PERFORMANCE AND BONE QUALITY OF MODERN BROILER CHICKS AS INFLUENCED BY HENS' AGE AND EGG STORAGE TIME

by

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(Under the Direction of Gene M. Pesti)

ABSTRACT

The Hens' age has been shown to influence bone quality, especially TD. Egg storage time has been shown to influence chick quality. Four experiments were conducted with chicks hatched from eggs laid by Ross × Ross 308 hens to investigate the influence of hens' age and egg storage time (0 vs. 10 d) on egg parameters, chick growth and bone abnormalities in progeny as influenced by diets formulated to induce tibial dyschondroplasia (TD) or rickets. The diets were based on corn, soybean meal and soybean oil. The TD-inducing diet contained 0.60 % Ca and 0.50 % available P and the Phosphorous (P) rickets-inducing diet contained 1.00 % Ca and 0.25 % available P. The hens' age had significant effects on 0 – 16 d chick growth and the score and incidence of TD. The egg storage time had significant effects on the score and incidence of P rickets. The 1α-OH cholecalciferol (vitamin D_3) had significant effects on the score and incidence of P rickts and chick mortality (%) for 16 d.

INDEX WORDS: P deficiency, Vitamin D deficiency, age, Egg Storage, Broilers

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B.S., Sahm Yook University, 2004

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2007

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DEDICATION

I would like to dedicate this thesis to my parents for their unconditional love and support.

ACKNOWLEDGEMENTS

I would like to thank Dr. Gene M. Pesti for allowing me to work under him, and Dr. Remzi Bakalli to stand my mistakes in the lab.

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CHAPTER 1

INTRODUCTION

Bone Abnormalities in Broiler Chickens

Fast growing broiler chickens are especially susceptible to bone abnormalities, causing major leg problems for broiler producers. The cortical bones of fast growing broiler chickens are highly porous which may lead to bone deformity (Thorp and Waddington 1997). Skeletal problems are recognized as one of the four major factors affecting the performance of meat-type birds (Day 1990). The most common leg problems are tibial dyschondroplasia (TD) and rickets.

The occurrence of TD as a spontaneously occurring cartilage abnormality in broiler chickens was first described by Leach and Nesheim (1965). Edwards (1984) stated that TD usually appears between 3 and 8 weeks of age, and is caused by a low level of dietary calcium and a high level of dietary phosphorus (Edwards 1983). The bone lesion is characterized by an abnormal white, opaque, un-mineralized, and unvascularized mass of cartilage occurring in the proximal end of the tibia (Farquharson and Jefferies 2000). The abnormal cartilage is irregular in shape and size. There is also a persistence of prehypertrophic cartilage that is not calcified and has not been invaded by vessels from the metaphysis below the growth plate (Riddell 1975; Edwards 1984).

Another bone abnormality that sometimes occurs in commercial flocks is rickets which is a disease of the young birds and animals characterized by continued growth of cartilage and failure of mineralization and calcification of cartilage (Jubb and Kennedy 1970). It is generally considered to be the result of an imbalance of vitamin D_3, calcium, and phosphorus or a deficiency of one of these nutrients. Itakura et al. (1979) provided a detailed description of an outbreak of rickets. Bones were soft but cortical bone was thickened with narrowing of the marrow cavity. There are two types of rickets:Hypocalcemic rickets (calcium deficiency) is characterized by an accumulation of proliferating cartilage, and hypophosphatemic (phosphorus deficiency) rickets in which the hypertrophic cartilage accumulate with normal metaphyseal vessel invasion (Lacey and Huffer 1982).

CHAPTER 2

LITERATURE REVIEW

Factors Influencing Leg Problems in Broiler Chickens

Nutritional Factors

Bone is a highly complex structure, and the composition varies according to the age and nutritional status of the animals. Nutrients of major concern to leg problems are calcium (Ca), phosphorus (P) and Vitamin D. It was difficult to interpret Ca studies with poultry prior to the discovery of vitamin D (Edwards 1992). The primary function of Vitamin D is to promote calcium absorption (Nicolaysen et al. 1953; Keane et al. 1956). Nicolaysen (1956) stated that the increased phosphorus absorption was secondary to calcium absorption, because phosphate transport is dependent on calcium transport (Harrison and Harrison 1961).

Ca and P in Bone

Ca and P are the two most abundant minerals in the bone of animals. Ca and P in normal bone are 370 and 170 g/kg bone ash, respectively (Doyle 1979). Most Ca in the bone combines with P to form calcium phosphate crystals or hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (Scott et al. 1982). Bone is in a constant state of flux as Ca and P are liberated via resorption by demand of the body, and deposited when the extracellular fluid become sufficient. This exchange of Ca and P between the bone and soft tissues is a continuous process and the process is especially important during egg production when high Ca is required (McDonald et al. 2002). Given the rigidity of dietary specifications for Ca and P, and the importance of avoiding excessive use of phosphorus to minimize pollution, dietary contents sometimes fail to meet specifications (Whitehead et al. 2004).

Dietary Ca and P levels

The Ca and P levels in bone of domestic animals are often discussed together due to the very close association between the two minerals. An excessive or deficient level of Ca or P in bone often leads to bone abnormalities due to the interaction between the two minerals. TD and rickets are associated with deficiencies and imbalances of Ca and P. Edwards and Veltmann (1983) were able to increase the incidence of TD in young chicks by feeding low levels of dietary Ca and high levels of dietary P. Long et al. (1984a; 1984b; 1984c) in his experiments described the histological changes in the growth plate during Ca and P rickets by feeding broiler chickens a phosphorus rickets-inducing diet (low level of P and normal Ca or high level of Ca and normal P) and a Ca rickets-inducing diet (vitamin D deficient diet or a Ca deficient diet). Some TD can still occur even under optimum calcium and phosphorus feeding, and some birds are genetically pre-disposed to getting TD. However, rickets should be preventable by correct diet formulation (Waldenstedt 2006).

Ca and P Requirements of Layer Chickens

The early work on Ca and P requirements were based on the slow-growing leghorn chickens bred for egg production. Leghorn chickens are physiologically adapted to absorb high levels of Ca for the formation of the egg shell in which calcium carbonate is deposited (Scott et al. 1982). The optimum ratio of Ca and P to obtain maximum growth and bone ash and the ratio of Ca to P was thought to be more important than the amounts of these minerals (Hart et al. 1930; Wilgus 1931; Nowotarski and Bird 1943).

Bethke et al. (1929) and Hart et al. (1930) showed that the optimum ratio of Ca to P was between 3:1 and 4:1 when a low level of vitamin D was fed. However, the ratios and levels of Ca and P were less important when adequate vitamin D was included in the diet (Bethke et al. 1929; Hart et al. 1930; Carver et al. 1946). Bethke et al. (1929) and Hart et al. (1930) also showed that when no vitamin D was present, ckicks grew poorer and bones were calcified less than when vitamin D was plentiful, unless the levels of dietary Ca and P were increased. However, when levels of Ca and P were high, performance and bone mineralization were further improved by the inclusion of vitamin D in the diet. Nowotarski and Bird (1943) showed that high dietary Ca and P could not completely make up for a lack of vitamin D in terms of growth and crooked breast bones of 10 week old chicks.

The levels of Ca used in the early studies with leghorns to maximize body weight and bone ash ranged between 2.0 and 3.5% (Bethke et al. 1929; Hart et al. 1930; Nowotarski and Bird 1943), while current National Research Coucil (1994) requirements for broiler chickens range between 0.8 and 1.0% Ca, less than half of these early requirement estimates. Ca and P requirements for birds that more closely resemble the modern meat-type chicken were first published by Simco and Stephenson (1961) using Vantress White Cornich × Arbor Acre Line 50 chicks.

Ca and P Requirements on Broiler Chickens

Broiler diets generally supply extra Ca and P (Waldenstedt 2006) Broiler chicks fed low levels of P with recommended levels of Ca (0.8 to 1.0%) have poorer performance and lower bone mineralization than normal broiler chicks and suffer from severe P rickets (Davis 1959; Simco and Stephenson 1961). This is probably due to P deficiency caused by the formation of calcium phosphate complexes in the gut (Hurwitz and Bar 1971).

Diets containing marginal levels of Ca in relation to P led to an increase in the incidence of TD in young growing chickens (Edwards and Veltmann 1983). They reported that a high P to Ca ratio in the diet was a major factor affecting the expression of TD and that the most severe TD was obtained at the lowest dietary Ca level (0.63%). This response may be linked to the cation-anion balance in the blood, because TD is increased after adding different anions to the diet (Halley et al. 1987). Long et al. (1984a) observed identical lesions for the two conditions (marginal Ca with adequate P and adequate Ca with excess P) suggesting that excess calcium forms insoluble $Ca_3(PO_4)_2$ in the intestine, therefore inducing phosphorus deficiency. When phytase or 1,25-$(OH)_2D_3$ were not fed, no single combination of Ca and P eliminated all incidences of TD and rickets, but above 0.99% Ca reduced the incidence of TD, and above 0.55% P reduced the incidence P rickets (Mitchell and Edwards 1996).

The growth performance of modern broiler chickens has changed considerably over recent years but broiler diets have changed little (Williams et al. 1998). Williams (1998) showed a disruption in the Ca and P ratios of the fast growing strains compared to a slower growing strain during the first 2 to 3 weeks of age; ratios approached values of almost 3 to 1 compared to the optimal 2 to 1. Based on growth plate assessment, Williams et al. (1999) suggested that diets should contain 1.1 to 1.3 % Ca and 0.3 to 0.6% available P.

For Ca, optimal requirements for bone calcification are higher than those for body weight gain, but for phosphorus, requirements for growth and bone mineralization seem similar (Bar et al. 2003). The Ca and P ratio given for poultry is generally within the range between 1:1 and 2:1. A normal content of starter diets is about 10g Ca and 4.5 g available P/kg feed, an approximate ratio of 2:1. However, Williams et al. (2000a; 2000b) showed that in modern broilers, optimal Ca and P ratios in the bone up to 11 days was up to 2.6:1, and that dietary contents of 12g Ca and 4.5g available P/kg feed gave the most normal growth plate morphology at 2 weeks of age. Producers generally feed much less calcium to maximize energy utilization and feed utilization efficiency.

Vitamin D

The antirachitic vitamin was named vitamin D by McCollum et al. (1922). Mellanby (1919) was the first to formally recognize rickets as a nutritional disease of humans. He thought it was caused by a deficiency of vitamin A present in cod-liver oil. However, when McCollum et al. (1922) destroyed the vitamin A in cod-liver oil, the oil still possessed the antirachitic effect. Then, the antirachitic substance was named vitamin D. Vitamin D is the general term applied to a number of fat-soluble sterol derivatives which are active for the prevention of rickets in chicks. Vitamin D is generally added to the diet, because most raw materials in broiler diet contain no or little Vitamin D.

Vitamin D metabolism in the bird is a complex process involving several metabolites. Vitamin D_3 or cholecalciferol was much more active than Vitamin D_2 for prevention of rickets in chicks (McChesney 1943). Workers in poultry production have been concerned about several problems, such as the biopotency and stability of the Vitamin D_3 preparations used for feed fortification (Yang et al. 1973). As Vitamin D_2 (ergocalciferol) has only about 10% potency of Vitamin D_3 for poultry, Vitamin D_3 is used (McDonald et al. 2002). Cholecalciferol (vitamin D_3) is absorbed from the diet or synthesized in the skin from dehydrocholesterol (Hart et al. 1925; Edwards et al. 1994). Dietary Vitamin D_3 is absorbed through the small intestine and transported in the blood to the liver where it is converted to 25-hydroxycholecalciferol (25-OHD_3). Then, 25-OHD_3 is transported to the kidneys where it is converted to 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) (Edwards 1992). 1,25-$(OH)_2D_3$ is the active metabolite and stimulates calcium absorption for bone development and modeling.

Vitamin D_3

The symptoms of Vitamin D_3 deficiency in chicks are similar to calcium deficiency of the tibia (Cheville and Horst 1981; Long et al. 1984c). Leach and Nesheim (1965) thought Vitamin D_3 might play a role to prevent TD. Vitamin D_3 might also have an important role to prevent defects causing field rickets in poultry (Olson et al. 1981; Bar et al. 1987). The metabolic acidosis in chickens fed high dietary chloride levels caused TD because of impaired bone mineralization resulting from alteration of vitamin D_3 metabolism (Mongin and Sauveur 1977).

The NRC (1994) recommends 200 IU of vitamin D_3/kg of diet for starters (1 to 21d), but the studies were conducted in environments excluding Ultraviolet light. The vitamin D_3 requirement in starter feeds (Elliot and Edwards 1997; Mitchell et al. 1997; Kasim and Edwards 2000; Fritts and Waldroup 2003) is higher than the NRC (1994) recommendation. The risk of toxicity is very minimal, because the dietary cholecalciferol toxicity levels were estimated to be 400,000 IU/kg by Morissey et al. (1977) and 500,000 IU/kg by Taylor et al. (1968).

Several factors might be influencing the growing chicks' quantitative requirement of vitamin D_3 for the maximum performance, maximum bone ash and minimum leg abnormalities. First, the presence of ultraviolet light decreases the requirement of chicks for vitamin D_3 (Edwards 1992; Edwards et al. 1992; Edwards et al. 1994; Edwards 2003). Second, the amount of cholecalciferol in the chicks from the hen or intake when they are hatching alters the requirement of vitamin D_3 (Bethke et al. 1936; Murphy et al. 1936; Griminger 1966). Third, the level of Ca and available P alters the requirement of vitamin D_3 (Waldroup et al. 1965; Baker et al. 1998). Fourth, the level of vitamins A and E also alters the requirement of vitamin D_3 (Aburto and Britton 1998a, b; Aburto et al. 1998).

The quantitative requirement of vitamin D_3 varies according to the criteria under evaluation. Usually the requirement is based on bone ash or the incidence of TD and rickets which have been found to be more sensitive indicators than growth rate. Edwards et al. (1994) used a corn-soybean diet containing 1.1% Ca and 0.61% available P in an environment excluding ultraviolet light, and he found that birds required cholecalciferol in ICU per kg of diet of 275 for growth, 503 for bone ash, 552 for blood plasma Ca and 904 for rickets prevention in the experiment 3 of the paper.

Waldroup et al.(1965) showed that the requirement of broiler chicks for vitamin D_3 depended on the dietary Ca and P levels. He reported that when birds were fed the levels of 1.00% Ca and 0.70% P, maximum body weight and bone ash were obtained with 90 ICU/lb (198 ICU/kg) of diet. This was in agreement with the vitamin D_3 requirement estimate by NRC (1960) at that time and the vitamin D_3 requirement is 200 ICU/kg by NRC (1994) now. However, when birds were fed a dietary Ca level of 1.00% and total P of 0.50%, 3600 ICU/lb (almost 8,000 ICU/kg) were needed for optimum body weight gain and bone ash. When the Ca level was 0.50%, the requirement of chicks for vitamin D_3 was decreased to 720 ICU/lb (1,600 ICU/kg).

Aburto and Britton (1998a; 1998b) and Aburto et el. (1998) reported that high levels of vitamins A and E negatively affected the utilization of vitamin D_3 or 25-OH$D_{3.}$ Therefore, the requirement for vitamin D_3 was increased by increasing the levels of vitamin A and E. They observed that 45,000 IU/kg of vitamin A decreased bone ash when vitamin D_3 was supplemented at 500 IU/kg to chicks in environments excluding Ultraviolet light. However, they couldn't observe any negative effect when D_3 was supplemented at 2,500 IU/kg. Similarly, vitamin E at 10,000 IU/kg decreased body weight, bone ash, plasma Ca and increased rickets incidence when vitamin D_3 was supplemented at 500 IU/kg in the environment excluding ultraviolet light. However, when vitamin D_3 was supplemented at 2,500 IU/kg in the same environments, no negative effect was observed.

Body weight gain, bone ash, and incidence of rickets might be changed by different sources of vitamin D_3 (Kasim and Edwards 2000). When the dietary vitamin D_3 supplementation increased in the diet, body weight gain and tibia ash are increased, and rickets incidence was decreased. The maximum level of vitamin D_3 used in this paper was 1200 IU/kg. Fritts and Waldroup (2003) observed a decrease in TD incidence and an even greater decrease in TD severity by supplementing vitamin D_3 up to 4,000 IU/kg of the diet of broiler chicks. More recently, McCormack et al. (2004) using vitamin D_3 at concentrations of 200, 800, 5,000, and 10,000 IU/kg reported that 10,000 IU can prevent TD incidence almost completely. Chicks were able to tolerate high levels of vitamin D_3 in diet by 50,000 IU/kg vitamin D_3 in the environment excluding ultraviolet light (Baker et al. 1998). As mentioned before, the risk of toxicity is very minimal, because the dietary cholecalciferol toxicity levels were estimated to be much higher than requirements, 400,000 IU/kg (Morissey et al. 1977) and 500,000 IU/kg (Taylor et al. 1968).

Even though 25-hydroxycholecalciferol (25-OHD_3) is currently used as a commercial feed additive and supplementation of the diet with 25-OHD_3

might significantly decrease the incidence and severity of TD, 25-OHD_3 is less effective than 1α-OHD_3 or 1-25$(OH)_2D_3$ (Boris et al. 1977; Edwards 1989). Yarger et al. (1995a; 1995b) indicated that 25-OHD_3 could be safely used as a source of cholecalciferol for commercial broiler rations. A significant increase in body weight occurred in 9 out of 10 studies and a significant improvement in adjusted feed efficiency occurred in 7 out of 10 studies when broiler were fed 25-OHD_3. They conducted two experiments using high levels of 25-OHD_3 (69,207 and 690 μg/kg, Experiment 2) (Yanger et al. 1995a). No toxic effects or improvement in performance were observed by increasing the level of 25-OHD_3 in experiment 1. In the second experiment, however, there was some evidence of renal calcification in birds fed 25-OHD_3 at 10 times the basal level, whereas dietary levels of vitamin D_3 at 50 times the basal level were required to show some evidence of renal calcification. They concluded that 25-OHD_3 is 5 to 10 times more toxic than vitamin D_3.

25-OHD_3 has been shown to improve bone ash and decrease the incidence and severity of TD and Ca rickets in broiler chicks (McNutt and Haussler 1973; Zhang et al. 1997; Fritts and Waldroup 2003; Ledwaba and Roberson 2003). However, other studies have not shown a decrease in TD incidence in broiler chicks selected for high incidence of TD by supplementing 25-OHD_3 in the diet (Zhang et al. 1997). Saunders and Blades (2004) supplemented broiler diets with 25-OHD_3 at 69μg/kg or vitamin D_3 at 3,000 IU/kg and observed faster body gain and greater breast and leg portions with 25-OHD_3. Even though when high levels of D sources were supplied the birds still developed rickets at 11 days of age.

Several studies have shown that 25-OHD_3 is more potent than D_3 (McNutt and Haussler 1973; Boris et al. 1977; Fritts and Waldroup 2003). However, when 25-OHD_3 is compared to vitamin D_3, its potency depends on the levels of vitamin D_3 tested.

Miscellaneous Factors

Genetics

Approximately two thirds of P in poultry diets is in the form of myo-inositol 1,2,3,4,5,6-hexakisphosphate or phytate P (PP) (Nelson et al. 1968; Reddy et al. 1982). PP utilization is different by different breeds of poultry, especially between layers and broilers. Gardiner (1969) conducted experiments with three different levels of dietary P and he found P retention, plasma P and bone ash deposition were significantly greater in single comb white leghorn (SCWL) chicks compared to commercial broiler chickens. The Athens Canadian Randombred (ACRB) chickens, an unselected random-mated chicken population, also utilize PP more efficiently than commercial broilers (Edwards 1983). Edwards (1983) also demonstrated SCWL were better able to utilize PP than commercial broilers when dietary Ca was high.

There is variation in PP utilization between broilers of the same strain related to growth, livability, and skeletal strength (Punna and Roland 1999). They conducted an experiment with corn-soybean diets with 0.95% Ca and 0.5% available P (aP), and collected feces after the second and fourth weeks revealed that those birds which demonstrated normal growth, no leg problems and no visible signs of a phosphorus deficiency were able to utilize phosphorus far more efficiently than birds which did display problems. Even PP utilization within single strains of chickens had differences (Carlos and Edwards 1998; Zhang et al. 1998; Smith et al. 2001).

Zhang et al. (2003) conducted an experiment to estimate the heritability of P (PBA), generic correlation of PBA, growth and feed utilization by establishing a pedigree base population from Athens-Canadian randombred (ACRB) chickens. Heritability for phytate P bioavailability (PBA) was estimated to be 0.10, which is a low heritability. Genetic correlations between PBA and body weight and feed consumption were moderate and negative, which indicates that selection for PBA would moderately and negatively affect growth. No genetic correlation was found between PBA and FCR. Ankra-Badu et al. (2004), using the same population of birds, found that faster growing birds utilized PP poorly compared to slow growing birds. It was suggested that faster growing birds have a reduced retention time compared to slow growing birds which reduces the exposure of PP in the feed to microbial and endogenous phytases and phosphatases. Broilers grow faster than layers. PBA was also positively correlated to energy utilization. Birds that utilized PP well may release more P for incorporation into the energy intermediate molecules adenosine diphosphate and adenosine triphosphate.

Breed differences are a source of variation between experiments. The meat-type chickens used in the different experiments had widely differing growth rates. Rapid growth is associated with a narrow feed to gain ratio while poor growth is associated with a wide feed to gain ratio. To maintain rapid growth, a diet must contain higher levels of nutrients to furnish the same nutrient intake per gram of gain as when poor growth and feed efficiency are obtained. Edwards et al. (1963) speculated that this might partially explain why chickens grown with a feed to gain ratio of approximately 1.9. Simco and Stephenson (1961) had a much lower Ca requirement than chickens grown by Edwards et al. (1963) with a feed to gain ratio approximately 1.5.

Chick's Age

Many experiments have found that mineral requirements were decreased with age when expressed as a proportion of the feed. The decrease in requirements may also be related to the increase in feed to gain ratio that occurs as birds' age. The proportions for maintenance increases (low requirement) and the proportion for growth decreases (high requirement). The P requirement of poultry were decreased with body size (Mitchell and McClure 1937; Mitchell 1947). The NRC (1994) P recommendations for chicks at the different ages 1 to 21, 22 to 42 and 43 to 56 days are 0.9%, 0.45% and 0.30% aP, respectively. The NRC (1994) Ca recommendations also decreased with increasing age as 1 to 21, 22 to 42 and 43 to 56 days were 1.0%, 0.8% and 0.35% Ca, respectively.

Ca and P requirements based on maximum bone ash were also different by age. Birds 0 - 6 wk old needed 1.04% Ca and 0.36% aP (Huyghebaert 1996). Birds 3 - 30 d old needed 1.00% Ca and 0.44% aP (Rama Rao et al. 1999). Birds 0 - 21 d old needed 1.00% Ca and 0.35-

0.44% aP (Waldroup et al. 2000). Birds 8 - 22 d old needed 1.04-1.3% Ca and 0.40-0.45% aP (Boling-Frankenbach et al., 2001). Birds 0 - 43 d old needed 1.39-1.57% Ca and 0.48-0.57% aP (Bar et al. 2003).

Egg Quality Factors

Breeder's Age

Fertility is decreasing from 95% to 90% by increasing breeder's age (from 27-29wks to 60wks), and hatchability is 10-15% lower than fertility (Wineland and Brake 1984). Previous studies have determined the effects of age of breeders on hatchability and chick hatching weight (Suarez et al. 1997; Lapao et al. 1999; Silversides and Scott 2001). Set weight, transfer weight, and hatch weight of eggs during incubation were increased by increasing age of female breeders (Suarez et al. 1997).

Smith and Bohren (1975) observed that the age groups have a significant effect on egg weight. This effect was entirely linear as the egg weight increases with the age of the pullets. The egg weight was a dominant factor affecting chick weight at hatch (Wilson 1991; Silversides and Scott 2001; Tona et al. 2002). From previous studies, Willey (1950), Tindell and Morris (1964), Merritt and Gowe (1965), and Morris et al. (1968) all reported significant, positive correlations between egg weight and broiler body weight at 63, 55, 67, and 84 days of age, respectively.

Egg Storage Time (pre-incubation storage)

Egg storage before incubation may affect embryonic life of the chick and thereafter the quality of the hatched chick and the growth potential posthatch. Previous studies have determined the effects of egg storage on hatchability and chick hatching weight (Reis et al. 1997; Lapao et al. 1999; Silversides and Scott 2001; Tona et al. 2003).

Bohren (1978) stated hatchability was not significant for the days of pre-incubation storage, and Reis et al. (1997) found no effect of preincubation storage on viability and hatchability in young breeders and viability was even higher in fresh eggs from older hens.

However, Wilson (1991) restated the often-heard comment that and egg is at its maximum hatching potential the moment it is laid. It is commonly recommended that hatching eggs be cooled immediately after they are laid. However, there are some instances in which immediate chilling of the eggs, especially of afternoon laid eggs, has been observed to increase embryonic abnormalities and to decrease hatch. Good quality hatching eggs kept under optimum conditions can be stored for about seven days with little loss of hatchability.

Jones and Musgrove (2005)collected forty-five dozen large eggs from a single inline processing facility weekly for 3 wk (replicates) and stored eggs in a 4°C cold room with 80% RH. They found a distinct decrease in egg weight during the first 5 wk of storage for the only second replicate from an average weight of 65.29 g to 58.03 g.

Albumen height and albumen pH were statistically unrelated in fresh eggs, but the association became larger as the storage period increased, suggesting that albumen height measures factors that are present when the egg is laid and changes during storage, whereas albumen pH measures only the effect of storage (Scott and Silversides 2000)

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CHAPTER 3

THE EFFECT OF DIET, HENS' AGE AND EGG STORAGE TIME ON BROILER PERFORMANCE AND BONE ABNORMALITIES[1]

[1]Shim, M. Y., G. M. Pesti, R. I. Bakalli and H. M. Edwards Jr. To be submitted to Poultry Science.

ABSTRACT

Two experiments were conducted with chicks hatched from eggs laid by Ross × Ross 308 hens to investigate the influence of hens' age (29 - 37 vs. 64 - 66 wk old) and egg storage time (0 vs. 10 d) on growth and bone abnormalities in chicks fed nutritionally adequate on deficient diets. The tibial dyschondroplasia (TD)-inducing diet contained 0.60 % Ca and 0.50 % available P (aP), and the P rickets-inducing diet contained 1.00 % Ca and 0.25 % aP.

In Experiment 1, hens' age had significant effects ($P<0.05$) on 0 – 16 d chick growth (373 ± 11 vs. 415 ± 10 for 29 and 66 wk old hens, respectively). The chicks from the older hens showed lower TD scores ($P=0.0018$) and incidence ($P=0.0025$), but hens' age did not affect P rickets score or incidence. The longer stored eggs resulted in lower P rickets scores ($P=0.0018$) and incidence ($P=0.0039$). The TD inducing diet caused a much higher incidence of TD in chicks from young (66%) vs. older (38%) hens (interaction $P = 0.0176$). The P rickets-inducing diet decreased P rickets in chicks from stored eggs (21%) vs. those from fresh eggs (39%) (interaction $P = 0.0039$). Bone ash was affected by diet, hens' age and egg storage time (3-way interaction $P = 0.0034$).

In Experiment 2, the control diet fed chicks had higher body weight gains (BWG) compared to those fed the P rickets inducing diet (442±7 vs. 372±4, respectively). Chicks from stored eggs had higher BWG than chicks from fresh eggs and fed the control diet. However, chicks fed the P deficient diet had lower BWG than the control diet. BWG, P rickets and bone ash were affected by diet at $P<0.01$.

Responses of chicks from the very young hens (28-29 wks) were different from those of the older hens (65-66 wks) in Experiment 1, but after peak (36-37wks) which responses were very similar to those from older hens (63-64 wks) in Experiment 2.

These experiments indicated that the incidence of bone abnormalities in broilers may be influenced by the hens' age, egg storage time and diet.

Key words: P deficiency, Vitamin D deficiency, age, Egg Storage, Broilers

INTRODUCTION

Fast growing broiler chicks are especially susceptible to bone abnormalities, causing major problems for broiler producers. Bone is a highly complex structure, and the composition varies according to the age and nutritional status of the animals. The most common bone abnormalities in commercial broiler production are tibial dyschondroplasia (TD) and rickets. Edwards and Veltmann (1983a) were able to increase the incidence of TD in young chicks by feeding low levels of dietary Ca and high levels of dietary P. On the other hand, severe phosphorus rickets (P-rickets) commonly occurs because broiler chicks are fed low levels of P with recommended levels of Ca. Waldenstedt (2006) concluded rickets should be totally preventable by correct diet formulation.

Nelson et al. (1992) conducted two experiments with chicks from Cobb 500 hens (27 vs. 57 wks of age) and reported that chicks from an older parent flock showed greater body weight gain (BWG) than chicks from a young parent flock, but age of maternal flock did not affect the incidence of TD. The TD incidence was 17% from old maternal flock (57 wks of age) and 25.9% from young maternal flock (27 wks of age). Nelson et al. (1992) fed 0.95% or 1.00% Ca with 0.50-0.52% available Phosphorus (aP) which might not induce TD.

Driver et al. (2006) conducted a series studies with a flock of hens to compare the incidence of TD in chicks from hens fed 250 or 2000 IU of vitamin D_3 per kg diet. The incidence of TD in the progeny declined from 85 to 24 % as the hens aged from 39 to 64 wks. Although the hen's age was a highly significant contribution to variation in progeny TD incidence, there was no way to distinguish hen's age effects from season effects or the influence of raising the different batcher of chicks under slightly different environment and nutritional conditions (different sources of macro ingredients).

Breeder age and egg storage time before incubation may affect the embryonic life of the chick and thereafter the quality of the hatched chick and the growth potential posthatch. Breeders' age and egg storage time are less-well-understood compared to nutritional and environmental factors. Egg storage time has been reported to have negative effects on chick quality, growth rate, and embryonic mortality and abnormalities (Merritt 1964), although some earlier studies in the 1940's and 1950's showed no large differences. However, the effects of egg storage time on bone abnormalities have not been studied.

There may be unknown, or at least uncontrolled, factors that are influencing the incidence of bone abnormalities in research and commercial settings. The observed variation in the incidence of TD within and between experiments seems high. For instance, Eliott and Edwards (1994) observed incidence of TD in control chicks of 43 and 69 % in seemingly identical treatment groups in different experiments; and Mitchell et al (1997) observed incidence of TD of 20 and 44 % in seemingly identical treatment groups in two experiments.

The specific objection of the study were to confirm the relationship between hen's age and TD observed by Driver et al (2006) using flocks of different ages so the chicks could be raised under identical conductions, and to determine if egg storage time could be a factor contributing to the observed variation in the incidence of leg abnormalities.

MATERIALS AND METHODS

General Procedures

Eggs laid by Ross × Ross 308 hens from older and young hens were stored at 12°C and 66% relative humidity for 0 or 10 d before setting. Each egg was individually marked with date of lay. Eggs not stored were collected the morning they were laid, and placed in egg trays at room temperature (20°C) for 12 h overnight before they were placed in the incubator. Eggs were set at 37.7°C and 55% RH in a single-stage NatureForm NMC-2000, 1,980 egg capacity setter (NatureForms Hatchery System, Inc., 925 North Ocean St., Jacksonville, FL 32202) with automatic temperature regulation (accurate to 0.10 °C), relative humidity (accurate to +/-2% relative humidity), and an automatic turning system (24 times/day).

For Experiment 1, on day 14, eggs were candled and all eggs with no of live embryos where removed. On day 19, eggs were transfered into hatcher baskets, and placed into the hatcher at 36.7-36.9°C and 70% relative humidity. For Experiment 2, on day 11, eggs were candled and all eggs without live embryos where removed. On day 18, eggs were transfered into hatcher baskets, and placed into the hatcher as above.

The chicks were housed in Petersime® wire-floored battery brooders for 16 days and were provided with water and the dietary treatments ad libitum. At the conclusion of each experiment, birds were weighed and sacrificed by asphyxiation using carbon dioxide.

Left tibias from all chicks were collected on day 16 for percentage tibia ash determination on a fat free dry weight basis (Association of Official Agricultural Chemists, 1995) while right tibias were sliced and scored for the severity of bone abnormalities including P rickets, Ca rickets and tibial dyschondroplasia (TD) as described by Edwards and Veltmann (1983b).

Experiment 1

Eggs laid by 29 vs. 66 wk old Ross × Ross 308 hens (1080 eggs each) were incubated. Four chicks from each hen age and egg storage time combination (16 chicks total) were placed in each of 5 pens per diet (20 pens total). The progeny were fed four diets based on corn, soybean meal and soybean oil (Table 1).

Experiment 2

720 Eggs each laid by Ross × Ross 308 hens 37 and 64 wk old were incubated. Ten chicks from each diet, hens' age and egg storage time combination were placed in each of 48 pens. The progeny were fed two corn-soybean basal diets (control vs. P deficient diet; Table 2).

Statistical Analysis

Analysis of variance was performed on all data for both experiments using the General Linear Model procedure of SAS (Version 8.02, 2001) appropriate for a randomized block design. Treatment means were separated using Duncan's Multiple Range Test at $P < 0.05$ (Duncan 1955).

RESULTS

In Experiment 1, with 37 wk difference in age of hens, there were significant differences in apparent fertility, 1 and 16 d body weights and 16 d mortality due to hen age (Table 3). Only day-old chick weights were different ($P<0.05$) due to hen age in Experiment 2 with a 27 wk difference in hen age. The difference in day-old chick weights due to hens' age was much more in Experiment 1 (7.7g) than Experiment 2 (4.5 g). Egg storage time was not a significant contributor to variation in any of the incubation or growth parameters in either experiment.

Diet contributed to variation in 16-d BWG in both experiments, but hens' age was only a significant contribution to 16-d BWG in Experiment 1. Diet was a significant contribution to variation in feed intake (FI) in Experiment 2. The P deficient diet decreased BWG and increased the score and incidence of P rickets in both experiments (Tables 5-7). Egg storage for 10 d decreased the incidence of P rickets in Experiment 1, but not in Experiment 2. The vitamin D deficient diet did not decrease growth or increase the incidence of rickets indicating that unsupplemented chicks were receiving a source of vitamin D from the eggs or some other component of the diet.

The TD inducing diet increased the incidence and severity of TD. This was especially marked in the chicks from the young hens. The three–way interaction ($P = 0.0034$) for the effects of hen age, diet, and egg storage time (ES time) emphasizes the complexity in determining the factors influencing leg problems in broilers (Tables 5-6). Egg storage time had its biggest effect on bone ash with chicks from older hens given the P rickets inducing diet. Bone ash was higher in chicks from young hens on all diets except the P rickets inducing diet, in which bone ash was higher in chicks from the older hens.

Mortality of chicks from younger hens (36-37 wk old) and eggs with longer (10d) and shorter (0d) egg storage times were 3.33% and 0.83%, and mortality of chicks from older hens (63-64 wk old) were 2.50% and 4.17%, respectively in Experiment 2.

BWG was significantly different between the eight treatments ($P<0.0001$; Table 7). FI and feed conversion ratio (FCR) were significantly different between the eight treatments ($P<0.05$). The control diet had higher body weight gain compared to P rickets inducing diet (442±7 vs. 372±4, respectively; Table 7). Chicks from eggs stored 10d had slightly higher BWG than chicks from fresh eggs fed the control diet, but it was not significantly different ($P=0.2832$).

P rickets and bone ash were affected by diet at $P<0.01$ (Table 8). Only the P deficient diet induced P rickets, but TD was present in chicks fed the control and P rickets inducing diets. Bone ash was higher with the control diet compared to P deficient diet (39.90±0.18 vs. 30.39±0.16, respectively).

DISCUSSION

Driver (2004) observed a decrease in the incidence of TD in chicks as hens' age increased. In those experiments, the different chick experiments were conducted at different times as the hens aged. Therefore, there were several factors that could have been confounded with hen ages and caused the results like feed ingredient source used in the hens and chick feeds. Experiment 1 demonstrates that the hen's age does affect TD in chicks since the hens' genotypes and rearing protocols were identical while chicks were raised under identical conditions co-mingled in the same pens. The severity of TD was reduced as the hens became older, which may be related to increased deposition of vitamin D or its metabolites in the eggs as the laying cycle progresses. Vitamin D metabolites have been shown to increase phytate P retention in broiler chicks (Edwards 1993) which would have increased the amount of P available to the bird.

Percentage hatch from very young hens (28-29 wks of age) were slightly higher and percentage hatch from young hens (36-37 wks of age) and old hens (63-64 and 65-66 wks of age) were lower than breeder expectations (Ross × Ross Management Guide, Aviagen, AL 35805), and lower than observations from Wineland and Brake (1984). Fertility decreased from 95% to 90% by increasing breeder age (from 27-29wks to 60wks), and hatchability was 10-15% lower than fertility (Wineland and Brake 1984). In the present studies, fertility was decreased from 97.21% to 80.17% with increasing breeder age (from 29-37wks to 64-66wk old), and hatchability of egg set was 11-27% lower than fertility.

Smith and Bohren (1975) observed that age groups have a significant effect on egg weight. This effect was entirely linear as the egg weight increased with the age of the pullets. The egg weight was a dominant factor affecting chick weight at hatch (Whiting 1982; Wilson 1991; Silversides and

Scott 2001; Tona et al. 2002). In the both Experiments 1 and 2, chick weights at hatch from older hens (64-66 wk old) were greater than younger hens (27-29 wk old). However, chick weights at hatch were not linearly increased by breeder age, because two experiments were conducted at different season with eggs from different maternal flocks.

Petek and Dikmen (2004) conducted experiments with two quail breeder ages (20 vs. 37 wk) and two egg storage times (5d vs. 15d). They had greater chick mortality (6.74 - 8.29%) than Experiment 1 and 2 and greater variation between replicates. Chick mortality in Experiments 1 and 2 from 0 to 6.25% could not be declared significantly different by age of breeders or egg storage. Longer storage times may result insignificant difference but the results would not be of practical significance.

In the Experiment 1, hen age had significant effects on 0 – 16 d chick growth confirmed the finding of other studies that the progeny from older hens had higher growth than the progeny from young hens (Whiting 1982; Reis et al. 1997).

Egg storage reduced apparent fertility, HES and HFE for 29 and 66 wk old hens in Experiment and 64 wk old hens in Experiment 2, but the differences were not big enough to be declared significant ($P>0.176$). Egg storage slightly increased these parameters in eggs from 37 wk old hens in Experiment 2 ($P>0.572$). No influence of egg storage on 0 or 16 d chick weights or mortality in either experiment. In Experiment 1, the longer egg storage time (10 d) resulted in lower P rickets score and incidence ($P=0.0381$, $P=0.0705$).

The incidence of P rickets is probably related to reducing bone ash. Bone ash was affected by diet, hens' age and egg storage time (3-way interaction). Egg storage time had its biggest effect on bone ash with chicks from older hens given the P rickets inducing diet. Bone ash was higher in chicks from young hens on all diets except the P rickets inducing diet, in which bone ash was higher in chicks from the older hens.

The second experiment did not show any significant differences between treatments except diets. The age of breeders (37 vs. 64 wk old) was closer than the age of breeders in Experiment 1 (29 vs. 66 wk old). Even hen age did not affect chick body weight gain. Chicks fed the control diet had higher body weight gain compared to those fed the P rickets inducing diet. Chicks from eggs stored for 10d had higher body weight gains than chicks from eggs not stored and fed the control diet. However, the P deficient diet did not affect body weights.

The vitamin D-Rickets inducing diets did not cause obvious symptoms in Experiment 1. Vitamin D-Rickets might have been prevented by several factors like ultraviolet light exposure of the breeders, nutrition of breeders or the levels of Ca and available P, the levels of vitamins A and E in the chick diets (Bethke et al. 1936; Murphy et al. 1936; Waldroup et al. 1965; Griminger 1966; Edwards et al. 1992; Edwards et al. 1994; Aburto and Britton 1998a, b; Aburto et al. 1998; Baker et al. 1998; Edwards 2003). Those were not strictly controlled in Experiment 1.

It is clear from the trials of Driver (2004) and Experiment 1 that hens' age can influence the incidence of TD. However, comparing Experiment 1 and

Experiment 2 makes it clear that large differences in hens' age are necessary to demonstrate significant differences. Very highly significant differences ($P<0.004$) in P rickets were observed in Experiment 1 due to egg storage when the hens are very young (28-29 wks of age), but not in another experiment when the hens have passed peak production (36-37 wks of age).

Mineral supplementation of breeders might have influenced chick performance. Many experiments have found that mineral requirements were decreased with age when expressed as a proportion of the feed. The decrease in requirements may also be related to the increase in feed to gain ratio that occurs as birds' age. The P requirement of poultry were decreased with body size (Mitchell and McClure 1937; Mitchell 1947).

Vitamin D_3 supplementation of breeders might have influenced egg and chick parameters. Leach and Nesheim (1965) thought Vitamin D_3 might play a role to prevent TD. Vitamin D_3 might also have an important role to prevent defects causing field rickets in poultry (Olson et al. 1981; Bar et al. 1987; Driver et al. 2006). BWG, bone ash, and incidence of rickets might be changed by different sources of vitamin D_3 (Kasim and Edwards 2000).

In our present experiments, we had only old and young hen ages and 0 and 10 d egg storage times. The results were too sparse to guess at the shape of the response curves. Only two points (two ages of hens or two egg storage times) can determine only straight lines, and more points would allow getting non-linear shapes. A comparison of Experiment 1 and 2 suggests pre- and post-peak may be the important distinction for maximum progeny bone health. If we had more samples from different hens' ages and several egg storage times, we might get nice graphs. Therefore, in the future experiments, we can compare chick performance with chicks from several breeder ages. We can also design egg and chick experiments with several egg storage times. The progeny of young broiler breeder flocks may benefit from higher maternal nutrient levels and/or higher or at least better balanced nutrients in their own feeds.

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TABLES AND FIGURES

TABLE 3.1. Composition of basal diets, experiments 1.

Ingredient	Control	Low P	Low Vit. D	TD Inducing
	------------------------(%)-------------------------			
Ground yellow corn	52.15	53.35	52.15	54.31
Soybean meal (dehulled)	37.73	37.52	37.73	37.35
Soy bean oil	5.97	5.57	5.97	5.26
Iodized sodium chloride	0.45	0.45	0.45	0.45
DL-Methionine	0.19	0.19	0.19	0.19
Vitamin premix[1]	0.25	0.25	0.00	0.25
Special vitamin premix[2]	0.00	0.00	0.25	0.00
Trace mineral premix[3]	0.08	0.08	0.08	0.08
Dicalcium Phosphate	1.91	0.57	1.91	1.90
Limestone	1.26	2.01	1.26	0.21
Calculated composition[4]				
ME, kcal	3.20	3.20	3.20	3.20
CP, %	23.00	23.00	23.00	23.00
Calcium, %	1.00	1.00	1.00	0.60
Phosphorus-total, %	0.74	0.49	0.74	0.74
nPP, %	0.50	0.25	0.50	0.50

[1]Vitamin mix provided the following (per kilogram of diet): Thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (*cobalamin*), 12.0 ug; pyridoxine-HCl, 2.7 mg; D-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-retinyl acetate, 5,500 IU; all-rac-tocopherol acetate, 11 IU; ethoxyquin, 150 mg.

[2] Special vitamin mix provided following (per kilogram of diet): Thiamin-mononitrate, 2.2 mg; riboflavin, 4.4 mg; Ca pantothenate, 12 mg; vitamin B_{12} (*cobalamin*), 0.01 mg; pyridoxine-HCl, 4.0 mg; niacin 44 mg; biotin, 0.3 mg; folic acid, 3 mg; menadione sodium bisulfate complex, 1.1 mg; choline chloride, 220 mg; vitamin A, 5500 mg; vitamin E, 11 mg; ethoxyquin, 150 mg.

[3]Trace mineral mix provides the following (per kilogram of diet): manganese ($MnSO_4.H_2O$), 60 mg; iron ($FeSO_4.7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4.5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 1.5 mg.

[4]Calculated from NRC (1994).

TABLE 3.2. Composition of basal diets, experiments 2.

Ingredient	Control	Low P
	----------------------(%)----------------------	
Ground yellow corn	52.15	53.35
Soybean meal (dehulled)	37.73	37.52
Soy bean oil	5.97	5.57
Iodized sodium chloride	0.45	0.45
DL-Methionine	0.19	0.19
Vitamin premix[1]	0.25	0.25
Trace mineral premix[2]	0.08	0.08
Dicalcium Phosphate	1.91	0.57
Limestone	1.26	2.01
Calculated composition[3]		
ME, kcal	3.20	3.20
CP, %	23.00	23.00
Calcium, %	1.00	1.00
Phosphorus-total, %	0.74	0.49
nPP, %	0.50	0.25

[1]Vitamin mix provided the following (per kilogram of diet): Thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (*cobalamin*), 12.0 ug; pyridoxine-HCl, 2.7 mg; D-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-retinyl acetate, 5,500 IU; all-rac-tocopherol acetate, 11 IU; ethoxyquin, 150 mg.

[2]Trace mineral mix provides the following (per kilogram of diet): manganese ($MnSO_4.H_2O$), 60 mg; iron ($FeSO_4.7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4.5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 1.5 mg.

[3]Calculated from NRC (1994).

Table 3.3. The influence of hens' age and egg storage (ES) time on egg and chick performance and suvival (Experiment 1)

Age of hens (wk)	ES Time[1] (d)	Apparent Fertility (%)	Hatchability HES[2] (%)	Hatchability HFE[3] (%)	Day-old chick weights (g)	16-d-old chick weights (g)	0-16 d Mortality (%)
29	Fresh eggs	97.21	86.41	88.89	38.2±0.4[4]	421±13	6.25±3.08
28	10 d	94.88	70.95	71.26	38.7±0.3`	403±19	6.25±3.08
66	Fresh eggs	80.17	65.16	81.27	46.0±0.3	455±13	1.25±1.25
65	10 d	78.87	59.84	75.43	46.3±0.4	467±16	0.00±0.00

Source	df[5]	Pr>F	Pr>F	Pr>F	df	Pr>F	Pr>F	Pr>F
Hens Age	1	0.0198	0.1933	0.8188	1	<0.0001	0.0022	0.0151
ES Time[5]	1	0.1760	0.2890	0.2964	1	0.2523	0.8495	0.7831
Hens Age*ES Time	1	—	—	—	1	0.7849	0.3379	0.7831
Error	1				44			

[1]ES Time=Egg Storage Time
[2]HES = Hatch of Egg Set
[3]HFE = Hatch of Fertile Eggs
[4]Main effect means ± standard errors of 5 pens of 4 birds each
[5]Degrees of Freedom

Table 3.4. The influence of hens' age and egg storage on egg and chick parameters (Experiment 2)

Age of hens (wk)	Egg storage (d)	Apparent Fertility (%)	Hatchability HES[1] (%)	Hatchability HFE[2] (%)	Day-old chick weights (g)	16-d-old chick weights (g)	0-16 d Mortality (%)
37	Fresh eggs	95.97	68.01	70.86	43.2±0.3[3]	450±11	0.83±0.83
36	10 d	96.46	71.68	74.31	43.0±0.3	457±15	3.33±1.88
64	Fresh eggs	87.17	66.76	76.59	47.6±0.5	447±13	4.17±1.49
63	10 d	82.87	59.95	71.34	47.5±0.4	457±14	2.50±1.31

Source	df[4]	Pr>F	Pr>F	Pr>F	df	Pr>F	Pr>F	Pr>F
Hens Age	1	0.1342	0.4323	0.7108	1	<0.0001	0.9088	0.3858
ES Time[5]	1	0.5722	0.8147	0.9341	1	0.6826	0.5161	0.7716
Hens Age*ES Time	1	—	—	—	1	0.9965	0.8959	0.1514
Error	1				44			

[1]HES = Hatch of Egg Set
[2]HFE = Hatch of Fertile Eggs
[3]Main effect means ± standard errors of 12 pens of 10 birds each
[4]Degrees of Freedom
[5]ES Time=Egg Storage Time

TABLE 3.5. The influence of hens', egg storage time and diets on bone quality (29 vs. 66 wk old), Experiment 1

Type of Diet	Hens' Age	ES[1] Time	n	BWG (g)	P Rickets Score	P Rickets Inc. (%)	Vit. D Rickets Score	Vit. D Rickets Inc. (%)	TD Score	TD Inc. (%)	TD3 Inc. (%)	Bone Ash (%)
Control	66	0	5	450±10	0.00±0.00[2]	0.0±0.0	0.05±0.05	5.0±5.0	0.00±0.00	0.0±0.0	0.0±0.0	41.06±0.32
Control	66	10	5	456±21	0.05±0.05	5.0±5.0	0.00±0.00	0.0±0.0	0.10±0.10	5.0±5.0	0.0±0.0	41.09±0.19
Control	29	0	5	412±18	0.00±0.00	0.0±0.0	0.00±0.00	0.0±0.0	0.35±0.15	15.0±6.1	5.0±5.0	40.77±0.22
Control	28	10	5	410±13	0.00±0.00	0.0±0.0	0.05±0.05	5.0±5.0	0.10±0.10	10.0±10.0	0.0±0.0	40.42±0.54
P Deficient	66	0	5	323±9	0.53±0.14	36.7±5.7	0.15±0.06	15.0±6.1	0.05±0.05	5.0±5.0	0.0±0.0	30.01±0.80
P Deficient	65	10	5	314±13	0.25±0.08	25.0±7.9	0.10±0.06	10.0±6.1	0.05±0.05	5.0±5.0	0.0±0.0	33.55±1.40
P Deficient	29	0	5	304±21	0.52±0.22	41.7±14.7	0.05±0.05	5.0±5.0	0.05±0.05	5.0±5.0	0.0±0.0	31.91±1.17
P Deficient	28	10	5	238±23	0.23±0.10	16.7±10.5	0.05±0.05	5.0±5.0	0.13±0.13	6.7±6.7	0.0±0.0	30.93±0.86
Vit. D Deficient	66	0	5	447±19	0.00±0.00	0.0±0.0	0.00±0.00	0.0±0.0	0.30±0.15	15.0±6.1	5.0±5.0	40.84±0.42
Vit. D Deficient	65	10	5	467±17	0.00±0.00	0.0±0.0	0.00±0.00	0.0±0.0	0.25±0.16	10.0±6.1	5.0±5.0	40.55±0.31
Vit. D Deficient	29	0	5	414±13	0.00±0.00	0.0±0.0	0.00±0.00	0.0±0.0	0.20±0.20	10.0±10.0	5.0±5.0	40.00±0.70
Vit. D Deficient	28	10	5	418±14	0.00±0.00	0.0±0.0	0.00±0.00	0.0±0.0	0.50±0.21	20.0±9.4	15.0±6.1	40.77±0.18
TD Inducing	66	0	5	416±11	0.00±0.00	0.0±0.0	0.10±0.06	10.0±6.1	1.00±0.41	40.0±12.8	30.0±14.6	37.58±1.48
TD Inducing	65	10	5	443±19	0.00±0.00	0.0±0.0	0.00±0.00	0.0±0.0	0.90±0.15	35.0±6.1	20.0±5.0	37.08±0.54
TD Inducing	29	0	5	402±12	0.00±0.00	0.0±0.0	0.18±0.08	18.3±7.6	1.78±0.35	66.7±9.1	45.0±17.4	36.59±0.62
TD Inducing	28	10	5	391±14	0.00±0.00	0.0±0.0	0.20±0.12	15.0±10.0	1.45±0.05	65.0±6.1	40.0±6.1	37.54±0.33

[1]ES Time=Egg Storage Time (d)
[2]Main effect means ± standard errors of five pens of four birds each

TABLE 3.6. The influence of hens' age, egg storage time and diet on bone quality (29 vs. 66 wk old), Experiment 1

Type of Diet	Hens' Age	ES[1] Time	n	BWG (g)	P Rickets Score	P Rickets Inc. (%)	Vit. D Rickets Score	Vit. D Rickets Inc. (%)	TD Score	TD Inc. (%)	TD3 Inc. (%)	Bone Ash (%)
Control			20	432±9	0.01±0.01[2]	1.3±1.3	0.03±0.02	2.5±1.7	0.14±0.06	7.5±3.2	1.3±1.3	40.83±0.17
P Deficient			20	295±11	0.38±0.07	30.0±5.2	0.00±0.00	0.0±0.0	0.07±0.04	5.4±2.5	0.0±0.0	31.60±0.58
Vit. D Deficient			20	439±9	0.00±0.00	0.0±0.0	0.09±0.03	8.8±2.7	0.31±0.09	13.8±3.8	7.5±2.6	40.54±0.22
TD Inducing			20	413±8	0.00±0.00	0.0±0.0	0.12±0.04	10.8±3.6	1.28±0.15	51.7±5.3	13.8±5.9	37.20±0.41
	Old		40	415±10	0.10±0.03	8.3±2.5	0.05±0.02	5.0±1.6	0.33±0.08	14.4±3.1	7.5±2.6	37.72±0.67
	Young		40	373±8	0.09±0.04	7.3±3.1	0.07±0.02	6.0±2.0	0.57±0.12	24.8±4.6	13.8±3.6	37.37±0.63
		0	40	396±9	0.13±0.05	9.8±3.3	0.07±0.02	6.7±1.9	0.47±0.12	19.6±4.2	11.3±3.7	37.35±0.69
		10	40	392±13	0.07±0.02	5.8±2.2	0.05±0.02	4.4±1.8	0.44±0.09	19.6±3.9	10.0±2.5	37.74±0.60

Source	df[3]	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Diet	3	<0.0001	<0.0001	<0.0001	0.0054	0.0240	<0.0001	<0.0001	0.0009	<0.0001
Hens Age	1	<0.0001	0.7248	0.6286	0.5184	0.6624	0.0018	0.0025	0.0136	0.2414
ES Time	1	0.6469	0.0381	0.0705	0.5184	0.3386	0.6692	0.9999	0.6106	0.1893
Diet*Hens Age	3	0.9372	0.9876	0.9677	0.0328	0.0506	0.0129	0.0176	0.0674	0.9945
Diet*ES Time	3	0.1551	0.0018	0.0039	0.9242	0.7254	0.3817	0.9350	0.3430	0.3725
Hens Age*ES Time	1	0.0847	0.8378	0.2895	0.1928	0.2589	0.7976	0.7038	0.6106	0.3228
Diet*Hens Age*ES Time	3	0.7276	0.9882	0.6570	0.8437	0.9006	0.3245	0.6102	0.7269	0.0034
Error	48									

[1]ES Time=Egg Storage Time
[2]Main effect means ± standard errors of five pens of four birds each
[3]Degrees of Freedom

TABLE 3.7. The influence of hens' age, egg storage time and dietary P level on body weight gain (BWG), feed intake and feed conversion ratio (37vs. 64 wk old), Experiment 2

Type of Diet	Hens' Age	ES Time [1]	n	BWG	Feed Intake	FCR
Control	64	0	6	429±16[2]	621±20	1.45±0.02
Control	63	10	6	452±9	635±11	1.41±0.02
Control	37	0	6	437±6	647±18	1.48±0.04
Control	36	10	6	451±19	655±18	1.46±0.03
P Deficient	64	0	6	369±9	584±32	1.58±0.07
P Deficient	63	10	6	367±10	555±13	1.52±0.05
P Deficient	37	0	6	376±10	589±18	1.57±0.03
P Deficient	36	10	6	377±5	567±13	1.51±0.03
		Pr>F		<0.0001	0.0408	0.0017
Control			24	442±7[a]	640±8	1.45±0.01
P Deficient			24	372±4[b]	574±10	1.54±0.02
	Old		24	404±9	599±12	1.49±0.02
	Young		24	410±9	615±11	1.50±0.02
		0	24	403±8	610±12	1.52±0.02
		10	24	412±10	603±11	1.47±0.02

Source	df[3]	Pr>F	Pr>F	Pr>F
Diet	1	<0.0001	0.0153	0.0068
Hens Age	1	0.5020	0.4009	0.8274
ES Time	1	0.2832	0.5030	0.0926
Diet*Hens Age	1	0.9204	0.5813	0.6012
Diet*ES Time	1	0.2447	0.1688	0.5483
Hens Age*ES Time	1	0.8312	0.9905	0.7932
Diet*Hens Age*ES Time	1	0.6850	0.7965	0.8171

[1]ES Time=Egg Storage Time
[2]Main effect means ± standard errors of n pens of 10 birds each
[3]Degrees of Freedom
[a-b]Means within the levels with no common superscript differ significantly (P<0.05); result of orthogonal contrast

TABLE 3.8. The influence of hens' age, egg storage time and dietary P level on bone quality (37vs. 64 wk old), Experiment 2

Type of Diet	Hens' Age	ES[1] Time	n	P Rickets Score	P Rickets Inc. (%)	TD Score	TD Inc. (%)	TD3 Inc. (%)	Bone Ash (%)
Control	64	0	6	0.00±0.00[2]	0.0±0.0	0.03±0.03	1.7±1.7	0.0±0.0	36.69±0.44
Control	63	10	6	0.00±0.00	0.0±0.0	0.09±0.05	6.9±3.4	1.7±1.7	40.66±0.46
Control	37	0	6	0.00±0.00	0.0±0.0	0.08±0.04	5.0±2.2	0.0±0.0	39.61±0.14
Control	36	10	6	0.00±0.00	0.0±0.0	0.05±0.04	3.5±2.2	0.0±0.0	39.67±0.10
P Deficient	64	0	6	0.57±0.10	44.6±7.1	0.00±0.00	0.0±0.0	0.0±0.0	30.44±0.48
P Deficient	63	10	6	0.51±0.14	34.8±8.8	0.03±0.02	3.3±2.1	0.0±0.0	30.44±0.24
P Deficient	37	0	6	0.58±0.05	40.7±5.2	0.02±0.02	1.7±1.7	0.0±0.0	30.22±0.18
P Deficient	36	10	6	0.55±0.06	47.0±3.3	0.00±0.00	0.0±0.0	0.0±0.0	30.45±0.36
		Pr>F		<0.0001	<0.0001	0.2911	0.2064	0.4456	<0.0001
Control			24	0.00±0.00[3]	0.0±0.0	0.06±0.02	4.3±1.2	0.4±0.4	39.90±0.18
P Deficient			24	0.55±0.04	41.8±3.2	0.01±0.01	1.3±0.7	0.0±0.0	30.39±0.16
	Old		24	0.27±0.07	19.9±5.0	0.04±0.02	3.0±1.1	0.4±0.4	35.31±1.04
	Young		24	0.28±0.06	21.9±4.8	0.04±0.02	2.5±0.9	0.0±0.0	34.99±0.16
		0	24	0.29±0.07	21.3±4.9	0.03±0.01	2.1±0.8	0.0±0.0	34.99±0.99
		10	24	0.27±0.07	20.5±4.9	0.04±0.02	3.4±1.2	0.4±0.4	35.30±1.03

Source	df[3]	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Diet	1	<0.0001	<0.0001	0.1014	0.2140	1.0000	<0.0001
Hens Age	1	0.9873	0.7884	0.3821	0.4293	1.0000	0.9470
ES Time	1	0.7607	0.8619	0.8425	0.5205	0.2132	0.8478
Diet*Hens Age	1	0.9799	0.6716	0.5793	0.6761	1.0000	0.2279
Diet*ES Time	1	0.6304	0.7834	0.9530	0.7180	0.3233	0.4040
Hens Age*ES Time	1	0.8834	0.4347	0.4005	0.3523	0.2133	0.1733
Diet*Hens Age*ES Time	1	0.8166	0.2194	0.7093	0.7675	0.1279	0.2320
Error	40						

[1]ES Time=Egg Storage Time
[2]Main effect means ± standard errors of n pens of 10 birds each
[3]Degrees of Freedom

CHAPTER 4

THE EFFECT OF HENS' AGE, EGG STORAGE TIME AND PHOSPHOROUS DEFICIENCY WITH 1α-OH VITAMIN D_3 ON THE PHOSPHORUS UTILIZATION OF BROILERS[1]

[1]Shim, M. Y., G. M. Pesti, R. I. Bakalli and H. M. Edwards Jr. To be submitted to Poultry Science.

ABSTRACT

Two experiments were conducted to confirm that variation in broiler phosphorous (P) utilization is due to hens' age and egg storage time. Experiment 1 was conducted with chicks hatched from eggs laid by Ross × Ross 308 hens (27 vs. 61 wk old) and stored for 0 or 10 d.

The age of hens had significant effects ($P<0.05$) on 0 – 16 d chick growth (379 ± 18 vs. 308 ± 19 for 27 and 61 wk old hens, respectively). The longer egg storage time of chicks from older hens resulted in higher P rickets score and incidence, but the longer egg storage time of chicks from younger hens resulted in lower P rickets score and incidence ($P=0.0455$). The longer egg storage time of chicks from older hens resulted in lower bone ash (%), but the longer egg storage time of chicks from younger hens resulted in higher bone ash (%).

Experiment 2 was conducted with chicks hatched from eggs laid by Ross × Ross 308 hens (26 vs. 60 wk old) and stored for 0 or 10 d. The diets were P deficient and with or without 5 μg/g 1α–OH Cholecalciferol (1α–OH vitamin D_3). Six replicates of 10 chicks from each hen age and egg storage time combination were raised in battery brooders.

The age of hens had significant effects ($P=0.0003$) on 0 - 16 d chick growth (272 ± 7 vs. 339 ± 8 for 26 and 60 wk old hens, respectively). The P rickets score increased with age of hens ($P=0.0186$), egg storage time ($P=0.1057$) and chick mortality ($P=0.0134$).

The factors influencing the incidence of P rickets in broilers should include hen's age and egg storage time as well as genetics and dietary levels of Ca, P and Vitamin D activity of the diets.

Key words: P deficiency, Vitamin D, age, Egg Storage, Broilers

INTRODUCTION

Chicks poorly utilize phytate Phosphorus (PP), because they do not have the microflora or endogenous enzyme activity to hydrolyse the phytate molecule. Approximately, two thirds of P in corn and soybean meal based chicks' diets is in the form of PP or myo-inositol 1,2,3,4,5,6-hexakisphosphate, which is unavailable to chicks (Nelson et al. 1968; Reddy et al. 1982). Even though chicks produce some phytase activity in their large intestine and cecum to dephosphorylate phytate and increase the availability of PP, it is too late in the gut for most of the P released to be absorbed. Therefore, it is necessary to supplement poultry diets with inorganic P in order to avoid a deficiency. However, this leads to the problem of excessively high levels of P entering the environment in some areas, especially under intensive production. The most effective way to reduce inorganic P in the feed, without compromising performance and leg quality, is to either use feedstuffs with higher P availability such as low-phytic acid corn, or to increase the digestibility of current ingredients. When altering the P content of the diet, it is necessary to reevaluate the requirement for Ca due to the numerous interactions between the two minerals within the body.

The egg storage time influence the score and incidence of P deficiency in rickets in the progeny (Chapter 3). When eggs were stored for 10d, young chicks tend to have lower rickets scores and incidence of P than eggs had not been stored. Bone ash was affected by diet, hens' age and egg storage time (3-way interaction; Chapter 3). Egg storage time had its biggest effect on bone ash with chicks from older hens given a P rickets-inducing diet. Bone ash was higher in chicks from young hens on all diets except the P rickets inducing diet, in which bone ash was higher in chicks from the older hens. The common factor between hens' age, ES time and diet seems to be reducing bone ash that leads to P rickets.

Vitamin D and several of its carbon-1, ɑ hydroxylated derivatives have been shown to improve PP and total P (tP) uptake in chicks. The mechanisms by which this occurs are as yet unclear; however, several theories have been suggested. Harrison and Harrison (1961) proposed that vitamin D stimulates a Ca-dependent P pump in cells lining the intestine by increasing the Ca content of these cells. There is also evidence that suggests that vitamin D acts as a P transport hormone stimulating P transport in many tissues in the body including the intestine (Tanka and DeLuca 1974). Cross and Peterlik (1988) and Sechman et al. (1996) concluded that the presence of high plasma 1,25-$(OH)_2D_3$ increases the number of Na-P transport proteins within the intestine of the chick.

Vitamin D and its derivatives may also increase P absorption simply by increasing Ca absorption. Less Ca increases the proportion of soluble P in contact with the gut mucosa as Ca forms the Ca_2PO_4 salt at the normal intestinal pH (Hurwitz and Bar 1971). Posphates in this insoluble state are unavailable for absorption.

The significant improvement in PP retention obtained with dietary 1,25-$(OH)_2D_3$ and 1ɑ-OH vitamin D_3 is consistently achieved when either is fed to modern fast growing chicks (Edwards 1993; Roberson and Edwards

1994; Biehl et al. 1995; Elliot et al. 1995; Mitchell and Edwards 1996; Biehl and Baker 1997).

The experiments reported here were conducted to test the hypotheses that; 1) hens' age, and egg storage can affect the incidence of P rickets and; 2) 1α-OH vitamin D_3 that may overcome the negative impacts of young hens and egg storage time.

MATERIALS AND METHODS

General Procedures

Eggs obtained from a commercial breeder company were stored at 12°C and 66% relative humidity for 0 or 10 d and warmed at room temperature (20°C) 12 h before setting. Each egg was individually marked with date of lay. All eggs were collected the morning they were laid, eggs not stored were placed in egg trays at room temperature (20°C) for 12 h overnight before they were placed in the incubator. Eggs were set at 37.7°C and 55% RH in a single-stage NatureForm NMC-2000, 1,980 egg capacity setter (NatureForms Hatchery System, Inc., 925 North Ocean St., Jacksonville, FL 32202) with automatic temperature regulation (accurate to 0.10 °C), relative humidity (accurate to +/-2% relative humidity), and an automatic turning system (24 times/day).

The chicks were housed in Petersime® wire-floored battery brooders for 16 d and were provided with water and the dietary treatments ad libitum. At the conclusion of each experiment, birds were weighed and sacrificed by asphyxiation using carbon dioxide.

Experiment 1

720 Eggs laid by Ross × Ross 308 hens (26 wk old stored, 27 wk old fresh, 60 wk old stored, and 61 wk old fresh) were incubated. On day 11, eggs were candled and all eggs with no live embryos where removed. On day 18, eggs were transfered into Hatcher baskets, and placed into the hatcher at 36.7-36.9°C and 70% relative humidity. Five chicks from each diet, hen age and egg storage time combination were placed in each of 48 pens. The progeny were fed two corn-soybean based diets (control vs. P deficient diet; Table 1).

Experiment 2

Eggs laid by 25 wk old (stored), 26 wk old (fresh), 59 wk old (stored), and 60 wk old (fresh) Ross × Ross 308 hens (720 eggs each) were incubated. On day 14, eggs were candled and all eggs without live embryos where removed. On day 19, eggs were transfered into hatcher baskets, and placed into the hatcher as above. Ten chicks from each diet, hens' age and egg storage time combination were placed in each of 48 pens. The progeny were fed a diet based on corn, soybean meal and soybean oil with or without 5 μg/g 1α–OH vitamin D_3 (Table 1). Feed and excreta samples were analyzed for phytate P (Latta and Eskin 1980) and chromic oxide (Brisson 1956). Phytate P retention was calculated using the methods of Edwards and Gillis (1959).

Statistical Analysis

Analysis of variance was performed on all data for both experiments using the General Linear Model procedure of SAS (Version 8.02, 2001) appropriate for a randomized block design. Treatment means were separated using Duncan's Multiple Range Test at $P < 0.05$ (Duncan 1955).

RESULTS

Hens' age and egg storage time (ES time) were not significant contributors to variation in any of the incubation parameters in either experiment (Tables 2-3). Day-old chick weights were different ($P<0.0001$) due to hens' age in both experiments. Chicks from the younger hens were smaller when the eggs were stored for 10 d in Experiment 1 but not Experiment 2. Hen's age effects were significant for day-old chicks in Experiment 1 and for 16 d old chicks in Experiment 2. Because of variation, the 38 g difference in 16 d weights was not significant at $P<0.05$ in Experiment 1, but a 33 g difference was significant in Experiment 2.

Hens' age contributed to variation in 16-d body weight gain (BWG) and feed intake (FI) in both experiments. The P deficient diet decreased BWG (418±14 vs. 270±13 for control and P deficient diets, respectively) in Experiment 1 (Table 4).

The P deficient diet increased the score and incidence of P rickets in Experiment 1 (Table 4). The longer egg storage time of chicks from older hens resulted in higher P rickets scores and incidences, but the longer egg storage time of chicks from younger hens resulted in lower P rickets score and incidence (Interaction $P=0.0455$; Table 5).

1α-OH vitamin D_3 increased 16 d BWG by 30.5 g for chicks from older hens, but only 15.0 g for chicks from younger hens ($P=0.0348$; Experiment

2; Table 6). The P deficient diet with 1α-OH vitamin D_3 increased BWG, bone ash and phytate P retention in Experiment 2 (Table 6-7). The P deficient diet with 1α-OH vitamin D_3 also decreased the score and incidence of P rickets, and chick mortality. Hens' age decreased P rickets score (Table 7). The chicks from the older hens showed higher P rickets scores (P=0.0186), but egg storage time did not affect P rickets score or incidence.

1 α-D_3 had significant effects on 0 - 16 d chick mortality (4.58±1.34 vs. 17.50±3.52 for the P deficient diet with and without 1α-OH vitamin D_3, respectively; P=0.0005). Hens' age also had significant effects on 0 - 16 d chick mortality (15.83±3.71 vs. 6.25±1.45 for 26 and 60 wk old hens, respectively; P=0.0071). Mortality of chicks from 10 d storage was higher (12.92±2.98) than those from fresh eggs (9.17±2.94), P=0.0134.

DISCUSSION

In Experiment 1, egg size was increasing rapidly so that the chick weights from the 26 wk old hens whose eggs had been stored for 10 d were considerably smaller than chicks from the 27 wk old hens that had not been stored. The fertility of this flock was also increasing rapidly, from 36 to 77 % over the 10 d storage period. In contrast, in Experiment 2, chick weights were very similar and actually slightly smaller for the 26 wk old hens and no egg storage than those from the 25 wk old hens with 10 d storage. Fertility in this flock was higher than from the slightly older flock in Experiment 1. Fertility increased from 58 to 90 % over the 10 d storage period. Therefore, although these flocks were raised under identical protocols, there are differences in their performance that may influence the results of progeny trials. Experiment 1 was conducted in the Fall and Experiment 2 was conducted in the Spring. In the Spring, male chickens were stimulated sooner and their fertility was higher. The weather might be a complicating factor which changes chicks' performance. Changes in temperature quickly affect consumption and egg size.

The significant improvement in PP retention obtained with dietary 1,25-$(OH)_2D_3$ and 1α-OH vitamin D_3 is consistently achieved when either is fed to modern fast growing chicks (Edwards 1993; Roberson and Edwards 1994; Biehl et al. 1995; Elliot et al. 1995; Mitchell and Edwards 1996; Biehl and Baker 1997) which would have increased the amount of P available to chicks. Experiment 2 demonstrates that 1α-OH vitamin D_3 does affect PP retention in chicks since the hens' genotypes and rearing protocols were identical while chicks were raised under identical conditions co-mingled in the same pens. The P deficient diet with 1α-OH vitamin D_3 decreased the score and incidence of P rickets, which is usually associated with increased PP retention in chicks. Thus the increase in P rickets from older hens and egg storage does not appear to be due to a lack of vitamin D or lack of an active hydroxylated vitamin D hormonal form.

Percentage hatch from very young hens (26 wks of age) were slightly higher and percentage hatch from young hens (27 wks of age) and old hens (60 and 61 wks of age) were lower than breeder expectations (Ross × Ross Management Guide, Aviagen, AL 35805), and lower than observations by Wineland and Brake (1984). In Experiment 1, fertility was increased from

77.41% to 79.26% when breeders' age increased (from 27 wk to 61 wk old) although these were different flocks. In Experiment 2, fertility decreased from 89.64% to 79.64% with increasing breeder's age (from 26wks to 60wk old) although again these were different flocks. HES was approximately 6-8% lower than fertility in both experiments.

Smith and Bohren (1975) observed that hen's age groups have a significant effect on egg weight. This effect was entirely linear as the egg weight increased with the age of the pullets. The egg weight was a dominant factor affecting chick weight at hatch (Whiting 1982; Wilson 1991; Silversides and Scott 2001; Tona et al. 2002). In Experiment 2, chick weights at hatch from older hens (60 wk old) were greater than younger hens (26 wk old). However, chick weights increased much more from 25 to 26 wks than from 26-27 wks in Experiment 2. Experiments 1 and 2 were conducted at different season with eggs from different maternal flocks, therefore there were differences between results.

Petek and Dikmen (2004) conducted experiments with two quail breeder ages (20 vs. 37 wk) and two egg storage times (5d vs. 15d). They had significantly lower chick mortality (6.74 - 8.29%) in their study, because of variation between replicates. Chick mortality in Experiments 1 and 2 from 3.33 to 19.17% could not be declared significantly different by age of quail breeders or egg storage.

In both experiments 1 and 2, hens' age had significant effects on 0 – 16 d chick growth confirming the findings of other studies that the progeny from older hens had higher growth than the progeny from young hens (Whiting 1982; Reis et al. 1997).

Vitamin D_3 supplementation of breeders might have influenced egg and chick parameters in Experiments 1 and 2. Bethke et al. (1936) found that the level of vitamin D_3 in the breeder diet influenced chick weight and bone calcification at five wks of age when fed a rachitic diet. Stadelman et al. (1950) and Stevens et al. (1984) have since shown that growth and calcification in turkey poults fed a vitamin D_3-free diet was directly proportional to the vitamin D_3 level in the diet of dams during the first four wks. Griminger (1966) reported that chicks from breeder hens fed different levels of vitamin D_3 showed no difference in weight after one wk of age. However, vitamin D_3 might also have an important role to prevent defects causing field rickets in poultry (Olson et al. 1981; Bar et al. 1987). BWG, bone ash, and incidence of rickets might be changed by different sources of vitamin D_3 (Kasim and Edwards 2000). In Experiment 2, the P deficient diet with 1α-OH vitamin D_3 increased BWG, bone ash, and phytate P retention. The P deficient diet with 1α-OH vitamin D_3 also decreased the score and incidence of P rickets, and chick mortality in Experiment 2.

In the present two experiments, hens' age influenced the score and incidence of P rickets. Chicks from older hens had more P rickets and more severe P rickets. In Experiment 1, hens' age and egg storage time influenced the score and incidence of P rickets. In past experiments, egg storage time influenced the score and incidence of P rickets in Experiment 1 (chapter 3). After eggs were stored, young chicks had lower scores and incidences of P rickets than the eggs not been stored; and the severity of TD was reduced as the hens became older. In conclusion, to maximize broiler performance, P requirement and Ca - P interaction need to be investigated with chicks from young and old hens. The influence of ES time on requirements may also need to be quantitated.

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TABLES AND FIGURES

TABLE 4.1. Composition of basal diets, Experiments 1 and 2.

Ingredient	Experiment 1		Experiment 2
	Control	P Deficient	P Deficient
	---------------------------(%)---------------------------		
Ground yellow corn	52.15	53.35	53.15
Soybean meal (dehulled)	37.73	37.52	37.55
Soy bean oil	5.97	5.57	5.64
Iodized sodium chloride	0.45	0.45	0.45
DL-Methionine	0.19	0.19	0.19
Vitamin premix[1]	0.25	0.25	0.25
Trace mineral premix[2]	0.08	0.08	0.08
Dicalcium Phosphate	1.91	0.57	0.57
Limestone	1.26	2.01	2.01
Cr_2O_3	-	-	0.10
Calculated composition[3]			
ME, kcal	3.20	3.20	3.20
CP, %	23.00	23.00	23.00
Calcium, %	1.00	1.00	1.00
Phosphorus-total, %	0.74	0.49	0.49
nPP, %	0.50	0.25	0.25

[1]Vitamin mix provided the following (per kilogram of diet): Thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (*cobalamin*), 12.0 ug; pyridoxine-HCl, 2.7 mg; D-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-retinyl acetate, 5,500 IU; all-rac-tocopherol acetate, 11 IU; ethoxyquin, 150 mg.

[2]Trace mineral mix provides the following (per kilogram of diet): manganese ($MnSO_4.H_2O$), 60 mg; iron ($FeSO_4.7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4.5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 1.5 mg.

[3]Calculated from NRC (1994).

Table 4.2. The influence of age of hens and egg storage on egg and chick parameters (Experiment 1)

Hens' age (wk)	Egg storage (d)	Apparent Fertility (%)	Hatchability HES[1] (%)	Hatchability HFE[2] (%)	Day-old chick weights (g)	16-d-old chick weights (g)	0-16 d Mortality (%)
27	0	77.41	71.85	92.82	44.2±1.2	368.0±27.1	10.0±3.9
26	10	36.30	27.41	75.51	37.0±0.6	330.3±27.7	13.3±4.5
61	0	79.26	71.48	90.19	46.0±0.3	419.4±30.4	3.3±3.3
60	10	89.59	81.04	90.46	46.5±0.5	431.5±22.5	5.0±2.6

Source	df[4]	Pr>F	Pr>F	Pr>F	df	Pr>F	Pr>F	Pr>F
Hens Age	1	0.4779	0.5044	0.6109	1	0.0934	0.1857	0.2032
ES Time[5]	1	0.6567	0.6349	0.5099	1	<0.0001	0.6378	0.4970
Hens Age*ES Time	—	—	—	—	1	<0.0001	0.3628	0.8205
Error	1				44			

[1]HES = Hatch of Egg Set
[2]HFE = Hatch of Fertile Eggs
[3]Main effect means ± standard errors of 5 pens of 4 birds each
[4]Degrees of Freedom
[5]ES Time=Egg Storage Time

Table 4.3. The influence of age of hens and egg storage on egg and chick parameters (Experiment 2)

Hens' age (wk)	Egg storage (d)	Apparent Fertility (%)	Hatchability HES[1] (%)	Hatchability HFE[2] (%)	Day-old chick weights (g)	16-d-old chick weights (g)	0-16 d Mortality (%)
26	0	89.64	81.09	88.60	36.7±0.3[3]	326.1±9.2	12.5±5.5
25	10	57.50	—	—	37.5±0.2	293.0±8.5	19.2±5.0
60	0	79.38	71.17	89.61	48.2±0.3	390.5±9.8	5.8±1.9
59	10	79.44	—	—	48.6±0.6	392.0±5.7	6.7±2.3

Source	df[4]	Pr>F	Pr>F	Pr>F	df	Pr>F	Pr>F	Pr>F
Hens Age	1	0.7785	—	—	1	<0.0001	<0.0001	0.2457
ES Time[5]	1	0.5012	—	—	1	0.1032	0.0672	0.3544
Hens Age*ES Time	—	—	—	—	1	0.6177	0.0456	0.4705
Error	1				44			

[1]HES = Hatch of Egg Set
[2]HFE = Hatch of Fertile Eggs
[3]Main effect means ± standard errors of 12 pens of 10 birds each
[4]Degrees of Freedom
[5]ES Time=Egg Storage Time

TABLE 4.4. The influence of age of hens, egg storage time and dietary P level on body weight gain (BWG), feed intake, feed conversion ratio and bone quality (27vs. 61 wk old), Experiment 1

Diet	Age of Hens	ES Time [1]	n	BWG (g)	Feed Intake (g)	FCR (g/g)	P Rickets Score	P Rickets Inc. (%)	Bone Ash (%)
Control	61	0	6	454±24[2]	592±31	1.30±0.01	0.00±0.00	0.0±0.0	35.93±0.51
Control	60	10	6	446±21	641±24	1.45±0.05	0.00±0.00	0.0±0.0	35.88±0.83
Control	27	0	6	407±18	556±16	1.37±0.04	0.00±0.00	0.0±0.0	37.86±1.75
Control	26	10	6	366±33	494±25	1.39±0.07	0.00±0.00	0.0±0.0	36.42±0.64
P Deficient	61	0	6	293±30	451±28	1.61±0.16	0.67±0.20	50.0±11.3	25.67±1.76
P Deficient	60	10	6	324±17	493±30	1.53±0.09	0.99±0.11	81.7±3.8	23.31±0.87
P Deficient	27	0	6	240±13	377±15	1.58±0.07	0.68±0.10	63.6±8.3	23.07±2.89
P Deficient	26	10	6	221±16	341±27	1.54±0.05	0.46±0.07	45.8±6.5	26.64±1.16
		Pr>F		<0.0001	<0.0001	0.0864	<0.0001	<0.0001	<0.0001
Control			24	418±14[a]	571±16[a]	1.38±0.02[b]	0.00±0.00[b]	0.0±0.0[b]	36.52±0.52[a]
P Deficient			24	270±13[b]	416±17[b]	1.57±0.05[a]	0.70±0.07[a]	60.3±4.7[a]	24.67±0.92[b]
	Old		24	379±18[a]	544±21[a]	1.47±0.05	0.41±0.10	32.9±7.8	30.20±1.31
	Young		24	308±19[b]	442±21[b]	1.47±0.03	0.28±0.07	27.4±6.4	30.99±1.56
		0	24	349±21	494±21	1.47±0.05	0.34±0.09	28.4±6.8	30.63±1.60
		10	24	339±20	492±25	1.48±0.03	0.36±0.09	31.9±7.4	30.56±1.26

Source	df[3]	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Diet	1	<0.0001	<0.0001	0.0023	<0.0001	<0.0001	<0.0001
Hens Age	1	0.0323	0.0362	0.7939	0.9775	0.5162	0.2083
ES Time	1	0.8984	0.4571	0.5383	0.9015	0.1633	0.0945
Diet*Hens Age	1	0.5552	0.9122	0.8825	0.8992	0.7769	0.3562
Diet*ES Time	1	0.3311	0.7875	0.2295	0.7837	0.5383	0.3484
Hens Age*ES Time	1	0.1911	0.0107	0.6900	0.1537	0.0251	0.1187
Diet*Hens Age*ES Time	1	0.7865	0.6474	0.4524	0.0359	0.0016	0.0659
Error	40						

[1]ES Time=Egg Storage Time
[2]Main effect means ± standard errors of n pens of 10 birds each
[3]Degrees of Freedom
[a-b]Means within the levels with no common superscript differ significantly (P<0.05); result of orthogonal contrast

TABLE 4.5. The influence of age of hens and ES time[1] on body weight gain (BWG), feed intake, feed conversion ratio and bone quality of chicks fed P deficient diet (27vs. 61 wk old), Experiment 1

Age of Hens	ES Time[1]	n	BWG (g)	Feed Intake (g)	FCR (g/g)	P Rickets Score	P Rickets Inc. (%)	Bone Ash (%)
61	0	6	293±30[2]	451±28	1.61±0.16	0.67±0.20	50.0±11.3	25.67±1.76
60	10	6	324±17	493±30	1.53±0.09	0.99±0.11	81.7±3.8	23.31±0.87
27	0	6	240±13	377±15	1.58±0.07	0.68±0.10	63.6±8.3	23.07±2.89
26	10	6	221±16	341±27	1.54±0.05	0.46±0.07	45.8±6.5	26.64±1.16
		Pr>F	0.0057	0.0018	0.9398	0.0568	0.0194	0.2661
Old		24	309±17[a]	472±21[a]	1.57±0.09	0.83±0.12[a]	65.8±7.4	24.29±1.00
Young		24	231±10[b]	359±16[b]	1.56±0.04	0.57±0.07[b]	54.7±5.7	26.25±0.67
	0	24	267±17	414±19	1.60±0.08	0.67±0.11	56.8±7.0	25.76±0.92
	10	24	273±19	417±30	1.54±0.05	0.73±0.10	63.8±6.5	24.97±0.85

Source	df[3]	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Hens' Age	1	0.0009	0.0003	0.9352	0.0536	0.1766	0.1596
ES Time	1	0.7610	0.9128	0.5602	0.6855	0.3918	0.5199
Hens Age*ES Time	1	0.2184	0.1406	0.8455	0.0455	0.0054	0.2071
Error	40						

[1]ES Time=Egg Storage Time
[2]Main effect means ± standard errors of n pens of 10 birds each
[3]Degrees of Freedom
[a-b]Means within the levels with no common superscript differ significantly (P<0.05); result of orthogonal contrast

TABLE 4.6. The influence of age of hens, egg storage time and P deficient diet with or without 1α-OH vitamin D_3 on body weight gain (BWG), feed intake and feed conversion ratio (26vs. 60 wk old), Experiment 2

1α-OH vitamin D_3 (μg/g)	Age of Hens	ES Time[1]	n	BWG (g)	Feed Intake (g)	FCR (g/g)
5	60	0	6	353±16[2]	510±11	1.46±0.07
5	59	10	6	356±7	537±15	1.51±0.04
5	26	0	6	288±17	444±10	1.57±0.13
5	25	10	6	272±10	397±12	1.47±0.04
0	60	0	6	332±9	546±25	1.65±0.09
0	59	10	6	314±21	515±18	1.67±0.10
0	26	0	6	291±10	448±17	1.55±0.05
0	25	10	6	239±11	414±31	1.73±0.10
		Pr>F		<0.0001	<0.0001	0.2090
5			24	317±10	472±13	1.50±0.04
0			24	294±10	481±16	1.65±0.04
	Old		24	339±8[a]	527±9[a]	1.57±0.04
	Young		24	272±7[b]	426±10[b]	1.58±0.04
		0	24	316±9	487±12	1.56±0.04
		10	24	295±11	466±16	1.59±0.04
Source			df[3]	Pr>F	Pr>F	Pr>F
1α-OH vitamin D_3			1	0.4808	0.2908	0.3196
Hens Age			1	0.0003	<0.0001	0.9756
ES Time			1	0.3708	0.4118	0.1833
1α-OH vitamin D_3*Hens Age			1	0.0348	0.1198	0.5300
1α-OH vitamin D_3*ES Time			1	0.1475	0.3937	0.2742
Hens Age*ES Time			1	0.1642	0.1536	0.9772
1α-OH vitamin D_3*Hens Age*ES Time			1	0.7173	0.1986	0.1790
Error			40			

[1]ES Time=Egg Storage Time
[2]Main effect means ± standard errors of n pens of 10 birds each
[3]Degrees of Freedom
[a-b]Means within the levels with no common superscript differ significantly (P<0.05); result of orthogonal contrast

TABLE 4.7. The influence of age of hens, egg storage time and P deficient diet with or without 1α-OH vitamin D_3 on bone quality and survival (26vs. 60 wk old), Experiment 2

1α-OH vitamin D_3 (μg/g)	Hens' Age	ES[1] Time	n	Phytate P retention (%)	P Rickets Score	P Rickets Inc. (%)	Bone Ash (%)	Mortality (%)
5	60	0	6	42.83±5.33[2]	0.54±0.13	39.8±7.3	31.78±0.49	3.33±2.11
5	59	10	6	36.58±2.27	0.71±0.13	48.5±6.9	30.91±0.87	5.00±3.42
5	26	0	6	35.08±5.39	0.48±0.08	37.8±4.9	30.99±0.37	3.33±2.11
5	25	10	6	37.63±2.25	0.63±0.09	45.7±6.6	31.29±0.39	6.67±3.33
0	60	0	6	28.72±4.26	1.08±0.09	68.4±6.4	28.61±0.47	8.33±3.07
0	59	10	6	34.56±4.70	1.23±0.13	78.3±2.7	28.22±0.55	8.33±3.07
0	26	0	6	28.27±4.56	0.83±0.16	58.3±8.6	29.23±0.77	21.67±9.80
0	25	10	6	24.24±2.91	0.80±0.12	62.1±9.0	28.38±0.49	31.67±6.01
		Pr>F		0.0647	0.0005	0.0010	<0.0001	0.0008
5			24	38.03±2.01^{a}	0.59±0.05^{b}	42.9±3.2^{b}	31.24±0.27^{a}	4.58±1.34^{b}
0			24	28.96±2.09^{b}	0.98±0.07^{a}	66.8±3.7^{a}	28.61±0.28^{b}	17.50±3.52^{a}
	Old		24	35.68±2.26	0.89±0.08^{a}	58.7±4.3	29.88±0.43	6.25±1.45^{b}
	Young		24	31.30±2.16	0.68±0.06^{b}	51.0±4.0	29.97±0.35	15.83±3.71^{a}
		0	24	33.72±2.60	0.73±0.07	51.1±4.2	30.15±0.37	9.17±2.94^{b}
		10	24	33.26±1.86	0.84±0.07	58.6±4.1	29.70±0.41	12.92±2.98^{a}

Source	df[3]	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
1α-OH vitamin D_3	1	0.0036	<0.0001	<0.0001	<0.0001	0.0005
Hens Age	1	0.1426	0.0186	0.1135	0.8225	0.0071
ES Time	1	0.7269	0.1057	0.2724	0.4719	0.0134
1α-OH vitamin D_3*Hens Age	1	0.8762	0.2057	0.1251	0.2796	0.2738
1α-OH vitamin D_3*ES Time	1	0.6393	0.5465	0.8811	0.6829	0.7135
Hens Age*ES Time	1	0.9248	0.5663	0.7194	0.6680	0.3933
1α-OH vitamin D_3*Hens Age*ES Time	1	0.1149	0.6348	0.7793	0.3207	0.5411
Error	40					

[1]ES Time=Egg Storage Time
[2]Main effect means ± standard errors of n pens of 10 birds each
[3]Degrees of Freedom

CHAPTER 5

GENERAL CONCLUSIONS

Results from the current work indicate a decrease in the incidence of TD in chicks as hens' age increased. From the past studies, the different chick experiments were conducted at different times as the hens aged. Therefore, there were several factors that could have been confounded with hens' age and caused the results. Possibilities include feed ingredient source used in the hen and chick feeds. Our current experiment demonstrates that the hens' age does affect TD in chicks since the hens' genotypes and rearing protocols were identical while chicks were raised under identical conditions co-mingled in the same pens. The severity of TD was reduced as the hens became older, which may be related to increased deposition of vitamin D or its metabolites in the eggs as the laying cycle progresses.

In the current experiments, hens' age had significant effects on 0 – 16 d chick growth confirmed the finding of other studies that the progeny from older hens had higher growth than the progeny from young hens. However, chick weights at hatch were not linearly increased by breeders' age, because four experiments were conducted at different seasons with eggs from different maternal flocks.

$1\alpha\text{-}OHD_3$ does affect Phytate Phosphorus (PP) retention in chicks. The significant improvement in PP retention obtained with dietary $1\alpha\text{-}OHD_3$ is consistently achieved when fed to modern fast growing chicks. A phosphorus deficient diet with $1\alpha\text{-}OHD_3$ decreased the score and incidence of P rickets, which may be related to increasing PP retention in chicks.

1 – 16 d chick mortality was affected by diets, hens' age and ES time. Mortality was decreased by adding 1α-OHD_3 in the diets and by increasing breeders' age, but it increased by storing eggs.

In our present experiments, we had only old and young hens' ages and 0 and 10 d egg storage times. The results were too sparse to guess at the shape of the response curves. Only two points (two hens' ages or two egg storage times) can determine only straight lines, and more points would allow getting non-linear shapes. If we had more samples from different hens' ages and several egg storage times, we might get nice graphs. Therefore, in the future experiments, we can compare chick performance with chicks from several breeders' ages (approximately 25, 35, 45, 55, 65 wks, etc). We can also design egg and chick parameters with several egg storage times (approximately 0, 3, 6, 9, 12 d).

APPENDICES

TABLE 1. Feed consumption data, experiments 1 from chapter 3.

Pen #	Added Feed (g)	Weigh Back Feed (g)	Feed Consumption (g)
1	12.116	2.870	9.246
2	12.564	3.715	8.849
3	13.110	4.043	9.067
4	12.846	6.831	6.015
5	13.185	4.471	8.714
6	12.762	3.230	9.532
7	12.539	5.847	6.692
8	12.267	3.841	8.426
9	12.474	3.521	8.953
10	12.670	6.181	6.489
11	12.692	6.930	5.762
12	13.017	3.925	9.092
13	12.151	3.535	8.616
14	12.400	3.657	8.743
15	12.668	3.626	9.042
16	12.551	5.867	6.684
17	12.649	3.797	8.852
18	12.618	3.627	8.991
19	12.934	4.212	8.722
20	12.781	4.688	8.093

TABLE 2. The influence of treatments on P Rickets (29 vs. 66 wk old), Experiment 1 from chapter 3.

Type of Diet	ES[1] Time	n	P Rickets Score	P Rickets Inc. (%)
Control	0	10	0.00±0.00[2]	0.00±0.00
Control	10	10	0.03±0.03	2.50±2.50
P Deficient	0	10	0.53±0.12[a]	39.17±7.46[a]
P Deficient	10	10	0.24±0.06[b]	20.83±6.36[b]
Vit. D Deficient	0	10	0.00±0.00	0.00±0.00
Vit. D Deficient	10	10	0.00±0.00	0.00±0.00
TD Inducing	0	10	0.00±0.00	0.00±0.00
TD Inducing	10	10	0.00±0.00	0.00±0.00

[1]ES Time=Egg Storage Time

[2]Main effect means ± standard errors of five pens of four birds each

[a-b]Means within the levels with no common superscript differ significantly ($P<0.05$); result of orthogonal contrast

TABLE 3. The influence of treatments on bone quality (29 vs. 66 wk old), Experiment 1 from chapter 3.

Type of Diet	Hens Age	n	Vit. D Rickets Score	Vit. D Rickets Inc. (%)	TD Score	TD Inc. (%)	TD3 Inc. (%)
Control	Old	10	0.03±0.03[1]	2.50±2.50	0.05±0.05	2.50±2.50[b]	0.00±0.00
Control	Young	10	0.03±0.03	2.50±2.50	0.23±0.09	12.50±12.50[a]	2.50±2.50
P Deficient	Old	10	0.00±0.00	0.00±0.00	0.05±0.03	5.00±3.33	0.00±0.00
P Deficient	Young	10	0.00±0.00	0.00±0.00	0.09±0.07	5.83±3.94	0.00±0.00
Vit. D Deficient	Old	10	0.13±0.04[a]	12.50±4.17[a]	0.28±0.10	12.50±4.17	5.00±3.33[b]
Vit. D Deficient	Young	10	0.05±0.03[b]	5.00±3.33[b]	0.35±0.15	15.00±6.67	10.00±4.08[a]
TD Inducing	Old	10	0.05±0.03[b]	5.00±3.33[b]	0.95±0.21[b]	37.50±6.72[b]	25.00±7.45[b]
TD Inducing	Young	10	0.19±0.07[a]	16.67±5.96[a]	1.62±0.18[a]	65.83±5.19[a]	42.50±8.74[a]

[1]Main effect means ± standard errors of five pens of four birds each
[a-b]Means within the levels with no common superscript differ significantly (P<0.05); result of orthogonal contrast