. In the on-farm free-range study, the variation in body weight and uniformity appears to reflect the effect of farm management. Performances varied from farm to farm. However, Flocks that have high uniformity showed overall better egg production than the other flocks. This pattern could be a model for other free-range flocks to attain good egg production.

3. Results from the experiment at research station showed that body weight and body weight uniformity, play an important role in egg production and egg quality. Maintaining flock uniformity will optimize the egg production with a concern of body weight at point of lay. Maintaining body weight at point of lay as breed standard recommendation resulted in higher and longer egg production, although there was no significant correlation between flock uniformity and egg production and egg quality parameters. Heavy body weight might have the potential to cause many negative effects on reproductive tract. CT is an accurate method to measure body composition.

4. Improving uniformity standards and compliance with breeder recommended growth rates at a commercial level will significantly increase production performance and egg quality traits, even from the current high standards.

5. The poor compliance of most free-range flocks with recommended growth rates and uniformity standards is a major constraint on the expression of genetic potential for current

commercial strains, and will significantly impair the economic performance of this sector until more standardisation is achieved.

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Appendices

Appendix A. Egg Quality Measurements between rearing flocks in a cage production system

Measurement	19 weeks		26 weeks		37 weeks		50 weeks		60 weeks	
	A	B	A	B	A	B	A	B	A	B
Shell Quality										
Translucency Score	2.57 ± 0.12	2.67 ± 0.11	2.57 ± 0.08	2.65± 0.08	2.7 ± 0.09	2.97 ± 0.10	2.1 ± 0.11	2.12 ± 0.09	2.7 ± 0.11	2.77 ± 0.13
Shell Reflectivity %	29.1 ± 0.4	27.7 ± 0.4	26.5 ± 0.3	27.3 ± 0.4	27.1 ± 0.4	30.8 ± 0.6	29.0 ± 0.5	29.7 ± 0.5	27.9 ± 0.4	28.6 ± 0.5
Egg Weight g	49.5 ± 0.5	51.4 ± 0.5	59.5 ± 0.4	57.8 ± 0.5	62.9 ± 0.5	59.4 ± 0.5	63.4 ± 0.4	62.7 ± 0.5	65.9 ± 0.5	63.8 ± 0.7
Breaking Strength N	45.4 ± 0.9	45.6 ± 0.7	43.7 ± 0.7	44.1 ± 0.7	41.7 ± 0.8	39.9 ± 0.9	40.8 ± 0.9	39.7 ± 0.8	41.6 ± 1.0	40.7 ± 1.0
Deformation µm	311.2 ± 2.9	311.2 ± 2.9	280.8 ± 3.5	288.7 ± 3.4	287.3 ± 4.7	280.5 ± 4.6	259.3 ± 4.2	248.5 ± 4.3	258.0 ± 4.0	255.3 ± 4.3
Shell Weight g	4.94 ± 0.05	4.97 ± 0.05	5.78 ± 0.04	5.6 ± 0.06	6.07 ± 0.06	5.6 ± 0.06	6.09 ± 0.06	6.01 ± 0.06	6.2 ± 0.07	5.99 ± 0.07
% Shell	10 ± 0.1	9.68 ± 0.08	9.74 ± 0.07	9.69 ± 0.07	9.64 ± 0.06	9.50 ± 0.08	9.60 ± 0.08	9.59 ± 0.07	9.37 ± 0.09	9.41 ± 0.10
Shell Thickness µm	398.2 ± 3.2	392.8 ± 3.0	413.0 ± 2.6	406.2 ± 2.9	398.2 ± 2.8	381.3 ± 3.2	409.9 ± 3.7	409.1 ± 2.9	409.2 ± 3.6	404.9 ± 4.5
Internal Quality										
Albumen Ht mm	10.9 ± 0.10	10.8 ± 0.10	9.6 ± 0.13	9.2 ± 0.11	9.2 ± 0.16	8.8 ± 0.16	9.2 ±0.17	8.8 ± 0.12	9.4 ± 0.16	9.1 ± 0.12
HU	105.2 ± 0.4	104.1 ± 0.5	97.5 ± 0.6	96.1 ± 0.5	94.3 ± 0.8	93.4 ± 0.9	95.0 ± 0.9	92.99 ± 0.7	95.4 ± 0.9	94.6 ± 0.7
Yolk Colour Score	10.5 ± 0.1	10.2 ± 0.1	11.2 ± 0.08	11.0 ± 0.07	11.4 ± 0.08	11.2 ± 0.1	11.7 ± 0.1	11.6 ± 0.6	11.7 ± 0.09	11.6 ± 0.10

Appendix A. Main effect of sheds on shell reflectivity before staining and spectrophotometric measurements (L*a*b*) before and after staining

Measurement	Sheds		P Value
	A	B	
Before staining			
Shell reflectivity			
L	60.25±0.29	60.50±0.32	ns
a	18.33±0.17	18.34±0.18	ns
b	28.23±0.23	29.23±0.17	ns
After staining			
L*	53.71±0.33	54.56±0.41	ns
a*	-0.69±0.49	0.40±0.44	ns
b*	30.71±0.16	30.61±0.14	ns
ΔL*	6.36±0.23[a]	5.94±0.19[b]	0.0454
Δa*	19.02±0.48	17.94±0.47	ns
Δb*	-1.76±0.28	-1.38±0.19	ns
Single score	20.54±0.51[a]	19.13±0.50[b]	0.0420

Appendix B. The main effect of flock age and rearing shed on the mammillary ultrastructure scores of the eggshell

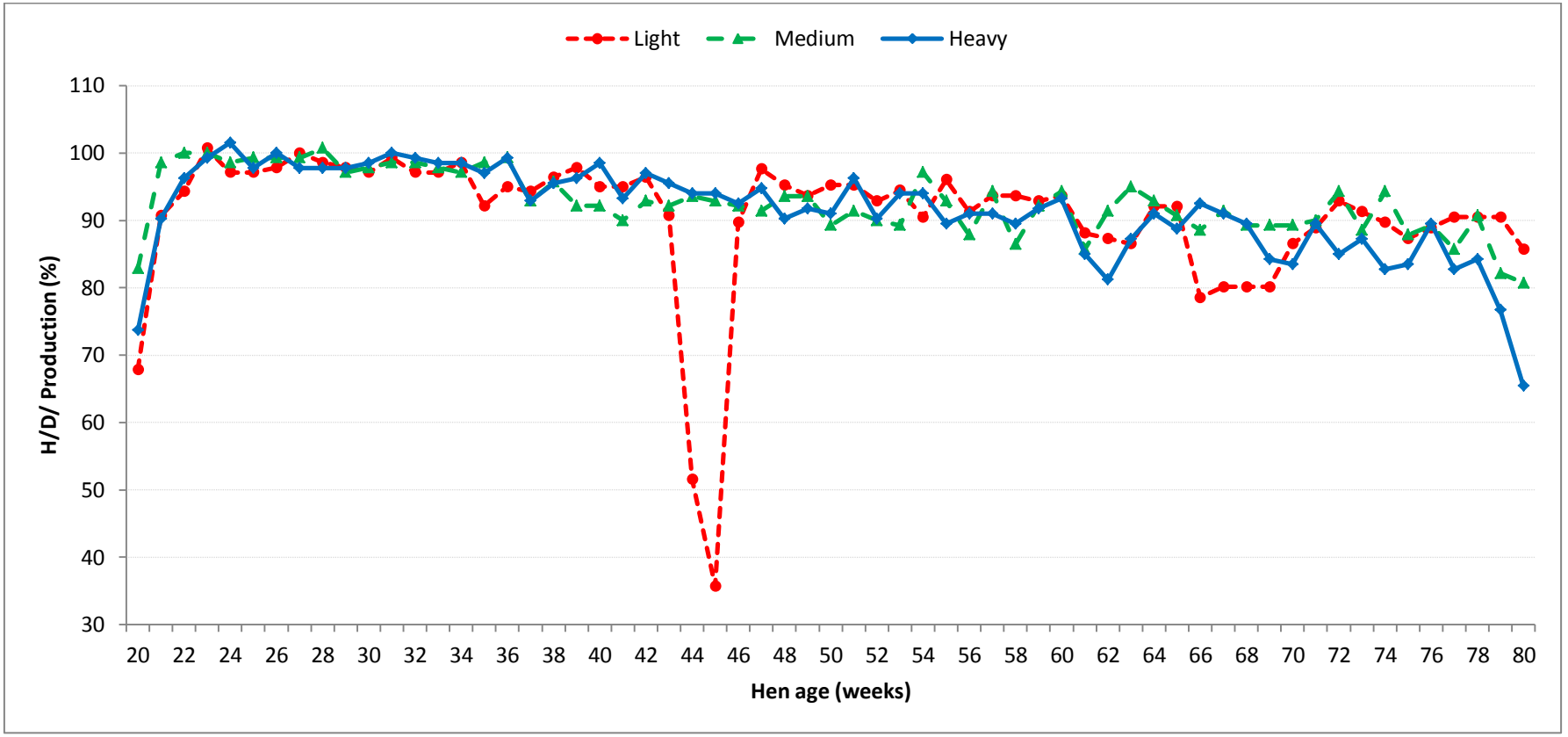
Ultrastructure features	Sheds	Flock age (weeks)				P Value		
		26	37	50	60	Flock	Age	F*A
Mammilary cap size	A	1.8±0.1	1.8±0.1	2.2±0.1	2.2±0.1	NS	<0.0001	0.05
	B	1.4±0.1	1.7±0.1	2.1±0.1	2.4±0.1			
Confluence	A	2.4±0.2	2.6±0.2	1.9±0.1	2.2±0.2	NS	0.0069	NS
	B	2.6±0.2	2.6±0.2	2.2±0.2	2.3±0.2			
Caps quality	A	2.2±0.1	2.2±0.1	2.6±0.1	2.9±0.1	NS	0.0102	0.034
	B	2.5±0.1	2.3±0.1	2.4±0.1	2.4±0.1			
Early fusion	A	3.5±0.1	3.5±0.1	3.5±0.1	2.9±0.2	NS	0.0004	NS
	B	3.5±0.1	3.6±0.2	3.2±0.1	3.0±0.2			
Late fusion	A	3.0±0.2	2.7±0.2	2.8±0.2	3.4±0.2	NS	<0.0001	0.039
	B	2.8±0.2	2.2±0.2	3.3±0.2	3.3±0.2			
Alignment	A	2.0±0.1	2.5±0.1	2.2±0.2	2.6±0.2	NS	<0.0001	0.0002
	B	2.0±0.1	1.9±0.1	2.8±0.1	2.9±0.2			
Type A	A	1.2±0.1	1.6±0.1	1.5±0.1	1.9±0.1	NS	0.0119	0.0269
	B	1.6±0.1	1.6±0.1	1.7±0.1	1.7±0.1			
Type B	A	1.8±0.1	2.0±0.04	2.2±0.1	2.7±0.1	NS	<0.0001	NS
	B	2±0.1	2.1±0.1	2.2±0.1	2.7±0.2			
Aragonite	A	1.0±0.04	1.0±0.01	1.4±0.1	1.7±0.2	NS	0.0222	0.0008
	B	1.2±0.1	1.3±0.1	1.1±0.1	1.2±0.1			
Cubics	A	1.1±0.1	1.0±0.04	1.1±0.1	1.1±0.1	NS	NS	NS
	B	1±0.0	1.1±0.1	1.04±0.04	1.04±0.04			
Cubic cone formation	A	1.1±0.1	1±0.0	1±0.0	1±0.0	NS	NS	NS
	B	1±0.0	1.04±0.04	1±0.0	1±0.0			
Cuffing	A	1.1±0.1	1.1±0.1	1.04±0.04	1.04±0.04	NS	NS	NS
	B	1±0.0	1.1±0.1	1±0.0	1±0.0			
Changed membrane	A	1±0.0	1.1±0.1	1.04±0.04	1±0.0	0.0005	<0.0001	<0.0001
	B	1.8±0.2	1.2±0.1	1±0.0	1±0.0			
Depression	A	1±0.0	1±0.0	1±0.0	1.1±0.1	NS	0.0004	NS
	B	1.04±0.04	1.1±0.1	1.04±0.04	1.3±0.1			
Erosion	A	1.1±0.1	1.04±0.04	1.1±0.1	1.4±0.1	NS	0.0002	NS
	B	1.1±0.1	1.3±0.1	1.2±0.1	1.5±0.1			

Appendix C. Interaction between free range flocks and flock age for egg quality measurements

Measureme	Flock age	FR 1	FR 2	FR 3	FR 4	FR 5	FR 6	FR 7	P Value
Translucency	26 week	3.62±	4.37	2.87	3.62	3.78	4.12	4.25	
	37 week	4.62	3.13	3.25	3.2	3.9	3.1	3.1	<0.0001
	50 week	3.1	3.83	3.55	3.67	2.62	3.18	3.2	
	60 week	3.43	3.6	3.95	3.28	3.48	3.48	3.78	
Reflectivity	26 week	25.93	27.808	27.26	30.95	28.95	28.79	26.36	
(%)	37 week	30.44	32.09	29.76	32.38	31.93	29.9	27.28	<0.0001
	50 week	32.78	33.65	30.89	29.13	30.47	25.85	26.2	
	60 week	33.43	29.96	31.58	30.95	33.85	28.94	28.07	
Egg weight	26 week	60.91	56.85	57.86	60.67	56.39	59.63	58.29	
(g)	37 week	63.26	65.41	58.76	62.33	59.88	61.27	60.89	<0.0001
	50 week	65	62.43	61.13	66.26	62.15	64.76	62.57	
	60 week	63.64	65.11	61.64	65.75	61.34	63.05	62.29	
Breaking	26 week	46.31	45.67	37.41	41.09	41.01	50.48	47.01	
(N)	37 week	45.87	41.91	39.71	38.89	44.11	47.57	46.6	<0.0001
	50 week	40.01	42.36	42.52	38.88	44.14	46.51	39.62	
	60 week	40.24	40.52	41.7	39.19	45.53	37.78	37.28	
Deformation	26 week	327.5	298	285.17	285.83	274	297.5	282.67	
(µm)	37 week	285.25	288.17	296.26	292	281.5	277.83	289.33	<0.0001
	50 week	257.17	280.33	274.67	259.5	273.33	280.83	260	
	60 week	270.83	268.33	253.67	261.67	272.33	241.17	257.27	
Shell weight	26 week	5.93	5.49	5.03	5.48	5.42	5.94	5.73	
(g)	37 week	5.98	5.99	5.34	5.53	5.69	6.03	5.87	<0.0001
	50 week	6.23	6.12	5.8	5.91	6.08	6.37	5.79	
	60 week	5.87	6.08	6.05	6.12	6.01	5.83	5.78	

Measureme	Flock age	FR 1	FR 2	FR 3	FR 4	FR 5	FR 6	FR 7	P Value
Percentage	26 week	9.76	9.65	8.7	9.04	9.61	9.98	9.83	
(%)	37 week	9.47	9.16	9.09	8.86	9.51	9.85	9.64	<0.0001
	50 week	9.6	9.82	9.51	8.94	9.79	9.84	9.27	
	60 week	9.24	9.37	9.83	9.33	9.81	9.26	9.3	
Shell	26 week	412.22	405.89	373.4	386.65	395.94	414.57	418.76	
(µm)	37 week	410.72	397.33	382.9	384.24	399.49	415.33	439.35	<0.0001
	50 week	415.89	415.62	404.19	396.53	448.87	425.24	404.26	
	60 week	399.66	408.53	421.1	404.84	421.67	398.29	401.1	
Albumen	26 week	9.8	8.81	8.66	9.67	8.64	7.75	9.42	
(mm)	37 week	8.13	7.56	5.69	6.63	7.33	6.48	7.97	<0.0001
	50 week	7.52	6.29	7.59	6.51	7.52	6.14	6.55	
	60 week	6.71	5.27	7.26	7.65	6.44	4.96	6.02	
Albumen	26 week	9.8	8.81	8.66	9.67	8.64	7.75	9.42	
	37 week	8.13	7.56	5.69	6.63	7.33	6.48	7.97	<0.0001
	50 week	7.52	6.29	7.59	6.51	7.52	6.14	6.55	
	60 week	6.71	5.27	7.26	7.65	6.44	4.96	6.02	
Yolk colour	26 week	10.63	10.7	9.37	10.43	10.18	9.87	11.08	
Score	37 week	11.3	9.38	9.37	8.87	9.87	9.75	11.43	<0.0001
	50 week	11.27	10.32	9.77	9.33	11.1	10.53	10.57	
	60 week	10.28	10.42	10.28	11.32	10.45	10.75	9.77	

Appendix D. Hen-day egg production (%) of hens at different body weights from 20 to 80 weeks of age (Chapter 5)



Appendix E. Season time of Free-range flocks when the first egg collected for analysis at the age of 26 weeks

Flock	Date at 26 weeks	Season
FR 1	17 May 2012	Autumn
FR 2	19 July 2012	Winter
FR 3	29 November 2012	Spring
FR 4	27 September 2012	Spring
FR 5	31 January 2013	Summer
FR 6	18 July 2013	Winter
FR 7	29 August 2013	Winter