ABSTRACT

FOSNAUGHT, MARY HELEN. Mediating Bone Mineral Status in Laying Hens by Feeding Increased Calcium during Rearing and the Lay Cycle. (Under the direction of Kenneth E. Anderson).

Trends to decrease the age of sexual maturity and point of lay without concurrent increases in dietary Ca may reduce the potential for full skeletal mineralization in pullets. During lay, the hen's increased genetic capacity to produce more eggs with less feed without respective increases in dietary Ca may further predispose hens to bone weakness leading to welfare and livability issues. Objectives of this research were to evaluate the effect of feeding increased calcium during rearing and the lay cycle as well as strain and density on laying hen performance and bone mineralization status. Pullets were grown to 16 wks in a grow house with 52 pullets/replicate and 28 replicates/treatment (5,824hens total) which were then moved to a lay house from 18-66 wks with either 24 or 36 hens/replicate (at 48 or 64 sq in) so that there was a total of 26 replicates/treatment (5,728 hens total). The 2 x 2 factorial arrangement of treatments during rearing were Leghorns strain: Hy-Line W-36 (H) and Babcock B-300 (B) and Ca:P ratios: elevated (RC+) Ca:P 2.14, 3.14, 4.14 and control (RC) Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively. In the lay cycle, the 2 x 2 x 2 x 2 factorial consisted of strain, rearing diet, layer dietary regimens: increasing Ca and P (LC+) and constant (LC) and cage densities: low, 64in^2/bird (LD) and high, 48in^2/bird (HD). All diets were isocaloric and fed *ad libitum.* Feed consumption (FC) and BW were monitored bi-weekly (by period) beginning at 2 weeks of age during the rearing and every 4 weeks during the layer phase. Mortality and egg production was recorded daily. During rearing, 5 femurs/trt and during laying, 3 femurs/trt were measured for dry fat-extracted bone weight (DFEW), % ash, volume, and bone breaking strength (BBS) from week 6-16 and from weeks 51-61, respectively. From week 0-17, FC was higher ($P \leq 0.01$) when feeding RC+ (5.11 kg) than RC (4.81 kg) otherwise there was no effect on Gain (1,017 and 1,029 g, respectively, $P=0.53$) or FE (0.199 and 0.214, respectively, $P=0.08$). Strain had no effect on FC, Gain, or FE. Mortality increased ($P \leq 0.03$) by period in the B compared to H strain. Layer performance was not affected by feeding the increased calcium during rearing or lay. Strain effected

($P \leq 0.05$) feed consumption, feed efficiency, egg production, and mortality. Feeding more Ca during rearing increased DFEW (RC+=0.94 g vs. RC=0.82 g, P=0.04) while strain effected bone volume (H=2.99 and B=2.37 cc, $P \leq 0.01$) and femoral BBS (B=8.55 vs. H=7.80 kg, P=0.01) of pullets. Feeding more Ca during lay did not effect bone status, but feeding it during rearing increased BBS (RC+=14.15 vs. RC=12.37 kg, $P \leq 0.01$) in older layers. Strain effected ($P \leq 0.001$) both BBS (H=14.26 vs. B=12.26 kg) and volume (H=5.90 vs. B=6.27 cc). These findings indicate that feeding increased Ca during rearing and laying impacts bone mineralization and may be a useful strategy to mitigate bone weakness and such related conditions as cage layer osteoporosis.

Mediating Bone Mineral Status in Laying Hens by Feeding Increased Calcium during Rearing and the Lay Cycle

by
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DEDICATION

To all who courageously battle chronic illness

BIOGRAPHY

Mary Fosnaught, daughter of Earl and Josephine Fosnaught, was raised on a farm in Paris, Ohio with her two brothers and two sisters. After graduating from Minerva High School, she completed a B.S. degree in Poultry Science at Ohio State University. During her undergraduate studies, she completed internships in the quality control laboratory of the Washington, Indiana Perdue Farms, Inc. Processing Plant and in Cookham, England through the International Farmers Exchange Program working in the turkey growout operation of Copas Brothers Farms. She then completed a certification program in broiler and broiler breeder management at Barneveld College, The Netherlands before accepting a flock supervisor position with Perdue Farms in Salisbury, MD. This exposure to the commercial poultry industry led Mary to pursue a M.S. in Poultry Nutrition focusing on Feeding Barley and Beta-Glucanase in Broiler Diets under the guidance of Dr. Jeannine Harter-Dennis at the University of Maryland Eastern Shore. From this point she pursued her Ph.D. in Poultry Nutrition with Dr. Kenneth Anderson focusing on Mediating Bone Mineral Status in Laying Hens by Feeding Increased Calcium during Rearing and the Lay Cycle.

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I thank my parents for valuing education and instilling in me a work ethic which would allow me to persevere despite many challenges. I thank my siblings, Joe, Julie, Tom, and Ann for being my first and foremost introduction to true camaraderie. For friends like Sandi and Tim Shell, Marisa Miller, Jeannette and Tom Snider, Meg Scott, Gail Frye, Nancy and Bill Dennis, Marcus Leyendecker, Aziz Conte, Rob Loomis, Jen Ciofani, Jan Maarsen, and Dan Kelly who have touched my heart in countless ways and have endured with me to this point.

All that my husband, Hans Slootweg, has sacrificed so that I could pursue this education against unfathomable obstacles is the greatest testament to devotion I have ever witnessed. You, dear friend, are a warrior, though not one taken to bravado, but rather able to toil in the background for the betterment of another. The salutation you deserve for your courage and steadfastness is beyond my ability to express in words.

I would like to acknowledge all the women that previously have opened doors so that I could access this education, I would like to recognize my country for valuing freedom from which rose the opportunity to pursue this study, and I would like to thank our Savior, Jesus Christ for always providing me with what I truly needed.

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INTRODUCTION

Mortality which occurs during the course of a lay cycle in commercial layer flocks is detrimental to overall flock productivity as the return on investment of rearing birds to point of lay is not fully materialized in the number of eggs per hen produced. Normal levels of lay cycle mortality are anticipated to be relatively low. North and Bell (1990) indicate lay cycle mortality under 1% while commercial breeders indicate 2-6%. During the North Carolina Layer Performance and Management Trial (NCLPMT) which is a side-by-side performance comparison of multiple strains produced under the same rearing and lay cycle conditions, it was observed that particular strains had a three-fold greater incidence of mortality in the lay cycle compared with certain peer strains in these studies (1994, 1996, and 1998). Performance of the birds in these studies with the higher mortality rates were not impaired, but rather these hens reached sexual maturity earlier, had greater egg production, and equal feed efficiency compared to those respective strains with the lowered mortality. Increased performance is atypical of most pathological etiologies which prompted further investigation.

In a survey regarding the health of U.S. layer flocks, the concern most noted of non-pathological origin was calcium depletion/tetany (Gingerich, 2007). It is understood that calcium deficiency can lead to mortality as observed by Roush *et al*., (1986) in which feeding low dietary calcium (2.5%) resulted in increased hen mortality. It may be that osteoporosis, a loss of mineralized bone, which is also associated with feeding low dietary calcium (Cheng and Coon, 1990a) may be a crucial component by which calcium deficiency leads to mortality. Cage Layer Fatigue (CLF), a condition involving leg weakness, paralysis, and acute death in caged, high-producing laying hens, results in bone loss (Bell and Siller, 1962) such that CLF may be an extreme consequence of osteoporosis (Whitehead and Fleming, 2000). In one study, osteoporosis contributed 35% (3.4 of 9.7%) of the total mortality in a commercial flock of 5,000 laying hens (McCoy *et al.*, 1996) while Rennie *et al.* (1997) found that the majority of hens from a commercial strain of layers were osteoporotic by the end of lay. In fact, research suggests that osteoporosis may be inevitable with the threshold of egg production now being reached by caged laying hens. Although calcium deficiency can lead to osteoporosis in laboratory settings, overt dietary calcium deficiency rarely occurs with

advances in modern feed formulation and with the quality of mineral sources used in commercial layer rations. Issues with calcium depletion may have more to do with metabolic calcium insufficiency than dietary deficiency.

With calcium being the most abundant mineral in the body and 99% of the body's calcium found in bones, it is not surprising that laying hens requiring daily bone turnover for eggshell calcification, are candidates for metabolic calcium deficiency. The hen will deposit in egg shells 30-40 times the calcium present in her own skeleton in one year of production (Simons, 1986). This magnitude of calcium cannot be supplied by dietary sources alone and forces the hen into a daily ritual of bone remodeling. Therefore, the reservoir of minerals amassed in the hen's skeletal system during puberty is crucial especially considering the calcium output associated with the record number of eggs currently being produced per bird in the lay cycle. For purposes of this paper, the mineral of focus in this discussion will be calcium although it is understood that calcium and phosphorous are dependent upon each other requiring to be maintained in a 2:1 ratio for proper bone development.

The calcium status of hens is known to be influenced by genetic factors and housing, but the effect of certain management practices without concurrent changes in dietary calcium have not been well studied especially when compounded by strain and housing. The rearing phase and lay cycle are two periods of extreme calcium mobilization with calcium deposition into bone (positive calcium balance) during rearing, while during the lay cycle, calcium is essentially "withdrawn" (negative calcium balance). For simplicity of this discussion, these two periods of rearing and laying will be considered independently of one another, although it is understood they are not mutually exclusive.

Any disruption in this early acquisition period of calcium in the bone reservoir could potentially have negative effects especially in later life when loss of calcium status due to constant bone remodeling. High rates of production have been shown to further impede the hen's bone due to diminished capacity to absorb calcium in the duodenal intestine (Al-Batshan, 1994). The early acquisition period in layers is the rearing phase where commercial trends have been to decrease the age of sexual maturity (20 to 17 weeks) and point of lay (age when 50% rate of lay is reached). With this decrease, the time available for skeletal development, mineralization, and growth important for sexual maturity is reduced. Bone, the

primary mineral reservoir, may not have the time to "fill" with adequate levels of calcium and phosphorous needed during the demands of peak egg production and the extensive lay cycle. This "filling" of the bone will include the formation of the woven medullary bone, a labile calcium reserve, which occurs with the onset of sexual maturity. The formation of medullary bone is a demanding period of calcium regulation and metabolism, but perhaps more importantly, this switch from structural (or cortical bone) to medullary bone may be a critical point for optimal bone mineralization.

Research indicates that early and late sexually maturing pullets respond differently to mineral levels with responses including osteoporosis and mortality (Rao *et al*., 1995, 1992). The potential for reduced skeletal mineralization due to earlier ages of sexual maturity may be exacerbated by the fact that recommendations for increasing calcium at earlier ages has not coincided in feeding guidelines by either NRC (1994) or commercial management guides. This incongruity has lead to suggestions of providing more calcium at lighting rather than first egg (Whitehead and Fleming, 2000) indicating a need to reconsider calcium requirements in light of changes in management and genetics of the pullet during the rearing phase.

Calcium requirements throughout the lay cycle may also need to be reconsidered. Recommendation for calcium requirements in layer diets has remained unchanged in the past 20 years (NRC, 1977 and 1994) although efficiency and productivity of layers has increased. During this time, the average body weight of white egg layers has dropped from 1.96 to 1.86 kg, feed conversion has improved from 2.69 to 2.15, while egg mass has increased from 16.3 to 19.9 kg/HH. This indicates more mineral output in eggshell with less mineral input in the feed and smaller mineral reserves in the skeletal mass. Calcium inclusion levels may need to be reconsidered in light of the genetic improvements of laying hens and the associated management practices from rearing through lay of this higher performing bird.

The influence of strain on the calcium status in laying hens may be an important management aspect for thwarting the propensity towards osteoporosis. It was Leeson *et al*. (1997) who indicated that despite the few number of commercial Leghorn strains available, "there is a surprising lack of information on comparative nutrition and their response to various feeding strategies". In this regard, the requirements for calcium respective to strain

may be no exception. It may be that a reduced calcium status among various strains which gives rise to CLO also drives mortality in particular strains. North and Bell (1990) suggest that mortality is often strain related and should not necessarily be dismissed as a management issue. Multiple studies have shown that bone breaking strength, a marker of adequate bone mineralization, is strain related (Anderson *et al.*, 1995). In a study by Silversides *et al.* (2006), it was observed that bone breaking strength was greater for an unselected line compared to two commercially available "selected" strains suggesting that selection may inadvertently be towards reduced bone density.

Metabolic calcium insufficiency may be a predisposing factor creating reduced calcium bone status which manifests as conditions such as osteoporosis contributing to the aforementioned lay cycle mortality observed in the NCLP& MT's. This daily ritual of bone remodeling for eggshell formation will occur on average of 275 times during the lay cycle which will represent 900 grams of calcium being shunted out of the hen's body in a lifetime. This constant restructuring of bone as a reservoir for egg shell calcium is a physiologically demanding process but does not explain the increased late age mortality observed during the lay cycle. Trends in management practices which include decreased age of sexual maturity without concurrent increases in mineral content of the feed may juxtapose with the hen's increased genetic capacity to produce more eggs with less feed (more calcium output and less input) such that bone mineralization is impaired during the rearing and/or the lay cycle giving rise to mortality issues stemming from calcium insufficiency and osteoporosis. Objectives of this research were to evaluate the influence of feeding increased dietary calcium during rearing and followed into the lay cycle of two strains of layers housed at two cage densities on the growth performance and bone mineralization of these hens in order to mitigate calcium insufficiency and lay cycle mortality.

LITERATURE REVIEW

Problem: Mortality in Laying Hens

Mortality which occurs during the lay cycle of commercial layer flocks is especially detrimental to overall flock productivity as the return on investment of rearing birds to point of lay is not fully materialized in the number of eggs produced on a hen housed basis. Normal levels of lay cycle mortality are anticipated to be relatively low. North and Bell (1990) indicate lay cycle mortality under 1% while commercial breeders indicate 2-6%. During the North Carolina Layer Performance and Management Trial (NCLP&MT) which is a side-by-side performance comparison of multiple strains produced under the same rearing and lay cycle conditions, it was observed that particular strains had a three-fold greater incidence of mortality in the lay cycle compared with certain peer strains in these studies (1994, 1996, and 1998). Performance of the birds in these studies with the higher mortality rates was not impaired, but rather these hens reached sexual maturity earlier, had greater egg production, and equal feed efficiency compared to those respective strains with the lowered mortality. Increased performance is atypical of most pathological etiologies which prompted further investigation as to a non-pathological source.

Relation between Calcium and Mortality

In a survey regarding the health of U.S. layer flocks, the concern most noted of non-pathological origin was calcium depletion/tetany (Gingerich, 2007). It is understood that calcium deficiency can lead to mortality as observed by Roush *et al.*, (1986) in which feeding low dietary calcium (2.5%) resulted in increased hen mortality. Increasing dietary calcium from 2.4 to 4.9% in layer diets from 66-77 weeks of age caused mortality to drop from 22.8% to 11.9% (Bar *et al.*, 2002). It is suggested that bone weakness in laying hens may be due to osteopenia, precursor to loss of bone mineral density, which could be caused by a combination of osteoporosis, severe loss of bone mineral density, and osteomalacia, incompletely mineralized bone (Randall and Duff, 1988). It is suggested these combined

types of bone weakness will respond to dietary treatments unlike the osteoporosis observed in older hens without osteopenia or osteomalacia (Knowles and Wilkins, 1998). Osteoporosis associated with feeding low dietary calcium (Cheng and Coon, 1990a) may be the bridge by which calcium deficiency leads to mortality. Cage Layer Fatigue (CLF), a terminology first coined by J.R. Couch in 1955 involving leg weakness, paralysis (due to weakened and collapsed spinal bone), and acute death in caged, high-producing laying hens, also results in bone loss (Bell and Siller, 1962). It is also being postulated that a new transient version of CLF caused by an intracellular calcium imbalance that impairs neuromuscular function is being observed which reproduces the paralysis, osteoporosis, and/or mortality of the traditional CLF (Cransberg *et al*., 2001). Cage Layer Fatigue may be an extreme consequence of osteoporosis (Whitehead and Fleming, 2000). In one study, osteoporosis contributed 35% (3.4 of 9.7%) of the total mortality in a commercial flock of 5,000 laying hens (McCoy *et al.*, 1996) while Rennie *et al.* (1997) found that the majority of hens from a commercial strain of layers were osteoporotic by the end of lay. The prevalence of osteoporosis in laying hens may be a marker for the larger issue of calcium depletion and the related mortality it induces. In fact, research suggests that osteoporosis may be inevitable with the threshold of egg production now being reached by caged laying hens.

Although calcium deficiency can lead to osteoporosis or mortality in research settings, overt dietary calcium deficiency rarely occurs due to advances in modern feed formulation and availability of quality mineral sources. Issues of calcium depletion may have more to do with metabolic insufficiency rather than dietary deficiency of calcium. For example, rickets can be due to metabolic calcium deficiency caused by malabsorption due to virus-induced mucosal damage in fast-growing broilers (Guy, 1998). Metabolic calcium insufficiency in laying hens may be a result of a concerted effect between certain management practices without concurrent increases in dietary calcium as well as genetic and cage density rather than a singular, etiological agent. Management issues include pullets reaching sexual maturity at earlier ages, greater egg production per bird with more extensive lay cycles, and hens that are genetically selected for lighter body weight, better feed efficiency, and increased egg production. This decreases the time for bone maturation (during rearing), increases the time for bone remodeling (during lay) while reducing the

capacity for calcium intake and calcium storage in the body. Furthermore, selection for increased egg production may be inadvertently selecting for decreased bone strength (Whitehead and Fleming, 2000) which is being exacerbated by the lack of bone-loading associated with the inactivity of hens in battery cages. A more comprehensive review of the issues that may give rise to metabolic calcium insufficiency will be discussed to better understand the predisposing factors for laying hens to develop inadequate calcium status leading to CLO or mortality.

Bone and Calcium Mineralization

In establishing a relation between metabolic calcium insufficiency and mortality in laying hens, bone is fundamental to the discussion. Calcium is the most abundant mineral in the body and 99% of the body's calcium is found in bones. Although calcium in bone cannot be considered independent of phosphorous as it is always present in a constant calcium and phosphorous ratio in bone and is the primary component of bone and eggshell ($CaCO_3$), it will be the mineral of focus for this discussion. The hen will deposit in egg shells 30-40 times the calcium present in her own skeleton in one year of production (Simons, 1986). This magnitude of calcium cannot be supplied by dietary sources alone and forces the hen into the daily ritual of bone remodeling. In fact, of the calcium in eggshell, 60-75% was found to be derived from dietary sources whereas 25-40% is from skeletal stores (Driggers and Comar, 1949). This makes the reservoir of minerals amassed in the hen's skeletal system during puberty critical especially with the extended production period of modern laying hens. Therefore, it is not surprising that research by van Niekerk and Reuvekamp (1994) found bone weakness in 13% of the mortality in commercial layer flocks they examined.

As bone is the primary calcium reservoir, a basic understanding of its physiology and anatomy can be useful for the purpose of this discussion. Bone and cartilage comprises the skeleton system which serves structural, protective, and metabolic functions. The bone metabolically is an ion reservoir of primarily calcium and phosphorous which facilitates serum homeostasis (Baron, 1996). The relationship between calcium metabolism and bone

physiology is critical component to establishing a relationship between poor calcium status and development of osteoporotic conditions. The axial skeletal system consists of flat bones like the skull and scapula whereas the appendicular skeletal system is composed of long bones like the femur and tibia. The appendicular skeletal system contains long bones which are the only bones that serve as mineral reservoirs for calcium mobilization. In these bones, endochondral histogenesis and ossification occurs which is critical for calcium deposition necessary for subsequent "withdrawals" for eggshell calcification. Basic bone anatomy includes the epiphysis (the wide ends), diaphysis (the central shaft), and metaphysic (developmental region between the epiphysis and diaphysis. The epiphysis and diaphysis is separated by the epiphyseal cartilage also referred to as the growth plate. The external portion of the bone is made of hard, dense calcified tissue called the cortical or compact bone which becomes progressively thinner towards the metaphysis where it forms the spongy cancellous or trabecular bone. There are two bone surfaces interfacing with soft tissue, the outer periosteum and the inner endosteum surfaces. These surfaces are both layered with osteogenic cells but differ in that the cortical bone of the periosteum is 80-90% calcified by volume while the endosteum is 15-25% calcified (the remainder occupied by bone marrow, blood vessels, and connective tissue). Thus, 70-85% of the interface with soft tissue occurs in the endosteum which will be the primary region for calcium mobilization while the cortical bone of the periosteum will primarily fulfill a mechanical function.

When bone is being formed, a protein-based matrix serves as the scaffolding for calcium to be deposited. The ground substances of the matrix are glycoproteins and proteoglycans which have a high ionic capacity (as they are highly anionic) contributing to calcification by fixing hydroxyapatite crystals ($(Ca_{10}(PO_4)_6(OH)_2$) to these collagen fibers. The preferential orientation of collagen fibers alternates in mature bone layer by layer resulting in a lamellar structure which allows the highest density of collagen per unit volume of tissue. If bone is formed rapidly (as with medullary bone) there is no preferential organization of the collagen fibers and woven bone results as compared to lamellar bone. In laying hens, it is normal to observe a thinning of cortical bone and reduction in cancellous bone with age although this was found to be accentuated in hens with CLF (Bell and Siller, 1962).

When the hen must access calcium from the bone to renew old bone, replace bone with microfractures, or maintain calcium homeostasis, a remodeling process takes place in which osteoclasts and osteoblasts work closely together in time and space (coupling) to remove and replace new bone (cancellous or cortical). Cancellous bone is remodeled about 5-10 times more rapidly than cortical bone which makes it the preferred bone besides medullary bone for the calcium demands associated with egg production. The remodelling process is regulated by hormonal and mechanical stimuli as also seen in growth and modeling processes. In each remodeling process, the ostoeblasts are activated to secrete collagenase which removes a thin layer of unmineralized bone typical of a resting bone surface. This exposes the mineralized bone underneath to the mobile osteoclasts which begin to resorb the bone until reaching the reversal or cement line, a narrow zone of hypermineralization that marks the termination point of resorption and initiation point of new bone formation. If coupling has occurred, osteoblasts produce osteoid (collagen and ground substance) until a certain thickness is reached and then mineralization occurs from the bottom osteoid tissue. At the end of the remodeling process, the bone surface is again covered with thin layer of mineralized bone becoming the resting surface ready for the next remodeling process to begin. However, especially with aging, there is a negative balance to the remodeling process such that bone mass is lost and cancellous bone network is disrupted. This thinning of cancellous bone allows the osteoclast to perforate more deeply which compounds the bone loss observed with aging. The third bone cell type is osteocytes which lie more deeply within bone tissue in tiny lacunae and serve a mechanostatic function. With movement these cells sense stress or strain on the bone and send signals to activate the osteoblasts and osteoclasts on the surface. In humans, studies have shown that strict bed rest leads to 1-2% loss of cancellous bone per week, and in laying hens, the inactivity of caged layers will also prove an important component of the loss of calcium status.

In the progression of osteoporosis, the cortical bone diminishes, and the trabeculae become a less cohesive system that is fewer, thinner, and less well-connected. This can be qualified through lower bone breaking strength, histomorphometric analysis, or by radiography of bones that contain little or no medullary bone. Other measures of osteoporosis include cancellous bone volumes (e.g. free thoracic vertebra (FTV) and

proximal tarsometatarsus (PTM)), radio -graphic densities of humerus and keel, and breaking strengths of humerus and tibia. When considering calcification of bones in laying hens, the bones being measured are almost as important as the variables being tested. Typically, the humerus and tibia are two bones of interest in the study of how calcium depletion manifests in laying hens. However, these two bones can have completely opposite responses based on housing system and type of activity. For example, bone strength of the humerus was found to significantly increase by keeping hens in furnished cages with perches while the tibia BS was unaffected by this change (Leyendecker *et al*., 2005). It is not surprising to observe these varied responses as the tibia can develop large amounts of medullary bone whereas the pneumatized humerus will usually contain no medullary bone.

Calcium Homeostasis

The regulatory "balancing-act" to maintain calcium homeostasis despite the voluminous turnover of calcium by laying hens for egg production, belies an impressive calcitropic effort which deserves mentioning in the scope of this discussion. Each hen during a lay cycle will produce 280 eggs which represents an almost daily removal of 2-3 grams of calcium in the form of eggshells being removed from the hens' calcium reserves (Gilbert, 1983). These 2-3 grams of calcium represents 10% of the birds total body calcium (Etches, 1987). Calcium homeostasis is created through a balance between intestinal calcium absorption, renal calcium excretion, and bone mineral metabolism to meet the bird's requirement (Elaroussi *et al.*, 1994). In order to mobilize calcium with speed and volume, the avian's physiology has two specialized adaptations. These are the calcium reservoirs of blood and bone. Blood calcium circulates in two forms, as diffusible ionized Ca and nondiffusible protein-bound calcium which is bound to plasma calcium-binding proteins (CBP) vitellogenin and albumin. A bird's concentration of calcium in the blood is 10.2 mg% when not producing eggs, but this will rise to 30 mg% when mobilized for shell calcification. Normal blood calcium levels are tightly controlled (usually between 10-11 mg%), and certain hormone levels correlate precisely with these calcium levels. The egg-layer is highly dependent on the endocrine system to regulate CBP and thus the capacity of the blood to

carry calcium. This enhanced calcium-carrying capacity of the blood essentially serves as a readily available vault of calcium easily accessible by the hen. The other major reservoir of calcium for the hen is bone, and it is the most important sight for control by the calcitropic hormones. The bone stores Ca and P as crystalline hydroxyapatite (Ca_{10} $(PO_4)_6$ $(OH)_2$) which is resorbed when dietary calcium cannot meet the hen's needs. The bone can be thought as offering two forms of calcium storage: fast and slow access. The fast access of calcium occurs through basic chemistry while the slow access occurs through the effects of hormones and bone cells. The bone cells are either osteoclasts (catabolic) or osteoblasts (anabolic). Part of how the hormones elicit their effects is through up regulating or down regulating production of these various cells. Medullary bone, another important receptacle for calcium storage, is unique to the avian's pneumatic bones, and it is highly regulated through the endocrine system. In fact it has been said that medullary bone is the most overtly estrogen sensitive of all vertebrate bone types. It is found in the endosteal cavities of the long bones and is a nonstructural type of woven bone (Bonucci and Gherardi, 1975) that offers little towards bone strength. The mineral phase of medullary bone is similar to cortical bone differing in the orientation of the hydroxyapatite crystal in respect to the organic matrix (Neuman and Neuman, 1958). Medullary bone is more heavily calcified than cortical bone with less collagen fibril (Taylor *et al.*, 1971) suggesting a higher concentration of more accessible calcium. Thus, medullay bone has a large surface area, is well vascularized, is better mineralized, and can be metabolized at a rate at least 10-15 times faster than cortical bone (Simkiss, 1967). Furthermore, there are greater numbers of osteoclasts in avian medullary bone compared to cortical bone indicating its advantage as a labile calcium source (van de Velde *et al*., 1984).

Several endocrine factors are responsible for calcium mobilization prior to the onset of egg production which is imperative to calcium homeostasis. The hormonal target tissues are bone, intestine, and kidney, but unique to egg-layers are the target tissues of medullary bone, shell gland, and liver. Those hormones of greatest import include Parathyroid Hormone (PTH), Calcitonin (CT), and Vitamin D serving primary roles and Estrogen, Thyroid Hormone, Testosterone, and Growth Hormone (GH) working in a secondary fashion. The integral role of PTH, CT, and Vitamin D in calcium regulation justifies further

explanation of their contribution as calcitropic hormones. Parathyroid Hormone is secreted in low levels by two pairs of parathyroid glands in response to a fall in plasma [Ca] which is the major physiological stimulus for PTH secretion (rise in [Ca] suppresses PTH (Kenny, 1986). The primary function of PTH is to maintain calcium levels, and it seems birds are especially sensitive to this PTH function. It is thought to have its primary action on calcium regulation by mobilizing skeletal calcium while its secondary roles involve decreased renal calcium secretion and improved calcium gut absorption via an indirect effect of Vitamin D. However, administering PTH to laying hens resulted in hypercalcemia within 8 minutes suggesting that PTH could not be eliciting its effect so rapidly just through the aforementioned processes (Candlish and Taylor, 1970). Possible theories of PTH's control on calcium include inhibition of net calcium uptake by the skeleton, inhibition of collagen synthesis in avian epiphyseal cartilage cells, and/or through enhancing cAMP (Shaw and Dacke, 1989). The main effect of PTH on bone is to increase resorption through a net result of indirect stimulation of osteoclastic bone resorptive activity and an accompanying inhibition of osteoblastic bone formation (Dacke *et al.*, 1993). PTH seems to be able to "activate" osteoblasts in the medullary bone as observed by the ruffled borders typical in the active form of these bone resorbing cells (Sugiyama and Kusuhara, 1996). Features of this ruffled border and osteoclasts (e.g. such as Ca^{2+}-ATPase on plasma membrane only) suggest further how PTH impacts calcium regulation at a more molecular level. Calcitonin works as an antagonist to PTH. Calcitonin (CT) is secreted from the ultimobranchial glands and has primarily a hypocalcemic affect in the bird functioning opposite that of PTH. As blood calcium increases beyond the normal 10.2 mg%, so does the release of CT. Calcitonin is released in response to hypercalcemia (Zeigler *et al.*, 1969), and it is known that CT infusion brings about hypocalcemia (Sommerville, 1978). Affects of CT on calcium seem to occur by protecting bone against resorption (rather than promoting active deposition) as well as at the kidney to modify mineral excretion (Taylor and Dacke, 1984). Like PTH, CT has its effect at osteoclasts (inhibitory), reducing ruffled borders (indicating non-active osteoclasts) and via cAMP. Vitamin D's role in calcium metabolism is fairly broad in part due to requirement that it be activated at multiple sites in the body. The two biologically active forms of Vitamin D are Vitamin D_2 (irradiated ergosterol, plant source) and D_3 (irradiated 7-dehydro-

cholesterol, animal source) with birds utilizing only ergosterol (DeLuca, 1979). Vitamin D is either absorbed from the intestines or made in the skin via ultraviolet radiation. This is an important consideration as many laying hens are raised in closed housing with minimal sunlight exposure requiring all Vitamin D to be supplied via the diet, and furthermore, avians have little ability to store this nutrient in their body. Vitamin D does not exist in the body in its active form and must be hydroxylated first in the liver and then the kidney. Hormonal control of Vitamin D occurs mainly in the kidney at the second step of the activation process. In the liver, it is hydroxylated by monoxygenase enzymes in the microsomes to produce 25-hydroxyvitamin D_3 (25-(OH)D_3). In the mitochondria of the kidney, 25-(OH)D_3 is transformed by the 1-hydroxylase enzyme to 1,25-$(OH)_2D_3$. Another hydroxylated Vitamin D metabolite produced in the kidney is 24,25-$(OH)_2D_3$ which had previously been thought to be inconsequential but is now being considered a necessary cofactor for 1,25-$(OH)_2D_3$ to reach its full potential during reproduction (Henry and Norman, 1984). Once in its active form, it is delivered in the blood to the target tissues of intestines, bone, and elsewhere in the kidney by plasma albumins. A Vitamin D receptor (VDR) has been identified in 10 avian tissues, and its gene-induced product, a Ca-binding protein referred to as calbindin-D28K, has been reported in 9 avian tissues (Norman, 1986). An increase in active transport of Ca across epithelial cells of the duodenum has been linked in part to calbindin-D28K protein (Cross and Peterlik, 1988). The intestinal expression of calbindin-D28K is enhanced by 1, 25-$(OH)_2D_3$ with Thyroid Hormone being thought to further enhance this relationship (Shirley et al., 2003). Vitamin D also enhances active calcium transport in the intestine by stimulating the synthesis of the epithelial calcium channels and the plasma membrane calcium pumps (Wasserman, 2004). Vitamin D is thought to be necessary for activities of other proteins including a brush border membrane alkaline phosphatase and a calcium-dependent ATPase in the basal lateral membrane. Bone carboxyglutamic acid protein is also stimulated by 1,25-$(OH)_2D_3$.

One of the most important and unique aspects of calcium acquisition for avians is the development of medullary bone which occurs in the rearing phase. It is in the final 10 days before egg-laying begins that medullary bone will be formed and transformational changes in calcium metabolism will take place. There is a concerted endocrine effort to bring about

sexual maturation which results in an increased capacity to mobilize calcium. There is also a concurrent maturation of the ovarian follicles (which produces estrogen and testosterone) which is necessary to stimulate medullary bone formation. Gonadal steroids are thought to act directly on the cells in the medullary cavity and independently of Ca intake. Photostimulation will cause increases in Follicle Stimulating Hormone (FSH) and Leutinizing Hormone (LH) that are necessary for the maturation of the ovary which will then drive production of estrogen and testosterone. The steady rise in circulating estrogen ultimately depresses the plasma levels of LH (with progesterone promoting ovulation through a biphasic effect on LH) at a time which coincides with the first ovulation. The cumulative effect of these efforts is especially important for calcium metabolism in order to increase estrogen levels. Several weeks before lay, total blood calcium increases due to estrogen stimulating lipogenesis in the liver resulting in the production of the calcium-binding proteins and yolk protein precursors which allows the protein-bound portion of the blood to transport more calcium. With the onset of sexual maturity, gradual increases in estrogen concentrations in the plasma are observed which increase more notably from 16-20 weeks of age (Whitehead and Fleming, 2000). Estradiol levels averaged 93 pg/ml in pullets at 7 wks of age, but spiked sharply 2-3 wks before lay and increased to 138 pg/ml when egg-laying commenced (Senior, 1974). Activity of renal 1-hydroxylase, an enzyme necessary for the conversion of a Vitamin D metabolite, increases just prior to the initiation of egg laying, at a time corresponding with an increase in circulating estrogen and total plasma calcium. It is not thought that 1,25-$(OH)_2D_3$ mediates the transfer of calcium to medullary bone in the 10 days prior to onset of lay (where estrogen and testosterone are active) as the appearance of this bone occurs 1-2 weeks prior to the increase in renal 1-hydroxylase activity and elevation of plasma total calcium. However, it is believed that 1, 25-$(OH)_2D_3$ is necessary for the formation of a fully mineralized medullary bone. The increase in 1-hydroxylase activity is under the control of estrogen and PTH (Soares, 1984). Serum [Ca] through the action of PTH is also related to the regulation of 1,25-$(OH)_2D_3$ synthesis (from 25-$(OH)D_3$) such that Vitamin D can be thought of as under the influence of PTH. It seems that 1, 25-$(OH)_2D_3$ has little effect on Calbindin D28K in immature and point of lay hens. In a study by Wu *et al.* (1993), trace amounts of Calbindin mRNA were detected in enterocytes in jejunal tissue

taken from immature and point of lay chickens but significantly higher in egg producing hens. Calcitonin also seems to make its debut before lay commences (normally CT is higher in males, but this changes with onset of lay) as shortly after ovulation CT levels are highest and fall as eggshell calcification proceeds. It is thought that gonadal steroids, especially testosterone, can largely influence circulating CT levels (Taylor and Dacke, 1984). These processes all work towards preparing the pullet for egg production by filling the medullary bone, an important calcium reservoir in avians.

Importance of Calcium in Laying Hens

The demands of egg shell formation for calcium carbonate are massive during the extensive lay cycle of the hen. For the calcification of a single egg, the shell gland will need to transport 2-2.5 g Ca within a period of 15 hr. During the ovulation-oviposition cycle, ionized Ca will peak at 0.057 mg/ml within 4 hr after oviposition, then drop during shell calcification to 0.049 mg/ml. This represents a sigmoidal curve with serum Ca peaking within 3-6 hours of oviposition and falling as shell calcification proceeds to a minimum 3-6 hr before the next oviposition (Parsons and Combs, 1981). If total plasma Ca is considered during this same period (ovulation-oviposition), only minimal fluctuation of 0.2-0.26 mg/ml will occur attesting to the exquisite hormonal regulation of this mineral. To summarize this hormonal regulation of Ca during the 24-hour egg laying cycle, when an egg is in the infundibulum, magnum, or isthmus of the oviduct, osteoblasts actively form medullary bone with the induction of estrogen secreted by matured follicles. When the egg enters the shell gland, mobilized Ca for the shell causes a decrease in plasma [Ca]. PTH responds to this lowered plasma [Ca] by stimulating osteoclastic bone resorption. After eggshell formation, CT inhibits osteoclastic bone resorption.

In the process of egg formation, the uterus is the portion of the avian oviduct where eggshell is formed from Ca obtained from the blood (Nys *et al*., 1999). If the gut cannot sustain calcium absorption or shell calcification occurs in the night when the gut is empty, blood calcium levels will drop. The drop is blood calcium stimulates PTH secretion by the parathyroid glands which will cause bone resorption. PTH levels are highest during the

period of eggshell calcification when Ca needs of the hen are greatest (Singh *et al*., 1986). Bone resorption by PTH mainly involves medullary bone but with Ca dietary deficiency, cortical bone may be utilized. Solubilized bone mineral releases more P_i ($Ca:P_i$ ratio = 2.5:1) than needed to form an egg ($Ca:P_i$ ratio = 20:1) which creates the potential for high blood P_i. This hyperphosphatemia could suppress blood Ca levels except that it is attenuated through PTH stimulation of urinary Pi excretion (Wideman *et al*., 1980). The action of PTH on the kidneys will further maintain Ca levels during the demands of egg shell calcification by reducing the Ca filtration rate and/or decreasing urinary Ca excretion (Candlish, 1970). These PTH effects are further complemented by estrogen-stimulated up regulation of kidney PTH receptors causing the kidneys of laying hens to be more sensitive to PTH (Forte *et al*., 1983). In turn, elevated PTH titers during shell calcification stimulates increased 1, 25 $(OH)_2D_3$ synthesis. Vitamin D's active form not only improves absorption of Ca and Pi from the gut, but is also thought to have a direct effect on the kidney (Forte *et al*., 1983). Interestingly, a PTH response in effect works as a buffer to the acidosis created during eggshell formation. Hydrogen and carbonate ions are generated by the shell gland when eggshell is formed. Typically in avian urine, salts of uric acid will buffer the acidic metabolites, but during eggshell formation, excess Pi serves as the predominate buffer (Prashad and Edwards, 1973).

Calcitonin produces hypocalcemia and hypophosphatemia by inhibition of bone resorption. In the kidney, CT has a reciprocal relationship with PTH in that PTH increases glomerular filtration rate, urine flow rate, and Pi and Ca clearance while there must be a balanced release of CT to maintain Ca homeostasis. It is thought that tissues where Ca transport is particularly important, there exists CT receptors. CT receptors are confirmed in the kidney and may also be in the shellgland. Increased binding affinity and capacity was observed for the CT receptor in the shell gland 12 hours before oviposition. This suggests CT may mediate its calcitropic effects at the site of the shell gland as well (Ogawa *et al.*, 2003). During reproduction, concentrations of CT and 1,25-$(OH)_2D_3$ in the plasma are high. It has been proposed that by opposing the resorptive action of Vitamin D on bone, CT preserves the integrity of the skeleton and directs the action of Vitamin D to the GI tract to

meet the need for Ca. Cholecystokinen and enteroglucagon also seem to contribute to regulation of CT through action on the gut.

As PTH regulates Ca via bone, Vitamin D regulates Ca via the gut. Activity of 1-hydroxylase activity increases during the ovulation-oviposition cycle at the time of ovulation. This increase in activity is followed by an increase in circulating concentrations of 1,25-$(OH)_2D_3$ 4 hr after ovulation and elevated levels persist until 10 hr after ovulation or after the initiation of eggshell formation (Castilloe *et al*., 1979). Vitamin D works at many levels to impact Ca transport. Wasserman and Faher (1977) isolated a Ca-binding protein from intestinal mucosa of birds that requires Vitamin D. DeLuca (1978) suggested that Ca-dependent ATPase, alkaline phosphatase, and actin may be induced by 1,25-$(OH)_2D_3$ implying the far-reach of this hormone. Bone cells respond to acitivated D metabolite by modulating an array of proteins including collagen an alkaline phosphatase, required for bone mineralization and remodeling. Large amounts of calbindin mRNA were detected in upper crypt and all villus enterocytes in tissue taken from laying hens with maximum levels found in the basal third of the villus (Wu *et al*., 1993). Calcium uptake in this tissue was found to be twice as high in laying hens verses immature and point of lay birds. Daily surges of estrogen occur approximately 4-6 hr prior to ovulation and coincide with the daily surges of LH and progesterone (Etches and Cheng, 1981). One direct effect of PTH is to increase acid production of osteoclasts by activating adenylate cyclase via a Gs type-protein. This stimulation of acidification by PTH and cAMP is blocked by estradiol to the point that estradiol worked similarly as CT (Gay *et al*., 1993). This may indicate one way in which estradiol can protect bone as associated with postmenopausal induction of osteoporosis.

Dietary Ca is another consideration impacting calcitropic hormones. Calcium is obtained for shell formation by absorption from of dietary sources in the intestine (mainly duodenum and upper jejunum) or resorption from bone (mainly medullary bone but cortical bone is used during Ca deficiency). The relative importance of these two Ca resources, gut and bone, is dependent on dietary Ca. Hens consume 25% more feed on days of eggshell formation. If concentration of Ca in the feed is greater than 3.6%, most of eggshell calcium will be derived from the intestine whereas if dietary Ca concentration is below 2.0%, bone will supply 30-40% of shell calcium. Obviously, with a completely Ca deficient diet, all Ca

will be derived from bone. These relationships likely depend on time of day. If hens have constant access to feed, most of feed intake occurs early in the photoperiod with remainder consumed at end of daylight. However, shell is formed predominately during the night when Ca contact of digestive tract is nominal resulting in medullary bone serving as the primary Ca reserve. Thus it follows that if the primary hormonal control of the gut is through 1, 25-$(OH)_2D_3$ and the bone is PTH, then their relative activity will follow consumption of dietary Ca. Level of CT positively correlates with dietary Ca and circulating Ca levels. By feeding Ca deficient diets, CT levels dropped through a reduction in number of secretory cells in the ultimobranchial glands. Dietary Ca also could impact medullary bone's contribution to Ca metabolism. During the ovulation-oviposition cycle, periods of intense medullary bone formation alternate with periods of severe bone depletion. Hens fed a high-Ca diet are generally able to replenish the Ca lost from medullary bone during shell calcification when shell formation is not taking place, but on low Ca diet the cortical bone of the femur is eroded, while medullary bone is maintained in a fairly constant amount. Under these conditions the new medullary bone that forms is only partially calcified, and an increase in the number of osteoblasts is indicative of a more rapid turnover rate.

Research indicates that bone loss in aging hens is not so much due to calcium deficiency but caused primarily by cellular factors affecting bone remodeling although it is cautioned to avoid calcium deficiency by elevating dietary calcium when birds are lighted rather than waiting until first egg (Rennie *et al*., 1997).

Strain and Calcium

Since the 1950's, great strides in the breeding programs for egg laying strains has resulted in high egg production, low body weight, and low feed intake. These genetic selection methods may have inadvertently selected for bone fragility as evidenced by the high fracture rates among laying hens. In a study by Gregory and Wilkins (1989), 29% of spent, older, caged layers had broken bones by the point of being shackled in a processing facility. This predisposition towards fracture is a marker of weakened bone (Knowles *et al*., 1993) which in part may be attributable to strain differences. Older unimproved, "heritage"

strains are relatively unaffected by bone weakness compared to modern hybrid layer strains (Rennie *et al*., 1997). A study comparing a white egg-laying, modern strain (Babcock B300), a brown egg-laying modern strain (ISA-Brown), and an unselected line of brown leghorns, found bone fracture rates of 11.1, 11.7, and 0.0%, respectively, in 72 week old spent hens at depopulation (Budgell and Silversides, 2004). In another study by Silversides *et al*., (2006) using these same strains, the unselected brown leghorns had lowered bone density at 25 weeks compared to commercial strains (likely associated with delayed sexual maturity) but had greater trabecular bone density later in the lay cycle. This might suggest that the lowered egg production of the unselected lines may be correlated to the reduction in bone density. However, in this same study, it was the ISA-Brown which had the highest rates of egg production, best feed efficiency, and yet had the best measures of bone strength throughout the study. Another study comparing brown (Shaver 579) and white (Shaver 2000) egg strains, found that although the white-egg strain laid more eggs (due to longer clutches), the brown-egg strain produced heavier eggs with more eggshell while maintaining greater bone areas and breaking strength in the femur and humerus (Riczu, *et al*., 2004). Total bone density between strains in this study was found to be greater in only the humerus and not the femur. This indicates that strains may also differ in preferential calcium resorption sites within the skeletal system. Although the relationship between strain and bone strength may not be easily discerned, it is understood that birds can be genetically selected for improved bone characteristics. Bishop *et al.* (2000) selected hens based on a Bone Index which measured both bone strength and density, and it was found that within three generations, those birds selected for a high Bone Index had a 25% greater tibia strength, 13% greater humeral strength, and 19% greater keel density with heritability of the Bone Index being 0.40. Furthermore, humerus fracture incidence was reduced significantly by 6-fold through selection for Bone Index. These findings indicate that breeding will be an important factor in resolving issues of calcium depletion which results in impaired bone quality.

Rearing and Calcium

When considering the nutritional needs of calcium in laying hens, the focus is primarily on the lay cycle. Both the rearing phase and lay cycle are two periods of extreme calcium mobilization with calcium being primarily "deposited" into bone (positive calcium balance) during rearing, while during the lay cycle, calcium is essentially "withdrawn" (negative calcium balance). For simplicity of this discussion, these two periods of rearing and laying will be considered independently of one another, although it is understood they are not mutually exclusive. A certain threshold of body, skeletal, and reproductive tract maturation must occur in pullets during rearing before the onset of lay will commence. This represents a significant nutritional need for calcium by the pubescent bird that is irrespective of the calcium demands of egg production. The inability of bone to acquire its full mineralization potential during the rearing period could be a contributing factor to metabolic calcium deficiency. Any disruption in this early acquisition period of calcium in the bone reservoir could potentially have dire effects especially in later life when the issue would be compounded by the hen's diminished capacity to mobilize calcium as she ages.

Many factors are being attributed to the bone characteristics that laying hens develop which give rise to calcium depletion. Body weight may need to be further considered and its implication for developing unfavorable bone characteristics. Commercial trends in the rearing phase have been to decrease the age of sexual maturity (20 to 17 weeks) and point of lay (age when 50% rate of lay is reached) at which time mature BW is reduced. In a study by Leeson *et al*. (1997) pullets selected as light weight (< 85% of mean 18-week body weight) or heavy weight (>115%) were compared, and light weight birds matured more slowly ($P<0.01$), ate less feed and produced less total egg mass ($P<0.05$), and had a 400 g lowered BW at 70 wk across all four commercial strains tested. This is especially of concern in Leghorn pullets that do not exhibit compensatory growth once they mature (Leeson *et al*., 1991). Strains that are early- versus late-maturing tend to have lower body weights that are persistent throughout the lay cycle (Robinson *et al*., 2001). The impact of lowered mature BW may be genetically linked to bone strength. Body weight is positively correlated with the humeral and tibial breaking strengths and keel radiographic density (bone characteristics associated with osteoporosis) (Fleming *et al*., 1996). This may help to explain why late-maturing pullets seem to have better bone status than their early-maturing counterparts (Rao

et al., 1995). Rao *et al*. (1995) also found that late-maturing pullets respond more dramatically to challenges in bone status (low dietary phosphorous) resulting in a greater decline in bone mineral content, bone density, and bone strength as well as the maximum levels of osteoporosis and mortality occurring in a late-maturing treatment group. This suggests that lowered body weight resulting from earlier sexual maturity does impact the reproductive capacity of the hen which may include negative manifestations of calcium depletion.

With a lower age for sexual maturity and the resulting lowered body weight, there is less capability for skeletal maturation. Time is needed for bones to grow and mineralize which includes constructing matrix and depositing calcium into it. With bone being the primary mineral reservoir, repercussions of this shortened period for calcium investment during rearing may not be observed until the extensive lay cycle when vast withdrawals of calcium are necessary. This issue is exacerbated by the fact that the onset of sexual maturity will induce the development of woven medullary bone, a crucial calcium resource during the lay cycle. However, medullary bone forms at the expense of cortical bone so that there is a depression in structural bone formation (Hudson *et al*., 1993). The impact of this switch from structural to medullary bone formation may be more far-reaching than just the moment of sexual maturation. There is observed a striking loss of cancellous bone with the concurrent increase in medullary bone volume during the first 10 weeks after sexual maturity which is well into the lay cycle (Fleming *et al.*, 1998b).

The potential for reduced skeletal mineralization due to earlier ages of sexual maturity may be exacerbated by the fact that recommendations for increasing calcium at earlier ages has not coincided in feeding guidelines by either NRC (1994) or commercial management guides. In the past there was reluctance to feed increased calcium to layers especially at earlier ages as it was found that feeding 3.25% calcium starting at 50 days of age increased the risk for hens to develop uroliths later in life (Wideman *et al*., 1985). However, it may be that genetically, the demands for calcium have changed such that this is no longer the problem it once was. It is advised by NRC (1994) to increase dietary calcium at 18 weeks which if pullets are mobilizing large amounts of calcium 10-14 days prior to the first egg, this may create a dietary shortcoming of calcium even for those birds reaching

sexual maturity at 18 weeks of age. However, not all pullets mature simultaneously such that those pullets which are early maturing have a greater risk for a temporary calcium insufficiency. This inconsistency has lead to suggestions of providing more calcium at lighting rather than first egg (Whitehead and Fleming, 2000) indicating a need to reconsider calcium requirements during the critical changes in calcium metabolism during the rearing phase.

Calcium Depletion and Housing

In exploring the relation between calcium depletion and housing, level of confinement confers considerable influence. Hens kept in more extensive aviary setting will demonstrate bone strength responses differently than birds in the more intensive situations of enhanced or modified cages as to those hens in standard battery cages which present the greatest levels of confinement. As over 95% of layers and 50% of replacement pullets are reared in cages in the U.S. (North and Bell, 1990), it is all the more important to consider the influence of confinement level on the propensity for laying hens to develop negative calcium balance.

Calcium depletion is characterized by diminished bone breaking strength (BBS) that is experienced by hens in cages more so than in more extensive rearing systems. Establishing the link between housing and bone strength is not easily discerned. Early on in the history of battery cage use, it was found that birds reared in floor pens had increased BBS and tibia ash than those in cages (Rowland *et al*., 1968). It was discerned that having access to litter allowing nutrients to be recycled which was not responsible for the advantages of floor pens on BBS (Rowland and Harms, 1970). Wire mesh flooring typical in cages was evaluated in floor pens indicating this surface was not responsible for the effects of cages on BBS (Rowland and Harms, 1970).

The improvements in BBS of hens reared in floor pens compared with cages may suggest that this effect is an artifact of space provided. Bone strength (measured as stress and elasticity) of 69-wk old hens increased within just 20 days of being moved from cages to an aviary (Newman and Leeson, 1998). However, the association between increasing area

per bird and increasing bone strength is not absolute. Although hens in both conventional and furnished cages were provided 450 cm^2 per bird from 20-64 wks, it was only hens in the furnished cages (which included both perches and dust baths) that had the greatest improvements in femoral, tibial, and humeral bone strength parameters (Jendral *et al*., 2008). This suggests that activity may be the link between housing type and bone strength. Improvements in bone strength of hens in floor pens or furnished cages could result in movements that incite mechanical demand on the bone. Wolff's Law states that bone in a healthy person or animal will adapt to the loads it is placed under (Marcus, 1996). If loading of a particular bone increases, the bone will remodel itself over time to become stronger and more able to resist the "load" with the external cortical portion of the bone becoming stronger. Conversely, if loading decreases the bone will become weaker. This is why in human study's, a completely immobilized person may lose 40% of their bone mass in one year although this can be prevented with just 30 minutes of standing upright with postural shifting (Marcus, 1996). However, it seems that this type of mechanical loading is very specific to the bone being considered and general exercise may not provide the loading necessary to prevent bone loss. In a study where exercise was provided to caged hens by the addition of a treadmill, there were no significant ($P<0.01$) improvements in tibia BBS as occurred with the floor reared birds as compared to unexercised, caged hens (Meyer and Sunde, 1974). Just as with humans in which bone mineral density is dramatically greater for the racquet arm verses the non-racquet arm of elite tennis players, specific activity will stimulate mechanical loading on a specific bone. This could partially explain why a higher cage height of 60 cm compared to 40 cm resulted in significant increases of humerus breaking strength although tibia BS was unaffected (Moinard *et al*., 1998).

As concerns for animal welfare begin to alter the recommendations for level of confinement of laying hens, simply increasing space allocations or exercise may not be enough to counteract calcium depletion experienced by laying hens. Also, level of confinement is associated with pressures on group social structures in hens which could impact well-being and performance traits (Adams and Craig, 1985). Therefore, behavior could be an integral component of the activity crucial for bone loading and the prevention of

calcium depletion. Designing housing to maximize productivity, bird welfare, and bone strength may be more intricate than previously realized.

Summary

Due to the rigorous demands of eggshell formation and egg production, over 900 grams of Ca will be shunted out of the hen's body in a lifetime. It is physiologically extraordinary to move so much Ca especially in a continuous daily ritual. In avians, the bone must serve as a reservoir of Ca in a constant state of readiness for withdrawal due to egg production. In this analogy, the endocrine system would be the bank-keeper of this vault which releases and invests Ca as needed. Maintenance of Ca homeostasis while withdrawing 2 g of Ca for each egg requires a very intricate regulatory system making it susceptible to even slight altercations. Genetic changes among modern commercial layers alongside changes in management without concurrent changes in dietary Ca may be enough to tip the scale towards Ca imbalance marked by CLO. With osteoporosis becoming more prevalent in commercial layers, further research may be necessary to discern the inter-relationship between factors such as dietary recommendations and strain, and how these juxtapose to influence the mineral status of the bone, the main mineral repository for egg production. Furthermore, welfare concerns involving laying hens has resulted in cage density changes which add another level in discerning how to best feed dietary calcium while maximizing bird performance and bone mineral status.

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CHAPTER 1

INFLUENCE OF CA:P LEVEL AND STRAIN ON REARING PHASE PULLET GROWTH PERFORMANCE

Trends in commercial layer production have decreased the age of sexual maturity and point of lay (age when 50% rate of lay is reached) without concurrent increases in dietary Ca recommendations by which pullets may be predisposed to develop osteoporosis later in the lay cycle. Our objective was to evaluate the influence of diet (containing an elevated and control ratio of calcium and phosphorous) and strain of layer on growth of pullets. Day old pullets were assigned to cages in a Quad-deck cage system in an environmentally-controlled brood-grow house with equal representation on each of 4 rows and 3 banks. Each cage contained 13 chicks (48 in^2/pullet), and four cages comprised a replicate (52 hens/replicate) with 28 replicates/treatment for a total of 5,824 hens. Pullets were reared to 17 weeks of age receiving a 3-phase feeding regimen *ad libitum* with diets formulated to be isocaloric. The experiment was a 2 x 2 factorial arrangement; of two commercial layer strains (Hy-Line W-36 (H) and Babcock 300 (B)); two Ca:P ratios, elevated rearing Ca:P ratios 2.14, 3.14, 4.14 (RC+) and the control rearing diets ratio of Ca:P 2.14, 2.14, 2.42 (RC) in growth phases of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively. Feed consumption and body weights were monitored bi-weekly beginning at 2 weeks of age. Mortality was recorded daily. As expected there were significant differences between the periods on pullet growth. From week 0-17, pullet feed consumption was higher ($P \leq 0.01$) in the RC+ regimen (5.11 kg) than RC regimen (4.81 kg). However, feeding RC+ compared to RC did not effect Gain (1,017 and 1,029 g, respectively, P=0.53) or FE (0.199 and 0.214, respectively, P=0.08). There was no effect of strain on feed consumption (H=4.92 and B=5.00 kg, P=0.37), gain (H=1,032 and B=1,014 g, P=0.98) or FE (H=0.210 and B=0.203 g, P=0.20). Overall, mortality was unaffected by the treatments in this study. Growth performance was not affected by rearing Ca:P dietary regimen or strain although results by period indicate that response to increasing Ca:P in the diet may be influenced by period (age) and strain.

INTRODUCTION

Trends in commercial layer selection and management have decreased age of sexual maturity and age at 50% production This has occurred without concurrent increases in dietary calcium recommendations which may predispose pullets to develop osteoporosis during the lay cycle. With earlier sexual maturity, the time available for skeletal development and mineralization is reduced especially as the bird switches from structural to medullary bone formation. This may be a predisposing factor for bone weakness observed in older laying hens (Knowles *et al*., 1993). Bone fragility leads to fractures and osteoporotic conditions associated with mortality (Knott *et al*., 1995). Osteoporosis has been found in 35% of all mortality in a commercial flock (McCoy *et al*., 1996). This level of osteoporosis, bone fragility, and mortality is indicative of a considerable economic and welfare issue in laying hens (Gregory and Wilkins, 1989). It may be that management and nutrition during the rearing phase is being overlooked as a crucial tool in affecting bone mineral status in the adult hen. Nutritional needs of calcium by pullets are often considered nominal in magnitude of quantity and import compared to those of laying hens. However, both the rearing phase and lay cycle are two periods of extreme calcium mobilization. The introduction of estrogen two weeks prior to sexual maturity and the formation of woven medullary bone, a labile calcium reserve, represent a great demand for calcium during rearing. As medullary bone forms at the expense of cortical bone, there is a depression in structural bone formation in pullets stimulated to come in to production earlier (Hudson *et al*., 1993). Furthermore, considerable amounts of cancellous bone are lost with the increases in medullary bone volume during the first 10 weeks after sexual maturity (Fleming *et al.*, 1998b).

Reduced skeletal mineralization due to earlier ages of sexual maturity may be exacerbated by the fact that recommendations for increasing calcium at earlier ages has not coincided in feeding guidelines by either NRC (1994) or commercial management guides. Increases in dietary calcium are not recommended by NRC (1994) until 18 weeks of age, however, pullets mobilize large amounts of calcium 10-14 days prior to the first egg. This may create a dietary shortcoming of calcium even for those birds reaching sexual maturity at 18 weeks of age, although earlier maturing pullets would be at an even greater risk for

calcium insufficiency. In the past there was reluctance to feed increased calcium to layers especially at earlier ages as it was found that feeding 3.25% calcium starting at 50 days of age increased the risk for hens to develop uroliths later in life (Wideman *et al*., 1985). Genetic and management changes may have made these concerns obsolete especially with improvements in feed efficiency. This inconsistency in calcium recommendations is being recognized as there are now suggestions for providing more calcium at lighting rather than first egg (Whitehead and Fleming, 2000).

Another factor influencing calcium deposition in pullets is the strain of layer. Bone characteristics have been demonstrated to have a high degree of heritability (Fleming *et al*., 1997). As laying hens have been intensively selected for high egg production, low body weight and feed intake, it may be that hens may have been inadvertently selected for bone fragility (Bishop *et al*., 2000). "Heritage" strains are relatively unaffected by bone weakness compared to modern hybrid layer strains (Rennie *et al*., 1997). Multiple studies have found that bone strength is strain related making it an important consideration in optimizing bone mineral status (Anderson *et al.*, 1995, Budgell and Silversides, 2004, and Fleming *et al*., 2006).

Trends in management practices resulting in decreased age of sexual maturity without concurrent increases in mineral content of the feed may combine with a pullet's genetics resulting in bones not prepared for the intensive demands of egg production. The modern hen produces more eggs for longer periods (persistency) with less feed, due to improved feed efficiency. This represents more calcium output with less calcium input with a shorter down time for bone remodeling. The inability of bone to reach its full mineralization potential during rearing could contribute to metabolic calcium insufficiency observed later in life as osteoporosis. Our objective was to evaluate the influence of elevated versus control constant ratio of calcium and phosphorous and strain of layer on growth and bone mineralization during the rearing phase while following the performance and bone mineralization of these birds into the lay cycle. This research will be presented in three parts with the first being the pullet performance during rearing, the second being the lay cycle performance of these same hens, and the final being the bone mineralization parameters measured during rearing and the latter portion of the lay cycle.

MATERIALS and METHODS

Animals and Housing

Eggs obtained from the respective commercial breeders of Hy-line W-36 (Hy-line International, Dallas Center, IA 50063) and Babcock B300 (ISA-Babcock, Inc., Ithaca, NY 14851) were set, hatched, and placed at the Piedmont Research Station, Poultry Unit in Salisbury, North Carolina. Day old chicks were assigned 13 chicks/cage in a quad-deck cage system in an environmentally-controlled, closed (light tight) brood-grow house with equal representation of treatments on each of 4 rows and 3 banks. Each cage was filled with 13 chicks (giving 48 in^2/pullet), and four cages comprised a replicate (52 pullets/replicate) with 28 replicates/treatment and 5,824 hens total. For statistical analysis, the replicates were assigned in a restricted randomized manner being that all treatments were approximately equally represented in all rows, levels, and rooms. All chicks were brooded in the same cage during the entire 16 wk rearing period. Pullets received a 3-phase feeding regimen throughout the 16 wk rearing period. The experiment was a 2 x 2 factorial arrangement of two strains Hy-Line W-36 (H) and Babcock 300 (B) and two Ca:P ratios, increasing 2.14, 3.14, 4.14 (RC+) and control constant (RC) 2.14, 2.14, and 2.42 (RC) in growth phases of starter (0-6 weeks), grower (7-12 weeks), and developer (13-17 weeks), respectively (Table 1). Treatment diets within a phase were formulated to be isocaloric. All feed was fed as a mash and was formulated to meet or exceed NRC (1994) on an *ad libitum* basis. The experiment was conducted during the summer and fall months in a negative-pressure building with side-wall fans for cooling. Chicks were started on 24 hours of light which was stepped down by an hour every 2 days until day 27 when 11 hours of light/day was provided. This photoperiod was maintained until week 15 when the photoperiod was dropped to 10 hours of light/day before increasing to 12h:12h light:darkness at 15 wk and 14h:10h light:darkness at 16 wk. Beak trimming occurred from day 14-17 using a Lyons Precision beak trimmer with a 14/64" guide hole. Chicks were vaccinated for Marek's at hatch (neck injection), for Newcastle and Bronchitis (aerosol) at day 10, 35, 63, and 105 days of age, and for Fowl Pox and Avian Encephalomyelitis (wing web) at day 70. Feeding occurred daily, by hand during the morning hours. Body weight and feed weigh backs were done on a bi-

weekly basis beginning at 2 weeks of age and all mortality was recorded daily. Mortality which occurred between day 1 through 8 was excluded if attributed to accidental death or removal of males. Each pullet placed was provided with 1 kg of starter feed per bird until 6 weeks. Thereafter, all birds were placed onto the grower diet on which they remained until 12 weeks of age. From 12 weeks to approximately 16 weeks of age, all strains were provided with the developer diet. Care of birds used in this research was approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Growth Performance

Feed consumption and body weights were monitored bi-weekly beginning at 2 weeks of age. From this information, pullet body weight (BW), gain/bird (GN), feed efficiency measured as gain:feed (FE), and feed consumption/bird (FC) was calculated. Mortality was recorded daily.

Experimental Design

This rearing phase study utilized a Randomized Complete Block design with main effects set up in a 2 X 2 factorial arrangement of treatments. There were 4 blocks based on location in the grow house with all treatments equally represented in each row and level within a block. The main effects were Strain (Hy-line W-36 and Babcock B-300) and Diet (Ca:P Plus and Ca:P Control providing 2.14, 3.14, 4.14 and 2.14, 2.14, 2.39 Ca:P levels in starter, grower, and developer, respectively).

Statistical Analyses

The data were subjected to an Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of the SAS Institute (1998) software with strain of hen and diet as main effects along with their interactions. Two-way interactions were included in the models but nonsignificant interactions were removed. Means were partitioned using Least

Squared Means (LSMEANS) and statements of statistical significance were based upon $P \bullet 0.05$ unless otherwise noted. Mean separation was accomplished using the PDIFF s.

RESULTS

Results will be first reviewed by period and then overall. Period affect was significant for all growth data. Of all growth parameters measured, the only significant interaction was associated with mortality at 2 weeks of age. This interaction will be discussed with the review of mortality, but otherwise, only main effect averages will be reported.

Growth Performance by Period

Ca:P Treatments

When measuring variance among the Ca:P treatments by period (every 2 weeks), feeding elevated Ca:P regimen was observed in wks 5-6 for BW and GN, in wks 7-8 for FE, in wks 12-16 for FC, and in wks 15-16 for FE. During wks 5-6, feeding the control vs. increased Ca:P increased BW (RC=0.381 and RC+=0.363, P=0.03) and GN (RC=169.2 and RC+=152.6, P<0.001) (See Table 1.2 and 1.3). In the next period (weeks 7-8), FE was improved by feeding the control dietary regimen (RC=0.260 and RC+=0.222, P<0.01) which was also observed in the final period (RC=0.142 and RC+=0.096, P<0.01). In the last three periods, FC was changed due to feeding rearing dietary Ca. From weeks 11-12, FC was higher for the control fed birds (RC=0.905 and RC+=0.843 kg/bird, P<0.001), while this reversed from weeks 13-14 (RC=0.804 and RC+=0.837 kg/bird, P=0.01) and weeks 15-16 (RC=0.863 and RC+=1.021kg/bird, P<0.001).

Strain

When measuring variance between the two strains on body weight by period, outside of the first 2 weeks, BW was numerically greater for the H vs. B strain of pullet, but it was statistically greater from weeks 9-10 (P<0.01), weeks 11-12 (P=0.01), and weeks 13-14 (P=0.03) with the H vs. B bird weighing 0.684 vs. 0.629, 0.841 vs. 0.802, and 0.968 vs. 0.936 kg/bird, respectively. Gain was also increased from week 9-10 due to strain (H=142.7 and B=102.3 g/bird, P<0.001) as well as FE (H=0.206 and B=0.148, P<0.01). This pattern in FE

was also observed from weeks 5-6 (H=0.350 and B=0.321, P<0.01). The only period in which FC was significantly affected by strain was the final period (weeks 15-16) when strain B consumed more than H (H=0.925 and B=0.959 kg/bird, P=0.03).

Overall Effect of Strain and Ca:P Treatments from 0-17 weeks

Bird weights were initially no different between the Ca:P dietary treatments (RC+=37.97 and RC=38.30 g, P=0.28) and Strain treatments (H=37.90 and B=38.37 g, P=0.13). The cumulative view of data (See Table 1.5), from week 0-16 found no interactions between rearing Ca:P diets and strain, but did find that feeding RC+ diet significantly increased FC (RC+=4.14 and RC=3.90 kg/bird, P<0.01) although it had no effect on GN (RC+=686 and RC=692 g, P=0.71) and FE (RC+=0.168 and RC=0.177, P=0.12). From weeks 0-16, strain had no effect on FC (H=4.92 and B=5.00 kg, P=0.37), GN (H=1,032 and B=1,014 g, P=0.98) or FE (H=0.210 and B=0.203 g, P=0.20).

Effect of Strain and Ca:P Treatments on Mortality by Period

Feeding the rearing Ca:P regimen had no significant effects on any period from 0-16 weeks, although numerically, the mortality was lower when feeding RC+ compared to RC diets from week 3-16 (See Table 1.4). From weeks 3-8 and weeks 11-12, there were significant (P<0.05) effects of strain on mortality with higher mortality observed in strain B than H. It was not until the final period (weeks 15-16) that disparity in mortality levels between strains was modified (H=3.28 and B=4.98, P=0.66). In the first period (weeks 1-2) there was an interaction (P=0.04) of Ca:P*Strain. Mortality levels for Strain B were higher when fed RC+ compared to RC (0.95 vs. 0.52%, respectively), but this response was in reverse for Strain H where feeding RC+ reduced mortality compared to feeding RC (0.08 vs. 0.42%, respectively).

DISCUSSION

Providing the increasing Ca:P dietary regimens used in this study did impact growth performance of pullets at different stages of growth as did strain. Differences due to pullet

age are expected especially with animals in the rapid growth portion of the rearing phase. These changes due to diet and strain at different points in the growth cycle may give clues to the growth curves of the two strains and the responsiveness of the bird to the dietary regimens at various points in its growth curve. Recalling that dietary changes in the Ca:P ratio occurred at 0-6, 7-12, and 13-16 wks, the period results will be accordingly discussed. The relative amount of Ca to P was the same (2.14) for the dietary treatments except that the RC+ diet provided 1.18 and 0.55% Ca:AvP and the RC diet supplied 0.90 and 0.42% Ca:AvP. The breeder guides for the B strain recommends 1.1 - 1.2% Ca to 0.50 - 0.55% AvP while the H strain recommends 1.0% Ca to 0.50% AvP during the first 6 weeks. This would indicate that the RC+ diet would be more suited to the B strain while the H strain might perform better on the RC diet. When reviewing the one interaction observed in this study involving mortality from week 0-2, an opposite response was observed with the H strain responding favorably to the higher levels of Ca and AvP (with a 5 fold reduction in mortality) while the B strain saw reduced (2 fold) mortality by feeding the lower level of Ca and AvP. Also when feeding the same Ca:AvP level of 2.14 from 0-6 weeks, there was the only age with a significant effect on body weight and gain which was increased from week 5-6 by feeding the control level of Ca and AvP. Feed consumption was unchanged during this period so that this effect would not be attributed to increased energy intake. These findings in this study indicate that very early calcium requirements may warrant further investigation in promoting earlier body weight gain as well as to provide a protective effect against early mortality.

Feed consumption was increased due to rearing Ca:P dietary treatments from 13-16 weeks. It was greater from 11-12 weeks when feeding the control Ca:P diet but from weeks 13-16, it was greater due to feeding the higher Ca:P diets. There was a change in Ca:AvP level at this point as 3.14 and 2.14 Ca:AvP was supplied in the RC+ and RC diets, respectively, from 6-12 weeks while 4.14 and 2.42 Ca:AvP was supplied from 13-16 weeks. This increase in FC did not yield increases in BW so that FE was significantly impaired by feeding the higher Ca:AvP level. Also, as day length was increased at week 15 (which would stimulate calcium mobilization in the body) the response from week 15-16 may have been associated with photo-stimulation. Also, from week 15-16, FC was also significantly greater

in the B strain. This late but more pronounced response of the B strain might attest that it is a late-maturing rather than early maturing bird as it reaches 50% production 1.15 weeks later than the H strain. The H strain was heavier than the B strain throughout the last 7 periods and was significantly heavier at week 10, 12, and 14. This is consistent with the management guides of the two breeders that the H strain would be heavier at this point. The H strain also gained significantly more during week 10 and had an improved ($P<0.01$) FE at both week 6 and 10 also supporting the tendency of this bird to be earlier maturing. Breeder recommendation for the H strain is that the first increase in Ca should come at week 16 whereas in this study it occurred at week 7. This may have proved beneficial to this strain at this point in its growth. The one period that feed consumption was significantly affected by strain was from 15-16 weeks in which the B strain consumed more feed than the H strain. This may indicate that the B Strain is a late maturing bird compared to the H strain. Mortality was significantly higher in the B strain compared to H strain for 5 periods which was not mediated by the Ca:P levels fed during rearing outside the interaction in the first period.

There is little current research focusing on effects of feeding higher calcium to pullets before the prelay period. In this study, feed consumption increased from week 0-16 by feeding higher levels of Ca:P during rearing although this did not translate as higher gain or feed. Research has indicated that elevated calcium in layers may depress feed intake (Hurwitz et al., 1969) which may indicate changes in need for calcium with current genetics and production. Although previous research has indicated adverse effects due to feeding high dietary calcium (Wideman *et al*., 1985), results in this study observed no negative effects on growth of pullets due to feeding higher levels of Ca:AvP. It has been observed by Keshavarz (1987) that feeding calcium-rich diets to non-laying pullets did not affect kidney weight and plasma concentrations of uric acid. This suggests that pullets may be able to tolerate more Ca:AvP in rearing than was previously recognized. There were no significant differences between strains on the growth parameters measured from 0-16 weeks of age although the H strain numerically consumed less feed, gained more, and had a slightly improved feed efficiency which correlate with breeder specifications. This may be artifact in part due to differences of earlier (H strain) compared to later (B strain) maturing strains. The

results of this study suggest that feeding a higher regimen of Ca:AvP would not be detrimental to bird performance. Growth performance was not affected by rearing Ca:P dietary regimen or strain although results by period indicate that the response to increasing Ca:P during rearing may be influenced by period (age) and strain. It remains to be seen how feeding more calcium during rearing will impact bone mineralization during the rearing and lay cycle.

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Table 1.1. Formulation and calculated analyses of Ca:P Control and Ca:P Plus Starter, Grower, and Developer diets[1] fed from 0-17 weeks of age[2].

Ingredients	Starter		Grower		Developer	
	Ca:P Control	Ca:P Plus	Ca:P Control	Ca:P Plus	Ca:P Control	Ca:P Plus
	%					
Corn , Yellow	55.43	56.41	63.94	65.95	67.34	70.03
Soybean Meal	23.64	25.18	21.54	23.14	16.0	18.69
Wheat Middlings	9.43	6.16	7.32	2.00	10.0	0.98
Corn Gluten Meal	5.11	4.68	----	----	----	----
Fat, Vegetable	2.23	2.30	2.30	2.20	2.01	2.13
Dicalcium Phosphate	1.55	2.27	2.07	2.29	1.77	2.34
Calcium Carbonate	1.37	1.73	1.64	3.19	1.82	4.64
Lysine 98%	0.37	0.33	0.12	0.05	0.12	0.08
D-L Methionine	----	----	0.06	0.05	0.12	0.10
Choline Chloride	0.27	0.27	0.17	0.17	0.04	0.04
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Sodium Bi-Carbonate	0.06	0.12	0.29	0.39	0.23	0.42
Layer Vitamin Premix[3]	0.10	0.10	0.10	0.10	0.10	0.10
Mold Inhibitor	0.10	0.10	0.10	0.10	0.10	0.10
Layer Mineral Premix[4]	0.05	0.05	0.05	0.10	0.05	0.05
Color Tracer	0.05	0.05	0.05	0.10	0.05	0.05
Calculated Nutrients						
Protein %	21.0	21.0	17.0	17.0	15.0	15.0
ME kcal/kg	2975.0	2975.0	2975.0	2975.0	2975.0	2975.0
Lysine %	1.30	1.30	0.97	0.97	0.85	0.85
TSAA %	0.75	0.75	0.65	0.65	0.65	0.65
Calcium %	0.90	1.18	1.10	1.73	1.10	2.28
Available Phosphorous	0.42	0.55	0.52	0.55	0.46	0.55
Ca:P	2.14	2.14	2.13	3.14	2.42	4.14

[1] Diets fed as mash

[2] Starter fed 0-6 wks, Grower fed 7-12 wks, and Developer fed 13-17 wks

[3] Vitamin mix supplied /lb: Vit. A, 5500 IU; Vit. D, 1300 IU; Vit. E, 13.33 mg; Vit. K-Menadione, 1.163 mg; Vit. B, 127.780 mcg; Biotin, 0.118 mg; Choline, 750 mg; Folic Acid, 0.668 mg; Niacin, 34.8 mg; Pantothenic Acid, 10.36 mg; Pyridoxine, 1.2 mg; Riboflavin, 3.686 mg; Thiamin, 2.087 mg

[4] Mineral premix supplied/lb. of diet: Mn, 79.642 ppm; Zn, 130.042 ppm; Fe, 0.034%; Cu, 20.403 ppm; I, 3.385 ppm; Cobalt, 0.174 ppm; Selenium, 0.398 ppm

[5] Selenium/lb. 0.06%

Table 1.2. Body weight and feed consumption of two strains of pullets from weeks 0-16 fed increased calcium during rearing[1].

	Week							
	2	4	6	8	10	12	14	16
Body Weight (kg/bird)								
Rear Ca:P								
Rear Plus	0.104	0.210	0.363[b]	0.529	0.658	0.823	0.960	1.05
Rear Control	0.104	0.212	0.381[a]	0.539	0.655	0.820	0.945	1.07
Strain								
Hyline W-36	0.103	0.215	0.378	0.542	0.684[a]	0.841[a]	0.968[a]	1.07
Babcock B-300	0.105	0.207	0.365	0.526	0.629[b]	0.802[b]	0.936[b]	1.05
SEM	0.003	0.007	0.008	0.012	0.017	0.015	0.015	0.019
	Probability							
Ca:P	0.98	0.80	0.03	0.44	0.86	0.84	0.30	0.52
Strain	0.59	0.25	0.10	0.20	<0.01	0.01	0.03	0.36
Ca:P*Strain	0.68	0.79	0.75	0.59	0.67	0.84	0.23	0.99
Cons/Bird (kg/bird)								
Rear Ca:P								
Rear Plus	0.135	0.340	0.480	0.763	0.691	0.843[b]	0.837[a]	1.021[a]
Rear Control	0.149	0.350	0.485	0.609	0.718	0.905[a]	0.804[b]	0.863[b]
Strain								
Hyline W-36	0.152	0.334	0.478	0.690	0.709	0.884	0.817	0.925[B]
Babcock B-300	0.132	0.350	0.488	0.681	0.701	0.864	0.825	0.959[A]
SEM	0.016	0.009	0.010	0.015	0.015	0.016	0.014	14.96
	Probability							
Ca:P	0.38	0.59	0.63	0.44	0.86	<0.001	0.01	<0.001
Strain	0.24	0.06	0.30	0.20	0.10	0.21	0.47	0.03
Ca:P*Strain	0.67	0.50	0.98	0.59	0.67	0.19	0.13	0.97

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$)

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 1.3. Feed efficiency (gain:feed) and gain of two strains of pullets from weeks 0-16 fed increased dietary calcium during rearing[1]

	Week							
	2	4	6	8	10	12	14	16
Gain:Feed								
Rear Ca:P								
Rear Plus	.496	.321	.327	.222[b]	.189	.195	.167	.096[b]
Rear Control	.489	.304	.345	.260[a]	.166	.184	.157	.142[a]
Strain								
Hyline W-36	.468	.329	.350[a]	.243	.206[a]	.179	.161	.113
Babcock B-300	.516	.296	.321[b]	.239	.148[b]	.200	.163	.125
SEM	.020	.014	.007	.010	.012	.008	.011	.011
	Probability							
Ca:P	0.82	0.40	0.10	<0.01	0.18	0.39	0.51	<0.01
Strain	0.10	0.11	<0.01	0.77	<0.01	0.07	0.91	0.46
Ca:P*Strain	0.36	0.54	0.61	0.74	0.82	0.39	0.06	0.24
Gain (g/bird)								
Rear Ca:P								
Rear Plus	66.3	105.7	152.6[b]	166.8	128.6	164.9	137.0	94.9
Rear Control	66.1	107.3	169.2[a]	157.6	116.4	164.9	124.7	122.4
Strain								
Hyline W-36	65.5	111.3	163.7	163.1	142.7[a]	156.5	127.4	101.3
Babcock B-300	66.9	101.7	158.0	161.3	102.3[b]	173.3	134.3	116.0
SEM	3.53	5.89	4.51	7.88	11.09	9.38	11.57	14.96
	Probability							
Ca:P	0.94	0.80	<0.001	0.25	0.28	0.99	0.29	0.07
Strain	0.69	0.25	0.21	0.83	<0.001	0.08	0.52	0.33
Ca:P*Strain	0.61	0.79	0.87	0.62	0.91	0.63	0.07	0.23

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$)

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 1.4. Mortality of two strains of pullets from weeks 0-16 fed increased dietary calcium during rearing[1].

	Week							
	2	4	6	8	10	12	14	16
Mortality (%)								
Rear Dietary Ca:P								
Rear Plus	0.52	0.76	1.75	2.16	2.52	2.14	3.24	3.98
Rear Control	0.47	1.01	2.24	2.64	3.10	3.63	3.59	4.27
Strain								
Hyline W-36	0.25	0.26[b]	1.33[b]	1.72[b]	2.14	2.67[b]	2.62	3.28
Babcock B-300	0.74	1.51[a]	2.65[a]	3.08[a]	3.48	3.10[a]	4.21	4.98
SEM	0.016	0.564	0.683	0.015	0.015	0.016	0.013	0.019
	Probability[2]							
Ca:P	0.83	0.91	0.37	0.43	0.47	0.53	0.62	0.13
Strain	<0.01	<0.001	0.02	0.03	0.06	0.05	0.09	0.66
Ca:P*Strain	0.04	0.21	0.47	0.50	0.65	0.60	0.63	0.73

[a-b] Means within the same column with no common superscript are significantly different ($P<0.05$)

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

[2] P-value derived from arc sin transformation

Table 1.5. Total performance of two strains of pullets fed increased dietary calcium during rearing[1] from 0-17 weeks of age.

	Gain kg/bird	**Feed Cons** kg/bird	**Feed Efficiency** Gn/Fd
Main Effects			
Rear Ca:P			
Rear Plus	1.017	**5.11**[a]	0.199
Rear Control	1.029	**4.81**[b]	0.214
SEM	13.39	0.063	0.00394
Strain			
Hyline W-36	1.032	4.92	0.210
Babcock B-300	1.014	5.00	0.203
SEM	13.39	0.063	0.00394
		Probability	
Ca:P	0.53	**<0.01**	0.08
Strain	0.35	0.37	0.20
Ca:P*Strain	0.43	0.17	0.43

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

CHAPTER 2

INFLUENCE OF FEEDING INCREASED CA:P LEVEL FROM REARING INTO LAY CYCLE ON LAYER PERFORMANCE OF TWO COMMERCIAL STRAINS REARED AT TWO DENSITIES

To fully realize the advantage of feeding increasing Ca:P regimens at an earlier age in pullet diets, these affects would need to carry-over into lay cycle performance. Gains made during rearing by feeding more calcium may also serve to offset mortality associated with cage layer osteoporosis which has been reported as high as 35% (McCoy *et al*., 1996). As hens consume more feed and lay more eggs at lower stocking densities, density would be another consideration to better understand calcium needs of layers especially with trends to lower density for improved animal welfare. Our objective was to evaluate layer performance during the first lay cycle as effected by layer Ca and P regimens, pullet Ca:P regimens, density, and strain. Hens were housed in environmentally-controlled layer houses in tri- or quad-deck cages from 18-66 wks of age. A 2 X 2 X 2 X 2 factorial arrangement with 5,728 hens (26 replicates/treatment) were used to evaluate the effects of rearing dietary regimen increasing Ca:P (RC+) and control (RC) regimens, strains Hy-Line W-36 (H) and Babcock 300 (B), layer dietary regimen increasing Ca and P (LC+) and constant (LC), and low density (LD) with 413 cm^2/hen and high density (HD) with 310 cm^2/hen on the lay cycle performance parameters. The lay cycle dietary calcium regimens were based upon the increases in feed intake that occur during the lay cycle; birds fed LC+ were on a fixed 4.1% Ca and 0.42% Av.P level throughout the study so that Ca intake increased during the lay cycle while birds fed LC had Ca and P that lowered from 4.0 to 3.5% Ca and 0.40 to 0.36 % AvP by the end of the study creating a constant intake of Ca and P. All diets were isocaloric at 2925 kcal/kg ME and fed *ad libitum*. Performance was monitored via measurements of feed consumption (FC) every 4 wks and daily collections of egg production and mortality. Performance measured as kg feed consumption/100 hens (FC/100), g eggs produced/g feed consumed (FE), average number of eggs produced/100 hens/day (HDProd) was not significantly (P>.05) effected by layer dietary regimen (LC+ or LC) or rearing dietary regimen (RC+ or RC). Mortality was not affected by rearing (P=0.27) or lay (P=0.81) dietary treatments, but the B strain had higher mortality than the H strain (20.4% vs. 5.3%,

(P<0.001) and LD resulted in higher mortality than HD (15.2 vs. 10.5%, P<0.001. Strain and density effected (P<0.05) most production parameters measured. The H strain compared to B had lower feed consumption/100 hens (9.7 vs. 10.3 kg/100 hens, P<0.01) and lower hen day production (80.1 vs. 81.3 %, P<0.01). The LD vs. HD showed lower feed cons/100 hens (10.4 vs. 9.5 kg, p<.0001) and higher hen day production (82.9 vs. 78.5 %, P<0.001). A significant three way interaction occurred between strain, lay cycle diets, and density (P<0.05). Hens responded similarly to the LC+ regimen when kept at HD regardless of strain. However, if these hens were housed at LD then the H strain responded favorably to the LC+ regimen. At LD, the H vs. B strain when fed LC+ consumed less feed (9.98 vs. 10.91 kg FC/100, P=0.05), had an improved FE (0.47 vs. 0.45 g egg/ g feed, P=0.05), elevated eggs/hen housed (272 vs. 265 eggs/HH, P=0.02) and increased egg income ($16.10 vs. $15.64, P=.04). The findings of this study suggest that to optimize Ca and P levels for improved lay cycle performance, an intricate balance exists between strain of layer, housing density, and calcium levels fed during the lay cycle.

INTRODUCTION

Although calcium demands during rearing are considerable, calcium mobilization during the lay cycle is relatively massive. Egg shell formation will demand the transport of 2-2.5g Ca within a 15 hr period across the shell gland for just one egg. This will drop circulating calcium to such a point as to stimulate bone resorption. Bone resorption is more so limited to medullary bone but may involve cortical bone especially with calcium insufficiency. During rearing, medullary bone, a labile calcium reserve set down with the onset of sexual maturity will form at the expense of structural bone (Hudson, 1993). As birds come into the lay cycle at younger ages due to trends towards increasing sexual maturity, they may begin the lay cycle with a lower bone mineralization status and at a metabolic disadvantage. As the demands of eggshell formation continue throughout an extensive lay cycle, the constant bone remodeling to supply calcium for the eggshell will eventually reveal any inadequacies in bone status. Furthermore, as laying hens are genetically selected for lighter body weight, better feed efficiency, and increased egg production, this would further exacerbate conditions of calcium insufficiency as the bird has less overall mineral reserves, less feed (thus calcium) intake, and more egg (or calcium) output. Genetic selection also

may be creating an impediment of calcium status during the lay cycle. As layers achieve increased egg production through genetic selection, they may inadvertently be selected for decreased bone strength (Whitehead and Fleming, 2000). Housing conditions of layers may also lead to impaired calcium status due to the lack of bone-loading associated with the inactivity of hens in high density battery cages. These issues working independently or jointly, may be enough to predispose laying hens towards inadequate calcium status which causes the hen to utilize the mineral content of the bone to fund its calcium demands. This would result in bones with less mineral per unit of osteoid tissue giving rise to osteoporotic conditions. The prevalence of osteoporosis in laying hens may be a marker for larger issues regarding calcium metabolism and depletion that may ultimately predispose layers to osteoporotic mortality. Osteoporosis which has been found to contribute 35% (3.4 of 9.7%) of the total mortality in a commercial flock of 5,000 laying hens (McCoy *et al.*, 1996) while it was observed by Rennie *et al.* (1997) that the majority of hens from a commercial strain of layers were osteoporotic by the end of lay. Reduced calcium status in layers may be mediated by increasing dietary calcium during the lay cycle especially in strains of layers or housing conditions that predispose the hen to increased demands for calcium. Furthermore, as the hen ages, her ability to absorb calcium declines which indicates that feeding more calcium during the lay cycle might offset the reduced calcium status of the aging laying hen (Al-Batshan *et al*., 1994). The objectives of this study was to determine if feeding an increasing or a constant level of calcium and phosphorous during the lay cycle to two commercial strains at two cage densities could improve overall laying hen performance while determining if the effects of feeding elevated calcium and phosphorous during rearing would be realized into the lay cycle as improved layer performance.

MATERIALS & METHODS

Animals and Housing

Further detail regarding the rearing of pullets used in this study can be found in the previous study (See Manuscript I). Birds used in this trial were hatched at the Piedmont Research Station, Poultry Unit in Salisbury, North Carolina after eggs were obtained from the respective commercial breeders of Hy-line W-36 (Hy-line International, Dallas Center, IA 50063) and Babcock B300 (ISA-Babcock, Inc., Ithaca, NY 14851). All chicks were

brooded in the same cage of the grow house during the entire 16 wk rearing period before being moved to quad-deck cages in the environmentally-controlled lay houses of this study where birds were housed at either low density (LD) with 413 cm^2/hen or 64 in^2/hen or high density (HD) with 310 cm^2/hen or 48in^2/hen from 18-66 wks of age. Replicate cages were all 40.5 cm deep but varied in a width of either 30.5 or 40.5 cm while keeping bird population constant at 4 hens/cage. Treatments were assigned to replicates in a restricted randomized manner with all treatments being approximately equally represented in all rows, levels, and cage sizes. All individual cages within each block of either 6 or 8 cages created a replicate containing either 24 or 32birds/replicate.

During the first 16 weeks, pullets received a 3-phase feeding regimen which consisted of the two dietary rearing treatments indicated in this study (two Ca:P ratios which were an elevated (RC+) and control (RC) ratio of Ca:P (2.14, 3.14, 4.14 and 2.14, 2.14, 2.42 respectively) fed in growth phases of starter (0-6 weeks), grower (7-12 weeks), and developer (13-17 weeks). This study also utilized two dietary lay treatments of feeding increasing calcium and a control or constant level of calcium. The increasing dietary regimen (HC) provided a fixed 4.1% calcium and 0.42% available phosphorous such that as feed consumption increased throughout the lay cycle so did calcium intake (See Table 2.1a and 2.1b) while the constant dietary regimen (LC) provided a drop from 4.0 to 3.5% calcium and 0.40 and 0.36% available phosphorous that reduced as feed consumption increased holding calcium intake constant (See Table 2.2a and 2.2b). Treatment diets within a phase were formulated to be isocaloric (2925 kcal/kg ME) and were fed at any given time to provide the nutrient intake appropriate as based on bird age, production stage, and average daily feed intake. All feed was fed as crumbles and was formulated to meet or exceed NRC (1994). Feed and water were provided *ad libitum*. The experiment was conducted in positive-pressure buildings with side-wall fans for cooling. Sensors monitored the inside temperatures and adjusted ventilation fans. Lighting was provided for 16.5 hours (7.5 hours darkness). Feed was weighed each 4 week period. Egg production and mortality was recorded daily. Care of birds used in this research was consistent with the Institutional Animal Care and Use Committee of North Carolina State University.

Growth Performance

Feed consumption was determined every 4 week period, and egg production was recorded daily along with mortality. All feed offered for consumption was recorded for each replicate and at 28 day intervals, feed not consumed was weighed back to calculate feed consumed. Daily feed intake was calculated as kg feed/100 hens/day. All potentially marketable eggs were collected and credited towards each test unit's (or replicate) egg production. Egg production was summarized at 28 day intervals and calculated on a hen-day basis. Egg weight was determined every 4 weeks by collecting all eggs within a 24 hour period which were weighed and sorted by size of Pee Wee, Small, Medium, Large, and Extra Large based on < 18, 18 – 21, 21 – 23.5, 23.5 – 27, and >27 ounces/dozen respectively. Percentages of eggs within each size category, average egg weight (g), and egg mass (g) were calculated and reported. Egg Quality was measured at 28 day intervals with all eggs produced within the previous 24 hours examined by candling light and graded according to current USDA standards for egg quality. Eggs were graded at the point of production with no handling prior to examination. Egg income was calculated using three-year regional average prices for farm value of eggs based on egg production and quality evaluation. Feed costs were based on the actual feed prices for each feed delivery which were summarized for the whole lay cycle. From these values, calculations were made for feed consumption (kg/100 hens/d), hen day egg production (average daily number of eggs produced per 100 hens/day), hen housed egg production (total number of eggs produced/number of birds housed at 18 weeks), egg mass (average daily production of egg mass in grams/hen day), mortality (% of birds to die between 18 – 66 wks), feed efficiency (g egg produced/g feed consumed), feed costs (feed costs per hen housed at 18 wks using the pounds/diet consumed and average price of each diet/ton which ranged from $155.60-$164.72/ton), and egg income (calculated income per hen housed at 18 wks from egg production using 3 year regional average egg prices as follows: Grade A Extra Large 80.2 cents/dozen, A Large 80.2, A Medium 64.8, A Small 49.1, A Pee Wee 24.6, all Grade B 24.6, and all Cracks 42.5 cents/dozen).

Experimental Design

This lay cycle study utilized a Completely Randomized Design with main effects set up in a 2 X 2 X 2 X 2 factorial arrangement of treatments. This factorial arrangement utilized 5,728

hens with 14 reps/trt (of 24 or 32 layers/rep depending on density). All treatments were equally represented in each row and level within each house. The main effects were Strain (Hyline W-36 (H) and Babcock B-300 (B)), Rearing Diet (Control Ca:P (RC) and elevated Ca:P (RC+) , Laying Diet ((increasing Ca and P (LC+) and costant (LC)), and Density (64 in^2/hen (LD) and 48in^2/hen (HD)).

Statistical Analyses

The data were subjected to an Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of the SAS Institute (1998) software with strain of hen and diet as main effects along with their interactions. Interactions were included in the models but nonsignificant interactions were removed. Means were partitioned using Least Squared Means (LSMEANS) and statements of statistical significance were based upon $P \bullet 0.05$ unless otherwise noted. Mean separation was accomplished using the PDIFF s.

RESULTS

Main effect averages will be reviewed where appropriate despite multiple two- and three-way interactions. Although feeding elevated dietary calcium in either the Rearing or the Lay Cycle had no significant main effects on layer performance, they did influence several two- and three-way interactions.

Rear

Rearing calcium dietary treatments (RC+ and RC) had no influence directly on layer performance (See Table 2.3 and Table 2.4) but did result in interactions involving egg size and grading (See Table 2.5 and 2.6). Feeding RC compared to RC+ did not affect lay cycle mortality (12.29 to 13.33%, P=0.27), feed consumption (9.99 and 9.99 kg/100 hens/day, P=0.97), feed efficiency (0.464 and 0.462, P=0.52), egg production (either as hen housed (256.1 and 256.2 eggs, P=0.83) or hen day (80.7 and 80.7 %, P=0.37)) (See Table 2.3). Egg mass (P=0.30), egg weight (P=0.73), and egg income (P=0.95) were also not affected by rearing dietary treatments (See Table 2.4). Egg sizes (See Table 2.5) were significantly affected by an interaction between rearing dietary treatments and density. Although percentage of Pee Wee (P=0.14) and Medium (P=0.77) size eggs were unaffected by feeding RC or RC+, Small (RC=7.65 and RC+=7.27 %, P=0.05), Large (RC=40.18 and RC+=39.56%, P=0.05), and Extra Large (RC=24.78 and RC+=25.60%, P=0.03) size eggs

were affected by an interaction between rearing diets and density. At LD, feeding elevated rearing calcium resulted in number of Small eggs decreasing from 7.71% to 6.59% respectively while at HD, feeding more rearing calcium caused number of small eggs to increase from 7.59% to 7.95% (See Fig. 2.9). As for Large eggs, feeding elevated rearing calcium resulted in number of Large eggs at LD to decrease from 41.91% to 39.79% respectively while at HD, feeding more rearing calcium caused number of Large eggs to increase from 38.45% to 39.34% (See Fig. 2.10). At LD, feeding elevated rearing calcium resulted in number of Extra Large eggs to increase from 25.16 to 27.89% respectively while at HD, feeding more rearing calcium caused number of Extra Large eggs to decrease from 24.41% to 23.32% (See Fig. 2.11). At HD, the RC diet yielded the most Small and Large eggs whereas the RC+ diet yielded the most Extra Large eggs at this density. The greatest number of Large eggs were produced at the HD feeding the RC diet (41.91%) while the greatest number of Extra Large eggs were produced also at HD but by feeding the RC+ diet (27.89%). Number of Grade A eggs and Checks (See Table 2.6) were significantly affected by an interaction between rearing dietary treatments, density, and strain. To discern this 3 way interaction, 2 way interactions will be reviewed (although 2 way interactions were not significant). Density by Rear Diet indicates that at LD number of Grade A eggs increases when feeding RC (RC+=97.99 and RC=98.09%), and it drops at HD (RC+=97.99 and RC=97.87%) (See Fig. 2.13). Density by Strain indicates that the H strain is responsive to density (LD=98.34 and HD=98.12%) while the B strain is not (LD=97.74 and HD=97.74%) regarding % Grade A eggs produced (See Figure 2.14). Rear diet by Strain suggests no interaction but finds that the H strain produces more Grade A eggs (98.23%) than the B strain (97.74%) (See Fig. 2.15). These results suggest that at higher production levels of Grade A eggs, there is an increased sensitivity especially at high stocking densities. Number of checks was higher in the B strain at HD with a greater response by feeding RC+ (See Figure 2.16).

Lay

Feeding the lay cycle dietary treatments (LC+ and LC) did influence feed efficiency, egg production, and egg income in a 3-way interaction. It did not affect feed consumption (LC=9.98 and LC+=10.00 kg/100 hens/day, P=0.74), HD Egg Production (LC=80.7 and

LC+=80.7 %, P=0.88), and mortality (LC=12.70 and LC+=12.93%, P=0.81) as well as the remaining parameters measured (See Table 2.3, 2.4, 2.5, and 2.6). To better understand the 3 way interaction between Strain*Density*Lay Diets, in the case of feed efficiency, a significant (P=0.05) Strain*Lay interaction gives indication of the relationship between these main effects. In this 2-way interaction, the Babcock strain responds favorably to LC while the Hy-line bird responds favorably to LC+ (See Fig. 2.1). To try and discern the affect of density, separate statistical analysis was completed for all treatments at low density as well at high density. When observing the same 2-way interaction of Strain*Lay at HD, there is no (P=0.99) interaction (Fig. 2.2), but at LD, the feed efficiency of the Babcock strain responds favorably to LC (P=0.04) while the Hy-line's feed efficiency is improved by feeding LC+ (See Fig. 2.3). This relationship also follows when analyzed across LD for Eggs/HH in which the Babcock averaged (P=0.02) 7 more eggs/bird when fed LC while the Hy-line bird produced 4.8 more eggs on LC+ (See Fig. 2.4). This relationship between Lay diets and Strain analyzed at LD also corresponds to the 3-way interaction (P=0.02) for Egg Income. Analyzing at LD, feeding LC+ to the Hy-line strain resulted in a $0.31 increase in egg income/bird while feeding LC diet to the Babcock strain increased egg income by $0.52/bird (See Fig. 2.7). Hen Day% egg production resulted in a 2-way interaction (P=0.05) between Strain*Density. The Babcock strain produces 5.34 % more eggs and the Hy-line produces 3.61% more eggs at LD compared to HD (See Fig. 2.5). This same Strain*Density 2-way interaction is observed (P=0.04) for Egg Mass with the Babcock and Hy-line producing more egg mass (3.8 and 2.6 g/bird/d respectively) at LD compared to HD (See Fig. 2.6), and it is observed (P=0.04) for Feed Cost/HH as feed costs are $0.80/HH higher for the Babcock and $0.56 higher for the Babcock at LD (See Fig. 2.8). In these three measures of growth, the responsiveness to density by the Babcock compared to Hy-line strain appeared greater.

Density

Most of the remaining performance parameters measured that did not have significant interactions had significant main effects of strain and density. Feed consumption was greater (P<0.001) for the Babcock compared to Hy-line strain (10.28 kg/100 birds/day vs. 9.71 kg/100 birds/day, respectively), and for the LD compared to HD housed birds (10.46 vs. 9.52 kg/100 birds/day). Mortality was not affected by rearing (P=0.27) or lay (P=0.81) dietary

treatments, but the B strain had higher mortality than the H strain (20.4% vs. 5.3%, P<0.001) and LD resulted in higher mortality than HD (15.2 vs. 10.5%, P<0.001) (See Fig. 2.12). Egg weight was also greater (P<0.001) for the Babcock compared to Hy-line strain (57.95 vs. 56.30 g/egg respectively), and for the LD compared to HD housed birds (57.49 vs. 56.76 g/egg). As for Egg Size, the Hy-line produced more (P<0.001) PeeWee and Medium eggs compared to the Babcock (4.98 and 2.74% Pee Wee and 25.11 and 21.55% Medium eggs respectively). Although there was an interaction involving Small and Extra Large size eggs, there were also main effects of strain with the Hy-line producing (P=0.04) more Small while the Babcock produced (P<0.001) more Extra Large eggs (See Table 2.5). There was a Density main effect on all egg sizes except Small, but there was an interaction involving density for the Small, Large, and Extra Large Egg Sizes. As for Egg Size, HD resulted in more (P<0.02) PeeWee and Medium eggs compared to LD (4.16 and 3.56% Pee Wee and 25.03 and 21.63% Medium eggs respectively). It is interesting to note that main effect of density for the Large and Extra Large Eggs was opposite that of smaller egg sizes with LD rather than HD that resulted in more (P<0.01) Large and Extra Large Eggs (40.84 and 38.89% Large and 26.53 and 23.86% Extra Large eggs respectively).

DISCUSSION

There were no main effects of period. Feeding more calcium during rearing or laying both were involved with layer performance influenced by the interactions of strain and density. There was minimal carryover effect of what was fed during rearing on lay cycle bird performance. Rearing dietary treatments were also demonstrated to have little long-term effect on adult characteristics by Leeson *et al.* (1997) although there were significant strain effects.

Small, Large and Extra Large egg sizes were affected by a rearing diet and density interaction. The number of Small and Large eggs increased when feeding RC+ to birds housed at HD and dropped at LD. Reduced feed consumption observed at HD may limit calcium intake which is being compensated by gains made in bone status through the increased calcium fed in the RC+ regimen. Number of Extra Large eggs increased at LD and decreased at HD when fed RC+. As birds produce more eggs at LD, this represents more calcium output as increased number of eggs and increased egg size due to the Extra Large

sizing which may represent a threshold of calcium demand such that the enhanced bone status acquired during rearing when feeding RC+ may assist the bird with the increased calcium demands due to egg size and numbers.

Feeding more calcium during the lay cycle influenced feed efficiency, egg production (HH), and egg income related to strain and density. However, feed consumption, HD Egg Production, and mortality were not affected by feeding more calcium during lay. Keshavarz and Nakajima (1993) also found no response of feeding increasing calcium during the lay cycle on feed consumption, egg production, and mortality. Some studies observe increased feed consumption with increased dietary calcium, but this response is observed more so when marginal calcium levels are being fed (Roland *et al*., 1996). In discussing the interaction between strain, density, and lay diets, the intricate nature of optimizing calcium levels in layers is realized. In this study, the Hy-line strain generally perform better on the LC+ diet while the Babcock strain performs better on the LC diet. This is observed with both feed efficiency, egg production (eggs/HH), and egg income. This influence between strain and lay calcium regimen is not observed at HD in which a reduction in egg production is observed. Egg production at LD would represent the greatest calcium output which would make the need for more dietary calcium more pronounced in the high-producing yet more feed efficient (less calcium intake) Hy-line strain. It is not surprising that these results translated into the observed increases in egg income/bird for the Hy-line strain as it was observed by Castillo et al. (2004) that 4.35% was the economic optimal level of calcium for maximum profitability. This value is in line with the 4.25% fed in the LC+ diet with both values being much higher than the 3.25% recommended in NRC (1994). Overall, increasing layer dietary calcium had no adverse effects on performance parameters measured in this study which suggests that there were no such negative consequences of feeding excess calcium such as metabolic alkalosis and uroliths (Wideman *et al*., 1985). However, it does raise the question if the inverse response of the Babcock strain to the LC+ diet was not partly attributed to an adverse response to higher calcium during lay albeit only at LD.

There were also several 2-way interactions involving strain and density. Both strains produced more egg mass at LD although the Babcock was more responsive in this regard. As for feed cost/HH, the higher levels of mortality associated with the Babcock strain may

contribute to its higher HH feed costs. The Babcock generally consumed more feed than the Hy-line but produced greater egg weights. Although the Babcock produced fewer numbers of eggs expressed as eggs/HH, it produced more eggs/HD% which took into account its higher levels of mortality. Also, it generally produced larger eggs which yielded higher egg mass. This makes the interaction involving strain and lay cycle calcium all the more surprising as it would have been thought that the Babcock might have responded favorably to the LC+ regimen. This suggests a nuance to calcium response that could be more strain dependent than previously recognized. Fleming et al. (2006) developed two lines of hens divergently selected for high or low bone strength. Changes in egg production and bone traits between these two lines somewhat simulate the varying responses of the Hy-line and Babcock strain which may indicate a "divergence" in calcium metabolism between these two commercial strains. This may also help to explain differences in sizes of eggs by the different strains although these numbers are also influenced by the Hy-line strain reaching peak production earlier than the Babcock.

These results indicate that rearing and lay cycle dietary calcium recommendations need to be made in light of strain and cage density. For example, hens housed at lower density generally consumed more feed and had greater egg production although the response was more pronounced in the Hy-line than Babcock strain. Fleming et al. (2006) noted that environment (cage vs. aviary) and genotype (high or low bone strength line of layers) were additive on bone strength which was similar in this study regarding high and low density and the Hy-line and Babcock strains. As animal welfare concerns result in shifts towards lowered stocking densities, calcium requirements for optimal lay cycle performance may need to be re-evaluated specific to egg production, feed intake, and type of bird being housed. It remains to be seen if feeding more calcium during rearing can improve bone mineral status such that even after the constant bone remodeling during the lay cycle, bone status would continued to be improved. This would suggest causality between bone modeling of rearing on the subsequent bone mineral status of laying and dietary calcium levels.

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Table 2.1a. Formulation and calculated analyses of constant (LC) calcium and phosphorous lay cycle dietary regimen fed from 18-66 weeks of age, Part 1.

Ingredient	LC Layer Diets				
	D	E	F	G	H
	Pounds Per Ton				
Corn	772.07	819.01	934.81	1000.11	1068.72
Corn Gluten Meal	100	75	85	90	90
Soybean Meal 48%	603.43	581.58	530.8	466.26	412.42
Wheat Middlings	145.62	150	100.14	109.38	110.93
Calcium Carbonate	200.49	194.3	188.83	184.03	178.56
DiCalcium Phosphate	21.2	23.15	24.36	24.02	24.91
Sodium Bi-Carbonate	16.74	16.66	17.72	17.54	17.52
Salt	5	5	5	5	5
Methionine	3.54	4.5	4.82	5.45	5.06
Lysine	*	1.31	*	1.9	2.66
Choline Chloride	5.49	5.35	5.27	5.2	5.1
Vitamin premix[1]	2	2	2	2	2
Mineral premix[2]	1	1	1	1	1
Fat	120.42	118.14	97.25	85.11	73.12
Mold Inhibitor	2	2	2	2	2
Tracer	1	1	1	1	1
Total[3]	2000	2000	2000	2000	2000
Calculated Analysis					
Protein %	22	21	20	19	18
ME kcal/kg	2925	2925	2925	2925	2925
Calcium %	4.1	4	3.9	3.8	3.7
Total Phosphorus %	0.59	0.6	0.59	0.58	0.58
Lysine %	1.14	1.15	1.02	1	0.95
Total Sulfur Amino Acids %	0.9	0.9	0.9	0.9	0.85

[1] Vitamin mix supplied /lb: Vit. A, 5500 IU; Vit. D, 1300 IU; Vit. E, 13.33 mg; Vit. K-Menadione, 1.163 mg; Vit. B, 127.780 mcg; Biotin, 0.118 mg; Choline, 750 mg; Folic Acid, 0.668 mg; Niacin, 34.8 mg; Pantothenic Acid, 10.36 mg; Pyridoxine, 1.2 mg; Riboflavin, 3.686 mg; Thiamin, 2.087 mg

[2] Mineral premix supplied/lb. of diet: Mn, 79.642 ppm; Zn, 130.042 ppm; Fe, 0.034%; Cu, 20.403 ppm; I, 3.385 ppm; Cobalt, 0.174 ppm; Selenium, 0.398 ppm

[3] Selenium/lb. 0.06%

Table 2.1b. Formulation and calculated analyses of constant (LC) calcium and phosphorous Lay Cycle dietary regimen fed from 18-66 weeks of age, Part 2.

Ingredient	LC Layer Diets					
	I	M	N	O	P	Q
	Pounds Per Ton					
Corn	1136.5	1211.9	1233.3	1215.7	1318.2	1390.2
Corn Gluten Meal	100	85	50	50	25	25
Soybean Meal 48%	346.86	314.14	300.8	223.71	216.04	162.24
Wheat Middlings	109.19	103.88	147.81	256.18	200	200
Calcium Carbonate	178.31	168.03	158.33	155.44	150.2	145.85
DiCalcium Phosphate	26	25.88	24.14	19.6	20.3	18.78
Sodium Bi-Carbonate	17.56	17.69	16.77	14.52	15.7	15.71
Salt	5	5	5	5	5	5
Methionine	4.56	4.32	3.36	2.2	1.97	1.58
Lysine	3.8	3.91	1.97	1.8	2.61	3.37
Choline Chloride	5.04	4.91	4.71	4.6	4.46	4.37
Vitamin premix[1]	2	2	2	2	2	2
Mineral premix[2]	1	1	1	1	1	1
Fat	61.19	49.3	47.79	45.26	34.79	21.94
Mold Inhibitor	2	2	2	2	2	2
Tracer	1	1	1	1	1	1
Total[3]	2000	2000	2000	2000	2000	2000
Calculated Analysis						
Protein %	17	16	15	14	13	12
ME kcal/kg	2925	2925	2925	2925	2925	2925
Calcium %	3.7	3.5	3.3	3.2	3.1	3
Total Phosphorous %	0.58	0.57	0.56	0.54	0.52	0.5
Lysine %	0.9	0.85	0.75	0.65	0.65	0.6
Total Sulfur Amino Acids %	0.8	0.75	0.65	0.55	0.5	0.45

[1] Vitamin mix supplied /lb: Vit. A, 5500 IU; Vit. D, 1300 IU; Vit. E, 13.33 mg; Vit. K-Menadione, 1.163 mg; Vit. B, 127.780 mcg; Biotin, 0.118 mg; Choline, 750 mg; Folic Acid, 0.668 mg; Niacin, 34.8 mg; Pantothenic Acid, 10.36 mg; Pyridoxine, 1.2 mg; Riboflavin, 3.686 mg; Thiamin, 2.087 mg

[2] Mineral premix supplied/lb. of diet: Mn, 79.642 ppm; Zn, 130.042 ppm; Fe, 0.034%; Cu, 20.403 ppm; I, 3.385 ppm; Cobalt, 0.174 ppm; Selenium, 0.398 ppm

[3] Selenium/lb. 0.06%

Table 2.2a. Formulation and calculated analyses of increasing (LC+) calcium and phosphorous Lay Cycle dietary regimen fed from 18-66 weeks of age, Part 1.

Ingredient	LC Layer Diets				
	D	E	F	G	H
	Pounds Per Ton				
Corn	910.4	971.2	1070.1	1132.5	1196.5
Corn Gluten Meal	100	100	150	150	150
Soybean Meal 48%	647	595	471	413	360
Wheat Middlings	*	*	*	*	*
Calcium Carbonate	193	193	193	194	194
DiCalcium Phosphate	29	30	30	31	31
Sodium Bi-Carbonate	4	4	4	4	4
Salt	5.4	5.1	5.1	5.1	5.1
Methionine	2.4	3	3	3.6	3.1
Lysine	*	*	0.9	3	3.7
Choline Chloride	6.5	6.4	6.6	6.5	6.3
Vitamin premix[1]	1	1	2	2	2
Mineral premix[2]	1	1	1	1	1
Fat	97	87	61	52	41
Mold Inhibitor	2	2	2	2	2
Tracer	1	1	1	1	1
Total[3]	2000	2000	2000	2000	2000
Calculated Analysis					
Protein %	22	21	20	19	18
ME kcal/kg	2925	2925	2925	2925	2925
Calcium %	4.1	4.1	4.1	4.11	4.1
Total Phosphorous %	0.63	0.63	0.61	0.61	0.6
Lysine %	1.2	1.12	1	1	0.95
Total Sulfur Amino Acids %	0.9	0.9	0.9	0.9	0.85

[1] Vitamin mix supplied /lb: Vit. A, 5500 IU; Vit. D, 1300 IU; Vit. E, 13.33 mg; Vit. K-Menadione, 1.163 mg; Vit. B, 127.780 mcg; Biotin, 0.118 mg; Choline, 750 mg; Folic Acid, 0.668 mg; Niacin, 34.8 mg; Pantothenic Acid, 10.36 mg; Pyridoxine, 1.2 mg; Riboflavin, 3.686 mg; Thiamin, 2.087 mg

[2] Mineral premix supplied/lb. of diet: Mn, 79.642 ppm; Zn, 130.042 ppm; Fe, 0.034%; Cu, 20.403 ppm; I, 3.385 ppm; Cobalt, 0.174 ppm; Selenium, 0.398 ppm

[3] Selenium/lb. 0.06%

Table 2.2b. Formulation and calculated analyses of increasing (LC+) calcium and phosphorous Lay Cycle dietary regimen fed from 18-66 weeks of age, Part 2.

Ingredient	LC Layer Diets					
	I	M	N	O	P	Q
	Pounds Per Ton					
Corn	1258.4	1321.3	1368.6	1386	1436.4	1487.3
Corn Gluten Meal	150	150	130	75	60	45
Soybean Meal 48%	307	254	235	270	239	208
Wheat Middlings	*	*	*	*	*	*
Calcium Carbonate	194	194	194	194	195	195
DiCalcium Phosphate	32	32	32	32.1	32.4	32.7
Sodium Bi-Carbonate	4	4	4	4	3.5	3.5
Salt	5.1	5.1	5.2	5.3	5.3	5.3
Methionine	2.7	2.2	0.8	*	*	*
Lysine	4.3	5	3.3	*	*	*
Choline Chloride	6.2	6.1	5.8	5.3	5.1	4.9
Vitamin premix[1]	2	2	2	1	1	12
Mineral premix[2]	1	1	1	1	1	1
Fat	31	21	16	23	18	13
Mold Inhibitor	2	2	2	2	2	2
Tracer	1	1	1	1	1	1
Total[3]	2000	2000	2000	2000	2000	2000
Calculated Analysis						
Protein %	17	16	15	14	13	12
Me kcal/kg	2925	2925	2925	2925	2925	2925
Calcium %	4.1	4.1	4.1	4.1	4.11	3
Total Phosphorous %	0.59	0.58	0.58	0.58	0.58	0.5
Lysine %	0.9	0.85	0.75	0.65	0.6	0.6
Total Sulfur Amino Acid %	0.8	0.75	0.65	0.57	0.53	0.45

[1] Vitamin mix supplied /lb: Vit. A, 5500 IU; Vit. D, 1300 IU; Vit. E, 13.33 mg; Vit. K-Menadione, 1.163 mg; Vit. B, 127.780 mcg; Biotin, 0.118 mg; Choline, 750 mg; Folic Acid, 0.668 mg; Niacin, 34.8 mg; Pantothenic Acid, 10.36 mg; Pyridoxine, 1.2 mg; Riboflavin, 3.686 mg; Thiamin, 2.087 mg

[2] Mineral premix supplied/lb. of diet: Mn, 79.642 ppm; Zn, 130.042 ppm; Fe, 0.034%; Cu, 20.403 ppm; I, 3.385 ppm; Cobalt, 0.174 ppm; Selenium, 0.398 ppm

[3] Selenium/lb. 0.06%

Table 2.3. Feed consumption, feed efficiency and egg production of two strains of layers from 18-66 weeks of age housed at two cage densities and fed increased dietary calcium regimens during rearing[1] and the lay cycle[2].

Main Effects	Feed Cons kg/100/day	Feed Efficiency g egg/g feed	Egg Production Hen Housed	Egg Production Hen Day%
Strain				
Hy-line W-36	**9.71**[b]	0.467	**263**[a]	**80.1**[b]
Babcock B-300	**10.28**[a]	0.459	**249**[b]	**81.3**[a]
SEM	0.068	0.0026	1.14	0.3
Cage Density				
48 in^2	**9.52**[b]	**0.468**[a]	**246**[b]	**78.5**[b]
64 in^2	**10.46**[a]	**0.458**[b]	**266**[a]	**82.9**[a]
SEM	0.69	0.0025	1.14	0.3
Lay Dietary Ca&P				
Lay Constant	9.98	0.464	256.4	80.7
Lay Increasing	10	0.462	256.2	80.7
SEM	0.068	0.0026	1.14	0.3
Rear Dietary Ca:P				
Rear Control	9.99	0.462	256.1	80.5
Rear Plus	9.99	0.464	256.5	80.9
SEM	0.69	0.0026	1.15	0.3
		Probability		
Strain (S)	**0.0001**	0.06	**<0.001**	**<0.01**
Density (D)	**0.0001**	<**0.01**	**<0.001**	**<0.001**
Lay (L)	0.74	0.54	0.90	0.88
Rear (R)	0.97	0.52	0.83	0.37
Strain*Density	0.46	0.48	**<0.001**	**0.05**
Strain*Lay	0.33	0.05	0.24	0.12
Strain* Rear	0.73	0.70	0.89	0.64
Density*Lay	0.54	0.54	0.52	0.81
Density*Rear	0.31	0.64	0.88	0.55
Lay*Rear	0.72	0.87	0.83	0.40
S*D*L	0.06	**0.05**	**0.02**	0.41
S*D*R	0.39	0.98	0.97	0.46
S*L*R	0.40	0.97	0.44	0.57
D*L*R	0.91	0.86	0.72	0.59

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$)

[1] Rear Plus=elevated rearing Calcium:Available Phosphorous (Ca:P) 2.14, 3.14, 4.14 and Rear Control=control rearing Ca:P 2.14, 2.14, 2.42 ratios fed in starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

[2] Lay Increasing=increasing lay cycle calcium and available phosphorous (Ca&P) regimen providing fixed 4.1% Ca and 0.42% P and Lay Control=constant lay cycle Ca&P regimen providing reducing increments of 4.0-3.5% Ca and 0.40-0.36% P based on increasing feed intake from 18-66 weeks of age.

Table 2.4. Egg Mass, Egg Weight, Egg Income, and Feed Cost of two strains of layers from 18-66 weeks of age housed at two cage densities and fed increased dietary calcium regimens during rearing[1] and the lay cycle[2].

Main Effects	Egg Mass g/bird/day	Egg Weight g/egg	Egg Income Income/HH	Feed Cost Feed Cost/HH
Strain				
Hyline W-36	**45.51[b]**	**56.30[b]**	**15.44[a]**	5.87
Babcock B-300	**47.51[a]**	**57.95[a]**	**14.98[b]**	5.8
SEM	0.211	0.11	0.076	0.043
Cage Density				
48 in^2	**44.91[b]**	**56.76[b]**	**14.49[b]**	**5.50[a]**
64 in^2	**48.10[a]**	**57.49[a]**	**15.93[a]**	**5.17[b]**
SEM	0.21	0.11	0.076	0.043
Lay Dietary Ca&P				
Lay Constant	46.53	57.19	15.23	**5.71[b]**
Lay Increasing	46.48	57.06	15.19	**5.96[a]**
SEM	0.211	0.11	0.076	0.043
Rear Dietary Ca:P				
Rear Control	46.35	57.09	15.2	5.85
Rear Plus	46.66	57.15	15.21	5.82
SEM	0.211	0.11	0.076	0.043
		Probability		
Strain (S)	**<0.001**	**<0.001**	**<0.001**	0.2
Density (D)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Lay (L)	0.88	0.43	0.68	**<0.001**
Rear (R)	0.3	0.73	0.95	0.68
Strain*Density	**0.04**	0.56	**<0.001**	**0.04**
Strain*Lay	0.16	0.56	0.09	0.38
Strain* Rear	0.79	0.74	0.84	0.88
Density*Lay	0.94	0.49	0.5	0.36
Density*Rear	0.19	0.09	0.72	0.5
Lay*Rear	0.37	0.51	0.72	0.95
S*D*L	0.95	0.11	**0.04**	0.35
S*D*R	0.32	0.28	0.85	0.7
S*L*R	0.3	0.24	0.97	0.92
D*L*R	0.95	0.18	0.9	0.9

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$)

[1] Rear Plus=elevated rearing Calcium:Available Phosphorous (Ca:P) 2.14, 3.14, 4.14 and Rear Control=control rearing Ca:P 2.14, 2.14, 2.42 ratios fed in starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

[2] Lay Increasing=increasing lay cycle calcium and available phosphorous (Ca&P) regimen providing fixed 4.1% Ca and 0.42% P and Lay Control=constant lay cycle Ca&P regimen providing reducing increments of 4.0-3.5% Ca and 0.40-0.36% P based on increasing feed intake from 18-66 weeks of age.

Table 2.5. Egg Size of two strains of layers from 18-66 weeks of age housed at two cage densities and fed increased dietary calcium regimens during rearing[1] and the lay cycle[2].

	Egg Size				
Main Effects	PeeWee	Small	Medium	Large	XLarge
Strain					
Hyline W-36	**4.98**[a]	**7.84**[a]	**25.11**[a]	40.32	**21.50**[b]
Babcock B-300	**2.74**[b]	**7.08**[b]	**21.55**[b]	39.41	**28.89**[a]
SEM	0.179	0.263	0.518	0.532	0.627
Cage Density					
48 in^2	**4.16**[a]	7.77	**25.03**[a]	**38.89**[b]	**23.86**[b]
64 in^2	**3.56**[b]	7.14	**21.63**[b]	**40.84**[a]	**26.53**[a]
SEM	0.179	0.262	0.517	0.533	0.627
Lay Dietary Ca&P					
Lay Constant	3.83	7.47	23.15	39.44	25.89
Lay Increasing	3.89	7.45	23.51	40.29	24.49
SEM	0.179	0.262	0.518	0.534	0.628
Rear Dietary Ca:P					
Rear Control	3.67	7.65	23.44	40.18	24.78
Rear Plus	4.05	7.27	23.23	39.56	25.6
SEM	0.179	0.262	0.517	0.53	0.628
	Probability				
Strain (S)	**<0.001**	**0.04**	**<0.001**	0.23	**<0.001**
Density (D)	**0.02**	0.09	**<0.001**	**0.01**	**<0.01**
Lay (L)	0.8	0.95	0.63	0.26	0.12
Rear (R)	0.14	0.31	0.77	0.42	0.36
Strain*Density	0.41	0.69	0.75	0.59	0.89
Strain*Lay	0.12	0.78	0.72	0.43	0.98
Strain* Rear	0.78	0.73	0.91	0.5	0.65
Density*Lay	0.37	0.58	0.99	0.78	0.79
Density*Rear	0.99	**0.05**	0.78	**0.05**	**0.03**
Lay*Rear	0.93	0.51	0.83	0.69	0.85
S*D*L	0.61	0.21	0.56	0.78	0.3
S*D*R	0.65	0.67	0.41	0.34	0.86
S*L*R	0.89	0.35	0.8	0.72	0.59
D*L*R	0.95	0.61	0.5	0.35	0.13

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$)

[1] Rear Plus=elevated rearing Calcium:Available Phosphorous (Ca:P) 2.14, 3.14, 4.14 and Rear Control=control rearing Ca:P 2.14, 2.14, 2.42 ratios fed in starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

[2] Lay Increasing=increasing lay cycle calcium and available phosphorous (Ca&P) regimen providing fixed 4.1% Ca and 0.42% P and Lay Control=constant lay cycle Ca&P regimen providing reducing increments of 4.0-3.5% Ca and 0.40-0.36% P based on increasing feed intake from 18-66 weeks of age.

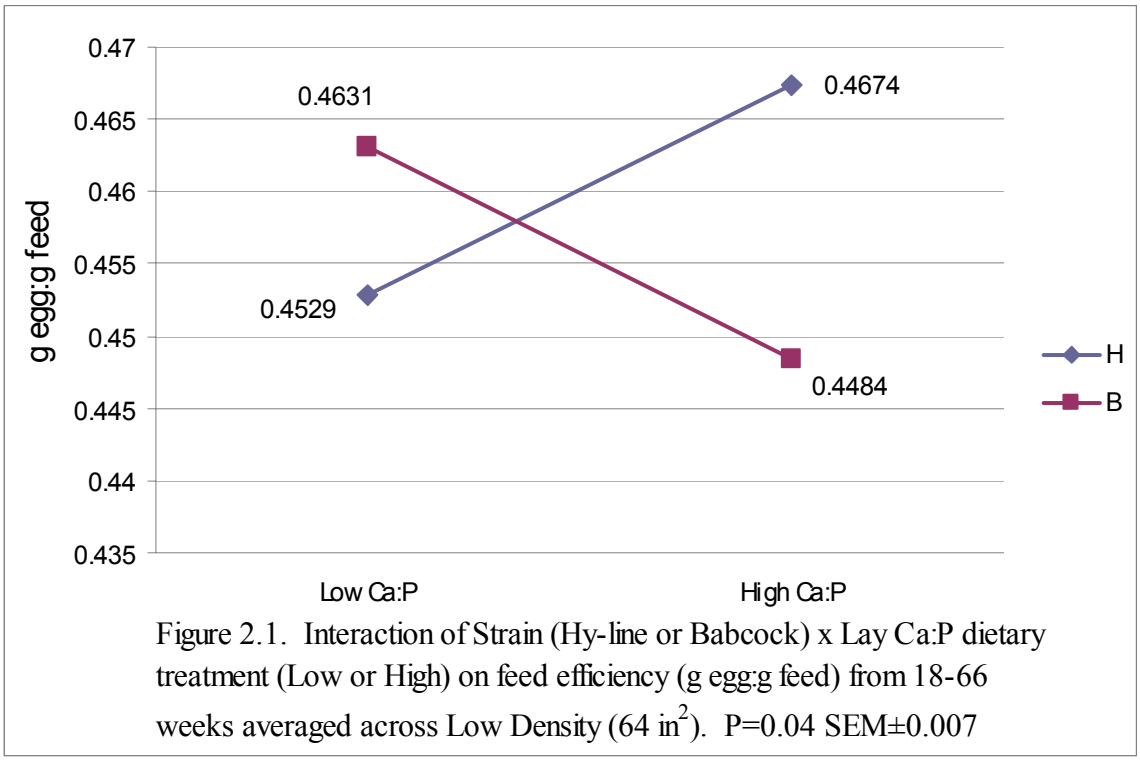

Figure 2.1. Interaction of Strain (Hy-line or Babcock) x Lay Ca:P dietary treatment (Low or High) on feed efficiency (g egg:g feed) from 18-66 weeks averaged across Low Density (64 in^2). P=0.04 SEM±0.007

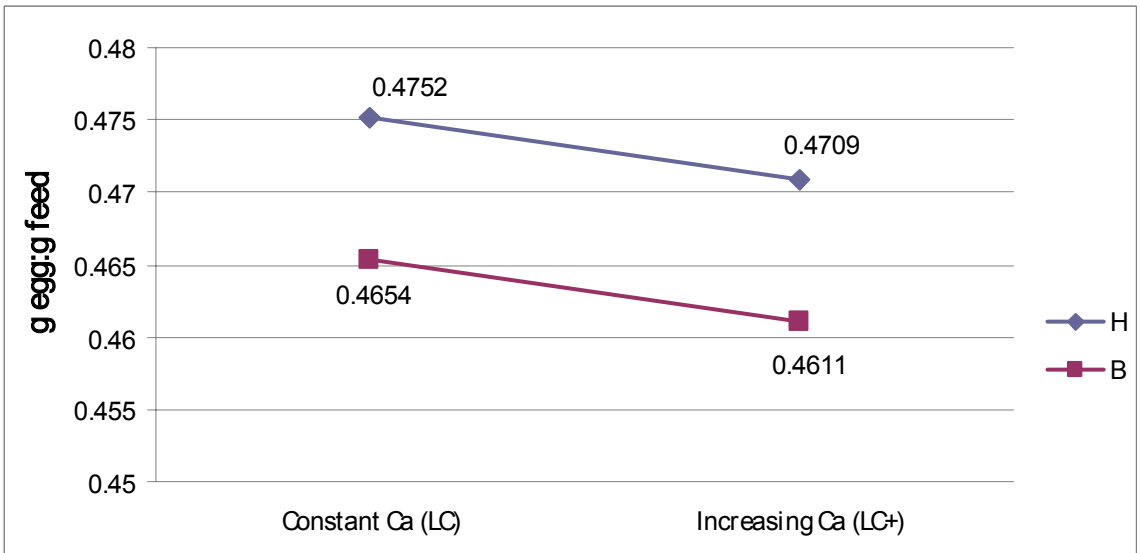

Figure 2.2. Interaction of Strain (Hy-line or Babcock) x Lay Ca:P dietary treatment (LC or LC+) on feed efficiency (g egg:g feed) from 18-66 weeks averaged across High Density (48 in^2). SEM ± 0.0034 P=0.99

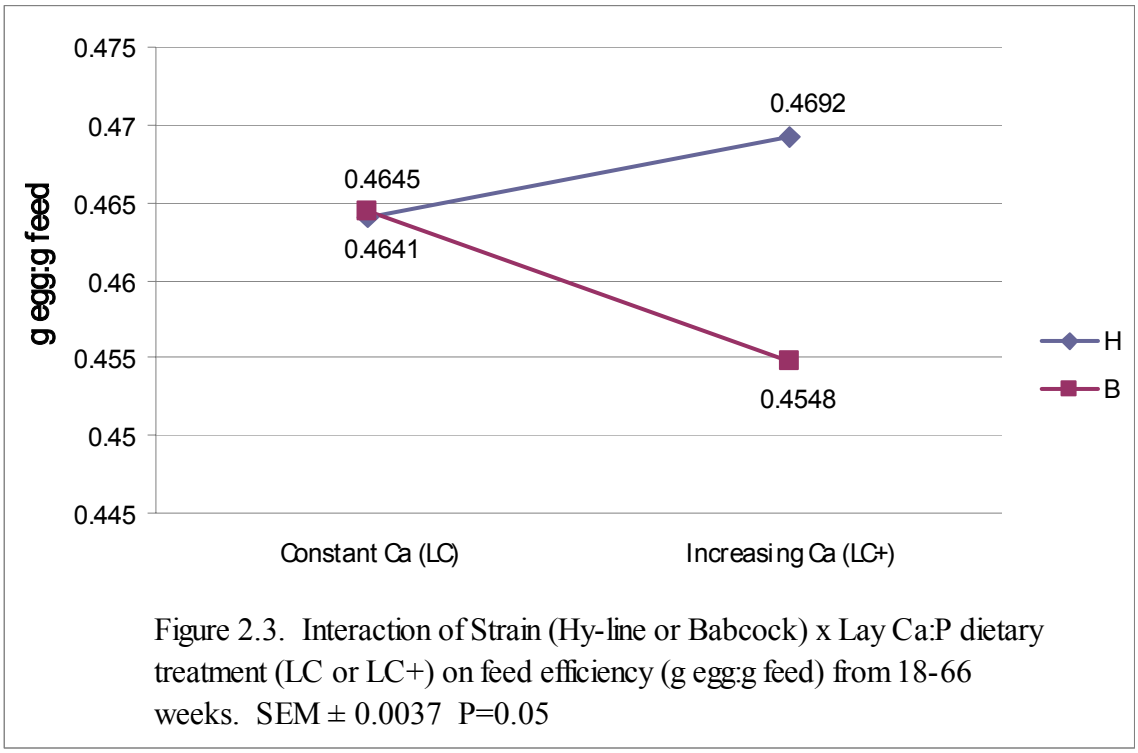

Figure 2.3. Interaction of Strain (Hy-line or Babcock) x Lay Ca:P dietary treatment (LC or LC+) on feed efficiency (g egg:g feed) from 18-66 weeks. SEM ± 0.0037 P=0.05

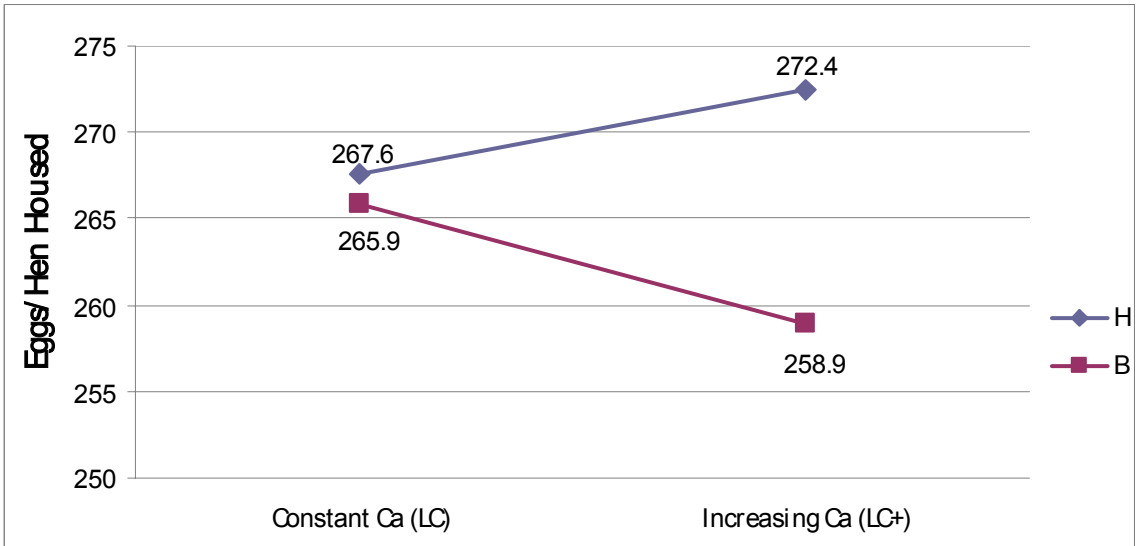

Figure 2.4. Interaction of Strain (Hy-line or Babcock) x Lay Ca:P dietary treatment (LC or LC+) on Egg Production (Eggs/HH) from 18-66 weeks at Low Density (64 in^2). SEM ± 2.48 P=0.02

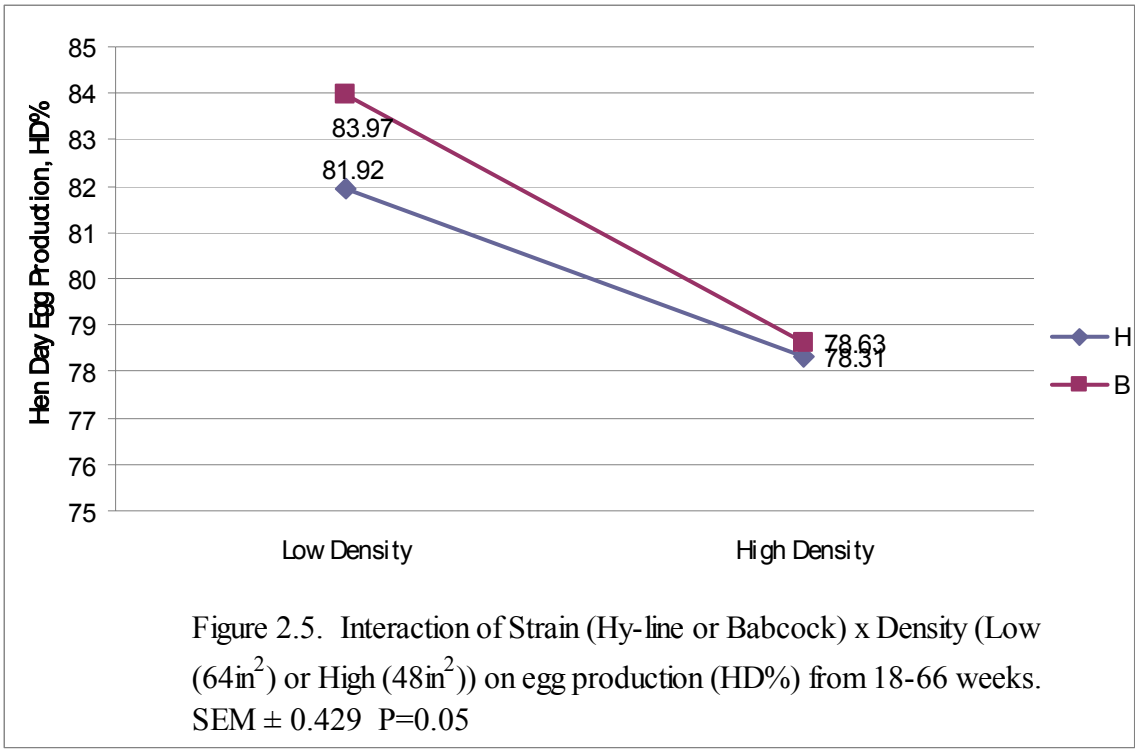

Figure 2.5. Interaction of Strain (Hy-line or Babcock) x Density (Low (64in^2) or High (48in^2)) on egg production (HD%) from 18-66 weeks. SEM ± 0.429 P=0.05

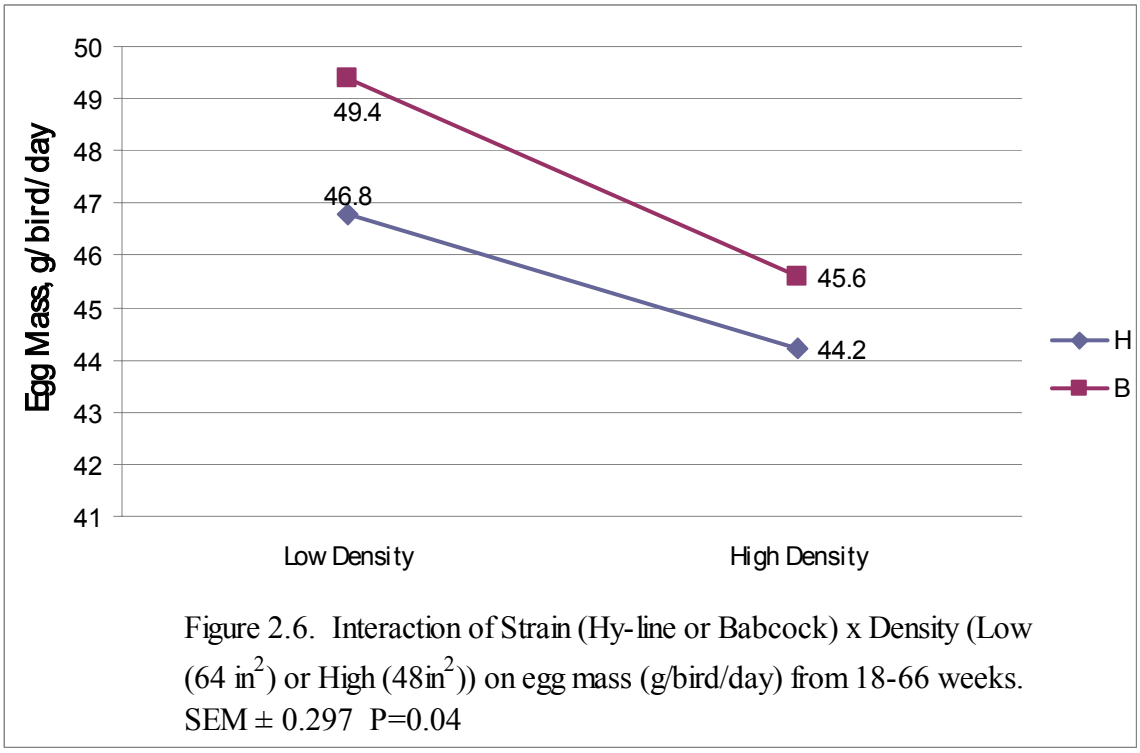

Figure 2.6. Interaction of Strain (Hy-line or Babcock) x Density (Low (64 in^2) or High (48in^2)) on egg mass (g/bird/day) from 18-66 weeks. SEM ± 0.297 P=0.04

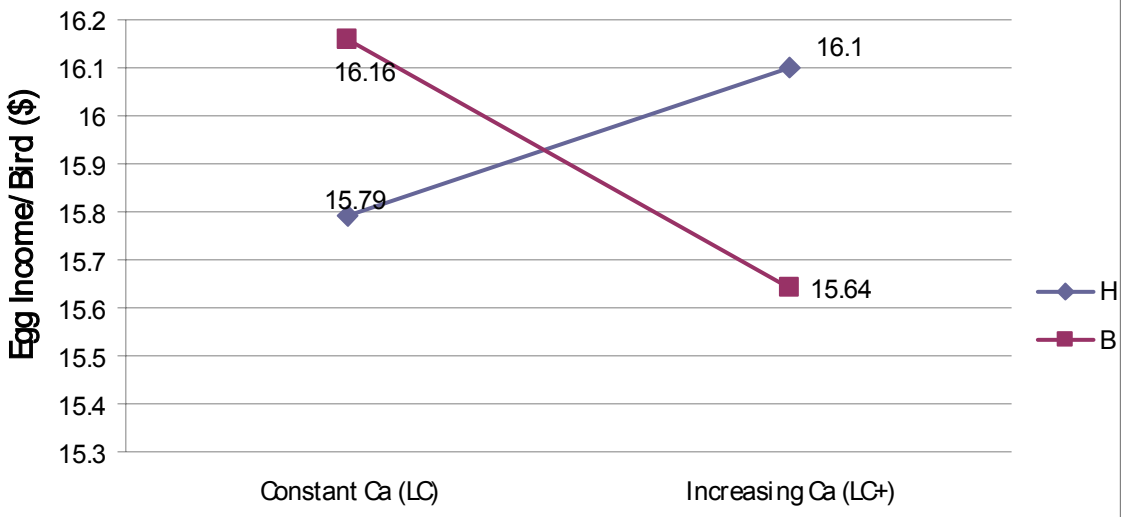

Figure 2.7 Interaction of Strain (Hy-line or Babcock) x Lay Ca:P dietary treatment (LC or LC+) on Egg Income (egg income ($)/bird at 36 weeks) from 18-66 weeks averaged across Low Density (64 in^2). SEM ± 0167 P =0.02

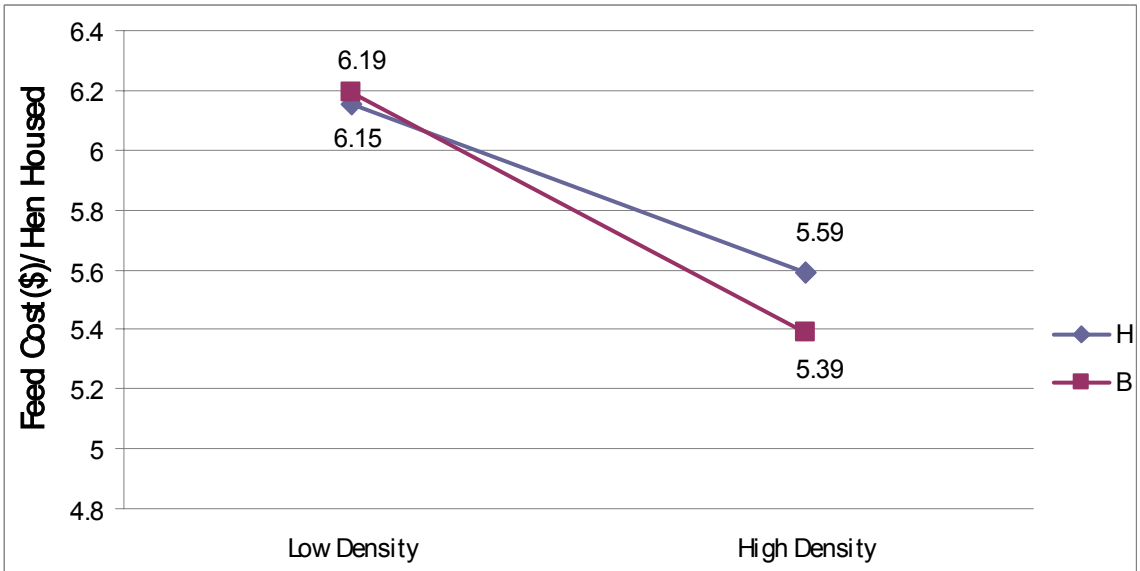

Figure 2.8. Interaction of Strain (Hy-line or Babcock) x Density (Low (64 in^2) or High (48in^2)) on feed cost (feed cost($)/HH) at 36 weeks. SEM ± 0.043 P=0.04

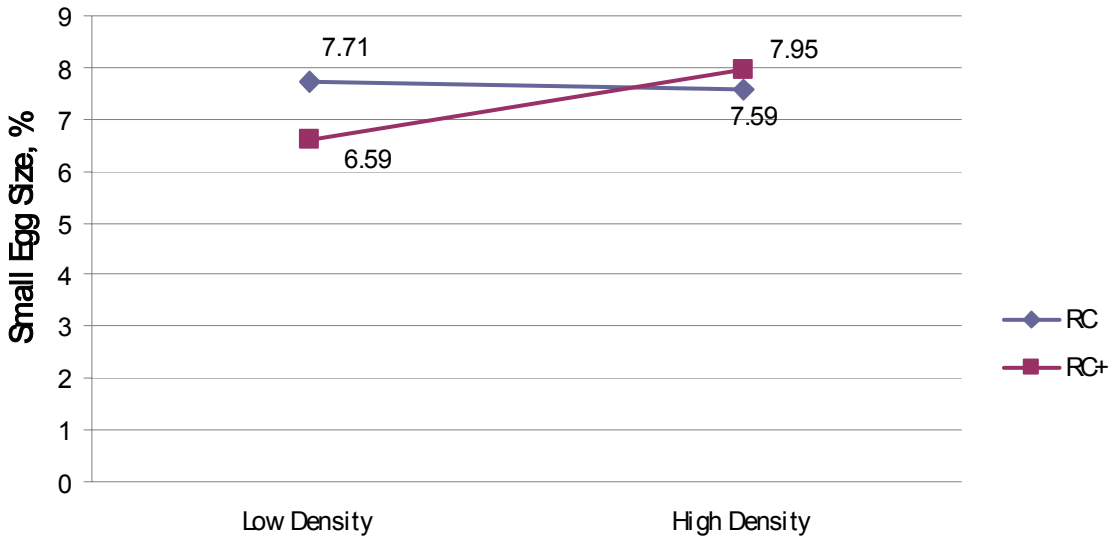

Figure 2.9. Interaction of Density (Low (64 $in^{2)}$ and High (48 in^{2}) x Rear Ca:P dietary treatment (RC (Control Ca:P) or RC+ (High Ca:P) on Small Egg Size. SEM ± 0.37 P=0.05

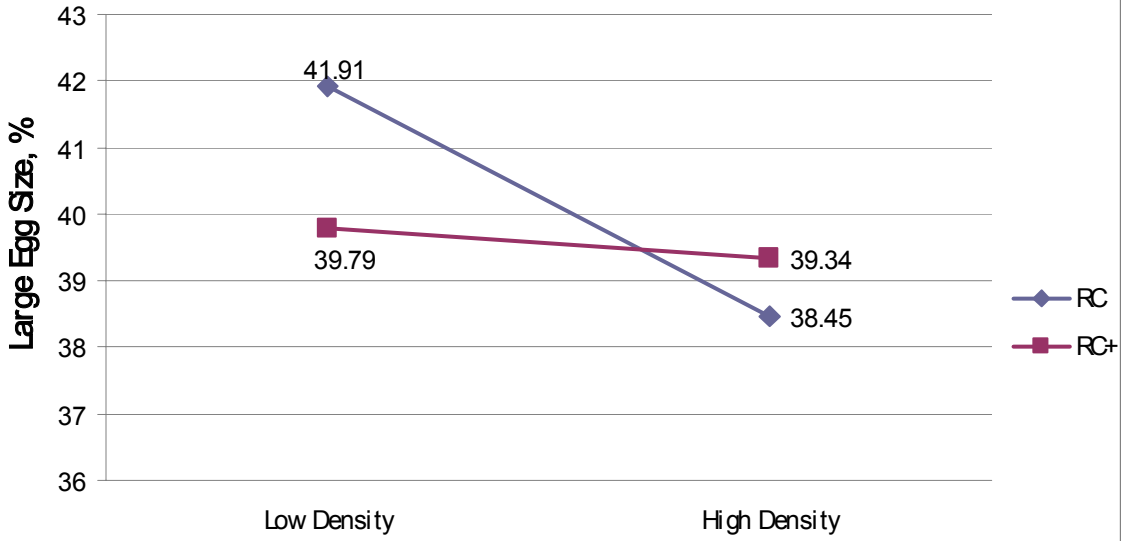

Figure 2.10. Interaction of Density (Low or High) x Rear Ca:P dietary treatment (RC (Control Ca:P) or RC+ (Elevated Ca:P) on Large Egg Size. SEM ± 0.75 P=0.05

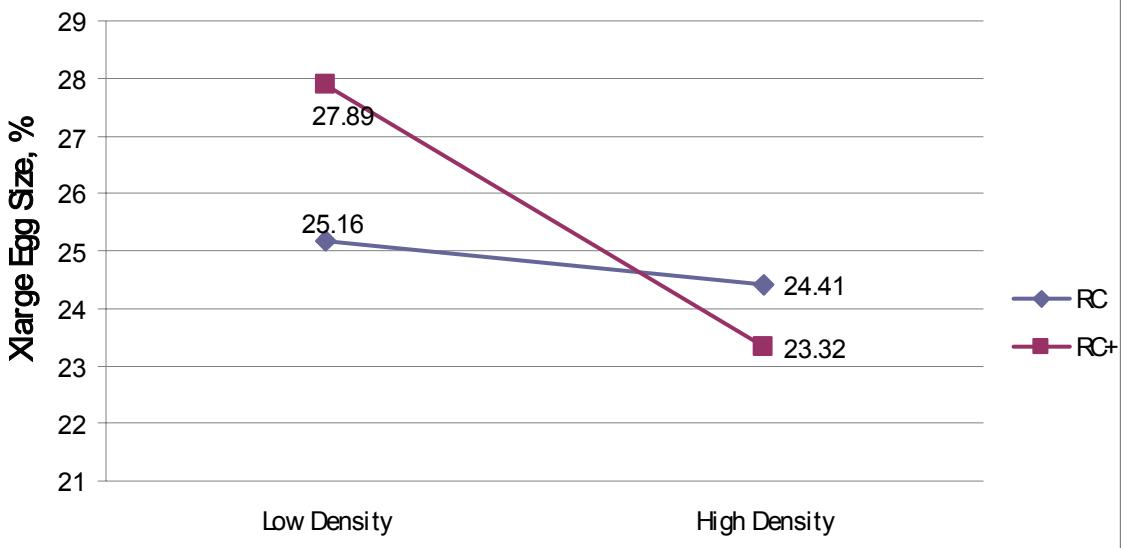

Figure 2.11. Interaction of Density (Low (64 $in^{2)}$ and High (48 in^2) x Rear Ca:P diets (RC (Control Ca:P) or RC+ (High Ca:P) on XLarge Egg Size. SEM ± 0.89 P=0.05

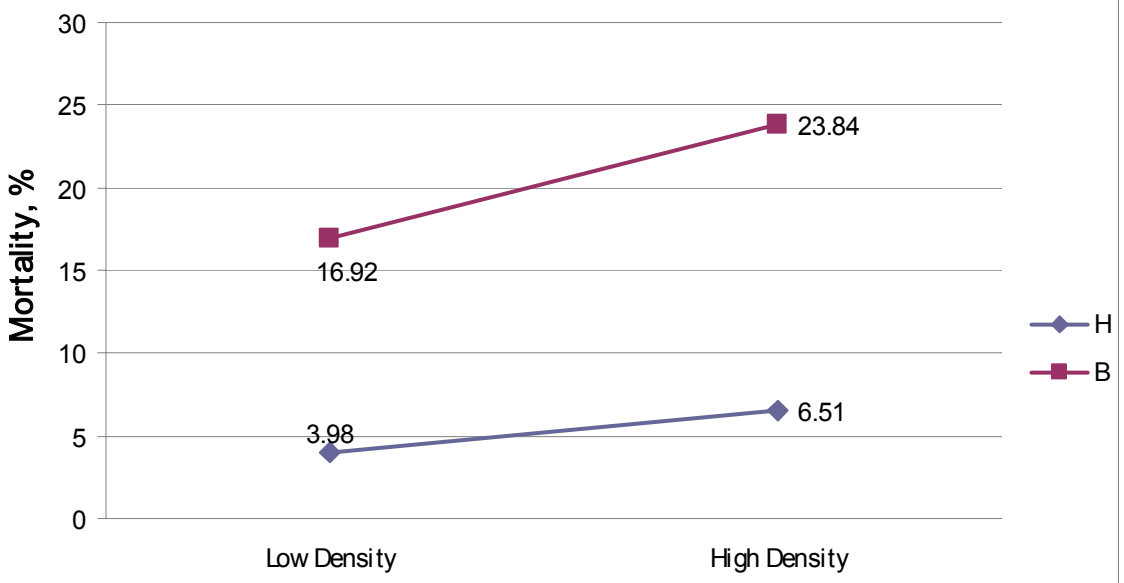

Figure 2.12 Interaction of Strain (Hy-line or Babcock) x Density (Low (64in^2) or High(48in^2) on Mortality (%) from 18-66 weeks. SEM ± 0.94 P=0.02

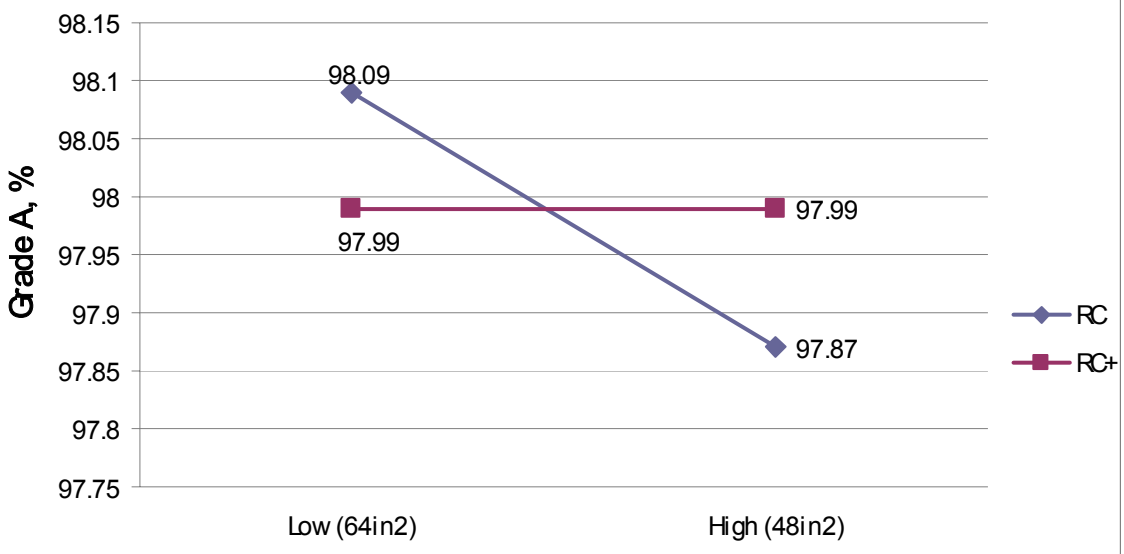

Figure 2.13. Interaction of Density (Low or High) x Rear Ca:P diets (RC (Control Ca:P) or RC+ (High Ca:P)) on Grade A Egg % (Understanding 3-way S*D*R). SEM ± 0.163 P=0.78

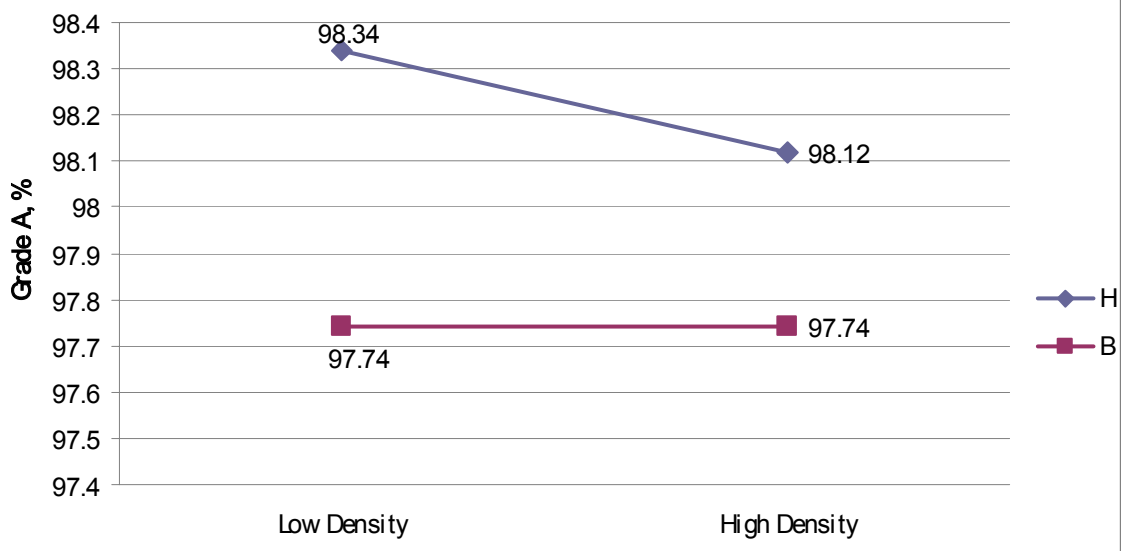

Figure 2.14. Interaction of Density ((Low (64in^2) or High(48in^2)) x Strain (Hyline or Babcock) on Grade A Egg % (Understanding 3-way S*D*R). SEM ± 0.16 P=0.85

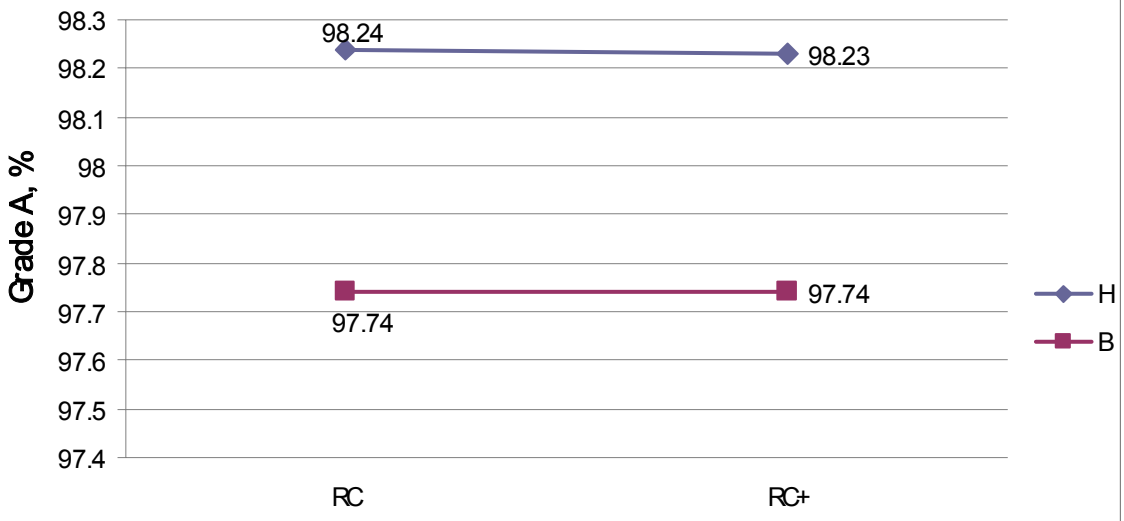

Figure 2.15. Interaction Rear Diets (RC (Control Ca:P) or RC+ (High Ca:P)) x Strain (H or B) on Grade A Egg % (Understanding 3-way S*D*R). SEM ± 0.16 P=0.78

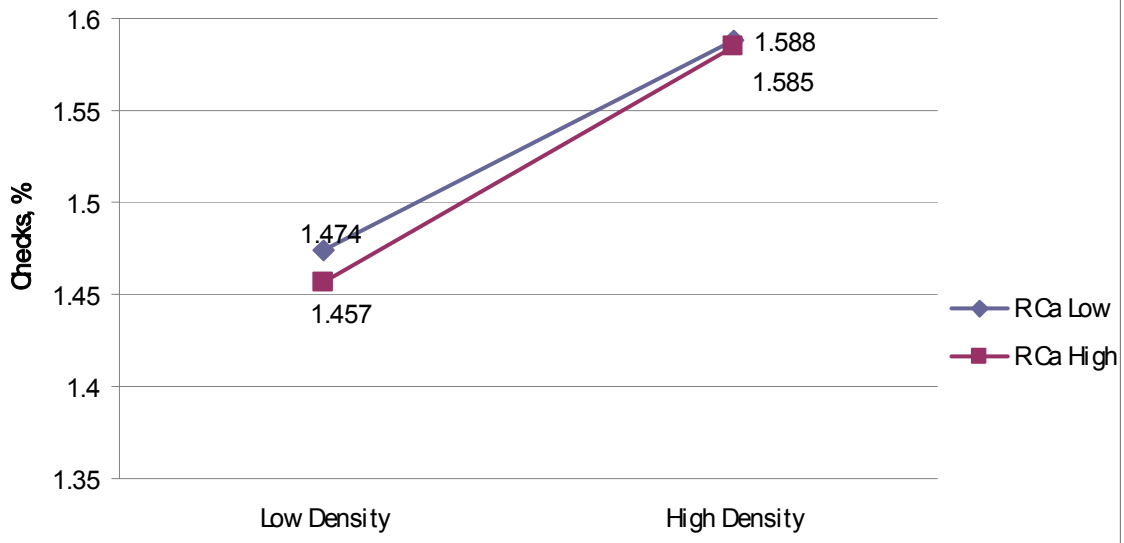

Figure 2.16. Interaction of Density ((Low (64in^2) or High(48in^2)) x Rear Diets (RC (Control Ca:P) or RC+ (High Ca:P) on Grade Checks Egg % (Understanding 3-way S*D*R). SEM ± 0.163 P=0.78

CHAPTER 3

IMPACT OF INCREASED CA AND P REGIMENS DURING REARING AND LAYING ON BONE MINERALIZATION STATUS OF TWO COMMERCIAL STRAINS OF LAYERS

Osteoporosis, a condition in which skeletal integrity is compromised by reduced bone mineralization is increasing in laying hens giving rise to economic, welfare, and livability concerns. Trends in dietary recommendations of calcium, management practices, and genetics may be working in concert to predispose the laying hen to reduced calcium status giving rise to osteoporosis. Objectives of this research were to evaluate the influence of diet (containing an elevated and control ratio of calcium and phosphorous) and strain of layer on growth and bone mineralization during the pullet phase and following these effects through to the first lay cycle. Pullets were reared in cages in an environmentally-controlled house at a density of 48 sq in/pullet 13 birds/cage with 4 cages/replicate (52 pullets/replicate), and 28 replicates/treatment (5,824hens total) from 0-17 wks during the pullet phase and moved to tri- or quad-deck cages in environmentally-controlled, layer house from 18-66 wks of age during layer phase of study with 5,728 hens (26 replicates/treatment). The 2 x 2 factorial arrangement of treatments in the rearing phase were two strains of Leghorns (Hy-Line W-36 (H) and Babcock 300 (B)) and two isocaloric, Ca and P regimens, which were an elevated (RC+) and control (RC) ratio of Ca:P (2.14, 3.14, 4.14 and 2.14, 2.14, 2.42 respectively) during a 3-phase feeding regimen of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks). In the layer phase, the 2 x 2 x 2 factorial arrangement was the same two strains (H and B) and the same two rearing dietary regimens (elevated Ca:P (RC+) and control (RC)) as in the pullet phase with two added layer dietary regimens (increasing Ca and P (LC+) and control (LC)). The lay cycle dietary regimens were based on increases in feed intake observed with age creating increasing (LC+) or constant (LC) Ca and P intake. All test diets were isocaloric and fed *ad libitum.* Feed consumption and BW were monitored bi-weekly beginning at 2 weeks of age during the rearing and every 4 weeks during the layer phase. Mortality and egg production was recorded daily. During rearing, 5 femurs and tibias/trt were assessed for dry fat-extracted bone weight (DFEW), % ash, volume, and bone breaking strength (BBS) beginning at 2 wks

of age and 3 femurs/trt were collected bi-weekly from 51-61 wks of age and also measured for bone mineralization parameters. Overall, there was a significant (P=0.04) effect of diet on the bone parameter of DFEW with pullets fed the RC+ vs. RC diet having higher DFEW (.94 g vs. .82 g, respectively). There was a significant (P=0<.01) effect of strain on bone volume with strain H vs. B having higher volumes (2.99 vs. 2.37 cc, respectively). However, femoral BBS was significantly (P=0.01) greater for strain B than H (8.55 vs. 7.80 g, respectively). There was no significant effect of treatments on mortality. Bone parameters were not significantly affected by layer dietary regimen, but rearing dietary regimen increased (P<0.01) BBS (RC+=14.15 vs. RC=12.37 kg) in the lay cycle. Strain effected (P<0.001) BBS (H=14.26 vs. B=12.26 kg) and volume (H=5.90 vs. B=6.27 cc). These results indicate that strain influences the performance of pullets and hens as well as mineralization status of their bones. Furthermore, these findings indicate that feeding more calcium during rearing and laying can have significant impacts of bone mineralization status although it does not overtly change bird performance.

INTRODUCTION

Trends in commercial layer production have decreased the age of sexual maturity and point of lay (age when 50% rate of lay is reached) in laying hens. With this decrease, the time available for skeletal development and mineralization is reduced. This may be a predisposing factor for bone weakness observed in older laying hens. Levels of broken bones in older layers have been reported as high as 29% at the beginning and 98% by the end of processing (Gregory and Wilkins, 1989). This predisposition towards fracture is marked by weakened bone (Knowles *et al*., 1993) and osteoporosis (Knott *et al*., 1995). Osteoporosis, a decrease in amount of structural bone, is becoming more prevalent among mature layers (Whitehead and Fleming, 2000). In a flock of laying hens, 35% of all mortality was attributed to osteoporosis with this osteoporotic mortality occurring on average 6 weeks earlier than non-osteoporotic mortality (McCoy *et al*., 1996). This level of osteoporosis, bone fragility, and mortality is indicative of a considerable economic and welfare issue in laying hens.

Many theories have been posed to explain the observed declining bone quality in

laying hens, but one that may be overlooked is the impact of management and nutrition during the rearing period on skeletal development. Nutritional needs of calcium by pullets are often considered nominal in magnitude of quantity and import compared to those of laying hens although both the rearing phase and lay cycle are two periods of extreme calcium mobilization. If considering bone as the storage "vault" of calcium, then rearing is the period when calcium is "deposited" (or positive calcium balance) while the lay cycle would be when calcium is "withdrawn" (or negative calcium balance). The introduction of estrogen two weeks prior to sexual maturity represents a demanding period for calcium metabolism. The main calcium deposit made during rearing is the formation of the woven medullary bone, a labile calcium reserve, which occurs with the onset of sexual maturity. Medullary bone forms at the expense of cortical bone so that there is a depression in structural bone formation in pullets stimulated to come in to production earlier (Hudson *et al*., 1993). There is observed a striking loss of cancellous bone with the concurrent increase in medullary bone volume during the first 10 weeks after sexual maturity (Fleming *et al.*, 1998b). This indicates causality between the bone modeling of rearing on the constant bone remodeling of the lay cycle on the ultimate outcome of bone health.

Reduced skeletal mineralization due to earlier ages of sexual maturity may be exacerbated by the fact that recommendations for increasing calcium at earlier ages has not coincided in feeding guidelines by either NRC (1994) or commercial management guides. Increases in dietary calcium are not recommended by NRC (1994) until 18 weeks of age, and if pullets mobilize large amounts of calcium 10-14 days prior to the first egg, this may create a dietary shortcoming of calcium even for those birds reaching sexual maturity at 18 weeks of age (although earlier maturing pullets would be at an even greater risk for calcium insufficiency). In the past there was reluctance to feed increased calcium to layers especially at earlier ages as it was found that feeding 3.25% calcium starting at 50 days of age increased the risk for hens to develop uroliths later in life (Wideman *et al*., 1985). Genetic and management changes may make these concerns obsolete. This inconsistency in calcium recommendations is being recognized with suggestions for providing more calcium at lighting rather than first egg (Whitehead and Fleming, 2000).

The responsiveness of the heritability of bone characteristics to genetic selection

indicate that with all the intensive genetic selection for high egg production, low body weight and feed intake, it may be that hens may have been inadvertently selected for bone fragility (Bishop *et al*., 2000). "Heritage" strains which are generally less selected are relatively unaffected by bone weakness compared to modern hybrid layer strains (Rennie *et al*., 1997). Hens selected for a high Bone Index, which measured both bone strength and density, were found within three generations of selection to have 25% greater tibia strength, 13% greater humeral strength, and 19% greater keel density than those peer treatments not selected for Bone Index; heritability of the Bone Index was 0.40 (Bishop *et al*., 2000) . A study comparing a white egg-laying, modern strain (Babcock B- 300), a brown egg-laying modern strain (ISA-Brown), and an unselected line of brown leghorns, found bone fracture rates of 11.1, 11.7, and 0.0%, respectively, in 72 week old hens (Budgell and Silversides, 2004). Multiple studies find that bone strength is strain related making it an important consideration in the outcome of bone health (Anderson *et al.*, 1995, Fleming *et al*., 2006).

This purpose of this research was to monitor the effect of feeding two strains of pullets and layers increased levels of calcium and phosphorous on the bone mineralization status in the rearing period and late portion of the lay cycle. Bone parameters will be measured to indicate bone mineral status and serve as markers of osteoporosis in order to ascertain if feeding more calcium to growing and/or mature laying hens will impact bone mineralization.

MATERIALS & METHODS

Animals and Housing

Further detail regarding the rearing of pullets and layers used in this study can be found in the previous studies (See Manuscript I and II). In order to track effects of feeding increasing calcium on bone status during rearing into the lay cycle, two strains of pullets Hyline W-36 (H) and Babcock B-300 (B) were fed an elevated calcium (RC+) and control calcium (RC) dietary treatments during rearing and then these same hens were fed an increasing calcium (LC+) and constant calcium (LC) dietary treatment throughout the lay cycle (See Tables 1.1, 2.1 and 2.2 for further dietary descriptions). During week 6-17, bones were sampled from pullets to measure bone parameters to quantify the impact of the RC+

and RC diets on bone mineralization, and during week 51-61, bones were sampled from layers to measure bone parameters to quantify the impact of not only the LC+ and LC diets on bone mineralization status, but to follow the impact of the RC+ and RC diets as well.

Bone Mineralization Status

To monitor bone mineralization during rearing, 5 pullets from different replicates (a maximum of one bird per sampling period per cage in a replicate) were euthanized by cervical dislocation and birds were weighed. From these pullets the right tibia and both femurs were collected at the same time and day beginning bi-weekly from 6-11 wks of age and then weekly from 12-17 wks of age (corresponding to marked increases in Ca:P in grower and developer dietary treatment s). No more than one bird was selected from a replicate each sampling as to minimize the impact of increased cage density. To monitor bone mineralization during the lay cycle, 3 hens were euthanized by cervical dislocation and birds were weighed. From these hens both femurs were collected at the same time and day beginning bi-weekly at 51 weeks until 61 weeks. During the period of week 51-61, all normal mortality were collected and also analyzed for bone mineral status.

All bones were excised and manually defleshed of connecting tissue and then placed in storage at -20 C until analyzed. From the right femur, dry, fat-extracted bone weight, and % ash was determined, and from the left femur and tibia (during the rearing period), bone breaking strength (BBS) was measured. Dry weight was determined by drying bones at 110 C for 16 hr, and fat extracting the dry bones with petroleum ether for 24 hour in a Soxhlet apparatus. Bones were allowed to dry in a forced-air oven at 90 C for 12 hours. Dry, fat-free extracted bone weight was then determined. The bones were then ashed at 700 C for 4 hr. Bone ash was expressed as a percentage of fat-free dry bone. External bone volume was determined on the hydrated bone by water displacement as described by Garlich et al. (1981). Briefly, bones and water were brought to room temperature. The femur was submerged in water in a filled 125 ml ungraduated cylinder (35 mm inside diameter x 150 mm deep). Overflow was collected in a tared beaker, and volume of water was quantitated by weighing to the nearest 0.01 g (with 1.00 g water equaling 1.00 cc). The evaluation was done in triplicate and averaged. Femur and tibias were thawed before BBS (breaking force divided

by bone weight expressed as kilograms per gram) was measured using an Instron Universal Testing Machine (Model 1011, Instron Corp., Canton, MA 02021) with a probe (15 mm dial) and speed of 200 mm/min. Each bone was supported by a fulcrum with a 3.35 cm span for femur and 8 cm span for tibia. A probe with a length of 6 cm and a round base was attached to a 500 kg load cell with a crosshead speed of 200 mm/min and used to determine breaking force.

Experimental Design

The first part of the study during rearing was conducted as a 2 x 2 factorial arrangement of treatments with the main effects of strain (H or B) and rearing calcium dietary treatments (RC+ and RC) while the second part of the study during the lay cycle (LC+ and LC) utilized a 2 x 2 x 2 factorial arrangement of treatments including the same strain and rearing calcium dietary treatments with added lay cycle calcium dietary treatments. Sampling during the pullet phase of the study would constitute a total of 20 bones (5 bone replicates per each of 4 treatments) per each of 9 sampling periods (week 6, 8, 10, 12, 14, 15, 16, and 17) for a total of 180 bones to be analyzed to ascertain pullet bone mineralization status. During the lay cycle, a total of 24 bones (3 bone replicates per each of 8 treatments) per each of the 5 bi-weekly sampling periods (week 51, 53, 55, 57, 59, and 61) resulted in a total of 120 bones to analyze for layer bone mineralization status. Also during the period of week 51-61, all normal mortality were collected and also analyzed for bone mineral status.

Statistical Analyses

As pullet bones mineralization was in a dynamic state due to growth, bones were analyzed by period whereas all lay cycle bones were collectively analyzed. The data were subjected to an Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of the SAS Institute (1998) software with strain of hen and diet as main effects along with their interactions. Mean separation was accomplished using the PDIFF s. Means were partitioned using Least Squared Means (LSMEANS) and statements of statistical significance were based upon $P \bullet 0.05$ unless otherwise noted.

RESULTS

Pullet Bone Status

The calcium status of the bird before egg production commences is perhaps the most useful to the egg producer. Thus, the results at week 17, the end point of the rearing portion of the study, will first be reviewed. There was a related interaction of Rear*Strain on % Ash (P=0.01) and Ash/Volume (P=0.05) at week 17. In this, % Ash was increased in the B strain by feeding more rearing calcium (RC=46.7 and RC+=51.7%) while in the H strain, % Ash decreased (RC=54.3 and RC+=52.5%). Ash/volume was increased in the B strain by feeding more rearing calcium (RC=0.22 and RC+=0.28 g/cc) while in the H strain, % Ash decreased (RC=0.33 and RC+=0.32%). In both these measures, the B strain was significantly lower in % ash or ash/volume until fed the RC+ diet. At both week 13 and 14, there was an interaction of Rear*Strain on Volume (P=0.05). At week 13, there was a drop in volume due to feeding RC+ to the B strain while at week 14 the drop in volume due to feeding RC+ was in the H strain (See Table 3.1).

There were several main effects of rearing calcium dietary treatments on bone parameters at week 17. All birds which had been sampled had been weighed as to discern that changes in bone measures were not artifact of body size; there were no differences (P=0.98) of bird weights sampled (See Table 3.1). Feeding the RC+ compared to RC diet increased Bone Weight (3.59 and 3.16 g, respectively, P=0.02), Ash Weight (1.87 and 1.60 g, respectively, P=0.01), Femur BS (22.07 and 17.45 kg, respectively, P<0.01), and Tibia BS (23.36 and 20.28 kg, respectively, P<0.01). In the remaining parameters measured, feeding RC+ resulted in numerical increases in % Ash (RC+=52.1 and RC=50.5%, P=0.22), Volume (RC+=6.2 and RC=6.1, P=0.46), and Ash/Volume (RC+=0.30 and RC=0.27 g/cc, P=0.07). When reviewing the main effect of rearing by period, at week 6, feeding RC+ compared to the RC diet increased both bone weight (0.94 and 0.82 g, respectively, P=0.04) and tibia BS (7.12 and 6.18 kg, respectively, P=0.05) (See Table 3.2). As this was a baseline value before Ca:P levels increased, it suggests that the differences in ratios of Ca:P actually had affected bone mineralization in the first 6 weeks.

This study also observed that whether rearing a Hy-line W-36 or Babcock B300 will influence the bone status of pullets. At week 17, bird weight of those pullets sampled was not different between strains indicating bird weight is not a contributing factor of the results observed (See Table 3.3). As mentioned previously, there was a significant interaction of Strain*Rear on % Ash and Ash/Volume in which the B strain when fed the RC diet had a lowered % Ash and Ash/Volume than the other three treatments in this comparison. There were significant main effects for the aforementioned Ash (P,0.01) and Ash/Volume (P<0.001) with the H strain compared to B strain having higher % Ash (53.4 and 49.2% respectively) and Ash/Volume (0.32 and 0.25 g/cc respectively), but there was also a main effect of volume due to strain with the B strain having a greater (P<0.001) bone volume than the H strain (B=6.96 and H=5.38%) (See Table 3.4). Strain had no effect at week 17 on bone weight (H=3.25 and B=3.49 g, P=0.16), Ash Weight (H=1.74 and B=1.73 g, P=0.94), Femur BS (H=19.2 and B=20.3 kg, P=0.47), and Tibia BS (H=21.7 and 22.0 kg, P=0.78).

When reviewing the main effects of strain by period, Bone Weight was greater (P<0.01) at week 13 in the B (2.60 g) compared to the H (2.25 g) strain. At week 12, % Ash was greater (P<0.01) in the B (46.1 %) compared to the H (43.3 %) strain. Volume itself was greater at week 6 (H=2.99 and B=2.37cc, P<0.01), week 10 (H=4.38 and B=4.92 cc, P=0.02), week 13 (H=4.97 and B=6.15cc, P<.001), and as previously mentioned at week 17 (H=5.38 and B=6.96cc, P<0.001). The Ash/Volume parameter was significantly different due to strain at week 6 (H=0.13 and B=0.18, g/cc, P<0.001), week 13 (H=0.20 and B=0.18, g/cc, P<0.01), week 15 (H=0.24 and B=0.22, g/cc, P=0.01), week 16 (H=0.29 and B=0.26, g/cc, P=0.03) and as previously mentioned at week 17 (H=0.32 and B=0.25, g/cc, P<0.001) (See Table 3.5). Femur BS was only affected by strain at week 6 (H=7.80 and B=8.55 kg, P=0.01) and tibia BS was only affected by week 13 where the H strain had a greater (P<0.01) tibia BS than the B strain (15.96 vs. 13.71 kg, respectively) (See Table 3.6 and 3.7).

Layer Bone Status

After analysis of all bones collected from 51-61 weeks of age, outside of one interaction involving Rear*Lay dietary treatments, there were no significant main effects of feeding increasing calcium in the lay cycle (See Table 3.8). In the interaction, when feeding

the constant LC diet, bone volume increased in response to feeding increased calcium during rearing (LCRC=5.91 and LCRC+=6.27) while feeding LC+ diets with or without more calcium during rearing resulted in the same response as just feeding more calcium during rearing (LC+RC=6.13 and LC+RC+=6.03). There were numerical increases due to feeding more calcium during the lay cycle with greater Bone Weight (LC+=3.76 and LC=3.68 g, P=0.35), Ash Weight (LC+=1.92 and LC=1.88 g, P=0.29), % Ash (LC+=53.21 and LC=52.68 %, P=0.39), and Femur BS (LC+=13.66 and LC=12.86 g, P=0.16). Femur BS was increased (P<0.01) by feeding RC+ as compared to the RC diet (14.15 and 12.37 kg, respectively). Feeding the RC+ diet also numerically improved Bone Weight (RC+=3.77 and RC=3.67 g, P=0.20), Ash Weight (RC+=1.92 and RC=1.88, P=0.51), and Volume (RC+=6.15 and RC=6.02 cc, P=0.20) although % Ash was decreased (RC+=52.71 and RC=53.19 %, P=0.44). There were significant main effects of strain on Femur BS (P<0.001) and Volume (P<0.001). The Hy-line bird affected a stronger Femur BS than the Babcock (14.26 and 12.26, respectively) while the Babcock had a greater bone volume than the Hy-line (6.27 and 5.90, respectively). The other parameters were numerically greater for the Babcock compared to the Hy-line bird. Ash weight was 1.93 g for the H bird and 1.87 g for the B strain (P=0.30), and % Ash were 52.65 % for the H strain and 53.25% for the B Strain (P=0.34). There was an approach of significance (P=0.07) for Bone Weight (H=3.64 g and B=3.80).

DISCUSSION

These results find that feeding increased calcium levels to pullets will impact favorably the bone parameters measured throughout the growth cycle and even into the lay cycle. There is limited research on bone mineralization of pullets before lay. The main effect of feeding increased rearing calcium tended to occur independent of strain except for the interaction involving % Ash (week 12 and 17), Volume (week 13), and Ash/Volume (week 17). Ash as expressed as a percent of fat-free, dry extracted bone has been indicated as a useful assessment of mineral status of growing birds (Waldroup *et al*., 1963), but Ash/Volume has been suggested to be more effective to detect pre-osteoporotic conditions in

the bone. Bone volume and the organic matrix of the bone can remain constant despite mineral deficiencies although bone ash is reduced (Garlich *et al*., 1982). In this study, the Ash/Volume parameter seemed especially sensitive in detecting the interaction of the rearing dietary treatment and strain. This corroborates findings in which bone ash/volume was found to better reflect changes of bone status in hens than just ash since volume is so highly correlated with other measures of bone status (Cheng and Coon, 1990 and Zhang and Coon, 1997). Ash/Volume was especially sensitive in this study to observe the bone growth due to strain as significant strain effects occurred at week 6, 13, 15, 16, and 17. It was noted that not one strain had consistently higher Ash/Volume ratio, but rather from week 6-12, the Babcock strain tended to have a higher Ash/Volume while from 12-17, the Hy-line was higher in Ash/Volume. As the Hy-line (21.8 weeks at 50% production) strain is a faster maturing bird than the Babcock (23 weeks at 50% production), it would be expected that rates of skeletal development would differ between strains. Shifts in the Ca:AvP levels fed in this study occurred at week 6 and 12 which correlates with some of the responses in the bone. It may be that the respective strain was being supplied with a more suitable calcium intake for the particular point it was on its growth curve. The interaction of strain and rearing diets at week 17 on % Ash and Ash/Volume indicates the differing responses of the two strains to feeding more rearing calcium. The Babcock had a significant increase in %Ash and Ash/Volume by being fed the RC+ diet while there was no significant response by the Hy-line strain when feeding RC+ or RC. Levels of calcium fed in this study from week 13-17, was over 1.0% higher than NRC (1994) which as pullets were photo-stimulated in this period, may be useful especially for the Babcock strain. It was Hurwitz (1964) who observed that feeding high Ca (4.1%) before the onset of lay (14 days) resulted in greater pre-lay storage of calcium in the end and cortical segments of bone. In this study, bone status was affected by rearing calcium diets as early as week 6 in which bone weight and tibia BS were increased when feeding RC+. This would indicate that the bone responded to the relative differences in Ca:AvP fed from 0-6 weeks (both treatments fed 2.14 Ca:AvP, the RC+ diet had a higher ratio (1.18:0.55) compared to the RC diet (0.90:0.42) of Ca:AvP). Research suggests that feeding more marginal levels of calcium creates sensitivity to lower phosphorus levels which may in part explain this response (Frost and Roland, 1990).

Even though in this trial, Ca and AvP levels were fed in excess of NRC (1994), the bone parameters measured were fairly sensitive in detecting changes in bone growth. This suggests these measures of bone mineralization are useful for situations beyond mineral deficiencies. It was also interesting to note that in this at week 17, the findings which tested significant for rearing diets (Bone Weight, Ash Weight, Femur BS, and Tibia BS) were not those that tested significant for strain (% Ash, Volume, Ash/Volume).

Results from the bone parameters measured from 51-61 weeks of age, indicate that there are impacts of feeding increased calcium during rearing that can impact into the lay cycle. The one interaction involving the Rear*Lay dietary treatments indicated that feeding more calcium during rearing (with or without more calcium during lay) improved Bone Volume over feeding no increased calcium during rearing or lay. This suggests that feeding more calcium during rearing can have a greater impact on bone volume in the lay cycle than what is fed in the lay cycle. Femur BS of laying hens from 51-61 weeks was also increased by feeding more calcium during rearing although only as a main effect. This is indicative of the importance of rearing calcium recommendations on subsequent bone mineralization status. It has long been understood in human medicine that acquiring greater bone mass during puberty is one of the best preventive strategies to avoiding osteoporosis observed with aging. The findings in this study suggest a similar response between pubertal bone acquisition and bone status later in life. Also, the importance of BBS may be of greater importance in mature rather than growing animals. Again, in the lay cycle, strain impacts bone mineralization as observed in the Hy-line's stronger femur BS and the Babcock's greater bone volume (although lower Ash/Volume). Previous research indicates that calcium absorption is a not contributing factor as no difference in calcium uptake were observed between the Hy-line and Babcock strain (Al-Batshan *et al*., 1994). As the Babcock produced greater egg mass, it may be that this strain is more susceptible to production-induced osteoporosis. Cransberg *et al*. (2001) suggests that production-induced osteoporosis can be induced by high production rates that results in a loss in BW from week 35-45. Effects observed during the lay cycle may be in part due to differences among phosphorous levels of the two rearing and laying dietary regimens. Sensitivity to phosphorous was observed by Keshavarz and Nakajima (1993) in reduced bone ash from weeks 52-64 when a step down

program of available phosphorous was fed to laying hens. Birds fed the step-down AvP from weeks 20-52 indicated no response to Ash such that the latter phase of lay production may be an especially critical time for feeding correct Ca:AvP levels.

The findings of this study indicate that rearing dietary treatments impact the bone status of developing pullets yielding a pullet at the end of the grow cycle with a better calcium status that can influence birds into the lay cycle. These results of this study indicate that there is causality between bone status during rearing that will ultimately impact the bone mineral status during laying and that this causality can be influenced by dietary calcium. Findings of this study suggest that recommendations for calcium should not be made independently of either the rearing or lay cycle periods. For example, if a bird has had a high calcium status during rearing, the bird may not require an increasing calcium regimen such as the LC+ diet. However, results of this study indicate that calcium needs cannot be considered irrespective of strain and housing density. This was also indicated by Fleming *et al*. (2006) who stated that genetics, environment, and nutrition had an additive effect on bone status. From the findings of this study it seems a reasonable speculation that feeding more calcium during rearing might facilitate bone reaching its full mineralization potential during the rearing period which should serve to prevent osteoporotic susceptibility in the lay cycle. It has been suggested that cage-layer osteoporosis is the most significant disease of the skeleton in mature chickens used for egg production (Riddell, 1981), and its prevalence is increasing (Whitehead and Fleming, 2000).

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Table 3.1. Final bone status at week 17 of two strains of pullets fed increased calcium during rearing[1].

Main Effects	Bird Weight	Bone Weight	Ash Weight	% Ash	Volume	Ash/ Volume	Femur BS	Tibia BS
	g	g	g	%	cc	g/cc	kg	kg
Main Effects								
Dietary Ca:P								
Plus	1111.3	**3.59[b]**	**1.87[a]**	52.09	6.24	0.301	**22.07[a]**	**23.36[a]**
Control	1115.1	**3.16[a]**	**1.60[b]**	50.50	6.10	0.271	**17.45[b]**	**20.28[b]**
Strain								
Hyline W-36	1126.5	3.25	1.74	**53.43[a]**	**5.38[a]**	**0.324[a]**	19.22	21.68
Babcock B-300	1104.9	3.49	1.73	**49.17[b]**	**6.96[b]**	**0.249[b]**	20.30	21.96
SEM	30.9	0.116	0.069	0.887	0.137	0.011	1.03	0.700
				Probability				
Ca:P	0.98	**0.02**	**0.01**	0.22	0.46	0.07	**<0.01**	**<0.01**
Strain	0.63	0.16	0.94	**<0.01**	**<0.001**	**<0.001**	0.47	0.78
Ca:P*Strain	0.89	0.38	0.08	**0.01**	0.46	0.05	0.20	0.06

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 3.2. Dry Fat Extracted Femur Weight from week 6-17 of two strains of layers fed increased calcium during rearing[1].

	Dry Fat Extracted Femur Weight (g)								
Week :	**6**	**8**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
Main Effects									
Dietary Ca:P									
Plus	**0.944**[a]	1.58	2.17	2.28	2.37	2.54	2.82	3.02	**3.59**[a]
Control	**0.823**[b]	1.62	2.06	2.39	2.48	2.64	2.82	2.96	**3.16**[b]
SEM	0.0385	0.0726	0.0766	0.0670	0.0761	0.0916	0.0847	0.0970	0.116
Strain									
Hy-line W-36	0.869	1.54	2.07	2.39	**2.25**[b]	2.53	2.75	3.09	3.25
Babcock B-300	0.898	1.66	2.17	2.28	**2.60**[a]	2.65	2.89	2.89	3.49
SEM	0.0385	0.0726	0.0766	0.0670	0.0761	0.0916	0.0847	0.0970	0.116
	Probability								
Ca:P	**0.04**	0.70	0.33	0.29	0.31	0.44	0.97	0.69	**0.02**
Strain	0.61	0.29	0.35	0.26	**<0.01**	0.38	0.24	0.15	0.16
Ca:P*Strain	0.70	0.60	0.68	0.25	0.58	0.41	0.33	0.68	0.38

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 3.3. Percent Ash from week 6-17 of two strains of layers fed increased calcium during rearing[1].

	Ash %								
Week :	**6**	**8**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
Main Effects									
Dietary Ca:P									
Plus	45.75	49.03	43.06	44.38	45.16	43.54	46.51	50.92	52.09
Control	47.76	46.92	44.49	44.01	42.32	44.21	47.60	51.04	50.50
SEM	1.08	1.18	1.03	0.634	1.07	1.07	0.972	0.889	0.887
Strain									
Hy-line W-36	45.79	49.10	44.04	**43.32**[b]	44.79	43.61	48.04	51.23	**53.43**[a]
Babcock B-300	47.72	46.85	44.51	**46.07**[a]	42.69	44.13	46.07	50.72	**49.17**[b]
SEM	1.08	1.18	1.03	0.634	1.07	1.07	0.972	0.889	0.887
	Probability								
Ca:P	0.21	0.22	0.33	0.68	0.08	0.67	0.44	0.92	0.22
Strain	0.23	0.20	0.35	**<0.001**	0.18	0.74	0.17	0.69	**<0.01**
Ca:P*Strain	0.83	0.08	0.60	**<0.01**	0.47	0.49	0.56	0.13	**0.01**

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 3.4. Femur Bone Volume from week 6-17 of two strains of layers fed increased calcium during rearing[1].

	Volume (cc)								
Week :	**6**	**8**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
Main Effects									
Dietary Ca:P									
Plus	2.74	3.53	4.57	4.87	5.36	5.14	5.65	5.43	6.24
Control	2.63	3.81	4.73	5.21	5.76	5.26	5.82	5.69	6.10
SEM	0.115	0.190	0.145	0.146	0.178	0.167	0.183	0.209	0.137
Strain									
Hy-line W-36	**2.99**[a]	3.62	**4.38**[b]	4.99	**4.97**[b]	**5.04**[b]	5.52	5.52	**5.38**[b]
Babcock B-300	**2.37**[b]	3.72	**4.92**[a]	5.09	**6.15**[a]	**5.37**[a]	5.95	5.61	**6.96**[a]
SEM	0.115	0.190	0.145	0.146	0.178	0.167	0.183	0.209	0.137
	Probability								
Ca:P	0.50	0.33	0.45	0.11	0.13	0.55	0.53	0.39	0.46
Strain	**<0.01**	0.70	**0.02**	0.62	**<0.001**	0.21	0.12	0.78	**<0.001**
Ca:P*Strain	0.54	0.59	0.48	0.94	**0.05**	**0.05**	0.70	0.78	0.46

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 3.5. Ash:Volume from week 6-17 of two strains of layers fed increased calcium during rearing[1].

	Ash:Volume (g:cc)								
Week :	**6**	**8**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
Main Effects									
Dietary Ca:P									
Plus	0.131	0.231	0.205	0.209	0.199	0.218	0.232	0.284	0.301
Control	0.152	0.205	0.195	0.201	0.185	0.222	0.231	0.266	0.271
SEM	.00440	.0123	.00555	.00501	0.00520	0.00756	0.00399	0.00773	0.113
Strain									
Hy-line W-36	**0.133**[b]	0.211	0.203	0.203	**0.203**[a]	0.220	**0.239**[a]	**0.288**[a]	**0.324**[a]
Babcock B-300	**0.180**[a]	0.225	0.196	0.207	**0.181**[b]	0.220	**0.224**[b]	**0.262**[b]	**0.249**[b]
SEM	.00440	.0123	0.00555	.00501	0.00520	0.00756	0.00398	0.00773	0.0113
	Probability								
Ca:P	0.15	0.16	0.22	0.33	0.07	0.75	0.93	0.12	0.07
Strain	**<0.001**	0.43	0.41	0.54	**<0.01**	0.98	**0.01**	**0.03**	**<0.001**
Ca:P*Strain	0.65	0.63	0.47	0.52	0.39	0.20	0.78	0.32	**0.05**

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 3.6. Femur Breaking Strength from week 6-17 of two strains of layers fed increased calcium during rearing[1].

	Femur Breaking Strength (kg)								
Week :	**6**	**8**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
Main Effects									
Dietary Ca:P									
Plus	8.40	13.64	15.71	14.25	14.16	15.62	14.08	16.71	**22.07**[a]
Control	7.96	14.32	14.70	14.44	13.21	13.78	14.14	15.16	**17.45**[b]
SEM	0.194	0.776	0.819	0.986	0.813	0.761	1.063	0.816	1.028
Strain									
Hyline W-36	**7.80**[b]	12.85	15.26	15.39	13.91	14.95	14.18	16.47	19.22
Babcock B-300	**8.55**[a]	15.12	15.15	13.31	13.46	14.45	14.04	15.39	20.30
SEM	0.194	0.776	0.819	0.986	0.813	0.761	1.063	0.816	1.03
	Probability								
Ca:P	0.12	0.55	0.40	0.15	0.42	0.11	0.89	0.20	**<0.01**
Strain	**0.01**	0.06	0.93	0.89	0.70	0.65	0.99	0.36	0.47
Ca:P*Strain	0.99	0.57	0.98	0.66	0.89	0.48	0.09	0.40	0.20

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 3.7. Tibia breaking strength from week 6-17 of two strains of layers fed increased calcium during rearing[1].

	Tibia Breaking Strength (kg)								
	6	**8**	**10**	**12**	**13**	**14**	**15**	**16**	**17**
Dietary Ca:P									
Plus	**7.12**[a]	10.92	13.58	14.81	15.26	16.71	18.83	20.27	**20.36**[a]
Control	**6.18**[b]	11.21	12.57	15.56	14.41	15.69	17.42	19.86	**20.28**[b]
Strain									
Hyline W-36	6.74	10.89	13.47	15.60	**15.96**[a]	16.73	18.80	20.53	21.68
Babcock B-300	6.56	11.25	12.68	14.77	**13.71**[b]	15.66	17.45	19.61	21.96
SEM	0.318	0.436	0.560	0.574	0.538	0.643	0.497	0.516	0.700
	Probability								
Ca:P	**0.05**	0.65	0.22	0.37	0.28	0.28	0.06	0.58	**<0.01**
Strain	0.69	0.57	0.34	0.32	**<0.01**	0.26	0.07	0.23	0.78
Ca:P*Strain	0.53	0.85	0.27	0.85	0.32	0.17	0.76	0.94	0.06

[a-b] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).
[1] Plus=elevated rearing calcium:available phosphorous (Ca:P) 2.14, 3.14, 4.14 and Control=control rearing Ca:P 2.14, 2.14, 2.42 ratio of starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

Table 3.8. Bone status measures from 51-61 weeks of age in two strains of layers fed increased calcium during rearing[1] and the lay cycle[2].

Main Effects	Bone Weight g	Ash Weight g	% Ash %	Volume cc	Femur BS kg
Strain					
Hy-line W-36	3.64	1.87	52.65	**5.90^{b}**	**14.26^{a}**
Babcock B-300	3.8	1.93	53.25	**6.27^{a}**	**12.26^{b}**
Rear Dietary Ca:P					
Rear Plus	3.77	1.92	52.71	6.15	**14.15^{A}**
Rear Control	3.67	1.88	53.19	6.02	**12.37^{B}**
Lay Dietary Ca&P					
Lay Increasing	3.76	1.93	53.21	6.082	13.66
Lay Control	3.68	1.87	52.68	6.085	12.86
SEM	0.0586	0.0402	0.437	0.0715	0.407
	Probability				
Strain	0.07	0.3	0.34	**<0.001**	**<0.001**
Rear Ca:P	0.2	0.51	0.44	0.2	**<0.01**
Lay Ca&P	0.35	0.29	0.39	0.97	0.16
Strain*Rear	0.14	0.04	0.16	0.69	0.16
Strain*Lay	0.66	0.91	0.52	0.66	0.06
Rear*Lay	0.35	0.53	0.6	**0.03**	0.85
Strain*Rear*Lay	0.37	0.68	0.74	0.11	0.53

[a-b] or [A-B] Means within the same column with no common superscript are significantly different ($P \leq 0.05$).

[1] Rear Plus=**e**levated rearing Calcium:Available Phosphorous (Ca:P) 2.14, 3.14, 4.14 and Rear Control=control rearing Ca:P 2.14, 2.14, 2.42 ratios fed in starter (0-6 weeks), grower (6-12 weeks), and developer (12-17 weeks), respectively.

[2] Lay Increasing=increasing lay cycle calcium and available phosphorous (Ca&P) regimen providing fixed 4.1% Ca and 0.42% P and Lay Control=constant lay cycle Ca&P regimen providing reducing increments of 4.0-3.5% Ca and 0.40-0.36% P based on increasing feed intake from 18-66 weeks of age.

SUMMARY AND CONCLUSIONS

Due to the rigorous demands of eggshell formation and egg production, over 900 grams of Ca will be shunted out of the hen's body in a lifetime. It is physiologically extraordinary to move so much Ca especially in the continuous daily ritual of egg production. Maintenance of Ca homeostasis while withdrawing 2 g of Ca for each egg requires a sophisticated regulatory system that may be reaching the threshold of its capacity due to genetic changes among modern commercial layers alongside changes in management without concurrent changes in dietary calcium. This may be tipping the scale towards Ca imbalance as marked by the increased prevalence of cage layer osteoporosis and related mortality. To better understand the need for Ca in laying hens, it is important to discern the inter-relationship between dietary Ca recommendations and strain, and how these juxtapose to influence the mineral status of the bone, the main mineral repository for egg production. Furthermore, welfare concerns involving laying hens has resulted in cage density changes which add another level in determining how to best feed dietary calcium while maximizing bird performance and bone mineral status. In this study, feeding more dietary calcium during rearing and the lay cycle contributed towards influencing bone mineralization status and bird performance. It was found that feeding more rearing dietary Ca effected bone mineralization status of birds up to 45 weeks later during the lay cycle. This indicates that Ca recommendations of pullets should not be overlooked as an important arena for improving bone density of older laying hens. This corroborates human studies which indicate that dietary Ca intake consumed during puberty rather than post-menopause can have a greater impact on bone density in this later age when issues of osteoporosis are present. This study has also found that Ca recommendations cannot be made irrespective of strain or cage density as they not only have strong independent effects but interactive effects with Ca level fed. As feeding more Ca influenced egg size and grading, a further consideration in choosing optimal dietary Ca levels would need to be done in light of target egg markets. Although mortality was not changed by feeding increased Ca in this study, the changes made in bone mineralization status do indicate that increased dietary Ca may be a useful preventative strategy to mediate the predisposing factors of bone weakness that give rise to osteoporosis in

laying hens. Just as it was observed by Castillo *et al*. (2004) that there is a biological and an economical optimal Ca level for laying hens that differ from one another, it may be that there is optimal Ca recommendation for bone mineralization status that differs from current dietary Ca recommendations. As genetic selection continues to improve laying hen performance and changes are made in housing and stocking density, dietary Ca recommendations may need to be as dynamic to not only optimize bone mineralization status but laying hen performance and welfare.