Life cycle changes in bone mineralization and bone size traits of commercial broilers

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ABSTRACT Life cycle changes in bone mineralization and bone size traits of the tibia and humerus were evaluated in commercial male and female broilers using dual-energy x-ray absorptiometry (DEXA). Experiment 1 evaluated weekly changes in bone traits from 2 to 7 wk of age, whereas experiment 2 compared the bone traits of 4 strains of commercial meat-type chickens from 4 to 8 wk of age. Birds were restrained without anesthesia, and the humerus and tibia were scanned in vivo. After each scan, individual BW was determined. From the DEXA scans, bone mineral density (BMD), bone mineral content (BMC), as well as bone length, width, and area were determined. Bone mineralization and size traits were analyzed as an analysis of covariance with BW as the covariate. If BW was NS as a covariate, then an ANOVA was used. The BMD reached its peak at 4 wk of age. The BMC of the humerus changed little from 2 to 8 wk of age, whereas tibial BMC increased as the birds aged, especially in males ($P < 0.0001$). In experiment 1, bone length, width, and area also increased with age ($P < 0.0001$), with the tibia growing in length at a faster rate than the humerus. In experiment 2, the BMD did not differ among the 4 strains of commercial broilers. Interactions with strain of chicken were also NS, indicating that all strains of chickens responded similarly with respect to age (4, 6, and 8 wk of age), sex, and type of bone (humerus vs. tibia). Coefficients of variation for BMD ranged from 15 to 16%, indicating a potential use of DEXA for selection to improve skeletal integrity. In conclusion, the tibia continued to grow, especially after the initiation of the growth spurt at 3 to 4 wk of age, as indicated by bone length, width, and BMC, but it did not become denser in mineral after 4 wk of age as its surface area increased.

Key words: bone mineralization, dual-energy x-ray absorptiometry, humerus, tibia, broiler

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INTRODUCTION

Leg problems due to valgus-varus deformities, tibial dyschondroplasia, rickets, and femoral head necrosis are costly to the United States broiler industry with economic losses estimated at $120 million annually (Cook, 2000). A national survey of broiler companies indicates that broiler flocks experience 1.1% mortality due to leg problems and that an additional 2.1% of the birds are condemned or downgraded during processing as a result of leg abnormalities. Birds with severe lameness experience pain (McGeown et al., 1999) and are unable to access feed and water, leading to BW loss.

Skeletal integrity is affected by rapid growth rate, genetics, the environment, management, nutrition, locomotive activity, toxins, age, and infectious diseases (Rath et al., 2000). These factors affect skeletal health in broilers and manifest themselves as leg deformities with compromised bone structure.

Rath et al. (2000) emphasized the importance of calcium as a nutritional factor in upholding bone strength. Continuous bone remodeling and turnover promotes bone health. Without adequate dietary sources of calcium, blood hypocalcemia occurs, leading to reduced bone strength and mineralization. As an example, adult White Leghorn hens consuming diets with decreasing calcium levels (5.4, 3.6, and 1.8% Ca) showed a linear decrease in tibial and humeral bone mineral density (**BMD**) and bone mineral content (**BMC**; Schreiweis et al., 2003).

Bone density is the mass of material per volume of bone (Rath et al., 2000), which includes both organic (collagen) and inorganic (mineral hydroxyapatite) components. Bone mechanical strength is affected by nutrition, genes for the expression of collagen and proteins, quantity and quality of the organic and inorganic material, content and size of the mineral material, and design and structure of the bone (Boskey et al., 1999).

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Several traits such as stress and strain, bending moment, moment of inertia, and modulus of elasticity can be used to evaluate the mechanical state of the bone. Bone breaking strength is measured by evaluating the reaction of the bone to stress and force. An increase in bone mineralization is accompanied by an increase in bone stress and bending moment values. When optimum mineralization is accomplished, maximum stress can be achieved with room for increasing bending moment. Higher increases in stress occur with smaller increases in ash content (Crenshaw et al., 1981). A decrease in bone mineralization is accompanied by a decrease in bone breaking force and bone ash weight. In chickens, bone mineralization as determined through dual-energy x-ray absorptiometry (**DEXA**) is positively correlated with bone breaking force ($r = 0.58$ to 0.68; $P < 0.001$) and bone ash weight ($r = 0.73$ to 0.99; $P < 0.001$; Onyango et al., 2003; Mazzuco and Hester, 2005; Schreiweis et al., 2005). Furthermore, as the BMD of the excised tibia decreased in White Leghorns, the incidence of bone breakage increased ($r = –0.54$; $P < 0.05$; Mazzuco and Hester, 2005). Because live scans using DEXA are highly correlated with scans of excised bones (Schreiweis et al., 2005), DEXA is a valuable tool for monitoring bone mineralization in vivo as birds age. Birds are restrained during the scan but do not have to be anesthetized (Hester et al., 2004) as is the case for quantitative computed tomography (**CT**), another in vivo measurement of skeletal integrity of poultry (Korver et al., 2004). However, a major advantage of quantitative CT over DEXA is that it can distinguish cortical from trabecular bone. The information retrieved from quantitative CT focuses on a specific site of a single bone, whereas DEXA can provide information on bone mineralization of an entire bone or multiple bones with a single scan. Both techniques are labor-intensive with quantitative CT requiring 15 to 20 min/scan (Korver et al., 2004) and DEXA taking 10 min/scan (Hester et al., 2004). Other in vivo measures of avian bone quality include digitized fluoroscopy and ultrasound (Fleming et al., 2004). Digitized fluoroscopy does not require that birds be anesthetized during assessment, but the procedure has an increased risk of x-ray exposure requiring technicians to use lead gloves and gowns. The x-ray exposure with DEXA and quantitative CT is minimal and does not require use of protective equipment or shielding. All equipment (DEXA, quantitative CT, and digitized fluoroscopy) is expensive and not easily portable. If used for genetic selection, equipment would have to be placed near the source of birds. Amplitude-dependent speed of sound ultrasound, on the other hand, is portable, relatively inexpensive, and health risks to technicians and animals are minimal, making it more applicable to field situations. Birds do not require anesthesia; however, ultrasound measures velocity through cortical bone and may not detect cancellous bone (Fleming et al., 2004).

The objective of the current study was to determine life cycle changes in bone mineralization and bone size traits of the tibia and humerus of male and female commercial meat-type chickens using DEXA. The in vivo monitoring of bone growth and mineralization as broilers age may help identify vulnerabilities in the life cycle whereby bone mineralization may be compromised, making birds more susceptible to bone fracture.

MATERIALS AND METHODS

Experiments were conducted under guidelines approved by the Purdue University Animal Care and Use Committee.

Experiment 1

One-day-old male and female chicks of a commercial strain of broilers, hatched from eggs set at Purdue University hatchery, were injected s.c. in the neck with Marek's vaccine, feather-sexed, wing-banded in the right wing, and housed in littered floor pens of Management House II at the Purdue University Poultry Research Farm. Males were raised separately from females. Three pens per sex were littered with wood shavings with 48 birds placed in each pen resulting in 774 cm^2 of floor space per bird. Broilers were vaccinated with the B1 strain of Newcastle and the Massachusetts-Connecticut strain of infectious bronchitis at 14 d of age. Feed and water were provided for ad libitum consumption. Broilers were fed a mash starter diet from 0 to 4 wk of age and a mash finisher diet for the remainder of the trial. Feed was formulated to meet or exceed the recommended nutrient requirements of broilers (NRC, 1994). Mortality was recorded daily.

Ambient room brooding was used with temperature maintained at approximately 35°C the first week of the chick's life. Subsequently, the temperature was reduced by 1.7 to 2.8°C each week until a temperature of approximately 21°C was reached; this temperature was maintained until termination of the experiment at 7 wk of age. Using incandescent lights, a photoperiod of 23L:1D at 10 lx was used the first 2 d of age followed by 20L:4D at 5 lx for the remainder of the trial.

Beginning at 1 wk of age and then at weekly intervals until 7 wk of age, 6 females and 6 males were randomly selected from a single pen. Birds selected at 1 wk of age were healthy with no apparent leg anomalies. Birds were restrained without anesthesia and their bones were scanned in vivo as described previously (Schreiweis et al., 2003). The humerus and tibia of each of the 12 birds were scanned using DEXA for determination of BMD, BMC, and the bone size traits of length, width, and area. After each scan, individual BW were determined and black spray paint was applied to the feathers on the bird's back to allow for easy identification and access of birds within a pen for future DEXA scans.

The BMD and BMC data were analyzed as an analysis of covariance with BW as the covariate (Steel et al., 1997). An ANOVA was used for BW and also for bone length, width, and area because BW was NS as a

covariate. Repeated measurements were used beginning at 2 wk of age because BMD and BMC could not be consistently detected with DEXA at 1 wk of age. Fixed effects included the sex, bone, and age of the bird. Bone was considered as a subplot for all analyses except BW. The mixed model procedure of SAS Institute (2003) was used. The Tukey-Kramer test was used to partition differences among means (Oehlert, 2000).

Experiment 2

Chicks of 4 commercial strains of broilers (A, B, C, and D) were hatched at Purdue University. Day-old chicks were injected s.c. in the neck with Marek's vaccine, sexed, wing-banded in the right wing, and housed in littered floor pens of Management House II at the Purdue University Poultry Research Farm. Males were raised separately from females. Each of the 4 commercial lines of male and female birds was assigned randomly to 3 replicate pens/strain per sex for a total of 24 pens. During brooding, 60 chicks were placed in each pen resulting in a stocking density of 619 cm^2 per bird. At 3 wk of age, bird numbers were reduced to 38 broilers per pen allowing for an equal density among pens of 975 cm^2 per bird.

Broilers were vaccinated with the B1 strain of Newcastle and the Massachusetts-Connecticut strain of infectious bronchitis at 10 d of age. Broilers were fed a crumbled starter diet from 1 to 21 d of age and a pelleted finisher diet for the remainder of the trial. Feed was formulated to meet or exceed nutrient recommendations of NRC (1994). Feed and water were provided for ad libitum consumption. Mortality was recorded daily.

Room temperature was maintained as described for experiment 1. Using incandescent lights, continuous light of 20 lx was used the first 2 d of age followed by 20L:4D at 5 lx for the remainder of the trial.

Starting at 4 wk of age, 3 birds were randomly selected from each pen and their tibia and humerus were scanned for BMD and BMC using DEXA. Birds selected at 4 wk of age were healthy with no apparent leg anomalies. Broilers were restrained without anesthesia and their bones were scanned in vivo as described previously (Schreiweis et al., 2003). Livestock paint was applied to the back feathers and plastic leg bands were used to allow for easy identification of birds 2 wk later because the same birds were scanned again at 6 and 8 wk of age. A total of 72 birds (9 chickens/strain per sex) were scanned repeatedly at 4, 6, and 8 wk of age. From each scan, bone length, width, and area were determined. Body weight was also determined after the scan.

Bone mineralization and size traits were analyzed as an analysis of covariance with BW as the covariate (Steel et al., 1997). Strain and sex of the bird were main plots. Type of bone (tibia and humerus) and age of the birds were the subplots. An ANOVA with repeated measures was used for BW with strain, sex, and age as fixed effects. The mixed model procedure of SAS Institute (2003) was used. The Tukey-Kramer test was used to partition differences among means (Oehlert, 2000).

RESULTS

Experiment 1

Males had higher BW than females beginning at 4 wk of age. This trend persisted until termination of the study at 7 wk of age (sex × age interaction; $P < 0.002$; Figure 1).

The BMD of the humerus and tibia peaked at 4 wk of age and remained at approximately peak values for the remainder of the study (to 7 wk of age) with 1 exception. The BMD of the humerus of male broilers decreased at 6 and 7 wk of age as compared with peak values at 4 and 5 wk of age, resulting in a bone × sex × age interaction ($P < 0.04$; Figure 2a).The BMC of the humerus of male and female broilers did not change from 2 to 7 wk of age. In contrast, the BMC of the tibia increased as the birds aged with the tibial BMC of males exceeding females at 6 and 7 wk of age (bone × sex × age interaction, $P < 0.0001$; Figure 2b).

Bone length increased with age ($P < 0.0001$) with the tibia growing at a faster rate than the humerus between 2 and 7 wk of age (bone × age interaction, $P < 0.0001$; Figure 3a). Bone width increased with age ($P < 0.0001$) with similar bone widths between the humerus and tibia at 2 and 3 wk of age. The bone width of the tibia was greater than the humerus from 4 to 7 wk of age (bone × age interaction, $P < 0.02$; Figure 3b). The bone area of the humerus and tibia increased with age ($P < 0.0001$) with the area of the tibia always greater than the humerus. Differences in area between bones became greater as the birds aged (bone × age interaction, $P < 0.0001$; Figure 3c).

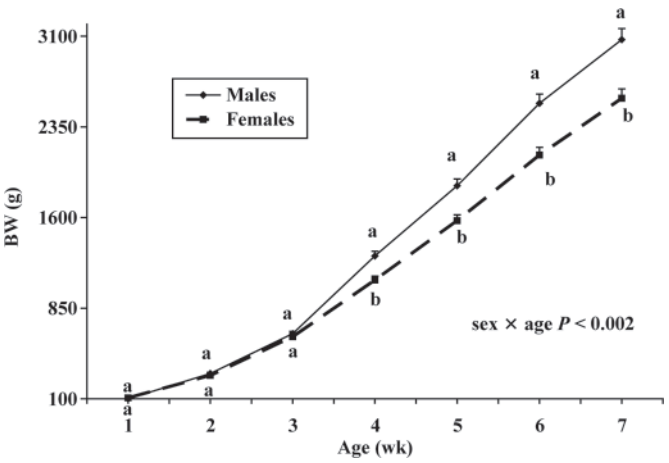

Figure 1. The effect of sex on weekly BW of a commercial strain of broilers (experiment 1). Each value represents the least squares mean of 6 observations per sex. Means within an age with no common letter differ significantly ($P < 0.05$).

Experiment 2

Bone mineralization did not differ among the 4 strains of commercial broilers (Table 1). All interactions with strain of chicken were NS, indicating that all strains of chickens responded similarly with respect to age (4, 6, and 8 wk of age), sex, and type of bone (humerus vs. tibia). Coefficients of variation for BMD and BMC ranged from 15 to 20%.

The BMD of broiler males decreased at 8 wk of age when compared with 4 and 6 wk of age, whereas the BMD of females showed little change from 4 to 8 wk of age, resulting in a significant sex × age interaction ($P < 0.02$, Figure 4a). The BMD of the tibia but not the humerus showed an age-related decline at 8 wk of age (bone × age interaction, $P < 0.05$, Figure 4b). The BMC of the female tibia increased with age, whereas the BMC of the male tibia increased from 4 to 6 wk with no further increase in BMC at 8 wk of age. The BMC of the humerus changed little from 4 to 8 wk of age, although the BMC of the male humerus was lower at 6 as compared with 8 wk of age (bone × sex × age interaction, $P < 0.02$, Figure 4c).

From 4 to 8 wk of age, both the humerus and tibia increased in length with age ($P < 0.0001$), but unlike the humerus, tibial growth slowed between 6 and 8 wk (bone × age interaction, $P < 0.0001$, Figure 5a). The width of the humerus increased from 4 to 6 wk of age with no subsequent increase at 8 wk of age. The width of the tibia did not change from 4 to 8 wk of age (bone × age interaction, $P < 0.0001$, Figure 5b). Bone area of both the humerus and tibia increased with age with the tibial area increasing more between 4 and 6 wk of age

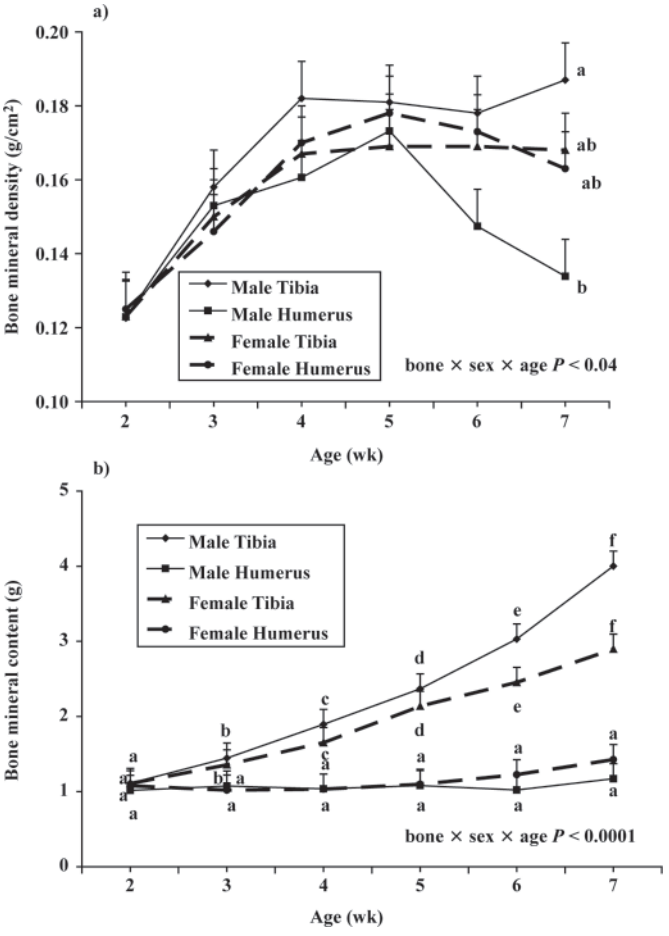

Figure 2. The effect of age on (a) bone mineral density and (b) bone mineral content of the tibia and humerus of a commercial strain of male and female broilers (experiment 1). Each value represents the least squares mean of 6 observations per bone and sex. Means (a) within an age and (b) within bone and sex with no common letter differ significantly ($P < 0.05$). Values were adjusted for BW.

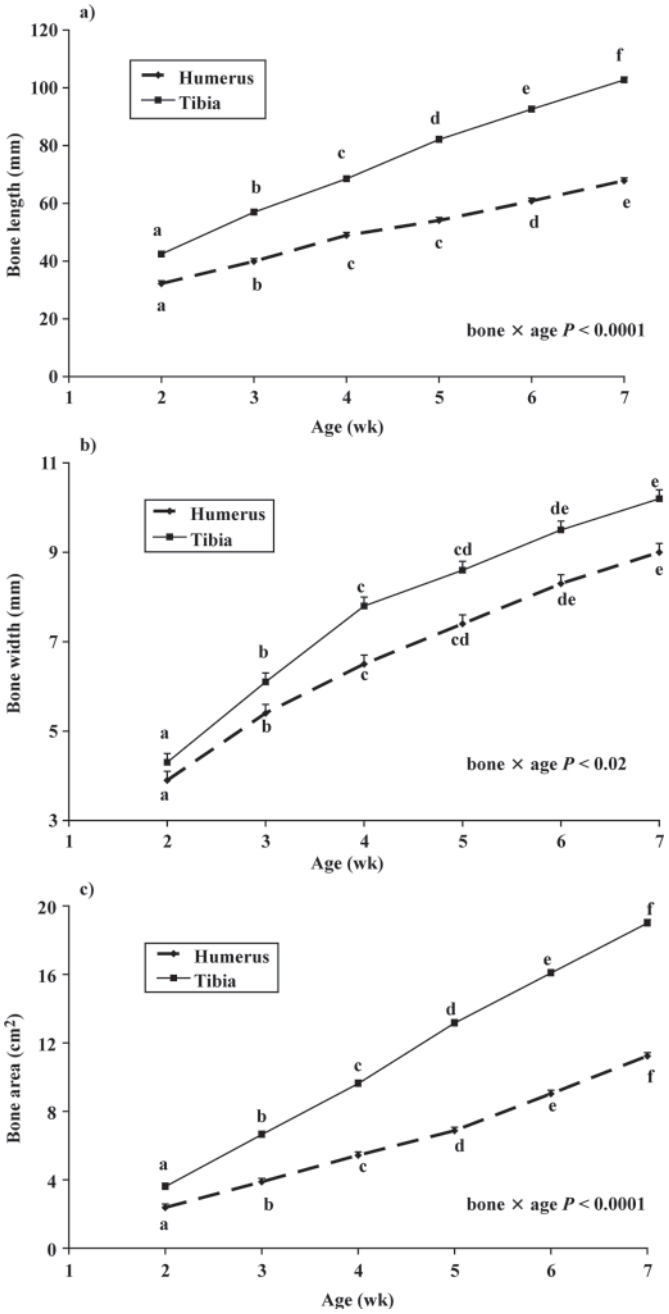

Figure 3. The effect of age on (a) length, (b) width, and (c) area of the humerus and tibia of a commercial strain of broilers (experiment 1). Each value represents the least squares mean of 12 observations per bone. Means within a bone with no common letter differ significantly ($P < 0.05$).

than the humerus (Figure 5c, bone × age interaction, $P < 0.0001$).

The BW of scanned male (Figure 6a) and female (Figure 6b) broilers showed an age-related increase ($P < 0.001$). There were no differences in BW among strains of chickens ($P = 0.66$). The BW of males was greater than females ($P < 0.02$).

DISCUSSION

Broilers experienced a dramatic increase in BW beginning at 3 to 4 wk of age (Figure 1) that continued to increase exponentially until termination of the experiment (Figures 6a and b). At about the same time broilers were initiating a growth spurt (Figure 1), the BMD of the tibia and humerus had already peaked (Figure 2a). No further increases in bone mineralization were noted after 4 wk of age with values remaining stable or lower until termination of the experiment at 7 or 8 wk of age (Figures 2a, 4a, and 4b). The tibia in particular continued to grow especially after the initiation of the growth spurt at 3 to 4 wk of age as indicated by bone length at 7 or 8 wk of age (Figures 3a and 5a, respectively), width (Figure 3b), and BMC at 6 or 7 wk of age (Figures 4c and 2b, respectively). It is apparent that the tibia did not become more dense in mineral after 4 wk of age as its surface area (length × width) increased (Figures 3c and 5c). Skeletal muscle deposition should place more load on bone, making it denser and stronger, but genetic selection in meat-type fowl has focused more on increasing breast muscle as opposed to leg muscle (Nestor et al., 1985, 1987; Nestor and Emmerson, 1990). Shifting emphasis to selecting birds for more leg muscle as opposed to breast muscle could place more load on the tibia and femur and cause a concomitant increase in BMD leading to stronger bones that are less susceptible to breakage.

Williams et al. (2003) provided evidence that rapid growth rates, rather than genotype, have a greater effect on bone mineralization. A fast-growing commercial line of broilers showed decreased tibial mineralization and increased bone porosity as compared with a slow-growing line of broilers. The fast growth rate of these broilers caused rapid bone deposition at the periosteal surface leading to increased circumferential expansion. The increased porosity was due to the inability of the osteoblasts to completely infill the lamella surrounding the Haversian canals of osteons. Feed restricting the fast-growing line of broiler improved mineralization and porosity to the level observed in the tibia of the slow-growing broilers, strongly suggesting that growth rate and not genotype was the main contributor to poorer bone quality (Williams et al., 2003). This increased bone porosity could lead to darkening of cooked meat around the bones due to the seepage of bone marrow heme pigments through the porous bones (Smith and Northcutt, 2004).

Reduced locomotive activity and flapping of the wings as broilers age may also contribute to the lack of increase in BMD after 4 wk of age. Broilers spend about 76% of their time lying, which increases with age. Walking also declines with age, with broilers spending about 3.3% of their time walking as they approach market age (Weeks et al., 2000). Walking ability of birds is generally better at 4 wk of age than at 6 and 7 wk of age (Sørensen et al., 2000). The BMD (Figures 2a and 4b) and BMC (Figures 2b and 4c) of the humerus of birds of the current study changed little after 4 wk of age even when it was growing in length (Figures 3a and 5a), width (Figures 3b and 5b), and area (Figures 3c and 5c) and may have been due to lowered use or activity.

The reason for the decrease in the BMD of the humerus at 7 wk of age in males of experiment 1 is unknown and may have been due to random chance (n = 6 observations, Figure 2a). The decrease in BMD observed in males at 8 wk of age of experiment 2 (Figure 4a) was most likely due in part to the inability of lame males to reach the feeders. Birds to be scanned (3 chickens per pen) were selected randomly at 4 wk of age and repeated measurements were done on the same birds throughout the study. Only healthy birds without defects were selected at 4 wk of age for DEXA scanning, but unfortunately, a few males developed leg abnormalities. When the BMD data of experiment 2 were reanalyzed excluding birds with broken wings and leg abnormalities, the sex × age interaction was NS ($P = 0.49$). The strain of birds used in experiment 1 did not have any lameness problems; therefore, the tibia BMD did not show a decrease after 6 wk of age (Figure 2a) as occurred in the males of experiment 2 (Figure 4a) .

Table 1. The bone mineralization (adjusted for BW) of 4 strains of commercial broilers (experiment 2)

Strain	Bone mineral density[1] (g/cm^2)	CV (%)	Bone mineral content[1] (g)	CV (%)	n[2]
A	0.212	16	2.89	20	108
B	0.218	15	3.03	19	104
C	0.209	16	2.90	20	106
D	0.207	16	2.88	20	106
SEM	0.003		0.06		
P-value	0.11		0.18		

[1]Values represent the least squares mean averaged across bone (humerus and tibia), sex of the bird, and age (4, 6, and 8 wk of age).

[2]Within a strain, n = the number of observations for each least squares mean.

Sexual dimorphism among bone traits was most apparent with tibial BMC after 6 wk of age, with males having greater values (Figures 2b and 4c). In general, previous reports have shown that heavier males have higher bone morphological and compositional values (Bond et al., 1991; Yalcin et al., 2001; Fanatico et al., 2005; Venalainen et al., 2006).

No differences in bone mineralization occurred among the 4 strains used in the current study (Table 1). Similar results were reported by Yalcin et al. (2001), who compared the mineral composition and morphological tibial traits of 2 strains of commercial broilers. Difference between strains in bone mineralization and anatomy were noted before 16 d of age (ages that we did not measure in experiment 2), but at the later ages of 32

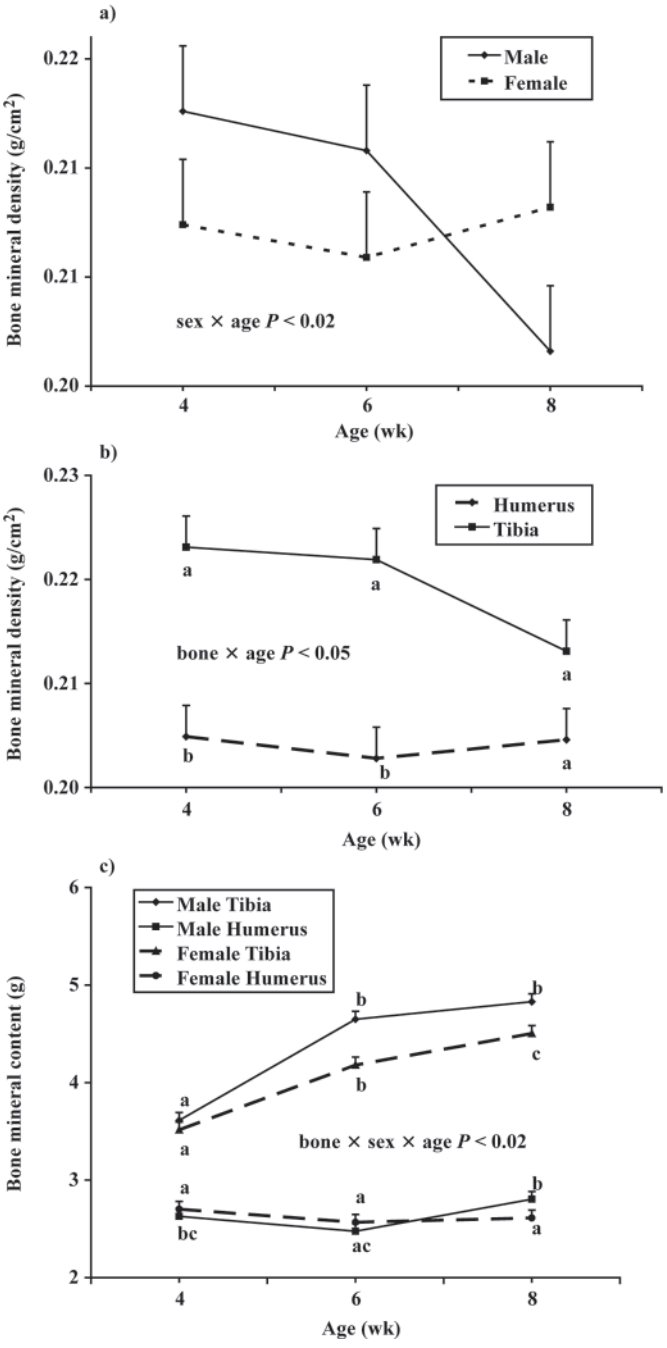

Figure 4. Mineralization of bones from commercial broilers at 4, 6, and 8 wk of age (experiment 2). Values were adjusted for BW. The bone mineral density of (a) bones from male and female chickens and (b) the humerus and tibia. Each value represents the least squares mean of 72 observations. Means within an age with no common letter differ significantly ($P < 0.05$). Panel c depicts the bone mineral content of the tibia and humerus of male and female broilers. Each value represents the least squares mean of 36 observations. Means within a bone and sex with no common letter differ significantly ($P < 0.05$).

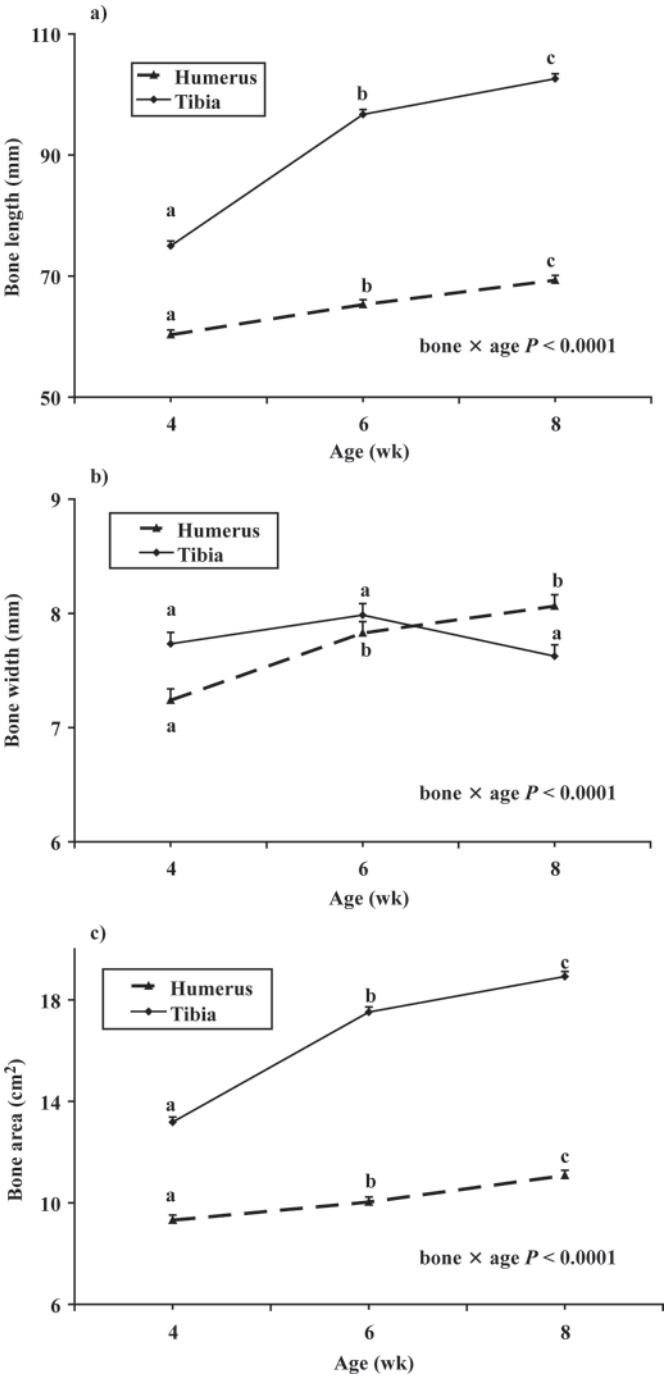

Figure 5. The (a) length, (b) width, and (c) area of the humerus and tibia of commercial broilers at 4, 6, and 8 wk of age (experiment 2). Each value represents the least squares mean of 72 observations. Means within a bone with no common letter differ significantly ($P < 0.05$). Values were adjusted for BW.

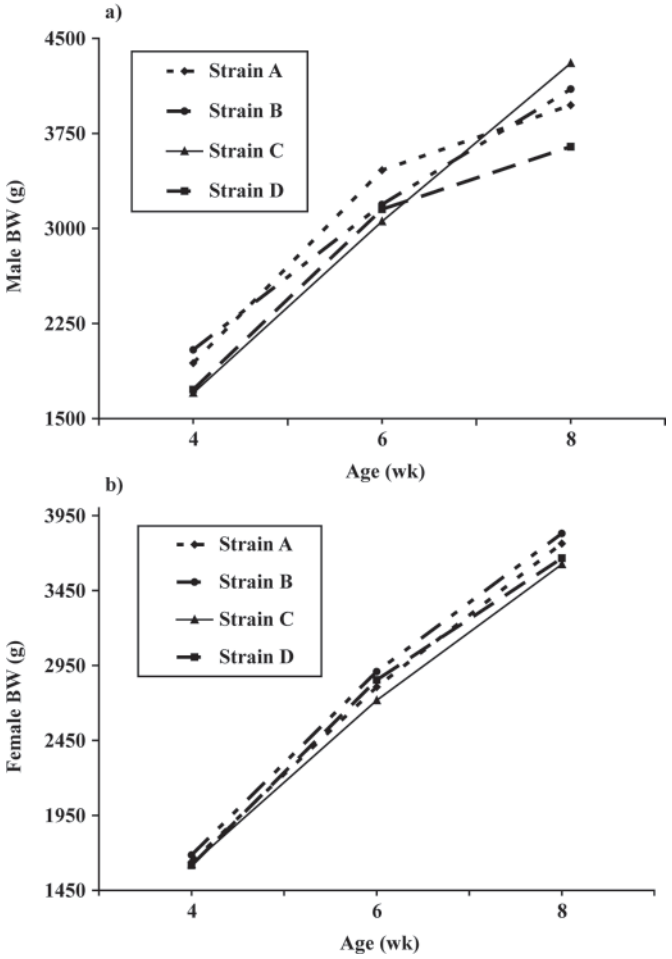

Figure 6. The BW of 4 strains of (a) male and (b) female commercial broilers from 4 to 8 wk of age (experiment 2). Each value represents the least squares mean of 9 observations. SEM = 206.

and 48 d of age, differences between strains dissipated with the exception of bone volume.

In conclusion, when considering the short life cycle of growing broilers, the BMD of the tibia and humerus reached its peak at 4 wk of age, with mineralization of bones being comparable among the 4 strains of commercial broilers. The tibia continued to grow, especially after the initiation of the growth spurt at 3 to 4 wk of age, as indicated by bone length, width, and BMC, but it did not become denser in mineral after 4 wk of age as its surface area increased. Coefficients of variation for BMD and content ranged from 15 to 20%, indicating a potential use of DEXA for selection to improve skeletal integrity.

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