

## **Nutritional factors that affect leg problems in meat poultry: A Review**

**Edgar O. Oviedo-Rondón and Peter R. Ferket**

*Department of Poultry Science, College of Agriculture and Life Sciences,  
North Carolina State University, Raleigh, NC., 27695-7608*

Skeletal disorders are one of the most prevalent welfare problems in broiler and turkey production; especially those that cause lameness that impair movement or bone breakage during catching and transportation (Julian, 2004; Mench, 2004). Leg problems are also a production problem, and it can be one of the most expensive diseases in terms of output at the farms and carcass condemnations during processing, resource wastage by culled birds, and treatment and prevention costs (Gregory and Wilkins, 1992; Pattison, 1992; Bennett *et al.*, 1999, Whitehead, 2003). Bone fragility and porosity also are correlated with the incidence of bone fragments in deboned meat products, and with discoloration of meat adjacent to bone due to leaching of blood; the product may be less appealing to consumers (Gregory and Wilkins, 1992).

In the USA, the last large survey about leg problems in broilers was done in 1993 (Morris). The results of this survey indicated that leg problems were responsible for 1.1% of broiler mortality and 2.1% of carcass condemnations and downgrades annually, and cost the broiler industry billions of dollars each year (Pattison, 1992). Since, the time of this survey great improvement has been done through out genetic selection, however leg problems continue to be affecting some broiler genetic lines under some growing conditions, but especially affects turkeys (Whitehead, 2003).

Currently, leg problems are a main concern turkey production world-wide. It has been estimated that 2-6% of all turkey flock display some observable signs of skeletal problems, while many more will be affected in a less visible way (Lilburn, 1994). Currently, the incidence of leg abnormalities may exceed 15% in some flocks and up to 5% of the mortality of a flock could be due only to leg problems, which costs the US turkey industry over 150 million per year. Turkeys exhibit more leg problems after 14 weeks of age than younger birds, and the incidence is greatest in winter than in summer seasons (Vaillancourt *et al.*, 1999, 2000; Woodward, 2004). However, the origin of these problems might start even during the embryonic period (Farquharson, 2003; Van der Eerden *et al.*, 2003).

The skeletal system consists of bones, cartilage, ligaments and tendons and accounts for about 20 percent of the body weight of most vertebrates. The syndromes that cause lameness in the birds of a flock can be affecting one or several of these tissues in the same bird or within the flock at the same time (Riddell, 1992; Thorp, 1992; Whitehead *et al.*, 2003; Crespo and Shivaprasad, 2003; Julian, 2004; Mench, 2004; Pines *et al.*, 2005).

In rough order of importance, leg problems include infected hocks (staphylococcal, coliform, viral arthritis, tendinitis), twisted legs (valgus/varus), tibial dyschondroplasia (TD), femoral head necrosis, spondylolisthesis (kinky back), osteomyelitis and pododermatitis (*E. coli*, *Salmonella* or *Staphylococcus*) and slipped tendon. In turkey flocks is most common to find femur spiral fractures (brittle bone), pododermatitis (Clark, 2002) and infectious agents that affect the musculo skeletal system such as *Staphylococcus*, *Mycoplasmas* (*M. meleagridis*, *M. synoviae*, *M. gallisepticum*), and *Ornithobacterium rhinotracheale* (ORT) (Reece, 1992).

Skeletal problems in breeders during lay include hypocalcemia, degenerative joint disease, staphylococcal or viral arthritis/tenosynovitis and keel bursitis (Hocking, 1992). The incidence of other leg defects, such as rotated toes and rickets, have decreased considerably in commercial flocks over the last 20 years due to genetic selection, improved nutrition and litter management.

Lameness is currently measured on-farm by scoring walking ability or willingness of the birds to stand (Mench, 2004). However, in most of the heavy older broilers with abnormal gait, no gross or histological lesions can be observed post-mortem in any of the tissues. The correlation between gait score and specific leg problems, such as TD, varus/valgus deformations, crooked toes, tibial rotation, tendon degeneration and scoliosis, is significant but low (Kestin *et al.*, 1999, Bokkers and Koene, 2000; Sanotra *et al.*, 2001). Part of the lameness problems may be due to conformation of fast-growing high-breast meat yield birds. These birds normally have short legs and large thigh muscle masses, and the development of large breast muscles moves their gravity cranially (Corr *et al.*, 2003a). To compensate for this variations, they walk slowly with their toes pointed outwards, and taking short, wide steps (Corr *et al.*, 2003b), and with aging they become less active (Kestin *et al.*, 2001). Then, solutions to leg problems can not be limited to just improve bone development, strength or density.

Many of the syndromes are poorly understood and multiple factors are involved in their etiology (Rath *et al.*, 2000; Whitehead *et al.*, 2003). Multitude of factors can cause bone development disorders, possibly by several distinct mechanisms, but each results in the occurrence of histological, anatomical and structural similar lesions (Farquharson, 2002; Pines *et al.*, 2005). Possible causes of skeletal defects include infection, malabsorption of nutrients due to enteritis, abnormal bone, cartilage or tendon development, or changes in metabolism as a result of genetic mutations or polymorphisms caused by human selection, or even inadequate nutrition or management, toxins or environmental factors. Litter conditions can play an important role on infectious related problems that can start by a pododermatitis (bumblefoot) and could cause hock or hip joint infectious (Clark *et al.*, 2002; Mayne, 2005).

The control and even treatment of skeletal abnormalities depends on the cause of the problem. If an infectious disease has been confirmed, antibiotics and/or improvements in biosecurity or vaccination programs may help, while nutritional defects may be corrected by attention to the balance diet and micronutrients for specific populations. However, very few skeletal problems are controlled solely by these means. Most of the skeletal disorders currently observed have strong correlation with genetics and management practices, so genetic selection programs and husbandry practices play an important role in their future control (Farquharson, 2002; Whitehead *et al.*, 2003), but nutrition might still solve some of the field cases.

## **Understanding bone development and regulation for better nutrition**

Since bone is the biggest part of the skeletal system, it has been the component of the skeletal system most commonly studied in avian species. Working with a fast grow animal is important to understand the regulation of bone growth. Nevertheless, it is important to remember that ligaments and tendons might have an important role.

**Bone composition, strength and mechanical properties.** Bone is a complex tissue made up of living cells (2%) enmeshed in a mineralized collagenous rich matrix (98%). This matrix is constituted by collagen (80-90%), which in its mature form is formed by pyridinium crosslinks (hydroxyllysyl pyridinoline and lysyl pyridinoline), and proteoglycans, lipids, and noncollagenous proteins (osteocalcin, osteonectin and osteopontins) that constitutes 10 to 15% of the organic matrix. The noncollagenous proteins contribute to a variety of functions of bone such as matrix stabilization, calcification, and other metabolic regulatory activities.

The mineral matrix is made up of calcium and phosphorus in the form of hydroxyapatite (60 – 70% of bone weight). The inorganic mineral provides shear strength (stiffness) and resists compression, whereas the organic collagen fibers withstand tension and torsion. Because the mineral matrix is the major component of the extracellular matrix, bone mineral density (BMD) is considered to reflect the status of bone health. High positive correlation ( $r = 0.81$ ) between bone breaking strength and BMD were reported by Frost and Rowland (1991) and Orban *et al.* (1993a). But, the correlation between shear force and BMD was only 0.56 and between ash and shear force 0.67 (Onyango *et al.*, 2003).

Rath *et al.* (1999) observed that bone strength showed positive correlations with its ash content, density, pyridinium crosslinks, and the fluorescence of the matrix. However, the correlation was stronger with both pyridinium crosslinks and the fluorescence of the organic matrix. These results suggest that bone strength is influenced by the content of its collagen crosslinks. Several other researchers have agreed that bone mineralization alone is not always the most related to mechanical properties and breaking strength (Ferretti *et al.*, 1984; Rath *et al.*, 2000; Massé *et al.* 2003)

**Bone growth mechanisms.** The terms osteogenesis and ossification are often used synonymously to indicate the process of bone formation. This process actually starts during the embryonic period and continues throughout life. There are two types of ossification: intramembranous and endochondral. Intramembranous ossification involves the replacement of sheet-like connective tissue membranes with bony tissue and is common in certain flat bones of the skull and some of the irregular bones. Then, their development is not discussed herein.

Endochondral ossification involves the replacement of hyaline cartilage with bony tissue. Most of the bones of the skeleton are formed in this manner. Bones grow in length at the epiphyseal plate by a process that is similar to endochondral ossification. The cartilage in the region of the epiphyseal plate next to the epiphysis continues to grow by mitosis. The chondrocytes, in the region next to the diaphysis, age and degenerate. Osteoblasts move in and ossify the matrix to form bone. This process continues throughout life after hatch until maturity when cartilage growth slows and finally stops. When cartilage growth ceases, the epiphyseal plate completely ossifies so that only a thin epiphyseal line remains and the bones can no longer grow in length (Farquharson, 2003).

The mechanisms of long bone growth are similar across animal species. But, there are, major variations in the growth rate between similar bones of different species, bones of an individual animal and the two growing regions (growth plates) of the same bone. In the domestic fowl, the fastest growing bone tip is the proximal tibia that grows at 0.86 mm/day (Kirkwood *et al.*, 1989). Human femur grows at 0.04, mm/day, and rats and rabbit only at 0.22 and 0.39 mm/day, respectively. The rate of bone growth attributed to a specific growth

plate is determined by an interaction between proliferative kinetics, matrix production in the transverse septa, and hypertrophic chondrocyte enlargement.

The growth plate matrix is formed by collagen type II, which interacts with collagen types IX and XI to form heterotypic fibrils that are distributed throughout the cartilage matrix. During chondrocyte maturation, collagen type II gene expression decreases and the hypertrophic chondrocytes initiate the synthesis of collagen type X-protein unique to this cell type in avian species. Some proteoglycans like aggrecan, and collagenous proteins, such as osteopontin and osteonectin, are also part of this growth plate matrix (Velleman, 2000). All constitute good markers for hypertrophy, which are down regulated in TD lesions (Farquharson, 2002).

Collagen intermolecular cross-links are very important to the mechanical performance of bone (Rath *et al.*, 1999, 2000). The cross-link pattern is determined and influenced by many factors, including the level of lysine hydroxylation, collagen turnover, molecular packing structure, and mineralization; thus, cross-linking is tissue specific rather than collagen type specific.

Terminally differentiated chondrocytes either differentiate into bone cells, proliferating with one daughter chondrocyte dying and the other becoming an osteoblast, or undergo the widely accepted route of programmed cell death. Cartilage mineralization is needed before vascular invasion of the growth plate can occur. This occurs by a series of physico-chemical and biochemical processes. Calcium and phosphorus, and latter hydroxyapatite are deposited in extracellular fluid. Mineralization rate depends on concentration of  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-4}$  in the extracellular fluid, pH, and the concentration of proteoglycans that promote mineralization. High concentrations of inorganic phosphate are attributed to the actions of the bone/liver/kidney form of alkaline phosphatase (ALP) (Farquharson and Jefferies, 2000, Farquharson, 2002, 2003). Any failure to accomplish these physiological processes of chondrocyte differentiation/maturation, vascular invasion and matrix mineralization causes TD, and it could be the origin for valgus and varus disorders as well as some breaking strength weaknesses that could cause higher susceptibility to future fractures.

**Hormonal control of bone growth.** Bone growth is under the influence of several endocrine hormones, such as growth hormone (GH), estrogens, Parathyroid Hormone-Related peptide (PTH/PTHrP), Vitamin D, and thyroid hormones ( $\text{T}_3$  and  $\text{T}_4$ ); and autocrine and paracrine factors (produced by bone cells), such as prostaglandins, cytokines, insulin-like growth factor-I (IGF-I), transforming growth factor-B superfamily including BMP, and fibroblast growth factor (FGF). Prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ) is known to have diphasic effects on bone formation; at low concentrations it stimulates formation, but a high concentration  $\text{PGE}_2$  inhibits formation (Raisz, 1993, Watkins *et al.*, 2000). Dietary saturated:unsaturated ratios and omega3:omega6 fatty acid balance, vitamin E and other antioxidant vitamins, and biotin can modulate  $\text{PGE}_2$  to obtain the desired effect on bone development (Watkins, 2002, Farquharson, 2003).

The  $\text{T}_3$  and Vitamin D actions are mediated by nuclear receptors ( $\text{T}_3$  and vitamin D receptors -  $\text{T}_3\text{R}$  and VDR) that are structurally homologous members of the steroid/thyroid hormone receptor superfamily, and they function as hormone-inducible transcription factors. The  $\text{T}_3\text{R}$  and VDR recognize similar hormone response element DNA sequences and preferentially bind to DNA as heterodimers with 9-cis-retinoic acid (Pcis-RA) receptors (RXR) (Van der Eerden *et al.*, 2003).

IGF-I stimulates proliferation, whereas  $T_3$  induces hypertrophic differentiation.  $T_4$  is a prohormone that is converted to  $T_3$  by iodothyronine deiodinase 5'-DIO (type 1 to 3), a selenoprotein. GH and glucocorticoids (GC) stimulates this conversion in specific tissues, like chondrocytes located in growth plates. But excessive levels of GH and GC inhibit chondrocyte differentiation in avian species (Monsonogo *et al.*, 1995).

The hormone  $T_3$  in physiological concentrations directly affects cartilage growth and maturation by several means: it stimulates the resting zone cells to differentiate; it is indispensable for chondrocyte hypertrophy (Burch and Lebovitz, 1982); it induces the expression of type II and X collagen and the activity of the differentiation marker ALP; and it is required for vascular invasion of the growth plate and metaphyseal trabecular bone formation (Robson *et al.*, 2002; Van der Eerden *et al.*, 2003). Shen *et al.* (2004) reported that broiler genetic lines susceptible to TD have inadequate gene expression of DIO type 2 in the growth plates and they concluded that this bone disorder is a result of tissue hypothyroidism. Hypothyroidism causes down-regulation of chondrocyte and osteoblastic cells IGF-I, failure of hypertrophic chondrocyte differentiation, absence of collagen-X expression, and increased PTHrP mRNA expression (Robson *et al.*, 2002). Early hypothyroidism in life may result in increased bone density at maturity, but increased fracture rate in adult animals.

***Normal parameters of bone growth in chickens and turkeys.*** Bone development in broiler chickens has been described by Skinner and Waldroup (1995), Rose *et al.* (1996), Leterrier and Constantin (1999); Rath *et al.* (1999), Williams *et al.* (2000, 2004), Yalçın *et al.* (2001), and Torres *et al.* (2005). There are a wide variety of experimental techniques and parameters available for evaluation of bone structure, microstructure, and biomechanics (Turner and Burr, 2001). Some of the parameters used to describe the biomechanical properties of poultry bones are described in Table 1. The status of bone may be poorly described by just one of these properties. For example, a highly mineralized bone (high BMD) will tend to be very stiff, but also brittle, resulting in reduced work to failure and increased risk of fracture. On the other hand, a bone from young animals will tend to be poorly mineralized and weak, but very ductile (large ultimate displacement), resulting in increased work to failure (Turner and Burr, 2001; Carter and Spengler, 2002).

Poultry researchers normally observe differences in bone development due to gender, genetic strain, and growth rates. These differences between males and females are evident after the second week of age with females having lower values. Females generally show lower diaphyseal diameters, which is counterbalanced by modifications in the composition of the matrix and in the porosity of the cortex, leading normally to equal biomechanical characteristics of tibiotarsi in both sexes.

Across broiler strains rapid bone formation occurs between 4 to 18 days and the highest rate of mineralization between 4 to 11 days (Williams *et al.*, 2000). The early rapid bone deposition at the periosteal surface is associated with decreased mineralization, changes in calcium:phosphorus ratios, increased cortical size and porosity, and altered biomechanical properties. The differences observed between strains for bone anatomy and bone mineralization during the rapid growth period around 16 d are not significant at later ages, with the exception of bone volume. Several authors conclude that growth rate, and not the genetic potential, of the fast growing birds is responsible for the rapid periosteal bone deposition and might cause some bone developmental problems (Williams *et al.*, 2004; Whitehead *et al.*, 2004).

**Table 1.** Normal physical, mechanical and biochemical parameters of broiler chicken tibias

Age (days)	Physical and chemical variables			Mechanical variables		
	Trait	Males	Females	Trait	Males	Females
21	Live body weight (g) <sup>1</sup>	786 ± 121 <sup>a</sup>	679 ± 97 <sup>b</sup>	Porosity (%) <sup>1</sup>	45.0 ± 1.7 <sup>ns</sup>	44.63 ± 1.96 <sup>ns</sup>
28		1326 ± 212 <sup>a</sup>	1071 ± 170 <sup>b</sup>		40.7 ± 1.8 <sup>ns</sup>	40.42 ± 2.35 <sup>ns</sup>
35		1559 ± 229 <sup>a</sup>	1287 ± 211 <sup>b</sup>		36.3 ± 2.3 <sup>ns</sup>	34.83 ± 3.03 <sup>ns</sup>
42		2305 ± 382 <sup>a</sup>	1865 ± 401 <sup>b</sup>		36.3 ± 2.9 <sup>ns</sup>	36.44 ± 2.84 <sup>ns</sup>
21	Tibia weight (g) <sup>1</sup>	6.9 ± 1.3 <sup>a</sup>	5.65 ± 0.77 <sup>b</sup>	Area (cm <sup>2</sup> ) <sup>1</sup>	0.21 ± 0.03 <sup>a</sup>	0.16 ± 0.02 <sup>b</sup>
28		11.7 ± 1.4 <sup>a</sup>	8.73 ± 1.16 <sup>b</sup>		0.29 ± 0.05 <sup>a</sup>	0.21 ± 0.03 <sup>b</sup>
35		14.7 ± 2.0 <sup>a</sup>	11.29 ± 1.63 <sup>b</sup>		0.29 ± 0.05 <sup>a</sup>	0.22 ± 0.03 <sup>b</sup>
42		20.6 ± 2.9 <sup>a</sup>	15.58 ± 2.12 <sup>b</sup>		0.41 ± 0.07 <sup>a</sup>	0.32 ± 0.09 <sup>b</sup>
21	Tibia length (mm) <sup>2</sup>	73 ± 0.3 <sup>a</sup>	71 ± 0.2 <sup>b</sup>	Maximum force(N) <sup>1</sup>	171.1 ± 24.9 <sup>a</sup>	132.0 ± 24.7 <sup>b</sup>
28		87 ± 0.3 <sup>b</sup>	84 ± 0.3 <sup>b</sup>		272.3 ± 49.4 <sup>a</sup>	190.1 ± 28.5 <sup>b</sup>
35		97 ± 0.3 <sup>a</sup>	94 ± 0.4 <sup>b</sup>		235.9 ± 67.2 <sup>a</sup>	178.9 ± 51.5 <sup>b</sup>
42		110 ± 1 <sup>a</sup>	103 ± 13 <sup>b</sup>		296.5 ± 61.4 <sup>a</sup>	226.8 ± 69.2 <sup>b</sup>
49		118 ± 1 <sup>a</sup>	114 ± 1 <sup>b</sup>	<b>Load at break (kg/mm<sup>2</sup>)<sup>4</sup></b>		
56		126 ± 1 <sup>a</sup>	121 ± 1 <sup>b</sup>	<b>26.66 ± 4.89</b>	<b>20.96 ± 1.82</b>	
21	Tibia width (mm) <sup>2</sup>	4.9 ± 0.2	4.5 ± 0.1	Flexion resistance (MPa) <sup>1</sup>	100. ± 15.3 <sup>ns</sup>	105.6 ± 17.4 <sup>ns</sup>
28		5.9 ± 0.2	5.6 ± 0.2		107.6 ± 22 <sup>ns</sup>	118.4 ± 15.0 <sup>ns</sup>
35		7.1 ± 0.2	6.6 ± 0.2		93.9 ± 16.6	102.2 ± 21.9
42		8.1 ± 0.2	7.0 ± 0.3		95.7 ± 20.3 <sup>ns</sup>	105.9 ± 24.6 <sup>ns</sup>
49		8.6 ± 0.2	7.6 ± 0.2			
56		9.5 ± 0.2	7.8 ± 0.3			
21	Density (g/cm <sup>3</sup> ) <sup>1</sup>	1.04 ± 0.1 <sup>ns</sup>	1.08 ± 0.07 <sup>ns</sup>	Inertia x 10 <sup>-10</sup> (m <sup>4</sup> ) <sup>1</sup>	5.19 ± 1.38 <sup>a</sup>	3.53 ± 1.05 <sup>b</sup>
28		1.12 ± 0.1 <sup>ns</sup>	1.16 ± 0.09 <sup>ns</sup>		10.82 ± 4.32 <sup>a</sup>	5.70 ± 1.44 <sup>b</sup>
35		1.25 ± 0.1 <sup>ns</sup>	1.30 ± 0.10 <sup>ns</sup>		12.81 ± 3.72 <sup>a</sup>	7.96 ± 3.11 <sup>b</sup>
42		1.14 ± 0.1 <sup>ns</sup>	1.17 ± 0.11 <sup>ns</sup>		21.30 ± 7.84 <sup>a</sup>	13.90 ± 7.91 <sup>b</sup>
21	Tibia Ash <sup>*</sup> (%) <sup>3,4‡</sup>	48.9 <sup>3</sup>	62 <sup>3</sup>	<b>Stress<sup>49a</sup> (kg/mm<sup>2</sup>)</b>	<b>1.76 ± 0.22<sup>4</sup></b>	<b>1.97 ± 0.40<sup>4</sup></b>
28		63 <sup>3</sup>	63 <sup>3</sup>			
35		64.98 ± 0.8 <sup>4</sup>	64.03 ± 1.1 <sup>4</sup>	<b>Strain<sup>49a</sup> (kg/mm<sup>2</sup>)</b>	<b>0.16 ± 0.02<sup>4</sup></b>	<b>0.21 ± 0.02<sup>4</sup></b>
49						
21	Tibia Ash (%) <sup>2‡</sup>	41.86 ± 0.80	43.62 ± 0.62	Young's module* (GPa) <sup>1</sup>	3.05 ± 0.50 <sup>b</sup>	3.75 ± 0.60 <sup>a</sup>
28		41.93 ± 0.45	43.59 ± 0.42		3.14 ± 0.89 <sup>b</sup>	4.69 ± 0.69 <sup>a</sup>
35		42.43 ± 0.34	44.13 ± 0.69		3.59 ± 0.73	5.01 ± 1.14
42		42.86 ± 0.78	44.35 ± 0.54	3.70 ± 0.79 <sup>b</sup>	5.105 ± 1.52 <sup>a</sup>	
49		43.43 ± 0.43	43.83 ± 0.66	<b>Modulus (kg/mm<sup>2</sup>)<sup>4</sup></b>	<b>26.60 ± 6.69</b>	<b>25.62 ± 5.34</b>
56		43.73 ± 0.61	45.49 ± 0.53			
21	Tibia Calcium (%) <sup>2</sup>	14.31 ± 0.52	14.92 ± 0.17	<b>Matrix biochemical constituents at 49 d<sup>4</sup></b> ----- (µg/mg of tibia bone) -----		
28		14.30 ± 0.19	14.49 ± 0.33	Collagen	225.51 ± 4.40	221.56 ± 4.88
35		14.36 ± 0.20	14.93 ± 0.59		Proteoglycan	1.56 ± 0.55
42		15.29 ± 0.30	17.70 ± 0.99			
49		15.45 ± 0.16	18.30 ± 0.25			
56		14.78 ± 0.59	20.08 ± 0.49			
21	Tibia phosphorus (%) <sup>2</sup>	7.25 ± 0.24	7.48 ± 0.21	<b>Bone collagen composition at 49 d<sup>4</sup></b> ----- (µg/mg of collagen) -----		
28		7.68 ± 0.28	7.97 ± 0.15	HP	0.026 ± 0.006	0.026 ± 0.006
35		7.56 ± 0.14	7.60 ± 0.28		LP	0.016 ± 0.003
42		7.72 ± 0.22	9.12 ± 0.57	Total pyridinolines		0.042 ± 0.003
49		7.43 ± 0.15	9.88 ± 0.74			
56		8.10 ± 0.21	9.94 ± 0.71			
21	Ca:P ratio (M)	1.97	1.99	Collagenase extractable fluorescence (AFU/mg bone)	1.96 ± 0.16	2.31 ± 0.17
28		1.86	1.82			
35		1.90	1.96			
42		1.98	1.94	Collagenase resistant matrix (%)	14.77 ± 2.30	21.08 ± 2.88
49		2.40	2.32			
56		1.82	2.02			

<sup>ab</sup>Means with the same superscript do not differ significantly by Tukey's test (P>0.05); <sup>ns</sup> Non-significant Sources: <sup>1</sup>Torres *et al.*, 2005; <sup>2</sup>Adapted from Skinner and Waldroup, 1995; <sup>3</sup>Boskey, 2002; <sup>4</sup>Rath *et al.*, 1999 - HP = hydroxylysylpyridinoline; LP = lysylpyridinoline; AFU = arbitrary fluorescence unit. \* Elasticity modulus. <sup>1</sup>Density (mm of aluminum equivalents) at 21 d 2.17 ± 0.03 (Soares da Silva *et al.*, 2003). <sup>\*</sup> Bones ashed at 750 °C for 22 h without fat extraction. <sup>‡</sup> Tibia ash after removing the fat by 20 hr extraction in hot alcohol and 20 hr in ether and ashed at 550 °C.

The general form of each bone is genetically determined, but the specific architecture that is critical for structural bone competence develops and persists only in response to continued exposure to loading. Loading is osteogenic, but this stimulus appears to saturate after a few daily loading cycles (Lanyon, 1992). Recently, Reich *et al.* (2005) demonstrated in an elegant study with broilers that moderate (10%) increments in body weight (by weight loading bags) at an early age and for short periods of time (2 – 4 days) accelerates cartilage resorption, increases osteopontin and matrix metalloproteinases expression, that enhances early mineralization resulting in narrower growth plates and shorter bones. This can explain differences in bone length and structure of chickens with different early body weight gains.

Some bone morphology and biomechanical parameters are commonly overlooked in poultry bone research, but they can explain some of the bone disorders observed in the field. For example, the cross-sectional moment of inertia (CSMI), which is a parameter of morphology, gives an idea about the distribution of bone material around a given axis in the cortical area. CSMI is calculated by the diameters of bone cross section and cortical thickness. The collagen fiber orientation is another parameter that is important according to the type of strain applied to the bone, tension or compression. Longitudinal orientation is better in tension and transversal in compression. Collagen fiber orientation is evaluated based on birefringence of bone under polarized light or circularly polarized filters (Turner and Burr, 2001; Carter and Spengler, 2002).

Mechanical evaluations, such as the three-point loading/bending tests, are common among poultry literature. However, other important evaluations like tensile testing, compressive testing, four point loading tests, torsion testing, pure shear tests and fatigue testing are not commonly evaluated and they can give important characteristics of bone integrity depending on the problem to be evaluated (Turner and Burr, 2001; Carter and Spengler, 2002).

We have noticed that there is high variability in the data published related to bone development due to the methodology of analyses, which was also discussed by Rath *et al.* (2000). Fresh bone gives better bone breaking strength correlated to the other bone parameters than dry or fat-free dry preparation (Kim *et al.*, 2004). At the moment of evaluating bone properties, it is important to keep the samples hydrated with a physiological saline solution, and at physiological temperature (40 – 41 °C), otherwise the mechanical properties change dramatically. Failure to control these two factors can cause variabilities in tensile strength (31%), toughness (55%) and fatigue (100%) in the experimental values. The rate at which loading is applied during biomechanical testing of bone affects the measured stiffness and strength. If one is trying to simulate physiological conditions, the strain rate should be between 0.01 and 0.08/s, which is within the range of strain rates that occurs *in vivo* (Turner and Burr, 2001).

Bonser and Casinos (2003) used microhardness testing as a probe for fine-scale regional variation in the mechanical performance of bones and present data showing the extent of regional variation in the femora and humeri of 7-week-old broiler birds. Ash content of dry bone was broadly correlated with microhardness, although there is some evidence that the relationship linking the two differs between the femur and the humerus. Regional variations in the properties of avian bones are widely overlooked in the literature. Awareness of them is vital and existing measures of bone 'strength' may be misleading if local variation in

properties is not taken into account when exploring the effects of nutrition and husbandry practices on bone mechanical performance.

***Genetic background, endocrine system and nutrition in modern poultry.*** Lameness, leg and skeletal problem incidence definitely can be diminished by appropriate multi-trait selection with a continued improvement in growth rate (Whitehead *et al.*, 2003). Some commercial chicken strains appear to have a genetic predisposition to skeletal abnormalities when management practices and their accelerated growth rates increases the amount of defects in their bones (Kestin *et al.*, 1999, 2001; Whitehead, 2003; Mench, 2004). On the other hand, Hafez *et al.* (2004) investigated five commercial meat turkey lines (Kelly Bronze, Nicholas 300, BUT 9, Nicholas 700, and BUT-Big 6) regarding leg disorders. Under commercial rearing and feeding conditions these turkey lines are characterized by different body weight development. The obtained results revealed that there was no correlation between turkey lines and the incidence of TD, fractures, and arthritis.

Modern broilers and turkeys have been genetically selected to grow faster, have higher feed intake and better feed conversion, and yield more breast meat, but at the same time some modifications in the endocrine mechanisms have occurred especially in thyroid and GH hormone production, elimination, tissue utilization and peripheral conversion (Gonzales *et al.*, 1999; Rahimi, 2005). Thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) has great influence over avian bone development (Bonser *et al.*, 2004), and even some common bone disorders, such as TD in broilers have recently been correlated to tissue-specific hypothyroidism (Shen *et al.*, 2004). Deficiency in the thyroid hormones and elevated corticosterone play a key deleterious role in the development of other metabolic disorders typical of fast-growing birds like the ascites syndrome (Luger *et al.*, 2002).

A more pulsatile GH secretion, common in commercial strains of poultry, can inhibit differentiation in avian growth-plate chondrocytes, thereby sustaining their proliferative state and maintaining their sensitivity to growth factors such as EGF (Monsonego *et al.*, 1995). Consequently, it is not surprising that chickens affected with TD have elevated levels of plasma GH (Vasilatos-Younken and Leach, 1986).

Additionally, several minerals and trace elements are essential for normal thyroid hormone metabolism, namely iodine, iron, selenium, and zinc. Coexisting deficiencies of these elements can impair thyroid function (Zimmermann and Kohrle, 2002). In the same way, the metabolism of zinc, copper, magnesium, calcium, manganese, and selenium, (Aihara *et al.*, 1984; Simsek *et al.*, 1997), as well as vitamin A (Aktuna *et al.*, 1993) is abnormal in thyroid diseases. Altered zinc turnover also may be also involved in depressed thyroid-thymus axis efficiency. Zinc turnover is under the control of zinc-bound metallothioneins (Zn-MTs) synthesis. Thyroid hormones, corticosterone and nutritional zinc affect Zn-MT induction (Mocchegiani *et al.*, 2002) and thyroid hormones mediate changes in membrane fluidity that might play an important role in modulating Zn<sup>2+</sup> transport activity of intestinal and renal brush-border membranes controlling its absorption and reabsorption (Prasad *et al.*, 1999). The T<sub>3</sub> receptor is thought to require zinc to adopt its biologically active conformation. Some of the effects of zinc deficiency, therefore, may be due to loss of zinc from the T<sub>3</sub> receptor and impairment of T<sub>3</sub> action (Freake *et al.*, 2001).

Thyroid function can also be altered by management practices (Yahav *et al.*, 2000), incubation conditions during the embryonic period (Christensen *et al.*, 2002; Decupere,

2005), nutritional levels, brooding temperatures and feeding programs ((Noy *et al.*, 2001; Proudman and Siopes, 2005). Plasma T<sub>3</sub> concentrations, which may mediate some of the intestinal effects of feed deprivation, were depressed in poult without access to feed (Noy *et al.*, 2001). High temperatures reduce T<sub>3</sub> levels and exposition to low temperatures increase T<sub>3</sub> (Haddad and Mashaly, 1989). Plasma T<sub>3</sub> is positively and significantly correlated with food intake (Yahav, 2000) Even, maternal thyroid function can affect thyroid function in the progeny and this can be modified by iodine supplementation (Christensen and Davis, 2001). Christensen *et al.* (1996) observed in turkey hens that increased (P < 0.05) T<sub>4</sub> was evidenced in embryos from youngest hens, whereas increased (P < 0.05) T<sub>3</sub> activity was evident in embryos from hens of older ages. Similar effects have been observed in broilers, affecting bone weight, ash content and bone volume on day of hatch, but the extent of this effect also depended on the strain (Yalçın *et al.*, 2001).

These evidences indicate that commercial broilers and turkeys can be exposed to constant variations in thyroid status that can affect bone development. It is interesting to note that the proven successful nutritional methods to resolve bone problems are also similar to the ones observed to improve developmental problems in hypothyroid animals.

## **Nutrition and skeletal problems in meat poultry**

Skeletal problems in poultry have been associated with the dietary levels of vitamins like Vitamins D, A, C and K and most B-vitamins, but especially pyridoxine and folic acid; minerals, such as calcium (Ca), phosphorus (P), sodium (Na), chloride (Cl), zinc (Zn), selenium (Se), copper (Cu), manganese (Mn); protein levels or amino acids, such as methionine, cystine, cysteine and the metabolite homocysteine; fatty acids; and total feed consumption. Bone development is also affected by metabolic acidosis induced by dietary electrolytes, toxic minerals like cadmium that contaminate other mineral sources, and mycotoxins coming by fungi proliferation on grains stored for long periods of time.

Some of the leg disorders observed in the field could be caused more frequently by feed processing factors than for inadequate feed formulation. Situations such as high variability on feed ingredient composition, feed mixing problems, fat and oil oxidization and uncertainty of nutrient digestibility and bioavailability are common in commercial feed mills. Improve feed quality control is essential to solve any of these problems.

### ***Vitamins***

***Vitamin D.*** Vitamin D is composed for a group of molecules similar to steroids called secosteroids, which include cholecalciferol (Vitamin D<sub>3</sub>), ergosterol (Vitamin D<sub>2</sub>), 24,25 (OH)<sub>2</sub>D<sub>3</sub>, and their photoisomers. These compounds play an important role in Ca homeostasis, regulation of cell differentiation (genomic responses) in several tissues such as the immune system, skin and different bone cell populations (Whitehead, 2002; Holick, 2004; Dusso *et al.*, 2005).

Vitamin D action is mediated throughout an interaction with the cellular nuclear receptors (VDR). In this way this pro-hormone regulate gene transcription with synthesis of typical proteins, but there are other effects of this vitamin that may result from non-genomic mechanisms, which are membrane-associated processes (Lu *et al.*, 2000; Dusso *et al.*, 2005). The genomic effects could last hours and involve in the bone genes for the bone protein

osteocalcin as well as the matrix GLA protein, and osteoclast generation. While the non-genomic effects last only minutes and involve  $\text{Ca}^{2+}$  channels and membrane receptors.

Vitamin  $\text{D}_3$  deficiency results in hypocalcemia and hypophosphatemia. Vitamin  $\text{D}_3$  is absorbed into the blood and stored in the liver, and so circulating levels of  $\text{D}_3$  are usually low. Circulating 25 (OH) $\text{D}_3$  levels in broilers should be at least 5 ng/ml (Goff, 1992). In vitamin  $\text{D}_3$  deficient chicks, bone resorption is inhibited after about 7 d, while the formation of osteoid continues, resulting in an increased total bone mass.

The biopotency of  $\text{D}_3$  sources is still controversial. In avian species, vitamin  $\text{D}_3$  is 30 to 40 more potent than vitamin  $\text{D}_2$ . 1,25(OH) $_2\text{D}_3$  is able to prevent rickets with five times the potency of vitamin  $\text{D}_3$ . Ferretti *et al.* (1984) concluded that in order of potency to promote bone strength, the sources can be ranked 1,25(OH) $_2\text{D}_3 > 25\text{-OH-}\text{D}_3 > \text{VIT-}\text{D}_3$  (Leeson *et al.*, 1995).

Fritts and Waldroup (2003) evaluated cholecalciferol (VIT- $\text{D}_3$ ) and 25-hydroxycholecalciferol (25-OH- $\text{D}_3$ ) in doses to provide 125, 250, 500, 1,000, 2,000, or 4,000 IU/kg of vitamin D activity in a nutritionally complete corn-soybean meal diets. They observed that the incidence and severity of TD was significantly lower for birds fed 25-OH- $\text{D}_3$  and was reduced by increasing levels of vitamin D, regardless of source. Results of this study show that 25-OH- $\text{D}_3$  is more metabolically potent on a per unit basis than vitamin  $\text{D}_3$  for support of body weight, tibia ash, and reduction in incidence and severity of TD. The differences were observed primarily at lower levels of vitamin D. At typical industry levels, few differences were observed between the two sources. The utilization of 25-OH- $\text{D}_3$  may allow for supplementation with lower levels or may provide a greater margin of safety, especially in enteritis problems because 25-OH- $\text{D}_3$  can be absorbed better than Vitamin  $\text{D}_3$ . Ledwaba and Roberson (2003) reported increased body weights and tibia ash and decreased TD incidence and severity as the dietary 25-OH- $\text{D}_3$ , but this response was dependent upon the calcium level.

The requirement for cholecalciferol ( $\text{D}_3$ ) varies according to the parameter evaluated and according to the dietary level of Ca or P (Leeson *et al.*, 1995; Whitehead *et al.*, 2004). High levels of vitamin  $\text{D}_3$  have failed to prevent rickets or TD incidence (Edwards 1990, 2003; Atencio *et al.* 2005), even there is little response to extra supplementation of vitamin  $\text{D}_3$  when Ca and P levels are adequate.

Genetics always plays a role in the responses to any nutrient supplementation in bone development as was discussed by Ewards (2000). The supplementation with high levels of three derivatives of Vitamin D ((25-(OH) $\text{D}_3$ , 1,25-(OH) $_2\text{D}_3$ , and 24R,25-(OH) $_2\text{D}_3$ ), or ultraviolet light can decrease incidence of TD in lines selected for low incidence of TD, while none of these treatments is able to reduce the incidence in strains with high incidence. It seems that this effect is mainly linked to fail in chondrocyte differentiation and expression of VDR (Berry *et al.*, 1996).

The amount of vitamin  $\text{D}_3$  stored in the yolk sac is very important for future growth and bone development. Atencio *et al.* (2005) observed chicks hatched from eggs laid by hens fed 2,000 or 4,000 IU of  $\text{D}_3$ /kg as the maximum level of vitamin  $\text{D}_3$  had the highest body weight gains and bone characteristics. Chicks fed 3,200 IU had the highest body weight and tibia ash and the lowest TD and Ca rickets incidences. The commercial levels used by the industry

in the US are commonly 9 times higher than the NRC recommendations for broilers (Table 2), and at least 3 times higher for turkeys (Table 3).

Exposure to ultraviolet light (285-365 nm) for short periods of time (11 to 45 minutes) is enough to produce the equivalent to 20 to 40 µg/kg of vitamin D<sub>3</sub> in the feed, and the 1,25-(OH)<sub>2</sub>D<sub>3</sub> or the photoisomers generated are able to reduce the incidence and severity of TD under adequate and inadequate dietary levels of Ca, P or vitamin D<sub>3</sub>. The same effect could not be observed even by increasing in 10 times the recommended (NRC, 1994) dietary level of Vit. D<sub>3</sub> (Edwards, 2000; 2003). The exposure to UV light is more effective when done at first day of age for 30 minutes than latter on in life and for longer periods of time (Edwards, 2003). Lewis *et al.* (2000) did not observe significant improvements in leg integrity or performance in male turkeys reared in environments supplemented with UV light, but with white light intensities higher than 10 lux.

**Other Lipid-soluble Vitamins and Bone Development.** Vitamin D is a lipid-soluble vitamin and competes for absorption with other vitamins like A and E. Oxidized fats degrade activity of vitamin D and all fat soluble vitamins. Very high dietary levels of vitamin A. (45,000 IU/kg) and E (10,000 IU/Kg) (Tables 2 and 3) may increase the requirement for cholecalciferol (Aburto and Britton, 1998; Aburto *et al.*, 1998); however, moderate levels of vitamin E (150 IU/kg) do not exacerbate cholecalciferol deficiency in young broilers (Bartov, 1997).

Vitamin E also affects bone formation in poultry. Supplemental vitamin E increased the bone formation rate in broiler chicks fed diets containing varying levels of unsaturated fatty acids (Xu *et al.*, 1994b). The status influences the amount of free radicals in plasma and liver of broilers. This is important because the mineralized region of growth cartilage may have limited capacity to handle oxidized lipids, since superoxide dismutase and catalase activities are low in this region of cartilage (Matsumoto *et al.*, 1991), and the osteoclastic bone resorption may be enhanced by free radicals (Garret *et al.*, 1990).

**Table 2.** Average vitamin fortification levels (*times of NRC 1994 recommendations*) for commercial market broilers according to feeding phase.

Vitamin	Units	NRC (1994)	% NRC supply by corn-soy <sup>†</sup>	Times NRC		
				Starter	Grower	Finisher
Retinol, A	IU/kg	1500	97	5.4	4.6	3.9
Cholecalciferol, D <sub>3</sub>	IU/kg	200	-	12.8	10.8	9.5
Tocopherol, E	mg/kg	10	121	2.2	1.6	1.4
Menadione, K <sub>3</sub>	mg/kg	0.5	80	3.4	2.9	2.6
<b>Thiamine, B<sub>1</sub>*</b>	<b>mg/kg</b>	<b>1.8</b>	<b>194</b>	<b>0.9</b>	<b>0.8</b>	<b>0.7</b>
Riboflavin, B <sub>2</sub>	mg/kg	3.6 – 3	50	1.9	1.6	1.7
<b>Pyridoxine, B<sub>6</sub>*</b>	<b>mg/kg</b>	<b>3.5 - 3</b>	<b>142</b>	<b>0.7</b>	<b>0.6</b>	<b>0.6</b>
Cyanocobalamin, B <sub>12</sub>	µg/kg	10 – 7	0	1.3	1.1	1.3
Niacin, B <sub>3</sub>	mg/kg	35 - 25	57	1.2	1.2	1.2
Panhotenic acid, B <sub>5</sub>	mg/kg	10	80	1.1	1.0	<b>0.9</b>
Folic acid	mg/kg	0.55 – 0.5	72	1.5	1.3	1.2
<b>Biotin, H*</b>	<b>mg/kg</b>	<b>0.15 – 0.12</b>	<b>120</b>	<b>0.6</b>	<b>0.5</b>	<b>0.6</b>

**Source:** Adapted from **BASF, 1998.** \*Vitamins that are fortified below NRC recommended levels. <sup>†</sup>Low bioavailability, Leeson *et al.*, 1995.

Vitamin A deficiency may not only act directly on the growth plate, but may also indirectly affect growth by systemic mechanisms. For example, retinoids appear to be required for GH secretion, and for thyroid hormone secretion and action (De Luca *et al.*, 2000). Dietary vitamin A within the range 2 to 4.5 mg retinol/kg (6600 - 15000 IU/kg) did not show any interaction with vitamin D<sub>3</sub> status at either age (Whitehead *et al.*, 2004).

Zhang *et al.*, (2003) concluded that to gain optimum bone quality and broiler performance, the concentration of vitamin K should be 8 mg/kg, 2 mg/kg, and 2 mg/kg, for the starter, grower and finisher phases, respectively. Furthermore, they showed that the starter period is an important phase for improving bone quality. In addition, this study validated the mechanism of vitamin K effects on bone quality. Vitamin K boosts the carboxylation of osteocalcin and decreases the concentration of serum under-carboxylated osteocalcin enhancing hydroxyapatite binding capacity of serum osteocalcin and improving bone quality.

**Table 3.** Average vitamin fortification levels (*times of NRC 1994 recommendations*) for commercial market turkeys according to feeding phase.

Vitamin	Units	NRC (1994)	Times NRC		
			Starter	Grower	Finisher
Retinol, A	IU/kg	4000	2.9	2.1	1.4
Cholecalciferol, D <sub>3</sub>	IU/kg	900	5.7	3.9	2.6
Tocopherol, E	mg/kg	12 – 10	4.6	2.4	1.1
Menadione, K <sub>3</sub>	mg/kg	1 – 0.8	2.4	2.1	2.1
<b>Thiamine, B<sub>1</sub>*</b>	<b>mg/kg</b>	<b>2</b>	1.4	<b>0.8</b>	<b>0.4</b>
Riboflavin, B <sub>2</sub>	mg/kg	4 – 3	2.6	2.2	1.7
<b>Pyridoxine, B<sub>6</sub>*</b>	<b>mg/kg</b>	<b>4.5 - 3.5</b>	1.04	<b>0.7</b>	<b>0.5</b>
Cyanocobalamin, B <sub>12</sub>	µg/kg	3	6.1	4.2	2.6
Niacin, B <sub>3</sub>	mg/kg	70 – 50	1.3	1.3	<b>0.9</b>
Panthenic acid, B <sub>5</sub>	mg/kg	11 – 9	1.9	1.6	1.2
Folic acid	mg/kg	1 – 0.8	2.1	1.6	1.0
<b>Biotin, H*</b>	<b>mg/kg</b>	<b>0.2 – 0.13</b>	1.1	<b>0.7</b>	<b>0.6</b>

**Source:** Adapted from **BASF, 1998.** \*Vitamins that are fortified below NRC recommended levels

**Vitamin C.** The benefits of supplementing Ascorbic Acid (AA) to prevent leg disorders and cartilage problems in broilers are highly variable. The dietary addition of AA has been observed to increase duodenal Ca-binding protein and plasma 1,25 (OH)<sub>2</sub>D<sub>3</sub>, as evidence of increased 1-hydroxylase activity (Weiser *et al.*, 1988) and AA co-induced with Vitamin D<sub>3</sub> chondrocyte differentiation and mineralization in avian species (Leach and Rosselot, 1992). The last effect could be due to increase in number of VDR (Farquharson *et al.*, 1998). Petek *et al.* (2005) observed reduction in incidence and severity of TD and improvement of the cortical thickness of tibiotarsus by supplementation (150 mg/l) of AA in water, and application of intermittent lighting (12L:3(1L:3D)) programs in broilers.

On the other hand, dietary supplementation at even higher levels of AA has not prevented TD in broilers (Leach and Burdette, 1985; Edwards, 1989b). No clear evidence of interaction has been observed between AA and Vitamin D<sub>3</sub> supplementation to improve bone developmental parameters or TD incidence (Edwards, 1989b; Roberson and Edwards, 1994).

Some benefit on reducing TD was observed when AA was supplemented to diets with only 2 µg/kg of 1,25-(OH)<sub>2</sub>D<sub>3</sub> (Whitehead *et al.*, 1994). Using even high levels (250, 500 or 1,000 mg/Kg of diet) of AA in diets devoid or with only 10 µg/kg of 1,25-(OH)<sub>2</sub>D<sub>3</sub> has not shown to improve TD even under low-Ca, high-P diets (Rennie and Whitehead, 1996).

This high variability in the response to AA supplementation could be due to the genetic variability of the broiler genetic strains used and the level of stress of those chickens during the experiment and the degree of stress during the experiment. The AA supplementation has been clearly linked to modulation of GC, such as corticosterone under heat stress conditions (Mahmoud *et al.*, 2004) and many other multiple stresses (McKee and Harrison, 1995). Ascorbic Acid improves the performance of chicks with experimentally induced hypothyroidism (Takashi *et al.*, 1991). Orban *et al.* (1993b) observed increments in plasma ionic Ca in AA-treated birds, femur strength improvement by 16% in birds fed 2,000 ppm of AA, but other bone characteristics were not affected.

Deficiencies of all **water-soluble vitamins** play important roles in skeletal disorders (Leeson *et al.*, 1995). Although a common corn-soybean meal (Tables 2 and 3) diet may contain enough of these vitamins, it has been proven that they are not sufficiently bioavailable for bone development of fast growing chickens. Sufficient vitamin fortification of diets with synthetic sources is necessary. Vitamin premixes are commonly not formulated to supply the minimum NRC (1994) recommended levels for thiamine, pyridoxine and biotin (Tables 2 and 3, BASF, 1998). Marginal **pyridoxine** deficiencies are involved in hyperhomocysteinemia and reduced activity of lysyl oxidase fundamental for elastin and collagen cross linking. Recent data suggest that homocysteine, folate, vitamins B<sub>6</sub> and B<sub>12</sub> affect bone metabolism, bone quality and fracture risk in humans. **Biotin** dietary levels are associated with pododermatitis in turkeys (Clark *et al.*, 2002; Hafez *et al.*, 2004, Mayne, 2005).

## Minerals

**Calcium and phosphorus.** Almost 99% of body Ca is contained in the skeleton, however is necessary to maintain a constant 2.5 mM or 10 mg/100 ml (non-laying birds) concentration of Ca<sup>2+</sup> in plasma and extracellular fluids. Variations in the molar Ca:P ratio of bone is likely to cause alterations in the bone mineral crystal structure and consequently in the mechanical competence (Thorp and Waddington, 1997). In commercial poultry diets, Ca does not seem to be a problem and current recommended levels are sufficient broiler starter diets, and even could be in excess in grower diets to achieve maximum growth and adequate bone development (Driver *et al.*, 2005).

The source of P and its availability affects bone strength (Hemme *et al.*, 2005). Presently, P requirements are based on consumption of nonphytate P (NPP), which does not account for the fact that NPP may not be completely available and that phytate P can be partially utilized to fulfill phosphorus requirements. Phosphorus retention values for feed ingredients, accounting for NPP and phytate P and total retainable phosphorus requirements, are needed to formulate diets that meet the P requirements for bone development of poultry without causing excessive amounts of P in poultry excreta (Leske and Coon, 2002).

Leske and Coon (2002) determined the retention of the P from different P. The maximum retentions of total P, NPP, and phosphorus from mono calcium phosphate (MCP) for the basal-MCP test diets were 67.6, 80.2, and 98%, respectively. The maximum retention of

dietary retainable P occurred with a 2:1 ratio of 0.48% Ca and 0.24% retainable P. The retainable P intakes for 10-to-15-d-old broilers required to provide a steady physiological state was 108 mg/d, as determined by two-line regression analysis. Retainable P requirements based on segmented line regression analysis using bone strength measurements for 0-to-3-wk-old chicks and 3-to-6-wk-old broilers were 0.39 and 0.30%, respectively. This indicates that current commercial diets might have enough P for bone development. Even, it had been suggested that maintaining a 2:1 Ca:NPP ratio during the 42 – 49 period enabled the removal of supplemental dicalcium phosphate with minimal effect of tibia strength or performance (Skinner and Waldroup, 1992). Chen and Moran (1992) confirmed no effects on live performance whenever the dicalcium phosphate was omitted (Ca:Total P = 1.6), however they observed an increased incidence of carcass defects after automated on-line processing.

Adequate levels of P and supplementation of phytase avoid tibia dischondroplasia (Qian *et al.*, 1996; Scheideler and Ferket, 2000). Sohail and Roland (1999) showed that phytase (300 FTU/kg) had greater influence on bone mineral content, bone density, bone breaking strength, and livability in broilers fed 0.225% NPP than in broilers fed 0.325% NPP. In diets containing marginal to deficient levels of either NPP or Ca or both, the addition of microbial phytase at 300 to 600 FTU/kg feed prevents P deficiency symptoms. Increasing phytase levels from 600 FTU/kg of feed provided no additional benefit. However, if dietary P is reduced relying in the phytase activity, and the mix uniformity of phytase is very bad (CV 103%) the bone ash and breaking strength, and Ca and P retention could be decreased (Johnston and Southern, 2000).

**Zinc.** Zinc is necessary for chondrocyte proliferation and differentiation. Proliferating chondrocytes may have a high requirement for zinc, VDR contain two zinc fingers (Zhongjian *et al.*, 2000). Supplementation of serum-free primary cultures of avian growth plate chondrocytes with 10-100  $\mu\text{M}$  Zn resulted in an increase in cell protein and greatly increased alkaline phosphatase (AP) activity (Litchfield *et al.*, 1998).

A short-term dietary zinc deficiency inhibits chondrocyte proliferation, differentiation, and induces cell apoptosis in the epiphyseal growth plate of young chickens (Wang *et al.*, 2002). The bioavailability of the microminerals is important and varies according to the source (Cao *et al.*, 2000), and the levels deposited in bone increase with the dietary level offered. Organic or chelated forms have been proven to be more efficient to support performance and health, despite of the level of inorganic source used (Kidd *et al.*, 1994a, b).

Congenital rickets observed during the first few days after hatch is usually linked to maternal nutrition or to problems of mineral absorption during the hatching process. Kidd *et al.* (1992) reported that the progeny of breeder hens fed diets supplemented with zinc-methionine had greater tibia bone ash content. Supplementation of 20 and 40 ppm of zinc and manganese as methionine chelate forms significantly reduced the incidence of shaky leg and angular defects in half in comparison to inorganic sulfate form (Ferket *et al.*, 1992).

**Selenium.** Selenium is involved in several metabolic processes such in transulfatation to form cysteine from methionine and in the deionization of thyroid hormones from T<sub>4</sub> to T<sub>3</sub> by 3,5,3' –triiodotironina. These processes are necessary for chondrocyte maturation. Iodothyronine deiodinases are selenoproteins contributing to systemic or local thyroid hormone homeostasis (Köhrle *et al.*, 2005). Selenoproteins are involved in bone metabolism as well as functions of the endocrine pancreas and adrenal glands. Jianhua *et al.* (2000)

showed that increasing dietary levels of Selenium increased 5' deiodinase activity and concentrations of T<sub>3</sub>, with an improvement in live performance.

Moreno-Reyes *et al.* (2001) investigated selenium deficiency on bone metabolism in growing male rats fed a selenium-deficient diet for two generations (Se-). In the SE- rats, erythrocyte glutathione peroxidase activity and plasma selenium concentration were strongly reduced as compared to pair-fed selenium-adequate rats (Se+). Weight and tail length were reduced by 31% and 13% in the Se- rats, respectively ( $p < 0.001$ ). The Se- diet was associated with a 68% reduction of pituitary growth hormone (GH;  $p = 0.01$ ) and a 50% reduction of plasma insulin-like growth factor I (IGF-I;  $p < 0.001$ ). Plasma calcium was lower and urinary calcium concentration was greater in Se- rats. This group had a 2-fold increase in parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>] in plasma. Plasma osteocalcin and urinary deoxypyridoline were reduced by 25% and 57% in the Se- rats ( $p < 0.001$ ). Selenium deficiency resulted in a 23% and 21% reduction in bone mineral density (BMD) of the femur and tibia ( $p < 0.001$ ) and this effect persisted after adjustment for weight in a linear regression model. A 43% reduction in trabecular bone volume of the femoral metaphysis ( $p < 0.001$ ) was found in Se- rats

**Other Minerals.** Data indicate that adding 25 mg/kg of dietary **nickel** to a poultry diet will have a positive influence on bone strength characteristics and performance (Wilson *et al.*, 2001). **Aluminum** in feed (0.3%) reduces tibiotarsal length (Johnson *et al.*, 1992), but levels as low as 0.1% reduced bone strength and concentrations of Ca, P, Mg and Zn. Supplementation of **boron** (5 -25 mg/kg of feed) to diets with inadequate (0.25 MIU) or adequate (2.0 MIU) vitamin D<sub>3</sub> content increases significantly the tibia calcium content and reduces its zinc content, resulting in stronger bones (Kurtoğlu *et al.*, 2005).

Deficiency of **copper**- Cu (< 1 ppm) was shown to decrease collagen crosslink formation and to lower mineralization (Osphal *et al.*, 1982). Banks *et al.* (2004) observed that even though the body weight gain was not different ( $P > 0.05$ ) among groups fed with Cu lysine (Cu-Lys), Cu sulfate (Cu-Sul), Cu citrate (Cu-Cit) and Cu chloride (Cu-Cl), the supplementation with 250 ppm of Cu from Cu-Lys resulted in chicks having greater toe and tibia ash weights and percentage as compared with birds supplemented with Cu-Sul. They also observed that supplementation with 250 ppm Cu from Cu-Cit or Cu-Sul resulted in decreased apparent P retention. Supplementation with 250 ppm Cu-Cl or Cu-lys, however, improved apparent P retentions as compared with Cu-Cit or Cu-Sul.

**Fluoride** in the form of sodium fluoride (~100 ppm) increases bone density and breaking strength in chickens (Merkely and Miller, 1983; Lundy *et al.*, 1992). Fluoride seems to increase the activities of osteoblasts and accelerate mineralization, while inhibit the activities of osteoclast (Dolegowska *et al.* 2003). Under normal dietary conditions fluoride levels do not cause risks related with bone fractures (Holick et al, 2004) as it has been previously suggested (Leeson *et al.*, 1995).

**Magnesium** (Mg) at dietary levels of 0.3% causes shortening, twisting and bowing of the tibiotarsus with a concomitant reduction in bone ash (Lee *et al.*, 1980). Microscopic evaluation showed rachitic-type lesions, with a widened and lengthened growth plate, excessive osteoid seams on endochondral bone and osteoid or capped metaphyseal blood vessels with few associated osteoblasts. It is important to limit the Mg content in the diets,

since commercial broiler diets using dolomitic limestone (20% Mg) can contain up to 0.4 – 0.5% of Mg.

**Cadmium** (Cd) is a heavy metal that is widely distributed in the environment and is considered the most toxic of the heavy metals with a maximum tolerable level of 0.5 ppm (NRC, 1980; 1994). Low-level long-term exposure to Cadmium (Cd) causes skeletal damage, suppressed humoral and cellular immunity, and thyroid dysfunction. In recent years, Cd poses a potential environmental hazard due to increases in its industrial use. Chickens are exposed to cadmium by feed and ground water contaminated by leaching (Rambeck and Guillot, 1990; 1996; Vodela *et al.*, 1997a, b; Linden *et al.*, 1999). Other potential sources include mining and smelting operations, corrosion of metal-plated iron, discarded cadmium-chloride products, and the use of urban sewage sludges to fertilize pastures or croplands.

The most likely source of contamination in the animal feed industry is in conjunction with the use of zinc sulfate or poorly processed zinc ores as sources of supplemental zinc. Addition of 60 ppm zinc to the diet would contribute only 0.1 ppm cadmium from the zinc oxide containing 1,290 ppm of the element and about 0.008 ppm from the oxide containing 79 ppm. Sources of zinc sulfate generally contain greater amounts of cadmium than oxide forms and should be analyzed carefully prior to use (NRC, 1980).

Relatively large quantities of Cd are found in commercial phosphate fertilizer and phosphate sources (0.1 - 67 ppm with an average of  $9.4 \pm 14$  ppm). Thus, the increases in soil and plant Cd contents may lead to increases in dietary Cd for poultry. For example, some soybeans can contain between 55.7 and 73.5 ng Cd/g (Zhang *et al.*, 1998). Addition of the highest cadmium-containing phosphate to poultry diets at 2% would add approximately 1.5 ppm cadmium while the average concentration (9.4 ppm) would add less than 0.2 ppm of the element. The defluorinated phosphates are practically devoid of cadmium, probably as a result of volatilization of element during thermal processing. In fact, the cadmium content of practical-type diets for poultry generally is about 0.05 to 0.35 ppm (NRC, 1980).

Gupta and Kar (1999) showed that the administration of cadmium chloride (2.5 mg/kg body weight/day) to chickens daily for 15 days decreased serum triiodothyronine (T<sub>3</sub>) concentration (by 68.75%) without altering the levels of serum thyroxine (T<sub>4</sub>). Hepatic 5'-monodeiodinase (5'D-I) and superoxide dismutase (SOD) activities were also decreased (by 90.47% and 20.81% respectively) with a concomitant increase in lipid peroxidation (LPO, by 206.25%). The administration of the antioxidant vitamin E at 5 mg/kg body weight on alternate days, to cadmium intoxicated chickens or rats restored thyroid function by maintaining normal hepatic 5'D-I activity and serum thyroid hormone concentrations (Gupta and Kar, 1999). Vitamin C administration restored serum T<sub>3</sub>, but no T<sub>4</sub> levels (Gupta and Kar, 1998). Both vitamins prevented cadmium-induced increase in LPO.

Mineral content of the liver is modified according to Cd level: iron, magnesium and selenium decreased while copper, zinc and manganese increased with increasing Cd levels. Iron is the most strikingly affected metal, falling to one-fifth of control values at high dietary Cd exposure. In this dosage group, selenium decreased to 36% of mean control concentrations while zinc increased to 168%. This mineral imbalance, especially depleted iron stores, can contribute, at least in part, to the Cd-associated risk of osteoporosis (Noel *et al.*, 2004). Cadmium also has an antagonistic activity in Ca metabolism and reduction in serum P

concentrations due to either increased P excretion because of tubular damage in kidney or suppressed absorption of this element or both (Uyanik *et al.*, 2001).

**Protein and specific amino acids.** Broilers and turkeys fed low crude protein diets normally show fewer leg defects (Leeson *et al.*, 1995). However, this effect might be caused by reduction of growth rate (Ferket and Sell, 1989; Waldroup *et al.*, 1998) and performance that is not recovered even after re-feeding higher amino acid levels.

Skinner *et al.* (1991) observed that when the total amino acid levels were increased by 20% above the requirements of the birds, a significant reduction in tibia mineralization, weight and length occurred among birds consuming diets containing 0,5% and 1,0% calcium. When the birds consumed 1.0% calcium in the diet, tibia weight increased with decreasing amino acid levels, however, when the diet contained 0.50% calcium no increase in tibia weight occurred with elevation in amino acid levels. Then, marginal levels of calcium in diets with higher levels of amino acids can affect bone development.

In a study of bone development of broiler chickens fed different amino acid and calcium levels with respective recommendations of 80, 100 and 120% and 50, 100 and 150% of NRC guidelines (1984), Sekine *et al.* (1994) noted an interaction between the factors evaluated and bone density. Diets containing 150% of recommended calcium levels did not promote a greater resistance of the tibia to fracture as compared to rations containing 120% amino acids. In addition, calcium levels affected the longitudinal growth of the tibia and promoted bone calcification. On the one hand, amino acid levels acted on transverse growth and facilitated the formation of bone matrix as well as calcification. On the other hand, several authors have failed to observed significant interactions between amino acids and minerals (Soares da Silva *et al.*, 2003). Soares da Silva *et al.* (2003) observed that regardless of the level of amino acids, the diets with low calcium level reduced bone density, tibia weight and spongy layer thickness for the Cobb strains but not for the Avian Farms genetic line demonstrating that some strains are more sensitive to a reduction in dietary calcium. Driver *et al.* (2005) concluded that current recommended level of 1.0% for broiler starter diets is adequate, and 0.9% is even excessive for grower diets independently of the level of protein in the diet.

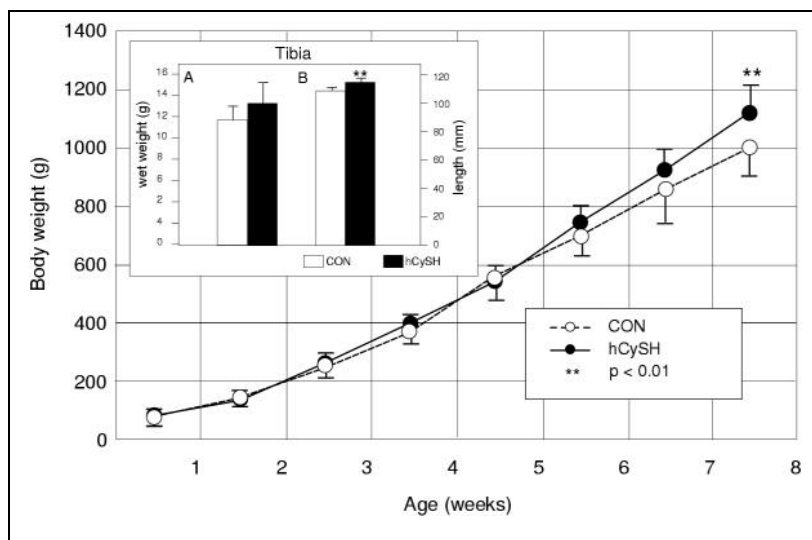
Marginal deficiencies of amino acids such as valine can occur when low crude protein diets are utilized. Farran and Thomas (1992) showed that birds fed a starter valine deficient diet have increased incidence of leg problems, and related this to reduced hydroxyproline availability and also to increased calcium excretion in the urine.

**Sulfur amino acid metabolism and leg problems.** Sulfur amino acids seem to play the main lead role on the effect of excess of protein in bone development. Diets with excess of sulfur amino acids can interfere with folic acid, pyridoxine, and vitamin A metabolism and increase their requirements. All these vitamins have been related to bone development and treatments for leg problems in avian species.

Diets with an excess of methionine and marginal deficiencies of folic acid and pyridoxine (Vitamin B<sub>6</sub>) cause hyperhomocysteinemia. Marginal deficiencies in vitamin B<sub>6</sub> is common in the poultry meat industry because it is commonly formulated under the NRC (1994) requirement (Tables 2 and 3) to promote better feed conversion. A recent study has shown that homocysteine could inhibit the conversion of retinal to retinoic acid in avian embryos (Limpach *et al.* 2000). In the same way, the requirements of vitamin B<sub>6</sub> for maximal growth

was found to increase ( $P < 0.01$ ) from 0.73 to 1.05 mg/kg, a 44% increase, when 10 g/kg excess methionine was present in the diet. Chicks were fed seven graded doses of supplemental pyridoxine (PN) in diets that contained either adequate (2 g/kg) or excess (12 g/kg) methionine. Indeed, this level of excess dietary methionine depressed ( $P < 0.01$ ) growth at all PN dose levels  $<1$  mg/kg, but not at PN doses of 1.2 or 1.4 mg/kg (Scherer and Baker, 2000).

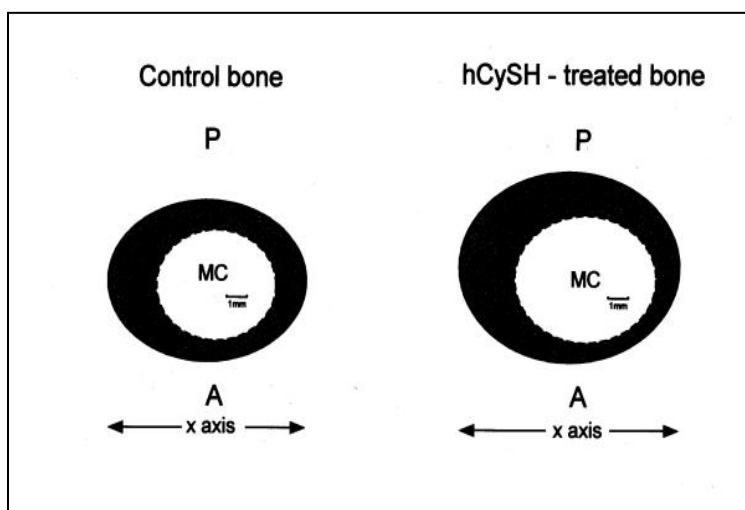
The hyperhomocysteinemic chickens have higher plasma levels of hCySH, methionine, cystathionine, and inorganic sulfate, but calcium, phosphate, and other indices of osteoblast metabolism are not different from controls (Massé *et al.* 1995, 1996). These hyperhomocysteinemic chickens grew faster, had heavier and longer tibias (Figure 1), and had accelerated radial and longitudinal bone growth with more advanced ossification of epiphyses and greater architectural efficiency of the diaphyseal cross-sectional design. Massé *et al.* (2003) observed that radiographs of the lower limbs showed generalized osteopenia with distinct metaphyseal and suprametaphyseal lucencies.



**Figure 1.** Growth curves (mean  $\pm$  SD of body weights) for control (CON) and hyperhomocysteinemic (hCySH) groups of broilers. hCySH-fed chickens were significantly heavier at the end of the 8-week experiment (\*\* $P < 0.01$ ). Inset: (A) tibial wet weight in grams; (B) length of tibia in millimeters (\*\* $P < 0.01$ ). Source: Massé *et al.*, 2003.

Hyperhomocysteinemia causes an abnormal bone collagen matrix defect, as chondrodysplastic cartilage changes in tibial cross-sectional contour (Figure 2), and the previously described radiological changes that indicate points of bone weakness. Although biomechanical testing of the tibiae, including maximal load to failure and bone stiffness, indicate stronger bone, strength was proportional to the increased length and cortical thickness in the hCySH-supplemented group. Bone ash weights and infra red-spectroscopy of cortical bone showed no difference in mineral content or mineral crystallization, but there were chemical abnormalities of bone mineral composition such as higher  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  and lower  $\text{Ca}^{2+}/\text{CO}_3^{2-}$  molar ratios than in controls.

These findings may have applications in the meat poultry industry where diets are formulated with high levels of methionine to maximize breast meat yield responses and guarantee good feathering, however some of the bone fractures and fragility could be due to excess of methionine and marginal deficiency of pyridoxine (Tables 2 and 3).



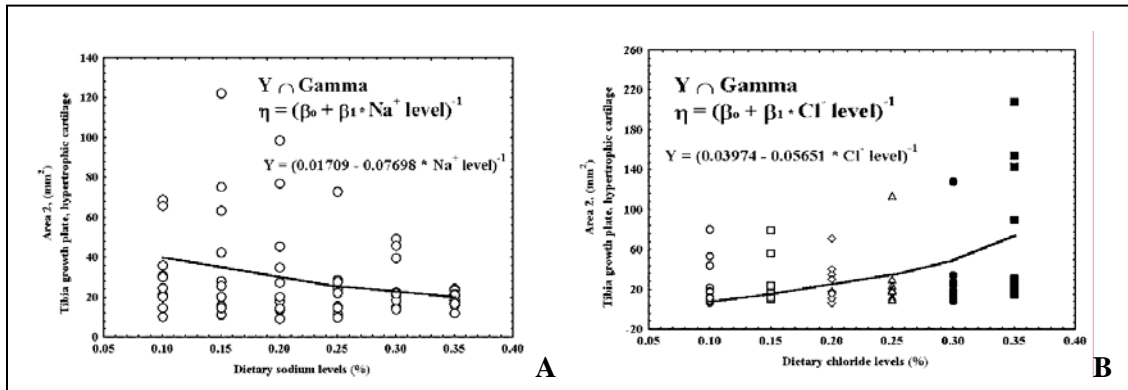
**Figure 2.** Mean mid-shaft tibial cross-sectional contours for control and homocysteine (hCySH)-treated chickens. hCySH-treated cortical bone is asymmetric with an eccentric medullary cavity. Its greater thickness on one side of the X axis was also noticeable on radiographs. A: anterior cortex of tensile side; P: posterior cortex of compressive side; MC: medullary cavity; — periosteal cortical bone; - - - endosteal cortical bone. Bar = 1 mm. **Source:** Massé *et al.*, 2003.

**Feeding and Management Practices.** Quantitative feed restriction and microelement supplementation at 7 days of age reduced mortality from ascites and leg problems and permitted compensatory growth sufficient to equal the production characteristics of the control group at 49 d of age (Camacho *et al.*, 2004). Feeding whole grain can decrease mortality and leg problems in turkeys (Bennett *et al.*, 2002), or no have clear effects on leg problems in broilers (Lippens *et al.*, 2000). Bruno *et al.* (2000) observed that feed restriction and high environmental rearing temperature reduce long bone growth. In their study bone breaking strength was not affected by bird age, feed restriction nor rearing ambient temperature, but the calculated BW/BL index was reduced by heat exposure. High environmental rearing temperature reduced bone length and width at 42 d of age. The common management practice of adding organic acids such as citric acid enhances dialysability of calcium, magnesium, manganese and zinc, but at the same time increases lead and cadmium dialysability (Walter *et al.*, 1998).

**Electrolyte balance.** The dietary electrolyte balance (DEB) value measured by the effect of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentration (mEq/Kg) affects the blood acid-base balance status of animals. Blood pH, partial pressure of  $\text{CO}_2$ , bicarbonate ( $\text{HCO}_3^-$ ),  $\text{CO}_2$  tension ( $\text{TCO}_2$ ), and base excess are significantly affected by small dietary modifications of  $\text{Na}^+$  or  $\text{Cl}^-$  (Oviedo-Rondón *et al.*, 2001; Murakami *et al.*, 2001). It is known that bone mineralization process are highly pH dependent (Carano *et al.*, 1993; Farquharson *et al.*, 2003, Bushinsky, 2004; Pines *et al.*, 2005).

Numerous poultry researchers have reported higher leg problem incidence in cases of metabolic acidosis in broilers and turkeys (Leeson *et al.*, 1995). For example, Halley *et al.* (1987) observed that chicks having the lowest base excess had higher incidences of both leg disorders. Whitehead (1989) cites evidence that metabolic acidosis, caused by high chloride levels in the feed, can reduce the production of  $1,25(\text{OH})_2\text{D}_3$  in the kidney and higher production of 24-25 DHCC. Additionally, chronic metabolic acidosis depletes proton buffers in bones, with phosphate exceeding that of  $\text{HCO}_3^-$  at the expense of the bone mineral (Bushinsky *et al.*, 2003). Oviedo-Rondón *et al.* (2001) and Murakami *et al.* (2001; 2003) have consistently observed in several experiments that incidence and severity of TD,

measured by the increment in the hypertrophic area of the the tibia growth plate, increases by  $\text{Cl}^-$  and is reduced by  $\text{Na}^+$  (Figure 3). We recommend that dietary DEB should be maintained around 250 mEq/kg for maximum growth and reduce TD incidence.



**Figure 3.** A. Nonlinear model (gamma distribution and link function inverse power) describing areas of hypertrophic region of tibial growing cartilage of broiler chickens in relation to dietary sodium levels at 22 d of age (A) or chloride levels during growing phase at 42 d of age. (A) Supplemental sodium (0.05 to 0.25%) was provided from sodium bicarbonate (0.19 to 0.93%). (B). The supplemental chloride (0.05 to 0.30%) was provided by sodium chloride (0 to 0.34%). **Source:** (A) Oviedo-Rondón *et al.*, 2001 and (B). Murakami *et al.*, 2001.

**Fatty acids.** Dietary lipids alter the fatty acid composition of bone polar and neutral lipids (Taylor, 1998), and the concentrations of prostaglandin and IGF-I in bone and consequently affect longitudinal bone growth (Watkins *et al.*, 2000, Watkins, 2002). Acidic phospholipids in matrix vesicles are an integral part of  $\text{Ca}^{2+}$  and Pi complexes initiating mineralization in epiphyseal cartilage. Growth cartilage chondrocytes of broiler chickens selectively accumulate fatty acids from the diet (Xu *et al.*, 1994a). Although Omega-3 polyunsaturated fatty acids are readily taken up by chondrocytes, the essential fatty acid linolate (omega-6) do not concentrate in these cells. This indicates that growth cartilage in young chickens may be sensitive to excessive amounts of certain unsaturated fats. Moderate amounts of saturated fats (Watkins *et al.*, 1993) and supplemental vitamin E (Xu *et al.*, 1994b) enhanced bone formation. These effects are due to more available arachidonic acid for PGE2 production, and altered concentrations of IGF-I, in plasma, cortical bone and epiphyseal cartilage.

Taylor (1998) observed that the lipid composition of the maternal diets stimulates growth rate, bone development and strength of the progeny. The tibiae diameter of chickens hatched from eggs collected from breeders fed diets containing poultry fat (3%) were significantly larger than the ones from breeders fed menhaden oil at 14 and 28 days of age. Chickens coming from breeders fed diets containing soybean oil had tibias with higher values for shear force and breaking strength than the ones coming from hens fed poultry fat and menhaden oil.

**Mycotoxins, production and immunological stresses.** Aflatoxins and ochratoxins cause bone fragility and affect vitamin D metabolism (Huff *et al.*, 1980; Duff *et al.*, 1987). While feed contamination with fumonisin B<sub>1</sub> (Wu *et al.*, 1995) and fusaric acid (Chu *et al.*, 1993) do not have good correlation with TD or any other leg disorders.

Environmental stress (Rath *et al.*, 2000) and immune response to bacterial challenge simulated by *E. coli* lipopolysaccharide injections (Mireles *et al.*, 2005) reduce tibia bone calcium content and breaking strength in chickens. The same responses could be expected in turkeys. The severity of this effect is mediated by glucocorticoids and cytokines, and is age dependent.

## Conclusions

Leg abnormalities in meat poultry is a very complex problem that can be influenced by many different factors. The genetic modifications of fast-growing broilers and turkeys to their hormonal balance (thyroid, GH and IGF) on systemic and tissue-specific functions, and the number of cellular vitamin D receptors in bone tissues could play important roles in the predisposition to different types of bone developmental disorders. Breeder nutrition, incubation conditions, management (brooding) practices, and the physiological responses to environmental stress and immunological responses might affect the field incidence of these developmental problems. Chicks and poults with high alterations in their bone regulatory mechanisms might not respond to any dietary manipulation directed to solve leg problems. Thus, genetic selection comes again as the less expensive and main strategy to solve these issues in the long term.

To understand a specific skeletal problem observed in the field and develop a plan to solve it, we must study several parameters. Although bone mineralization and density are good markers of bone development, it is very important to evaluate the biochemical composition of bone collagen and the biomechanical properties of the bone by testing tensile, torsion and fatigue. Along with good feed quality and sufficient dietary nutrient levels typically used in the poultry industry, attention should be placed to levels of highly bioavailable sources of zinc, selenium, copper, pyridoxine, biotin, and sources of vitamin D. Moreover, appropriate levels of Ca, P, Na, Cl and good balance between  $\Omega 3$ :  $\Omega 6$  fatty acids according to the stage of development are important. It is noteworthy to assess the effects of high methionine-low pyridoxine diets on bone development and resistance to fractures in broilers and turkeys under commercial conditions. Additionally, strategies to avoid enteritis, fat and oil rancidity, mycotoxins, and cadmium contamination should be placed at the feed mill and at the farms to reduce skeletal disorders in poultry.

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