GENETICS OF SKELETAL INTEGRITY IN MEAT TYPE CHICKENS

by

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(Under the Direction of SAMUEL E. AGGREY)

ABSTRACT

Genetic selection in meat-type chickens has led to an impressive improvement of important traits such as growth rate, feed efficiency and meat yield. Unfortunately, this improvement in production performance has led to the increased incidence of metabolic disorders such as leg problems. Furthermore, it has been indicated that the optimal growth and development of the skeleton have been compromised. Leg problems and low quality bones impact negatively the economy of the meat-type chicken industry and also affect the welfare of the birds. Although those skeletal issues have been directly associated with improvement of growth rate, the genetic association among leg problems, bone quality traits and growth rate has not been conclusively evidenced. In order to devise practical breeding strategies it is necessary to ascertain adequately the nature of that genetic relationship.

We carried out different analyses to study the genetic basis of leg problems such as tibial dychondroplasia (TD) and varus-valgus deformities (VVD), and several bone quality traits, and studied the genetic association among leg problems, bone quality traits and growth rate. The leg problems and the bone quality traits that we studied have an additive genetic component. It was found that the genetic associations of TD and VVD with growth rate and bone quality traits were

weak. The results suggested that genetic selection for growth rate impact negatively some traits that are related with the integrity of leg bones.

INDEX WORDS: Genetics, leg problems, bone quality, broiler chickens

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DEDICATION

To my mother…

Elena Cerón-Rodríguez

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CHAPTER 1

INTRODUCTION

It is estimated that, between 2011 and 2013, about 840 million people suffered chronic hunger in the world. A more productive agriculture and higher food availability could contribute to hunger reduction (FAO, 2013a). Poultry is an important component of the system that feed people globally; it is estimated that there are 20 billion of chickens around the world which represent 80% of all livestock (FAO, 2013b). Genetic selection programs applied to meat type chickens during the last 6 decades have achieved an accelerated growing rate and a shorter time to slaughter (Havenstein et al., 2003). However, these improvements have come accompanied by some skeletal defects (Velleman, 2000) that affect the performance and the welfare of the birds (Julian, 2005).

Bone ash (Hall et al., 2003), bone ash concentration (Cheng and Coon, 1990), bone mineral content and density (Schreiweis et al., 2003), and bone breaking strength (Rath et al., 2000) represent indicator traits for the assessment of bone status in poultry. Skeletal integrity can also be compromised by leg problems such as tibial dyschondroplasia and varus-valgus deformities, two common lesions in broiler chickens (EC, 2000). Although it has been demonstrated that some indicator traits of bone status and leg problems possess an additive genetic component (de Verdal et al., 2013; Rekaya et al., 2013), their genetic relationship with growth rate seems not conclusive (Leterrier et al., 1998; Williams et al., 2000; Zhang et al., 1995; Kuhlers and McDaniel, 1996) or has not been documented. On the other hand, genomic tools could help to the uncovering of the molecular basis of the phenotypes observed in domestic animals (Andersson, 2001) and the

probable identification of the genes underlying those (Siegel et al., 2006). In this sense, there exists evidence of efforts directed to the understanding of skeletal integrity traits in molecular genetics terms (Zhou et al., 2007; Hu et al., 2013; Chen et al., 2011).

The objectives of the current dissertation were to study the genetic basis of leg problems and diverse indicator traits of bone quality. Likewise, it was pursued to investigate the additive genetic association among those skeletal integrity traits and also their relationship with growth rate. The design of practical breeding strategies directed to the joint optimization of broiler traits and fitness characteristics, such as skeletal integrity traits, requires of the knowledge of their genetic parameters.

CHAPTER 2

LITERATURE REVIEW

Growth

Growth can be defined as the increase in size and weight of an individual over time (Marple, 2003), and it is characterized by the processes of hyperplasia and hypertrophy (Trenkle and Marple, 1983). Along with these changes, animals also experience changes in their form because of adjustments in physiological needs as they become bigger and mature. These modifications, related to the physiological homeostasis, are important in terms of animal's survival (Lawrence et al., 2012). Growth has driven a significant part of the improvement that has been achieved by broiler companies which has resulted in a reduction in market age thereby reducing the cost of production (Griffin and Goddard, 1994; Havenstein et al., 2003). The growth pattern of chickens can be described by mathematical models such as the Logistic, Gompertz and Richards growth equations (Trenkle and Marple, 1983; Aggrey 2002). Growth curve functions are considered the most suitable mean for description of growth pattern given that they summarize data into a few parameters that can be interpreted in a biological way (Aggrey, 2002; Goliomytis et al., 2003; Nahashon et al., 2006). Growth in animals is allometric instead of isometric, which means that it is proportional with respect to tissues and organs of the body (Scanes, 2003); and this pattern has been confirmed in broiler chickens (Gous et al., 1999).

Bone growth and development

The skeleton is a structural support system that adapts to compressive and bending stress through mechanisms of bone growth and remodeling (Lawrence et al., 2012). Bone tissue consists of cells interacting in a matrix formed by hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and collagen. While the mineral part gives strength and resistance to compression, the latter helps to deal with tension and torsion. Basic organization of bones comprises of the thin, dense, and compact outer cortical bone, the marrow cavity that contains hematopoietic elements and the trabecular bone which is spongy (Currey, 2002). Ossification results from the proliferation of osteoblasts that deposit the osteoid, an extracellular matrix comprised by collagen, mucopolysaccharide and glycoprotein, which in turn eventually encloses them. Osteoblasts mature to non-dividing osteocytes and the organic matrix is impregnated with hydroxyapatite (Lian and Stein, 2006). Bone growth is characterized by two components: endochondral growth (length) and appositional growth (width). Long bones, such as femur, tibia, and humerous, growth in length by the former of those components in addition to the activities of the cartilaginous growth plate, the region of bone elongation (Robling et al., 2001; Boskey, 2006). Farquharson (2003), Khurana and Safadi (2010), and Lawrence et al. (2012) describe the processes of bone growth and remodeling. Appositional growth of the bone is related to the increment in width which allows dealing with the stress resulting from the progressive increment in body weight. Two processes can be distinguished. First, osteoblasts in the bone periosteum participate in the laminar deposition of bone which forms the outer circumferential lamellae. In this process, blood vessel and periosteal elements are trapped and covered by bone to form primary osteons, the structural unit of bone. Second, osteoclasts act via bone resorption in the endosteum. Thus, these coupled processes lead to the increment of the

diameter of the bone and of the bone marrow. Increments in the diameter of the bone and in the cross-sectional area of the cortical part affect bone strength.

Endochondral growth results from the process of chondrocytes maturation and changes in their extracellular matrix in the growth plate that eventually lead to the replacement of the cartilage template by bone. The extracellular matrix is comprised by proteoglycans, mainly aggrecan and collagen of the types I, II, IX and XI. Bone morphogenetic proteins (BMPs) and the 1,25-D metabolite of vitamin D_3 stimulate cartilage matrix formation. Based on the maturational stage of chondrocytes, the growth plate can be divided in the next zones: resting, proliferative, maturing and hypertrophic. The resting zone allocates undifferentiated stem cells that will start cartilage formation or chondrogenesis by differentiation into chondrocytes of the proliferative zone. Cells in this zone carry out cell division and reach the stage of pre-hypertrophic chondrocyte that in turn finally differentiates into hypertrophic chondrocyte. Posterior matrix mineralization and vascularization eventually facilitate to the formation of the bone. Growth hormone (GH), insulin-like growth factor I (IGF-I), parathyroid hormone-related peptide (PTHrP), morphogen indian hedgehog (Ihh), transforming growth factors-β (TGF-β), Smad proteins, BMPs, fibroblast growth factor, and the 1,25-D metabolite act as mediators of chondrocyte differentiation.

Hypertrophic chondrocytes are characterized by greater volume and height than cells in previous stages; they also have greater matrix production, higher membrane alkaline phosphatase (ALP) activity, expression of collagen type X, chondrocalcin, osteonectin, osteopontin, but lower expression of collagen type II. Subsequently, extracellular matrix is mineralized which will allow the process of vascularization. Matrix mineralization is increased by ALP activity and can be observed in two phases. First, calcium and phosphorus form a complex that is transformed into hydroxyapatite. Later, hydroxyapatite penetrates matrix vesicles to start the formation of additional

crystal. This process is dependent of concentration of Ca^{2+}, PO_4^{3-}, pH, and proteoglycans. TGF-β, BMPs, and the 1,25-D metabolite participate in matrix mineralization. Vascularization takes place at the chondro-osseous junction; angiogenesis starts with the migration and replication of vascular endothelial cells. Furthermore, hypertrophic chondrocytes express angiogenic factors such as the vascular endothelial growth factors (VEGFs). Vascularization facilitates the recruitment of osteoclast precursor cells and perivascular osteoblast progenitors. This permits resorption of terminal chondrocytes and part of the mineralized septa by osteoclasts. The mineralized septa that is not resorbed is used for the osteoid deposition by osteoblasts. Later, osteoid is mineralized to form the primary spongiosa which in turn will be replaced by secondary spongiosa made of lammelar bone. During growth, bone is continuously under mechanical and metabolic variations that induce bone remodeling. While mechanical loading enhances bone formation, reduced loading by immobilization or weightlessness induce bone loss. Mechanical stress alters expression of genes such as the receptor activator of NF-κB (RANK) that in turn induce osteoclast differentiation and bone resorption. This allows vascularization and osteogenic precursor cells recruitment that in turn start the ossification process.

Non-genetic factors affecting leg bone health

Indicator traits of bone status in broiler chickens, such as bone breaking strength, bone ash content, and bone length, have showed an additive genetic component with heritability ranging from 0.4 to 0.7 (Mandour et al., 1989; de Verdal et al., 2013; Merritt, 1966). Tibial dyschondroplasia (TD) and varus-valgus deformities (VVD) have shown heritability ranging from 0.10 to 0.38 (Sheridan et al., 1978; Mercer and Hill, 1984; Le Bihan-Duval et al., 1996, 1997; Kapell et al., 2012; Rekaya et al., 2013). However, the whole health of the leg results from the interaction of those heritable factors with other ones such as nutrition and management (Edwards,

Jr., 1992). This situation is even more relevant in those cases where it is known that the cause of a leg problem is not of infectious origin (Reiter and Bessei, 1998).

Nutrition

Edwards (1992, 2000) and Fleming (2008) reviewed the role of nutrition on bone health in poultry, highlighting its importance for production performance maximization and the negative effect of imbalance and deficiency of nutrients on bone health. These authors have pointed out the relevant role of cholecalciferol ($1,25(OH)_2D_3$), calcium, and phosphorus in cell differentiation in bone development and bone mineralization. Likewise, vitamins A and E, nicotinic acid, folic acid, ascorbic acid, magnesium, chloride, sodium, zinc, copper, boron, fluoride, silicon, vanadium, cysteine, cysteine, homocysteine, fatty acids, protein, and energy are mentioned as nutritional factors of interest for bone health.

Management

Classen (1992) and Bradshaw et al. (2002) highlighted the relevance of the rapid growth rate of modern broiler chickens on the incidence of leg problems. It is mentioned that the modification of some management factors has been investigated as strategy to promote the correct development of the skeleton. Restriction of the growth, through light management, feed restriction or alteration of feed quality, is one of the strategies used. From a survey of commercial broiler chicken flocks, Knowles et al. (2008) corroborated the aforementioned authors and suggested that more natural lighting patterns, and modifications in stock density and feed consumption by changing the physical presentation of the feed, can contribute to a better leg health but with the cost of a reduced growth rate.

Indicator traits of bone status

Evaluation of bone status in poultry has been assessed with a variety of methods (Kim et al., 2004) that include bone ash (Hall et al., 2003), bone ash concentration (Cheng and Coon, 1990), bone mineral content and density (Schreiweis et al., 2003), and bone breaking strength (Rath et al., 2000). It has been pointed out that in genetic selection programs, noninvasive methods are more useful than the invasive ones (Hester et al., 2004). This is important because studies in animal models (Klein, 2002; Alam et al., 2011), humans (Havill et al., 2007; Wagner et al., 2013), and poultry (Shim et al., 2012) have demonstrated that bone traits possess a strong genetic component.

Bone breaking strength (BBS)

Currey (2002) defines bone strength as the load that the bone can bear before breaking. BBS is influenced by properties of the bone such as: shape, size, mass, composition and structure (Rath et al., 2000). Since composition of the bone includes calcium phosphate crystals in addition to collagen (Currey, 2002), bone mineralization values, such as ash content and mineral density, are used as bone strength indices (Rath et al., 2000). Poor mechanical properties, that is, high porosity and low mineralization, increase the risk of cortical bone fractures in fast growing birds (Williams et al., 2004). In contrast, it has been observed that BBS is positively correlated with bone ash content (Lewis et al., 2009), bone mineral density (Frost and Roland, 1991; Hester et al., 2004), and bone weight (Rath et al., 2000). Several of the studies that have evaluated this bone trait in poultry in recent years have used material test devices that applied certain force (Newton or kg) on the bone until it is broken (Yalcin et al., 2001; Williams et al., 2004; Hemme et al., 2005; McDevitt et al., 2006; Shahnazari et al., 2007; Lewis et al., 2009; Shaw et al., 2010).

Bone ash content (BAC)

Bone ash content (BAC) is a parameter used as indicator of bone mineralization (Shaw et al., 2010), being tibia ash the main variable used in poultry (Hall et al., 2003). Methods such as the AOAC (2005) and the Bolin-Frankenbach et al. (2001) are examples of the techniques used for the determination of bone ash. The methodology used in different poultry investigations indicate that the sample bones are oil extracted, dried at 80-105°C for a variable period of time (from 4 hours to overnight) and then they are ashed in muffle furnaces at about 520-600°C for 12-18 hours. The weight of the ash is determined and then BAC is expressed as proportion of bone dry mass (Williams et al., 2000; Yalcin et al. 2001; McDevitt et al., 2006; Shahnazari et al., 2007). BAC has been positively correlated with BBS in poultry (Lewis et al., 2009).

Bone mineral content (BMC)

Bone mineral content (BMC) refers to the amount of bone mineral in a specific region of the bone (Dalén et al., 1976; Cauley et al., 2005), thus it can be seen as the ash, Ca and P content in the fat free dry matter (Hemme et al., 2005). BMC can be assessed by the dual-energy X-ray absorptiometry (DXA) methodology, a noninvasive tool that allows the monitoring of the skeletal integrity of live birds (Hester et al. 2004; Shahnazari et al. 2007). It has been observed that BMC is positively correlated with BAC (Hester et al., 2004).

Bone mineral density (BMD)

Bone mineral density (BMD) has been measured as the proportion of the bone ash weight to the bone volume (Yalcin et al., 2001) or by the quantitative computed tomography (QCT) methodology (Korver et al., 2004); however, the dual-energy X-ray absorptiometry (DXA) methodology has been more commonly used in poultry studies in recent years (Hester et al. 2004;

Shahnazari et al. 2007; Shim et al., 2012). Under DXA methodology, BMD is defined as the value of BMC (i.e. mg) divided by the area (i.e. centimeters squared) of a specific region of the bone (Cauley et al., 2005). In poultry, BMD is positively correlated with BBS (Frost and Roland, 1991).

Leg morphology

Julian (2005) states that broiler chickens have short and thick bones, and Deeb and Lamont (2002) suggested that long legs facilitate leg problems because of the heavy weight broilers have to bear.

Growth rate and indicator traits of bone status

Poultry breeding companies have applied genetic selection methods for about 60 years which has resulted in an accelerated growing rate that in turn has reduced the time to slaughter (Havenstein et al., 2003). Likewise, chicken body's conformation has suffered changes that can be realized in the current proportion of breast with respect to the total body weight; while in 1950's it was about 9%, currently it can be around 18% (Schmidt et al., 2009). Along with the improvement of broiler traits a higher incidence of skeletal defects has been observed (Velleman, 2000) and it seems that it is generally accepted that those skeletal problems are directly related to the growth rate of modern broiler chicken (Korver, 2004; Julian, 2005).

Experiments that have investigated the effect of reducing growth, through low energy diets, on the status of the bone have concluded that that strategy do not improve the quantity or quality of cortical bone in terms of tibial volume, length, ash content, or density (Leterrier et al., 1998). In contrast, other studies have evidenced that fast growing birds have lower bone mineral content (Leterrier and Nys, 1992a), lower ash content and greater cortical bone porosity (Williams et al., 2000; Williams et al., 2004), and weaker tibiotarsi bones with reduced stiffness and lower

resistance to fracture (Rawlinson et al., 2009; Shim et al., 2012); thus, fast growing birds are in disadvantage with respect to the load they need to support.

Genetic parameters of bone traits

A main function of leg bones is to support body mass (Korver et al., 2004). Surveys at commercial flocks have evidenced that modern broiler chickens frequently show locomotor problems whose origin is related with genetic factors (Kestin et al., 1992). Other results have confirmed differential quality of traits associated to leg weakness among strains of chickens (Kestin et al., 1999; McDevitt et al., 2006; Lewis et al., 2009). In meat type chicken literature on genetic parameters of indicator traits of leg bones status and their genetic correlation with growth rate is rather scarce. Heritability of BBS related traits in broilers has been estimated between 0.67 and 0.80 when the traits humerus elastic force (kg), humerus stress (kg/cm^2) (Mandour et al., 1989), and tibia breaking strength (TBS) (de Verdal et al., 2013) were studied. Heritability of traits related to BAC such as tibia ash content (TAC) and relative tibia ash weight (TAW) has ranged between 0.41 and 0.52 (de Verdal et al., 2013). Apparently there are no reports on the heritability of BMC in poultry; however, Havill et al. (2007) stated that from 40 to 50% of the observed variance in BMC in humans could be explained by genetics. Studies in turkeys, humans and mice have demonstrated that BMD is a heritable trait with heritability values ranging from 0.35 to 0.74 (Havenstein et al., 1988; Klein et al., 1998; Ng et al., 2006; Park et al., 2012; Wagner et al., 2013). The heritability of morphological traits in meat type chickens have ranged from 0.03 to 0.09 in the case of shank length (El-Ibiary and Shaffner et al., 1951) and from 0.38 to 0.74 when tibia weight (TW), tibia length (TL) and tibia diameter (TD) were analyzed (de Verdal et al., 2013).

The additive genetic correlation of indicator traits of bone status with live weight has showed both favorable and unfavorable relationships between them. Merritt (1966) estimated

genetic correlations ranging from 0.77 to 0.81 between shank length and body weight at 42 and 63 days of age in both sexes. On the other hand, results by de Verdal et al. (2013) showed that genetic correlations of body weight at 23 days of age with tibia ash content, relative tibia weight, relative tibia ash weight, tibia length, tibia diameter, and tibia breaking strength were -0.22, 0.69, 0.38, -1.00, -0.95, and 0.93, respectively. Globally, these estimations would indicate that selection for heavier chickens would lead to birds with shorter and narrower tibias with less ash content but also would lead to larger shanks, and heavier and stronger tibias. However, Havenstein et al. (1994) have documented that the modern broiler chicken is radically distinct than 40 years ago. Furthermore, commercial live weight is not reached at 23 days of age (Talaty et al., 2010). Thus, investigations described above would not be conclusive about the additive genetic relationship of growth rate with indicator traits of bone status in broiler chickens.

Leg bone problems

It is considered that the improvement of broiler traits by selection has been accompanied by a greater incidence of metabolic diseases such as skeletal problems that mainly affect the locomotors system (EC, 2000; Kalmar et al., 2013). Metabolic diseases have been associated to high metabolic demands that result from a high work-load in specific organs or a particular system that eventually lead to the failure of the body (Julian, 2005). Thus, skeletal pathologies commonly found in young birds selected for fast growth are indicatives of the genetic and production pressure put on the skeleton and it is believed that they frequently are the result of abnormalities in the growth plate or in bone modelling (Thorp, 1994). Leg problems reduce or even prevent the ability of the bird to walk, thus affected chickens are unable to access feed and water. Furthermore, they can also suffer pain, receive attacks from other birds and eventually die (Julian, 1998, 2005). Likewise, the number of consumers worried about animal health and welfare in animal production

systems is increasing (Tuyttens et al., 2008). Different reports (Sullivan, 1994; Julian, 1998; Cook, 2000; Julian, 2005) have mentioned the variety of leg problems that can be observed in poultry; however, tibial dyschondroplasia, and varus-valgus deformities have been recognized among the most common forms of leg problems and have received attention at commercial level (Kapell et al., 2012).

Tibial dyschondroplasia (TD)

Tibial dyschondroplasia (TD) is a metabolic disease mainly observed in meat-type poultry (Almeida Paz et al., 2005; Leach and Monsonengo-Ornan, 2007) and first described by Leach and Nesheim (1965). TD is recognized as an abnormality in the growth plate that mainly occurs in the proximal end of the tibia (Hocking, 2011), having a bilateral incidence (Lynch et al., 1992; Farquharson and Jefferies, 2000). TD is the most common lesion in broiler chicken leg bones (EC, 2000). It is estimated that it causes up to 50% of lameness in some flocks (Julian, 2005) and about 30% of the birds that reach market-weight each year are affected in some degree for this condition (Velleman, 2000). In broiler chickens, it has been stated that TD develops between 2-3 weeks of age (Hocking, 2011), and between 2-5 weeks of age (Lynch et al., 1992). TD is characterized by a mass of avascular opaque cartilage located in the epiphyseal growth plate that in turn results from the accumulation of non-calcified chondrocytes. The size of the lesion is variable, going from a small cartilaginous plug to a lesion that occupies widely the metaphysis under the growth plate (Farquharson and Jefferies, 2000). Depending on the severity of the lesion, two types of problems can be observed: 1) leg deformities or 2) fracture through the growth plate (Bradshaw et al., 2002). Histological examination is considered the most reliable technique for the assessment of TD; however macroscopic examination scoring and bone mineral density readings by optical radiographic densitometry are significantly reliable about the status of the growth plate as well

(Almeida Paz et al., 2005). Edwards and Veltmann (1983) established a four categories scale that is applied to a tibia that previously has been exposed through a longitudinal cut. The categories 0, 1, 2, and 3 corresponded to: a) normal cartilage with little irregularities, b) the cartilage is thickened or show considerable irregularities, c) the cartilage is thickened and have deep irregularities, and d) large mass of cartilage in the proximal end of the tibia, respectively. For the assessment *in vivo*, Bartels et al. (1989) proposed a methodology based on the usage lixiscope which is a real-time skeletal imaging device. The evaluation of the images obtained by the lixiscope allows the scoring of the TD lesion in one of three categories: 1) normal, 2) suspect or 3) pathologic, depending on the status of the cartilage. The Edwards and Veltmann (1983) method has been used in studies that evaluated TD in slow- and fast-growing chickens (Shim et al., 2012) and the lixiscope has been used in studies that have estimated genetic parameters of TD in broiler chicken populations (Kuhlers and McDaniel, 1996). Although the natural etiology of TD is not known (Rath et al., 2004), it has been accepted that growth rate is a major component influencing its occurrence (Julian, 1998; Leach and Monsonego-Ornan, 2007). However, it is also know that TD is influenced by genetic and nutritional factors (Thorp, 1994; EC, 2000). These latter ones include electrolyte balance, calcium to phosphorus ratio, 1,25-dihydroxy vitamin D3, and ascorbic acid; however, TD can also be induced by dithiocarbamates like thiram and mycotoxins like *Fusarium* (Praul et al., 2000). With respect to the genetic component, several reports have informed about genetic parameters of TD in broiler chicken populations (Kuhlers and McDaniel, 1996; Akbas et al., 2009; Kapell et al., 2012; Rekaya et al., 2013). Thus, it is believed that this combination of mechanical, genetic and nutritional factors alters normal chondrocyte differentiation (Praul et al., 2000). In general, TD is the result of a disruption of the chondrocyte maturational sequence in the bone growth plate, meaning that there is an arresting of the pre-hypertrophic phase (Farquharson and

Jefferies, 2000; Almeida Paz et al., 2005; Velada et al., 2011) which is associated to a failed mineralization and penetration of the metaphyseal blood vessels and angiogenesis. Signaling systems related to the modulation of growth plate such as: growth hormone, insulin-like growth factors, the Indian hedgehog/parathyroid hormone-related peptide pathway, fibroblast growth factors, and the transforming growth factor family would be involved in changes in chondrocyte differentiation observed in TD (Leach and Monsonengo-Ornan, 2007).

Valgus-varus deformity (VVD)

Valgus-varus deformity (VVD) is the lateral or medial deviation of the distal end of the tibia and the metatarsal bone. The deviation can result in two conditions: 1) hocks-in/feet-out or valgus (VL) or 2) hocks-out/feet-in or varus (VR) (Julian, 1984; Bisaillon et al., 1988). While VR is more often unilateral, VL is rather bilateral (Leterrier and Nys, 1992b). Likewise, the incidence of VL has been found higher than that of VR (Le Bihan-Duval et al., 1996). VVD is the most common long bone distortion in broiler chickens (EC, 2000). VVD affects from 0.5 to 25% of the flock and it is more often observed between 1 and 4 weeks of age (Julian, 1984) but it appears even until 7 weeks of age (Leterrier and Nys, 1992b). Depending on the severity of the condition, affected birds experience difficulties to walk or they are unable to walk so that they can't access feed and water (Hunter et al., 2008). Affected chickens are frequently culled before they go to market (Newbrey et al., 1988), but legs from those birds that reach market weight may be discarded because of hemorrhage, increased fluid in the joint or bone fractures (Julian, 1984). Microscopic lesions are not evident so that evaluation of the condition is by assessing leg conformation (Hunter et al., 2008). Julian (1984) stated that the description of VVD should mention if the affection is unilateral or bilateral; likewise, a 4 categories scale, based on the degrees deviated of the distal tibia-metatarsal bone, could be used in order to assess the severity of the lesion. Such categories

would be: 0 = normal, less than 5° deviation, 1 = mild, 5-20° deviation, 2 = moderate, 20-50° deviation, and 3 = severe, more than 50° deviation. The precise etiology of VVD is unknown but it is considered that the combination of fast growth, nutrition, and genetics influence its incidence (Hunter et al., 2008). Furthermore, biomechanical tension on long bones may lead to angulation (Lanyon and Baggott, 1975) by influencing columnar orientation of chondrocytes in the bone growth plate (Haye and Simons, 1978). However, it has been suggested that VR and VL have different etiological origins (Leterrier and Nys, 1992b).

Growth rate and leg bone problems

It has been recognized that the relationship between leg problems and body weight is not clear and the evidence is not conclusive (Zhang et al., 1995; Kuhlers and McDaniel, 1996). For instance, in an experiment by Cook et al. (1984) was found that body weight and severity of leg deformity are independent, and in a multi-strain experimental design by Hocking et al. (2009), TD was observed in both broiler and traditional strains so that it was thought that TD does not result because of selection for growth *per se*. Likewise, Wong-Valle et al. (1993a) did not find differences in body weight at 7 weeks of age between lines divergently selected for TD incidence. On the other hand, Havenstein et al. (1994) compared the performance of the 1957 Athens-Canadian Random Breed Control (ACRBC) and the 1991 Arbor Acres (AA) strain, and found that AA strain body weights were between 2.7 and 4.2 times larger than those corresponding to the ACRBC but also AA strain had higher TD incidences (25.6-48.6 vs 1.2%) so that it was concluded that the faster the growth rate and body weight had influence on the incidence of leg problems and walking ability. In contrast, Zhang et al. (1995) observed an inverse relationship between TD incidence and body weight at 7 weeks of age in lines of broilers selected for high and low incidence of TD.

Genetic parameters of leg bone problems

Leach and Nesheim (1965, 1972) were the first to report the influence of a genetic component in broiler chickens affected by tibial dyschondroplasia (TD). Since then, several defects such as VVD has also been studied. Sheridan et al. (1978) showed results corresponding to a breeding program directed to the development of a line of broilers with high TD incidence at 7 weeks of age. After 4 generations they estimated TD heritabilities of 0.26 and 0.18 corresponding to females and males, respectively. On the other hand, genetic correlations between TD incidence and body weight at 7 weeks of age were 0.29 and 0.28 for each sex as well. Burton et al. (1981) studied the incidence of TD in the commercial broiler industry in Australia. Estimated TD heritabilities were 0.26 and 0.30 when the leg condition was scored as binomial and 4 categories scale, respectively. In the same manner, genetic correlations between TD and body weight at 7 weeks of age were -0.18 and -0.12, respectively. Mercer and Hill (1984) estimated genetic parameters for skeletal defects in broiler chickens and concluded that leg problems and live weight at 6 weeks of age seemed genetically related. Estimated genetic correlations of live weight with varus and valgus were 0.26 and 0.10, respectively. Wong-Valle et al. (1993b) estimated a realized heritability, corresponding to TD incidence at 7 weeks of age, of 0.437 in a line selected for high TD incidence. Zhang et al. (1995) estimated heritabilities and genetic correlations between incidence of TD and live body weight (BW) at 7 weeks of age by using high and low incidence of TD lines obtained after 7 generations of selection and a control randombred line. TD incidence and BW showed negative genetic correlations (-0.65 to -0.46). Heritability of TD was 0.37. Kuhlers and McDaniel (1996) reported heritabilities of 0.37 and 0.42 for TD at 4 and 7 weeks of age, respectively. On the other hand, genetic correlations between body weight at 4 weeks and TD at 4 and 7 weeks of age were 0.10 and 0.08, respectively. Those corresponding to weight and TD

at 7 weeks and TD at 4 weeks and weight at 7 weeks were 0.07 and -0.01, respectively. Le Bihan-Duval et al. (1996) estimated genetic parameters for varus and valgus angulations in 2 commercial broiler strains, named A and B, at 6 weeks of age. Valgus heritabilities were 0.16 and 0.29 for lines A and B, respectively. On the other hand, heritabilites for varus were 0.21 and 0.24 for lines A and B, respectively. Le Bihan-Duval et al. (1997) reported estimations of genetic parameters for varus and valgus at 6 weeks of age in 2 broiler strains. Heritabilities estimated from the sire/maternal-grandsire component were 0.15 and 0.28 for valgus and 0.21 and 0.23 for varus in lines A and B, respectively. On the other hand, genetic correlations between susceptibility to leg deformities at 6 weeks of age and body weight at 3 and 6 weeks of age were between -0.10 and 0.09. Zhang et al. (1998) showed results corresponding to the development of 2 broiler chicken lines divergent for tibial dyschondroplasia (TD). Heritability of TD at 4 weeks of age in the high incidence line was 0.65 for females and 0.52 for males; while in the low incidence line the values were 0.50 and 0.40 for females and males, respectively. On the other hand, heritability of TD at 7 weeks of age in the high incidence line was 1.06 for females and 0.86 for males; while in the low incidence line the values were 0.10 and 0.05 for females and males, respectively. Akbas et al. (2009) estimated heritabilities corresponding to tibial dyschondroplasia (TD) and valgus-varus deformity in Hubbard broiler chickens. Estimated heritabilities on binomial scale were 0.06 and 0.26 for TD and valgus-varus deformity, respectively. Once transformed to continuous scale, the heritabilites were 0.21 and 0.72 for TD and valgus-varus deformity, respectively. Kapell et al. (2012) reported heritabilities from 0.04 to 0.07 for deformities of the long bones (LD) and 0.10 to 0.27 for tibial dyschondroplasia (TD) in a commercial population of chickens. Genetic correlations between these two latter traits were -0.26 to 0.16 and between these traits and body weight at 5 weeks of age were 0.16 to 0.22 and 0.09 to 0.25 for LD and TD, respectively. In an analysis with broilers

by Rekaya et al. (2013), heritabilities of 0.12, 0.23 and 0.26 for TD, valgus and varus, were found, respectively. On the other hand, genetic correlations between these three traits and body weight at 6 weeks -0.03, -0.20 and -0.06, respectively.

Molecular genetics of indicator traits of bone status and leg bone problems

Genomics has been seen as a mean to uncover the molecular basis of phenotypes in domestic animals (Andersson, 2001). Knowledge of the chicken genome sequence (Hillier et al., 2004) and current biochemical based techniques that allow direct access to the bird's genome could provide knowledge about the genetic value without environmental interference but also they represent a way for the biological understanding of traits (Hocking, 2010; Thiruvenkadan et al., 2011) and of the genotype-phenotype relationship which include the probable identification of the genes underlying traits of economic interest (Siegel et al., 2006). In consequence, it has been warned that with the advent of genomics, quantitative geneticists are going to need a better understanding of the biochemical processes underlying phenotypes (Dodgson, 2007). It is considered that genomics could have a significant impact mainly on those traits where conventional genetic selection techniques would have some limitations, that is, traits that are difficult or expensive to measure or that have low heritability (Bulfield, 2004). QTL mapping (Jansen, 2001; Hoeschele, 2001), genome-wide association studies (McCarthy et al., 2008), and genomic selection (Meuwissen et al., 2001) are elements of the genomics toolbox.

Quantitative trait loci

A quantitative trait locus (QTL) is a segment of the genome that could harbor one or more genes that influence a quantitative trait (Geldermann, 1975). Even though QTL detection and its precise location are important tasks in QTL mapping (Andersson, 2001), the discovery of the

responsible genes underlying the QTL is the major challenge in QTL experiment (Andersson and Georges, 2004; Hocking, 2005). At present, the Animal QTLdb database (Hu et al., 2013) contains 3923 QTLs corresponding to the chicken. From this QTL set, 228 QTLs correspond to bone strength, bone mineral content, bone mineral density, bone morphology traits, leg bowing, leg twisting or tibial dyschondroplasia and they are distributed on chromosomes 1-9, 11-15, 17-24, 26, 27 and Z.

Genome wide association studies (GWAS)

Genome-wide association studies (GWAS), based on current collections of SNPs in the whole genome together with pedigree and phenotype information, and the development of methods for the analysis of the data that they provide, allow a mean for higher resolution mapping (Andersson, 2001; Zhang et al., 2012), and the identification of molecular markers and genes related to complex traits (Liu et al., 2013). In an attempt for summarizing results from chicken GWAS in bone traits, it was found that, apparently, there are not yet studies conducted in this field. The searching was conducted via PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) at the end of September 2013 by using the words "genome-wide association", "single nucleotide polymorphisms", "chicken", and "poultry", in combination with each one of the traits previously described, that is, bone, BBS, BAC, BMC, BMD, leg bone morphology traits, TD, and VVD.

Genomic selection

Genomic selection is a method directed to the prediction of the genetic value of an animal based on information from dense marker maps (Meuwissen et al., 2001) and its posterior usage in making selection decisions (Dodgson, 2007). Currently, this technique is based on data from a large number of SNPs in the bird's genome in addition to phenotypic information (Kranis et al.,

2013). Genomic selection has demonstrated being advantageous when categorical scored traits in broiler chickens are analyzed. Chen et al. (2011) found that genomic evaluation, implemented with BLUP methodology adapted for the simultaneous usage of pedigree and genomic information, achieved about 50% higher breeding value accuracies than phenotypic BLUP methodology when a low heritability leg score trait was analyzed along with two additional normal distributed traits.

Candidate genes

The gene-phenotype relationship can be tackled through the knowledge and the understanding of 2 features: 1) the functions that gene's products perform and 2) trait physiology (Womack et al., 2012). Thus, candidate gene approach is a powerful tool when the gene corresponds to a true causative one (Andersson, 2001). Results from QTL mapping and gene expression studies in poultry have suggested candidate genes related with bone traits. Zhou et al. (2007) studied chicken skeletal integrity through a whole genome QTL analysis and proposed positional candidate genes to be explored for association with bone in traits in chickens. Those genes were: fibroblast growth factor 9 (FGF9), Sox-10, bone morphogenetic protein receptor type II, transforming growth factor-β receptor 3, bone proteoglycan 2, osteocrin, angiotensin II, progesterone receptor, tumor necrosis factor-α, calcitonin receptor, and type IIb Na-P co-transporter. Rubin et al. (2007) compared global RNA expression in femoral bone from adult White Leghorn and Red Junglefowl birds by using cDNA-microarrays and the authors highlighted that among the genes differentially expressed there are some involved in bone metabolism, such as: WD-repeat containing protein 5 (WDR5), Wnt inhibitory factor 1 (WIF1), syndecan 3 (SDC3), and immunoglobulin-like receptor CHIR-A (CHIR-A).

Although the precise cellular defect underlying tibial dyschondroplasia (TD) and valgus-varus deformities is not known, it has been stated that TD is the result of an impairment of

chondrocytes for reach terminal differentiation that in turn leads to vascularization and mineralization (Farquharson and Jefferies, 2000). Thus, the characterization of candidate genes for TD has been mainly focus on genes associated to cell development, chondrocyte differentiation and hypertrophy, extracellular matrix, calcium transport, matrix mineralization, vascular invasion, apoptosis, hypoxia response, and heat shock proteins. Identification of the genes directly involved in the process that lead to TD condition would allow the management of the problem by genetic selection means (Farquharson and Jefferies, 2000). Table 2.1 summarizes genes that have been studied in poultry with respect to bone mineralization, length, and weight, and tibial dyschondroplasia.

Conclusions

It has been demonstrated that skeletal integrity traits in meat type chickens have an additive genetic component; however, indicator traits of bone status have not received the same attention than tibial dyschondroplasia and valgus-varus deformities by researchers. Moreover, the information reviewed indicates that the genetic relationship between growth rate and skeletal integrity traits is not conclusive. Several chromosomal regions related to skeletal integrity traits have been identified and molecular information seems to improve the accuracy of estimated breeding values of leg bone traits. Likewise, bone development knowledge has allowed the investigation of the role of several genes in skeletal integrity phenotypes. Those genes are mainly related to cell development, chondrocyte differentiation, vascularization and mineralization of the bone. Nevertheless these efforts, the molecular genetics of skeletal integrity traits is not conclusive either.

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Table 2.1. Genes studied in bone integrity research in poultry.

Trait	Gene	Reference
Bone Mineral Content and/or Bone Mineral Density	Fas cell surface death receptor (Fas)	Wu et al. (2003)
	Transforming growth factor-β2 and -β4 (TGF-β2 and β4)	Li et al. (2003)
	Phosphatase PHOSPHO1	Houston et al. (2004)
	Very low density apolipoprotein-II (apoVLDL-II)	Li et al. (2005)
	Vitamin D receptor (VDR)	Bennett et al. (2006)
Bone Length	Recessive shankless mutation (shl)	Langhorst and Fechheimer (1985)
	Growth hormone receptor (GHR)	Burnside et al. (1992)
	Osteopontin (OPN), and matrix metalloproteinases (MMP)-9 and -13	Reich et al. (2005)
	Collagen type X	Mauro et al. (2010)
	Visfatin	Han et al. (2011)
	Retinoblastoma 1 (RB1)	Zhang et al. (2011)
	Homeobox D11 (Hoxd11) and D12 (Hoxd12)	Kamiyama et al. (2012)
Bone Weight	Insulin-like growth factor-I (IGF1)	Zhou et al. (2005)
Tibial Dyschondroplasia	C-myc proto-oncogene and the transforming growth factor-β3 (TGF- β3)	Loveridge et al. (1993)
	Osteopontin (OPN) and alkaline phosphatase	Knopov et al. (1995)
	Transforming growth factors-β1-3 (TGF- β1-3)	Law et al. (1996)
	Collagen type X	Zhang et al. (1996)
	Collagen types II and X, and osteopontin	Pines et al. (1998)
	Collagen type X	Reginato et al. (1998)
	Parathyroid receptor (PTH/PTHrP)	Ben-Bassat et al. (1999)
	Elongation factor 2 (EF2), galline extracellular fatty acid binding protein (Ex-FABP), calcium-dependent cell adhesion molecule B-cadherin, and cell surface antigen H7	Jefferies et al. (2000)
	Calbindin or cholecalciferol receptor	Shirley et al. (2003)
	Collagen type X, Indian hedgehog (Ihh), and parathyroid hormone-related protein (PTHrP)	Webster et al. (2003)
	Iodothyronine deiodinases types 1 (DIO1), 2 (DIO2) and 3 (DIO3)	Shen et al. (2004)

Table 2.1. (Continuation) Genes studied in bone integrity research in poultry.

Trait	Gene	Reference
Tibial Dyschondroplasia	Collagen types II and X, aggrecan, glyceraldehyde 3 phosphate dehydrogenase (GAPDH), transglutaminase 2 (TGMse), runt-related transcription factor (Runx2), matrix metalloproteinases (MMP)-2 and -13, and vascular endothelial growth factor (VEGF)	Rath et al. (2005)
	Aggrecan (AGC1)	Ray et al. (2006)
	Inducible nitric oxide synthase (iNOS-Alu I), macrophage migration inhibitory factor (MIF), and transforming growth factor-β3 (TGF-β3)	Ye et al. (2006)
	Vascular endothelial growth factor (VEGF), its receptor Flk-1, and the metalloproteinases (MMP)-9 and -13	Gay et al. (2007)
	Endothelial growth factor (VEGF), its receptors (VEGFR1 and VEGFR2) and an antiapoptotic protein (Bcl-2)	Rath et al. (2007)
	Metalloproteinases (MMP)-2, -9 and -13	Simsa et al. (2007)
	Hypoxia-inducible factor-1α (HIF-1α), heat-shock proteins 90 (Hsp90) and 70 (Hsp70)	Genin et al. (2008)
	Metalloproteinases (MMP)-2, -3, -9 and -13	Hasky-Negev et al. (2008)
	Metalloproteinases (MMP)-2, -3, -9 and -13	Dan et al. (2009)
	Pro-alpha-1 collagen type I (Col I α1), collagen types IX (Col IX), and X (Col X), NADH dehydrogenase (NADH DH), cytochrome C oxidase subunit III (COX III), enolase 1-alpha (ENO1), carbonic anhydrase II (CA2) and heat shock protein 90 (Hsp90), Matrilin 3 (MATN3) and chondromodulin-I (ChM-I)	Tian et al. (2009)
	Collagens types II, IX, X, and XI, aggrecan, and metalloproteinases (MMP)-9, -10, -11, and -13	Velada et al. (2011)

CHAPTER 3

GENETIC ANALYSIS OF LEG PROBLEMS AND GROWTH IN A RANDOM MATING BROILER POPULATION[1]

[1] F. González-Cerón, R. Rekaya, N. B. Anthony, and S. E. Aggrey. Submitted to *Poultry Science*, 06/23/2014.

Abstract

Improvement in growth has been widely reported as the cause of increased incidence of leg problems in broiler chickens. We report herein the genetic relationship between growth and leg problems in a random mating broiler control population. The traits studied were valgus (VL), varus (VR), and tibial dyschondroplasia (TD) which were expressed on a binary scale of 0 (normal) and 1 (abnormal) and growth rates from 0 to 4 (BWG 0-4), from 0 to 6 weeks of age (BWG 0-6) and residual feed intake from 5 to 6 weeks of age (RFI 5-6). A threshold-linear mixed model was employed for the joint analysis of the categorical and linear traits. Incidences of VL, VR, and TD were 26, 4 and 2%, respectively. Heritability of leg problems ranged from 0.11 to 0.13. Phenotypic correlations alluded to an unfavorable relationship between growth and leg problems, however, the genetic relationship between growth and leg problems was extremely weak ranging from -0.01 to 0.08. There is therefore a basis for genetic improvement in leg problems, however, improved management practices would also be important to reduce incidence of leg problems in broiler chickens.

Keywords: Leg problems, growth, genetics

Introduction

During the last six decades poultry production has experienced an extraordinary improvement in performance. This advance has resulted from a better knowledge and understanding of nutrition, physiology, housing environment, and successful broiler breeding programs. Unfortunately, the improvement of some of the production traits has been accompanied with an incidence of metabolic disorders including skeletal problems (EC, 2000; Kalmar et al., 2013; Rekaya et al., 2013).

Skeletal disorders reduce the general mobility of broilers and in extreme cases limit their ability to walk to the feeder and drinker. Leg weakness has been described as the primary cause of mortality in late stages of the growing period (Kestin et al., 1999; Knowles et al., 2008). Chickens with these problems are at a disadvantage and many receive attacks from other birds and eventually die (Julian, 1998; 2005). Furthermore, animal health and welfare in animal production systems are of great importance to the poultry industry (Tuyttens et al., 2008).

Multiple reports highlight the skeletal problems in poultry (Sullivan, 1994; Julian, 1998; Cook, 2000; Julian, 2005). Tibial dyschondroplasia (TD), and varus-valgus deformations (VVD) are important examples of these issues. TD describes an avascular lesion characterized by an abnormal cartilage formation resulting from an accumulation of immature chondrocytes, with an extended lifespan, in the proximal metaphyses of the tibiotarsus and tarsometatarsus (Leach and Nesheim, 1965; Leach and Monsonego-Ornan, 2007). On the other hand, VVD refers to a lateral or medial angulation of the shaft of the distal tibiotarsal bone resulting in deviation of the lower part of the leg (Randall and Mills, 1981; Julian, 1984). More specifically, valgus and varus deformations are outward and inward deviations of the tibiometatarsus, respectively (Hunter et al., 2008).

Although high growth rates have been associated with higher incidence in leg deformities (Sorensen, 1992; Julian, 2005; Shim et al., 2012), the direct genetic relationship between these two factors is not conclusive (Zhang et al., 1995; Kuhlers and McDaniel, 1996). Some results indicate that the relationship does not exist (Cook et al., 1984) or that the incidence of leg deformities is not a direct result of selection for broiler traits (Hocking et al., 2009). However, the definition of a breeding strategy for practical purposes requires adequate knowledge of the genetic relationship among traits of economic importance (Le Bihan-Duval et al., 1996).

Several studies have demonstrated that leg problems are heritable (Sheridan et al., 1978; Mercer and Hill, 1984; Le Bihan-Duval et al., 1997; Kapell et al., 2012; Rekaya et al., 2013). However, genetic correlations between leg problems and growth have been contradictory in the literature. In the case of TD, these values have been positive (Sheridan et al., 1978; Kapell et al., 2012), close to zero (Kuhlers and McDaniel, 1996; Rekaya et al., 2013) or negative (Burton et al., 1981; Zhang et al., 1995). Similar results were reported for VVD (Mercer and Hill, 1984; Le Bihan-Duval et al., 1997; Kapell et al., 2012; Rekaya et al., 2013).

Some of the inconsistencies in the relationship between growth parameters and leg problems as described by Rekaya et al. (2013) are due in part to the categorical nature of the data and the methodological and computational challenges associated with joint analyses of these traits. Also the nature of the population used in the study could affect the relationships; e.g. the study of Rekaya et al. (2013) was conducted using a commercial line which is under selection. The objective of this study was to estimate genetic variance-covariance parameters for leg problems, growth and feed efficiency in a random mating broiler control population.

Materials and methods

Experimental population and husbandry

In the present study 2,301 pedigreed broiler chickens, produced in 8 consecutive hatches from mating 24 sires and 72 dams, were used. The Arkansas random mating population which is an unselected broiler control population was used (Aggrey et al., 2010). Once hatched, chicks were sexed and placed in pens (0.071 m^2/bird) with litter. From hatching to 18 days the chickens received a mash starter diet containing 225 g/kg protein, 52.8 g/kg fat, 25.3 g/kg fiber, 12.90 MJ ME/kg, 9.5 g/kg calcium (Ca), and 7.2 g/kg total phosphorus (P) (4.5 g/kg available P). Henceforth chickens were fed a pelleted grower diet containing 205 g/kg protein, 57.6 g/kg fat, 25.0 g/kg fiber, 13.20 MJ ME/kg, 9.0 g/kg Ca and 6.7 g/kg total P (4.1 g/kg available P). At 28 days of age, birds were transferred to individual metabolic cages (width = 20.32 cm; length = 60.96 cm; and height = 30.48 cm). They were allowed to acclimate for one week prior to measuring feed efficiency from 5 to 6 wk. Water and feed were provided *ad libitum* for the duration of the study. Birds were kept on a 20L:4D light regimen.

Data

Weekly body weight (BW) was recorded for each bird from hatch till 6 weeks. Feed intake was measured from week 5-6, and legs were scored VVD at week 4 and TD at week 6. Body weight gain (BWG 0-4) from week 0-4 and (BWG 0-6) from week 0-6 was calculated. Residual feed intake from 5 to 6 weeks of age (RFI 5-6) was calculated according to Aggrey et al. (2010). Methods described by Leterrier and Nys (1992) were followed in order to score VVD in each leg at week 4. Depending on the angle size of tibia-metatarsus, 4 categories and scores of VVD were defined: normal (score = 0), mild (10-25° angle; score = 1), intermediate (25-45° angle; score =

2), and severe (>45° angle; score = 3). Methods described by Edwards and Veltmann (1983) were followed in order to score TD in the right tibia. A longitudinal cut across the tibia was made and the white cartilage plug abnormality was observed. Depending on the severity of the cartilage abnormality, 4 categories and scores of TD were defined: normal (score = 0), mild (score = 1), intermediate (score = 2), and severe (score = 3). Bird handling and experimental protocols were in line with the University of Georgia Animal Use and Care Guidelines.

Statistical analysis

After data editing, there were 2,257 birds recorded for 8 traits: valgus in the right leg (VLR), valgus in the left leg (VLL), varus in the right leg (VRR), varus in the left leg (VRL), TD, BWG 0-4, BWG 0-6, and RFI 5-6. However, VLR and VLL were merged into valgus (VL), and VRR and VRL were merged into varus (VR) for the analysis. To ameliorate the grossly unequal representation of leg incidences in different hatch groups, and also for computational feasibility, the leg data was expressed on a binary scale of 0 (normal) and 1 (abnormal) for VL, VR, and TD so that birds having no leg problems were scored as 0 and those birds having leg problems in either one of both legs were scored as 1. Descriptive statistics were obtained using PROC UNIVARIATE procedures of SAS version 9.1.3 (SAS Institute, 2006). A threshold-linear mixed model similar to that one used by Rekaya et al. (2013) was implemented for the joint analysis of the categorical (leg problems) and linear traits (growth and feed efficiency). The threshold-linear mixed model used was:

$$y_{ijnk} = H_i + S_j + u_n + e_{ijnk} \qquad [1]$$

where y_{ijnk} was the observed BWG 0-4, BWG 0-6, RFI 5-6, or the liabilities for the leg problems (VL, VR, TD) for bird n, H_i (i = 1-8) was the fixed effect of the hatch class i, S_j was the fixed

effect of sex j (j = 1-2) of bird n, u_n was the random additive effect of bird n, and e_{ijnk} was the random residual term. Assuming normality conditionally on the model parameters, the joint distribution of the liability for binary scores and continuous traits is expressed in matrix notation as:

$$\mathbf{y} \mid \boldsymbol{\beta}, \mathbf{u}, \mathbf{R_0} \sim N(\mathbf{X\beta} + \mathbf{Zu}, \mathbf{R_0} \otimes \mathbf{I}) \qquad [2]$$

where $\mathbf{y} = (\mathbf{l}_1', \mathbf{l}_2', \mathbf{l}_3', \mathbf{y}_1', \mathbf{y}_2', \mathbf{y}_3')'$ is the vector of liabilities ($\mathbf{l}$) and continuous responses ($\mathbf{y}_i$); $\boldsymbol{\beta}$ and $\mathbf{u}$ are vectors of systematic and random effects, respectively; and $\mathbf{R_0}$ is an 6 × 6 residual (co)variance matrix with the first 3 diagonal elements, corresponding to the categorical traits, fixed to 1. $\mathbf{X}$ and $\mathbf{Z}$ are known incidence matrices. Rekaya et al. (2013) stated that in the Bayesian implementation of a model like the observed in [1] via Markov chain Monte Carlo (MCMC) methods, the direct sampling of $\mathbf{R_0}$ is not feasible due to the fixation of some of its diagonal elements. To overcome this problem of sampling of the residual (co) variance matrix, the methods described by Rekaya et al. (2013) were used.

The multiplication of model [1] by a diagonal matrix $\mathbf{D} = \mathbf{D_0} \otimes \mathbf{I}$, where $\mathbf{D_0}$ is an 6 x 6 diagonal matrix and $\mathbf{I}$ is an identity matrix with appropriate dimensions, yields an equivalent model:

$$\mathbf{y}^* = \mathbf{X\beta}^* + \mathbf{Zu}^* + \mathbf{e}^* \qquad [3]$$

where

$\boldsymbol{\beta}^* = (\boldsymbol{\beta}_1^{*\prime}, \boldsymbol{\beta}_2^{*\prime}, \ldots, \boldsymbol{\beta}_t^{*\prime})'$,

$\mathbf{u}^* = (\boldsymbol{u}_1^{*\prime}, \boldsymbol{u}_2^{*\prime}, \ldots, \boldsymbol{u}_t^{*\prime})'$,

with $\boldsymbol{\beta}_i^* = \boldsymbol{\beta}_i d_{ii}$ and $\boldsymbol{u}_i^* = \mathbf{u}_i d_{ii}$, where $\boldsymbol{\beta}_i$ and $\mathbf{u}_i$ are vectors of fixed and random effects for the trait *i* in the identifiable model in [2], respectively, and d_{ii} is the diagonal element *i* of matrix $\mathbf{D_0}$. However, model in [3] is not identifiable because of **D** is not known. The residual (co)variance matrix of the non-restricted model in [3] is given by:

$$\text{var}(\mathbf{e}^*) = \mathbf{DRD}' = \sum = \sum_0 \otimes \mathbf{I}, \qquad [4],$$

a non-restricted residual (co)variance matrix, where **R** is the original restricted residual (co)variance matrix in [2], and $\sum_0$ is an 6 x 6 residual (co)variance matrix of the non-identifiable model in [4]. Thus, estimates of the restricted residual covariance matrix, **R**, could be easily obtained using equation [4] and the non-restricted matrix $\sum_0$. The lack of restriction in $\sum$ facilitates enormously the Bayesian implementation via MCMC methods. However, in order to obtain the parameters of the identifiable model in [2] from the draws of the non-identifiable parameters, **D** needs to be defined. The identifiable parameters, based on expressions in [3] and [4], can be retrieved as:

$$\boldsymbol{\beta}_i = \frac{1}{d_{ii}} \boldsymbol{\beta}_i^*; \qquad [5]$$

$$\mathbf{u}_i = \frac{1}{d_{ii}} \boldsymbol{u}_i^*; \qquad [6]$$

$$\mathbf{R} = \mathbf{D}^{-1}\sum\mathbf{D}'^{-1} \text{ and } \mathbf{R_0} = D_0^{-1} \sum_0 D_0^{-1} \qquad [7].$$

Given that the diagonal elements of $\mathbf{R_0}$ corresponding to the binary responses are fixed to 1, the first 3 diagonal elements of the matrix $\mathbf{D}_0$ must be equal to the square root of their corresponding elements in $\sum_0$, and the 3 diagonal elements of $\mathbf{D}_0$ corresponding to the continuous traits are set equal to one as indicated by Rekaya et al. (2013).

To complete the Bayesian formulation, the following priors were assumed to the unknowns in the model,

$p(\boldsymbol{\beta}^*) \sim U[-10^6, 10^6]$

$p(\mathbf{u} \mid \mathbf{A}, \mathbf{G}) \sim N(0, \mathbf{A} \otimes \mathbf{G_0})$

and for the elements of matrix $\mathbf{G_0}$,

$p(g_{ii}) \sim U[0, 10^5]$ for $i = 1,2,\ldots, 6$,

and $p(g_{ij}) \sim U\left[-\sqrt{\sigma^2_{u1}\sigma^2_{u2}}, \sqrt{\sigma^2_{u1}\sigma^2_{u2}}\right]$ for $i \neq j = 1,2,\ldots,6$,

where $\mathbf{A}$ is the additive genetic relationship between birds, $\mathbf{G_0}$ is the additive genetic (co)variance matrix, and g_{ii} is the genetic variance for the trait i. Similar priors are assumed for $\sum_0$ but with 10^6 for the upper bound of the uniform distribution for the diagonal elements.

The resulting full conditional distributions needed for the implementation of Gibbs sampling for the systematic and random effects, liabilities, and genetic and residual (co)variance matrices were in closed form being normal, truncated normal and scaled inverted Wishart, respectively. A unique chain of 200,000 samples was implemented where the first 50,000 samples were discarded as burn-in period based on visual inspection of the behavior of the chain. In each iteration of the sampling process, the transformations indicated in equations [5], [6] and [7] were applied to draws of the non-identified model in order to obtain samples corresponding to the identifiable parameters in model [2]. Computer software developed by Rekaya et al. (2013) was used for analysis.

Results

Incidence (%) of leg problems and phenotypic correlations of these traits with growth rate are shown in Table 3.1. Descriptive statistics of the traits studied are summarized in Table 3.2. Incidence of VL was greater ($P<0.01$) than VR. Phenotypic correlations of leg problems with growth rate were unfavorable and ranged from 0.02 to 0.10 but only those of VL and VR were significant ($P<0.01$). Heritability of leg problems and growth and feed efficiency traits are presented in Table 3.3. While heritability values corresponding to leg problems ranged from 0.11 to 0.13, those of growth and feed efficiency traits ranged from 0.12 to 0.27. Genetic and residual correlations among the traits are presented in Table 3.4. Genetic correlation between VVD was low and negative (-0.02) but between these traits and TD was positive (0.01 to 0.05) but weak. Genetic correlations of VL and VR with growth rate were unfavorable although small and ranged from 0.02 to 0.08. In contrast, the genetic association of TD with growth rate tended to be still close to zero (-0.01). Genetic correlations of leg problems with RFI 5-6 ranged from 0.01 to 0.02.

Discussion

The observed differential incidence between VL and VR was in concordance with previous studies (Le Bihan-Duval et al., 1996; Cook, 2000). Phenotypic correlations suggested that the higher the growth rate during the first four weeks of age, the greater the incidence of VL and VR, a similar pattern was observed between VL and BWG 0-6. This observation would coincide with the association between growth and leg problems that has been discussed by some authors (Sorensen, 1992; Julian, 2005). Heritability of TD was lower than values (0.22 to 0.36) reported by Sheridan et al. (1978) and Burton et al. (1981); however, it was within the range (0.10 to 0.27) informed in more recent investigations (Kapell et al., 2012; Rekaya et al., 2013). Heritability of VL and VR in previous reports ranged from 0.21 to 0.38 and from 0.22 to 0.29, respectively

(Mercer and Hill, 1984; Le Bihan-Duval et al., 1996, 1997; Rekaya et al., 2013). These estimates are higher than the heritability values of VVD that were found in the present study. The low heritability estimates of leg problems imply that the expression of these traits is highly influenced by environmental causal effects (Visscher et al., 2007). Therefore, in addition to genetic improvement, management strategies are needed to reduce the incidence of leg problems (Rekaya et al., 2013). Heritability of growth rate was lower than values (0.39 to 0.52) from different age periods (Pym et al., 1991; Druyan et al., 2007). Likewise, heritability of RFI 5-6 was low compared to the value (0.26) recently estimated in a commercial broiler population for the period 6 to 7 weeks of age (Rekaya et al., 2013). Taken together with other studies, it appears an extremely weak genetic association between growth and VVD. The weak negative genetic association estimated between VVD was similar to that of Le Bihan-Duval et al. (1996) even though they used a generalized linear model and a multinomial logistic transformation model. Rekaya et al. (2013) reported a rather high negative genetic relationship (-0.70) between VR and VL, however, in the current study an extremely weak association was observed. This might be due to the fact that, the commercial population used by Rekaya et al. (2013) has undergone genetic improvement for growth whereas the population used in this study is a control population.

The favorable genetic association between VVD and TD was also observed by Kapell et al. (2012) in two of three broiler lines that they studied (0.10). Overall, the genetic correlations of leg problems and growth rates were similar to earlier reports in the literature. While genetic correlations of VVD with growth rate were unfavorable, those of TD with growth seemed slightly favorable. This latter result would tend to the additive genetic association of TD with body weight at 7 weeks (-0.12 to -0.46) reported by Burton et al. (1981) and Zhang et al. (1995). However, current results are different from reports of Sheridan et al. (1978) and Kapell et al. (2012) who

found unfavorable genetic correlations (0.16 to 0.38) of TD with body weight. On the other hand, the genetic association between VVD and growth found in the current study differs from other reports. For example, Rekaya et al. (2013) reported negative genetic correlations of body weight at 6 weeks with valgus (-0.20) and varus (-0.06). Likewise, Le Bihan-Duval et al. (1997) provided estimates of favorable genetic correlations of VVD with body weight at 3 (BW3) and 6 weeks (BW6), in two broiler lines. Genetic correlation of valgus and varus with BW3 ranged between -0.02 and -0.03 and between 0 and -0.10, respectively in one line. The other line showed a favorable genetic correlation of varus with BW6 (-0.06). In contrast, the current weak unfavorable genetic correlation of VVD with growth rate is similar to what was reported by Mercer and Hill (1984) from commercial broiler strains with genetic correlation of valgus and varus with BW6 ranging from 0.02 to 0.25 and between 0.28 and 0.34, respectively, when estimated from half-sib analysis. Furthermore, using a full-sib analysis genetic correlation of valgus and varus with BW6 ranged between 0.07 and 0.11 and between 0.17 and 0.25, respectively. Other unfavorable genetic correlations between VVD and BW6 were reported by Le Bihan-Duval et al. (1997). Genetic correlation of valgus with BW6 ranged between 0.01 and 0.09; and in one of the broiler lines that they studied, the genetic correlation of varus with BW6 was 0.08. Recently, Kapell et al. (2012) also reported unfavorable genetic associations of VVD with body weight at 6 weeks which ranged from 0.09 to 0.25 in the 3 broiler lines that they studied. Genetic correlations of VL and TD with RFI 5-6 were similar to the values reported (0.04 and 0.02, respectively) by Rekaya et al. (2013), however, the genetic correlation between VR and RFI was contradictory. Residual correlations were low, except those between growth traits. In fact, the posterior means of residual correlations between leg soundness traits were practically equal to zero. However, their associated posterior SD were large. A further inspection of these results showed that although the Markov Chain Monte

Carlo chain has good mixed and converged to the target distribution (Figure 3.1), the marginal distributions of these correlations (Figure 3.2) are somewhat flat and covered almost the whole parametric space of a correlation coefficient (-1, 1). This is very likely due to the small size of the data and the low incidence of these traits. Thus, point estimates such as the posterior mean; although unbiased should be interpreted with caution.

From all the aforementioned results, the genetic correlations between growth and leg problems is very low varying from slightly negative to slightly positive. Considering the standard deviations associated with such estimates, it becomes practically impossible to determine the true genetic relationship between growth and leg problems with certainty. Despite this uncertainty, Kapell et al. (2012) outlined a strategy for the simultaneous improvement of these traits which considers the discarding of breeder candidates with clinical symptoms and the identification, through breeding values, of breeder candidates that have not developed clinical symptoms, but have propensity to the problems. Therefore in order to achieve a simultaneous improvement, it has been suggested the inclusion of the fitness traits in the general breeding goal along with a sufficient weight for each trait (Wall et al., 2007; Berglund, 2008). The management strategies under which these birds are raised would contribute more significantly in reducing the incidence of leg problems, even though Kapell et al. (2012) clearly demonstrated that genetic selection for reduced leg problems can also achieve favorable results.

Conclusions

From the current study and studies already reported in the literature, it is amply clear that leg problems have genetic component, however, the additive genetic variation is small. Despite the favorable response of leg problems to genetic improvement, optimal management strategies could go a long way to reduce the incidence of leg problems in broilers. The current study showed that

there is genetic variability underlying varus, valgus and TD, but their association with increased growth is extremely weak at best.

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Table 3.1. Incidence (%) of leg problems[1] and phenotypic correlations between these traits and growth rate[1] in the Arkansas randombred chicken population.

	VL	VR	TD
Incidence	26.23	4.25	2.13
N	592	96	48
BWG 0-4	0.10**	0.08**	0.02
BWG 0-6	0.09**	0.03	0.05

[1]VL = valgus (right and left legs combined); VR = varus (right and left legs combined); TD = tibial dyschondroplasia; N = number of birds with each leg problem; BWG 0-4 = body weight gain from 0 to 4 weeks of age; BWG 0-6 = body weight gain from 0 to 6 weeks of age. **($P<0.01$).

Table 3.2. Descriptive statistics of leg problems and growth and feed efficiency traits in the Arkansas randombred chicken population.

Trait[1]	N	Mean	SD	Minimum	Maximum
VL	2,257	0.26		0.00	1.00
VR	2,257	0.04		0.00	1.00
TD	2,257	0.02		0.00	1.00
BWG 0-4 (kg)	2,257	0.82	0.12	0.18	1.21
BWG 0-6 (kg)	2,257	1.65	0.21	0.71	2.36
RFI 5-6 (g)	2,257	0.00	0.11	-0.53	0.85

[1]VL = valgus (right and left legs combined); VR = varus (right and left legs combined); TD = tibial dyschondroplasia; BWG 0-4 = body weight gain from 0 to 4 weeks of age; BWG 0-6 = body weight gain from 0 to 6 weeks of age; RFI 5-6 = residual feed intake from 5 to 6 weeks of age.

Table 3.3. Posterior means (posterior SD) of genetic, residual variances, and heritability of leg problems and growth and feed efficiency traits in the Arkansas randombred chicken population.

Trait[1]	Genetic variance	Residual variance	Heritability
VL	0.170 (0.141)	1.000	0.13 (0.10)
VR	0.128 (0.112)	1.000	0.11 (0.08)
TD	0.172 (0.142)	1.000	0.12 (0.11)
BWG 0-4	0.003 (0.003)	0.0082 (0.0004)	0.27 (0.04)
BWG 0-6	0.006 (0.006)	0.0216 (0.0009)	0.23 (0.04)
RFI 5-6	0.002 (0.002)	0.0116 (0.0004)	0.12 (0.02)

[1]VL = valgus (right and left legs combined); VR = varus (right and left legs combined); TD = tibial dyschondroplasia; BWG 0-4 = body weight gain from 0 to 4 weeks of age; BWG 0-6 = body weight gain from 0 to 6 weeks of age; RFI 5-6 = residual feed intake from 5 to 6 weeks of age.

Table 3.4. Genetic (above diagonal) and residual[2] (below diagonal) correlations of leg problems and growth and feed efficiency traits in the Arkansas randombred chicken population.

Trait[1]	VL	VR	TD	BWG 0-4	BWG 0-6	RFI 5-6
VL		-0.02 (0.11)	0.01 (0.15)	0.08 (0.14)	0.08 (0.14)	0.02 (0.08)
VR	0.00 (0.27)		0.05 (0.13)	0.02 (0.14)	0.02 (0.14)	0.02 (0.09)
TD	0.02 (0.30)	0.02 (0.29)		-0.01 (0.16)	-0.01 (0.15)	0.01 (0.10)
BWG 0-4	0.00 (0.12)	0.00 (0.11)	0.00 (0.11)		0.84 (0.00)	0.23 (0.02)
BWG 0-6	0.00 (0.14)	0.00 (0.13)	0.00 (0.13)	0.67 (0.02)		0.26 (0.02)
RFI 5-6	0.00 (0.07)	0.00 (0.07)	0.00 (0.07)	-0.01 (0.03)	-0.05 (0.03)	

[1]VL = valgus (right and left legs combined); VR = varus (right and left legs combined); TD = tibial dyschondroplasia; BWG 0-4 = body weight gain from 0 to 4 weeks of age; BWG 0-6 = body weight gain from 0 to 6 weeks of age; RFI 5-6 = residual feed intake from 5 to 6 weeks of age. [2]Most of the residual correlations appears as zero due to the rounding to two decimal places, however, all they are different from zero.

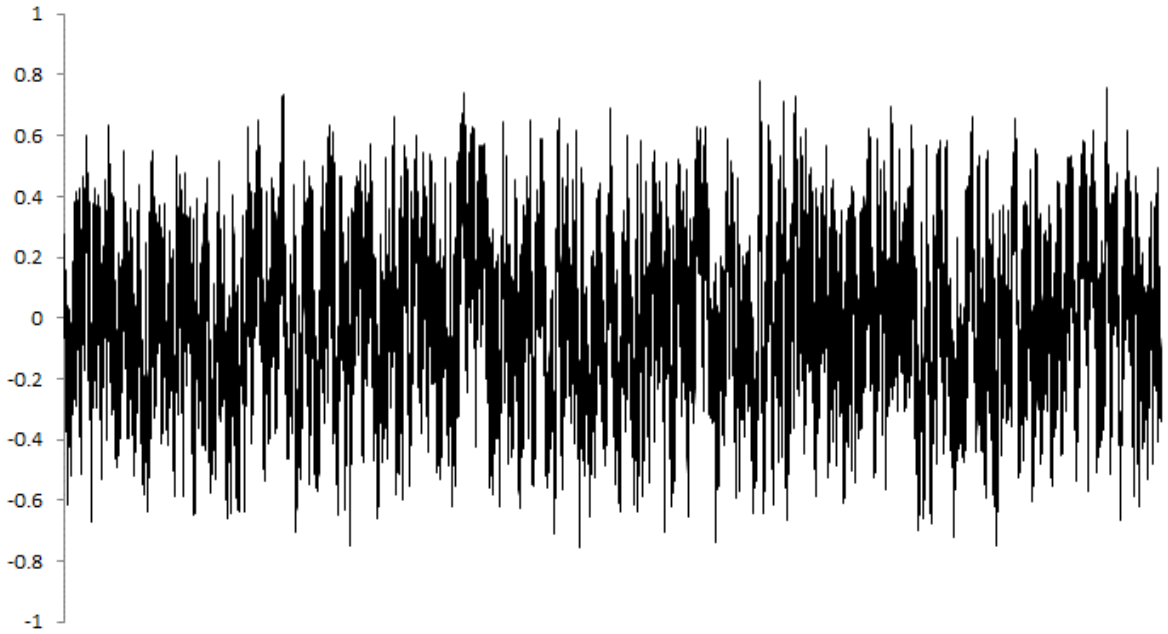

Figure 3.1. Trace plot of the sampling process of the residual correlation between valgus (VL) and varus (VR).

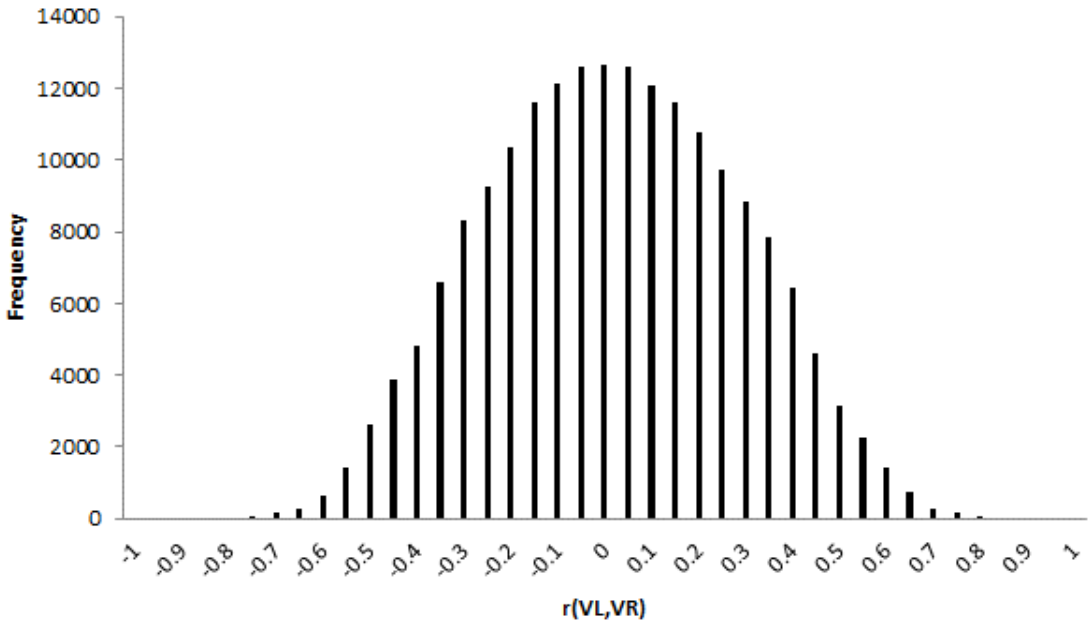

Figure 3.2. Marginal posterior distribution of the residual correlation between valgus (VL) and varus (VR).

CHAPTER 4

GENETIC ANALYSIS OF BONE QUALITY TRAITS AND GROWTH IN A RANDOM MATING BROILER POPULATION[2]

[2] F. González-Cerón, R. Rekaya, and S. E. Aggrey. Submitted to *Poultry Science*, 08/13/2014.

Abstract

We report herein the genetic relationship between growth and bone quality traits in a random mating broiler control population. Traits studied were growth rates from week 0-4 (BWG 0-4), from week 0-6 (BWG 0-6), and residual feed intake from week 5-6 (RFI 5-6). Bone quality traits were obtained at 6 weeks of age. These traits were weight (SW), length (SL), and diameter (SDIAM) of the shank; weight (TW), length (TL), and diameter (TDIAM) of the tibia. Likewise, tibia was used to obtain its density (TDEN), breaking strength (TBS), mineral density (TMD), mineral content (TMC), and ash content (TAC). At phenotypic level, growth traits were positively correlated with most of the bone quality traits except with TDEN and TAC which tended to show unfavorable associations (-0.04 to -0.31). Heritability of bone quality traits ranged from 0.08 to 0.54. The additive genetic associations of growth traits with weight, length, and diameter of shank and tibia were positive (0.37 to 0.80). A similar pattern was observed with TMD and TMC (0.06 to 0.65). In contrast, growth traits showed unfavorable genetic associations with TDEN, TBS, and TAC (-0.03 to -0.18). It is concluded that bone quality traits have an additive genetic background and they can be improved by means of genetic tools. It appears that selection for growth is negatively correlated with some traits involved in the integrity, health, and maturity of leg bones.

Keywords: Bone quality, growth

Introduction

The broiler chicken industry has improved growth to increase meat yield through genetic selection (Williams et al., 2004). Selection for growth has arguable resulted in a correlated effect on skeletal integrity (Wise, 1970; Letterier and Nys, 1992; Yalcin et al., 2001). It has been stated that selection for broiler traits has negatively impacted leg health (Reiland et al., 1978) leading to leg problems (Sanotra et al., 2001), mainly to those related to the structure of the bone (Lilburn, 1994). Others have argued that the skeletal system of modern broilers is not harmonious with their overall growth and development (Rath et al., 2000; Dibner et al., 2007; Shaw et al., 2010) and that, the distribution of the muscle mass could represent an excessive physical load for the bones (Yalcin et al., 2001). Low bone quality, is expected to lead to a greater propensity to bone deformity, fragility, risk of fractures, and economical and welfare issues (Williams et al., 2004; McDevitt et al., 2006; Shaw et al., 2010).

Femur, tibia and shank constitute the main bones of the leg (Sharman et al., 2007); and their quantitative assessment for quality and integrity include bone breaking strength (BBS), mineralization level (Hester et al., 2004) and morphological variables (Rath et al., 2000). Bone strength is influenced by several different properties (Turner, 2002; Currey, 2003; Davidson et al., 2006; Seeman and Delmas, 2006) such as shape, size, mass, structure, and composition (McDevitt et al., 2006; Lewis et al., 2009; Shaw et al., 2010). Dual-energy x-ray absorptiometry (DXA) methodology has been used for rapid measurement of bone mineral content (BMC) and density (BMD). While BMC is the measure of the mineral in the bone, BMD is a mathematical ratio of the BMC in a defined area of bone (Licata and Williams, 2014). However, given the body mass that legs have to support, bone morphological traits have been related to the propensity to leg problems (Deeb and Lamont, 2002). Studies in poultry show that, at phenotypic level, BBS is

positively correlated with BMD, bone ash content (BAC) and bone weight (Frost and Roland, 1991; Rath et al., 1999; Yalcin et al., 2001). As a result, bone mineralization-related traits are often used as strength indices (Rath et al., 2000; Hester et al., 2004; Lewis et al., 2009). Recently, Shim et al. (2012) using BBS, BAC and BMD as bone quality indicators observed that bone quality of slow growing broilers was better compared to the fast growing ones and further asserted that faster chickens were disadvantaged by the body weight they had to support.

There is a suggestion that bone mechanical properties could be alleviated through modulation of growth rate (Williams et al., 2000; Williams et al., 2004), however, a report by Leterrier et al. (1998) refutes such an approach. It has been shown that there is additive genetic component that underlies bone quality in turkey (Havenstein et al., 1988), and laying hens (Bishop et al., 2000) but the genetic relationship between growth rate and leg skeletal integrity in meat type chickens has not been sufficiently documented (Merritt, 1966; de Verdal et al., 2013) even though the inclusion of leg quality traits in broiler breeding programs has been alluded to (Whitehead, 2007).

The objective of the current study was to study the genetic basis of skeletal integrity in broiler chickens and ascertain the genetic relationship between growth rate and leg skeletal integrity and bone quality in a randombred broiler chicken population.

Material and methods

Experimental population and husbandry

A data base of 2,301 pedigreed broiler chickens, produced in 8 consecutive hatches from mating 24 sires and 72 dams, was used in this study. The data corresponded to the Arkansas random mating population which is an unselected broiler control population (Aggrey et al., 2010). Once hatched, chicks were sexed and placed in pens (0.071 m^2/bird) with litter. From hatching to 18 days the chickens received a mash starter diet containing 225 g/kg protein, 52.8 g/kg fat, 25.3 g/kg fiber, 12.90 MJ ME/kg, 9.5 g/kg calcium (Ca), and 7.2 g/kg total phosphorus (P) (4.5 g/kg available P). Henceforth chickens were fed a pelleted grower diet containing 205 g/kg protein, 57.6 g/kg fat, 25.0 g/kg fiber, 13.20 MJ ME/kg, 9.0 g/kg Ca and 6.7 g/kg total P (4.1 g/kg available P). At 28 days of age, birds were transferred to individual metabolic cages (width = 20.32 cm; length = 60.96 cm; and height = 30.48 cm). They were allowed to acclimate for one week prior to measuring feed efficiency from 5 to 6 wk. Water and feed were provided *ad libitum* for the duration of the study. Birds were kept on a 20L:4D light regimen.

Data

Weekly body weight was recorded for each bird from hatch till 6 weeks, and feed intake was measured from week 5-6. Body weight gain (BWG 0-4) from week 0-4 and (BWG 0-6) from week 0-6 was calculated. Residual feed intake (RFI 5-6) from week 5-6 was computed according to Aggrey et al. (2010). At 6 weeks, chickens were killed by exsanguination, scalded, de-feathered and eviscerated. Carcasses were chilled on ice in a cooler at 5° overnight. At the next day, carcasses were processed and both femurs were dislocated to remove the legs from the frame. In order to obtain morphological traits, shanks were measured for weight (SW), length (SL), and diameter

(SDIAM). Values from both legs were averaged to obtain a single value for each trait. On the other hand, only right tibia was measured for weight (TW), length (TL), diameter (TDIAM), and breaking strength (TBS), and only left tibia was measured for mineral density (TMD), mineral content (TMC), and ash content (TAC). Tibia diameters were measured at the narrowest and widest points, and then averaged. Assuming the tibia as a cylinder (volume = $\pi \cdot \text{radius}^2 \cdot \text{height}$), TW, TL and TDIAM measurements were used to derive the variable tibia density (TDEN), that is, TDEN = tibia weight/tibia volume. Meat from both tibias was removed before conducting all the measurements.

TBS was measured with an Instron Materials Tester (model 5500, Instron Corp., Canton, MA) with Automated Materials Test System software version 4.2. The deformation rate was 5 mm/min. Tracing of force was recorded at a constant rate. The graphs showed plateau curves of maximal force (kg) reached to measure of the energy stored in the bone.

TMC and TMD were measured by dual-energy x-ray absorptiometry (DXA). DXA scans were performed by using a Lunar Prodigy densitometer (GE Medical Systems, Waukesha, WI) operated in the small animal mode. TMC and TMD measurements by DXA are defined as the amount of bone mineral in grams in the scan region, and the amount of TMC normalized to the scan region in centimeters squared, respectively (Cauley et al. 2005; Foutz et al., 2007).

After DXA assessment, left tibia was used for determination of percentage of ash on a fat-free dry weight basis, according to AOAC International (2005; method 932.16). Bird handling and experimental protocols were in line with the University of Georgia Animal Use and Care Guidelines.

Statistical analysis

After editing 2,257 birds were recorded for BWG 0-4, BWG 0-6, and RFI 5-6. Number of birds with records for SW, SL, SDIAM, TW, TL, TDIAM, TDEN, TBS, TMD, TMC, and TAC ranged from 1,783 to 2,051. Descriptive statistics were obtained using PROC UNIVARIATE procedures of SAS version 9.1.3 (SAS Institute, 2006).

The genetic analysis was carried out using a Bayesian approach implemented via Gibbs sampling. After exploring the data with a multiple-trait animal mixed model for the joint analysis of growth and bone quality traits, it was decided to implement single trait analyses to obtain heritability estimates and two-trait analyses, considering all possible combinations of traits, to obtain genetic correlations. The linear mixed model used was:

$$y_{ijnk} = H_i + S_j + u_n + e_{ijnk} \qquad [1]$$

where y_{ijnk} was the observed BWG 0-4, BWG 0-6, RFI 5-6, SW, SL, SDIAM, TW, TL, TDIAM, TDEN, TBS, TMD, TMC, and TAC, for bird n, H_i (i = 1,2,…8) was the fixed effect of the hatch class i, S_j was the fixed effect of sex j (j = 1,2) of bird n, u_n was the random additive effect of bird n, and e_{ijnk} was the random residual term. Assuming normality conditionally on the model parameters, the joint distribution of each pair of traits is expressed in matrix notation as:

$$\mathbf{y} \mid \boldsymbol{\beta}, \mathbf{u}, \mathbf{R_0} \sim N(\mathbf{X\beta} + \mathbf{Zu}, \mathbf{R_0} \otimes \mathbf{I}) \qquad [2]$$

where $\mathbf{y} = (\mathbf{y}_1', \mathbf{y}_2')'$ is the vector of responses ($\mathbf{y}_i$); $\boldsymbol{\beta}$ and $\mathbf{u}$ are vectors of systematic and random effects, respectively; and $\mathbf{R_0}$ is a 2 × 2 residual (co)variance matrix. $\mathbf{X}$ and $\mathbf{Z}$ are known incidence matrices. The Bayesian implementation, via MCMC methods, of the model observed in [1] was

carried out following Rekaya et al. (2013). The following priors were assumed to the unknowns in the model,

$p(\boldsymbol{\beta}) \sim U[-10^6, 10^6]$

$p(\mathbf{u} \mid \mathbf{A}, \mathbf{G}) \sim N(0, \mathbf{G_0} \otimes \mathbf{A})$

and for the elements of matrix $\mathbf{G_0}$,

$p(g_{ii}) \sim U[0, 10^5]$ for $i = 1,2$,

and $p(g_{ij}) \sim U\left[-\sqrt{\sigma^2_{u1}\sigma^2_{u2}}, \sqrt{\sigma^2_{u1}\sigma^2_{u2}}\right]$ for $i \neq j = 1,2$,

where **A** is the additive genetic relationship between birds, $\mathbf{G_0}$ is the additive genetic (co)variance matrix, and g_{ii} is the genetic variance for the trait i. Similar priors are assumed for $\mathbf{R_0}$ but with 10^6 for the upper bound of the uniform distribution for the diagonal elements. In the single-trait model, var (**u**) = **A** g_{ii} for i = 1,2,…14, and var(**y**) = $\mathbf{I}\sigma^2_e$, where σ^2_e is the residual variance and **I** is the identity matrix with the appropriate dimensions.

The resulting full conditional distributions needed for the implementation of Gibbs sampling for the systematic and random effects, and genetic and residual (co)variance matrices were in closed form being normal, and scaled inverted Wishart, respectively. A unique chain of 200,000 samples was implemented where the first 50,000 samples were discarded as burn-in period based on visual inspection of the behavior of the chain. Computer software developed by Rekaya et al. (2013) was used for analysis.

Results

Descriptive statistics and phenotypic correlations among traits are summarized in Tables 4.1 and 4.3, respectively. At phenotypic level, BWG 0-4 and BWG 0-6 were positively correlated with both shank and tibia weights, lengths and diameters ranging from 0.35 to 0.79. In contrast, TDEN showed negative associations with both growth traits (-0.24 and -0.31). While BWG 0-4 and BWG 0-6 had positive correlations with TBS, TMD and TMC (0.08 to 0.72), they were negatively associated with TAC (-0.04 and -0.13, respectively); however, the relationship of BWG 0-6 with TAC was not significant ($P>0.05$). In general, the phenotypic association of RFI 5-6 with bone quality traits was weak (-0.02 to 0.08). Correlations among weight, length, and diameter of both shank and tibia bones were strong ranging from 0.45 to 0.78 with the exception of those associations of SDIAM with TW and TL (0.16 and 0.28, respectively). While the association of TW with TDEN was weak and positive (0.15), the correlations of this latter trait with the rest of the bone morphological traits were negative, ranging from -0.07 to -0.58. Phenotypic associations of bone morphological traits with TBS and mineralization-related traits were positive, ranging from 0.24 to 0.75; however, those correlations with TAC were negative or close to zero, ranging between -0.12 and 0.06. TDEN showed negative correlations with TBS, TMC, TMD, and TAC (-0.08 to -0.35). Correlations among TBS and the mineralization-related traits were all positive (0.09 to 0.90). These relationships show that TBS was strongly associated with TMD (0.70) than with TAC (0.23).

Heritability and genetic correlations of the traits are shown in Tables 4.2 and 4.3, respectively. Heritability of BWG 0-4, BWG 0-6, and RFI 5-6 were 0.26, 0.19, and 0.14, respectively. Heritability of morphological traits of bones ranged from 0.23 to 0.54 except for SDIAM which had a value of 0.09. TBS and the mineralization-related traits had heritability

ranging from 0.08 to 0.26. Genetic relationship of growth with weight, length and diameter of shank and tibia ranged from 0.37 to 0.80. In contrast, growth traits showed unfavorable genetic associations with TDEN, TBS, and TAC (-0.03 to -0.18). The mineralization-related traits TMD and TMC had positive genetic correlations with growth traits (0.06 to 0.65) but these latter ones showed an unfavorable association with TAC (-0.09 and -0.14).

In general, the genetic correlations of RFI 5-6 with the bone quality traits were positive or close to zero. Genetic associations among the weight, length and diameter of shank and tibia were positive (0.34 to 0.99). In contrast, all these latter morphological traits (SW, SL, SDIAM, TW, TL, TDIAM) had unfavorable genetic correlations with TDEN (-0.04 to -0.97). While TL showed unfavorable genetic associations with TBS and TAC (-0.23 and -0.88, respectively), TDIAM had an opposite genetic relationship with those tibia traits (TBS and TAC) (0.35 and 0.51, respectively). Shank and tibia weights, lengths and diameters showed positive genetic correlations with TMD and TMC (0.09 to 0.85). Tibia density showed unfavorable genetic associations with TBS, TMD, TMC, and TAC (-0.13 to -0.51). Mineralization-related traits (TMD, TMC, TAC) and TBS had positive genetic correlations (0.28 to 0.99).

Discussion

The main objective was to study the genetic basis of bone quality traits and their relationship with growth in broiler chickens. However, important phenotypic trends were also identified.

Growth was positively associated with size and weight of leg bones, tibia breaking strength and with tibia mineralization level measured with DXA methodology (TMD and TMC). This should be expected, as bones are also components of the overall body weight of the bird. Sharman

et al. (2007) and Tsudzuki et al. (2007) also reported positive phenotypic correlations between growth and size, and weight of leg bones. Zhou et al. (2007) reported a positive relationship between growth and TMD, and TMC. Similarly, Lewis et al. (2009), and Barreiro et al. (2011) observed a positive association between growth and leg bone breaking strength. However, the size and weight of bones do not necessarily reflect quality. In the current study, bone quality was negatively correlated to growth. Fast growing birds had relatively less tibia mineralization compared to slow growing birds, when the bone ash was used as indicator for bone mineral content. This has also been observed by Rath et al. (2000), but Frost and Roland (1991) and Seeman (1999) reported that bone strength was also related to its mass.

There are studies that suggest that growth rate is inversely related to bone mineralization, and that there is better mineralization (Corr et al., 2003; Brickett et al., 2007), density (Letterrier and Nys, 1992) and biomechanical properties (Pitsillides et al., 1999; Williams et al., 2004; Rawlinson et al., 2009; Shim et al., 2012) of bones from slow growing birds compared to their fast growing counterparts. Thus, the rate of growth could also explain the negative phenotypic correlations of growth with TAC and TDEN. It has been argued that the improvement on the mineral phase of the bone, under a slow growth rate, is due to the fact that skeleton is allowed to adapt to the increasing body mass (Brikett et al., 2007) in such a way that osteoblasts can optimally perform the filling of the bone during its formation (Williams et al., 2004).

Tibia breaking strength was positively associated with all of the bone traits except with bone density. Bone strength is not determined by a single factor but by a series of bone characteristics (Turner, 2002; Currey, 2003; Davidson et al., 2006; Seeman and Delmas, 2006) including mass, ash content, mineral density, (Rath et al., 2000), size, shape (Rath et al., 1999; Turner, 2006), and loading-induced modifications (Foutz et al., 2007; Rawlinson et al. 2009). Corr

et al. (2003) reported that heavier birds had broader bones, but these bones had smaller mineral content compared to lighter chickens. This could also explain the negative association between growth and TAC and TDEN. Therefore, the bone mineralization indicators (TMD, TMC and TAC) could be considered as important parameters in assessing biomechanical integrity of bones. Among the three, TMD is the easiest to measure and also has the strongest phenotypic correlation with TBS.

Heritability of weight and length of both leg bones were within the range of values (0.16-0.62) estimated from chicken (Merritt, 1966; de Verdal et al., 2013) and turkey populations (Abplanalp and Kosin, 1952; Kondra and Shoffner, 1955; Johnson and Asmundson, 1957; McCartney, 1961; Krueger et al., 1972; Havenstein et al., 1988). In contrast, heritability of shank and tibia diameters were lower than reported estimates of 0.33-0.46 for shank diameter and 0.74 for tibia diameter in turkeys (Nestor et al., 1985; Havenstein et al., 1988) and broiler chickens (de Verdal et al., 2013). Heritability of tibia density was lower than 0.62-0.65 reported by Havenstein et al. (1988) for turkeys, however, it should be pointed out that they used a different method to assess these traits. Likewise, heritability of tibia breaking strength was lower than 0.30-0.80 reported for broiler chickens (Mandour et al., 1989; de Verdal et al., 2013) and White Leghorn hens (Bishop et al., 2000). We are not aware of any heritability estimates on TMD and TMC in broiler chicken but studies in mice (Klein et al., 1998) and humans (Ng et al., 2006; Park et al., 2012; Wagner et al., 2013) have reported heritability of bone mineral density, corresponding to either the whole body or several different types of bones, ranging from 0.35 to 0.84. Heritability for TAC was lower than reported estimate of 0.41 (de Verdal et al., 2013).

In general, the heritability estimates for bone traits in the current study imply that they can be improved by means of genetic selection. However, given the low heritability of SDIAM and

TAC other non-genetic strategies may be beneficial than genetic improvement. Heritability of the growth traits ranged from 0.19 to 0.26, and that of RFI was 0.14 were was lower than the estimate reported by Rekaya et al. (2013) for commercial broiler population for the period 6 to 7 weeks of age.

Estimate genetic correlations between length and weight of each leg bone (shank and tibia) followed the positive pattern observed at phenotypic level and also were in line with genetic association values of 0.72-0.84 reported by Havenstein et al. (1988) from a turkey population. Therefore, weight and length of the bone would be influenced by common genetic factors.

Current genetic correlations of growth with shank length and tibia weight followed the pattern of values (0.77 to 0.81, and 0.69, respectively) reported previously in broiler chickens populations (Merritt, 1966; de Verdal et al., 2013). Likewise, our results were in line with genetic correlations of growth with length and weight of shank and tibia (0.27 to 0.94), informed in investigations with turkeys (Johnson and Asmundson, 1957; McCartney, 1961; Krueger et al., 1972; Havenstein et al., 1988). The genetic association of growth with tibia length and tibia diameter found in the current study differs from the study by de Verdal et al. (2013) who reported negative genetic correlations of body weight of broiler chickens at 23 days of age with tibia length (-1.00) and tibia diameter (-0.95). Overall, our observations indicated that genetic factors influencing faster growth also lead to heavier, longer and wider leg bones.

Current results indicated that although the genetic association between TMD and TMC is positive but it is not as strong as the observed association at phenotypic level in the present study (0.90). Tibia mineral content had a stronger association with the direct assessment of the mineralization level of the bone (TAC), than that of TMD with TAC. Thus TMC would be a more

suitable none invasive method for improvement of bone quality. The genetic correlations of growth with tibia ash content followed the negative pattern (-0.22) reported by de Verdal et al. (2013) from a broiler chickens population. Although the other mineralization-related traits (TMD and TMC) showed a positive genetic association with growth traits, the above mentioned association of TAC with growth would be more relevant given the strong genetic association of TAC with TBS (0.99) and the importance of TBS as indicator trait of skeletal integrity (Turner, 2006; Rath et al., 2000). Likewise, this negative association would support the idea that genetic selection for growth rate has negative effects on leg soundness (Lilburn, 1994; Webster, 1995), due to the fact that bone development is not in synch with the body mass (Rath et al., 2000; Dibner et al., 2007; Shaw et al., 2010). Therefore, the general observation would indicate that faster growth affects negatively the mineralization level of the leg bone.

Unlike previous research with broiler chickens (de Verdal et al., 2013) and laying hens (Bishop et al., 2000), which found a favorable genetic association between growth and tibia breaking strength (0.93 and 0.33, respectively), current results clearly suggested that genetic factors that favor greater growth rates do have an opposite effect on TBS. Likewise, our observations also indicated that TBS would not be favored when selecting for better feed efficiency. A similar trend (0.54) was previously reported by de Verdal et al. (2013).

Genetic correlations of tibia breaking strength with tibia length and tibia weight resembled the pattern (-0.70, and 0.69, respectively) found in a previous study with broiler chickens (de Verdal et al., 2013). Therefore, genetic factors favoring tibia weight do have a similar effect on TBS. This fact confirms our observations at phenotypic level and other results found in poultry (Frost and Roland, 1991). The importance of the weight of the bone for its breaking strength relies on the fact that the mineral phase constitutes around 70% of the bone mass and the mineral content

of the bone is a main determinant of its biomechanical properties (Rath et al., 2000; Currey, 2003). In contrast, promoting larger tibias by selection would have detrimental effects on TBS.

Current phenotypic results and observations from other studies in poultry (Rath et al., 1999; Onyango et al., 2003; Kim et al., 2004) confirmed the relevance of the mineral phase of the bone (TMD, TMC, TAC) on its biomechanical properties (TBS). This association was reaffirmed by current genetic correlations of TBS with TMD, TMC and TAC. Our results also suggested that practically TAC and TBS are influenced by the same genetic factors. However, given their genetic association, TMC would represent a good indicator of TBS when invasive methods for mineralization assessment are not feasible.

Conclusions

Bone quality traits showed additive genetic variation, implying that they can be improved through selection. It appears that genetic selection for growth is negatively correlated with some traits involved in the integrity, health, and maturity of leg bones. The improvement of the mineralization level of the bone would enhance its quality and strength which would be reflected in an improved welfare.

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Table 4.1. Descriptive statistics of growth, feed efficiency, and bone quality traits in the Arkansas randombred chicken population.

Trait[1]	N	Mean	SD	Minimum	Maximum
BWG 0-4 (kg)	2,257	0.82	0.12	0.18	1.21
BWG 0-6 (kg)	2,257	1.65	0.21	0.71	2.36
RFI 5-6 (g)	2,257	0.00	0.11	-0.53	0.85
SW (g)	2,048	73.49	11.83	37.00	111.00
SL (mm)	2,049	76.70	5.79	51.12	93.47
SDIAM (mm)	2,051	10.57	1.81	5.80	17.22
TW (g)	1,840	13.14	2.69	5.00	24.00
TL (mm)	1,950	100.26	5.39	68.15	116.34
TDIAM (mm)	1,950	7.49	0.81	5.02	9.97
TDEN (mg/mm^3)	1,832	3.01	0.51	1.48	5.07
TBS (kg)	1,783	25.30	6.76	5.92	94.96
TMD (g/cm^2)	1,947	0.12	0.02	0.07	0.18
TMC (g)	1,947	1.03	0.27	0.20	2.03
TAC (%)	1,965	40.13	3.04	24.88	79.73

[1]BWG 0-4 = body weight gain from 0 to 4 weeks of age; BWG 0-6 = body weight gain from 0 to 6 weeks of age; RFI 5-6 = residual feed intake from 5 to 6 weeks of age; SW = shank weight; SL = shank length; SDIAM = shank diameter; TW = tibia weight; TL = tibia length; TDIAM = tibia diameter; TDEN = tibia density; TBS = tibia breaking strength; TMD = tibia mineral density; TMC = tibia mineral content; TAC = tibia ash content.

Table 4.2. Posterior means (posterior SD) of genetic, residual variances, and heritability of growth, feed efficiency, and bone quality traits in the Arkansas randombred chicken population.

Trait[1]	Genetic variance	Residual variance	Heritability
BWG 0-4	0.00295 (0.00000)	0.00830 (0.00036)	0.26 (0.03)
BWG 0-6	0.00541 (0.00000)	0.02221 (0.00097)	0.19 (0.04)
RFI 5-6	0.00179 (0.00000)	0.01094 (0.00040)	0.14 (0.03)
SW	24.40240 (25.49707)	37.82934 (3.05476)	0.39 (0.07)
SL	6.94273 (2.11967)	12.84962 (0.91417)	0.35 (0.06)
SDIAM	0.10653 (0.00122)	1.05051 (0.04123)	0.09 (0.03)
TW	0.99229 (0.04994)	2.16205 (0.14838)	0.31 (0.06)
TL	12.24331 (5.25709)	10.39515 (1.28144)	0.54 (0.07)
TDIAM	0.13644 (0.00072)	0.24140 (0.01703)	0.36 (0.06)
TDEN	0.03505 (0.00006)	0.11566 (0.00608)	0.23 (0.05)
TBS	6.46112 (2.53353)	28.99568 (1.41306)	0.18 (0.04)
TMD	0.00012 (0.00000)	0.00081 (0.00003)	0.13 (0.01)
TMC	0.01277 (0.00001)	0.03532 (0.00183)	0.26 (0.04)
TAC	0.49280 (0.02831)	5.41462 (0.21402)	0.08 (0.03)

[1]BWG 0-4 = body weight gain from 0 to 4 weeks of age; BWG 0-6 = body weight gain from 0 to 6 weeks of age; RFI 5-6 = residual feed intake from 5 to 6 weeks of age; SW = shank weight; SL = shank length; SDIAM = shank diameter; TW = tibia weight; TL = tibia length; TDIAM = tibia diameter; TDEN = tibia density; TBS = tibia breaking strength; TMD = tibia mineral density; TMC = tibia mineral content; TAC = tibia ash content.

Table 4.3. Genetic (above diagonal) and phenotypic (below diagonal) correlations of growth, feed efficiency, and bone quality traits in the Arkansas randombred chicken population.

Trait[1]	BWG 0-4	BWG 0-6	RFI 5-6	SW	SL	SDIAM	TW	TL	TDIAM	TDEN	TBS	TMD	TMC	TAC
BWG 0-4		0.82	0.17	0.73	0.59	0.40	0.67	0.72	0.37	-0.03	-0.18	0.06	0.47	-0.14
BWG 0-6	0.77**		0.27	0.80	0.64	0.52	0.79	0.74	0.50	-0.10	-0.04	0.18	0.65	-0.09
RFI 5-6	-0.01	0.00		0.21	0.20	0.12	0.13	0.22	-0.03	0.09	0.11	0.01	0.08	0.08
SW	0.60**	0.79**	0.00		0.87	0.95	0.98	0.84	0.55	-0.10	-0.20	0.23	0.71	-0.40
SL	0.48**	0.64**	0.01	0.68**		0.34	0.93	0.99	0.39	-0.10	-0.12	0.11	0.53	-0.98
SDIAM	0.35**	0.46**	0.00	0.46**	0.62**		0.84	0.36	0.58	-0.24	-0.02	0.09	0.60	-0.33
TW	0.39**	0.61**	0.05*	0.78**	0.50**	0.16**		0.91	0.86	-0.33	0.02	0.20	0.85	-0.21
TL	0.50**	0.63**	0.08*	0.64**	0.71**	0.28**	0.70**		0.37	-0.04	-0.23	0.10	0.58	-0.88
TDIAM	0.45**	0.68**	-0.01	0.75**	0.59**	0.45**	0.68**	0.49**		-0.97	0.35	0.29	0.80	0.51
TDEN	-0.24**	-0.31**	0.05*	-0.20**	-0.35**	-0.51**	0.15**	-0.07*	-0.58**		-0.34	-0.13	-0.42	-0.51
TBS	0.08*	0.40**	0.04	0.37**	0.33**	0.30**	0.38**	0.24**	0.53**	-0.29**		0.28	0.42	0.99
TMD	0.28**	0.55**	0.01	0.58**	0.43**	0.38**	0.47**	0.31**	0.64**	-0.33**	0.70**		0.33	0.08
TMC	0.50**	0.72**	-0.02	0.75**	0.58**	0.43**	0.61**	0.50**	0.74**	-0.35**	0.58**	0.90**		0.45
TAC	-0.13**	-0.04	0.03	-0.12**	0.02	0.06*	-0.10**	-0.02	-0.03	-0.08*	0.23**	0.23**	0.09*	

[1]BWG 0-4 = body weight gain from 0 to 4 weeks of age; BWG 0-6 = body weight gain from 0 to 6 weeks of age; RFI 5-6 = residual feed intake from 5 to 6 weeks of age; SW = shank weight; SL = shank length; SDIAM = shank diameter; TW = tibia weight; TL = tibia length; TDIAM = tibia diameter; TDEN = tibia density; TBS = tibia breaking strength; TMD = tibia mineral density; TMC = tibia mineral content; TAC = tibia ash content. **P<0.0001; *P<0.05

CHAPTER 5

GENETIC ANALYSIS OF LEG PROBLEMS AND BONE QUALITY TRAITS IN A RANDOM MATING BROILER POPULATION[3]

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Abstract

It has been suggested that genetic selection for broiler traits has a role as causative factor in leg problems and reduced bone quality. We report herein the genetic association between leg problems and bone quality traits in a random mating broiler control population. The studied leg problems traits were valgus (VL), varus (VR), and tibial dyschondroplasia (TD). Leg problems were expressed as binary traits of 0 (normal) and 1 (abnormal). Bone quality traits were obtained at 6 weeks of age. These traits were weight (SW), length (SL), and diameter (SDIAM) of the shank; weight (TW), length (TL), and diameter (TDIAM) of the tibia. Likewise, tibia was used to obtain its density (TDEN), breaking strength (TBS), mineral density (TMD), mineral content (TMC), and ash content (TAC). A threshold-linear mixed model was employed for the joint analysis of the categorical and linear traits. A Bayesian approach, via the Gibbs sampling method, was used in the implementation. At phenotypic level, chickens affected by VL, VR or TD shared the fact of having less mineralized tibias in terms of TAC. Furthermore, birds with either VL or VR problem had lighter and less dense tibias compared to healthy individuals. Heritabilities of leg problems ranged from 0.10 to 0.13. Heritability of bone quality traits ranged from 0.10 to 0.77. Genetic correlations of leg problems with bone quality traits tended to be weak ranging from -0.06 to 0.11. Additionally, these estimates had large posterior standard deviations (0.04 to 0.21) due to the relatively small size of the used dataset. Leg problems and bone quality traits showed to have an additive genetic basis. However, the low heritability of leg problems and some of the bone quality traits suggest that, for their improvement, management strategies would be also important.

Keywords: Leg problem, bone quality, breeding

Introduction

Genetic selection for broiler traits has had a relevant contribution to the production performance that characterizes the modern broiler chicken. However, it has been claimed that these improvements are not exempt of negative side effects such as metabolic disorders (EC, 2000) and imbalances that affect the development of different systems in the body of the chicken (Shaw et al., 2010). Leg problems, such as tibial dyschondroplasia (TD) and varus-valgus deformities (VVD) have been pointed out as important examples of those negative effects (Julian, 2005). Although the precise etiology of these leg problems is not known (Rath et al., 2004; Hunter et al., 2008), it has been suggested that low quality bones could aggravate the leg problems by increasing the propensity to bone deformity, fragility and risk of fractures (Shaw et al., 2010). Bone quality can be assessed through the measurement of bone traits such as breaking strength (BBS), mineralization level (Hester et al., 2004), and size (Rath et al., 2000). On the other hand, the heritable nature of TD and VVD has been reported from several studies (Sheridan et al., 1978; Le Bihan-Duval et al., 1997; Kapell et al., 2012; Rekaya et al., 2013). In contrast, it appears that neither the heredity of traits related to the integrity of leg bones (Merritt, 1966; de Verdal et al., 2013), nor their genetic relationship with leg problems has been explored.

The objective of this investigation was to study the genetic relationship between leg problems and bone quality traits, in broiler chickens, by means of the estimation of genetic parameters.

Materials and methods

Experimental population and husbandry

The present study used data from 2,301 pedigreed broiler chickens, produced in 8 consecutive hatches from mating 24 sires and 72 dams. The Arkansas random mating population, an unselected broiler control population, was used (Aggrey et al., 2010). Once hatched, chicks were sexed and placed in pens (0.071 m^2/bird) with litter. From hatching to 18 days, the chickens received a mash starter diet containing 225 g/kg protein, 52.8 g/kg fat, 25.3 g/kg fiber, 12.90 MJ ME/kg, 9.5 g/kg calcium (Ca), and 7.2 g/kg total phosphorus (P) (4.5 g/kg available P). Henceforth chickens were fed a pelleted grower diet containing 205 g/kg protein, 57.6 g/kg fat, 25.0 g/kg fiber, 13.20 MJ ME/kg, 9.0 g/kg Ca and 6.7 g/kg total P (4.1 g/kg available P). At 28 days of age, birds were transferred to individual metabolic cages (width = 20.32 cm; length = 60.96 cm; and height = 30.48 cm). Water and feed were provided *ad libitum* for the duration of the study. Birds were kept on a 20L:4D light regimen.

Data

The scoring of the legs for VVD and TD was performed at weeks 4 and 6, respectively. The VVD scores were obtained using the methods described by Leterrier and Nys (1992). Depending on the angle size of tibia-metatarsus, 4 categories of scores were defined. VVD were defined as: normal (score = 0), mild (10-25° angle; score = 1), intermediate (25-45° angle; score = 2), and severe (>45° angle; score = 3). Methods described by Edwards and Veltmann (1983) were followed in order to score TD in the right tibia. A longitudinal cut across the tibia was made and the white cartilage plug abnormality was observed. Depending on the severity of the cartilage

abnormality, 4 category scores for TD were defined: normal (score = 0), mild (score = 1), intermediate (score = 2), and severe (score = 3).

Bone quality traits were obtained at 6 weeks of age when the chickens were killed by exsanguination, scalded, de-feathered and eviscerated. During processing both femurs were dislocated to remove the legs from the frame. All flesh and skin were removed from the tibia prior to measurement. To obtain morphological traits, both shanks were measured for weight (SW), length (SL), and diameter (SDIAM). Values from both legs were averaged to have a single value for each trait. In the case of tibia, only the right limb was used to measure weight (TW), length (TL), diameter (TDIAM), and breaking strength (TBS). Assuming the tibia as a cylinder, TW, TL and TDIAM measurements were used to derive the tibia density (TDEN). The left tibia was used to measure mineral density (TMD), mineral content (TMC), and ash content (TAC). Meat from both tibias was removed before conducting all the measurements.

Tibia breaking strength was obtained with an Instron Materials tester (model 5500, Instron Corp., Canton, MA) with Automated Materials Test System software version 4.2. Tibia diameters were measured at the narrowest and widest points, and then averaged. The deformation rate was 5 mm/min. Tracing of force was recorded at a constant rate. The graphs showed plateau curves of maximal force (kg) reached to measure the energy stored in the bone. Tibia mineral content and TMD were assessed by dual-energy x-ray absorptiometry (DXA). DXA scans were performed by using a Lunar Prodigy densitometer (GE Medical Systems, Waukesha, WI) operated in the small animal mode. Bone density measurement by DXA is based on projection of bone density and is reported as grams per squared centimeters. After DXA assessment, left tibia was used for determination of percentage of ash on a fat-free dry-weight basis, according to AOAC

International (2005; method 932.16). Bird handling and experimental protocols were in line with the University of Georgia Animal Use and Care Guidelines.

Statistical analysis

After removing outliers (individuals with values greater than 3 SD from the mean), there were 2,257 birds recorded for five leg problems traits: valgus in the right leg (VLR), valgus in the left leg (VLL), varus in the right leg (VRR), varus in the left leg (VRL), and TD. However, VLR and VLL were merged into valgus (VL), and VRR and VRL were merged into varus (VR) for the analysis. To ameliorate the grossly unequal representation of leg incidences in different hatch groups, and also to eliminate extreme case problems (all observations within a class of the fixed effects have the same discrete response) and improve the computational stability of the analyses, the leg data were reclassified as binary responses 0 (normal) and 1 (abnormal) for each leg. On the other hand, the number of birds with records for SW, SL, SDIAM, TW, TL, TDIAM, TDEN, TBS, TMD, TMC, and TAC ranged from 1,783 to 2,051. Descriptive statistics were obtained using PROC UNIVARIATE procedures of SAS version 9.1.3 (SAS Institute, 2006). Differences in bone quality traits between normal and leg affected chickens were analyzed by *t* test. A threshold-linear mixed model similar to the model used by Rekaya et al. (2013) was implemented for the joint analysis of leg problems (binary scale) and the continuous bone quality traits. The threshold-linear mixed model used was:

$$y_{ijnk} = H_i + S_j + u_n + e_{ijnk} \qquad [1]$$

where y_{ijnk} was the observed SW, SL, SDIAM, TW, TL, TDIAM, TDEN, TBS, TMD, TMC, and TAC, or the liabilities for the leg problems (VL, VR, TD) for bird n, H_i (i = 1,2,..8) was the fixed effect of the hatch class i, S_j was the fixed effect of sex j (j = 1,2), u_n was the random additive

effect of bird n, and e_{ijnk} was the random residual term. Assuming normality conditionally on the model parameters, the joint distribution of the liability for binary scores and continuous traits is expressed in matrix notation as:

$$\mathbf{y} \mid \boldsymbol{\beta}, \mathbf{u}, \mathbf{R_0} \sim N(\mathbf{X\beta} + \mathbf{Zu}, \mathbf{R_0} \otimes \mathbf{I}) \qquad [2]$$

where $\mathbf{y} = (\mathbf{l}_1', \mathbf{l}_2', \mathbf{l}_3', \mathbf{y}_1', \mathbf{y}_2', \ldots, \mathbf{y}_{11}')'$ is the vector of liabilities ($\mathbf{l}$) and continuous responses ($\mathbf{y}_i$); $\boldsymbol{\beta}$ and $\mathbf{u}$ are vectors of systematic and random effects, respectively; and $\mathbf{R_0}$ is an 14 × 14 residual (co)variance matrix with the first 3 diagonal elements, corresponding to the categorical traits, fixed to 1. $\mathbf{X}$ and $\mathbf{Z}$ are known incidence matrices. Rekaya et al. (2013) stated that in the Bayesian implementation of a model like the observed in [1] via MCMC methods, the direct sampling of $\mathbf{R_0}$ is not feasible due to the fixation of some of its diagonal elements. To overcome the problem of sampling of the residual (co) variance matrix and in order to perform the Bayesian implementation of the model via Gibbs sampling, the methods described by Rekaya et al. (2013) were used.

The full Bayesian implementation of the model needed for the implementation of Gibbs sampling for the systematic and random effects, liabilities, and genetic and residual (co)variance matrices were in closed form, truncated normal and scaled inverted Wishart, respectively (Rekaya et al., 2013). A unique chain of 200,000 samples was implemented where the first 50,000 samples were discarded as burn-in period based on visual inspection of the behavior of the chain. Computer software developed by Rekaya et al. (2013) was used for analysis.

Results

Means of bone quality trait and category of leg problem are summarized in Table 5.1. Heritability and genetic correlations of leg problems and bone quality traits are shown in Tables

5.2 and 5.3, respectively. There were differences in bone quality traits (SW, SL, TW and TAC) between normal and chickens affected by VL, VR or TD (Table 5.1). In addition to these four traits, TDEN was also different between normal birds and chickens affected by VL or VR. Chickens affected by VL had higher values for shank traits (SW, SL, and SDIAM), TDIAM, and TMC in comparison to normal birds. However, those chickens with VL problems had lower values for some tibia traits (TW, TDEN, and TAC) compared to their healthy counterparts. Birds with TD had significant higher values for shank and tibia traits (weight, length and diameter) compared to their healthy counterparts. Chickens with TD also had lover TAC compared to their non-TD counterparts.

Therefore, chickens affected by VL, VR or TD appeared to have less mineralized tibia as evidenced by their TAC values compared to the healthy birds. Further, individuals with either VL or VR problem had lighter and less dense tibias comparing to healthy individuals. These negative associations of TAC with the three leg problems and those of TW and TDEN with VL and VR were confirmed by the phenotypic correlations in Table 5.3.

Heritability of VL, VR, and TD were 0.10, 0.12, and 0.13, respectively. Heritability of bone quality traits ranged from 0.10 to 0.77. Genetic correlations of leg problems with bone quality traits tended to be weak ranging from -0.06 to 0.11. Additionally, posterior standard deviations of these estimates were large (0.04 to 0.21). While genetic associations of shank traits and varus-valgus deformities (VVD) followed an unfavorable trend (0.00 to 0.11), those with TD showed a slightly favorable tendency (-0.06 - 0.01). Tibia weight, TL, and TDIAM had unfavorable genetic associations with VVD (0.02 - 0.11) and close to zero with TD (-0.03 - 0.00). Tibia density and TBS showed a slight favorable genetic correlation with VL (-0.04 and -0.05, respectively) and TD (-0.03 and -0.03, respectively). In contrast, the corresponding genetic associations of TDEN and

TBS with VR were close to zero (0.00 and 0.02, respectively). Genetic correlations of VL and TD with mineralization-related traits (TMD, TMC, TAC) tended to be slightly favorable (-0.06 - 0.00). On the contrary, VR showed an unfavorable genetic association with TMD, TMC, and TAC (0.00 - 0.09).

Genetic correlations among bone quality traits varied from -0.91 to 0.99. Posterior standard deviations of these estimates were low (≤0.03). Genetic correlations among weight, length and diameter of leg bones were strong and positive (0.39 - 0.99). TDEN showed negative genetic associations with all other bone traits (-0.91 to -0.10). TBS was favorably associated to the other bone quality traits (0.07 - 0.95) except with TL and TDEN (-0.04 and -0.64, respectively). The mineralization-related traits TMD and TMC were favorably associated to weight, length and diameter of leg bones (0.15 - 0.86). In contrast, TAC showed positive associations with SDIAM, TW, and TDIAM (0.10 to 0.52) but had negative correlations with SW, SL, and TL (-0.26 - -0.09). Mineralization-related traits (TMD, TMC, TAC) showed favorable genetic association among them (0.46 - 0.55).

Discussion

The main objective of this study was to investigate the genetic relationship between leg problems (VL, VR, and TD) and bone quality traits. At phenotypic level, VL, VR, and TD affected birds appeared to be associated to lower values of TAC compared to normal birds. This was on contrast with other nutritional-related study that reported no apparent relationship between TAC and TD (Edwards and Veltmann, 1983; Leach and Lilburn, 1992) or with varus-valgus deformities (VVD) (Leterrier et al., 1998). However, the aforementioned phenotypic relationship in concordance with reports by Edwards (2003), Whitehead (2007) and Khan et al. (2010). Likewise, Letterier and Nys (1992) also found that VVD affected birds had lower (shank) ash content

comparing to normal chickens. Contrary to our results, Hocking et al. (2009) found lighter tibias in TD affected chickens and Walser et al. (1982) showed that tibia length was not associated to TD. In the current study, we observed that varus affected birds has shorter tibia which was similar with the results of Leterrier and Nys (1992).

There are conflicting reports on leg abnormalities in chickens. This may be due to several factors including the definition of the trait, incidence of the malady in the population studied, the population size, and the analytical method. However, it is clear that chickens affected by VL, VR or TD showed an important phenotypic difference in TAC compared to normal birds. It seems that tibia weight and density is related to incidence of VVD problems in the chicken population under study. As mentioned by Turner (2006) and Seeman and Delmas (2006) bone integrity is a function of several traits, however, in the current study, tibia ash content tend to have a major influence on bone quality.

Heritability of TD was 0.13 which was in concordance with that reported by Kapell et al. (2012) and Rekaya et al. (2013) using different commercial populations. The heritability for TD was lower than the 0.22 reported by Sheridan et al. (1978) and Burton et al. (1981). The heritability for VVD was low, about 0.10, which was lower than previous estimates (Le Bihan-Duval et al., 1997; Rekaya et al., 2013). The heritability of shank traits were moderate ranging from 0.37 to 0.46 which was in concordance with previous reports (Merritt, 1966; Tsudzuki et al., 2007). Our heritability estimate of TW was 0.52 which was higher than the estimate reported by de Verdal et al. (2013) in a broiler chicken population but estimates for TL and TDIAM were similar. Bone breaking strength is an important trait directly relating to the bone's ability to support weight. Tibia bone strength is moderately heritable (0.29). Information in the literation on similar heritability

estimate is scarce. However, heritability estimates of humerus elastic force and humerus stress ranged from 0.67 to 0.80 (Mandour et al. 1989; de Verdal et al. 2013).

The heritability of TMD and TMC were 0.17 and 0.48, respectively. There appears to be no previous reports and the heritability of these traits in the poultry literature. However, in mouse and human studies, heritability of bone mineral density ranged from 0.35 to 0.84 (Klein et al., 1998; Ng et al., 2006; Park et al., 2012; Wagner et al., 2013). Tibia mineral content has moderate heritability, which is similar to reports in humans (Havill et al., 2007) which imply that, as a trait, it will respond favorably to genetic selection. The low heritability values of leg maladies and some bone quality traits such as TAC suggest that the phenotypic expressions of these traits are influenced mostly by non-genetic factors. Therefore, management strategies would be also necessary in order to observe their improvement (Rekaya et al., 2013). In addition to the magnitude of non-genetic factors influencing these traits, the accuracy of genetic parameter estimates are low. This, could be due in part to inaccuracies in the measurement of these traits and their low incidence levels in the populations that have been studied thus far.

From the genetic correlations, both shank and tibia length and weight contribute towards the development of varus-valgus deformities. Breeders can include these traits in their selection programs to reduce the incidence of VVD. However, both shank and tibia length and weight tend to have insignificant effect of TD. Even though a negative association was reported between TD and TW (Hocking et al., 2009), it was on the phenotypic level while Walser et al. (1982) reported no phenotypic relationship between these two traits. From the mineralization data, it appears that improvements in TMD, TMC and TAC would contribute towards reductions in incidences of VL and TD (Table 5.3). In general, there is a very low negative genetic relationship between bone traits and VL. As a result, improvement in bone quality may slightly reduce the incidence of

valgus. On the other hand, VR have a slightly positive genetic relationship in bone quality. This is expected since there is a very low negative genetic correlation between VL and VR. Even with low additive genetic factors that underlie both VL and VR, the genetic basis for these two leg abnormalities may be different. Tibia ash content was highly correlated genetically with TBS, therefore, tibia ash could be considered as a better indicator of bone quality than the in-vitro measurement, TMC which had a genetic correlation of 0.6 with TBS. TAC has a low negative genetic correlation with VL and TD suggesting that improving the ash content of bones could have some positive effects on reducing the incidences of these maladies.

The current study suggests that improvement in bone quality could reduce the incidences of VL and TD, but have slight detrimental effect on VR. This is supported by the weak positive genetic relationships between bone quality and VR, and the negative genetic correlation between VR and VL. Even though some of the genetic correlations were small, the trends in the interrelationships among the bone quality traits and bone maladies were obvious.

Conclusions

Leg problems and bone quality traits have underlying additive genetic effects even though it is relatively small compared to growth and meat quality traits. However, genetic improvement is still possible. Efforts should be made to increase bone mineralization as it seem to improve bone quality and bone breaking strength which has welfare implications. The low genetic factors affecting leg problems also make management and nutritional strategies equally important in ameliorating these issues.

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Table 5.1 Mean values of bone quality traits between normal (score = 0) and leg affected (score = 1) broiler chickens in the Arkansas randombred chicken population.

Leg Problems[1]	Score	Bone Quality Traits[1]										
		SW (g)	SL (mm)	SDIAM (mm)	TW (g)	TL (mm)	TDIAM (mm)	TDEN (mg/mm^3)	TBS (kg)	TMD (g/cm^2)	TMC (g)	TAC (%)
VL	0	73.16	76.42	10.45	13.28	100.20	7.46	3.08	25.41	0.12	1.02	40.54
	1	74.46*	77.52*	10.90**	12.78*	100.44	7.55*	2.85**	25.02	0.12	1.05*	38.96**
VR	0	73.64	76.76	10.56	13.21	100.38	7.49	3.03	25.40	0.12	1.03	40.18
	1	69.72*	75.13*	10.71	11.53**	97.42**	7.46	2.72**	23.06*	0.11*	1.00	38.91*
TD	0	73.39	76.60	10.54	13.10	100.18	7.48	3.01	25.28	0.12	1.03	40.15
	1	77.90*	80.74**	11.50*	14.72**	103.68**	7.73*	3.04	25.83	0.12	1.10	39.13*

[1]VL = valgus (right and left legs combined); VR = varus (right and left legs combined); TD = tibial dyschondroplasia; SW = shank weight; SL = shank length; SDIAM = shank diameter; TW = tibia weight; TL = tibia length; TDIAM = tibia diameter; TDEN = tibia density; TBS = tibia breaking strength; TMD = tibia mineral density; TMC = tibia mineral content; TAC = tibia ash content. Number of observations by bone quality trait within normal and leg affected birds ranged from 1,284 to 2,003 and from 46 to 529, respectively. Within each leg problem, * and ** indicate significant difference from normal birds at $P<0.05$ and $P<0.0001$, respectively.

Table 5.2 Posterior means (posterior SD) of genetic, residual variances, and heritability of leg problems and bone quality traits in the Arkansas randombred chicken population.

Trait[1]	Genetic variance	Residual variance	Heritability
VL	0.12 (0.010)	1.000	0.10 (0.07)
VR	0.15 (0.021)	1.000	0.12 (0.08)
TD	0.16 (0.026)	1.000	0.13 (0.09)
SW	38.17 (92.752)	29.272 (5.481)	0.56 (0.11)
SL	9.80 (2.558)	10.952 (1.057)	0.47 (0.06)
SDIAM	0.23 (0.005)	0.982 (0.049)	0.19 (0.05)
TW	1.90 (0.092)	1.708 (0.175)	0.52 (0.06)
TL	15.33 (2.918)	8.639 (1.040)	0.64 (0.05)
TDIAM	0.39 (0.007)	0.110 (0.042)	0.77 (0.10)
TDEN	0.14 (0.001)	0.063 (0.014)	0.68 (0.09)
TBS	10.75 (4.001)	26.206 (1.462)	0.29 (0.05)
TMD	0.0002 (0.000)	0.0008 (0.000)	0.17 (0.02)
TMC	0.03 (0.000)	0.028 (0.002)	0.48 (0.06)
TAC	0.59 (0.009)	5.398 (0.191)	0.10 (0.02)

[1]VL = valgus (right and left legs combined); VR = varus (right and left legs combined); TD = tibial dyschondroplasia; SW = shank weight; SL = shank length; SDIAM = shank diameter; TW = tibia weight; TL = tibia length; TDIAM = tibia diameter; TDEN = tibia density; TBS = tibia breaking strength; TMD = tibia mineral density; TMC = tibia mineral content; TAC = tibia ash content.

Table 5.3 Genetic (above diagonal) and phenotypic (below diagonal) correlations of leg problems and bone quality traits in the Arkansas randombred chicken population.

Trait[1]	VL	VR	TD	SW	SL	SDIAM	TW	TL	TDIAM	TDEN	TBS	TMD	TMC	TAC
VL		-0.03	0.02	0.02	0.04	0.00	0.02	0.04	0.04	-0.04	-0.05	-0.02	0.00	-0.06
VR	-0.10**		0.02	0.11	0.10	0.09	0.11	0.10	0.05	0.00	0.02	0.03	0.09	0.00
TD	0.04	-0.03		-0.03	0.01	-0.06	-0.03	0.01	0.00	-0.03	-0.03	-0.03	-0.04	-0.03
SW	0.05*	-0.06*	0.06*		0.86	0.87	0.96	0.84	0.53	-0.17	0.07	0.29	0.76	-0.09
SL	0.08*	-0.05*	0.11**	0.68**		0.58	0.92	0.99	0.51	-0.23	0.12	0.24	0.70	-0.10
SDIAM	0.11**	0.02	0.08*	0.46**	0.62**		0.83	0.52	0.70	-0.41	0.29	0.40	0.79	0.19
TW	-0.08*	-0.12**	0.10**	0.78**	0.50**	0.16**		0.87	0.66	-0.32	0.28	0.37	0.86	0.10
TL	0.02	-0.11**	0.10**	0.64**	0.71**	0.28**	0.69**		0.39	-0.10	-0.04	0.15	0.59	-0.26
TDIAM	0.05*	-0.01	0.05*	0.75**	0.59**	0.46**	0.68**	0.49**		-0.91	0.66	0.45	0.76	0.52
TDEN	-0.20**	-0.12**	0.01	-0.20**	-0.36**	-0.51**	0.15**	-0.07*	-0.59**		-0.64	-0.34	-0.48	-0.53
TBS	-0.03	-0.07*	0.01	0.38**	0.33**	0.30**	0.38**	0.24**	0.53**	-0.29**		0.50	0.60	0.95
TMD	0.03	-0.05*	0.03	0.58**	0.43**	0.38**	0.47**	0.31**	0.64**	-0.33**	0.69**		0.55	0.46
TMC	0.06*	-0.02	0.04	0.75**	0.58**	0.44**	0.61**	0.51**	0.74**	-0.35**	0.59**	0.90**		0.47
TAC	-0.23*	-0.08*	-0.05*	-0.12**	0.02	0.07*	-0.11**	-0.02	-0.03	-0.08*	0.23**	0.24**	0.09*	

[1]VL = valgus (right and left legs combined); VR = varus (right and left legs combined); TD = tibial dyschondroplasia; SW = shank weight; SL = shank length; SDIAM = shank diameter; TW = tibia weight; TL = tibia length; TDIAM = tibia diameter; TDEN = tibia density; TBS = tibia breaking strength; TMD = tibia mineral density; TMC = tibia mineral content; TAC = tibia ash content. **P<0.0001; *P<0.05.

CHAPTER 6

CONCLUSIONS

These studies confirmed that the leg problems tibial dyschondroplasia (TD) and varus-valgus deformities (VVD) are influenced by an additive genetic component, thus they are susceptible of improvement by means of genetic selection. However, it was also evidenced that the additive genetic influence is small and the application of optimal management strategies are important on reducing the incidence of these leg problems in meat type chickens. On the other hand, it was also showed that the genetic association of TD and VVD with growth rate is extremely weak at best. The bone quality traits studied in this research showed to have an additive genetic background as well. Importantly, it was evidenced that some of these traits, which are directly related with the integrity of the bone, have a negative genetic correlation with growth rate. Likewise, it was found that the genetic correlations of the leg problems TD and VVD with bone quality traits tended to be weak but they were estimated with large posterior standard deviations given the size of the dataset available for the analysis; thus, these trends must be observed with caution and more investigation would be necessary in this field. In general, given the heritability values estimated for leg problems and bone quality traits it is clear that even though they can be improved by genetic selection, management strategies play a major role on their improvement. Genetic selection for growth rate appears not be *per se* a determinant factor in the incidence of leg problems. However, current results suggested that selection for growth rate could affect negatively some aspects of the bone compromising its integrity, health and maturity. It seemed that mineralization level of the bone and, in consequence, its breaking strength, would be traits

importantly compromised. Thus, strategies directed to the improvement of the mineralization of the bone could yield legs of greater soundness which would be also reflected on the welfare of the birds.