LEG PROBLEMS OF MODERN BROILERS AS AFFECTED BY INCUBATION TEMPERATURE, FLUORIDE AND FAST GROWTH

by

MI YEON SHIM

(Under the Direction of GENE M. PESTI)

ABSTRACT

Fast growing broiler chickens are especially susceptible to bone abnormalities, causing major problems for broiler producers. In this research, the impacts of environmental (incubation temperature), nutritional (Fluoride) and genetic (random-bred population) factors were studied. It would be of great benefit to manipulate the development of leg bone by making simple changes to incubation temperature such that chickens are better able to reduce the incidence of abnormalities. No main effects or interactions between incubation temperature or time and bone abnormalities were detected. However, It is important to note that eggs hatched at different times in our study. As little as 1ºC for 3 days during early incubation ED 4 – 7 affected hatching time and weight, confounding results. Fluoride (F) has been shown to have varying degrees of beneficial effects on bone mineralization and strength, despite its toxic effects on growth and leg disorders. Some studies have demonstrated an increase in bone ash due to F supplementation. Even low levels of F like those used here have the potential to create measurable effects. Experiments were also conducted to test the hypothesis that fast growing (FG) broiler chickens

are susceptible to bone abnormalities, causing major leg problems. Growth rate was negatively associated with the twisted legs syndrome and a bone abnormality (TD) in a random-bred population. After all parameters were calculated per unit of final body weight at 6 wks, tibia density and bone ash percent of FG broiler chickens were lower than those of SG broiler chickens.

INDEX WORDS: Incubation temperature, Broiler, Hatchability, Tibial dyschondroplasia, Rickets, Fluoride, BW gain, Bone ash, Valgus, Varus, Arkansas randombred, Shank, tibia, Drum stick, Tibia breaking strength, Tibia mineral density, Tibia mineral content.

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

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2010

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December 2010

DEDICATION

I dedicate this dissertation to my husband, parents and sister. Their sincerity, patience, strength and encouragement have always inspired me to do the best that I can. Thank you.

ACKNOWLEDGEMENTS

I would first like to thank my husband, parents and sister for their unconditional love. I wish to express my deepest gratitude to my adivisor, Gene M. Pesti, for his mentoring, support and encouragement. Without him, none of this would have been possible. I would like to thank Samuel E. Aggrey, Michael J. Azain, Lynne Billard, Abhyuday Mandal for serving on my committee and for their advice. Finally, I would like to thank all those individuals who helped me develop academically and personally over the course of my study in University of Georgia.

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CHAPTER 1

INTRODUCTION

A variety of health and reproductive problems occur in broilers (Reddy, 1996) and some of the most common health problems observed are skeletal problems (Kestin et al., 1992). It is evident from the high incidence of leg problems observed among modern high-yield broiler chickens. The modern high-yield broiler chicken has been selected very successfully to reduce the time taken to reach target body weight and feed efficiency. However, there have been several indirect consequences of the selection programs with evidence of adverse effects on the skeletal systems in broilers by the fast growth at a given age (Wise, 1970, 1975; Pierson and Hester, 1982; Sørensen, 1992; Hester, 1994; Lilburn, 1994; Thorp and Waddington, 1997). Cortical bones of fast growing (FG) broiler chickens are highly porous which may easily lead to bone deformities. Acute and chronic pain, and mortality resulting from osteoporotic fractures pose serious animal welfare concerns (Webster, 2004) and increase economic loss (Cook, 2000) by increased mortality resulting from skeletal disease (Sullivan, 1994; Thorp, 1994).

Small differences in incubation temperature applied throughout incubation have been shown to influence growth of the long bone in the chick leg (Brookes and May, 1972). Formation of bone starts during embryonic days (ED) 4 – 7. Raising the temperature of the eggs by just one degree, from 37.5ºC to 38.5ºC, during ED 4 – 7 can increase the length of tibia and tarsus bones in Leghorns (Hammond et al., 2007). Oviedo-Rondon et al. (2008) concluded that

tibias of broilers were longest at 38ºC compared to 36, 37 or 39ºC. They also concluded that temperatures greater than 37ºC should be avoided to ensure optimal bone development at hatching. Incubation temperature can influence the development both of muscle and of bone, but the mechanisms by which this is occurring are unclear (Brookes and May, 1972; Maltby et al., 2004).

Fluoride (F) has been shown to have varying degrees of beneficial effects on bone mineralization and strength, despite its toxic effects on growth and leg disorders. Since growth depression is generally accepted as a good indicator of F toxicity (NRC, 1994), the lower limits of "safe" levels have been studied based on growth rates. Weber et al. (1969) concluded that chicks can tolerate F levels of up to 3600 mg/kg, but many researchers agree that the upper limit of the "safe" level of dietary F is about 300 mg/kg for broilers (Weber et al., 1969; Suttie et al., 1984; Huyghebaert et al., 1988; Rama Rao and Ramasubba Reddy, 2001).

Tibia dyschondroplasia usually appears between 3 and 8 weeks of age, and is caused by low levels of dietary calcium (Edwards 1984) and high levels of dietary phosphorus (Edwards, 1983). The bone lesion is characterized by an abnormal white, opaque, unmineralized, and unvascularized mass of cartilage occurring in the proximal end of the tibia (Farquharson and Jefferies, 2000). Many studies concluded that twisted legs are heritable (Hartmann and Flock, 1979; Leenstra et al., 1984; Mercer and Hill, 1984; Akbas et al., 2009). It is likely that a simplified selection scheme based on the presence or absence of twisted legs would reduce valgus deformity because of its higher incidence, while changes in incidence of varus would most probably be small or even unfavorable because of the negative genetic correlation between the two defects (LeBihanDuval et al., 1996). Despite the increase in incidences of leg

abnormalities during different decades (Hartmann and Flock, 1979) there is no direct association of frequency of leg abnormality and growth within the same strain.

Bone measurements such as bone breaking strength (Merkley, 1981; Ruff and Hughes, 1985; Park et al., 2003; Kim et al., 2006), bone density (Watkins and Southern, 1992; Kim et al., 2006), bone mineral content (Akpe et al., 1987; Kim et al., 2006) and bone ash (Garlich et al., 1982; Cheng and Coon, 1990; ; Park et al., 2003; Shim et al., 2008) have been used as indicators of bone status in the mineral nutrition of poultry. Because earlier studies on growth rate and bone quality were from different strains over a period of years and even decades, there is no conclusive data that bone measurements and growth rate are related.

REFERENCES

Akbas, Y., S. Yalcin, S. Ozkan, F. Kirkpinar, C. Takma, Y. Gevrekci, H. C. Guler, and L. Turkmut. 2009. Heritability estimates of tibial dyschondroplasia, valgus-varus, foot-pad dermatitis and hock burn in broiler. Archiv Fur Geflugelkunde 73:1-6.

Akpe, M. E., P. E. Waibel, K. Larntz, A. L. Metz, S. L. Noll, and M. M. Walser. 1987. Phosphorous availability bioassay using bone ash and bone densitometry as response criteria. Poult. Sci. 66:713-720.

Brookes, M., and K. U. May. 1972. The influence of temperature on bone growth in the chick. J. Anat. 111:351-363.

Cheng, T. K., and C. N. Coon. 1990. Sensitivity of various bone parameters of laying hens to different daily calcium intakes. Poult. Sci. 69:2209-2213.

Cook, M. E. 2000. Skeletal deformities and their causes: introduction. Poult. Sci. 79:982-984.

Edwards, H. M., Jr. 1983. Phosphorus. 1. Effect of breed and strain on utilization of suboptimal levels of phosphorus in the ration. Poult. Sci. 62:77-84.

Edwards, H. M., Jr. 1984. Studies on the etiology of tibial dyschondroplasia in chickens. J. Nutr. 114:1001-1013.

Farquharson, C., and D. Jefferies. 2000. Chondrobytes and logitudinal bone growth: The development of tibial dyschondroplasia. Poult. Sci. 79:994-1004.

Garlich, J., J. Brake, C. R. Parkhurst, J. P. Thazton, C. Morris, and G. W. Morgan. 1984. Physiological profile of caged layers during one production year, molt, and postmolt: Egg production, egg shell quality, liver, femur, and blood parameters. Poult. Sci. 63:339-343.

Hammond, C. L., B. H. Simbi, and N. C. Stickland. 2007. In ovo temperature manipulation influences embryonic motility and growth of limb tissues in the chick (Gallus gallus). J. Exp. Biol. 210:2667-2675.

Hartmann, W., and D. K. Flock. 1979. Line and family effects on the incidence of 'twisted legs' in meat chickens. Paper read at The British Poultry Breeders Roundtable.

Hester, P. Y. 1994. The role of environment and management on leg abnormalities in meat-type fowl. Poult. Sci. 73:904-915.

Huyghebaert, G., G. De Groote, R. Froyman, and J. Derijke. 1988. Effect of dietary fluoride on

performances and bone characteristics of broilers and the influence of drying and defatting on bone–breaking strength. Poult. Sci. 67:950–955.

Kestin, S. C., T. G. Knowles, A. E. Inch and N. G. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet. Rec. 131:190-194.

Kim, W. K., L. M. Donalson, A. D. Mitchell, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2006. Effects of alfalfa and fructooligosaccharide on molting parameters and bone qualities using dual energy x-ray absorptiometry and conventional bone assays. Poult. Sci. 85:15-20.

LeBihanDuval, E., C. Beaumont, and J. J. Colleau. 1996. Genetic parameters of the twisted legs syndrome in broiler chickens. Genetics Selection Evolution 28:177-195.

Lilburn, M. S. 1994. Skeletal growth of commercial poultry species. Poult. Sci. 73:897-903.

Leenstra, F., A. Van Voorst, and U. Haye. 1984. Genetic aspects of twisted legs in a broiler sire strain. Ann. Agric. Fenn. 23:261-270.

Maltby, V., A. Somaiya, N. A. French, and N. C. Stickland. 2004. In ovo temperature manipulation influences post-hatch muscle growth in the turkey. Br. Poult. Sci. 45:491-498.

Merkley, J. W. 1981. A comparison of bone strengths from broilers reared under varios conditions in cages and floor pens. Poult. Sci. 60:98-106.

Mercer, J. T., and W. G. Hill. 1984. Estimation of genetic-parameters for skeletal defects in broiler-chickens. Heredity 53:193-203.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Oviedo-rondon, E. O., J. Small, M. J. Wineland, V. L. Christensen, P. S. Mozdziak, M. D. Koci, S. V. L. Funderburk, D. T. Ort, and K. M. Mann. 2008. Broiler embryo bone development is influenced by incubator temperature, oxygen concentration and eggshell conductance at the plateau stage in oxygen consumption. Br. Poult. Sci. 49:666-676.

Park, S. Y., S. G. Birkhold, L. F. Kybena, D. J. Nisbet, and S. C. Ricke. 2003. Effect of storage condition on bone breaking strength and bone ash in laying hens at different stages in production cycles

Pierson, F. W. and P. Y. Hester. 1982. Factors influencing leg abnormalities in poultry: a review. World's Poult. Sci. J. 38:5-17.

Rama Rao, S. V., and V. Ramasubba Reddy. 2001. Utilisation of different phosphorus sources in relation to their fluoride content for broilers and layers. Br. Poult. Sci. 42:376–383.

Reddy, R. P. 1996. Symposium: The effects of long-term selection on growth of poultry. Poult. Sci. 75:1164-1167.

Ruff, C. R., and B. L. Hughes. 1985. Bone strengh of height restricted broilers as affected by levels of calcium, phosphorous and manganese. Poult. Sci. 64:1628-1636.

Shim, M. Y., G. M. Pesti, R. I. Bakalli, H. M. Edwards Jr. 2008. The effect of breeder age and egg storage time on phosphorus utilization by broiler progeny fed a phosphorus deficiency diet with 1α-OH vitamin D_3. Poult. Sci. 87:1138-1145.

Sørensen, P. 1992. The genetics of leg disorders. Pages 213-229 in Bone biology and skeletal disorders in poultry. C. C. Whitegead, ed. Abingdon, Oxfordshire, England.

Sullivan, T. W. 1994. Skeletal problems in poultry: estimated annual cost and description. Poult. Sci. 73:879-882.

Suttie, J. W., D. L. Kolstad, and M. L. Sunde. 1984. Fuoride Tolerance of the young chick and turkey poult. Poult. Sci. 63:738–743.

Thorp, B. H. 1994. Skeletal disorders in the fowl: a review. Avian Pathol. 23:203-236.

Thorp, B. H., and D. Waddington. 1997. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res. Vet. Sci. 62:67-73.

Watkins, K. L., and L. L. Southern. 1992. Effect of dietary sodium zeolite A and graded levels of calcium and phosphorous on growth, plasma, and tibia characteristics of chicks. Poult. Sci. 71:1048-1058.

Weber, C. W., A. R. Doberenz, and B. L. Reid. 1969. Fluoride toxicity in the chick. Poult. Sci. 48:230–235.

Webster, A. B. 2004. Welfare implications of avian osteoporosis. Poult. Sci. 83:184-192.

Wise, D. R. 1970. Comparisons of the skeletal systems of growing broiler and laying strain chickens. Br. Poult. Sci. 11:333-339.

Wise, D. R. 1975. Skeletal abnormalities in table poultry. Avian Pathol. 4:1-10.

CHAPTER 2

LITERATURE REVIEW

GENERAL INTRODUCTION OF SKELETAL PROBLEMS IN POULTRY

Bone Abnormalities in Broilers

Fast growing broiler chickens are especially susceptible to bone abnormalities, causing major 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). The most important production problems facing the broiler industry are sudden death syndrome, ascites, scabby hip syndrome, and leg abnormalities. 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). Figure 1 shows imbalance of Ca and P levels to induce TD and rickets (Pesti et al., 2005). The TD bone lesion is characterized by an abnormal white, opaque, unmineralized, 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 (Edwards, 1984;

Riddell, 1975). The incidence of TD in many broiler and turkey flocks is about 30% (Riddell, 1992). One report indicated that the incidence of TD in turkeys was unacceptably high at 87% (Hirt et al., 1997).

Another bone abnormality that sometimes occurs in commercial flocks is rickets which is a disease of 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) which is characterized by an accumulation of proliferating cartilage, and hypophosphatemic rickets (phosphorus deficiency) which occurs where the hypertrophic cartilage accumulates with normal metaphyseal vessel invasion (Lacey and Huffer, 1982).

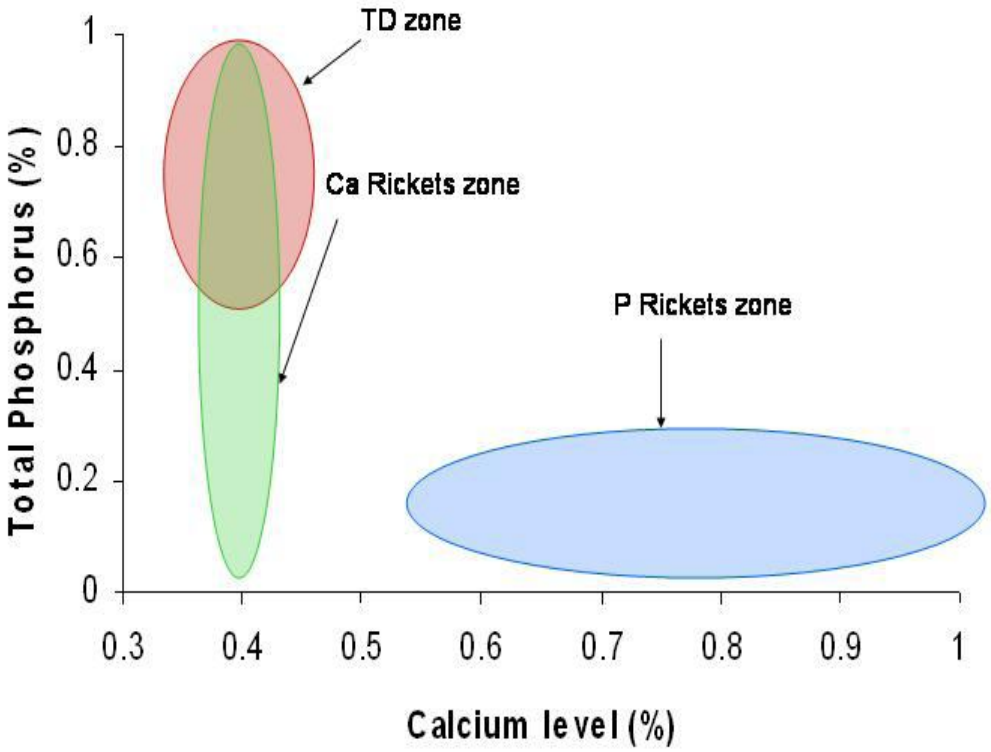


Figure 2.1. Combinations of dietary calcium and phosphorus that produce high (over 90%) incidence of tibial dyschondroplasia, calcium deficiency rickets, and phosphorus deficiency rickets (Pesti et al., 2005).

Valgus-Varus

No explanation has been found yet for the origin of the twisted legs syndrome (LeBihanDuval et al., 1996). However, twisted legs have appeared at higher frequencies concomitantly to the selection of meat-type chickens that has been aimed mainly at increasing growth rate. Hartmann and Flock (1979) compared the incidence of twisted legs in commercial lines between 1963-1968 and 1977-1978. Between these two periods, the incidence measured on male offspring at slaughter had increased from 20 to 32% (70% when including slight

deformities). It is highly probable that some of the genes coding for bone, tendon or cartilage growth and quality may be involved in variations of susceptibility to these disorders.

It is unclear whether the term 'twisted leg' refers to an angular deformity, or torsional deformity or both. Angular and torsional deformities can occur independently (Duff and Thorp, 1985a; Randall and Mills, 1981). Twisted legs were first described in broilers by Osbaldiston and Wise (1967) and referred to lateral angulation of tibiotarsal articulation. Randall and Mills (1981) and Julian (1984) later suggested that the imprecise term of 'twisted legs' should be replaced by valgus-varus angulation. Valgus or varus angulation has been mainly associated with tibial deviation (Sørensen, 1989) and rotation of the shallow distal condyle groove of the tibiotarsus (Cruickshank and Sim, 1986). A few studies also indicate deformations of the femora (Haye and Simons, 1978; Duff and Thorp, 1985a, 1985b; Sørensen, 1989). These discrepancies between observations suggest that intertarsal joint angulations may vary between strains or could be the expression of different angulation causes. The majority of affected birds in their studies were fed *ad libitum* and kept in batteries. When the defect is unilateral, the right leg is more commonly affected. Valgus deformation is more common than varus deformation (Riddell, 1983). The major change in the structure of the bone is a deviation at the growth zone (Hunter et al., 2008).

Many studies conclude that twisted legs are heritable (Hartmann and Flock, 1979; Leenstra et al., 1984; Mercer and Hill, 1984; Akbas et al., 2009). It is likely that a simplified selection scheme based on the presence or absence of twisted legs would reduce valgus deformity because of its larger incidence, while changes in incidence of varus would most

probably be small or even unfavorable because of the negative genetic correlation between the two defects (LeBihanDuval et al., 1996).

BONE BIOLOGY, FORMATION AND DEVELOPMENT

Bone biology

The bones in the skeleton are not all solid. The outside cortical bone is solid bone with only a few small canals. This tissue gives bones their smooth, white, and solid appearance, and accounts for 80% of the total bone mass of skeleton. Cortical bone may also be referred to as compact bone or dense bone. The insides of the bone contain trabecular bone which is like scaffolding or a honey-comb. The spaces between the bones are filled with fluid bone marrow cells, which make the blood, and some fat cells. Trabecular bone accounts for the remaining 20% of total bone mass but nearly ten times the surface area of cortical bone.

There are three special types of cells that are found only in the bone (Sommerfeldt and Rubin, 2001). Osteoclasts are large cells that dissolve the bone. They come from the bone marrow and are related to white blood cells. They are formed from two or more cells that fuse together, so the osteoclasts usually have more than one nucleus. They are found on the surface of the bone mineral next to the dissolving bone. Osteoblasts are the cells that form new bone. They also come from the bone marrow and are related to structural cells. They have only on nucleus. Osteoblasts work in teams to build bone. They produce new bone called “osteoid” which is made of bone collagen and other protein. Then they control calcium and mineral deposition. They are found on the surface of the new bone. When the team of osteoblasts has finished filling in a cavity, the cells become flat and look like pancakes. They line the surface of the bone. These old

osteoblasts are also called lining cells. They regulate passage of calcium into and out of the bone, and they respond to hormones by making special proteins that activate the osteoclasts. Osteocytes are cells inside the bone. They also come from osteoblasts. Some of the osteoblasts turn into osteocytes while the new bone is being formed, and the osteocytes then get surrounded by new bone. They are not isolated, however, because they send out long branches that connect to the other osteocytes. These cells can sense pressures or cracks in the bone and help to direct where osteoclasts will dissolve the bone.

Bone formation

The bones of the body originate as soft cartilage tissue but become ossified, or hardened. The ossification process begins during the embryonic period and continues for some bones. The rate of ossification differs considerably among various bones as well as among strains. Embryological development of bones is described by two main types of bone formation: intramembranous and endochondral ossification. Firstly, membrane bone or intramembranous ossification as exemplified by the skull. The second and more metabolically important pathway of the bone formation is by endochondral ossification which is relevant to most species. New bone forms on a core of mineralized cartilage by the action of lining cells or osteoblasts which have a mesenchyme tissue. These are distinct from osteoclasts which are the main bone resorbing cells and are derived from haemopoetic cell lines. Osteoblasts form a continuous monolayer on the surface of the mineralized matrix and first start synthesizing an organic matrix composed mostly of collagen. This sequence of appositional bone formation is quite important spatially because the older part of the bone will be the fully mineralized part and the mostly

newly formed bone will be the unmineralized organic layer next to the osteoblasts. Thus the active osteoblasts are gradually moving away from the matrix that they have laid down earlier. As the osteoblasts move away from the newly synthesized matrix they leave behind some cells which become engulfed first in the organic matrix and are considered to be the preosteocytes. As the matrix mineralizes around these cells they can be termed osteocytes which have numerous cell processes which are in contact with one another through the bone canaliculi (Aaron, 1976; Sommerfeldt and Rubin, 2001).

Osteoblasts produce the organic matrix of the osteoid in a highly organized manner. The matrix of bone contains many diverse materials the relative proportions of which are dependent on the species, age and the site of bone and often even the position in a single long bone. Various proteins, glycoproteins, peptides, carbohydrates, lipids are present in bone but the bulk (over 90%) of the organic component is made up of a single protein, collagen. Collagen is in various shapes and sizes and to date at least twelve different types of collagen have been described in different tissues. Type I collagen is widely distributed and is the major component of bone. Bone is also known to include type V and type XII collagen (Nimni, 1983; Nimni and Harkness, 1988; Miller, 1988; Burgeson, 1988). New bone formed by the laying down of organic matrix of osteoid which is predominantly composed of collagen fibrils. Most of the rest of the organic matrix is composed of non-collagenous proteins (Sommerfeldt and Rubin, 2001). Triffitt (1980) has worked out and expressed the relative concentrations of these. It is important to remember that firstly, 15 to 20% of the non-collagenous proteins are cell constituents rather than the matrix material. Secondly, as distinct from cartilage matrix, bone has very little proteoglycan amounting to less than 0.1% by weight.

Bone development

A long bone, such as the tibia, first contains a primary center of ossification. The secondary centers of ossification referred to as epiphyses appear at the two ends of bone. Basically a long bone grows inward from its epiphyseal ends. An x-ray of a rapidly growing bone shows a fairly wide white band, representing large concentrations of calcium. With increased age and growth this band becomes narrower. When the bone of the main shaft finally reaches the bone of the epiphysis, growth is no longer possible. The epiphysis is said to be closed or fused (Tanner, 1978; Sommerfeldt and Rubin, 2001). Bone growth occurs from the metaphysis to the epiphysis (Hochberg, 2002). The growth in width of bone does not involve cartilage, but new layers of bone are deposited on the outside of existing bone (Zaichkowsky et al., 1980).

BONE MINERALIZATION AND INTEGRITY

Bone mineralization

Bone mineralization has been studied with respect to process as well as chemical and physical structure since mineralization in the bone matrix is highly important, affecting bone strength which enables the skeleton to withstand the gravity and additional loading. Bone strength is determined not only by the volume of bone tissue and the microarchitectural organization of this bone, but also by the degree of mineralization of bone matrix (Boivin and Meunier, 2002). By the observation of the bone formation, once osteoblasts which are derived from primitive mesenchymal cell in bone marrow via osteoprogenitor cells (Owen and Ashton,

1986; Beresford, 1989) form the extracellular matrix of bone, various steps are followed in order to develop fully mineralized bone.

Microcrystal formation occurs in calcifying cartilage while the soluble constituent ions like Ca and P are complexed to form an insoluble compound. First, this makes small solid particles. Second, the particles grow or reorganize so that the first crystals of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] have been generated. The formation of the first crystals needs to overcome the thermodynamic equilibrium in the stage of calcifying cartilage because subsequently bone is laid on to spicules of calcified cartilage in the metaphysis. Third, in calcified cartilage the number of crystals with a change of their sizes increases, and the crystals subsequently grow and proliferate in the osteoid. Forth, if it is required, further solid phase changes or transformations occur, sometimes involving dissolution and recrystallization. Then finally, stable, organized and structurally sound bone mineral is developed (Glimcher, 1976; Posner, 1969).

The calcifying cartilage in long bones of growing chickens contains chondrocytes which elaborate matrix vesicles that initiate *de novo* mineralization (Wuthier, 1988). Matrix vesicles are also present in developing lone bones of the embryonic chick (Anderson, 1989). The matrix vesicle rapidly accumulates Ca and P to form apatite (Wuthier, 1988).

Bone remodeling

In the skeletally mature animal, remodeling of bone takes place whereby the osteoclasts responsible for resorbing bone remove bone matrix (Bain and Watkins, 1993; Parfitt, 1979) and this loss is made good by an equal amount of bone formation (Sommerfeldt and Rubin, 2001). Remodelling allows the skeleton to respond to alterations in both the internal and external

environments. This balanced process was first described by Frost (1973) and is referred to as coupling and it is the uncoupling of bone formation from resorption that leads to certain forms of skeletal pathology.

In contrast to mammals, chick embryonic cartilage does not calcify prior to resorption and the chick lacks a distinct growth plate (Roach and Shearer, 1989). Gay (1988) described the actions of avian osteoclasts on *in vitro* bone resorption in the late-stage embryo (15 to 19 days), the rapid growth phase (hatching to eight weeks old), and in medullary bone in laying hens. Reabsorption of medullary bone (osteolysis) is under the influence of enlarged osteocytes during eggshell calcification. In the case of the hen being in negative Ca balance, when she is not receiving enough dietary Ca to meet her requirements for shell formation, secretion of parathyroid hormone is markedly increased. This results in Ca being mobilized from cortical bone, to help meet requirements. However, with an acute calcium deficiency, egg production ceases, and medullary bone is gradually resorbed. An imbalance of Ca in remodeling with subsequent increases in cortical bone porosity is associated with increased risk of fracture (Bell et al., 1999).

DIRECT AND INDIRECT MEASURES OF SKELETAL STRENGTH

Bone strength: Breaking bones

Bone breaking strength (Merkley, 1981; Ruff and Hughes, 1985; Park et al., 2003; Kim et al., 2006) has been used as indicators of bone status in the mineral nutrition of poultry by measuring tibia or femur bone breaking strength. Park et al. (2003) concluded bone breaking strength was different between bones by refrigerated and frozen storage. When breaking

strengths of the tibias held in refrigerated storage were compared to those of tibias held in frozen storage, values from 72-wk-old hens were stronger than those obtained from tibias held in frozen storage ($P < 0.05$). A reduction of approximately 30% in bone strength for 72-wk-old laying hens was affected by frozen storage. Therefore, tibias were refrigerated before measuring bone breaking. The meat was removed before bone-breaking strength analysis of the tibias by Instron Materials tester (Model 5500, Instron Corp., Canton, MA) with Automated Materials Test System software version 4.2. The weights, diameters, and lengths of tibia were measured. The deformation rate was 5mm/min. Tracing of force was recorded at constant rate. The graph shows plateau curve of maximal force (kgf) is reached which measure of the energy stored in the bone.

Scoring: Valgus-varus

Varus angulation of tarsal joint appeared between 2 and 15 days of age (Leterrier and NYS, 1992). It occurred in 1 to 3 % of chicks reared on floor and its incidence increased up to 11% in caged chicks. This deformity may be caused by a lack of remodelling (Julian, 2005). The limb deformity was unilateral in 80% of cases and affected predominantly the right limb (80 to 100% of chicks by varus angulation; Leterrier and NYS, 1992). Varus angulation was always associated with inward displacement of the tendon and severe lameness. Valgus angulation was classified as mild (tibia-metatarsus angle between 10 and 25º), intermediate (angle between 25 and 45º) and severe (angle greater than 45º). Clinical, epidemiological and anatomical differences suggest that valgus and varus angulations of the tarsal joint result from different aetiology. Early tendonous displacement seems determinative in varus deformity and deformations of tibial diaphysis are likely to be an adaptive consequence of the luxation. In

contrast, valgus angulation appeared progressively with age in birds and could lead to secondary tendonous slipping (Leterrier and NYS, 1992; Riddell, 1992).

Scoring: TD and Rickets

TD and rickets incidences are determined by making a longitudinal cut across the right tibia at the end of the experiments. The TD bone lesion is characterized by an abnormal white, opaque, unmineralized, and unvascularized mass of cartilage occurring in the proximal end of the tibia (Farquharson and Jefferies, 2000). It scores from 1 (mild) to 3 (severe) depends on cartilage plug abnormality. Rickets are diseases 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 also scores from 1 (mild) to 3 (severe) depends on unmineralized and uncalcificated mass of cartilage.

Bone mineral density (BMD)

Bone mineral density which is the weight of mineral per volume of the bone (g/cm3) is determined by two factors: how many mineral atoms are deposited within the bone matrix, and how porous the matrix is. Since these two factors are highly related to the bone strength, the bone mineral density can indirectly show the bone strength. This method, however, cannot be used to measure bone density in living animals. In people, bone density can be measured using a technique called dual-energy x-ray absorptiomety, or DXA to predict osteoporosis without taking samples (Koo, 2000; Bolotin, 2007). Other methods of measuring bone mass in living persons or

animals include ultrasound, or quantitative computed tomography (QCT; Carter et al., 1992; Rizzoli et al., 1995; Roschger et al., 2008).

Bone Ash: bone mineral content measurement

Bone ash is a critical measurement how much mineralized the bone is. Mineralized percent bone ash is higher than unmineralized percent bone ash because of bone disorder (Shim et al., 2008). In order to measure mineralization, the left tibia was removed for bone ash determination on the fat-free dry bone. Fat is extracted from cleaned meat-free bones by ethanol and ether for 48 hours in order, and these procedures make wet bones to dry fat-free bones. Each step takes overnight. Remove organic matter by ashing at 600°C overnight in a furnace. Bone contains only minerals (the major ones being Ca and P) after ashing.

NUTRITIONAL AND GENETIC FACTORS AFFECTING SKELETAL DEVELOPMENT AND BONE QUALITY

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 body of animals. The bone is a reservoir for minerals. The bone stores 99% of the body's calcium and 85% of the phosphorus. They are often discussed together due to their function in bone formation. 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; Sommerfeldt and Rubin, 2001). Calcified bone contains about 70% inorganic mineral (hydroxyapatite), 25% organic matrix, cells (2-5%), and water (5%; Sommerfeldt and Rubin, 2001). 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). Also, the blood level of Ca should remain within a narrow range. As it increases or decreases beyond a certain level, the muscles and nerves do not function properly.

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. Ca and P have an antagonistic relationship like increasing dietary Ca reduced P absorption, and also reduced the utilization of phytate P (Davis, 1959; Waldroup et al., 1963; Edwards and Veltmann, 1983). 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 intake is critically important to the maintenance of skeletal integrity and the avoidance of fractures. Advances in the noninvasive measurement of bone density have led to studies directly relating calcium intake to bone mass (Marcus, 1987). If the supplies of Ca and P are inadequate, it is possible that they are obtained by increased bone resorption or by reduced bone formation within the osteons of cortical bone (Stevens and Lowe, 1992). Any less Ca intake could lead to osteoporosis (Kolata, 1986). On the other hand, Riggs and Melton (1986) fail to show a relationship between Ca intake and osteoporosis.

Ca and P with hormones

Hormones are important to bone strength. Hormones are chemicals made by glands. The hormones travel throughout the body and have many effects on growth, maturation, energy, weight, and bone strength. Bone is in a constant state of flux as Ca and P are liberated via resorption in times of demand by the body, and deposited when levels in the extracellular fluid become sufficient. This exchange of Ca and P between the bone and soft tissues is continuous

but is particularly important during lactation and egg production when Ca requirements are high (McDonald et al., 1995).

Blood Ca levels are regulated by hormones produced in the parathyroid gland and the thyroid gland (Frandson and Spurgeon, 1992). The parathyroid gland produces parathyroid hormone (PTH) from chief cells when blood Ca becomes too low. The hormone raises circulating Ca in three ways. Firstly, PTH increases mobilization of Ca from the skeleton by activating osteocytes to destroy bone matrix which releases trapped Ca while also stibulating osteoclasts to resorb bone tissue. Secondly, PTH promotes the absorption of Ca from the digestive tract by up-regulating the production of 1,25-$(OH)_2$ colecalciferol (1,25-$(OH)_2D_3$), the hormonal form of vitamin D_3. Finally, PTH stimulate the kidneys to excrete P while retaining Ca through re-absorption (Frandson and Spurgeon, 1992; Lee and Lorenzo, 1999).

The hormone calcitonin has opposite effect of PTH and inhibits the resorption of bone and decreases the release of Ca and P from the bone into the blood (Frandson and Spurgeon, 1992). Calcitonin is produced and relased from the parafollicular C cells of the thyroid gland and secretion is in direct proportion to the rise in blood Ca content. As calcitonin does not affect Ca and P deposition in the bone but only resorption, the net effect of calcitonin is the increase of Ca and P in the bone with a concomitant decrease of these minerals in the blood (Frandson and Spurgeon, 1992).

Ca and P Requirements of Layers

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). Hens long bone marrow cavities contain medullary bone as a temporary store of calcium for eggshell formation (Fleming, 2006). 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; Nowotarski and Bird, 1943; Wilgus, 1931).

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 Broilers

Broiler diets generally supply extra Ca and P (Waldenstedt, 2006) to maximize bone ash, to determining Ca and P concentrations required to eliminate bone disease and improve carcass quality. 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 (Duclos et al., 2007), 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. (2000) 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. Since a dietary deficiency of calcium can result in decreased feed intake and increased basal metabolic rate (Leeson and Summers, 2001), 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 (Edwards et al., 1994; Hart et al., 1925). 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-$(OH)_2$ D_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 (Bar et al., 1987; Olson et al., 1981). 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; Fritts and Waldroup, 2003; Kasim and Edwards, 2000; Mitchell et al., 1997) 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, 2003; Edwards et al., 1992, 1994). 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; Griminger, 1966; Murphy et al., 1936). Third, the level of Ca and available P alters the requirement of vitamin D_3 (Baker et al., 1998; Waldroup et al., 1965). Fourth, the level of vitamins A and E also alters the requirement of vitamin D_3 (Aburto and Britton, 1998a, 1998b; 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-OHD_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., 1995). 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 (Fritts and Waldroup, 2003; Ledwaba and Roberson, 2003; McNutt and Haussler, 1973; Zhang et al., 1997). 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 (Boris et al., 1977; Fritts and Waldroup, 2003; McNutt and Haussler, 1973). However, when 25-OHD_3 is compared to vitamin D_3, its potency depends on the levels of vitamin D_3 tested.

Fluoride Effect on Poultry

Fluoride (F) exists naturally in water sources and is derived from fluorine, the 13th most common element in the Earth's crust (Tokalioglu et al., 2001). In 1972, F was added to the list of essential elements when it was demonstrated that the growth rate of rats on a fluorine-free purified diet, could be increased up to 30% by addition of the element (Leeson and Summers, 2001). However, the importance of F in the prevention of dental cares had been demonstrated many years previously (Chankanka et al., 2010). F can be a very toxic element resulting in reduced appetite, and pitted and worn teeth, in certain farm animals (Leeson and Summers, 2001). There is little evidence that F is a required element for poultry.

Modern-day agricultural practices are just one of the many ways by which environmental

F can easily contaminate poultry diets (Chinoy, 2003). In addition, F is intentionally added to public water sources at very low levels in the United States. Environmental and dietary F may be unnoticed or ignored in the poultry industry, but F has the potential to affect flock health and growth. Unaccounted-for F in water and feed ingredients can have an unforeseen effect on poultry research, as well.

Several studies have been conducted on the toxic and beneficial effects of F in poultry since the 1960s, but the results are sometimes contradictory and often inconsistent. First of all, since growth depression is generally accepted as a good indicator of F toxicity, the lower limits of "safe" levels have been studied based on growth rates. Weber et al. (1969) claimed that chicks can tolerate F levels of up to 3600 ppm, but many researchers agree that the lower limit of the "safe" level of dietary F is about 300 ppm for broilers (Weber et al., 1969; Suttie et al., 1984; Huyghebaert et al., 1988; Rama Rao and Ramasubba Reddy, 2001). Also, 500-600 ppm has been accepted as the lowest toxic level of F in the broiler diet (Weber et al., 1969; Chan et al., 1976; Suttie et al., 1984; Rama Rao and Ramasubba Reddy, 2001).

Several studies, however, show that even lower levels of dietary of F have toxic effects. For example, significant increases in tibial dyschondroplasia (TD) have been observed at levels as low as 50 ppm (Merkley, 1979). Huyghebaert et al. (1988) reported that TD incidence increased with increasing levels of dietary F, but noted that few other studies have reported similar results. On the contrary, Japanese quail actually experience improved growth when fed a diet containing 0.177% F from hatch (Chan et al., 1973), although the toxic effects of dietary F seem to differ among avian species.

F has also been shown to have varying degrees of beneficial effects on bone

mineralization and strength, despite its toxic effects (Louw et al., 2002) on growth and leg disorders. Some studies have demonstrated an increase in percent bone ash due to F supplementation, which indicates an increase in bone mineralization and strength (Merkley and Miller, 1983; Huyghebaert et al., 1988). Dietary F in high amounts is known to stimulate new bone formation and inhibit bone resorption by formating of fluoroapatite which is more resistant to dissolution (Zipkin, 1970). However, studies on bone strength of F-supplemented birds are inconsistent. Suttie et al. (1984) reported no change in the breaking strength of bones of broilers supplemented with F compared to that of broilers on a F-free diet. Rennie et al. (1997) reported that the addition of F to a dicalcium-phosphate-based layer diet was almost as effective at increasing the percentage of the hens; medullary bone as was the use of oyster shell in place of dicalcium phosphate in the diet. Contrary to this study, Fleming et al. (2003) reported that the addition of F to the diet of laying hens had no beneficial effects on bone strength.

The biochemical mechanism by which F works is somewhat unclear. The most consistent result of adding F to chicken diets, in addition to decreased growth at toxic level, is increased calcium retention in bone, and Chan et al. (1976) presented their speculations as to how this may happen physiologically.

When dietary F is absorbed in the gastrointestinal tract of birds, it increases Ca uptake by bone and decreases Ca release from bone by binding Ca into the compound fluorapatite ($Ca_5(PO_4)_3F$). Fluorapatite is more resistant to osteoclastic activity than hydroxyapatite ($Ca_5(PO_4)_3(OH)$). The net effect of these two mechanisms is increased Ca retention in the bone. Fluoride may also directly increase pyrophosphatase activity, which in turn increases pyrophosphate levels in the bone. An increase in pyrophosphate levels has three effects: 1) it

decreases new bone formation, 2) it decreases synthesis of vitamin D intermediates (cholecalciferols), and 3) it increases parathyroid hormone (PTH) levels. PTH causes the distal convoluted tubules in the nephrons of the kidneys to increase Ca absorption. Normally, in birds that are not provided with dietary F, PTH would also stimulate the release of Ca from the bone to the bloodstream, but fluorapatite resists osteoclastic activity to a certain degree. The overall effect of fluoride on bone is an increased level of Ca retention. This explains at least part of the increased tibial bone ash that we observed in our F–supplemented birds, and helps to explain why none of our birds had Ca–deficiency rickets.

Genetic Factors

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; Smith et al., 2001; Zhang et al., 1998).

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. 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.

The chances of bone fractures occurring in modern hybrids of laying hens are high compared to slow-growing leghorn. Fleming (2004) started selection in G3 without initial information on sires by assigning birds to High (H) or Low (L) bone index (BI) line on the basis of their dam's BI value in G2 based on standard selection index theory (Bishop et al., 2000). He found keel deformities were eliminated completely in G7 hens in the H line (Fleming et al., 2004).

Mischellaneous Factors

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, 1947; Mitchell and McClure, 1937). 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).

The basis of age-related bone loss is remodeling, a continuous and inherently unbalanced process of coupled breakdown (resorption) and renewal (formation). The amount of bone formed during a remodeling cycle does not completely replenish that which has been removed by resorption. In the skeletally mature animal, remodeling of bone takes place whereby the cells responsible for resorbing bone remove bone matrix and this loss is made good by an equal amount of bone formation. Remodeling allows the skeleton to respond to alterations in both the internal and external environment (Frost, 1973). Alterations in remodeling activity are the means by which mechanical, hormonal and dietary factors modulate the rate of bone loss (Marcus, 1987).

Incubation Temperature

Bone development and growth plate differentiation starts during embryo development (Ballock and O'Keefe, 2003). In fact, the highest growth rate of bones occurs days prior to hatch and a few days post-hatch (Church and Johnson, 1964; Applegate and Lilburn, 2002). Consequently, bone developmental disorders could have origins during incubation (Hammond et al., 2007; Yalçin et al., 2007).

Temperature conditions during incubation are known to affect yolk utilization, intestinal maturation (Wineland et al., 2006a), thyroid metabolism (Wineland et al., 2006b), heart (Christensen et al., 2004), and muscle development (Christensen et al., 2006). Thyroid hormones have a critical role in growth plate chondrocyte differentiation (Shao et al., 2006); And yolk lipids, trace minerals and vitamins are involved in bone modeling are remodeling (Oviedo-Rondon et al., 2006). Embryonic mortalities or chick weight were not influenced by incubation temperature (Yalçin et al., 2010). As a result, alterations in development caused by incubation conditions like temperature may affect bone development. Actually, small differences in incubation temperature applied throughout incubation have been previously shown to influence growth of the long bone in the chicks' legs (Brookes and May, 1972). Formation of bone starts during embryonic days (ED) 4 – 7. Raising the temperature of the eggs by just on degree, from 37.5ºC to 38.5ºC during embryonic days (ED) 4 – 7 can increase the length of tibia and tarsus bones in Leghorns (Hammond et al., 2007). Oviedo-Rondon et al. (2008) concluded that tibias of broilers were longest at 38ºC compared to 36, 37 and 39ºC. They also concluded that temperatures greater than 37ºC should be avoided to ensure optimal bone development at hatching. Incubation temperature can influence the development both of muscle and of bone, but the mechanisms by which this is occurring are unclear (Brookes and May, 1972; Maltby et al., 2004). In the several studies cited above, all measured different parameters, but no one measured direct parameters such as are bone scoring and ash.

GROWTH AND WELFARE CONSEQUENCES OF SKELETAL DEFORMITIES

P contamination

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 when manure containing high levels of P is used as a fertilizer. P contamination results from the ability of the mineral to bind tightly to soil particles. The mineral therefore does not leach from the soil but it is instead carried out into rivers and streams through the process of erosion (Loer, 1984). A large quantity of P entering a water system can cause large-scale deterioration of the water quality by promoting algal blooms and other forms of eutrophication. This rapid growth and increased metabolic activity of the aquatic ecosystem in-turn leads to increased plant decay. The result of this on-going cycle of respiration and decomposition is a decline in the oxygen content of the water. When the depletion becomes excessive, the entire aquatic ecosystem may die. On the other hand, 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). The most effective way to reduce inorganic P in livestock feed, without compromising performance and leg quality, is to either use feed stuffs with higher P availability such as low-phytic acid corn, or to increase the digestibility of current ingredients by phytase supplementation. 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.

Bone strength effects in processing plant

Bone breakage in older birds results as a consequence of impaired calcification of the skeleton over time (Leeson and Summers, 2001). Few live birds have broken bones in the cage, the major problem occurring when these birds are removed from their cages. Bone disease leads to broken bones when farmers catch birds and when they arrive live at processing plants and are handled and shackeled. If angulation at the hocks and TD are severe, the legs can be deformed to the extent that it is difficult to position the bird properly in the shackle. What usually happens in this situation is that the birds are suspended part of the way up the shackle and the joints counteract on another to give an inflexible carcass. This malpositioning and inflexibility contribute towards some of the plucker damage (Meineche et al., 1980; Burton et al., 1981). Forcing such birds to hang properly in the shackles is potentially painful and should not be encouraged (Gregory and Wilkins, 1990). Broken bones prove problematic during the mechanical deboning of the muscles (Leeson and Summers, 2001).

Impacts of bone problems on the poultry meat industry

Leg weakness is a major economic and welfare problem in the broiler industry. It can increase mortality, downgrades and number of culls (Su et al., 1999; Weeks et al., 2000; Venalainen et al., 2006). Since modern broilers are too heavy for normal locomotion, they tend to spend 76% to 86% of the day lying, and the proportion of broilers lying increased with age and lameness (Morris, 1993). Thus, sitting behavior is strongly associated with poor walking ability and is of serious welfare consequence as birds suffer pain (Julian, 1998), have difficulty reaching for food and water and may lose weight (Yalçin et al., 1998). Previous studies

demonstrated that exercise can improve bone strength and decrease leg disorders (Balog et al., 1997; Falcone et al., 2004). The growth and development of a skeletal structure is a pre-requisite for a bird which is grown primarily for meat production. Leg problems including leg weakness and lameness can result in a loss of 0.5% to 5% of a flock during the growing period and in the downgrading of carcass (Julian, 1984; Riddell and Springer, 1985). Good carcass conformation can only be achieved from a bird with well balanced skeleton. Metabolic and infectious diseases of bones and muscles cause downgrading meat quality due to bruising, breast blisters, emaciation and joint infections.

Impacts of bone problems on the egg production

Caged domestic hens have high incidences of bone fractures (Whitehead and Wilson, 1992). For example, in the UK each year about 36 million laying hens are slaughtered and by the time they are killed about 30% have suffered broken bones (Gregory and Wilkins, 1989). Fractures occurred in 10% of hens during their life time in batteries and a further 17% during depopulation and transport (Gregory et al., 1994).

GENERAL CONCLUSIONS

There is no doubt that the preventive measures that can be taken against leg problems are well known, and they must be continually and in some cases more effectively employed to minimize the numbers of birds suffering from leg problems.

The geneticists working for primary breeding companies must continue to select against tibial dyschondroplasia, and the angular deformities, both valgus and varus. This can be difficult

as these conditions are not controlled by single genes and are influenced by environmental and nutritional factors. Some compromise may be also haven to be made in the selection for commercial characteristics of growth rate, food conversion ratio, and meat yield if the maximum effect against leg problems is to be achieved. However, it is hard to ensure that birds maintain satisfactory leg strength.

Broilers raised under identical experimental conditions and fed diets mixed according to the same formulations and fed diets mixed according to the same formulations performed differently and achieved different degrees of bone mineralization and pathology. Identifying and controlling sources of variation allows for a greater understanding of the true relationships and interaction involved in Ca and P nutrition. Many of the nutritional factors which influence leg problems are at least understood in general terms and this knowledge must be updated and used by industry as appropriate.

REFERENCES

Aaron, J. E. 1976. Histology and microanatomy of bone. Edited by B. E. C. Nordin, Calcium, phosphate and magnesium metabolism London: Churchill and Livingstone.

Aburto, A., and W. M. Britton. 1998a. Effects of different levels of vitamin A and E on the utilization of cholecalciferol by broiler chickens. Poult. Sci. 77:570-577.

Aburto, A., and W. M. Britton. 1998b. Effets and interactions of dietary levels of vitamins A and E and cholecalciferol in broiler chickens. Poult. Sci. 77:666-673.

Aburto, A., H. M. Edwards, Jr., and W. M. Britton. 1998. The incidence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, and 1,25 dehydroxycholecalciferol in young broiler chickens. Poult. Sci. 77:585-593.

Akbas, Y., S. Yalcin, S. Ozkan, F. Kirkpinar, C. Takma, Y. Gevrekci, H. C. Guler, and L. Turkmut. 2009. Heritability estimates of tibial dyschondroplasia, valgus-varus, foot-pad dermatitis and hock burn in broiler. Archiv Fur Geflugelkunde 73:1-6.

Anderson, H. C. 1989. Biology of disease: Mechamism of mineral formation in bone. Lab. Invest. 60:320-330.

Ankra-Badu, G. A., S. E. Aggrey, G. M. Pesti, R. I. Bakalli, and H. M. Edwards, Jr. 2004. Modeling of parameters affecting phytate phosphorus bioavailability in growing birds. Poult. Sci. 83:1083-1088.

Applegate, T. J., and M. S. Lilburn. 2002. Growth of the femur and tibia of a commercial broiler line. Poult. Sci. 81:1289-1294.

Bain, S. D., and B. A. Watkins. 1993. Local modulation of skeletal growth and bone modeling in poultry. J. Nutr. 123:317-322.

Baker, D. H., R. R. Biehl, and J. L. Emmert. 1998. Vitamin D_3 requirement of young chicks receiving diets varying in calcium and available phosphorus. Br. Poult. Sci. 39:413-417.

Ballock, T., and R. J. O'Keefe. 2003. The biology of the growth plate. J. Bone Joint Surg. 85:715-726.

Balog, J. M., G. R. Bayyari, N. C. Rath, W. E. Huff, and N. B. Anthony. 1997. Effect of intermittent activity on broiler production parameters. Poult. Sci. 76:6-12.

Bar, A., J. Rosenberg, R. Perlman, and S. Hurwitz. 1987. Field rickets in turkeys: Relationship to vitamin D. Poult. Sci. 66:68-72.

Bar, A., D. Shinder, S. Yosefi, E. Vax, and I. Plavnik. 2003. Metabolism and requirements for calcium and phosphorus in the fast-growing chickens as affected by age. Br. J. Nutr. 89:51-69.

Bell, K. L., N. Loveridge, J. Power, N. Garrahan, B. F. Meggitt, and J. Reeve. 1999. Regional differences in cortical porosity in the fractured femoral neck. Bone 24:57-64.

Beresford, J. N. 1989. Osteogenic stem cells and the stromal system of bone and marrow. Clin. Orthop. Relat. Res. 240:270-280.

Bethke, R. M., D. C. Kennard, C. H. Kick, and G. Zinzalian. 1929. The calcium-phosphorus relationship in the nutrition of the growing chick. Poult. Sci. 8:257-265.

Bethke, R. M., P. R. Record, O. H. M. Wilder, and C. H. Kick. 1936. Effect of different sources of vitamin D on the laying bird. II. Storage of vitamin D in the egg and chick and mineral composition of the mature embryo. Poult. Sci. 15:336-344.

Bishop, S. C., R. H. Fleming, H. A. Mccormack, D. K. Flock, and C. C. Whitehead. 2000. Inheritance of bone characteristics affecting osteoporosis in laying hens. Br. Poult. Sci. 41:33-40.

Boivin, G., and P. J. Meunier. 2002. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. Calcif. Tissue Int. 70:503-511.

Bolotin, H. H. 2007. DXA in vivo BMD methodology: An erroneous and misleading research and clinical gauge of bone mineral status, bone fragility, and bone remodelling. Bone 41:138-154.

Boris, A., J. F. Hurley, and T. Trmal. 1977. Relative activities of some metabolites and analogs of cholecalciferol in stimulation of tibia ash weight in chicks otherwise deprived of vitamin D. J. Nutr. 107:194-198.

Brookes, M., and K. U. May. 1972. The influence of temperature on bone growth in the chick. J. Anat. 111:351-363.

Burgeson, R. E. 1988. New collagens, new concepts. Annu. Rev. of Cell Biol. 4:551-577.

Burton, R. W., A. K. Sheridan, and C. R. Howlett. 1981. The incidence and importance of tibial chondroplasia to the commercial broiler industry in Australia. Br. Poult. Sci. 22:153-160.

Carlos, A. B., and H. M. Edwards, Jr. 1998. The effects of 1,25-dihydroxycholecalciferol and phytase on the natural phytate phosphorus utilization by laying hens. Poult. Sci. 77:850-858.

Carter, D. R. , M. L. Bouxsein, and R. Marcus. 1992. New approaches for interpreting projected bone density data. J. Bone Miner. Res. 7:137-145.

Carver, J. S., R. J. Evans, and J. McGinnis. 1946. Calcium, phosphorus, and vitamin D interrelationships in the nutrition of the growing chick. Poult. Sci. 25:294-297.

Chan, M. M., R. B. Rucker, and R. S. Riggins. 1976. Effects of fluoride and ethane-1-hydroxy-1,1-diphosphonate on bone metabolism in the growing chick. J. Nutr. 106:802-811.

Chan, M. M., R. B. Rucker, F. J. Zeman, and R. S. Riggins. 1973. Fluoride and its effect on bone metabolism in the Japanese Quail. J. Nutr. 103:1431-1440.

Chankanka, O., S. M. Levy, J. J. Warren, and J. M. Chalmers. 2010. A literature review of aesthetic perceptions of dental fluorosis and relationships with psychosocial aspects/oral health-related quality of life. Community Dentistry and Oral Epidemiology 38:97-109.

Cheville, N. F., and R. L. Horst. 1981. Pathology of experimental vitamin D deficiency in chickens and effects of treatment with vitamin D metabolites. Vet. Pathol. 18:638-651.

Chinoy, N. J. 2003. Fluoride in medicine, biology and toxicology. Ahmedabad, India: Gujarat University.

Christensen, V. L., M. J. Wineland, J. L. Grimes, E. O. Oviedo, P. S. Mozdziak, D. T. Ort, and K. M. Mann. 2006. Effect of incubator temperature and oxygen concentration at the plateau stage in oxygen consumption on turkey embryo muscle growth and development. Int. J. Poult. Sci. 6:406-412.

Christensen, V. L., M. J. Wineland, I. Yildrum, D. T. Ort, and K. M. Mann. 2004. Incubator temperature and oxygen concentration at the plateau stage affect cardiac health of turkey embryos. J. Anim. Vet. Adv. 3:52-65.

Church, L. E., and L. C. Johnson. 1964. Growth of long bones in chicken – rates of growth in length + diameter of humerus tibia + metatarsus. Am. J. Anat. 114:521-.

Cruickshank, J. J., and J. S. Sim. 1986. Morphomeric and radiographic characteristics of tibial bone of broiler chickens with twisted leg disorders. Avian Dis. 30:699-708.

Davis, G. K. 1959. Effects of high calcium intakes on the absorption of other nutritions. Fed. Proc. 18:1119-1123.

Day, E. J. 1990. Further research needs focus on new, old problems. Feedstuffs July 23:12-15.

Doyle, J. J. 1979. Toxic and essential elements in bene - A review. J. Anim. Sci. 49:291-606.

Duclos, M. J., C. Berri, and E. Le Bihan-Duval. 2007. Muscle growth and meat quality. J. Appl. Poult. Res. 16:107-112.

Duff, S. R. I., and B. H. Thorp. 1985a. Patterns of physiological bone torsion in the pelvic appendicular skeletons of domestic fowl. Res. Vet. Sci. 39:307-312.

Duff, S. R. I., and B. H. Thorp. 1985b. Abnormal angulation/torsion of the pelvic appendicular skeletons in broiler fowl: morphological and radiological findings. Res. Vet. Sci. 39:313-319.

Edwards, H. M., Jr. 1983. Phosphorus. 1. Effect of breed and strain on utilization of suboptimal levels of phosphorus in the ration. Poult. Sci. 62:77-84.

Edwards, H. M., Jr. 1984. Studies on the etiology of tibial dyschondroplasia in chickens. J. Nutr. 114:1001-1013.

Edwards, H. M., Jr. 1989. The effect of dietary cholecalciferol, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol on the development of tibial dyschondroplasia in broiler chickens in the absence and presence of disulfiram. J. Nutr. 119:647-652.

Edwards, H. M., Jr. 1992. Nutritional factors and leg disorders. 23rd Poultry Science Symposium Chapter 10 In: Bone Biology and Skeletal Disorders in Poultry 167-193.

Edwards, H. M., Jr. 2003. Effects of u.v. irradiation of very young chickens on growth and bone development. Br. J. Nutr. 90:151-160.

Edwards, H. M., Jr., M. A. Elliot, and S. Sooncharernying. 1992. Effect of dietary calcium on tibial dyschondroplasia interaction with light, cholecalciferol, 1,25-dihydroxycholecalciferol, protein, and synthetic zeolite. Poult. Sci. 71:2041-2055.

Edwards, H. M., Jr., M. A. Elliot, S. Sooncharernying, and W. M. Britton. 1994. Quantitative requirement for cholecalciferol in the absence of ultraviolet light. Poult. Sci. 73:228-294.

Edwards, H. M., Jr., J. E. Marion, H. L. Fuller, and J. C. Driggers. 1963. Studies on cacium requirements of broilers. Poult. Sci. 42:699-703.

Edwards, H. M., Jr., and J. R. Veltmann, Jr. 1983. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. J. Nutr. 113:1568-1575.

Elliot, M. A., and H. M. Edwards, Jr. 1997. Effect of 1,25-dihydroxycholecalciferol, cholecalciferol, and fluorescent lights on the development of tibial dyschondroplasia and rickets in broiler chickens. Poult. Sci. 76:570-580.

Falcone, C., J. A. Mench, and P. Wakenell. 2004. Can perches and platforms affect the incidence of gait abnormalities in broiler chickens? J. Anim. Sci. 82:362-362.

Farquharson, C., and D. Jefferies. 2000. Chondrocytes and longitudinal bone growth: The development of tibial dyschondroplasia. Poult. Sci. 79:994-1004.

Fleming, R. H. 2006. Towards the genetic improvement of bone quality in laying hens. Lohmann Information, 57-63.

Fleming, R. H., H. A. McCormack, L. McTeir, and C. C. Whitehead. 2003. Effects of dietary particulate limestone, vitamin K 3 and fluoride and photostimulation on skeletal morphology and osteoporosis in laying hens. Br. Poult. Sci. 44:683-689.

Fleming, R. H., H. A. Mccormack, L. Mcteir, and C. C. Whitehead. 2004. Incidence, pathology and prevention of keel bone deformities in the laying hen. Br. Poult. Sci. 45:320-330.

Frandson, R. D., and T. L. Spurgeon. 1992. Endocrinology. 5th ed, Anatomy and physiology of farm animal. Philadelphia: Lea and Febiger.

Fritts, C. A., and P. W. Waldroup. 2003. Effect of source and level of vitamin D on live performance and bone development in growing broilers. J. Appl. Poult. Res. 12:45-52.

Frost, H. M. . 1973. Bone remodelling and its relationship to metabolic bone disease. C. C. Tomas, ed. Tomas, Springfield, Illinois.

Gardiner, E. E. 1969. Response of two breeds of chickens to graded levels of dietary phosphorus. Poult. Sci. 48:986-993.

Gay, C. V. 1988. Avian bone resorption at the cellular level. CRC Crit. Rev. Poult. Biol. 1:197-210.

Glimcher, M. J. 1976. Comparison, structure and organization of bone and other mineralised tissues and the mechanism of calcification. Edited by R. O. Greep and E. B. Astwood, Handbook of Physiology: Endocrinology. Washington D.C. Am. Physiol. Soc.

Gregory, N. G., and L. Wilkins. 1989. Broken bones in domestic fowl: handling and processing in end-of-lay battery hens. Br. Poult. Sci. 30:555-562.

Gregory, N. G., and L. J. Wilkins. 1990. Broken bones in chickens: Effect of stunning and processing in broilers. Br. Poult. Sci. 31:53-58.

Gregory, N. G., L. Wilkins, T. G. Knowles, P. Sorensen, and T. Van Niekerk. 1994. Incidence of bone fractures in European layers. Paper read at The 9th European Poultry Conference.

Griminger, P. 1966. Influence of maternal vitamin D intake on growth and bone ash of offspring. Poult. Sci. 45:849-851.

Halley, J. T., T. S. Nelson, L. K. Kirby, and Z. B. Johnson. 1987. Effects of altering dietary mineral balance on growth, leg abnormalities, and blood base excess in broiler chicks. Poult. Sci. 66:1684-1692.

Hammond, C. L., B. H. Simbi, and N. C. Stickland. 2007. In ovo temperature manipulation influences embryonic motility and growth of limb tissues in the chick (Gallus gallus). J. Exp. Biol. 210:2667-2675.

Harrison, H. E., and H. C. Harrison. 1961. Intestinal transport of phosphate: Action of vitamin D, calcium, and potassium. Am. J. Physiol. 201:1007-1012.

Hart, E. B., H. T. Scott, O. L. Kline, and J. G. Halpin. 1930. The calcium-phosphorus ratio in the nutrition of growing chicks. Poult. Sci. 9:296-306.

Hart, E. B., S. Steenbock, S. W. F. Lepkovsky, J. G. Kletzien, J. G. Halpin, and O. N. Johnson. 1925. The nutritional requirment of the chicken, V. The influence of ultra-violet light on the production, hatchability, and fertility of the egg. J. Biol. Chem. 65:579-595.

Hartmann, W., and D. K. Flock. 1979. Line and family effects on the incidence of twisted legs in meat type chickens. Pages 1-8 in: Proceedings of 21st British Poultry Breeders Roundtable. Glasgow, U.K.

Haye, U, and P. C. M. Simons. 1978. Twisted legs in broilers. Br. Poult. Sci. 19:549-557.

Hirt, H., M. Reinmann, and H. Oester. 1997. Leg problems in fattening turkeys. In 5th Eropean Symposium on Poultry Welfare, edited by P. Koene and H. J. Blokhuis. Wageningen, Wageningen Agricultural University and the Institute of Animal Science and Health, The Netherlands, pp. 80-83.

Hochberg, Z. 2002. Endocrine control of skeletal maturation. Basel: Karger.

Hunter, B., A. Whiteman, B. Sanei, and A. Dam. 2008. Valgus/varus leg deformities in poultry.

http://www.agbiosecurity.ca/healthybirds/fckeditor/editor/filemanager/connectors/aspx/healthybirds/userfiles/file/Valgus%20Varus%20Leg%20Deformities%20in%20Poultry.pdf Accessed Mar. 2010.

Hurwitz, S., and A. Bar. 1971. Calcium and phosphorus interrelationships in the intestine of the fowl. J. Nutr. 101:677-686.

Huyghebaert, G. 1996. Effects of dietary calcium, phosphorus, Ca/P-ratio and phytase on zootechnical performances and mineralization in broiler chicks. Arch. Geflugelk. 62:53-61.

Huyghebaert, G., G. De Groote, R. Froyman, and J. Derijke. 1988. Effect of dietary fluoride on performances and bone characteristics of broilers and the influence of drying and defatting on bone-breaking strength. Poult. Sci. 67:950-955.

Itakura, C. , M. Takecji, Y. Miyakawa, and M. Goto. 1979. Pathology of naturally occurring vitamin D_3-deficient rickets in growing broiler chickens in Japan. Jap. J. vet. Sci. 41:517-530.

Jubb, K. V. F., and P. C. Kennedy. 1970. Pathology of domestic animals. 2 vols. Vol. 1. New York and London: Academic Press.

Julian, R. 2005. Production and growth related disorders and other metabolic diseases of poultry - A review. Vet. J. 169:350-369.

Julian, R. J. 1984. Valgus-varus deformity of the intertarsal joint in broiler-chickens. Can. Vet. J. 25:254-258.

Julian, R. J. 1984. Valgus-varus deformity of the intertarsal joint in broiler chickens. Can. Vet. J. 25:254-258.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult. Sci. 77:1773-1780.

Kasim, A. B., and H. M. Edwards, Jr. 2000. Evaluation of cholecalciferol sources using broiler chick bioassays. Poult. Sci. 79:1617-1622.

Keane, K. W., R. A. Collins, and M. B. Gillis. 1956. Isotopic tracer studies on the effect of vitamin D on calcium metabolism in the chick. Poult. Sci. 35:1216-1222.

Kim, W. K., L. M. Donalson, A. D. Mitchell, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2006. Effects of alfalfa and fructooligosaccharide on molting parameters and bone qualities

using dual energy x-ray absorptiometry and conventional bone assays. Poult. Sci. 85:15-20.

Kolata, G. 1986. How important is dietary calcium in preventing osteoporosis. Sci. 233:519-520.

Koo, W. W. K. 2000. Body composition measurements during infancy. Ann. N. Y. Acad. Sci. 904:383-392.

Lacey, D. L., and W. E. Huffer. 1982. Studies on the pathogenesis of avian rickets I. Changes in epiphyseal and metaphyaseal vessels in hypocalcemic and hypophophatemic rickets. Am. J. Pathol. 109:288-301.

Leach, R. M., and M. C. Nesheim. 1965. Nutritional, genetic and morphological studies of an abnormal cartilage formation in young chicks. J. Nutr. 86:236-244.

LeBihanDuval, E., C. Beaumont, and J. J. Colleau. 1996. Genetic parameters of the twisted legs syndrome in broiler chickens. Genet. Sel. Evol. 28:177-195.

Ledwaba, M. P., and K. D. Roberson. 2003. Effectiveness of twenty-five-hydroxycholecalciferol in the prevention of tibial dyschondroplasia in Ross Cockerels depends on dietary calcium level. Poult. Sci. 82:1769-1777.

Lee, S. K., and J. A. Lorenzo. 1999. Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. Endocrinology 140:3552-3561.

Leenstra, F., A. Van Voorst, and U. Haye. 1984. Genetic aspects of twisted legs in a broiler sire strain. Ann. Agric. Fenn. 23:261-270.

Leeson, S., and J. D. Summers. 2001. Minerals. Edited by M. L. Scott. 4th ed, Scott's nutrition of the chicken. Ithaca, N. Y.: M.L .Scott and Associates.

Leterrier, C., and Y. NYS. 1992. Clinical and anatomical differences in varus and valgus deformities of chick limbs suggest different etiopahogenesis. Avian Pathol. 21:429-442.

Loer, R. C. 1984. Pollution control for agriculture. Orlando, Fl: Academinc press, Inc.

Long, P. H., S. R. Lee, G. N. Rowland, and W. M. Britton. 1984a. Experimantal rickets in broilers: Gross, microscopic, and radiographic lesions. I. Phosphorus deficiency and calcium excess. Avian Dis. 28:460-474.

Long, P. H., S. R. Lee, G. N. Rowland, and W. M. Britton. 1984b. Experimental rickets in broilers: Gross, microscopic, and radiographic lesions. II. Calcium deficiency. Avian Dis. 28:921-932.

Long, P. H., S. R. Lee, G. N. Rowland, and W. M. Britton. 1984c. Experimental rickets in broilers: Gross, microscopic, and radiographic lesions. III. Vitamin D deficiency. Avian Dis. 28:933-943.

Louw, A. J., S. R. Grobler, W. Van, and T. J. Kotze. 2002. Degree of fluorosis in areas of South Africa with differing levels of fluoride in drinking water. Gen. Dent. 50:352-356.

Maltby, V., A. Somaiya, N. A. French, and N. C. Stickland. 2004. In ovo temperature manipulation influences post-hatch muscle growth in the turkey. Br. Poult. Sci. 45:491-498.

Marcus, R. 1987. Calcium intake and skeletal integrity: Is there a critical relationship? J. Nutr. 117:631-635.

McChesney, E. W. 1943. Studies of calcium and phosphorus metabolism in the chick. I. The comparative effect of vitamin D_2 and D_3 and dishydrotachysterol given orally and intramuscularly. J. Nutr. 26:81-94.

McCollum, E. V., N. Simonds, J. E. Backer, and P. G. Shipley. 1922. Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J. Biol. Chem. 53:293-312.

McCormack, H. A., L. McTeir, R. H. Fleming, and C. C. Whitehead. 2004. Prevention of tibial dyschondroplasia by high dietary concentrations of vitamin D_3. Paper read at XXII World's Poultry Congress, at Istanbul, Turkey.

McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan. 1995. Minerals, Animal Nutrition. Singapore: Longman Singapore Publishers (Pte) Ltd.

McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan. 2002. Animal Nutrition. Edited by P. Education, Major Mineral elements. Harlow, England. London. New York. Reading, Massachusetts. San Francisco. Toronto. Don Mills, Ontario. Sydney. Tokyo. Singapore. Hong Kong. Seoul. Taipei. Cape Town. Madrid. Mexico City. Amsterdam. Munich. Paris. Milan.

McNutt, K. W., and M. R. Haussler. 1973. Nutritional effectiveness of 1,25-dihydroxycholecalciferol in preventing rickets in chicks. J. Nutr. 103:681-689.

Meineche, C. F. , J. K. Skelles, E. L. Stephenson, and T. S. Nelson. 1980. An intermittent problem with tibial dyschondroplasia. Avian Dis. 24:517-519.

Mellanby, E. 1919. An experimental investigation of rickets. Lancet. 2:407-412.

Mercer, J. T., and W. G. Hill. 1984. Estimation of genetic-parameters for skeletal defects in broiler-chickens. Heredity 53:193-203.

Merkley, J. W. 1979. Tibial dyschondroplasia in broilers. Poult. Sci. 58:1021.

Merkley, J. W. 1981. A comparison of bone strengths from broilers reared under varios conditions in cages and floor pens. Poult. Sci. 60:98-106.

Merkley, J. W., and E. W. Miller. 1983. The effect of sodium fluoride and sodium silicate on growth and bone strength of broilers. Poult. Sci. 62:798-804.

Miller, E. J. 1988. Collagen Types: structure, distribution and functions in collagen. Edited by M. E. Nimni, Biochemistry I. Boca Raton, Florida: CRC Press Inc.

Mitchell, H. H. 1947. The mineral requirements of farm animals. J. Anim. Sci. 6:365-377.

Mitchell, H. H., and F. J. McClure. 1937. Mineral nutrition of fram animals. National Research Council No. 99.

Mitchell, R. D., and H. M. Edwards, Jr. 1996. Effects of phytase and 1,25-dihydroxycholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci. 75:95-110.

Mitchell, R. D., H. M. Edwards, Jr., and G. R. McDaniel. 1997. The effect of ultraviolet light and cholecalciferol and its metabolites on the development of leg abnormalities in chickens genetically selected for a high and low incidence of tibial dyschondroplasia. Poult. Sci. 76:346-354.

Mongin, P., and B. Sauveur. 1977. Interrelationship between mineral nutrition, acid-base balance, growth and cartilage abnormalities. In growth and poultry meat production. Edited by K. N. Boorman and B. J. Wilson, British Poultry Science Ltd. Edinburgh, Scotland.

Morissey, R. L., R. M. Cohn, R. N. Empson, Jr., H. L. Greene, O. D. Taunton, and Z. Z. Ziporin. 1977. Relative toxicity and metabolic effects of cholecalciferol and 25-hydroxycholecalciferol in chicks. J. Nutr. 107:1027-1034.

Morris, M. P. 1993. National survey of leg problems. edited by B. Industry.

Murphy, R. R., J. E. Hunter, and H. C. Knandel. 1936. The effect of vitamin D intake of the hen on the bone calcification of the chick. Poult. Sci. 15:284-289.

Nelson, T. S., L. W. Ferrara, and N. R. Storer. 1968. Phytate phosphorus content of feed ingredients derived from plants. Poult. Sci. 47:1372-1374.

Nicolaysen, R., N. Egg-Larsen, and O. J. Malm. 1953. Physiology of calcium metabolism. Physiol. Rev. 33:424-444.

Nimni, M. E. 1983. Collagen-structure, fuction, and metabolism in normal and fibrotic tissues. Semin. Arthritis Rheum. 13:1-86.

Nimni, M. E., and R. D Harkness. 1988. Molecular structures and functions of collagen. Edited by M. E. Nimni. Vol. Biochemistry I, Collagen. Boca Raton, Florida: CRC Press Inc.

Nowotarski, J. S., and H. R. Bird. 1943. Dietary calcium and phosphorus levels and the vitamin D requirement of the chick. Poult. Sci. 22:72-78.

Olson, W. G., H. E. Dziuk, M. M. Walser, G. F. Hanlon, P. E. Waibel, J. B. Stevens, and N. A. Jorgensen. 1981. Field rickets in turkey poults: Biochemical findings. Avian Dis. 25:550-554.

Osbaldiston, G. W., and D. R. Wise. 1967. Spondylolisthesis and leg weakness in chicken - A comon aetiology. Vet. Rec. 80:320-322.

Oviedo-Rondon, E. O., P. R. Ferket, and G. B. Havestein. 2006. Understanding long bone development in broilers and turkeys. Avian and Poult. Biol. Rev. 17:77-88.

Oviedo-rondon, E. O., J. Small, M. J. Wineland, V. L. Christensen, P. S. Mozdziak, M. D. Koci, S. V. L. Funderburk, D. T. Ort, and K. M. Mann. 2008. Broiler embryo bone development is influenced by incubator temperature, oxygen concentration and eggshell conductance at the plateau stage in oxygen consumption. Br. Poult. Sci. 49:666-676.

Owen, M., and B Ashton. 1986. Osteogenic differentiation of skeletal cell populations. Edited by S. Y. Ali, Cell mediated calcification and matrix vesicles. Amsterdam: Elsevier.

Parfitt, A. M. 1979. Quantum concept of bone remodeling and turnover: implications for the pathogenesis of osteoporosis. Calcif. Tissut Int. 28:1-5.

Park, S. Y., S. G. Birkhold, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2003. Effect of strage condition on bone breaking strength and bone ash inlaying hens at different stages in production cycles. Poult. Sci. 82:1688-1691.

Pesti, G. M., R. I. Bakalli, J. P. Driver, A. Atencio, and E. H. Foster. 2005. Poultry nutrition and feeding. Victoria, Canada: Trafford publising.

Posner, A. N. 1969. Crystal chemistry of bone mineral. Physiol. Rev. 49:760-791.

Punna, S., and D. A. Roland, Sr. 1999. Variation in phytate phosphorus utilization within the same broiler strain. J. Appl. Poult. Res. 8:10-15.

Rama Rao, S. V. , V. Ramasubba Reddy, and V. Ravindra Reddy. 1999. Non-phytin phosphorus requirements of commercial broilers and white leghorn layers. Anim. Feed Sci. Technol. 80:1-10.

Rama Rao, S. V., and V Ramasubba Reddy. 2001. Utilisation of different phosphorus sources in relation to their fluoride content for broilers and layers. Br. Poult. Sci. 42:376-383.

Randall, C. J., and C. P. J. Mills. 1981. Observations on leg deformity in broilers with particular reference to the intertarsal joint. Avian Pathol. 10:407-431.

Reddy, N. R., S. K. Sathe, and D. K. Salunkhe. 1982. Phytates in legumes and cereals. Adv. Feed. Res. 28:1-92.

Rennie, J. S., R. H. Fleming, H. A. McCormack, C. C. McCorquodale, and C. C. Whitehead. 1997. Studies on effects of nutritional factors on bone structure and osteoporosis in laying hens. Br. Poult. Sci. 38:417-424.

Riddell, C. 1983. Pathology of the skeleton and tendons of broiler chickens reared to roaster weights. I. Crippled birds. Avian Dis. 27:950-962.

Riddell, C. 1975. The development of tibial dyschondroplasia in broiler chickens. Avian Dis. 19:443-462.

Riddell, C. 1992. Non-infectious skeletal disorders of poultry: an overview. Edited by C. C. Whitehead, Bone biology and skeletal disorders in poultry. Abingdon, Oxfordshire, England: Carfax Publishing Co.

Riddell, C., and R. Springer. 1985. An epixootiological study of acute death syndrome and leg weakness in broiler-chickens in western canada. Avian Dis. 29:90-102.

Riggs, B. L., and L. J. Melton. 1986. Involutional osteoporosis. N. Engl. J. Med. 314:1676-1686.

Rizzoli, R., D. Slosman, and J. P. Bonjour. 1995. The role of dual energy X-ray absorptiometry of lumbar spine and proximal femur in the diagnosis and follow-up of osteoporosis. Am. J. Med 98:33S-36S.

Roach, H. I., and J. R. Shearer. 1989. Cartilage resorption and endochondral bone formation during the development of long bones in chick embryos. Bone miner. 6:289-309.

Roschger, P., E. P. Paschalis, P. Fratzl, and K. Klaushofer. 2008. Bone mineralization density distribution in health and disease. Bone 42:456-466.

Ruff, C. R., and B. L. Hughes. 1985. Bone strengh of height restricted broilers as affected by levels of calcium, phosphorous and manganese. Poult. Sci. 64:1628-1636.

Saunders, J. L., and D. R. Blades. 2004. Effect of vitamin D source on broiler production and carcass composition. J. Anim. Sci. 82, Suppl. 1:268.

Scott, M. L., M. C. Nesheim, and R. J. Young. 1982. Nutrition of the chicken. New York: Scott, M. L. and Associates.

Shao, Y. Y., L. Wang, and R. T. Ballock. 2006. Thyroid hormone and the growth plate. Rev. Endocr. Metab. Disord. 7:265-271.

Shim, M. Y., G. M. Pesti, R. I. Bakalli, and H. M. Edwards. 2008. The effect of breeder age and egg storage time on phosphorus utilization by broiler progeny fed a phosphorus deficiency diet with 1 alpha-OH vitamin D-3. Poult. Sci. 87:1138-1145.

Simco, T. F., and E. L. Stephenson. 1961. Re-evaluation of the calcium-phosphorus requirements of the chick. Poult. Sci. 40:1188-1192.

Smith, T. N., S. E. Aggrey, R. I. Bakalli, and G. M. Pesti. 2001. Influence of genetics on phytate phosphorus utilization. Poult. Sci. 80. Suppl. 1:343 (Abstr.).

Sommerfeldt, D. W., and C. T. Rubin. 2001. Biology of bone and how it orchestrates the form and function of the skeleton. Eur. Spine J. 10:S86-S95.

Sørensen, P. 1989. Broiler selection and welfare. In: Proceedings of the 3rd European Symposium on Poultry Welfare Tours. pp. 45-58.

Stevens, A., and J. S. Lowe. 1992. Histology. London, New York: Gower Medical Publishing.

Su, G., P. Sorensen, and S. C. Kestin. 1999. Meal feeding is more effective than early feed restriction at reducing the prevalence of leg weakness in broiler chickens. Poult. Sci. 78:949-955.

Suttie, J. W., D. L. Kolstad, and M. L. Sunde. 1984. Fuoride Tolerance of the young chick and turkey poult. Poult. Sci. 63:738-743.

Tanner, J. M. 1978. Fetus into man. Cambridge: Harvard University Press.

Taylor, T. G., K. M. L. Morris, and J. Kirklry. 1968. Effects of dietary excesses of vitamin A and D on some constituents of the blood of chicks Br. J. Nutr. 22:713-721.

Thorp, B. H., and D. Waddington. 1997. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res. Vet. Sci. 62:67-73.

Tokalioglu, S., U. Sahin, and S. Kartal. 2001. Determination of fluoride and some metal ion levels in the drinking waters in Kayseri province. Turkish J. Chem. 25:113-121.

Triffitt, J. T. 1980. The organic matrix of bone tissue. . Edited by M. R. Urist, Fundamental and clinical bone physiology. New York: Academic Press.

Venalainen, E., J. Valaja, and T. Jalava. 2006. Effects of dietary metabolisable energy, calcium and phosphorus on bone mineralisation, leg weakness and performance of broiler chickens. Br. Poult. Sci. 47:301-310.

Waldenstedt, L. 2006. Nutritional factors of importance for optimal leg health in broilers: A review. Anim. Feed Sci. Tech. 126:291-307.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms. 1963. Calcium and phosphorus requirements of finishing broilers using phosphorus sources of low and high availability. Poult. Sci. 42:752-.

Waldroup, P. W., J. H. Kersey, E. A. Saleh, C. A. Fritts, F. Yan, H. L. Stilborn, R. C. Crum, Jr., and V. Raboy. 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytate. Poult. Sci. 79:1451-1459.

Waldroup, P. W., J. E. Stearns, C. B. Ammerman, and R. H. Harms. 1965. Studies on the vitamin D_3 requirement of the broiler chick. Poult. Sci. 44:543-548.

Weber, C. W., A. R. Doberenz, and B. L. Reid. 1969. Fluoride toxicity in the chick. Poult. Sci. 48:230-235.

Weeks, C. A., T. D. Danbury, H. C. Davies, P. Hunt, and S. C. Kestin. 2000. The behaviour of broiler chickens and its modification by lameness. Appl. Anim. Behav. Sci. 67:111-125.

Whitehead, C. C., H. A. McCormack, L. McTeir, and R. H. Fleming. 2004. High vitamin D_3 reqirements in broilers for bone quality and prevention of tibial dyschondroplasia and interactions with dietary calcium, available phosphorus and vitamin A. Br. Poult. Sci. 45:425-436.

Whitehead, C. C., and S. Wilson. 1992. Characteristics of osteopenia in hens. Edited by C. C. Whitehead, Bone biology and skeletal disorders in poultry Abingdon: Carfax Publishing.

Wilgus, H. S. Jr. 1931. The quantitative requirement of the growing chick for calcium and phosphorus. Poult. Sci. 10:107-117.

Williams, B., S. Solomon, D. Waddington, B. Thorp, and C. Farquharson. 2000. Skeletal development in the meat-type chicken. Br. Poult. Sci. 41:141-149.

Williams, B., D. Waddington, and C. Farquharson. 1999. Influence of dietary calcium and phosphorus on skeletal quality of the modern broiler chicken. Edited by J. Danks, C. Dacke, G. Flik and C. Gay, Calcium Metabolism: Comparative Endocrinology. San Francisco, CA, USA: BioScientifia.

Williams, B., D. Waddington, S. Solomon, and C. Farquharson. 2000. Dietary effects on bone quality and turnover, and Ca and P metabolism in chickens. Res. Vet. Sci. 69:81-87.

Williams, B., D. Waddington, S. Solomon, B. Thorp, and C. Farquharson. 1998. Determining broiler bone life history. Br. Poult. Sci. 39 (Suppl.):S59-S60.

Wineland, M. J., V. L. Christensen, I. Yildrum, B. D. Fairchild, D. T. Ort, and K. M. Mann. 2006a. Incubator temperature and oxygen concentration at the plateau stage in oxygen consumption affects intestinal maturation of broiler chicks. Int. J. Poult. Sci. 5:229-240.

Wineland, M. J., V. L. Christensen, I. Yildrum, B. D. Fairchild, D. T. Ort, and K. M. Mann. 2006b. Incubator environment interacts with genetic line of broiler at the plateau stage to affect embryo plasma thyroxine and triiodothyronine concentrations. Int. J. Poult. Sci. 5:714-722.

Wuthier, R. E. 1988. Machanism of matirx vescle-mediated mineralization of cartilage. ISI Atlas Sci. Biochem. 1:231-241.

Yalçin, S., E. Babacanoğlu, H. C. Güler, and M. Akşit. 2010. Effects of incubation temperature on hatching and carcass performance of broilers. World's Poult. Sci. J. 66:87-94.

Yalçin, S., H. B. Molayoğlu, M. Baka, O. Genin, and M. Pines. 2007. Effect of temperature during the incubation period on tibial growth plate chondrocyte differentiation and the incidence of tibial dyschondroplasia. Poult. Sci. 86:1772-1783.

Yalcin, S., P. Settar, and O. Dicle. 1998. Influence of dietary protein and sex on walking ability and bone parameters of broilers. Br. Poult. Sci. 39:251-256.

Yang, H. S., P. E. Waibel, and J. Brenes. 1973. Evaluation of vitamin D_3 supplements by biological assay using the turkey. J. Nutr. 103:1187-1194.

Yanger, J. G., C. L. Quarles, B. W. Hollis, and R. W. Gray. 1995. Safety of 25-Hydroxycholecalciferol as a Source of Cholecalciferol in Poultry Rations. Poult. Sci. 74:1437-1446.

Yanger, J. G., J. L. Saunders, C. L. McNaughton, C. L. Quarles, B. W. Hollis, and R. W. Gray. 1995. Comparison of dietary 25-hydroxycholecalciferol and cholecalciferol in broiler chickens. Poult. Sci. 74:1159-1167.

Zaichkowsky, L. D., L. B. Zaichkowsky, and T. J. Martinek. 1980. Chapter 2. Physical growth and development, Growth and development. St. Louis, Toronto, London: The C. V. Mosby Company.

Zhang, W., S. E. Aggrey, G. M. Pesti, H. M. Edwards, Jr., and R. I. Bakalli. 2003. Genetics of phytate phosphorus bioavailbility: Heritability and genetic correlations with growth and feed utilization traits in a randombred chicken population. Poult. Sci. 82:1075-1079.

Zhang, X., G. Liu, G. R. McDaniel, and D. A. Roland. 1997. Responses of broiler lines slected for tibial dyschondroplasia incidence to supplementary 25-hydroxycholecalciferol. J. Appl. Poult. Res. 6:410-416.

Zhang, X., G. R. McDaniel, and D. A. Roland. 1998. Genetic variation of phytate phosphorus utilization from hatch to three weeks of age in broiler chicken lines selected for incidence of tibial dyschondroplasia. Poult. Sci. 77:386-390.

Zipkin, I. 1970. Interrelation of fluoride with other components of calcified tissue. Edited by K. B. 15 vols, Biological interrelations and nutrition. Zurich/Feldback: Bibl. Nutrtio et dieta.

CHAPTER 3

EFFECTS OF INCUBATION TEMPERATURE ON THE BONE HEALTH OF BROILERS[1]

[1] M. Y. Shim and G. M. Pesti. Submitted to Poultry Science.

ABSTACT

It would be of great benefit to manipulate the development of leg bone by making simple changes to incubation temperature such that chickens are better able to reduce the incidence of abnormalities. Raising the temperature of the eggs by just one degree, from 37.5ºC to 38.5ºC, during embryonic days (ED) 4 – 7 was reported to increase the length of tibia and tarsus bones in Leghorns (Hammond et al., 2007). Oviedo-Rondon et al. (2008) concluded that tibias of broilers were longest at 38ºC compared to 36, 37 or 39ºC. They also concluded that temperatures greater than 37ºC should be avoided to ensure optimal bone development at hatching. This study tested the hypothesis that increasing or decreasing the temperature of chick incubation by 1ºC for 3 days during early incubation ED 4 – 7 affects hatchability, growth and leg abnormalities of Cobb 500 broilers fed TD (tibial dyschondroplasia), Ca–, and P– deficiency rickets inducing diets. In Experiment 1, eggs hatched earlier, and more hatched, at 38.5ºC (92.77%) compared to 37.5ºC (86.22%). BW was lower in chicks incubated at the higher temperature (44.66 g vs. 42.92 g). In Experiment 2, egg setting times were plus 17 h for 36.5ºC eggs and minus 9 h for 38.5ºC compared to standard setting at 37.5ºC (506 h). Hatchability of fertile eggs (92.92%) was highest at 37.5ºC and decreased at 36.5ºC (89.82%) and 38.5ºC (81.55%). BW were lower (48.98g) at 36.5ºC than those (49.57g) at 37.5ºC and those (50.56g) at 38.5ºC. A third factorial experiment separated effects of incubation temperature and incubation time and was conducted with control and Ca–deficiency rickets inducing diets. No main effects or interactions between incubation temperature or time and bone abnormalities were detected. It is important to note that eggs hatched at different times in our study. As little as 1ºC for 3 days during early incubation ED 4 – 7 affected hatching time and weight, confounding results.

Key words: Incubation temperature, Broiler, Hatchability, Tibial dyschondroplasia, Rickets

INTRODUCTION

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 deformities (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) observed that TD usually appears between 3 and 8 weeks of age, and is caused by a low level of dietary calcium with a high level of dietary phosphorus (Edwards, 1983). The bone lesion is characterized by an abnormal white, opaque, unmineralized, 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 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, Ca, and P or a deficiency of one of these nutrients. Itakura et al. (1979) provided a detailed description of an outbreak of rickets in broilers. Bones were soft but cortical bone was thickened with narrowing of the marrow cavity. There are two types of rickets: Hypocalcemic rickets (Ca–deficiency) is characterized by an

accumulation of proliferating cartilage, and hypophosphatemic (P–deficiency) rickets in which the hypertrophic cartilage accumulates with normal metaphyseal vessel invasion (Lacey and Huffer, 1982).

Bone development and growth plate differentiation starts during embryo development (Ballock and O'Keefe, 2003). In fact, the highest growth rate of bones occurs days prior to hatch and a few days post–hatch (Church and Johnson, 1964; Applegate and Lilburn, 2002). Consequently, bone developmental disorders could have origins during incubation (Hammond et al., 2007; Yalçin et al., 2007).

Optimum incubation temperature is normally defined as that required to achieve maximum hatchability. However, Ducuypere and Michels (1992) have argued that the quality of the hatching chicks should be considered. The effect of temperature on length of incubation has been observed in several studies (Romanoff, 1935, 1936; Romanoff et al., 1938; Michels et al., 1974; French, 1994a) and on the rate of embryo growth (Romanoff et al. 1938; Ducuypere et al., 1979). Temperature conditions during incubation are known to affect yolk utilization, intestinal maturation (Wineland et al., 2006a), thyroid metabolism (Wineland et al., 2006b), heart (Christensen et al., 2004), muscle development (Christensen et al., 2006), hatching chick's thermoregulatory and posthatching growth rate (Wilson, 1991; Decuypere, 1994). Freguson (1994) has suggested that temperature may be able to alter the sex ratio by altering the phenotypic sex of a proportion of chick embryos. Optimum incubation temperature for the hatchability of poultry species is between 37 and 38ºC demonstrated (Lundy, 1969; Wilson, 1991). Recent studies suggest that optimum temperature may differ between poultry strain (Ducuypere, 1994; Christensen et al., 1994) or eggs of different sizes (French, 1994b). Thyroid

hormones have a critical role in growth plate chondrocyte differentiation (Shao et al., 2006) and yolk lipids, trace minerals and vitamins are involved in bone modeling and remodeling (Oviedo-Rondon et al., 2006). As a result, alterations in development caused by incubation conditions like temperature may affect bone development. Actually, small differences in incubation temperature applied throughout incubation have been shown to influence growth of the long bone in the chick leg (Brookes and May, 1972). Formation of bone starts during embryonic days (ED) 4 – 7. Raising the temperature of the eggs by just one degree, from 37.5ºC to 38.5ºC, during ED 4 – 7 can increase the length of tibia and tarsus bones in Leghorns (Hammond et al., 2007). Oviedo-Rondon et al. (2008) concluded that tibias of broilers were longest at 38ºC compared to 36, 37 or 39ºC. They also concluded that temperatures greater than 37ºC should be avoided to ensure optimal bone development at hatching. Incubation temperature can influence the development both of muscle and of bone, but the mechanisms by which this is occurring are unclear (Brookes and May, 1972; Maltby et al., 2004). The following experiments were conducted to test the hypothesis that incubation temperature could influence the incidence of leg abnormalities in susceptable growing broilers.

MATERIALS AND METHODS

Incubation Procedures

Experiment 1

354 day-old Cobb 500 broiler eggs were collected the morning they were laid, eggs were stored at 12ºC and 66% relative humidity for 1 d and warmed at room temperature (20ºC) for 12 h before setting. Eggs were set at 37.5ºC and 55% RH in a single–stage NatureForm NMC-2000,

1,980 egg capacity setter (NatureForms Hatchery System Inc., Jacksonville, FL) with automatic temperature regulation (accurate to 0.10 ºC), relative humidity (accurate to ± 2%), and an automatic turning system (24 times/d) for 454 h. Half of the eggs were moved to an identical setter and incubated at 38.5ºC during ED 4 – 7. After 72 h, the eggs were returned to the original setter. Temperatures and humidity within the incubators were measured with a LogTag HAXO–8 Humidity and Temperature recorder (LogTag Recorders, Auckland, NZ). After 454 h of incubation, all eggs were transferred to a hatching compartment at 36.1^0C and 70% RH for 54 h (total 508 h).

The chicks were randomly allocated into treatment groups (control and TD inducing diets) with twelve replicates of each treatment and ten chicks per replicate.

Experiment 2

Experiment 2 was conducted with control, TD and P–rickets inducing diets for 16 d with 450 Cobb 500 broiler chicks hatched from 705 eggs and incubated at 36.5, 37.5 and 38.5ºC during ED 4 – 7 with setting time adjustments. Setting time was adjusted based on the Cobb Hatchery Management Guide (2008, page 10) relating incubation temperature to hatching time. Eggs kept at 36.5, 37.5, and 38.5ºC for 525, 508, and 498 h, respectively.

The chicks were randomly allocated into treatment groups (control , TD and P–deficiency rickets inducing diets) with five replicates of each treatment and ten chicks per replicate.

Experiment 3

Experiment 3 was conducted with control, and Ca rickets inducing diets for 16 d with 480 Cobb 500 broiler chicks hatched from 900 eggs and incubated at 36.5, 37.5 and 38.5ºC during ED 4 – 7 with setting time adjustments. Setting time was adjusted based on Cobb hatchery management guide (2008). Eggs were incubated at 36.5ºC for 508 and 520 hours, 37.5ºC for 508 hours, and 38.5ºC for 505 and 508 hours.

The chicks were randomly allocated into treatment groups (control and Ca–deficiency rickets inducing diets) with four replicates of each treatment and ten chicks per replicate.

General Procedures

Chicks were identified by metal wing bands and were housed in electrically heated Petersime raised-wire-floor battery brooders for 16 d. Feed from each pen was weighed prior to and after the experiment. Each treatment group was provided with water and experimental diets *ad libitum* (Table 3.1).

At 16 d, chicks were killed by carbon dioxide asphyxiation. The right tibia of each bird was split and scored to evaluate the incidence of Ca–deficiency rickets, P–deficiency rickets, and TD according to the methods described by Edwards and Veltmann (1983). The left tibia of each bird was collected for percentage tibia ash determination on a fat–free dry–weight basis according to AOAC International (2005) method 932.16. The left tibia lengths were measured in Experiment 3 before ashing. Diet samples as fed were analyzed in duplicate for DM and percent ash using AOAC International (2005) methods 934.01 and 942.05, respectively. Feed conversion ratios were adjusted for mortality (AFCR).

Statistical Analysis

Analysis of variance was performed for each experiment using the General Linear Model procedure of SAS (SAS Institute, 2006) appropriate for one–way or two–way designs. The one–way ANOVA model was:

$$Y_{ij} = \mu + Temp_i + e_{ij}$$

where Y_{ij} was the dependent variable, μ was the overall mean, and $Temp_i$ was temperature effects (i = 37.5 or 38.5ºC in Experiment 1 and i = 36.5, 37.5 or 38.5ºC in Experiment 2). Linear and quadratic trends were analyzed for the quantitative factor (temperature).

The two–way ANOVA model was:

$$Y_{ijk} = \mu + Temp_i + Time_j + e_{ijk}$$

where Y_{ij} was the dependent variable, μ was the overall mean, $Temp_i$ was temperature effects (i = 36.5, 37.5 or 38.5ºC in Experiment 3), and $Time_j$ was setting time effects (j = 505, 508, 520 h in Experiment 3). Linear and quadratic trends were analyzed among quantitative factors (temperature and setting time).

RESULTS

Incubation

Hatchability in all three experiments was high, over 85%. In Experiment 1, eggs hatched earlier, and more hatched with high ED 4 – 7 heat (92.77%) compared to controls (86.22%; Table 3.2). That resulted in lower BW (42.92g) at 38.5ºC than those (44.66g) at 37.5ºC ($P <$ 0.01), because they spent more time out of their shells in the hatcher and dried out.

In Experiment 2, hatchability of fertile (HFE) of eggs (92.92%) was highest for control eggs and decreased when ED 4 – 7 temperature was 1ºC less (89.82%) or 1ºC more (81.55%; Table 3.3). Hatched BW was lower from eggs with 1ºC less (48.98g) or control (49.57g) compared to 1ºC more temperature (50.56g). One d BW increased with increasing temperature as a linear trend ($P < 0.01$) in Experiment 2.

In Experiment 3, HFE of eggs (91.33%) was highest for controls or those with 1ºC more ED 4 – 7 temperature, and decreased at 36.5ºC (88.33%; $P < 0.01$; Table 3.4). Hatched BW showed a quadratic trend with increasing temperature ($P < 0.01$). One d BW showed a significant difference due to setting time ($P < 0.01$), and showed linear and quadratic trends to increasing setting time ($P < 0.01$).

Performance

In Experiment 1, The only significant difference due to temperature noted was noted after d 1 was that AFCR for control chicks was better than those with extra ED 4 – 7 temperature (1.20 vs. 1.25; Table 3.5). Chicks fed the TD inducing diet exhibited signs of TD as expected.

In Experiment 2, after d 1, incubation temperature had no significant influence on any parameter measured and there were no diet × temperature interactions (Tables 3.6 and 3.7). Average BW gain for 16 d was lowest for chicks fed the P–deficiency rickets inducing diet (325 g) compared to those fed control (469 g) and TD inducing diets (467 g; $P < 0.01$; Table 3.6). AFCR was worst in chicks fed the P–deficiency rickets inducing diet (1.21) compared to chicks fed the control (1.14) and TD inducing diets (1.12; $P = 0.0463$).

In Experiment 3, average 16 d BW gain was lower in chicks fed the Ca–deficiency rickets inducing diet (396 g) than those fed the control diet (437 g; $P = 0.0121$; Table 3.8). Some parameters were close to significantly different at $\alpha = 0.05$, but there were no siginificant diet × temperature or diet × time ($P > 0.3837$) interactions. Tibia length (61.04mm) was highest in embryos with 505 hours of incubation and decreased at 508 hours (60.22mm) and 520 hours (59.25mm); there was a linear trend ($P = 0.0564$; Table 8). Ca score (1.14) was highest in embryos incubated for 505 hours and decreased in those incubated for 520 hours (1.11) and 508 hours (0.96); There was a quadratic trend ($P = 0.0512$; Table 3.9). Bone ash (mg/tibia: MBA; 414 mg/tibia) was lowest in embryos incubated at 36.5ºC and increased at 37.5ºC (438 mg/tibia) and 38.5ºC (450 mg/tibia); there was a quadratic trend ($P = 0.0599$).

DISCUSSION

Despite incubation temperature influencing bone development (Brookes and May, 1972; Hammond et al., 2007; Oviedo-Rondon et al., 2008), changes in incubator temperature during ED 4 – 7 did not affect leg abnormalities at 16 d based on score and bone ash ($P > 0.05$; Tables 3.5, 3.7 and 3.9). Variation in the incidences of TD, P–deficiency rickets, and Ca–deficiency rickets did not appear to be related to variation in incubation temperature during ED 4 – 7 in Experiments 1–3.

Hatchability may be affected by environmental factors in the incubator like sound, air velocity, light intensity, CO_2 and O_2 concentration, humidity and temperature (Romanoff, A. L., and A. J. Romanoff, 1949). The most important climatic incubation factor is temperature (Decuypere and Michels, 1992). Romanoff (1960) demonstrated that when hatching eggs from

the same source and equal weight (60g) were incubated under different temperatures and RH, the chick weights on d of hatch ranged from 21.9 to 41.4 g. Raising the temperature of the eggs by just one degree, from 37.5 to 38.5ºC during ED 4 – 7 causes profound changes in the development of leghorn embryos (Hammond et al., 2007). Interpretation of incubator temperature studies is difficult because they use incubator operation temperature as the temperature treatment applied to the egg. The temperature indicated on the incubator control may be significantly different from the temperature of the air surrounding the egg because the embryos are a source of heat.

When the embryo's incubated at the higher temperature during ED 4 – 7 hatched earlier in Experiment 1, we realized that the time chicks stayed in the incubator could confound any growth results. Chicks hatching earlier would be expected to dry out more, and have reduced growth potential. Yalçin et al. (2010) stated that embryonic mortalities or chick weight were not influenced by incubation temperature. However, a d 1 BW difference was demonstrated in Experiments 1 – 3 ($P < 0.01$).

The factorial Experiment 3 separated the effects of incubation time and temperature and suggests that earlier conclusions that temperature influences tibial length were due to indirect effects of temperature on hatching time. Tibia length (61.04mm) was highest with 505 hours of incubation and decreased at 508 hours (60.22mm) and 520 hours of incubation (59.25mm) (linear trend, $P = 0.0564$). However, changes in incubator temperature during ED 4 – 7 did not affect tibia length ($P = 0.4880$) when the probabilities were calculated using Type III Sums of Squares. This was different from Hammond et al. (2007) in that raising the temperature of the eggs by just one degree, from 37.5ºC to 38.5ºC, during embryonic days (ED) 4 – 7 increased the

length of tibia and tarsus bones in Leghorns. However, Hammond et al. (2007) did not distinguish separate effects of temperature and hatching time or total incubation time. Oviedo-Rondon et al. (2008) also concluded that tibias of broilers were longest at 38ºC compared to 36, 37 and 39ºC without regard to total incubation and hatching time.

Differences in responses of leghorn (Hammond, 2007) and broiler chicks (Oviedo-Rondon et al., 2008) to extra heat during ED 4 – 7 need to be reconciled. The decrease in performance observed by Oviedo-Rondon et al. with extra heat during ED 4 – 7 may be explained by a stressing effect of heat either in the incubator or the longer time the chicks spend in the incubator after they have hatched. In any case, Experiments 1 – 3 demonstrate that extra heat during ED 4 – 7 has no effect on subsequent TD or P deficiency induced rickets, but it will affect hatching time and hatching chick weights.

REFERENCES

AOAC International, 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. AOAC Int., Arlington, VA.

Applegate, T. J., and M. S. Lilburn. 2002. Growth of the femur and tibia of a commercial broiler line. Poult. Sci. 81:1289-1294.

Ballock, T., and R. J. O'Keefe. 2003. The biology of the growth plate. J. Bone Joint Surg. 85:715-726.

Brookes, M., and K. U. May. 1972. The influence of temperature on bone growth in the chick. J. Anat. 111:351-363.

Christensen, V. L., M. J. Wineland, I. Yildrum, D. T. Ort, and K. M. Mann. 2004. Incubator temperature and oxygen concentration at the plateau stage affect cardiac health of turkey embryos. J. Anim. Vet. Adv. 3:52-65.

Christensen, V. L., M. J. Wineland, J. L. Grimes, E. O. Oviedo, P. S. Mozdziak, D. T. Ort, and K. M. Mann. 2006. Effect of incubator temperature and oxygen concentration at the plateau stage in oxygen consumption on turkey embryo muscle growth and development. Int. J. Poult. Sci. 6:406-412.

Church, L. E., and L. C. Johnson. 1964. Growth of long bones in chicken – rates of growth in length + diameter of humerus tibia + metatarsus. Am. J. Anat. 114:521-538.

Cobb hatchery management guide, 2008. http://www.cobb-vantress.com/contactus/brochures/Hatchery_Guide_2008.pdf Accessed April, 2009.

Day, E. J. 1990. Further research needs focus on new, old problems. Feedstuffs. July 23:12-15.

Decuypere, E. 1994. Incubation temperature and postnatal development. Pages 407-410 in: Proceedings of 9th European Poultry Conference. Vol. II. World's Poultry Science Association, Glasgow, UK.

Decuypere, E., and H. Michels. 1992. Incubation temperature as a management tool: A review. World's Poult. Sci. J. 48:28-38.

Decuypere, E., E. J. Nouwen, E. R. Kühn, R. Geers, and H. Michels. 1979. Iodohormones in the

serum of chick embryos and post-hatching chickens as influence by incubation temperature. Relationship with the hatching process and thermogenesis. Ann. Biol. Anim. Biochem. Biophys. 19:1713-1723.

Edwards, H. M., Jr. 1984. Studies on the etiology of tibial dyschondroplasia in chickens. J. Nutr. 114:1001-1013.

Edwards, H. M., Jr., and J. R. Veltmann, Jr. 1983. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. J. Nutr. 113:1568-1575.

Farquharson, C., and D. Jefferies. 2000. Chondrobytes and logitudinal bone growth: The development of tibial dyschondroplasia. Poult. Sci. 79:994-1004.

Ferguson, M. W. J. 1994. Temperature dependent sex determination and growth in reptiles and manipulation of poultry sex by incubation temperature. Pages 380-382 in: Proceedings of 9th European Poulrty Conference Vol. II, World Poultry Science Association, Glasgow, UK.

French, N. A., 1994a. Effect of incubation temperature on the gross pathology of turkey embryos. Br. Poult. Sci. 35: 363-371.

French, N. A., 1994b. Do incubation temperature requirements vary between eggs? Pages 395-398 in: Proceedings of 9th European Poultry Conference Vol II, World Poultry Science Association, Glasgow, UK.

Hammond, C. L., B. H. Simbi, and N. C. Stickland. 2007. In ovo temperature manipulation influences embryonic motility and growth of limb tissues in the chick (Gallus gallus). J. Exp. Biol. 210:2667-2675.

Itakura, C., M. Takecji, Y. Miyakawa, and M. Goto. 1979. Pathology of naturally occurring vitamin D_3-deficient rickets in growing broiler chickens in Japan. Jap. J. Vet. Sci. 41:517-530.

Jubb, K. V. F., and P. C. Kennedy. 1970. Pathology of domestic animals. Academic Press, New York, NY, and London, UK.

Lacey, D. L., and W. E. Huffer. 1982. Studies on the pathogenesis of avian rickets. I. Changes in epiphyseal and metaphyseal vessels in hypocalcemic and hypophophatemic rickets. Am. J. Pathol. 109:288-301.

Leach, R. M., and M. C. Nesheim. 1965. Nutritional, genetic and morphological studies of an abnormal cartilage formation in young chicks. J. Nutr. 86:236-244.

Maltby, V., A. Somaiya, N. A. French, and N. C. Stickland. 2004. In ovo temperature manipulation influences post-hatch muscle growth in the turkey. Br. Poult. Sci. 45:491-498.

Oviedo-Rondon, E. O., P. R. Ferket, and G. B. Havestein. 2006. Understanding long bone development in broilers and turkeys. Avian and Poultry Biology Reviews 17:77-88.

Oviedo-rondon, E. O., J. Small, M. J. Wineland, V. L. Christensen, P. S. Mozdziak, M. D. Koci, S. V. L. Funderburk, D. T. Ort, and K. M. Mann. 2008. Broiler embryo bone development is influenced by incubator temperature, oxygen concentration and eggshell conductance at the plateau stage in oxygen consumption. Br. Poult. Sci. 49:666-676.

Riddell, C. 1975. The development of tibial dyschondroplasia in broiler chickens. Avian Dis. 19:443-462.

Romanoff, A. L. 1935. Influence of incubation temperature on the hatchability of eggs, post-natal growth and survival of turkeys. J. Agric. Sci. 25:318-325.

Romanoff, A. L. 1936. Effects of different temperatures in the incubator on the prenatal and postnatal development of the chick. Poult. Sci. 15: 311-314.

Romanoff, A. L. 1960. The avain embryo. Structural and functional development, Macmillan, New York, NY, USA.

Romanoff, A. L., L. L. Smith, and R. A. Sullivan. 1938. Biochemistry and biophysics of the developing hen's eggs. 3. Influence of temperature. Mem. Cornell Univ. Agric. Exp. Stn. 216:1-42.

Romanoff, A. L., and A. J. Romanoff. 1949. The Avian eggs. John Wiley and Sons, New York, NY.

SAS Institute, 2006. SAS User's Guide: Statistics. Version 9.1.3 ed. SAS Inst. Inc., Cary, NC.

Shao, Y. Y., L. Wang, and R. T. Ballock. 2006. Thyroid hormone and the growth plate. Rev. Endocr. Metab. Disord. 7:265-271.

Suarez, M. E., H. R. Wilson, B. N. McPherson, F. B. Mather, and C. J. Wicox. 1996. Low temperature effects on embryonic development and hatch time. Poult. Sci. 75:924-932.

Thorp, B. H., and D. Waddington. 1997. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res. Vet. Sci. 62:67-73.

Van der Eerden, B. C. J., M. Karperien, and J. M. Wit. 2003. Systemic and local regulation of

the growth plate. Endocrinology Reviews. 24:782-801.

Wilson, H. R. 1991. Physiological requirements of the developing embryo: Temperature and turning. Chapter 9. Pages 145-156 in: Avian Incubation. S. G. Tullett, ed. Butterworth-Heinemann, London, UK.

Wineland, M. J., V. L. Christensen, I. Yildrum, B. D. Fairchild, D. T. Ort, and K. M. Mann. 2006a. Incubator temperature and oxygen concentration at the plateau stage in oxygen consumption affects intestinal maturation of broiler chicks. Int. J. Poult. Sci. 5:229-240.

Wineland, M. J., V. L. Christensen, I. Yildrum, B. D. Fairchild, D. T. Ort, and K. M. Mann. 2006b. Incubator environment interacts with genetic line of broiler at the plateau stage to affect embryo plasma thyroxine and triiodothyronine concentrations. Int. J. Poult. Sci. 5:714-722.

Yalçin, S., H. B. Molayoğlu, M. Baka, O. Genin, and M. Pines. 2007. Effect of temperature during the incubation period on tibial growth plate chondrocyte differentiation and the incidence of tibial dyschondroplasia. Poult. Sci. 86:1772-1783.

Yalçin, S., E. Babacanoğlu, H. C. Güler, and M. Akşit. 2010. Effects of incubation temperature on hatching and carcass performance of broilers. Worlds Poult. Sci. J. 66 :87-94.

TABLE 3.1. Composition of the basal diets.

Ingredient	All experiments Control	Experiment 1 TD Inducing	Experiment 2 P deficient	Experiment 3 Ca deficient
	----------(%)----------			
Ground yellow corn	52.20	54.36	53.41	57.06
Soybean meal (dehulled)	37.73	37.35	37.52	36.85
Soy bean oil	5.97	5.26	5.57	4.35
Limestone	1.26	0.21	2.01	0.39
Dicalcium Phosphate	1.91	1.90	0.57	0.44
Iodized sodium chloride	0.40	0.40	0.40	0.40
DL-Methionine	0.19	0.19	0.19	0.19
Vitamin premix[1]	0.25	0.25	0.25	0.25
Trace mineral premix[2]	0.08	0.08	0.08	0.08
Calculated composition[3]				
ME, kcal	3.20	3.20	3.20	3.20
CP, %	23.00	23.00	23.00	23.00
Calcium, %	1.00	0.60	1.00	0.40
Phosphorus-total, %	0.74	0.74	0.49	0.47
nPP, %	0.50	0.50	0.25	0.23
Analyzed Composition				
DM, %	88.63	88.67	88.90	87.95
Ash, %	5.98	5.00	5.59	3.72

[1] Vitmain 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 g; 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-reinyl 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 ($MnSO_4.H_2O$), 101 mg; iron ($FeSO_4.7H_2O$), 20 mg; zinc (ZnO), 80 mg; copper ($CuSO_4.5H_2O$), 3 mg; iodine (ethylene diamine dihydroiodide), 0.75 mg; magnesium (MgO), 20 mg; selenium (sodium selenite), 0.3 mg.

[3] Calculated from NRC (1994).

Table 3.2. Influence of incubation temperature on egg and chick parameters (Experiment 1).

Incubator Temperature (ED4-ED7)	Fertility (%)	Hatchability (%)		BW (d 1)
		HES[1]	HFE[2]	(g)
37.5ºC	87.92±1.64[3]	64.17±3.49	86.22±3.38	44.66±0.33
38.5ºC	85.55±3.06	69.45±2.18	92.77±2.54	42.92±0.36

ANOVA			Type III SS		
Parameters	df	*P*–value	*P*–value	*P*–value	*P*–value
Temperature	1	0.5119	0.2286	0.1527	0.0051

[1] HES = hatch of egg set.
[2] HFE = hatch of fertile eggs.
[3] Main effect means ± standard errors of 177 eggs.

Table 3.3. Influence of incubation temperature on egg and chick parameters (Experiment 2).

Incubator Temperature (ED4-ED7)	Setting Time (hours)	Fertility (%)	Hatchability (%)		BW (d 1)
			HES[1]	HFE[2]	(g)
36.5ºC	525	91.84±1.93[3]	82.42±2.53	89.82±2.38	48.98±0.41
37.5ºC	508	91.91±2.17	85.42±2.74	92.92±1.87	49.57±0.29
38.5ºC	498	90.15±1.36	73.34±1.99	81.55±2.90	50.56±0.46

ANOVA			Type III SS		
Parameters	df	*P*–value	*P*–value	*P*–value	*P*–value
Temperature	2	0.7514	0.0058	0.0092	0.0308
Linear Temp.	1	0.5314	0.0156	0.0248	0.0098
Quadratic Temp.	1	0.6905	0.0198	0.0236	0.6761

[1] HES = hatch of egg set.
[2] HFE = hatch of fertile eggs.
[3] Main effect means ± standard errors of 150 eggs.

Table 3.4. Influence of incubation temperature on egg and chick parameters (Experiment 3).

Incubator Temperature (ED4-ED7)	Setting Time (hours)	Fertility (%)	Hatchability (%)		BW (d 1) (g)
			HES[1]	HFE[2]	
36.5ºC	508	87.33±4.14[3]	78.00±5.83	88.96±3.17	50.51±0.37
37.5ºC	508	91.33±2.12	86.33±2.02	94.58±1.06	49.14±0.30
38.5ºC	508	90.00±1.83	84.00±3.40	93.22±2.29	50.21±0.14
36.5ºC	520	89.33±2.45	84.67±2.26	94.80±0.85	47.68±0.18
38.5ºC	505	92.67±1.94	86.00±2.87	92.82±2.55	50.33±0.26
36.5ºC		88.33±2.29	81.33±3.15	91.88±1.83	49.10±0.51
37.5ºC		91.33±2.12	86.33±2.02	94.58±1.06	49.14±0.30
38.5ºC		91.33±1.33	85.00±2.12	93.02±1.62	50.27±0.14
	505	92.67±1.94	86.00±2,87	92.82±2.55	50.33±0.26
	508	90.00±1.51	83.67±1.99	92.83±1.16	49.75±0.22
	520	89.33±2.45	84.67±2.26	94.80±0.85	47.68±0.18

ANOVA			Type III SS		
Parameters	df	*P*–value	*P*–value	*P*–value	*P*–value
Temperature	2	0.5249	0.1734	0.1109	0.0031
Linear Temp.	1	0.5124	0.2384	0.1646	0.5152
Quadratic Temp.	1	0.3568	0.1416	0.1092	0.0009
Time	2	0.7124	0.3886	0.1646	<0.0001
Linear Time	1	0.9079	0.5124	0.1500	0.0001
Quadratic Time	1	0.4195	0.2288	0.2073	0.0003

[1] HES = hatch of egg set.
[2] HFE = hatch of fertile eggs.
[3] Main effect means ± standard errors of 80 eggs.

Table 3.5. Influence of incubation temperature on d 1 BW, BW gain, adjusted feed conversion ratio and bone quality (Experiment 1).

Diets	Incubator Temperature (ED4-ED7)	BW (d 1) (g)	BW gain (g)	AFCR	TD[1] Score	TD Incidence (%)	Bone Ash (%)	Bone Ash (mg/tibia)
Control	37.5ºC	44.37±0.35[2]	432.37±8.45	1.21±0.02	0.02±0.02	1.85±1.85	38.74±0.09	566.10±12.64
Control	38.5ºC	43.82±0.50	420.04±11.68	1.28±0.02	0.00±0.00	0.00±0.00	38.21±0.32	536.16±18.48
TD	37.5ºC	45.18±0.43	414.37±3.90	1.19±0.02	0.81±0.25	29.63±8.92	34.94±0.34	457.56±6.48
TD	38.5ºC	42.52±0.49	399.72±15.89	1.22±0.02	0.58±0.16	30.16±5.90	35.41±0.30	453.13±17.71
Control		44.09±0.30	426.20±7.12	1.25±0.02	0.01±0.01	0.93±0.93	38.48±0.18	551.13±11.59
TD		43.85±0.51	407.05±8.10	1.20±0.01	0.69±0.14	29.89±5.10	35.17±0.23	455.34±9.01
	37.5ºC	44.78±0.29	423.37±5.20	1.20±0.01	0.42±0.17	15.74±6.03	36.84±0.60	511.83±17.71
	38.5ºC	43.17±0.39	409.88±9.89	1.25±0.02	0.28±0.11	15.08±5.34	36.81±0.47	494.64±17.48
ANOVA					Type III SS			
Parameters	df	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value
Diet	1	0.5914	0.0942	0.0370	0.0002	<0.0001	<0.0001	<0.0001
Temperature	1	0.0018	0.2302	0.0220	0.3656	0.9042	0.9141	0.2541
Diet × Temperature	1	0.0289	0.9161	0.3324	0.4326	0.8286	0.0944	0.3938

[1] TD = Tibial Dyschondroplasia.

[2] Main effect means ± standard errors of 6 pens of 10 birds.

Table 3.6. Influence of incubation temperature on d 1 BW, BW gain, adjusted feed conversion ratio (Experiment 2).

Diets	Incubator Temperature (ED4-ED7)	Setting Time (hours)	BW (d 1) (g)	BW gain (g)	AFCR[1]
Control	36.5ºC	525	49.06±0.67[2]	468.54±10.29	1.18±0.03
Control	37.5ºC	508	49.82±0.68	479.88±6.60	1.14±0.03
Control	38.5ºC	498	50.32±0.52	458.41±3.63	1.10±0.01
TD	36.5ºC	525	47.98±1.02	467.38±4.50	1.15±0.02
TD	37.5ºC	508	49.42±0.26	466.72±5.66	1.08±0.05
TD	38.5ºC	498	50.20±0.61	467.06±6.68	1.12±0.02
P deficient	36.5ºC	525	49.06±0.32	337.47±12.35	1.15±0.02
P deficient	37.5ºC	508	49.78±0.47	318.92±18.00	1.17±0.02
P deficient	38.5ºC	498	50.82±0.42	318.77±14.24	1.31±0.11
Control			49.73±0.36	468.94±4.58	1.14±0.02
TD			49.20±0.45	467.05±3.04	1.12±0.02
P deficient			49.89±0.29	325.05±8.38	1.21±0.04
	36.5ºC	525	48.70±0.41	424.46±17.23	1.16±0.01
	37.5ºC	508	49.67±0.27	421.84±20.46	1.13±0.02
	38.5ºC	498	50.45±0.29	414.75±18.83	1.18±0.04

ANOVA Parameters	df	*P*–value	Type III SS *P*–value	*P*–value
Diet	2	0.3412	<0.0001	0.0463
Temperature	2	0.0038	0.4904	0.4399
Linear Temp.	1	0.0009	0.2516	0.6418
Quadratic Temp.	1	0.8129	0.7588	0.2347
Diet × Temperature	4	0.9247	0.5680	0.0944

[1] AFCR = Feed conversion ratio adjusted for mortality.
[2] Main effect means ± standard errors of 5 pens of 10 birds.

Table 3.7. Influence of incubation temperature on bone quality (Experiment 2).

Diets	Incubator Temperature (ED4-ED7)	Setting Time	TD[1] Score	TD Incidence (%)	P Rickets Score	P Rickets Incidence (%)	Bone Ash (%)	Bone Ash (mg/tibia)
Control	36.5ºC	525	0.14±0.09[2]	6.00±4.00	0.00±0.00	0.00±0.00	39.54±0.18	595.12±18.51
Control	37.5ºC	508	0.42±0.12	10.00±0.00	0.00±0.00	0.00±0.00	39.92±0.36	610.14±16.11
Control	38.5ºC	498	0.84±0.55	14.00±4.00	0.00±0.00	0.00±0.00	39.88±0.32	576.10±5.26
TD	36.5ºC	525	0.68±0.20	28.00±5.83	0.00±0.00	0.00±0.00	36.83±0.41	539.80±7.05
TD	37.5ºC	508	0.86±0.20	34.00±6.00	0.00±0.00	0.00±0.00	37.39±0.30	527.80±6.10
TD	38.5ºC	498	0.52±0.12	20.00±5.48	0.00±0.00	0.00±0.00	37.45±0.39	533.08±11.50
P deficient	36.5ºC	525	0.02±0.02	2.00±2.00	2.76±0.11	93.78±2.55	26.58±0.53	281.33±11.64
P deficient	37.5ºC	508	0.00±0.00	0.00±0.00	2.62±0.14	88.56±5.31	27.64±0.49	292.04±12.68
P deficient	38.5ºC	498	0.15±0.09	5.08±3.15	2.51±0.13	83.75±4.33	27.04±0.41	275.35±8.29
Control			0.47±0.19	10.00±1.95	0.00±0.00	0.00±0.00	39.78±0.16	593.79±8.59
TD			0.69±0.10	27.33±3.45	0.00±0.00	0.00±0.00	37.22±0.21	533.56±4.75
P deficient			0.06±0.03	2.36±1.28	2.63±0.07	88.70±2.51	27.09±0.28	282.90±6.18
	36.5ºC	525	0.28±0.10	12.00±3.80	0.92±0.35	31.26±11.84	34.32±1.51	472.08±37.23
	37.5ºC	508	0.43±0.12	14.67±4.24	0.87±0.33	29.52±11.28	34.98±1.43	476.66±36.63
	38.5ºC	498	0.50±0.19	13.03±2.83	0.84±0.32	27.92±10.64	34.79±1.50	461.51±35.80
ANOVA					Type III SS			
Parameters		df	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value
Diet		2	0.0042	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Temperature		2	0.4518	0.7177	0.4075	0.2571	0.1120	0.2738
Linear Temp.		1	0.2177	0.7566	0.1854	0.1017	0.1459	0.2722
Quadratic Temp.		1	0.8239	0.4544	0.8949	0.9678	0.1265	0.2375
Diet × Temperature		4	0.3013	0.1081	0.4628	0.2501	0.8716	0.5290

[1] TD = Tibial Dyschondroplasia.

[2] Main effect means ± standard errors of 5 pens of 10 birds.

Table 3.8. Influence of incubation temperature on d 1 BW, BW gain, adjusted feed conversion ratio and tibia length (Experiment 3).

Diets	Incubator Temperature (ED4-ED7)	Setting Time	BW (d 1) (g)	BW gain (g)	AFCR[1]	Tibia Length (mm)
Control	36.5ºC	508	50.68±0.28[2]	434.13±4.69	1.10±0.01	61.36±0.39
Control	37.5ºC	508	49.14±0.24	441.93±17.89	1.11±0.03	61.69±0.30
Control	38.5ºC	508	50.70±0.30	454.35±10.73	1.08±0.01	60.10±1.50
Control	36.5ºC	520	47.83±0.54	398.93±31.89	1.11±0.07	58.92±1.74
Control	38.5ºC	505	49.88±0.20	451.43±11.63	1.10±0.01	61.97±0.32
Ca deficient	36.5ºC	508	50.63±0.48	383.13±8.29	1.13±0.01	60.59±0.40
Ca deficient	37.5ºC	508	49.23±0.26	392.15±6.96	1.13±0.01	58.70±1.02
Ca deficient	38.5ºC	508	50.40±0.33	409.65±18.62	1.16±0.03	59.05±1.53
Ca deficient	36.5ºC	520	48.08±0.39	386.78±12.63	1.15±0.02	59.55±1.50
Ca deficient	38.5ºC	505	50.58±0.43	412.95±9.61	1.13±0.01	60.08±1.42
Control			49.56±0.25	437.11±8.67	1.10±0.01	60.98±0.40
Ca deficient			49.69±0.24	396.13±4.99	1.14±0.01	59.44±0.55
	36.5ºC		49.30±0.40	400.74±9.51	1.12±0.02	60.13±0.58
	37.5ºC		49.18±0.17	417.04±11.28	1.12±0.01	60.17±0.55
	38.5ºC		50.39±0.17	432.09±7.97	1.11±0.01	60.30±0.64
		505	50.23±0.25	432.19±10.08	1.11±0.01	61.04±0.72
		508	49.89±0.17	418.67±7.00	1.12±0.01	60.22±0.39
		520	47.95±0.31	392.85±16.04	1.13±0.03	59.25±1.14

ANOVA			Type III SS		
Parameters	df	*P*–value	*P*–value	*P*–value	*P*–value
Diet	1	0.2204	0.0121	0.1632	0.1092
Temperature	2	<0.0001	0.3852	0.9922	0.4880
Linear Temp.	1	0.7897	0.1781	1.0000	0.2322
Quadratic Temp.	1	<0.0001	0.7872	0.9013	0.9246
Time	2	<0.0001	0.6544	0.8856	0.1606
Linear Time	1	<0.0001	0.5116	0.6422	0.0564
Quadratic Time	1	<0.0001	0.5215	0.8768	0.8654
Diet × Temperature	2	0.8355	0.9799	0.3944	0.4572
Diet × Time	2	0.3837	0.5193	0.6817	0.7908

[1] AFCR = Feed conversion ratio adjusted for mortality.
[2] Main effect means ± standard errors of 4 pens of 10 birds.

Table 3.9. Influence of incubation temperature on bone quality (Experiment 3).

Diets	Incubator Temperature (ED4-ED7)	Setting Time (hours)	Ca Score	Ca Incidence (%)	TD[1] Score	TD Incidence (%)	Bone Ash (%)	Bone Ash (mg/tibia)
Control	36.5ºC	508	0.00±0.00[2]	0.00±0.00	0.28±0.11	15.55±5.21	38.21±0.28	511.28±7.49
Control	37.5ºC	508	0.00±0.00	0.00±0.00	0.48±0.16	21.25±6.93	38.32±0.22	543.44±13.61
Control	38.5ºC	508	0.00±0.00	0.00±0.00	0.10±0.07	7.50±4.79	38.90±0.23	546.70±14.03
Control	36.5ºC	520	0.00±0.00	0.00±0.00	0.25±0.13	15.83±6.55	38.03±0.26	495.54±18.30
Control	38.5ºC	505	0.00±0.00	0.00±0.00	0.25±0.07	17.50±4.79	38.87±0.32	552.05±9.16
Ca deficient	36.5ºC	508	1.68±0.23	75.00±8.66	0.53±0.14	17.50±4.79	30.04±0.11	325.43±6.69
Ca deficient	37.5ºC	508	1.99±0.13	75.00±3.27	0.69±0.10	27.50±4.53	30.02±0.36	332.55±7.69
Ca deficient	38.5ºC	508	2.00±0.33	75.00±8.66	0.65±0.13	25.00±6.46	30.30±0.67	345.50±21.56
Ca deficient	36.5ºC	520	2.23±0.11	82.50±4.79	0.58±0.03	20.00±0.00	29.28±0.48	324.43±12.45
Ca deficient	38.5ºC	505	2.28±0.13	87.50±6.29	0.85±0.29	32.50±11.09	30.41±0.57	355.53±10.74
Control			0.00±0.00	0.00±0.00	0.30±0.06	16.48±2.88	38.44±0.13	532.07±7.25
Ca deficient			2.03±0.09	78.33±2.60	0.66±0.06	25.00±2.69	30.01±0.20	336.00±5.32
	36.5ºC		0.98±0.26	39.38±10.43	0.41±0.06	17.22±2.20	33.89±1.10	414.16±23.72
	37.5ºC		0.99±0.26	37.50±9.81	0.58±0.09	24.38±4.08	34.17±1.09	437.99±28.25
	38.5ºC		1.07±0.29	40.63±10.82	0.46±0.11	20.63±4.03	34.62±1.12	449.94±26.52
		505	1.14±0.43	43.75±16.79	0.55±0.18	25.00±6.27	34.64±1.63	453.79±37.71
		508	0.96±0.18	37.50±6.91	0.48±0.06	20.38±2.56	34.26±0.76	435.11±18.89
		520	1.11±0.42	41.25±15.75	0.41±0.09	17.91±3.13	33.66±1.67	409.98±33.92

ANOVA				Type III SS			
Parameters	Df	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value
Diet	1	<0.0001	<0.0001	0.0030	0.1133	<0.0001	<0.0001
Temperature	2	0.4163	1.0000	0.2421	0.2850	0.4205	0.1436
Linear Temp.	1	0.2691	1.0000	0.8769	0.9689	0.2571	0.0599
Quadratic Temp.	1	0.4687	1.0000	0.0956	0.1156	0.5070	0.5719

Time	2	0.1191	0.2893	0.5545	0.4577	0.5267	0.7312
Linear Time	1	0.5063	0.6999	0.4779	0.4625	0.3912	0.4322
Quadratic Time	1	0.0512	0.1286	0.4134	0.3132	0.4622	0.9731
Diet × Temperature	2	0.4163	1.0000	0.4657	0.5148	0.8591	0.6034
Diet × Time	2	0.1191	0.2893	0.9613	0.9720	0.7677	0.8645

[1] TD = Tibial Dyschondroplasia.

[2] Main effect means ± standard errors of 4 pens of 10 birds.

CHAPTER 4

THE EFFECTS OF DIETARY FLUORIDE ON GROWTH AND BONE MINERALIZATION IN BROILER CHICKS[1]

[1] M. Y. Shim, C. Parr and G. M. Pesti. Submitted to Poultry Science

ABSTACT

Fluoride (F) has been shown to have varying degrees of beneficial effects on bone mineralization and strength, despite its toxic effects on growth and leg disorders. Some studies have demonstrated an increase in bone ash due to F supplementation. The purpose of the present study was to determine whether low levels of dietary F had any beneficial effect on bone strength and leg disorders of young chicks. Effects on BW and feed efficiency were also observed to monitor for F toxicity. Day–old Cobb × Cobb broiler chicks were weighed and randomly allocated into treatment groups with ten chicks per replicate in two experiments. Chicks were housed in electrically heated Petersime raised–wire–floor battery brooders. All chicks were provided with water and experimental diets for *ad libitum* consumption. Diets were formulated with two different phosphorus (P) sources: Treatment 1 contained feed grade dicalcium phosphate to simulate a commercial diet. Treatment 2 contained purified dicalcium phosphate to represent a diet with minimal F (~ 0.46 mg/kg). Treatments 3 and 4 used purified dicalcium phosphate as the P source and contained 10 and 20 mg/kg F from NaF, respectively. Four more treatments were added for the Experiment 2: Treatments 5, 6, 7 and 8 used purified dicalcium phosphate as the P source and contained 30, 40, 50 and 60 mg/kg F from NaF, respectively. Chicks fed purified calcium phosphate grew better in Experiment 1 ($P < 0.05$) and had a lower incidence of P–deficiency rickets in Experiment 2 ($P < 0.01$). Percent bone ash was increased by increasing F level in the diets in Experiment 1, but not Experiment 2. Even low levels of F like those used here have the potential to create measurable effects.

Key words: Fluoride, Broiler, Phosphorus rickets, BW gain, Bone ash

INTRODUCTION

Fluoride (F) exists naturally in water sources and is derived from fluorine, the 13th most common element in the Earth's crust (Tokalioglu et al., 2001). In 1972, F was added to the list of essential elements when it was demonstrated that the growth rate of rats on a F–free purified diet, could be increased up to 30% by addition of the element. However, the importance of F in the prevention of dental cares had been demonstrated many years previously (Chankanka et al., 2010). F can be a very toxic element resulting in reduced appetite, and pitted and worn teeth, in certain farm animals. There is little evidence that F is a required element for poultry.

Current agricultural practices are just one of the many ways by which environmental F can easily contaminate poultry diets (Chinoy, 2003). In addition, F is intentionally added to public water sources at very low levels in the United States. Environmental and dietary F may be unnoticed or ignored by the poultry industry, but F has the potential to affect flock health and growth. Unaccounted–for F in water and feed ingredients may have unforeseen effects on poultry research, as well.

Several studies have been conducted on the toxic and beneficial effects of F in poultry since the 1960s, but the results were inconsistent and often contradictory. First, since growth depression is generally accepted as a good indicator of F toxicity (NRC, 1994), the lower limits of "safe" levels have been studied based on growth rates. Weber et al. (1969) concluded that chicks can tolerate F levels of up to 3600 mg/kg, but many researchers agree that the upper limit of the "safe" level of dietary F is about 300 mg/kg for broilers (Weber et al., 1969; Suttie et al., 1984; Huyghebaert et al., 1988; Rama Rao and Ramasubba Reddy, 2001).

Several studies, however, show that even lower levels of dietary of F have toxic effects. For example, significant increases in tibial dyschondroplasia (TD) have been observed at levels as low as 50 mg/kg (Merkley, 1979). Huyghebaert et al. (1988) reported that TD incidence increased with increasing levels of dietary F, but noted that few other studies have reported similar results. Also, 500 – 600 mg/kg has been accepted as the lowest toxic level of F in broiler diets (Weber et al., 1969; Chan et al., 1976; Suttie et al., 1984; Rama Rao and Ramasubba Reddy, 2001). On the contrary, Japanese quail experienced improved growth when fed a diet containing 1770 mg/kg F from hatch (Chan et al., 1973). The toxic effects of dietary F seem to differ among avian species.

In humans, F has also been shown to have varying degrees of beneficial effects on bone mineralization and strength, despite its toxic effects on growth and leg disorders (Louw et al., 2002). Some studies have demonstrated an increase in percent bone ash due to F supplementation, which indicates an increase in bone mineralization and strength (Merkley and Miller 1983; Huyghebaert et al., 1988). Dietary F in high amounts is known to stimulate new bone formation and inhibit bone resorption by forming fluoroapatite which is more resistant to dissolution (Zipkin, 1970). However, studies on the bone strength of F–supplemented birds are inconsistent. Suttie et al. (1984) reported no change in the breaking strength of bones of broilers supplemented with F compared to that of broilers on a F–free diet. Rennie et al. (1997) reported that the addition of F to a dicalcium–phosphate–based layer diet was almost as effective at increasing the percentage of the hens' medullary bone as was the use of oyster shell in place of dicalcium phosphate in the diet. Contrary to this study, Fleming et al. (2003) reported that the addition of similar levels of F to the diet of laying hens had no beneficial effects on bone

strength.

We have observed considerable experiment to experiment variation in the bone ash and leg problems in broilers fed seeming identical basal diets (Table 4.1; Shirley, 2003; Shim, 2007; Liem, 2009). The purpose of the present study was to determine whether low levels of dietary F can have any beneficial effects on bone strength and leg disorders of young chicks fed TD or P–deficiency ricket inducing diets. Effects on BW and feed efficiency were also analyzed to monitor for F toxicity.

MATERIALS AND METHODS

General Procedures

Cobb 500 × Cobb 500 straight–run broiler chicks were obtained from a commercial hatchery, weighed, and randomly allocated into four treatment groups with six replicates of each treatment and ten chicks per replicate (240 chicks for Experiment 1 and 400 chicks for Experiment 2). Chicks were identified by metal wing bands and were housed for 16 days in electrically heated Petersime raised–wire–floor battery brooders (Petersime Incubator Co., Gettysburg, OH) in 95 cm × 35 cm × 23 cm pens. Ultraviolet irradiation was eliminated from the chick room by fitting Arm–a–Lite sleeves (Arm–a–Lite Thermoplastic Processes, Sterling, NJ) to all fluorescent fixtures in the room and battery brooders (Edwards et al., 1994). The lights were on 24 h each day. The temperature of the room was maintained at 22ºC. Mortality was monitored daily. The procedures performed were approved by the University of Georgia Institutional Animal Care and Use Committee.

Each treatment group was provided with water and experimental diets for *ad libitum* consumption. The F content of tap water was reported to be 0.73 – 0.87 mg/kg by the Athens Clarke County Utilities Department. The highest EPA allowed level is 4 mg/kg (Duck, 2009).

At the conclusion of each experiment, live chicks and leftover feed were weighed. Chicks were killed by carbon dioxide asphyxiation. The right tibia of each bird was split and scored to evaluate the incidence of calcium deficiency rickets, phosphorus deficiency rickets, and TD according to the methods described by Edwards and Veltman (1983). The left tibia of each bird was collected for percentage tibia ash determination on a fat–free dry–weight basis according to AOAC International (2005) method 932.16. Diet samples as fed were analyzed in duplicate for DM, percent ash, Ca, total P, and F using AOAC International (2005) methods 934.01, 942.05, 968.08, 965.17 and 984.37, respectively.

Diets

Experiment 1

Diets were formulated with two different phosphorus sources (Table 4.2): Treatment 1 contained feed grade dicalcium phosphate to simulate a commercial diet. Treatment 2 contained purified dicalcium phosphate (Dibasic power; Baker Chemicals Inc, Houston, TX) to represent a diet with minimal F (~ 0.46 mg/kg). Treatments 3 and 4 used purified dicalcium phosphate as a phosphorus source and contained 10 and 20 mg/kg F from NaF, respectively.

Experiment 2

Experiment 2 was conducted 3 months after Experiment 1. Diet formulation for Treatments 1 – 4 were identical to Experiment 1. However, different lots of the ingredients were used in mixing. Treatments 3, 4, 5, 6, 7 and 8 used purified dicalcium phosphate as a phosphorus source and contained 10, 20, 30, 40, 50 and 60 mg/kg F from NaF, respectively.

Statistical Analysis

Analysis of variance was performed on all data for both experiments using the General Linear Model procedure of SAS (SAS Institute, 2006) appropriate for a one–way design. The one–way ANOVA model was

$$Y_{ij} = \mu + Trt_i + e_{ij}$$

where Y_{ij} was the dependent variable, μ was the overall mean, and Trt_i was treatment effects (i = feed grade, purified 0, 10, and 20 mg/kg F or i = purified 0, 10, and 20 mg/kg F). Linear and quadratic trends were analyzed among quantitative factors (F levels). Next, orthogonal contrasts were computed to compare the feed grade and purified diets with or without F.

RESULTS

The recovery of analyzed F (mg/kg) was 71.77% based on F addition (mg/kg) and the R^2 was 0.9928 (Figure 4.1). The basal diet contained 0.46 mg/kg F based on the intercept in Figure 4.1 (= 0.3333 / 0.7177).

In Experiment 1, there was a significant difference in BW gain ($P < 0.05$) found between chicks fed feed grade and purified diets (Treatments 1 and 2; Table 4.3). Birds fed the diet

containing purified dicalcium phosphate grew significantly better than those fed the diet with feed grade dicalcium phosphate. There were no apparent differences when purified dicalcium phosphate treatments were compared. When feed grade and purified diets were compared, the only even suggestive difference among the diets was for feed efficiency in Experiment 1 (Table 4.3; $P = 0.0940$).

In Experiment 2, the probability that observed differences in BW gain were due to chance was nearly 1 in 20 ($P = 0.0517$; Table 4.4). There was a linear trend in BW gain when percentage of F from NaF increased ($P = 0.0446$). There were significant effects of feed grade versus purified dicalcium phosphate diets (Treatments 1 and 2) on P rickets score ($P < 0.05$) and incidence ($P < 0.01$; Table 4.4). Adding F from NaF with the purified dicalcium phosphate source also increased the incidence of P rickets (Table 4.4; $P < 0.05$; Figure 4.2).

DISCUSSION

We routinely observe experiment to experiment variation in the bone ash of chicks fed the same diets (23 – 31% vs. 35 – 41%; Table 4.1), leading to our hypothesis: uncontrolled F levels in mineral supplements from feed grade dicalcium phosphate influences chicks' bone ash. In the present experiments, again, identical treatments with feed grade dicalcium phosphate showed different bone ash values: 28.2 ± 0.3% in Experiment 1 vs. 26.6 ± 0.5% in Experiment 2. Bone ash values were much more similar when purified $CaHPO_4$ was fed (28.9 ± 0.6% vs. 28.1 ± 0.6%).

Huyghebaert et al. (1988) found that physical tests of bone–breaking strength are inconsistent in F studies. The group speculated that when bones are dried, the F content of bones

affects drying time in a non–linear manner, thus changing the brittleness of bones in an unpredictable way. Because of the uncertainty surrounding this test, we did not utilize it in our study, and instead relied on tibia ash as an indicator of bone strength.

The comparison of bone ash between purified dicalcium phosphate diets in Experiment 1 (Table 4.3) suggested that an increase in dietary F results in an increase in bone mineralization and strength, which is consistent with previous studies (Chan et al., 1976; Merkley and Miller, 1983; Huyghebaert et al., 1988). The variation between the percentage of bone ash in feed grade and purified dicalcium phosphate diets suggests ($P < 0.05$) that something in feed grade dicalcium phosphate may reduce bone mineralization. However, there were no significant differences of bone ash between purified dicalcium phosphate in Experiment 2 (Table 4.4). We cannot explain the difference in the trials, but they may be related to unexplained differences previously observed (Table 4.1).

The significant ($P < 0.05$) reduction in BW gain observed when chicks were fed a feed grade dicalcium phosphate based diet compared to purified dicalcium phosphate diet may indicate that F (or some other contaminant) has the potential to negatively impact growth even at very low levels (Treatments 1 vs. 2; Table 4.3). No F was added to Treatment 1; this raises the question of whether the growth of broiler chicks in the industry may be restricted by residual F in the feed or water. There was no significant difference between Treatments 1 and 2 in BW gain in Experiment 2 (Table 4.4; $P > 0.05$), but chicks fed a feed grade dicalcium phosphate diet (Treatment 1) weighted 11g less than purified dicalcium phosphate diet (Treatment 2). If the data from Experiments 1 and 2 are pooled, the probability of a difference in BW gain due to P source is 0.3132 (Table 4.5). Therefore, the data from both experiments suggest no difference in BW

gain between the two P sources.

The difference in percent bone ash between chicks fed the purified dicalcium phosphate diets is approximately 0.5%, while the difference among replicates of the same treatment was as high as 3.5% in Experiment 1. The confidence in mean values could be reduced simply by repeating the experiment with a greater number of replications. However, there was no trend in bone ash shown in Experiment 2. The only conclusion that can be drawn from the bone quality parameters is that the feed grade dicalcium phosphate source decreased bone mineralization.

Assuming that water consumption is roughly twice feed consumption (Pesti et al., 1985), F coming from the water was approximately 1.6 mg/kg [= (0.73 + 0.87) / 2 × 2] or about 4 times as much as coming from the basal diet. This amount is relatively low compared to the lowest treatment level of 10 mg F / kg of diet. Therefore, differences in water consumption between trials should not have been a contributing factor.

The influence of the chicks' water source on F consumption should also be taken into account in future studies. Our chicks were provided with tap water, which contains F, instead of deionized water. F content of tap water was reported 0.73 – 0.87 mg/kg by Athens Clarke County Utilities department. This unaccounted–for addition may have simply shifted our percent bone ash values. If water consumption changed with growth rate, it is also possible that it brought each treatment group closer to the same F intake level. If the latter is the case, our curve would have a steeper slope (and thus more variation among treatments) with the administration of deionized water than it did with tap water. To determine the true effects of dietary F contamination and a true nutritional need for F, it would be necessary to provide chicks with deionized water.

It is important to note that even low levels of F like those used in these studies have the potential to create measurable effects. Merkley (1979) noted that as little as 50 mg/kg F in the diet of broiler chicks, far below the accepted "safe" level, was enough to cause a significant increase in the incidence of TD. Such an effect can have an unforeseen influence on poultry research, particularly in TD and rickets studies, and may even reduce profitability in the industry.

REFERENCES

AOAC International, 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. AOAC Int., Arlington, VA.

Chan, M. M., R. B. Rucker, F. J. Zeman, and R. S. Riggins. 1973. Fluoride and its effect on bone metabolism in the Japanese Quail. J. Nutr. 103:1431–1440.

Chan, M. M., R. B. Rucker, and R. S. Riggins. 1976. Effects of fluoride and ethane–1–hydroxy–1,1–diphosphonate on bone metabolism in the growing chick. J. Nutr. 106:802–811.

Chankanka, O., S. M. Levy, J. J. Warren, and J. M. Chalmers. 2010. A literature review of aesthetic perceptions of dental fluorosis and relationships with psychosocial aspects / oral health–related quality of life. Community Dent. Oral Epidemiol. 38:97–109.

Chinoy, N. J. 2003. Fluoride in medicine, biology and toxicology. Ahmedabad, India: Gujarat University.

Duck, L. G. 2009. Athens clarke county public utilities department. http://www.athensclarkecounty.com/documents/pdf/publicutilities/ccr.pdf Accessed July 2010.

Edwards, H. M., Jr., and J. R. Veltmann, Jr. 1983. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. J. Nutr. 113:1568–1575.

Edwards, H. M., M. A. Elliot, S. Sooncharernying, and W. M. Britton. 1994. Quantitative requirement for cholecalciferol in the absence of ultraviolet light. Poult. Sci. 73:288–294.

Fleming, R. H., H. A. McCormack, L. McTeir, and C. C. Whitehead. 2003. Effects of dietary particulate limestone, vitamin K 3 and fluoride and photostimulation on skeletal morphology and osteoporosis in laying hens. Br. Poult. Sci. 44:683–689.

Huyghebaert, G., G. De Groote, R. Froyman, and J. Derijke. 1988. Effect of dietary fluoride on performances and bone characteristics of broilers and the influence of drying and defatting on bone–breaking strength. Poult. Sci. 67:950–955.

Liem, A. 2009. Dietary factors influencing calcium and phosphorus utilization by broiler chicks. PhD Diss. Univ. Georgia, Athens.

Louw, A. J., S. R. Grobler, W. Van, and T. J. Kotze. 2002. Degree of fluorosis in areas of South Africa with differing levels of fluoride in drinking water. Gen. Dent. 50:352–356.

Merkley, J.W. 1979. Tibial dyschondroplasia in broilers. Poult. Sci. 58:1021.

Merkley, J. W., and E. W. Miller. 1983. The effect of sodium fluoride and sodium silicate on growth and bone strength of broilers. Poult. Sci. 62:798–804.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Pesti, G. M., S. V. Amato, and L. R. Minear. 1985. Water–consumption of broiler–chickens under commercial conditions. Poult. Sci. 64:803–808.

Rama Rao, S. V., and V. Ramasubba Reddy. 2001. Utilisation of different phosphorus sources in relation to their fluoride content for broilers and layers. Br. Poult. Sci. 42:376–383.

Rennie, J. S., R. H. Fleming, H. A. McCormack, C. C. McCorquodale, and C. C. Whitehead. 1997. Studies on effects of nutritional factors on bone structure and osteoporosis in laying hens. Br. Poult. Sci. 38:417–424.

SAS Institute, 2006. SAS User's Guide: Statistics. Version 9.1.3 ed. SAS Inst. Inc., Cary, NC.

Shim, M. Y. 2007. Performance and bone quality of modern broiler chicks as influenced by hens' age and egg storage time. MS Diss. Univ. Georgia, Athens.

Shirley, R. B. 2003. Evaluation of phytase, vitamin D3 derivatives, and broiler breed differences on nutrient utilization, broiler performance, leg disorders, and the Experimentression of intestinal calbidin–28 kd mRNA and protein. PhD Diss. Univ. Georgia, Athens.

Suttie, J. W., D. L. Kolstad, and M. L. Sunde. 1984. Fuoride Tolerance of the young chick and turkey poult. Poult. Sci. 63:738–743.

Tokalioglu, S., U. Sahin, and S. Kartal. 2001. Determination of fluoride and some metal ion levels in the drinking waters in Kayseri province. Turkish Journal of Chemistry 25:113–121.

Weber, C. W., A. R. Doberenz, and B. L. Reid. 1969. Fluoride toxicity in the chick. Poult. Sci. 48:230–235.

Zipkin, I. 1970. Interrelation of fluoride with other components of calcified tissue. Edited by K. B. 15 vols, Biological interrelations and nutrition. Zurich/Feldback: Bibl. Nutrtio et dieta.

Table 4.1. Comparison of bone ash values from different Experiments when broilers were fed seemingly identical diets (%).

Shirley, 2003					
Diets	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
P Deficient	26.0±0.5	27.8±0.3	28.5±0.5	25.8±0.2	25.3±0.4
Shim, 2007					
Diets	Experiment 1	Experiment 2	Experiment 3		
Control	41.1±0.3	36.7±0.4	35.9±0.5		
P Deficient	30.0±0.8	30.4±0.5	25.7±1.8		
Liem, 2009					
Diets	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
P Deficient	31.7±0.5	35.1±0.5	32.8±0.8	31.8±0.1	27.5±0.2

Table 4.2. Composition of diets, Experiments 1 and 2.

	Experiment 1	Experiment 2						
	Feed Grade	Purified						
Ingredient	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8
	%							
Corn	53.32	53.66	53.66	53.66	53.66	53.66	53.66	53.66
Soybean Meal	37.52	37.46	37.46	37.46	37.46	37.46	37.46	37.46
Soy Oil	5.58	5.47	5.47	5.47	5.47	5.47	5.47	5.47
Limestone	2.03	1.97	1.97	1.97	1.97	1.97	1.97	1.97
Purified $CaHPO_4$	—	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Dicalcium Phosphate	0.58	—	—	—	—	—	—	—
Salt	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Vitamin Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL–Methionine[2]	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Mineral Premix	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
NaF (mg/kg)	—	—	22	44	66	88	110	132
Calculated Composition[3]								
ME, kcal	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
CP, %	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
Calcium, %	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Phosphorus, %	0.49	0.39	0.39	0.39	0.39	0.39	0.39	0.39
nPP, %	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Fluoride, mg/kg	—	—	10	20	30	40	50	60
Analyzed Composition								
DM, %	88.82	88.68	88.02	88.16	87.93	88.04	87.97	87.98
Ash, %	5.68	5.44	5.57	5.36	5.14	5.46	5.41	5.42
Ca, %	1.08	1.07	1.06	0.96	0.96	1.02	0.90	1.05
Total P, %	0.54	0.52	0.51	0.49	0.49	0.50	0.51	0.50
Fluoride, mg/kg[4]	ND[5]	ND	7	14	22	31	36	42

[1]Vitmain 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 g; 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–reinyl 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 ($MnSO_4.H_2O$), 101 mg; iron ($FeSO_4.7H_2O$), 20 mg; zinc (ZnO), 80 mg; copper ($CuSO_4.5H_2O$), 3 mg; iodine (ethylene diamine dihydroiodide), 0.75 mg; magnesium (MgO), 20 mg; selenium (sodium selenite), 0.3 mg.

[3]Calculated from NRC (1994).

[4]Fluoride analyzed by Maxxam analytics, Mississauga, Ontario.
[5]ND = Not detected.

Table 4.3. The effects of fluoride on production and bone strength (Experiment 1).

Treatments	$CaHPO_4$ source	F (mg/kg)	BW gain (g)	AFCR (%)	P Rickets Incidence (%)	P Score (1 to 3)	TD[1] Incidence (%)	Bone Ash (%)	Bone Ash (mg/tibia)
1	Feed Grade	0	329±8[2]	1.45±0.02	67.8±4.8	2.25±0.14	0.17±0.17	28.21±0.33	294±6
2	Purified	0	359±9	1.43±0.05	72.4±7.0	2.10±0.17	0.33±0.21	28.85±0.56	310±7
3	Purified	10	362±11	1.34±0.01	63.9±5.3	2.28±0.26	0.25±0.25	29.63±0.59	309±9
4	Purified	20	344±3	1.37±0.04	60.9±10.7	2.30±0.14	0.33±0.21	30.09±0.67	313±8
ANOVA						Type III SS			
Source		df	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value
Treatments		3	0.0326	0.1825	0.7294	0.8213	0.9216	0.1022	0.2677
Purified									
2 – 4		2	0.2527	0.3597	0.6081	0.6740	0.9613	0.3499	0.9277
Linear F		1	0.1902	0.3553	0.3408	0.4160	1.0000	0.1582	0.7388
Quad F		1	0.3024	0.2755	0.8165	0.7479	0.7827	0.8498	0.8548
Orthogonal Contrasts									
1 vs. 2		1	0.0129	0.6040	0.6639	0.5210	0.5598	0.4071	0.1377
1 vs. 2, 3, 4		1	0.0091	0.0940	0.8194	0.9054	0.5598	0.0499	0.0609

[1] TD =tibial dyschondroplasia

[2] Means ± standard errors of 6 pens of 10 birds

Table 4.4. The effects of fluoride on production and bone strength (Experiment 2).

Treatments	$CaHPO_4$ source	F (mg/kg)	BW gain (g)	AFCR (%)	P Rickets Incidence (%)	P Score (1 to 3)	TD[1] Incidence (%)	Bone Ash (%)	Bone Ash (mg/tibia)
1	Feed Grade	0	251±10[2]	1.30±0.00	91.6±2.1	2.52±0.09	4.00±2.45	26.58±0.54	203±10
2	Purified	0	262±7	1.34±0.02	66.7±3.5	1.93±0.18	8.89±2.22	28.06±0.55	225±6
3	Purified	10	237±2	1.33±0.02	77.9±3.5	2.03±0.12	2.78±2.78	27.36±0.49	196±5
4	Purified	20	247±3	1.32±0.02	80.3±6.2	2.39±0.12	2.22±2.22	27.10±0.45	203±4
5	Purified	30	247±11	1.35±0.03	84.4±9.4	2.38±0.37	6.25±6.25	27.13±1.12	210±15
6	Purified	40	248±12	1.30±0.00	82.9±7.9	2.20±0.16	0.00±0.00	28.58±0.52	213±12
7	Purified	50	245±8	1.38±0.02	93.1±4.2	2.37±0.17	6.94±4.17	27.59±0.45	211±11
8	Purified	60	237±12	1.36±0.04	89.6±5.3	2.47±0.18	2.50±2.50	27.81±0.73	213±15
ANOVA						Type III SS			
Source		df	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value
Treatments		7	0.0517	0.6067	0.0844	0.1929	0.7173	0.4133	0.4299
Purified									
2 – 8		6	0.0831	0.8082	0.1506	0.2906	0.6382	0.5197	0.3506
Linear F		1	0.0446	0.3109	0.0295	0.0852	0.4389	0.4934	0.9517
Quad F		1	0.7137	0.5601	0.5361	0.3789	0.7040	0.3399	0.5693
Orthogonal Contrast									
1 vs. 2		1	0.5538	0.2469	0.0065	0.0267	0.2490	0.1037	0.1575
1 vs. 2 – 8		1	0.0828	0.1571	0.1319	0.1574	0.9780	0.1777	0.7762

[1] TD = tibial dyschondroplasia

[2] Means ± standard errors of 5 pens of 10 birds

Table 4.5. The effects of fluoride on production and bone strength (Pooled data).

Treatments	$CaHPO_4$ source	F (mg/kg)	BW gain (g)	AFCR (%)	P Rickets Incidence (%)	P Score (1 to 3)	TD[1] Incidence (%)	Bone Ash (%)	Bone Ash (mg/tibia)
1	Feed Grade	0	315±16[2]	1.39±0.03	69.8±4.0	2.02±0.09	4.22±1.65	28.49±0.39	271±14
2	Purified	0	294±14	1.38±0.03	78.6±4.6	2.37±0.09	1.91±1.21	27.47±0.39	252±15
3	Purified	10	300±25	1.33±0.01	70.9±4.0	2.15±0.14	1.51±1.38	28.49±0.56	253±22
4	Purified	20	300±16	1.35±0.02	69.8±6.9	2.34±0.09	1.19±1.00	28.83±0.62	263±18
5	Purified	30	247±11	1.35±0.03	84.4±9.4	2.38±0.37	6.25±6.25	27.13±1.12	210±15
6	Purified	40	248±12	1.30±0.00	82.9±7.9	2.20±0.16	0.00±0.00	28.58±0.52	213±12
7	Purified	50	245±8	1.38±0.02	93.1±4.2	2.37±0.17	6.94±4.17	27.59±0.45	211±11
8	Purified	60	237±12	1.36±0.04	89.6±5.3	2.47±0.18	2.50±2.50	27.81±0.73	213±15
ANOVA						Type III SS			
Source		df	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value	*P*–value
Treatments		7	0.0176	0.5258	0.0693	0.1929	0.3997	0.4264	0.1020
Purified									
2 – 8		6	0.0831	0.8082	0.1506	0.2906	0.6382	0.5197	0.3506
Linear F		1	0.0446	0.3109	0.0295	0.0852	0.4389	0.4934	0.9517
Quad F		1	0.7137	0.5601	0.5361	0.3789	0.7040	0.3399	0.5693
Orthogonal Contrast									
1 vs. 2		1	0.3132	0.9135	0.2045	0.0267	0.3233	0.1356	0.3765
1 vs. 2 – 8		1	0.1539	0.2243	0.7910	0.1574	0.4795	0.2342	0.2652

[1] TD = tibial dyschondroplasia
[2] Means ± standard errors

Figure 4.1. F recovery based on analyzed F vs. calculated F (mg/kg).

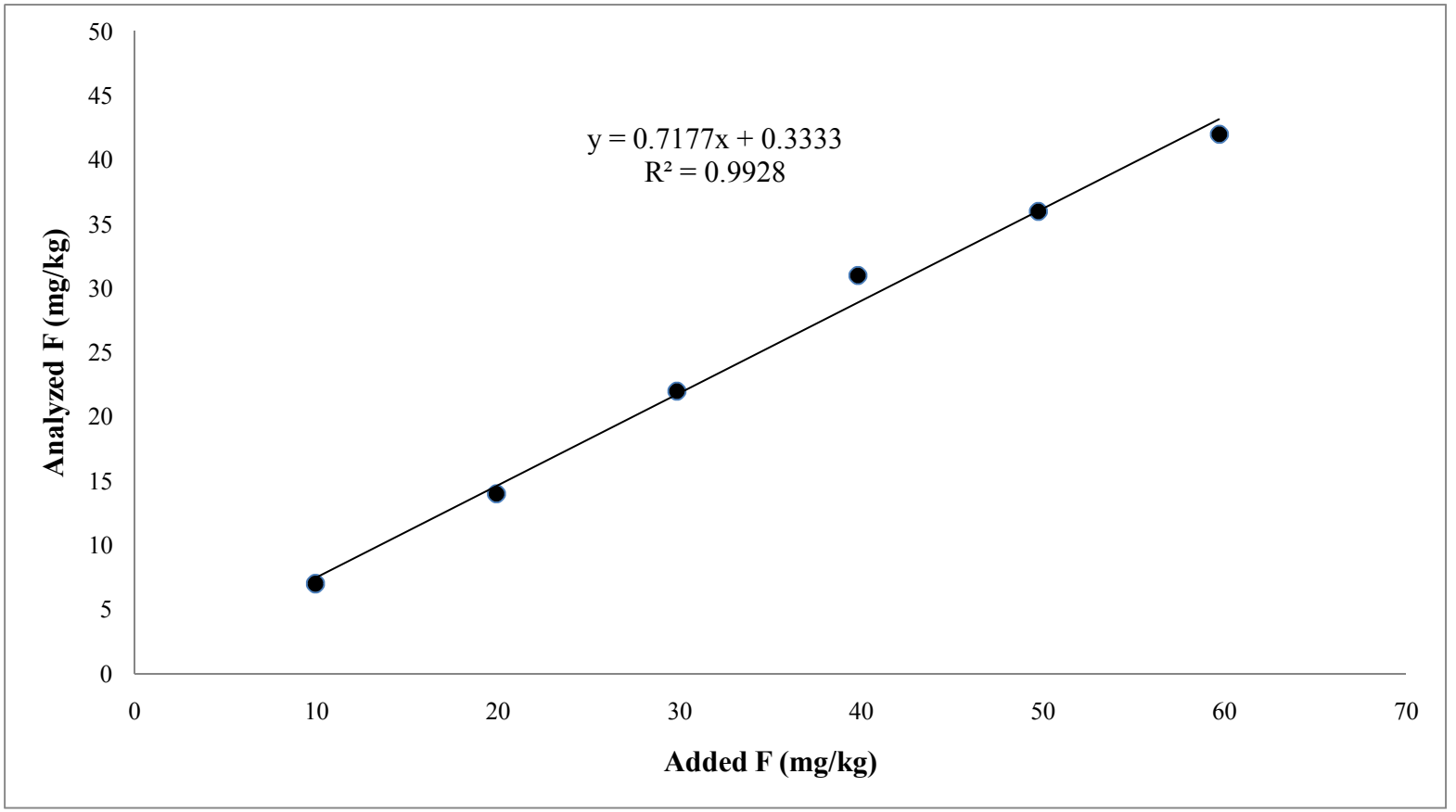
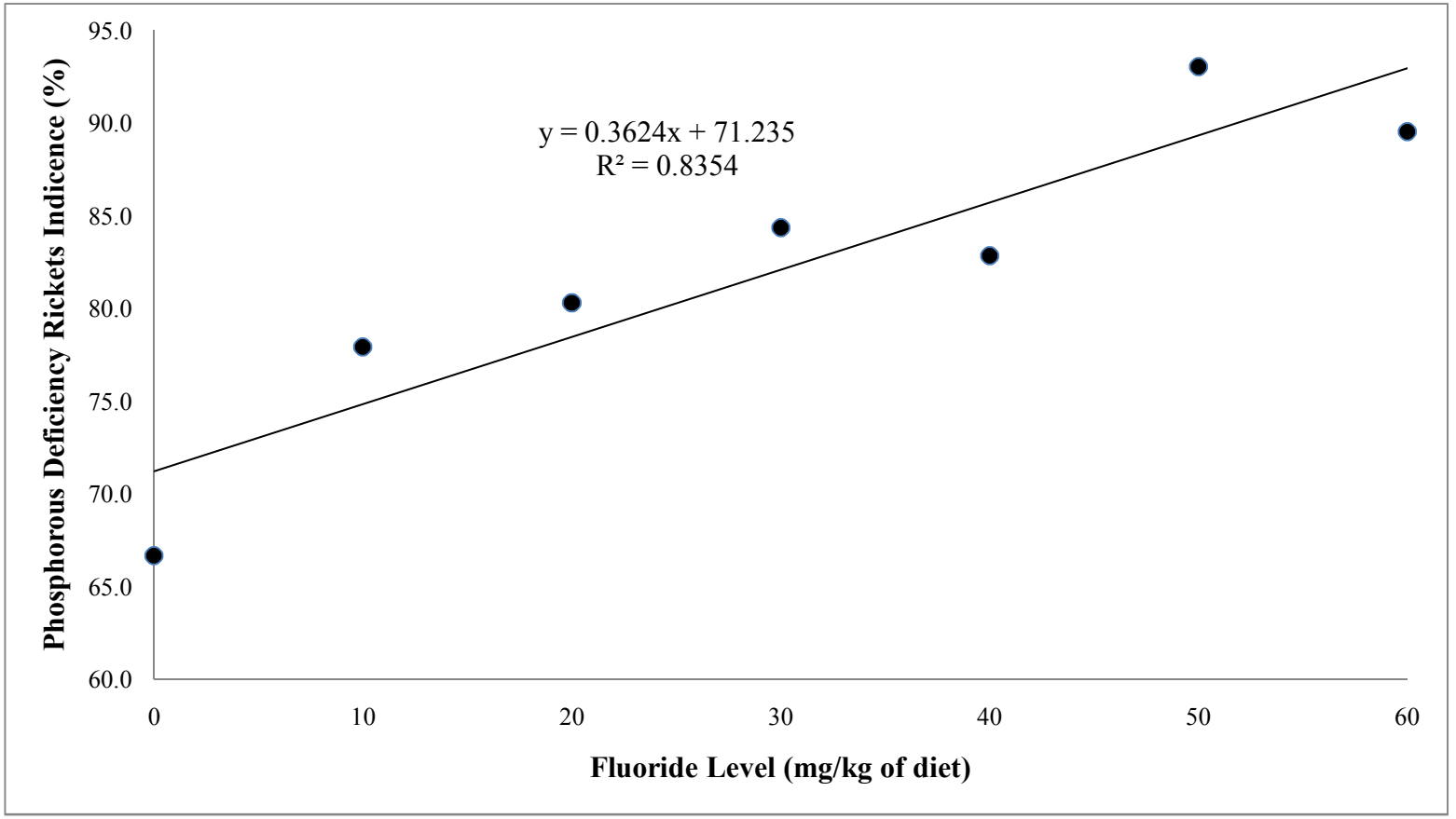


Figure 4.2. Phosphorus deficiency rickets incidence of tibias at levels of dietary fluoride in Experiment 2.



CHAPTER 5

THE EFFECTS OF BROILER CHICKEN GROWTH RATE ON VALGUS, CARUS AND TIBIAL DYACHONDROPLASIA[1]

[1] M. Y. Shim, A. B. Karnuah, N. B. Anthony, G. M. Pesti and S. E. Aggrey. To be submitted to Poultry Science.

ABSTACT

An experiment was conducted to test the hypothesis that fast growing (FG) broiler chickens are susceptible to bone abnormalities, causing major leg problems. Leg angulations, described in the twisted legs syndrome as 'valgus' and bilateral or unilateral 'varus', were investigated in 4 wk old Arkansas random-bred broiler chickens. Valgus angulation was classified as mild (tibia-metatarsus angle between 10 and 25º), intermediate (25 to 45º) or severe (> 45º). Body weight (BW) was measured at hatch and weekly to 6 weeks of age. Two subpopulations, slow growing (SG) and FG, were created based on their growth rate from hatch till 6 weeks of age. There were 581 and 585 individuals in the SG and FG populations, respectively. At 6 weeks of age, tibial dyschondroplasia (TD) incidences were determined by making a longitudinal cut across the right tibia. The TD bone lesion is characterized by an abnormal white, opaque, unmineralized, and unvascularized mass of cartilage occurring in the proximal end of the tibia. It was scored from 1 (mild) to 3 (severe) depending on cartilage plug abnormality size. Mean lesion scores of left and right valgus and TD (0.40, 0.38 and 0.06) of FG broilers were higher than those (0.26, 0.28, and 0.02) of SG broilers (P = 0.0002, 0.0037 and 0.0269). Growth rate was negatively associated with the twisted legs syndrome and a bone abnormality (TD) in this random-bred population.

Key words: Valgus, Varus, Tibial dyschondroplasia, Arkansas randombred, broilers

INTRODUCTION

Bone abnormalities, causing major leg problems in meat-type chickens, are thought to be related to their growth potential (Thorp and Waddington, 1997). Cortical bones of fast growing (FG) broiler chickens are highly porous which may easily lead to bone deformities. The occurrence of tibia dyschondroplasia (TD) as a spontaneously occurring cartilage abnormality in broiler chickens was first described by Leach and Nesheim (1965). Tibia dyschondroplasia usually appears between 3 and 8 weeks of age, and is caused by low levels of dietary calcium (Edwards 1984) and high levels of dietary phosphorus (Edwards, 1983). The bone lesion is characterized by an abnormal white, opaque, unmineralized, 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 pre-hypertrophic cartilage that is not calcified and has not been invaded by vessels from the metaphysis below the growth plate (Edwards, 1984; Riddell, 1975a). The incidence of TD in many broiler and turkey flocks is about 30% (Riddell, 1992). However a later report indicated that the incidence of TD in some turkey flocks was unacceptably high at 87% (Hirt et al., 1997).

No explanation has been found yet for the origin of the twisted legs syndrome (LeBihanDuval et al., 1996). However, twisted legs have appeared at higher frequencies concomitantly to the selection of meat-type chickens aimed mainly at increasing growth rate. Hartmann and Flock (1979) compared the incidence of twisted legs in commercial lines between 1963-1968 and 1977-1978. Between these two periods, the incidence measured on male offspring at slaughter had increased from 20 to 32% (70% when including slight deformities). It

is highly probable that some of the genes coding for bone, tendon or cartilage growth and quality may be involved in variation in susceptibility to these disorders.

It is not always clear whether the term 'twisted leg' refers to angular or torsional deformity or both. Angular and torsional deformities can occur independently (Duff and Thorp, 1985a; Randall and Mills, 1981). Twisted legs were first described in broilers by Osbaldiston and Wise (1967) and referred to lateral angulation of tibiotarsal articulation. Randall and Mills (1981) and Julian (1984) later suggested that the imprecise term of 'twisted legs' should be replaced by valgus-varus angulation. Valgus or varus angulation has been mainly associated with tibial deviation (Sørensen, 1989) and rotation of the shallow distal condyle groove of the tibiotarsus (Cruickshank and Sim, 1986). A few studies also indicate deformations of the femora (Haye and Simons, 1978; Duff and Thorp, 1985a, 1985b; Sørensen, 1989). These discrepancies between observations suggest that intertarsal joint angulations may vary between strains or could be the expression of different degrees of angulation. The majority of affected birds fed *ad libitum* and kept in batteries (Riddell, 1983). When the defect is unilateral, the right leg is more commonly affected. Valgus deformation is more common than varus deformation. The major change in the structure of the bone is a deviation at the growth zone (Hunter et al., 2008).

Many studies concluded that twisted legs are heritable (Hartmann and Flock, 1979; Leenstra et al., 1984; Mercer and Hill, 1984; Akbas et al., 2009). It is likely that a simplified selection scheme based on the presence or absence of twisted legs would reduce valgus deformity because of its higher incidence, while changes in incidence of varus would most probably be small or even unfavorable because of the negative genetic correlation between the two defects (LeBihanDuval et al., 1996).

Despite the increase in incidences of leg abnormalities during different decades (Hartmann and Flock, 1979) there is no direct association of frequency of leg abnormality and growth within the same strain. Our objective was to study the incidences of varus, vargus, and TD in two sub-populations of the same strain that differ markedly in growth rate.

MATERIALS AND METHODS

The Arkansas random-bred chicken population which is a random mating broiler control line was used in this study. Chicks were sexed at hatch and placed in pens (0.074 m^2/bird) with litter covered floors. Each chick was provided with water and diets for *ad libitum* consumption (Table 5.1). The birds were kept on an 20L:4D light regimen. BW was measured at hatch and hereafter weekly until 6 weeks of age. Two subpopulations slow growing (SG) and FG were created based on their growth rate from hatch till 6 wk of age. There were 581 and 585 individuals in the SG and FG populations, respectively. The growth rates were 230.563g/wk and 318.399g/wk for the SG and FG populations, respectively.

Scoring: Valgus-varus

Varus angulation is always associated with inward displacement of the tendon and severe lameness. Valgus-varus angulation was classified as normal (score=0), mild (tibia-metatarsus angle between 10 and 25º; score=1), intermediate (angle between 25 and 45º; score=2) and severe (angle greater than 45º; score=3) when the birds were 4 wk old.

Scoring: TD

The right tibia of each bird was split and scored to evaluate the incidence of TD according to the methods described by Edwards and Veltmann (1983) when the birds were 6 wk old. TD incidences were determined by making a longitudinal cut across the right tibia. The tibia were scored according to the white cartilage plug abnormality (0=normal; 1=mild; 2= intermediate; and 3=severe).

Statistical Analysis

The statistical model used to analyze the data was:

$Y_{ij} = \mu + Population_i + \varepsilon_{ij}$

Where, Y_{ij} is the leg severity scored on a categorical scale; μ = overall mean, $Population_i$ is the sub-population, SG and FG, and ε_{ij}= random residual error. The mean score of valgus, varus and TD was not normally distributed, so a weighted least squares method for estimating parameters was performed with PROC CATMOD (SAS Institute, 2009). CATMOD is specifically designed for categorical data that can be represented by contingency tables. Significant differences between the two populations were determined by the Chi-square distribution within the CATMOD procedure.

RESULTS AND DISCUSSION

The incidences (%) of valgus, varus and TD in SG and FG populations are given in Table 5.2. The incidence of varus in the left leg was similar in both the SG and FG populations. However, the incidence of varus in the right leg was higher (P=0.09) in the FG population

compared to the SG population. Varus angulation of the tarsal joint has been observed in chicks between 2 and 15 days of age (Leterrier and Nys, 1992). They observed that varus incidence could be up to 3% in chicks reared in floor pens and up to 11% in chicks raised in cages. This suggests that the rearing environment could make contribution towards the incidence of varus. However, the disproportionate incidence between the left and right leg cannot be explain, but according to Duff and Thorp (1985), a pattern of limb dominance exists in birds, and that the right pelvic limb is deformed more frequently and to a greater extent than the left limb. However, if the less deformed limb bears more body weight, then over loading can initiate or exacerbate physeal osteochondrosis (Randall and Mills, 1981). A similar observation was made by Leterrier and Nys (1992). They observed that limb deformity was unilateral in 80% of cases and affected predominantly the right limb (80 to 100%) of chicks by varus angulation and Julian (2005) points out that varus deformity may be caused by a lack of remodeling.

The incidence of valgus was significantly higher for both legs in the FG population compared to the SG population. Valgus is more prevalent in growing birds than varus. The incidence of valgus was about 29% in the FG population compared to 19% in the SG population (Table 5.2). Leterrier and Nys (1992) observed that in a growing bird population, the incidence of valgus was 30-40%. Clinical, epidemiological and anatomical differences suggest that valgus and varus angulations of the tarsal joint result from different aetiologies. Early tendonous displacement seems determinative in varus deformity and deformations of tibial diaphysis are likely to be an adaptive consequence of the luxation. In contrast, valgus angulation appeared progressively with age in birds and could lead to secondary tendonous slipping (Leterrier and Nys 1992; Riddell, 1992). Leg deformities in chickens can lead to abnormal posture, and Duff

and Thorp (1985) emphasized that abnormal limb posture could originate from femoral torsion and as a result, the whole pelvic limb need to be assessed. Studies by Le Bihan-Duval et al. (1995) and Akbas et al (2009) suggest a genetic basis for varus and valgus, however, whether, these syndromes are controlled major genes or polygenes are yet to be determined.

In broiler chickens, TD incidence varies from less than 1% to 40% (Throp and Maxwell, 1993). Sauveur and Mongin (1978) reported a TD incidence of 30-40 in a broiler flock. The incidence of TD could also be as high as 70 to 90% under experimental conditions (Edwards, 1989). The TD incidence was 1.03% in the FG chickens compared to 2.56% in the SG chickens (Table 5.2). The mean TD lesion score was 0.06 in the FG population which was higher (P=0.0269) than the lesion score of 0.02 in the SG population (Table 5.3). These results are similar to another study where the fast-growing genotype had more effect than slow-growing genotype on TD incidence (Fanatico et al., 2008). Other studies have suggested that growth rate could influence incidence and severity of TD (Sheridan et al., 1978; Lillburn et al., 1984). However, studies by Riddell (1975b) showed that excessive weight on the proximal tibia was not a primary factor in TD. Similarly, no differences were noted in live body weight of birds with or without TD by Timms et al. (1986). In birds with TD, there is a failure of hypertrophy, mineralization, vascular invasion and removal of cartilage of the growth plate, and pathogenesis is less understood (Riddell, 1975a). There are studies that point to genetic basis of TD and incidence of TD respond to selection (Sorensen, 1992; Akbas et al. 1995; Zhang et al. 1995).

There were significantly higher valgus and TD incidence in the FG chickens compared to with the SG chickens. Leg problems in meat-type chickens might be related to their growth potential (Thorp and Waddington, 1997), because cortical bones of fast growing broiler chickens

are highly porous which may easily lead to bone deformities. There is some evidence from the literature that suggest that incidence of leg abnormalities has been increasing (Hartmann and Flock, 1979), and Sorensen and Su (2001) reported an unfavorable genetic correlation between body weight and walking ability. But the genetic relationship between body weight and incidence of leg disorders is very low (Whitehead et al. 2003), and the direct estimate of the genetic correlation between growth rate and incidence has not yet been reported. Nevertheless, the current study shows that the incidence of leg abnormalities is higher in fast growing chickens than slow growing chickens, and since all the aforementioned leg abnormalities have some genetic basis, breeding strategies could be devised to slow down growth for proper skeletal development before fleshing meat type birds to meet market demands.

Leg disorders also have economic and welfare implications. Birds with serious leg disorders are not able to walk to the feeders and feed properly and as result have reduced body weight and consequently are culled. Decreasing leg disorders would in principle also improve the wellbeing and welfare of birds.

REFERENCES

Akbas, Y., S. Yalcin, S. Ozkan, F. Kirkpinar, C. Takma, Y. Gevrekci, H. C. Guler, and L. Turkmut. 2009. Heritability estimates of tibial dyschondroplasia, valgus-varus, foot-pad dermatitis and hock burn in broiler. Archiv Fur Geflugelkunde 73:1-6.

Cruickshank, J. J., and J. S. Sim. 1986. Morphomeric and radiographic characteristics of tibial bone of broiler chickens with twisted leg disorders. Avian Dis. 30:699-708.

Duff, S. R. I., and B. H. Thorp. 1985a. Patterns of physiological bone torsion in the pelvic appendicular skeletons of domestic fowl. Res. Vet. Sci. 39:307-312.

Duff, S. R. I., and B. H. Thorp. 1985b. Abnormal angulation/torsion of the pelvic appendicular skeletons in broiler fowl: morphological and radiological findings. Res. Vet. Sci. 39:313-319.

Edwards, H. M., Jr. 1983. Phosphorus. 1. Effect of breed and strain on utilization of suboptimal levels of phosphorus in the ration. Poult. Sci. 62:77-84.

Edwards, H. M., Jr. 1984. Studies on the etiology of tibial dyschondroplasia in chickens. J. Nutr. 114:1001-1013.

Edwards, H. M., Jr. 1989. The effect of dietary cholecalciferol, 25-hydroxycholecalciferol and 1,25 dihydroxycholecalciferol on the development of tibial dyschondroplasia in broiler chickens in the absence and presence of disulthiram. J. Nutr. 119:647.

Edwards, H. M., Jr., and J. R. Veltmann, Jr. 1983. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. J. Nutr. 113:1568-1575.

Fanatico, A. C., P. B. Pillai, P. Y. Hester, C. Falcone, J. A. Mench, C. M. Owens, and J. L. Emmert. 2008. Performance, livavility, and carcass yield of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci. 87:1012-1021.

Farquharson, C., and D. Jefferies. 2000. Chondrobytes and logitudinal bone growth: The development of tibial dyschondroplasia. Poult. Sci. 79:994-1004.

Julian, R. J. 1984. Valgus-varus deformity of the intertarsal joint in broiler-chickens. Canadian Veterinary Journal-Revue Veterinaire Canadienne 25:254-258.

Hartmann, W., and D. K. Flock. 1979. Line and family effects on the incidence of 'twisted legs' in meat chickens. Paper read at The British Poultry Breeders Roundtable.

Haye, U, and P. C. M. Simons. 1978. Twisted legs in broilers. Br. Poult. Sci. 19:549-557.

Hirt, H., M. Reinmann, and H. Oester. 1997. Leg problems in fattening turkeys. In Proceedings of the 5th Eropean Symposium on Poultry Welfare, edited by P. Koene and H. Blokhuis. Wageningen, Wageningen Agricultural University and the Institute of Animal Science and Health.

Hunter, B., A. Whiteman, B. Sanei, and A. Dam. 2008. Valgus/varus leg deformities in poultry. Keeping Your Birds Healthy.

Leach, R. M., and M. C. Nesheim. 1965. Nutritional, genetic and morphological studies of an abnormal cartilage formation in young chicks. J. Nutr. 86:236-244.

LeBihanDuval, E., C. Beaumont, and J. J. Colleau. 1996. Genetic parameters of the twisted legs syndrome in broiler chickens. Genetics Selection Evolution 28:177-195.

Leenstra, F., A. Van Voorst, and U. Haye. 1984. Genetic aspects of twisted legs in a broiler sire strain. Ann. Agric. Fenn. 23:261-270.

Leterrier, C., and Y. NYS. 1992. Clinical and anatomical differences in varus and valgus deformities of chick limbs suggest different etiopahogenesis. Avian Pathol. 21:429-442.

Lillburn, M., J. J. Lauterio, K. Rilling, J. H. Smith, and C. Scanes. 1984. Effects of growth rate and genetics on the incidence of tibial dyschondroplasia. Poult. Sci. 63:S138.

Mercer, J. T., and W. G. Hill. 1984. Estimation of genetic-parameters for skeletal defects in broiler-chickens. Heredity 53:193-203.

Thorp, B. H., and D. Waddington. 1997. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res. Vet. Sci. 62:67-73.

Osbaldiston, G. W., and D. R. Wise. 1967. Spondylolisthesis and leg weakness in chicken - A comon aetiology. Vet. Rec. 80:320-322.

Randall, C. J., and C. P. J. Mills. 1981. Observations on leg deformity in broilers with particular reference to the intertarsal joint. Avian Pathol. 10:407-431.

Riddell, C. 1975a. The development of tibial dyschondroplasia in broiler chickens. Avian Dis. 19:443-462.

Riddell, C. 1975b. Studies on the pathogenesis of tibial dyschondroplasia in chickens. III. Effect of body weight. Avian Dis. 19:497-504.

Riddell, C. 1983. Pathology of the skeleton and tendons of broiler chickens reared to roaster weights. I. Crippled birds. Avian Dis. 27:950-962.

Riddell, C. 1992. Non-infectious skeletal disorders of poultry: an overview. Edited by C. C. Whitehead, Bone biology and skeletal disorders in poultry. Abingdon, Oxfordshire, England: Carfax Publishing Co.

Sauveur, B., and P. Mongin. 1978. Tibial dyschondroplasia, a cartilage annormality in poultry. Annual Animal Biochemistry Biophysics. 18:87-98.

Sheridan, A. K., C. R. Howlett, and R. W. Burton. 1978. The inheritance of tibial dyschondroplasia in broilers. Br. Poult. Sci. 19:491-499.

Sørensen, P. 1989. Broiler selection and welfare. In: Proceedings of the 3rd European Symposium on Poultry Welfare, Tours, 45-58.

Throp, B. H. and M. H. Maxwell. 1993. Health problems in broiler production. In: Proceedings of the 4th European Symposium on Poultry Welfare, edited by C. J. Savory and B. O. Hughes. Potters Bar, Universities Federation for Animal Welfare.

Table 5.1. Composition of the basal diets.

Ingredient	Starter Diet (0-17 d)	Grower Diet (18-42 d)
	----------(%)----------	
Ground yellow corn	58.45	60.12
Soybean meal (dehulled)	34.49	32.72
Poultry fat	3.50	3.67
Limestone	0.90	0.93
Dicalcium Phosphate	1.58	1.45
Iodized sodium chloride	0.54	0.54
DL-Methionine	0.19	0.19
Vitamin premix[1]	0.25	0.25
Trace mineral premix[2]	0.08	0.08
Calculated composition[3]		
Crude Protein	22.5	20.50
Crude Fat	5.28	5.76
Fiber	2.53	2.50
Energy (kcal ME/kg)	3080	3150
Ca	0.95	0.90
Total P	0.72	0.67
Available P	0.45	0.41

[1] Vitmain 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 g; 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-reinyl 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 ($MnSO_4.H_2O$), 101 mg; iron ($FeSO_4.7H_2O$), 20 mg; zinc (ZnO), 80 mg; copper ($CuSO_4.5H_2O$), 3 mg; iodine (ethylene diamine dihydroiodide), 0.75 mg; magnesium (MgO), 20 mg; selenium (sodium selenite), 0.3 mg.

[3] Calculated from NRC (1994).

Table 5.2. Incidence (%) of Valgus, varus and TD

	Valgus(L)	Valgus(R)	Varus(L)	Varus(R)	TD
SG	18.93	19.97	1.89	3.27	1.03
FG	29.40	27.69	1.54	5.13	2.56

[1] TD = Tibial Dyschondroplasia

Table 5.3. Mean lesion score

Population	Valgus Left	Valgus Right	Varus Left	Varus Right	TD[1]
SG	0.2633	0.2754	0.0207	0.0344	0.0207
FG	0.4000	0.3829	0.0171	0.0564	0.0633
χ^2	13.72	8.44	0.17	2.82	4.90
Pr > χ^2	0.0002	0.0037	0.6816	0.0933	0.0269

[1] TD = Tibial Dyschondroplasia

CHAPTER 6

THE EFFECTS OF GROWTH RATE ON LEG MORPOHLOGY, TIBIA BREAKING STRENGTH, TIBIA DENSITY, TIBIA MINERALCONTENT AND TIBIA ASH OF BROILER CHICHENS[1]

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ABSTRACT

Fast growing (FG) broilers are especially susceptible to bone abnormalities, causing major problems for broiler producers. The cortical bones of fast growing broilers are highly porous which may lead to leg deformities. Leg problems were investigated in 6 wk old Arkansas randombred broilers. Body weight was measured at hatch and at 6 weeks. Two subpopulations, slow growing (SG) and FG broilers, were created based only on their growth rate from hatch till 6 weeks of age. There were 511 and 545 individuals in the SG and FG populations, respectively. At 6 weeks of age, the chickens were slaughtered and chilled at 4^{o}C overnight prior to deboning. Shank (78.2734±8.06), drum stick (190.9190±16.91), and thigh weights (233.8830±22.66) of FG broilers were higher than those of SG broilers (54.3933±6.86, 135.3910±15.45 and168.5010±21.13; $P < 0.0001$). Tibia weights (15.3550±2.28) of FG were also greater than those of SG broilers (11.2318±1.81; $P < 0.0001$). Shank length (81.5003±4.71) and tibia length (104.3360±4.45) of FG broilers were longer than those of SG broilers (71.8797±4.66 and 95.9749±4.85; $P < 0.0001$). Shank diameter (11.5933±1.60) and tibia diameter (8.1987±0.62) of FG broilers were wider than those of SG broilers (9.4526±1.74, 6.8151±0.58; $P < 0.0001$). Tibia breaking strength (28.4193±6.37) of FG broiler chickens was higher than those of SG broiler tibia (21.8073±5.89; $P < 0.0001$). Tibia density and content (0.1287±0.01 and 1.2900±0.23) of FG broiler chickens were higher than those of SG broiler tibia (0.1066±0.01 and 0.7888±0.18; $P < 0.0001$). Tibia ash content (39.7556±2.81) of FG broiler chicken was lower than those of SG broiler chicken (39.9920±2.67; $P = 0.1725$). FG broiler chickens had longer, wider, heavier and stronger bones than SG ones. After all parameters were calculated per unit of final body weight

at 6 wks, tibia density and bone ash percent of FG broiler chickens were lower than those of SG broiler chickens.

Key words: Shank, tibia, drum stick, broilers, bone breaking

INTRODUCTION

It is evident that there is a high incidence of leg problems observed among modern high-yield broiler chickens (Kestin et al., 1992). The modern high-yield broiler chicken has been selected very successfully to reduce the time taken to reach target body weight and feed efficiency. However, there have been several indirect consequences of the selection programs. There is evidence of adverse effects on the skeletal systems in broilers caused by the fast growth to a given age (Wise, 1970, 1975; Pierson and Hester, 1982; Sørensen, 1992; Hester, 1994; Lilburn, 1994; Thorp and Waddington, 1997). Cortical bones of fast growing (FG) broiler chickens are highly porous which may easily lead to bone deformities. Acute and chronic pain, and mortality resulting from osteoporotic fractures pose serious animal welfare concerns (Webster, 2004) and increase economic loss (Cook, 2000) by increased mortality resulting from skeletal diseases (Sullivan, 1994; Thorp, 1994).

Bone mineralization has been studied with respect to process as well as chemical and physical structure since mineralization of bone matrix is highly important. Mineralization affects bone strength which enables the skeleton to withstand the gravity and additional loading. Bone strength is determined not only by the volume of bone tissue and the microarchitectural organization of this bone, but also by the degree of mineralization of bone matrix (Boivin and Meunier, 2002). From the observation of the bone formation it has been concluded that, once osteoblasts which are derived from primitive mesenchymal cells in bone marrow via osteoprogenitor cells (Owen and Ashton, 1986; Beresford, 1989) form the extracellular matrix of bone, various steps are followed in order to develop fully mineralized bone.

Bone measurements such as bone breaking strength (Merkley, 1981; Ruff and Hughes, 1985; Park et al., 2003; Kim et al., 2006), bone density (Watkins and Southern, 1992; Kim et al., 2006), bone mineral content (Akpe et al., 1987; Kim et al., 2006) and bone ash (Garlich et al., 1982; Cheng and Coon, 1990; Park et al., 2003; Shim et al., 2008) have been used as indicators of bone status in the mineral nutrition of poultry.

Bone density, which is the weight of mineral per volume of the bone (g/cm3), is determined by two factors: how many mineral atoms are deposited within the bone matrix, and how porous the matrix is. Since these two factors are highly related to the bone strength, the bone density can indirectly be used to determine the bone strength. This method, however, cannot be used to measure bone density in living beings. In humans, bone density can be measured using a technique called dual-energy x-ray absorptiomety, or DXA to predict osteoporosis without taking samples (Koo, 2000; Bolotin, 2007). It has been shown that dual-energy X-ray absorptiomety (DXA) measurement of total body bone mineral content is highly correlated with total body ash content of the pig (Mitchell et al., 1996). Other methods of measuring bone mass in humans or animals include ultrasound, or quantitative computed tomography (QCT) (Carter et al., 1992; Rizzoli et al., 1995; Roschger et al., 2008).

Bone ash is a critical measurement of how mineralized the bone is. Chicks with bone disorder usually have lower percentage of bone ash than healthy ones. The amount of ash (inorganic material) present in bone is proportional to its degree of hardness or compression strength (Bonser and Casinos, 2003), the organic component of bone is important in providing tensile strength and flexibility (Velleman, 2000), and it is the balance between these two components of bone that contribute to its breaking strength (Rath ert al.,1999) . It has been

suggested that the relatively low bone mineral content seen in some intensively selected broiler genotypes is because current diets are not formulated to meet the increased mineral requirements of these FG genotypes (Thorp and Waddington, 1997; Williams et al., 2000).

Because earlier studies on growth rate and bone quality were from different strains over a period of years and even decades, there is no conclusive data that bone strength and growth rate are related. Our objective was to study leg morphology, tibia breaking strength, tibia density, tibia mineral content, and tibia ash, in two sub-populations of the same strain that differ markedly in growth rate.

MATERIALS AND METHODS

The Arkansas randombred chicken population which is a random mating broiler control line was studied. One-day old chicks were hatched and placed in pens (0.074 m^2/bird). Each chick was provided with water and diets *ad libitum* (Table 6.1). The birds were kept on a 20L:4D light regimen. BW was measured at hatch and hereafter weekly until 6 weeks. Two subpopulations, slow growing (SG) and FG, were created based on their growth rate from hatch till 6 wk of age. There were 511 and 545 individuals in the SG and FG populations, respectively. The growth rates averaged 230.563g/wk and 318.399g/wk for the SG and FG populations, respectively. Birds were handled according to the University of Georgia Animal Use and Care Guidelines. On d 42, birds were slaughtered after 2-4 hours of feed and water withdrawal. The birds were stunned with 14 V, 60 Hz AC for 9 s by a commercial stunner (Simmons model SF-7001, Simons Engineering Co., Dallas, GA) and then killed by manually cutting the carotid artery and jugular vein on the side of the neck. After exsanguination, the birds were scalded at

54°C for 120 s and picked for 30 s. The scalder (Cantrell Model SS300CF, Cantrell Machine Co., Inc., Gainesville, GA), picker (Cantrell Model CPF-60, Cantrell Machine Co., Inc., Gainesville, GA) and eviscerator (Cantrell Model Mark 4, Cantrell Machine Co., Inc., Gainesville, GA) in the automated line system used imposed most of the physical stresses normally encountered under commercial processing conditions with the exception that the warm eviscerated carcasses were static-chilled in slush-ice for four hours rather than by convective agitation. Carcasses were then chilled and individually wrapped in plastic bags and placed in a cool room (5°C) until they were dissected on d 43. Carcasses were suspended on cones and both femurs were dislocated in order to remove the thighs from the frame. For the detailed meat yield assessment parts were weighed and analyzed as actual and per unit of live weight for this trial: both thigh (skin, meat and bone), both drum sticks (skin, meat and bone), and both shanks (skin and bone).

Bone strength: Breaking bones

The meat was removed before bone-breaking strength analysis of the right tibias by using an Instron Materials tester (Model 5500, Instron Corp., Canton, MA) with Automated Materials Test System software version 4.2. The weights, diameters, and lengths of tibia were measured. Tibia diameters were measured at narrowest and widest places, then average. The deformation rate was 5mm/min. Tracing of force was recorded at a constant rate. The graphs showed plateau curves of maximal force (kgf) reached to measure of the energy stored in the bone.

Bone density and bone mineral content

After the left tibia was thawed and muscle tissue was cleaned off, bone density and geometric parameters of the harvested tibia were measured by the use of dual-energy x-ray absorptiomerty (DXA). Dual-energy X-ray absorptiometry scans was performed on tibia, using a Lunar DPXL densitometer (GE Medical Systems, Waukesha, WI).

Bone Ash

After measuring bone density and bone mineral content, the left tibia of each bird was collected for percentage tibia ash determination on a fat–free dry–weight basis according to AOAC International (2005) method 932.16.

Statistical Analysis

Analysis of variance was performed on all data for both Experiments using the General Linear Model procedure of SAS (SAS Institute, 2006) appropriate for a one–way design. The one–way ANOVA model was

$$Y_{ij} = \mu + Trt_i + e_{ij}$$

where Y_{ij} was the dependent variable, μ was the overall mean, and Trt_i was treatment effects (i = SG or FG).

RESULTS

The SG broilers had significantly lower amounts (weights, lengths and diameter) of shank, drum stick, thigh, tibia than the FG ones (Table 6.2). The SG broilers had significantly

lower amounts of tibia breaking strength, tibia mineral density, and tibia mineral content, but there was no difference in tibia ash content. Overall there were positive relationship between traits and live weights except percent tibia ash (Figure 6.1–6.12; R^2= 0.0610–0.8238). When expressed on a per unit of live weight basis, there were some negative relationships between traits and live weights (Figure 6.13–6.24; R^2=0.0002–0.8653). When expressed on a per unit of live weight basis, SG broilers had significantly higher amounts of shank length and diameter, drumstick weight, tibia length and diameter than the FG chickens, but there were no differences in weights of shank, thigh or tibia (Table 6.2). When expressed on a per unit of live weight basis, SG broilers had significantly higher amounts of tibia breaking strength, tibia density, and tibia ash contents, but SG broilers had significantly lower tibia mineral contents. Percent tibia ash had higher correlation between percent tibia ash and live weight when expressed on a per unit of live weight basis than those between actual percent tibia ash and live weight (R^2=0.0610 → 0.8653; Figure 6.12 and 6.24).

DISCUSSION

Results from this study clearly show the relationship between bone quality and growth in broiler chickens. Selection for growth over the past 50 years has resulted in increased in rate of growth and absolute values of tibia weight, length, density, mineral composition, ash content and breaking strength. However, when the shank and tibia are considered in terms of the load they need to support, i.e., expressing the absolute values in terms of unit of live weight, the bone quality of the SG population was significantly better than the FG population. The tibia breaking strength, tibia ash and tibia density were higher in the SG population than the FG population.

These tibia breaking strength, tibia density and tibia ash per unit of live weight results are similar to those of Newman and Leeson (1999) who also concluded that bone breaking strength of tibias may indicate the influence of genetic selection on the biomechanical indicators of bones. It is generally accepted that leg strengths are lower in modern high-yield broiler chickens than unselected broiler chickens (Pierson and Hester, 1982; Sørensen, 1992; Hester, 1994; Lilburn, 1994). Since the 1970s, questions were raised about the bone quality of broilers (Wise, 1970; Kestin et al., 1999; Yalci et al., 2001). Bone breaking strength was greater in a SG experimental cross than in a commercial FG broiler (Leterrier an Nys, 1992). The bones of the FG broiler chickens were more porous, with a lower mineral content, than the bones of SG broiler chickens which were considered to be of poor quality. Bones of FG broiler would have a lower effective bone breaking strength based on lower bone density (Williams et al., 2000) compared to their slow growing counterparts. In contrast, McDevitt et al. (2006) insisted tibias from the selected high-yield broiler chickens were twice as strong as those of the unselected chickens at the same age and were equally strong at the same body mass. They also concluded that the selected broilers had significantly heavier tibia with more than twice as much ash and organic matter per unit length of bone than did the unselected broilers at the same age. In general fast growing birds have higher absolute values of tibia and shank morphology. However, when considered in terms of the load (BW) these birds have to carry, it is abundantly clear that FG birds have a higher risk of bone breakage than SG birds.

The broiler breeding industry has thought in the recent past to set a balance between rapid growth and skeletal development by incorporating skeletal integrity in its selection programs (Sørensen, 1992; Williams et al., 2000). One of the difficulties of this approach is that the causes

of skeletal disorders are varied and include genetics, nutrition and management systems (Kestin et al., 1992; Thorp, 1994; Williams et al., 2000) and the interactions between these factors. In addition, there are only a few genetic parameter estimates of skeletal parameters and their relationships with other production parameters. Skeletal structure carrying muscle weight than it can bear would lead to weakened and unhealthy legs. A study by Kestin et al. (1992) showed that unselected broiler chickens had significantly better leg health than four commercial broiler strains based on gait scores. Selection for fast growth may not be the only cause for increased incidence of poor bone quality. In caged hens where the opportunity for movement and load-bearing exercises are limited, birds are particularly vulnerable to osteoporosis, lower tibia and femur mineral density, bone mass, cortical area and mass, and bone breaking strength compared to their counterparts raised in modified cages with nest box and perches (Jendral et al. 2008).

The chickens used in the current study had enough space to walk and exercise on litter. Besides both populations were raised together, thus making the plausible reason for the differences in leg quality, genetic. Leg abnormalities are the major single cause of economic losses in the chicken house. Approximately, 2-6% of all broilers display signs of skeletal problems (Day, 1990). Leg abnormalities result in culling, mortality, reduced feed efficiency and growth, and down-grading in the processing plant. In the US alone, skeletal problems are estimated at $80-120 million in broilers (Sullivan, 1994). Leg maladies are not only an economic concern, but also welfare. Birds suffering from leg abnormalities have limited locomotion and reduced welfare.

This study confirms from a single study the hypothesis that bone breaking strength, as evidenced by tibia breaking strength per unit of body weight, is negatively associated with

growth rate. This relationship was earlier suspected from retrospective evidence. This requires a proper balance between breeding for increased growth and leg strength.

REFERENCES

AOAC International, 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. AOAC Int., Arlington, VA.

Akpe, M. E., P. E. Waibel, K. Larntz, A. L. Metz, S. L. Noll, and M. M. Walser. 1987. Phosphorous availability bioassay using bone ash and bone densitometry as response criteria. Poult. Sci. 66:713-720.

Beresford, J. N. 1989. Osteogenic stem cells and the stromal system of bone and marrow. Clin. Orthop. Relat. Res. 240:270-280.

Boivin, G., and P. J. Meunier. 2002. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. Calcif. Tissue Int. 70:503-511.

Bolotin, H. H. 2007. DXA in vivo BMD methodology: An erroneous and misleading research and clinical gauge of bone mineral status, bone fragility, and bone remodelling. Bone 41:138-154.

Bonser, R. H. C. and A. Casinos. 2003. Regional variation in cortical bone properties from broiler fowl – a first look. Br. Poult. Sci. 44:350-354.

Carter, D. R. , M. L. Bouxsein, and R. Marcus. 1992. New approaches for interpreting projected bone density data. J. Bone Miner. Res. 7:137-145.

Cheng, T. K., and C. N. Coon. 1990. Sensitivity of various bone parameters of laying hens to different daily calcium intakes. Poult. Sci. 69:2209-2213.

Cook, M. E. 2000. Skeletal deformities and their causes: introduction. Poult. Sci. 79:982-984.

Day, E. J. 1990. Future research needs focus on new, old problems. Feedstuffs, July 23:12-15.

Garlich, J., J. Brake, C. R. Parkhurst, J. P. Thazton, C. Morris, and G. W. Morgan. 1984. Physiological profile of caged layers during one production year, molt, and postmolt: Egg production, egg shell quality, liver, femur, and blood parameters. Poult. Sci. 63:339-343.

Hester, P. Y. 1994. The role of environment and management on leg abnormalities in meat-type fowl. Poult. Sci. 73:904-915.

Kestin, S. C., T. G. Knowles, A. E. Inch and N. G. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet. Rec. 131:190-194.

Kestin, S. C., G. Su, and P. Sørensen. 1999. Different commercial broiler crosses have different susceptibilities to leg weakness. Poult. Sci. 78:1085-1090.

Kim, W. K., L. M. Donalson, A. D. Mitchell, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2006. Effects of alfalfa and fructooligosaccharide on molting parameters and bone qualities using dual energy x-ray absorptiometry and conventional bone assays. Poult. Sci. 85:15-20.

Koo, W. W. K. 2000. Body composition measurements during infancy. Ann. N. Y. Acad. Sci. 904:383-392.

Leterrier, C., and Y. NYS. 1992. Clinical and anatomical differences in varus and valgus deformities of chick limbs suggest different etiopahogenesis. Avian Pathol. 21:429-442.

Lilburn, M. S. 1994. Skeletal growth of commercial poultry species. Poult. Sci. 73:897-903.

McDevitt, R. M., G. M. McEntee and K. A. Rance. 2006. Bone breaking strength and apparent metabolisability of calcium and phosphorus in selected and unselected broiler chicken genotypes. Br. Poult. Sci. 47:613-621.

Merkley, J. W. 1981. A comparison of bone strengths from broilers reared under varios conditions in cages and floor pens. Poult. Sci. 60:98-106.

Mitchell, A. D., J. M. Conway, and A. M. Scholz. 1996. Incremental changes in total and regional body composition of growing pigs measured by dual-energy X-ray absorptiometry. Growth Dev. Aging 60:95-105.

Newman, S. and S. Leeson. 1999. The effect of dietary supplementation with 1,25-dihydroxycholecalciferol or vatamin C on the characteristics of the tibia of older laying hens. Poult. Sci. 78:85-90.

Owen, M., and B Ashton. 1986. Osteogenic differentiation of skeletal cell populations. Edited by S. Y. Ali, Cell mediated calcification and matrix vesicles. Amsterdam: Elsevier.

Park, S. Y., S. G. Birkhold, L. F. Kybena, D. J. Nisbet, and S. C. Ricke. 2003. Effect of storage condition on bone breaking strength and bone ash in laying hens at different stages in production cycles

Pierson, F. W. and P. Y. Hester. 1982. Factors influencing leg abnormalities in poultry: a review. World's Poult. Sci. J. 38:5-17.

Rath, N. C., G. R. Huff, W. E. Huff, G. B. Kulkarni and J. F. Tierce. 1999. Comparative difference in the composition an biochemical properities of tibiae of seven- and seventy-two week-old male and female broiler breeder chickens. Poult. Sci. 78:1232-1239.

Rizzoli, R., D. Slosman, and J. P. Bonjour. 1995. The role of dual energy X-ray absorptiometry of lumbar spine and proximal femur in the diagnosis and follow-up of osteoporosis. Am. J. Med 98:33S-36S.

Roschger, P., E. P. Paschalis, P. Fratzl, and K. Klaushofer. 2008. Bone mineralization density distribution in health and disease. Bone 42:456-466.

Ruff, C. R., and B. L. Hughes. 1985. Bone strengh of height restricted broilers as affected by levels of calcium, phosphorous and manganese. Poult. Sci. 64:1628-1636.

SAS Institute, 2006. SAS User's Guide: Statistics. Version 9.1.3 ed. SAS Inst. Inc., Cary, NC.

Sommerfeldt, D. W., and C. T. Rubin. 2001. Biology of bone and how it orchestrates the form and function of the skeleton. Eur. Spine J. 10:S86-S95.

Sørensen, P. 1992. The genetics of leg disorders. Pages 213-229 in Bone biology and skeletal disorders in poultry. C. C. Whitegead, ed. Abingdon, Oxfordshire, England.

Sullivan, T. W. 1994. Skeletal problems in poultry: estimated annual cost and description. Poult. Sci. 73:879-882.

Thorp, B. H. 1994. Skeletal disorders in the fowl: a review. Avian Pathol. 23:203-236.

Thorp, B. H., and D. Waddington. 1997. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res. Vet. Sci. 62:67-73.

Velleman, S. G. 2000. The role of the extracellular matrix in skeletal development. Poult. Sci. 79:985-989.

Watkins, K. L., and L. L. Southern. 1992. Effect of dietary sodium zeolite A and graded levels of calcium and phosphorous on growth, plasma, and tibia characteristics of chicks. Poult. Sci. 71:1048-1058.

Webster, A. B. 2004. Welfare implications of avian osteoporosis. Poult. Sci. 83:184-192.

Williams, B., S. Solomon, D. Waddington, B. Thorp and C. Farquharson. 2000. Skeletal development in the meat-type chicken. Br. Poult. Sci. 41:141-149.

Wise, D. R. 1970. Comparisons of the skeletal systems of growing broiler and laying strain chickens. Br. Poult. Sci. 11:333-339.

Wise, D. R. 1975. Skeletal abnormalities in table poultry. Avian Pathol. 4:1-10.

Yalcin, S., S. Ozkan, E. Coskuner, G. Bilgen, Y. Delen, and Y. Kurtulmus. 2001. Effects of strain, maternal age and sex on morphological characteristics and composition of tibial bone in broilers. Br. Poult. Sci. 42:184-190.

Table 6.1. Composition of the basal diets.

Ingredient	Starter Diet (0-17 d)	Grower Diet (18-42 d)
	----------(%)----------	
Ground yellow corn	58.45	60.12
Soybean meal (dehulled)	34.49	32.72
Poultry fat	3.50	3.67
Limestone	0.90	0.93
Dicalcium Phosphate	1.58	1.45
Iodized sodium chloride	0.54	0.54
DL-Methionine	0.19	0.19
Vitamin premix[1]	0.25	0.25
Trace mineral premix[2]	0.08	0.08
Calculated composition[3]		
Crude Protein	22.5	20.50
Crude Fat	5.28	5.76
Fiber	2.53	2.50
Energy (kcal ME/kg)	3080	3150
Ca	0.95	0.90
Total P	0.72	0.67
Available P	0.45	0.41

[1] Vitmain 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 g; 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-reinyl 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 ($MnSO_4.H_2O$), 101 mg; iron ($FeSO_4.7H_2O$), 20 mg; zinc (ZnO), 80 mg; copper ($CuSO_4.5H_2O$), 3 mg; iodine (ethylene diamine dihydroiodide), 0.75 mg; magnesium (MgO), 20 mg; selenium (sodium selenite), 0.3 mg.

[3] Calculated from NRC (1994).

Table 6.2. Leg and tibia morphology, tibia breaking strength, tibia density, tibia mineral content and tibia bone ash of broiler chickens based on growth rate

Trait	Slow growing population (N=511)	Fast growing population (N=545)	Pr >F
Shank weight, g	[1]54.3933 ± 6.86	78.2734 ± 8.06	<0.0001
Shank length, mm	71.8797 ± 4.66	81.5003 ± 4.71	<0.0001
Shank diameter, mm	9.4526 ± 1.74	11.5933 ± 1.60	<0.0001
Drumstick weight, g	135.3910 ± 15.45	190.9190 ± 16.91	<0.0001
Thigh weight, g	168.5010 ± 21.13	233.8830 ± 22.66	<0.0001
Tibia weight, g	11.2318 ± 1.81	15.3550 ± 2.28	<0.0001
Tibia length, mm	95.9749 ± 4.85	104.3360 ± 4.45	<0.0001
Tibia Diameter, mm	6.8151 ± 0.58	8.1987 ± 0.62	<0.0001
Tibia breaking strength, kg	21.8073 ± 5.89	28.4193 ± 6.37	<0.0001
Tibia mineral density, g/cm^3	0.1066 ± 0.01	0.1287 ± 0.01	<0.0001
Tibia mineral content	0.7888 ± 0.18	1.2900 ± 0.23	<0.0001
Tibia ash content, %	39.9920 ±2.67	39.7556 ± 2.81	0.1725
Shank weight per unit of live weight	3.8118 ± 0.47	3.9974 ± 0.36	<0.0001
Shank length per unit of live weight	5.0790 ± 0.43	4.1873 ± 0.01	<0.0001
Shank diameter per unit of live weight	0.6681 ± 0.13	0.5954 ± 0.00	<0.0001
Drumstick weight per unit of live weight	9.5306 ± 0.92	9.7940 ± 0.71	<0.0001
Thigh weight per unit of live weight	11.8495 ± 1.20	12.0018 ± 0.98	0.0232
Tibia weight per unit of live weight	0.7901 ± 0.12	0.7889 ± 0.11	0.8772
Tibia length per unit of live weight	6.7656 ± 0.48	5.3608 ± 0.29	<0.0001
Tibia diameter per unit of live weight	0.4801 ± 0.05	0.4209 ± 0.03	<0.0001
Tibia breaking strength per unit of live weight	1.5326 ± 0.41	1.4607 ± 0.33	0.0033
Tibia mineral density per unit of live weight	0.0075 ± 0.00	0.0066 ± 0.00	<0.0001
Tibia mineral content per unit of live weight	0.0554 ± 0.01	0.0661 ± 0.01	<0.0001
Tibia ash content per unit of live weight	2.8316 ± 0.32	2.0438 ± 0.17	<0.0001

[1]Main effect means ± standard deviation.

Figure 6.1. Shank weight (g)

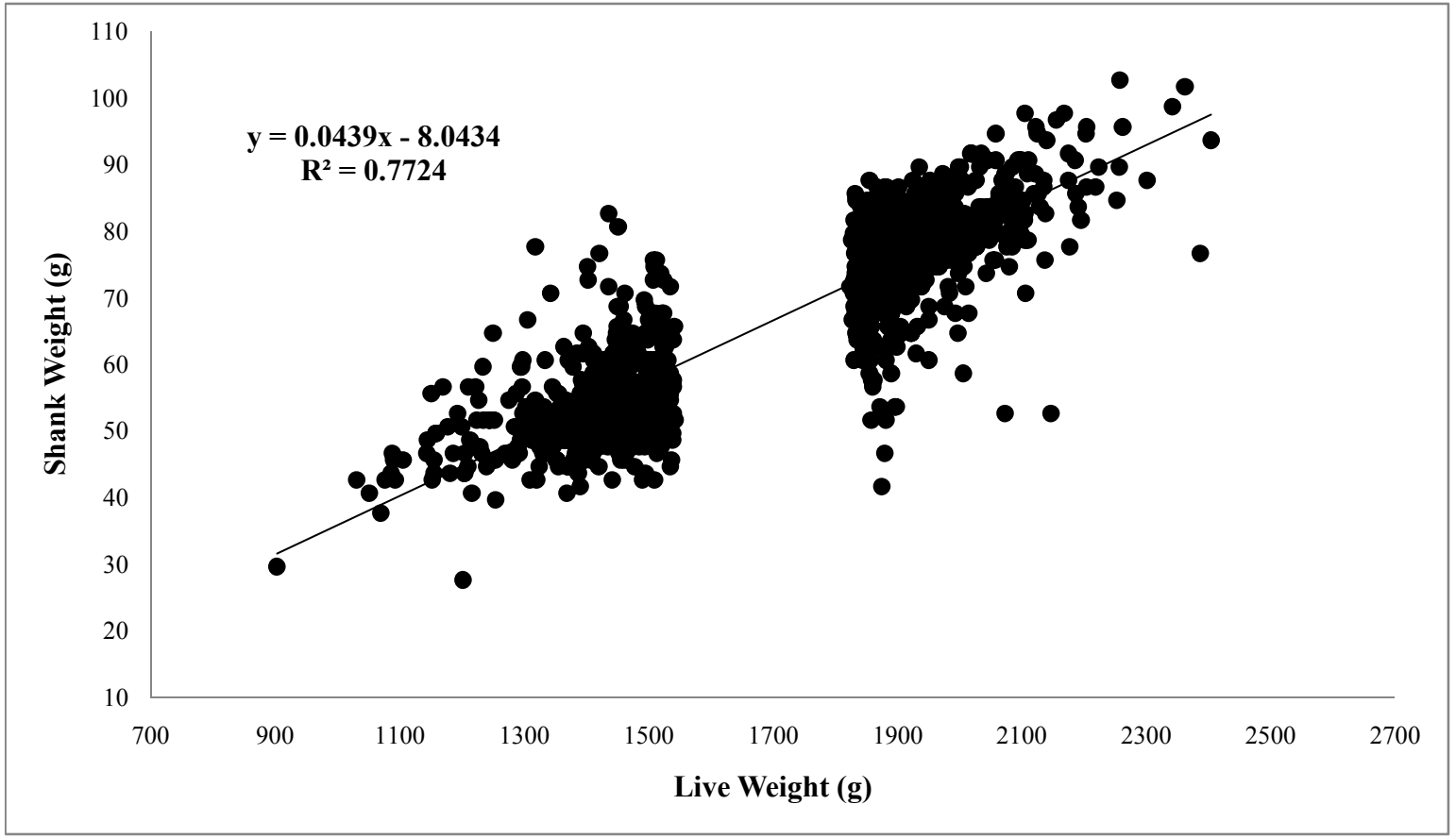


Figure 6.2. Shank length (mm)

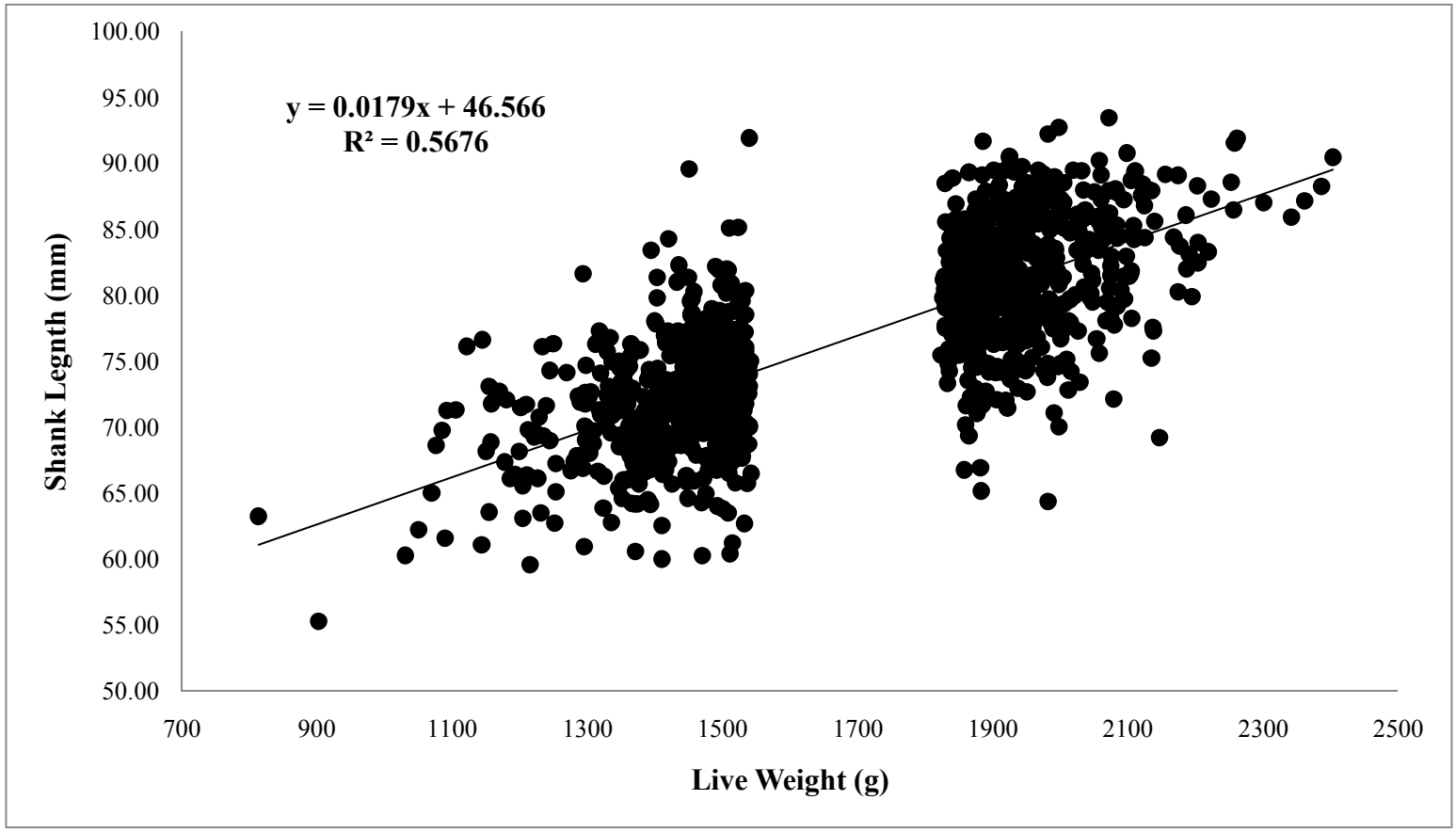


Figure 6.3. Shank diameter (mm)

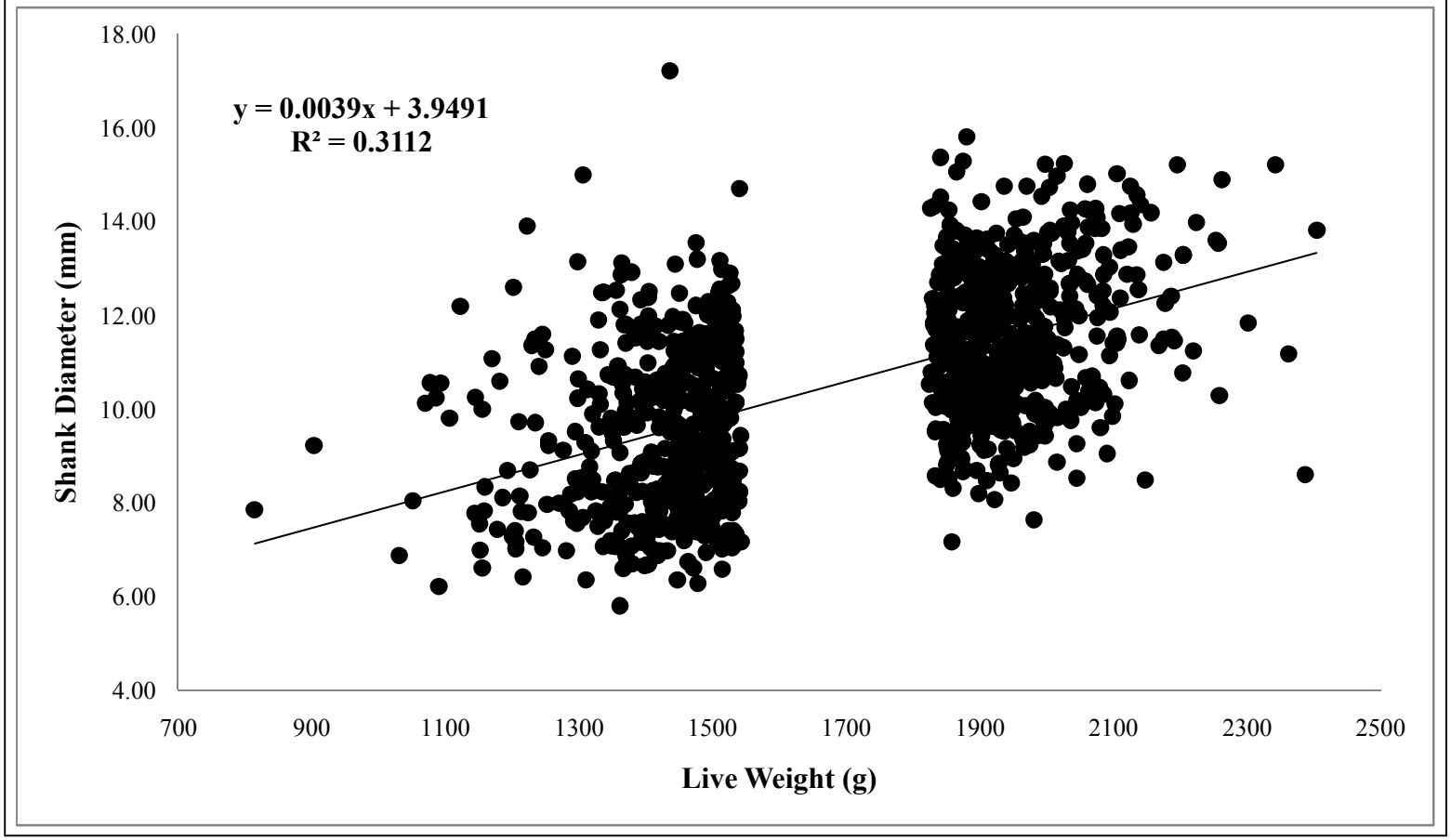


Figure 6.4. Drum stick weight (g)

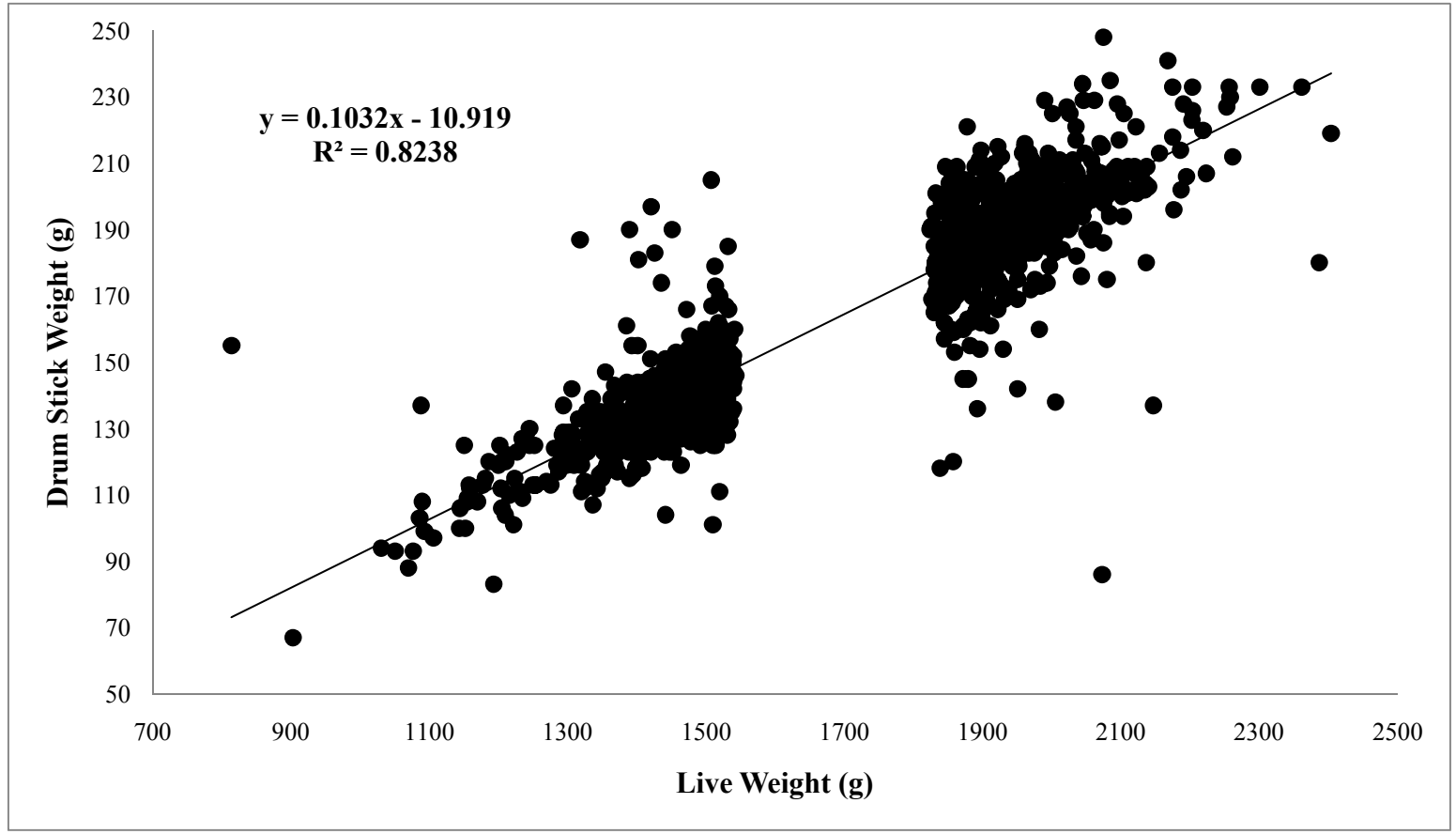


Figure 6.5. Thigh weight (g)

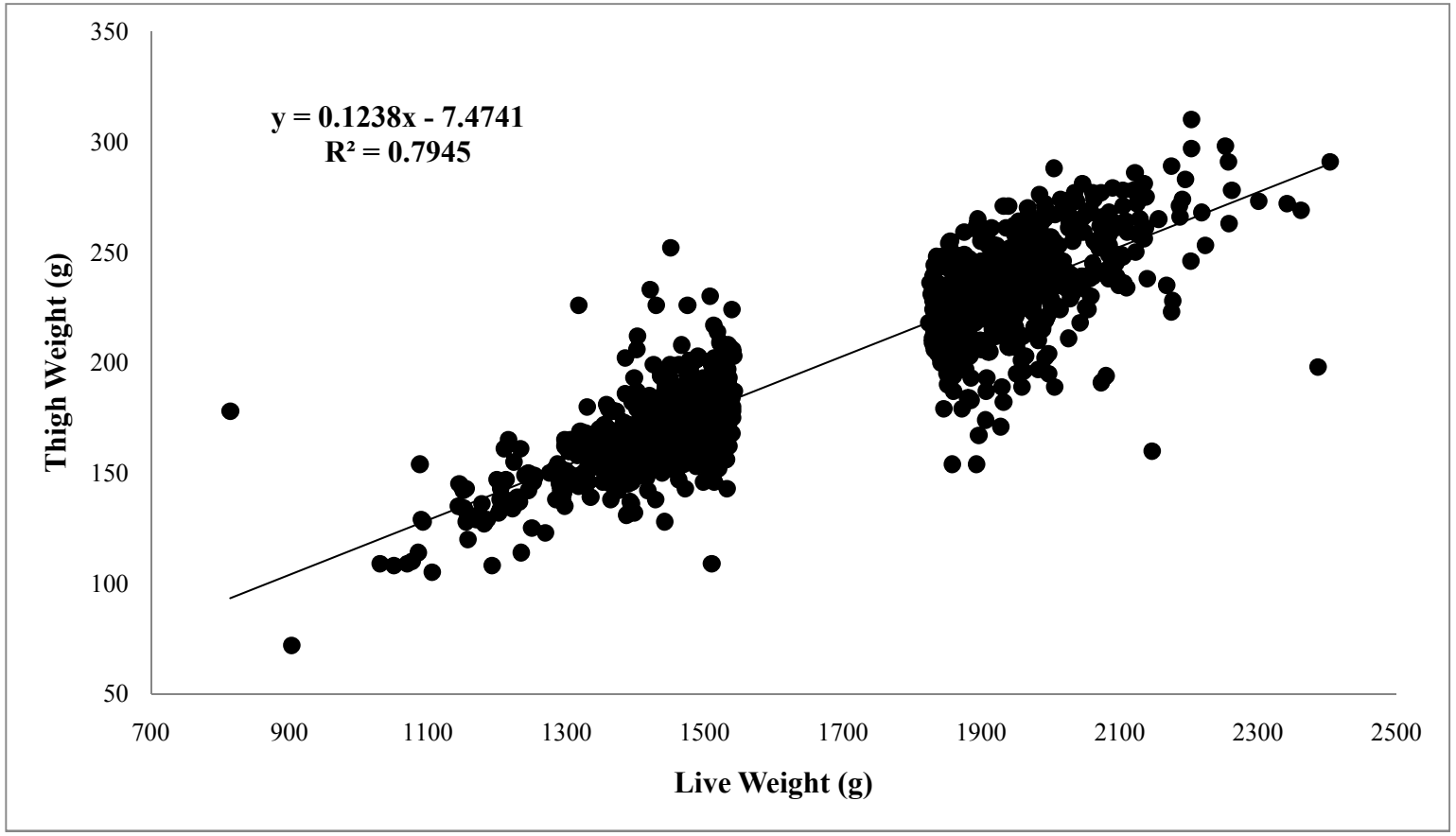


Figure 6.6. Tibia weight (g)

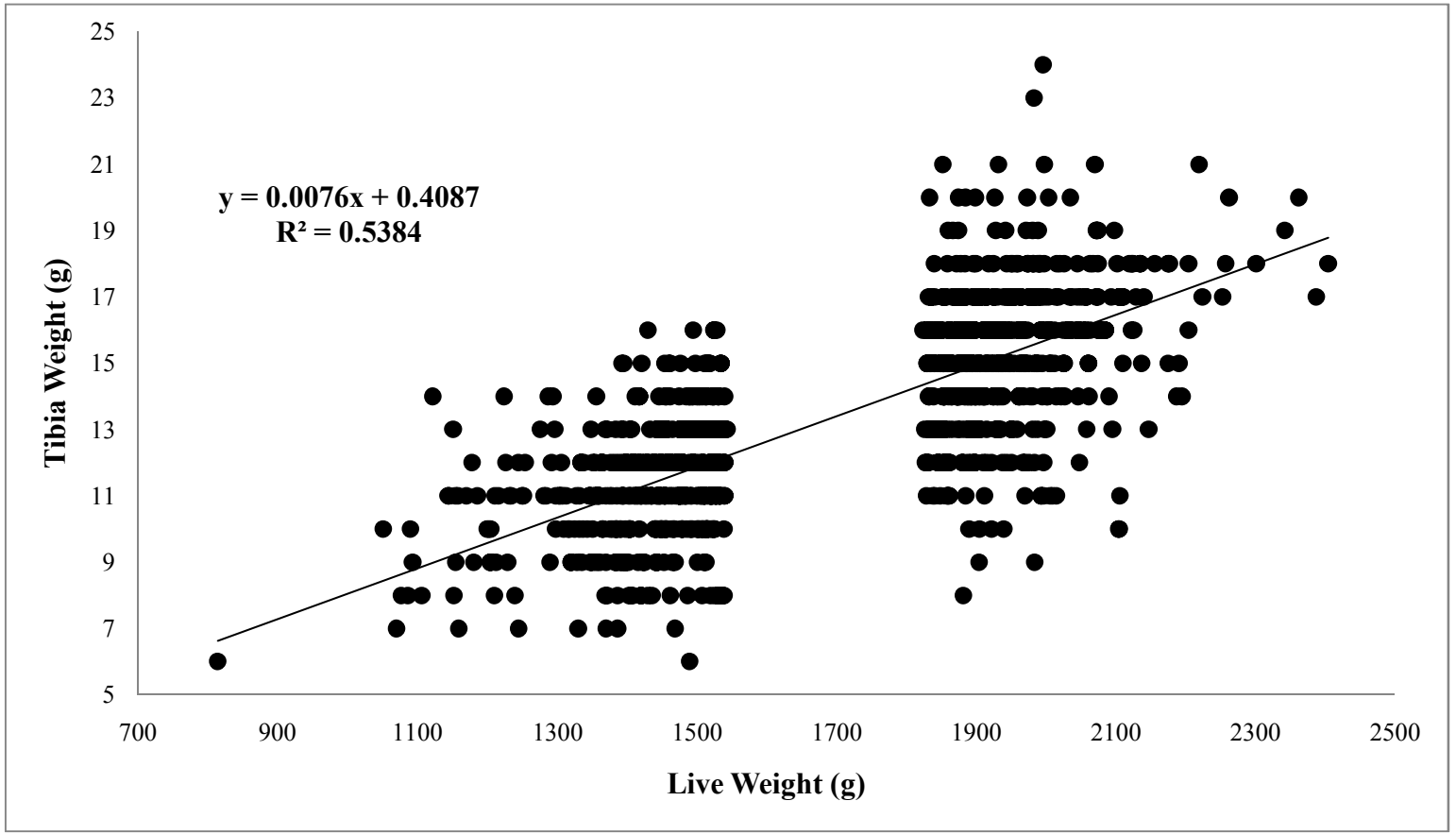


Figure 6.7. Tibia length (mm)

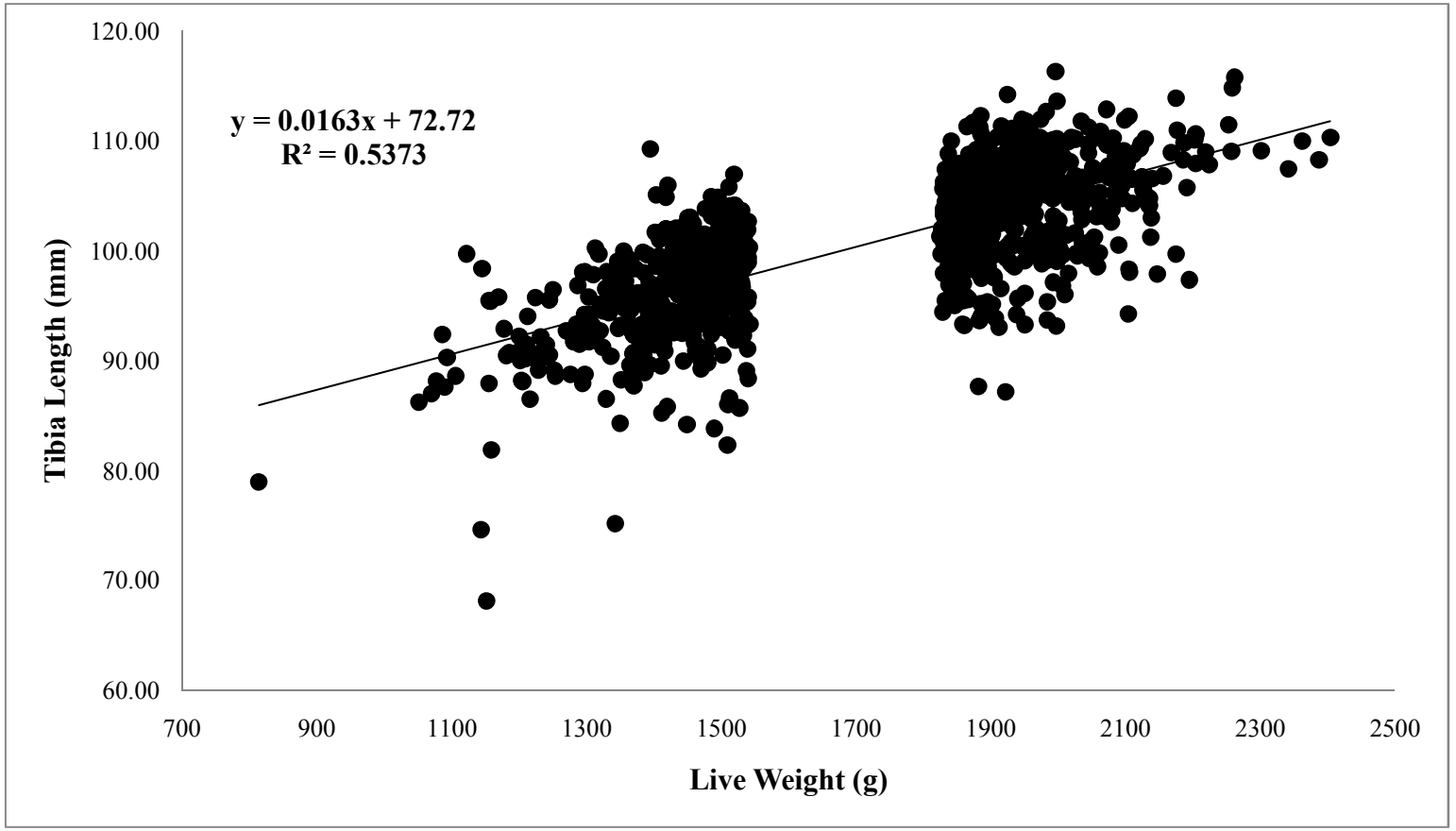


Figure 6.8. Tibia diameter (mm)

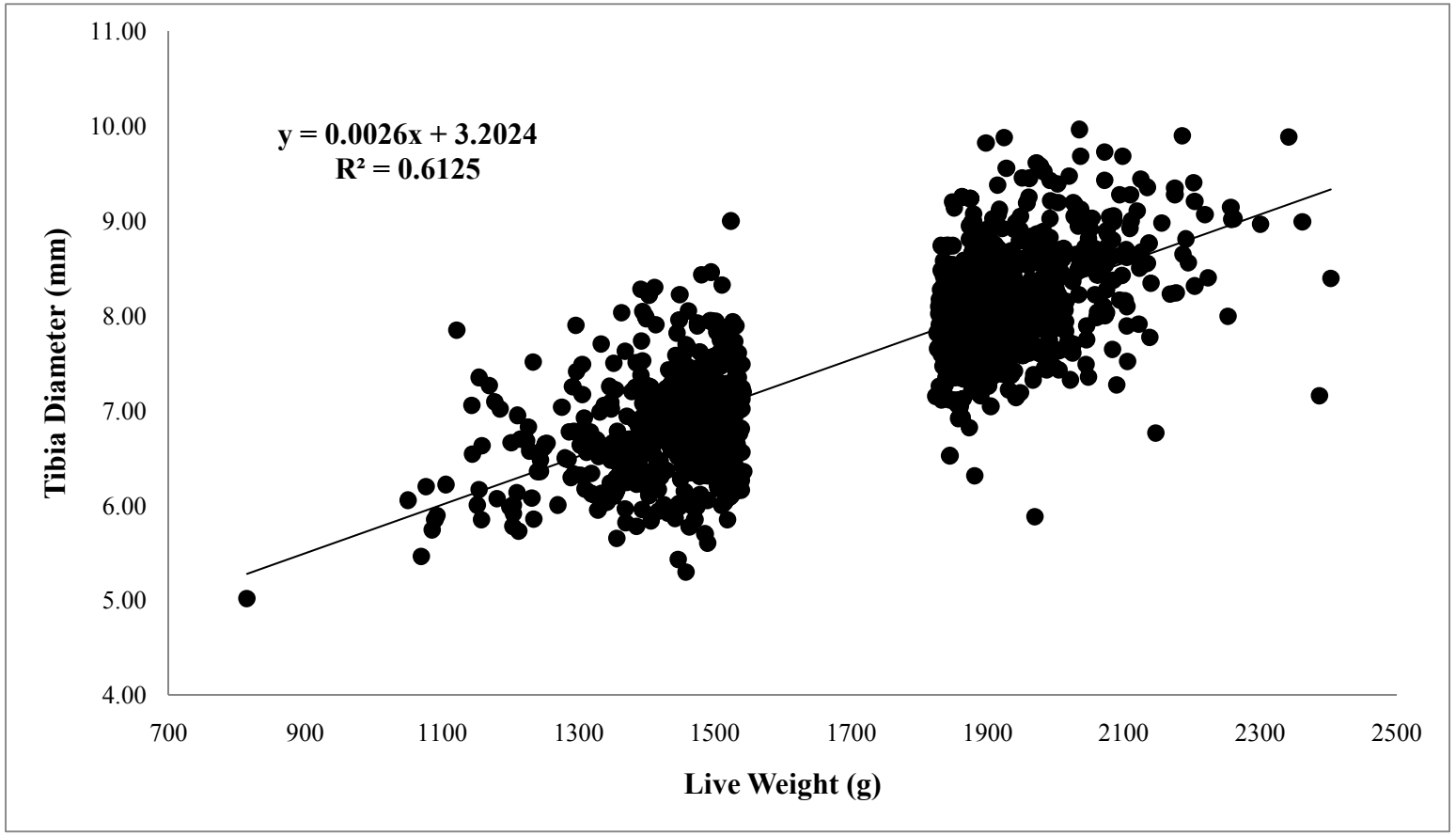


Figure 6.9. Tibia breaking strength (kgf)

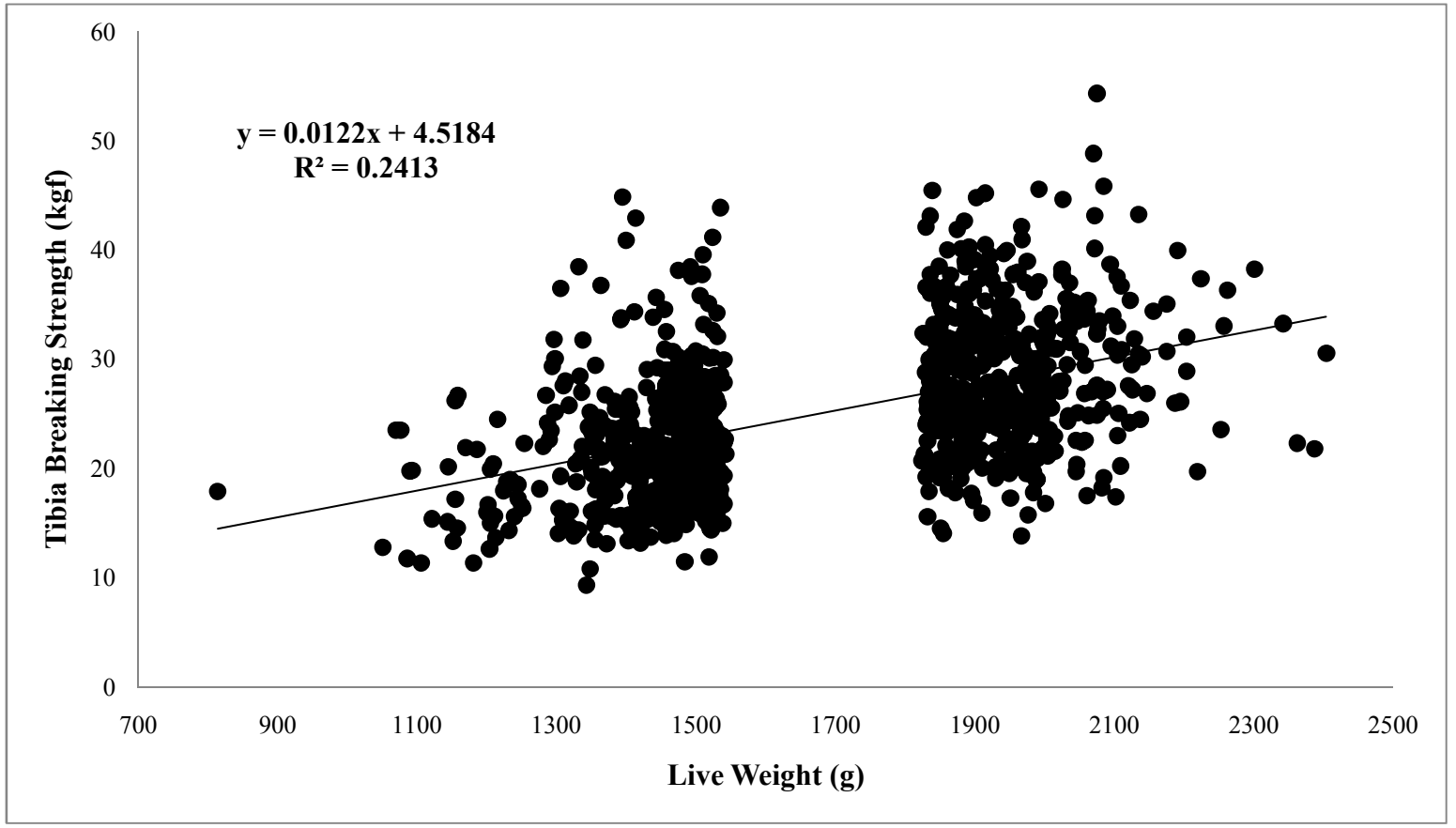


Figure 6.10. Tibia mineral density (g/cm^3)

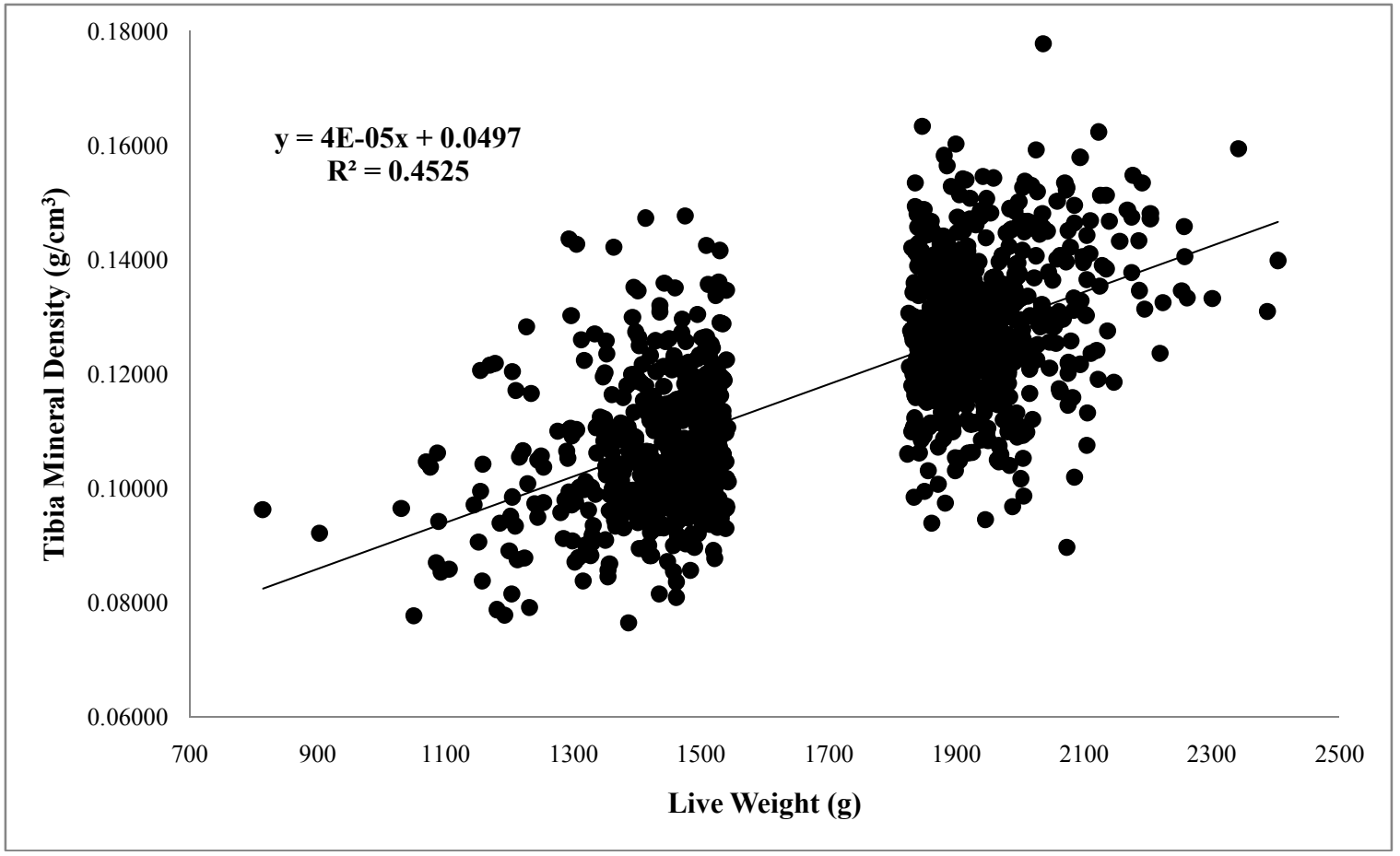


Figure 6.11. Tibia mineral content

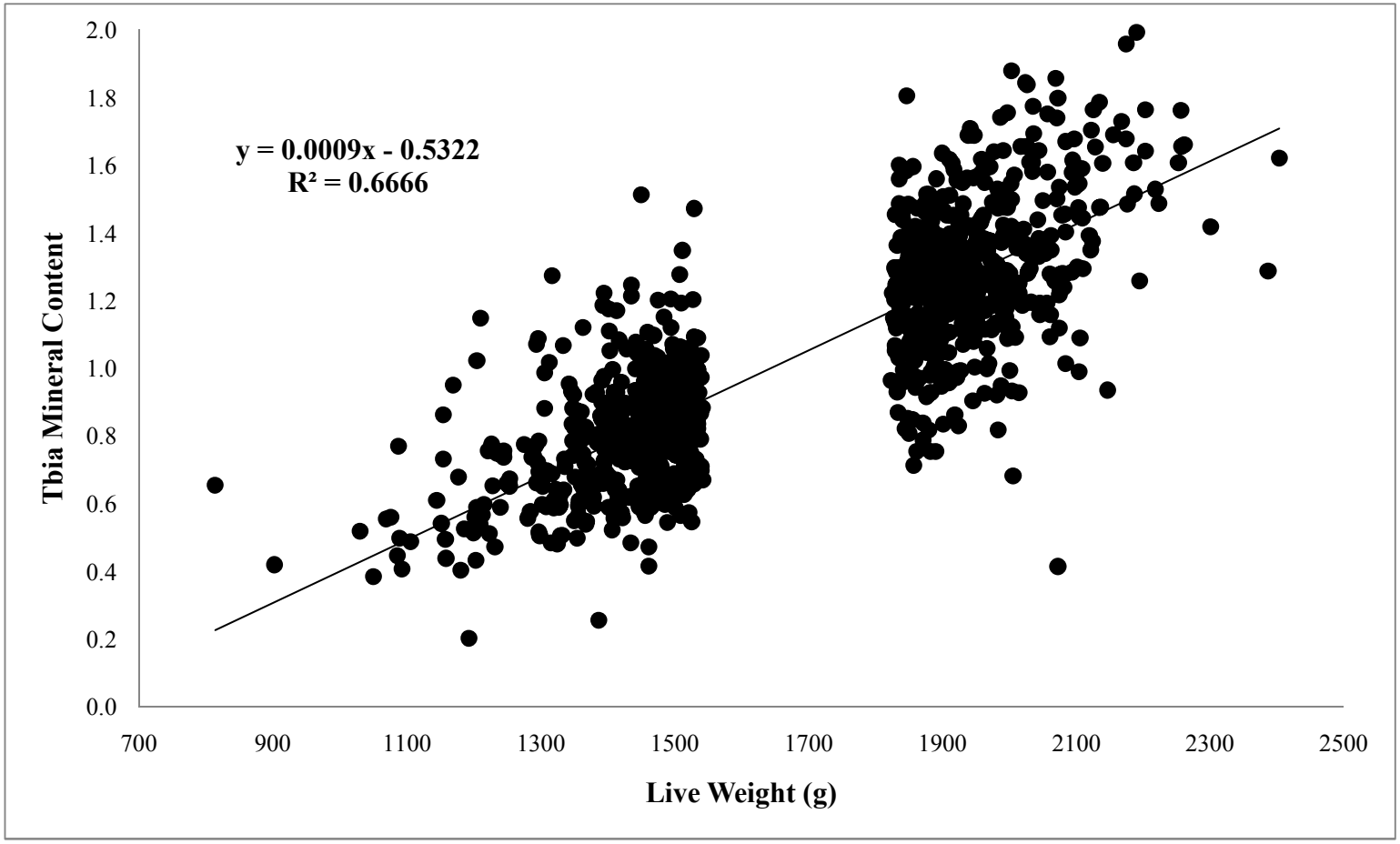


Figure 6.12. Percent tibia ash

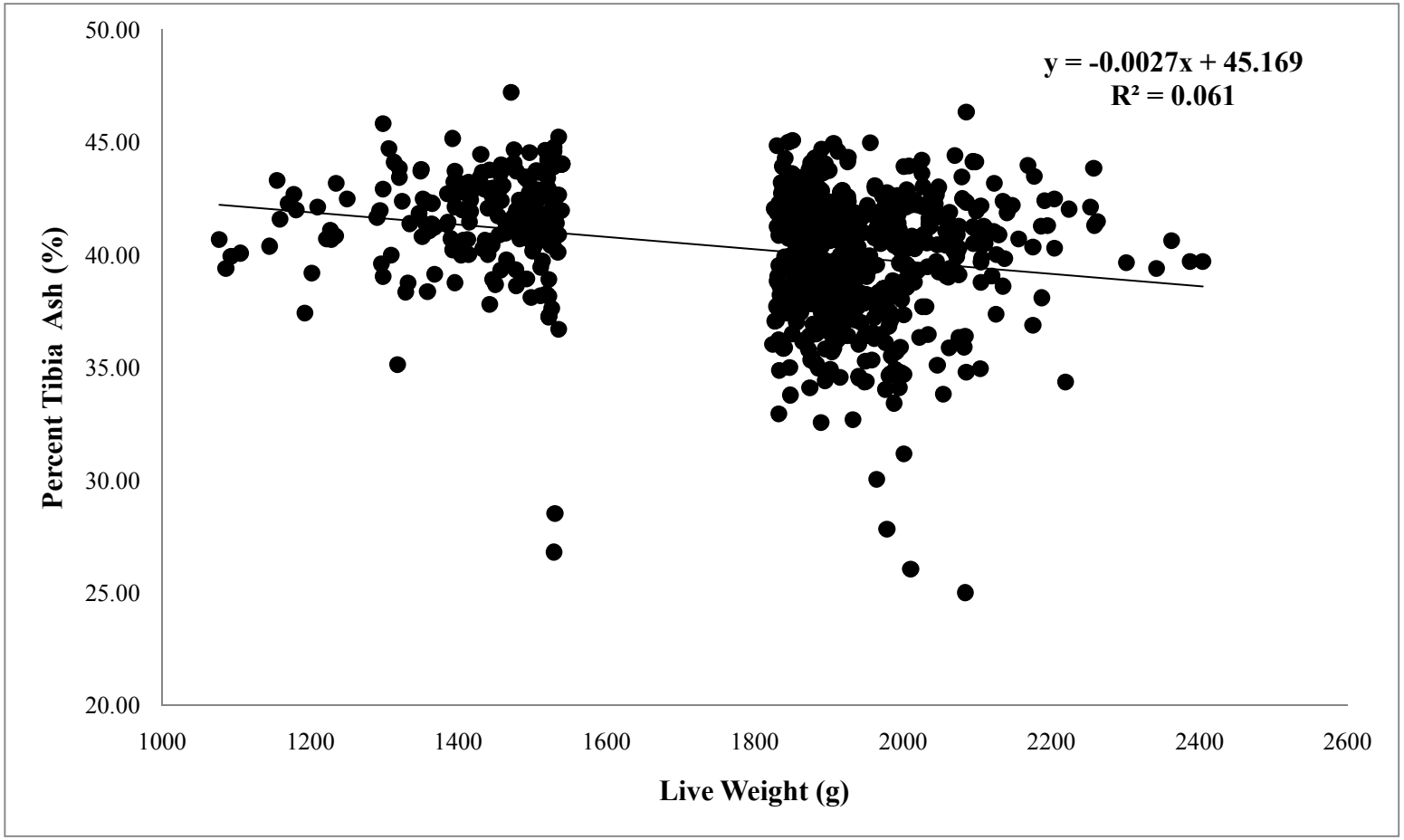


Figure 6.13. Shank weight per unit of live weight

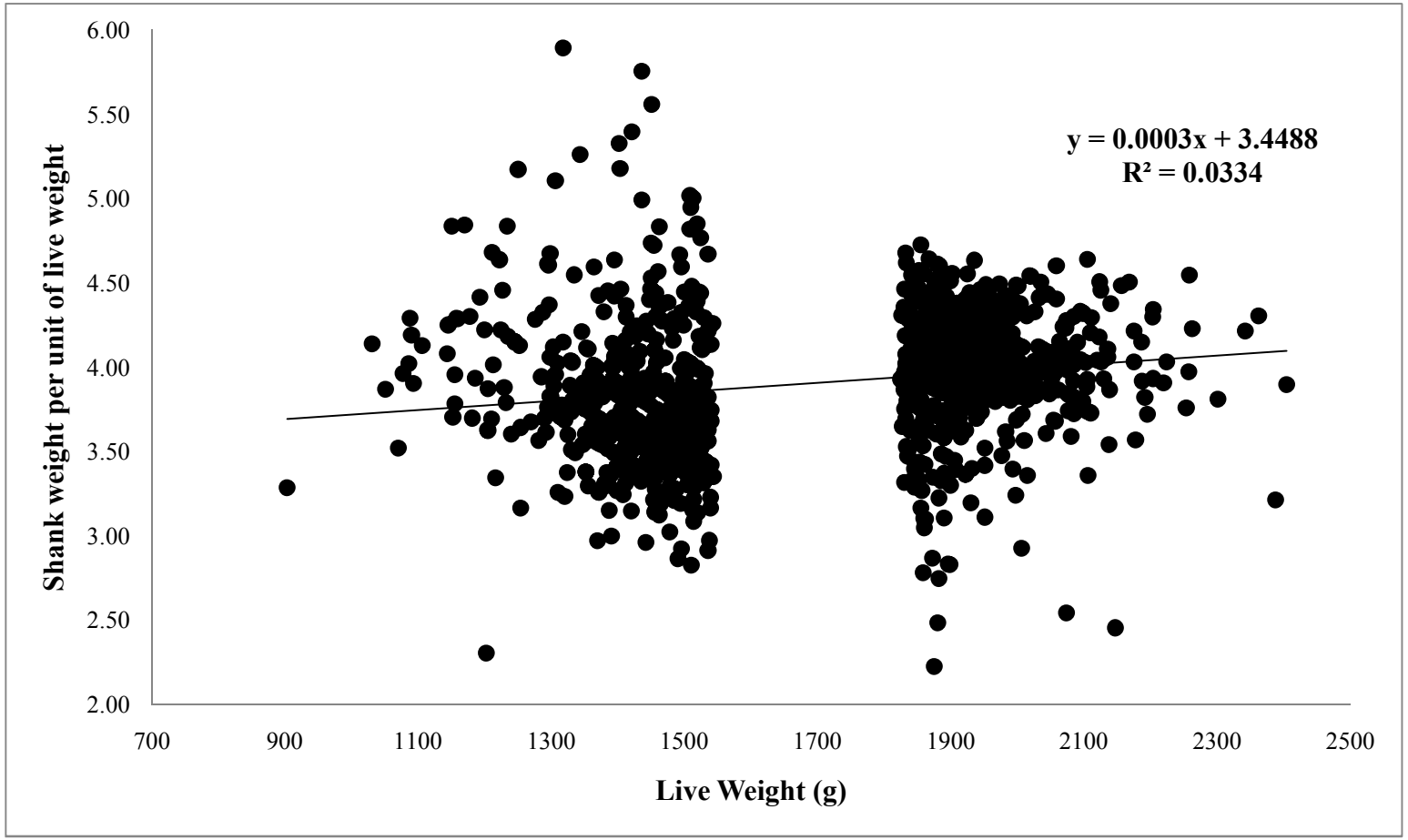


Figure 6.14. Shank length per unit of live weight

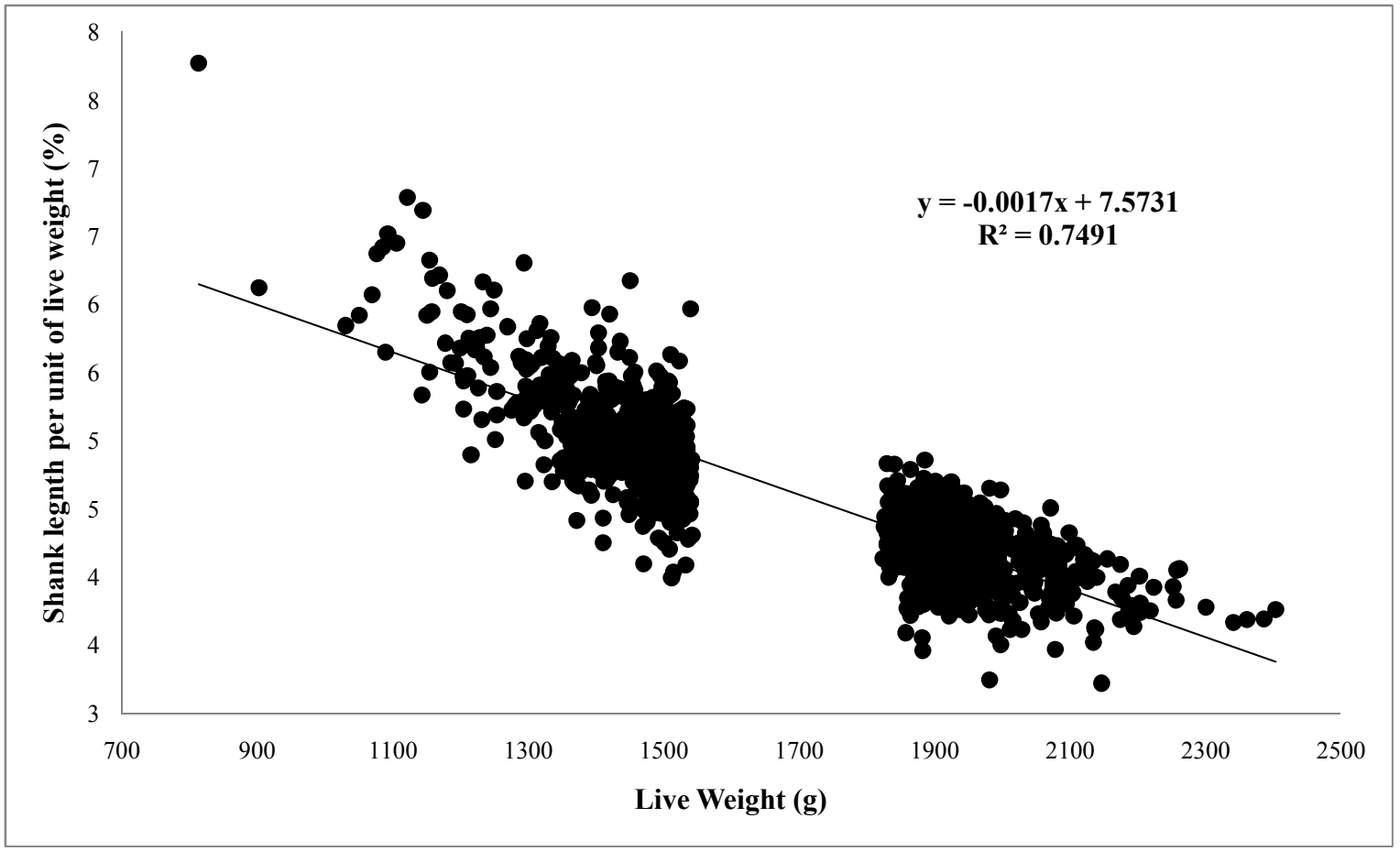


Figure 6.15. Shank diameter per unit of live weight

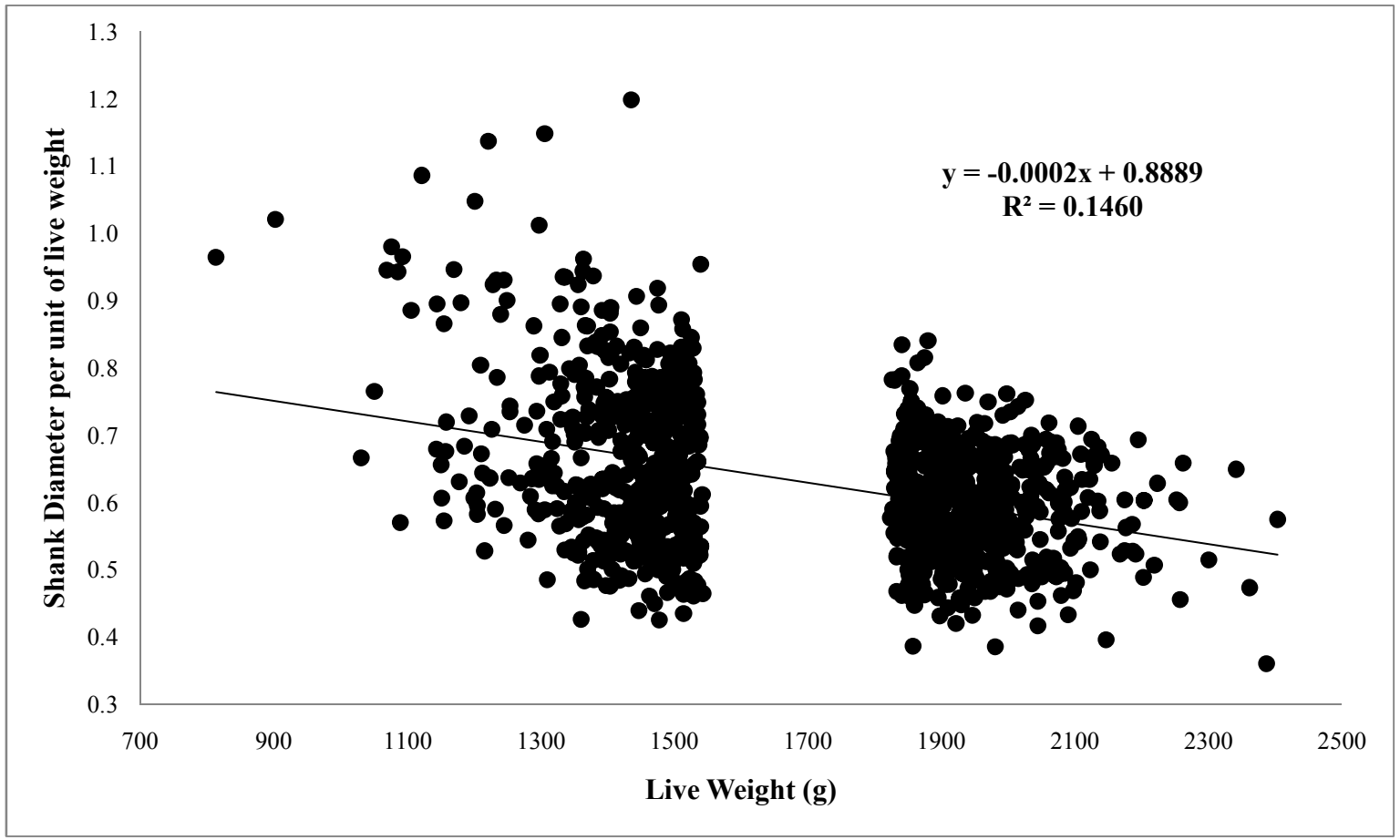


Figure 6.16. Drum stick weight per unit of live weight

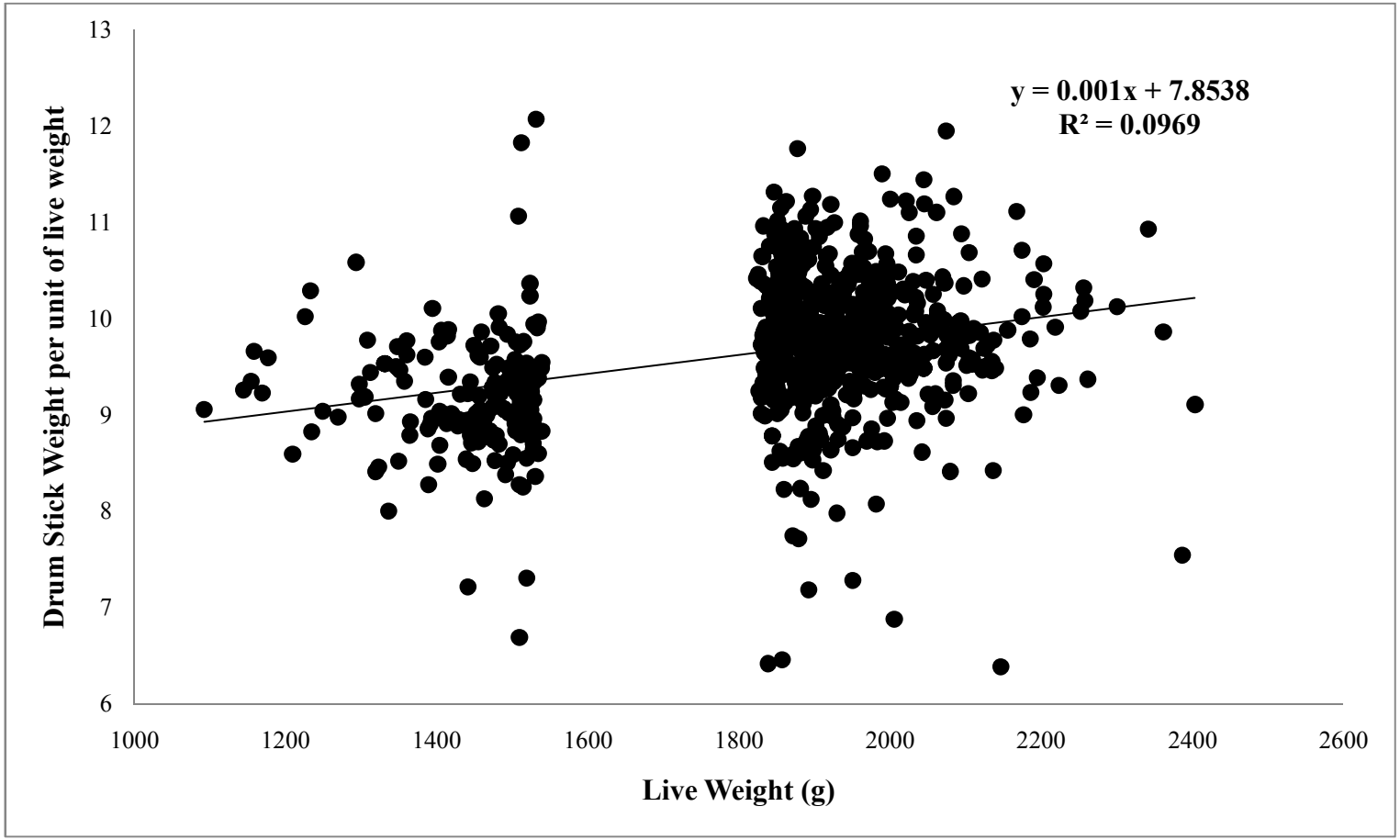


Figure 6.17. Thigh weight per unit of live weight

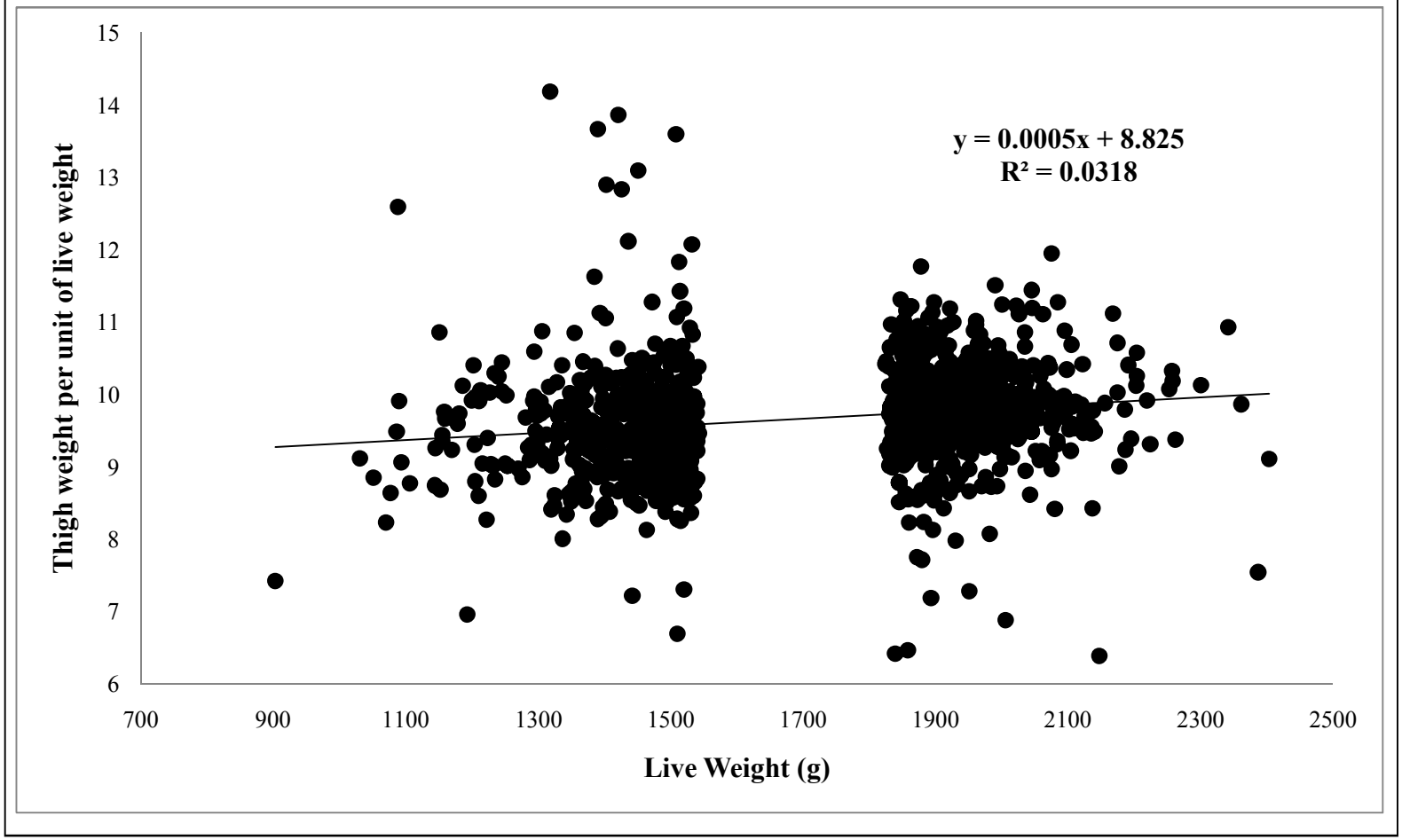


Figure 6.18. Tibia weight per unit of live weight

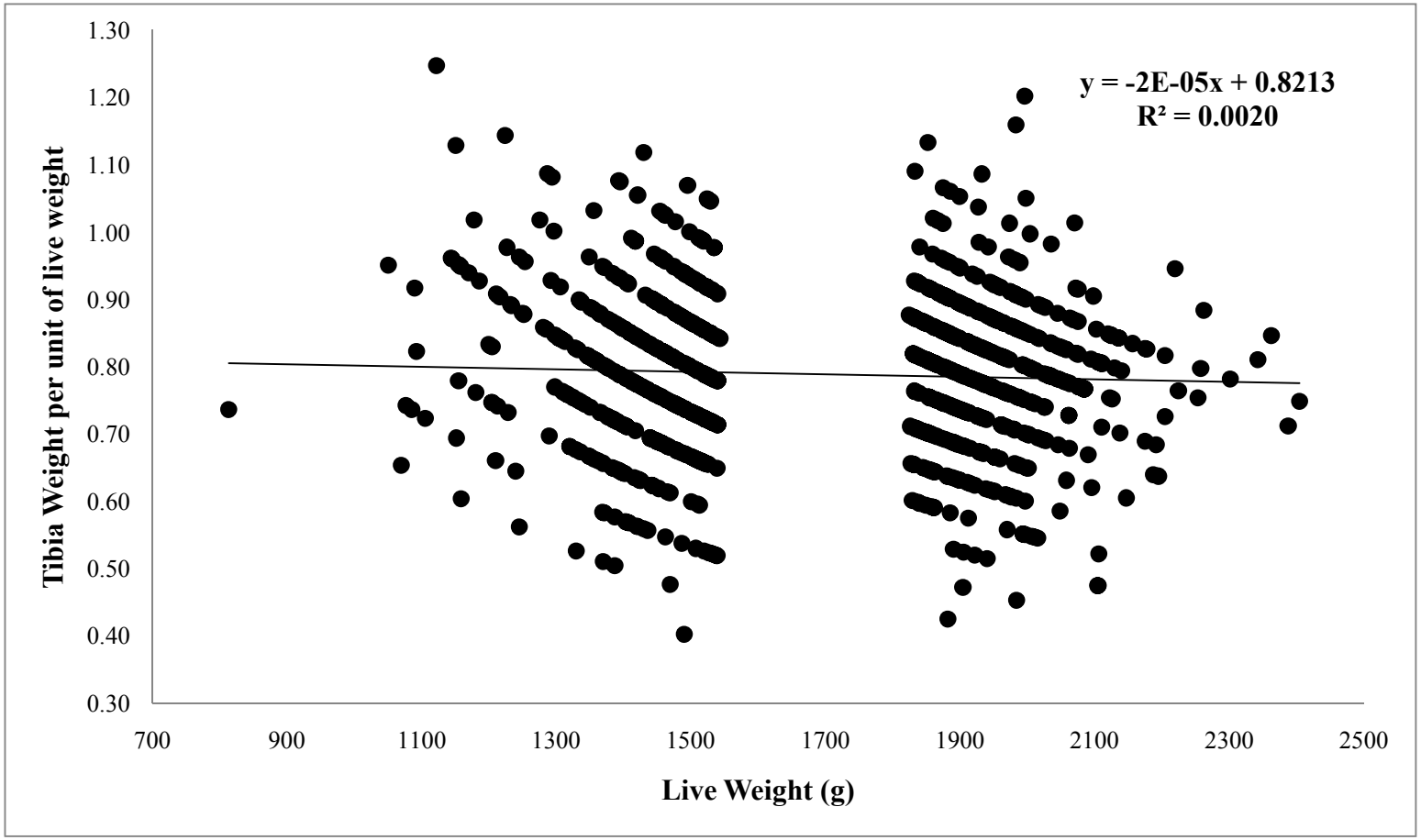


Figure 6.19. Tibia length per unit of live weight

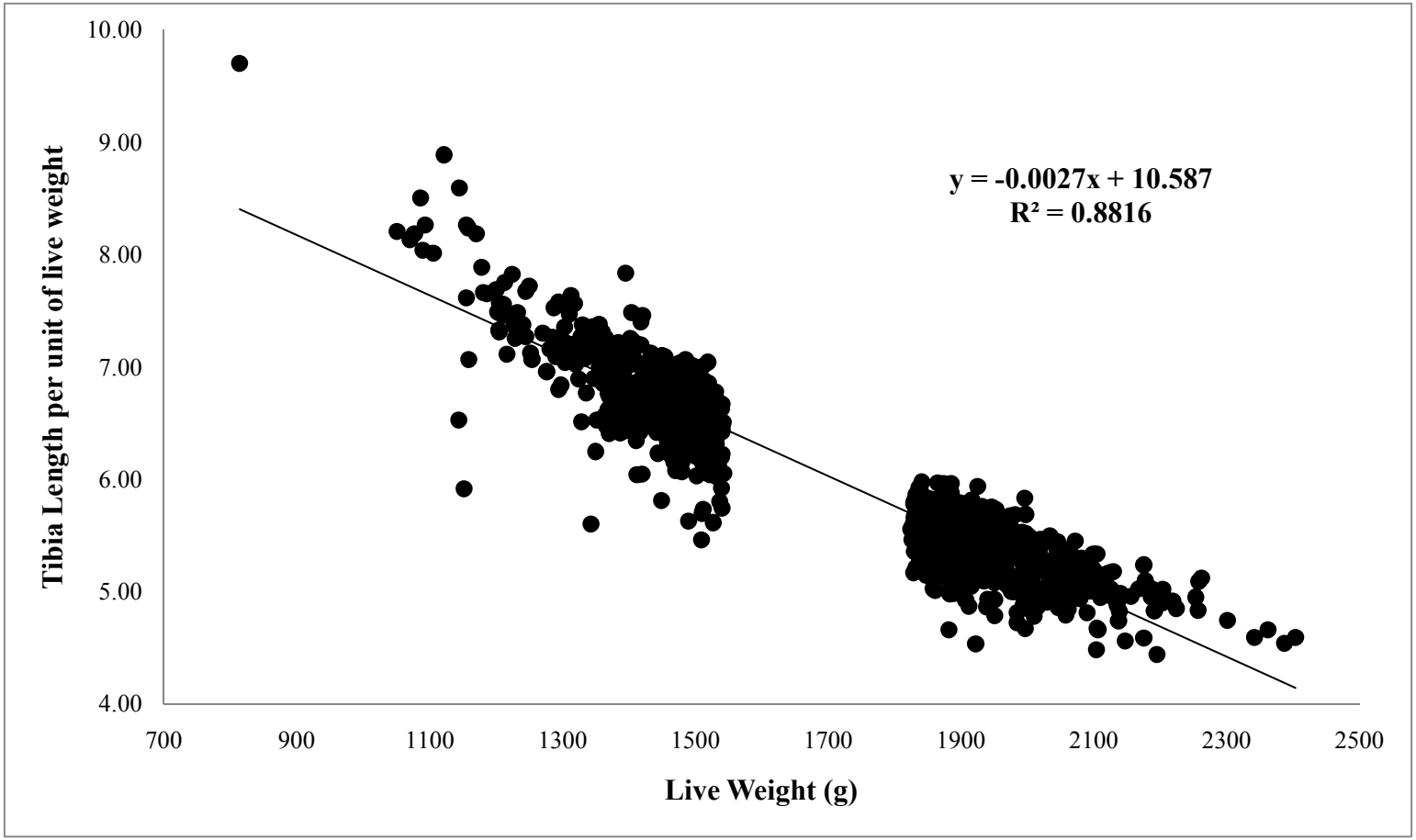


Figure 6.20. Tibia diameter per unit of live weight

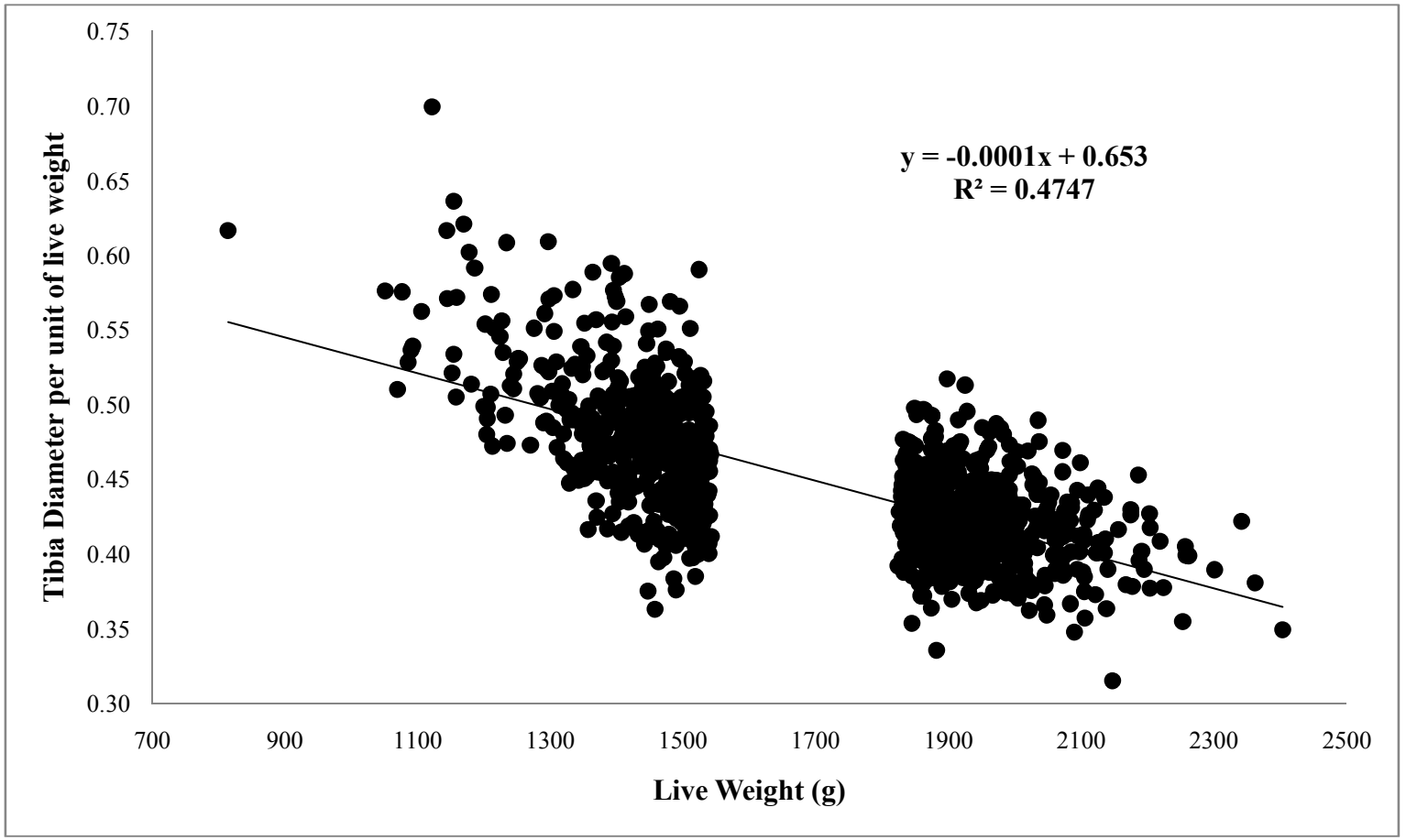


Figure 6.21. Tibia breaking strength per unit of live weight

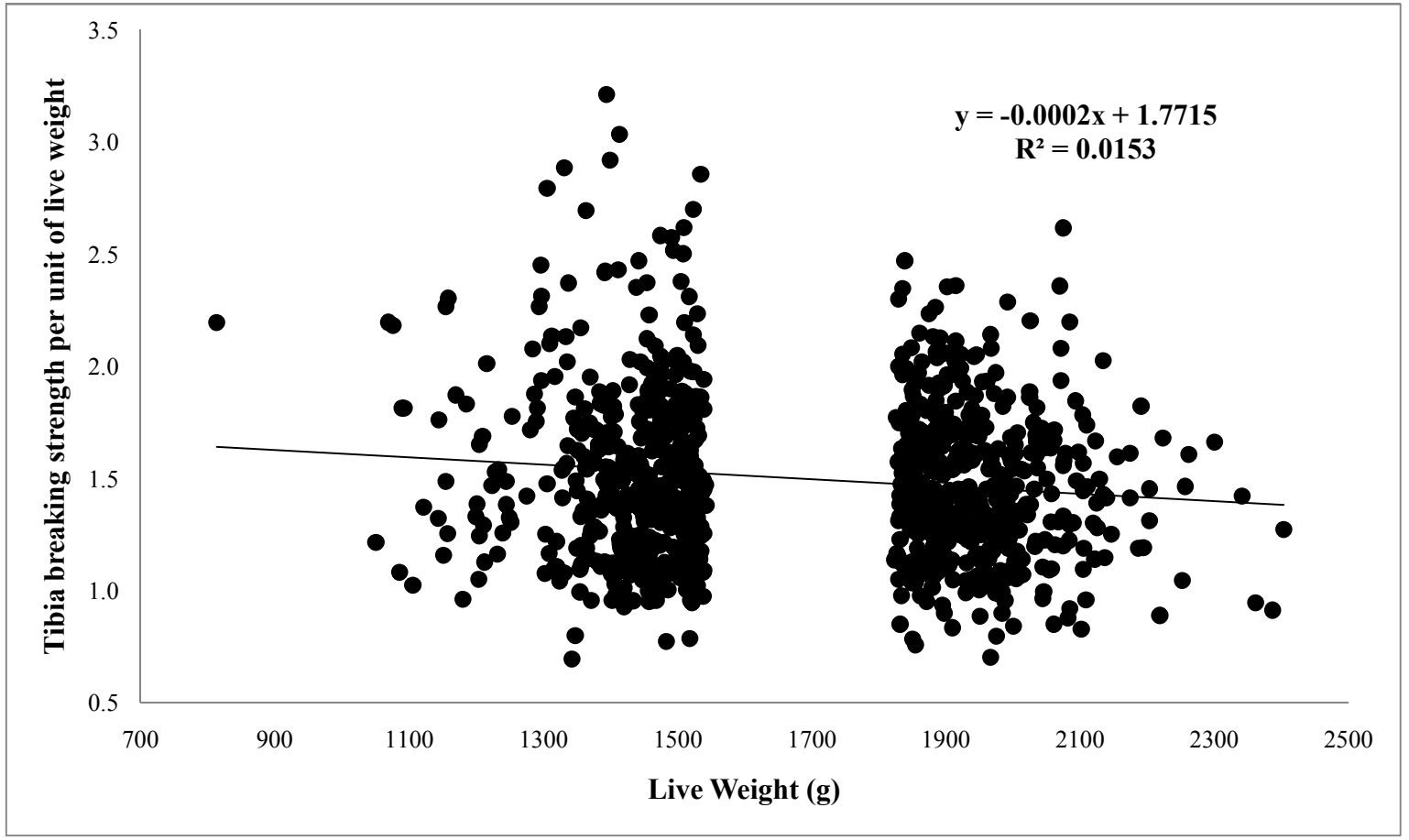


Figure 6.22. Tibia density per unit of live weight

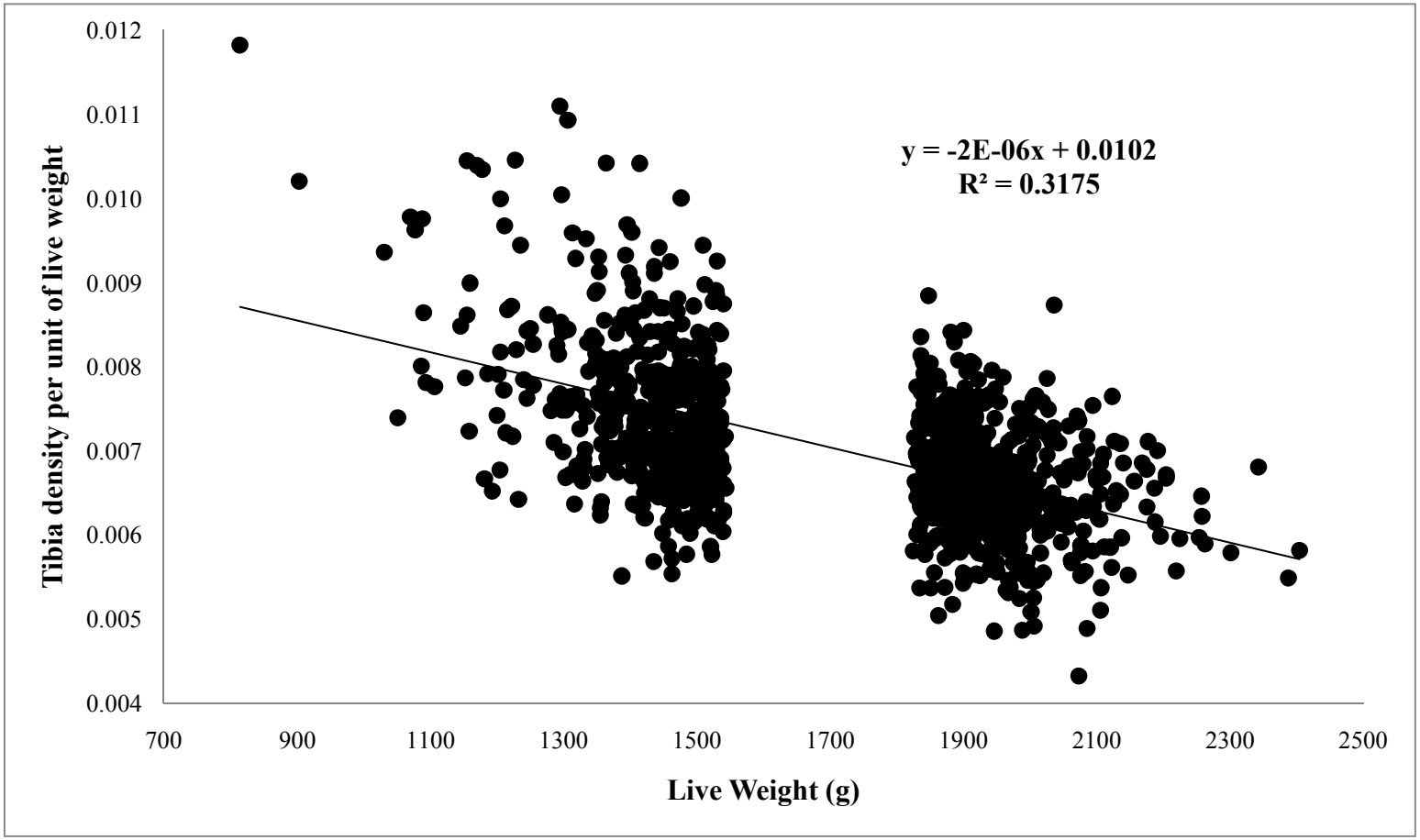


Figure 6.23. Tibia mineral content per unit of live weight

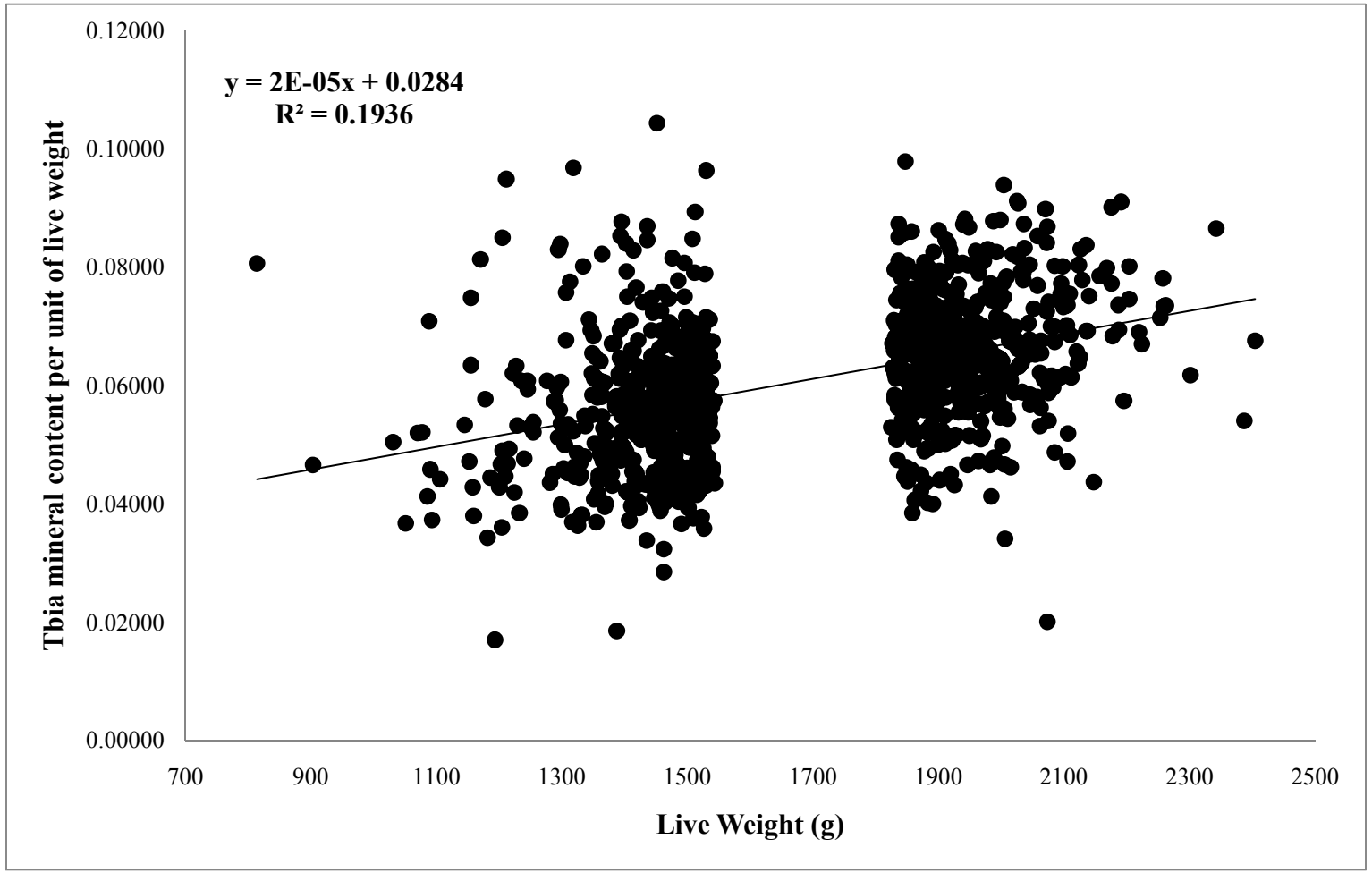
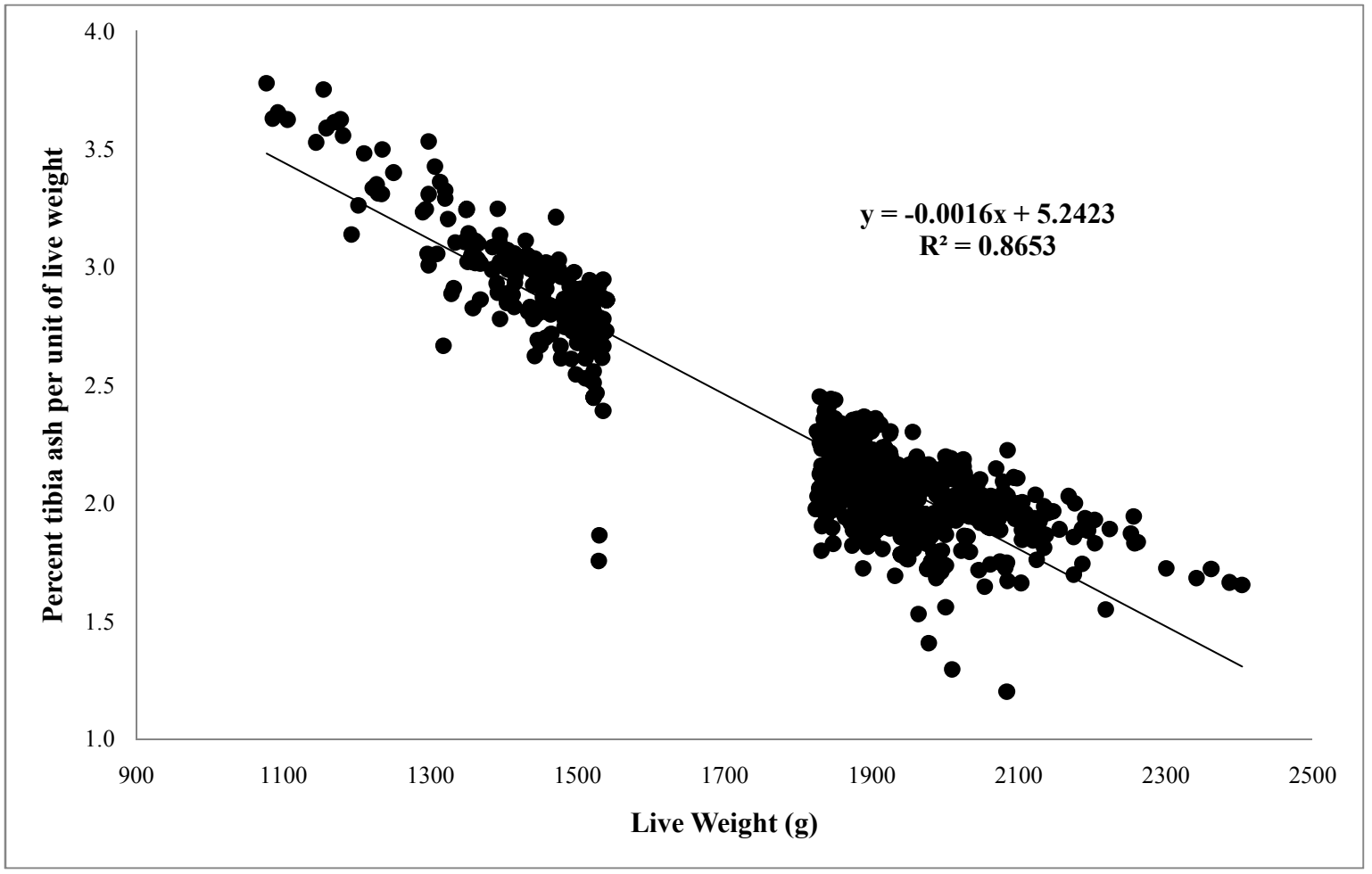


Figure 6.24. Tibia ash per unit of live weight



CHAPTER 7

GENERAL CONCLUSIONS

There is no doubt that the preventive measures that can be taken against leg problems are well known, and they must be continually, and in some cases more effectively, employed to minimize the numbers of birds suffering from leg problems.

Broilers raised under identical experimental conditions and fed diets mixed according to the same formulations and fed diets mixed according to the same formulations performed differently and achieved different degrees of bone mineralization and pathology. Chapter 3 demonstrates that extra heat during ED 4 – 7 has no effect on subsequent TD or P deficiency induced rickets, but it will affect hatching time and hatching chick weights. Identifying and controlling sources of variation allows for a greater understanding of the true relationships and interaction involved in Ca and P nutrition. Low levels of F like those used in Chapter 4 have the potential to create measurable effects. Many of the nutritional factors which influence leg problems are at least understood in general terms and this knowledge must be updated and used by industry as appropriate. Such an effect can have an unforeseen influence on poultry research, particularly in TD and rickets studies, and even reduce profitability to the industry.

The geneticists working for primary breeding companies must continue to select against tibial dyschondroplasia, and the angular deformities, both valgus and varus. This can be difficult as these conditions are not controlled by single genes and are influenced by environmental and

nutritional factors. Some compromise may be also have to be made in the selection for commercial characteristics of growth rate, food conversion ratio, and meat yield if the maximum effect against leg problems is to be achieved. However, it is hard to ensure that birds maintain satisfactory leg strength. Chapter 5 showed that the incidence of leg abnormalities is higher in fast growing chickens than slow growing chickens, and since all the aforementioned leg abnormalities have some genetic basis, breeding strategies could be devised to slow down growth for proper skeletal development before fleshing meat type birds to meet market demands. Chapter 6 confirmed from a single study the hypothesis that bone breaking strength, as evidenced by tibia breaking strength per unit of body weight, is negatively associated with growth rate. This relationship was earlier suspected from anectodal evidence.

Leg disorders also have economic and welfare implications. Birds with serious leg disorders are not able to walk to the feeders and feed properly and as result have reduced body weight and consequently are culled. Decreasing leg disorders would in principle also improve the wellbeing and welfare of birds.

Appendix A. Frequency of qualitative data (valgus, varus and TD score) in Chapter 6

	Valgus Left				Valgus Right				Varus Left				Varus Right				TD[1]			
Population	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
SG	471	67	43	0	465	72	44	0	570	10	1	0	562	18	1	0	575	2	2	2
FG	413	110	62	0	423	100	62	0	576	8	1	0	555	27	3	0	570	3	2	10

[1] TD = Tibial Dyschondroplasia