

The morphological, material-level, and ash properties of turkey femurs from 3 different genetic strains during production

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ABSTRACT Femoral fractures are observed in selective-bred commercial turkeys; however, the etiology of such fractures is unknown. The current study investigated the whole bone morphological, material-level mechanical, and bone ash properties to determine the effect of selective breeding on bone strength. Femora from 3 divergent strains of turkeys, a commercial line, a different selectively bred heavy line (F-line), and a lighter age or weight matched random-bred line (RBC2/R-EQ, respectively), were compared. Bone geometric properties were measured with micro-CT and bone mechanical properties were measured using 3-point bending tests. Whole bone ash quantities were also recorded. Statistics were run using a general linear model multivariate ANOVA (GLM ANOVA). Results showed that at similar ages, the faster growing birds (commercial and F-line) had femurs twice the size of the RBC2 line as measured by cross-sectional area as early as 8 wk into the study. The femurs of the commercial and F-lines also exhibited as much as 20% greater mechanical

strength than femurs from the RBC2 line at 16 and 20 wk of age as measured by properties such as elastic modulus and ultimate tensile strength. However, at similar BW, the slower growing R-EQ line had higher mechanical properties than the other lines, with the elastic modulus being 40% greater and the ultimate tensile strength being 37% greater at weights equivalent to those of the commercial and F-lines at 12 wk of age. Moreover, it was observed that the morphological properties (i.e., cross-sectional area, moments of inertia) are largely governed by BW, as there is little difference in the amount gained per week of age across the different lines. Conversely, the mechanical properties, as well as the related ash content, appear to be governed at least in part by time. Therefore, whereas modulation of bone geometry is the key responder for changes in BW, sufficient time for matrix mineralization or maturation or both to occur is also essential for mechanical competence of bone.

Key words: turkey, femur, bone geometry, bone mechanics, genetic strain

2012 Poultry Science 91:2736–2746
<http://dx.doi.org/10.3382/ps.2012-02322>

INTRODUCTION

Genetic selection has been successfully applied in developing commercial turkey genotypes that reach market weight at lower ages with significantly increased breast muscle yield (Abourachid, 1993; Corr et al., 2003a,b; Havenstein et al., 2007). However, Havenstein et al. (1988) showed that genetic selection for BW

can lead to large weight gains without similar gains in skeletal integrity. Further analysis revealed that genetic selection for BW led to disproportional gains in fat relative to muscle. Moreover, because the associated increase in the body mass has not been accompanied by proportional increases in the skeletal support system, this has led to a concomitant increase in skeletal disorders (Julian, 2005; Dibner et al., 2007). Many skeletal disorders are initiated early in life with the consequences appearing later in the production cycle (van der Eerden et al., 2003; Dibner et al., 2007; Oviedo-Rondon et al., 2008; Ferket et al., 2009). In controlled studies, reducing growth rate at 15 to 20 d of age significantly reduced the incidence of valgus-varus and kinky back deformities (Classen and Riddell, 1989;

©2012 Poultry Science Association Inc.

Received March 20, 2012.

Accepted July 17, 2012.

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Julian, 2005). This emphasizes the importance of early relative growth on overall skeletal health.

Femoral fractures make up an increasing proportion of total skeletal problems in commercial turkeys. Crespo et al. (1999) were among the first to report femoral breakage in commercial breeder toms, and investigated the role of compositional and mechanical properties in fracture incidence (Crespo et al., 2000, 2002). In one study (Crespo et al., 2000), both femurs were collected from adult toms that had a confirmed fracture in one of the femurs. In those toms with heavier BW and a broken femur, the mechanical properties of the non-broken femur were not significantly greater when compared with the femurs from lighter toms without a fracture, whereas the heavier toms without a broken femur had significantly higher mechanical properties than the lighter toms. Additionally, geometric measurements showed increased cortical thickness in heavier toms compared with the lighter toms (Crespo et al., 2000). The heavier turkeys with fractures also had reduced Ca content and altered collagen fibril orientation in their femurs, as well as increased porosity within the callus region at the medio-caudal site compared with heavier toms without fractures. Moreover, heavier toms without complete fractures had callus, implying a chronic prefracture condition (Crespo et al., 2002).

Many of the previously cited studies focused on quantifying the geometric properties of bone and bone ash content, but did not measure the actual material-level properties of bone directly (i.e., elastic modulus, yield stress/strain, failure stress/strain; Crespo et al., 2002; Corr et al., 2003a; Havenstein et al., 2007). In those studies, material properties were derived from whole bone tests, which are prone to error because the results are dependent on how thoroughly the geometry was assessed, and the theories linking load-displacement to stress-strain behavior are limited for irregularly shaped bone geometries (Levenston et al., 1994; Crespo et al., 2000; Ferket et al., 2009). Body weight was often cited as a potential factor in modulating the geometric and material-level properties of bone, but the correlations between BW and material-level properties are unknown. Therefore, it is unclear whether growing turkey bones adapt to loading by modulating the geometry alone or whether the material-level properties are also associated with changes in BW as well. By using a modeling approach, Soboyejo and Nestor (2000) showed that bone geometric properties can be some of the best predictors of mechanical quality. The results from Crespo et al. (2000), Havenstein et al. (2007), and others raise the question of what specific effects ultimately lead to a bone skeletal fractures in turkeys: increases in BW alone, or a more complicated combination of factors such as muscle to fat ratios that would be influenced by genetics? It is difficult to distinguish between the 2 by measuring whole bone mechanical properties because these properties are influenced by a large number of factors such as growth rate and shape. However, by

measuring material-level properties, a more accurate indicator of bone quality can be obtained (Currey, 1970).

The purpose of the current study was to analyze the relationships between BW and the morphological, material, and compositional properties of femora in growing turkeys with widely divergent growth rates. We hypothesized that the increased loads in commercial turkey femora would be compensated for by the modulation of cross-sectional geometry and at the expense of material level properties.

MATERIALS AND METHODS

Specimens

Three turkey lines ($n = 32/\text{line}$, $n = 16$ in R-EQ) were reared at the Ohio Agricultural Research and Development Center (OARDC), Wooster, with the approval of the Institutional Animal Care and Use Committee. These were current generation commercial turkeys, a randombred control line (RBC2) that is representative of commercial turkeys of the late 1960s, and a sub-line of the RBC2 that has been continuously selected since 1969 for a single trait, BW at 16 wk (F-line; Havenstein et al., 2007). The R-EQ toms were weight matched RBC2 toms to commercial and F-line toms at 8 and 12 wk, which roughly equated to 11 and 23 wk for the RBC toms, respectively. The F-line grows at a comparable rate to commercial toms but has considerably different conformation and muscle characteristics (Updike et al., 2005). Commercial turkeys have 4 to 5% increased breast meat yield compared with the F-line and RBC2, which have similar proportions of breast meat. The differences in breast yield suggest correlated effects on conformation (i.e., posture), although it is difficult to sort out BW versus conformation effects on skeletal development (Havenstein et al., 2007). At 1 d of age, the toms from each line were divided equally among 8 replicate pens with litter floors in one room at the OARDC Poultry Research Center. Each pen contained a turkey tube feeder and Plasson turkey drinker (Plasson Company, Or-Akiva, Israel). For the entire experiment, all toms were ad libitum fed the OARDC standard turkey diets. The stocking density at the start of the experiment was 5 ft^2 (0.46 m^2) per bird. One bird per pen was randomly selected for sampling at each age with the exception of the R-EQ toms, which were selected from pens containing RBC2 toms at the appropriate BW. At the time of sampling, both femurs were collected from each turkey, and the surrounding muscle and connective tissue were subsequently removed. The left femur was selected for mechanical and geometric analysis, whereas the right femur was selected for whole bone ashing. Left femoral heads were removed with a low-speed saw (Buehler Isomet 1000, Buehler, Lake Bluff, IL) and the diaphyses were wrapped with damp tissue paper and stored in a -20°C freezer until later analysis.

Bone Ash

The right femur was analyzed for ash content at Michigan State University. The femur was cut into several pieces using an autopsy saw (Stryker, Kalamazoo, MI), wrapped in cheesecloth, and placed into a soxhlet for ether extraction. After 72 h, the femurs were removed from the soxhlet, dried, and weighed. All the extracted pieces from each femur were placed into crucibles and dried at 105°C for 24 h in an American Scientific DN-81 constant temperature oven (Portland, OR). Crucibles were hot weighed and then placed in the ash oven (Thermolyne 30400, Barnstead International, Dubuque, IA) at 600°C for 8 h. Crucibles were hot weighed again and the ash content was determined.

MicroCT Imaging

The cross-sectional images of left femurs were obtained by microCT (micro-computed tomography, SCANCO μ CT 40, Scanco USA Inc., Wayne, PA) at Purdue University. Scans were taken at the middle of the diaphysis, as well as 10 mm proximal to and 10 mm distal from the middle of the diaphysis (Figure 1A). The image matrix was 1,024 \times 1,024 pixels. Samples were scanned at 30- μ m resolution at 70-kVp beam energy and 114- μ A beam intensity with 200-ms integration time.

Geometric properties (cross-sectional area and moments of inertia) were calculated by a customized MATLAB (The MathWorks Inc., Natick, PA) based image-processing script (Akkus et al., 2004). The microCT scans were rotated such that the vertical and horizontal axes were aligned with the anterior-posterior and lateral-medial axes, respectively (Figure 2). The 8-bit grayscale images were set at a threshold level of

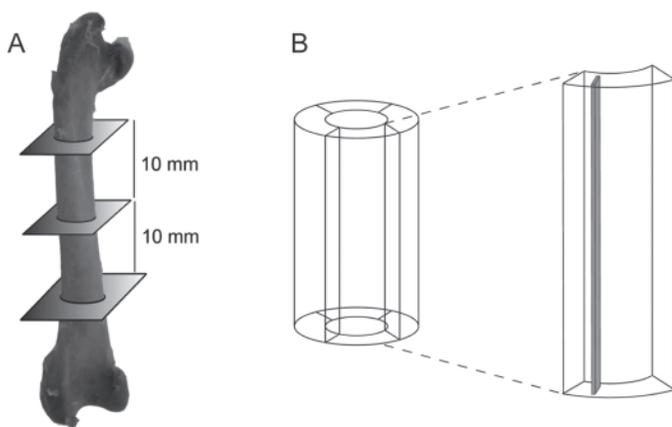


Figure 1. Placement of measurements for geometric and mechanical properties. Diagram indicating where measurements were taken with microCT for geometric properties (A) and how beams were milled for 3-point bending in calculating mechanical properties (B). In 1A, scans were taken at the center of the diaphysis, and 10 mm distal and proximal to the center. The plane represents the plane of the scan, as samples can be seen in Figure 2. In 1B, the femoral heads were removed, and the diaphysis was divided into quadrants. Beams were milled from these quadrants as shown.

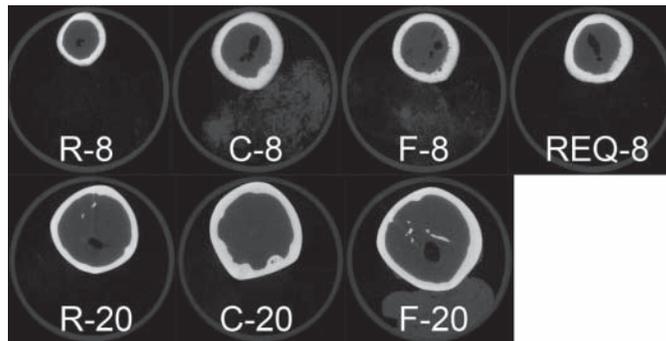


Figure 2. MicroCT slices of femurs from 3 turkey lines. Representative mid-diaphysis microCT slices of femurs from 3 turkey lines (R = randombred control line 2, C = commercial, F = F-line), at 8 and 20 wk of age, and the R-EQ line at 8 wk.

110/255 to isolate only the cortical bone. For each image, the centroid of the cross-section was calculated for the x and y axes. Lateral (*lat*)-medial (*med*), anterior (*ant*)-posterior (*post*), and polar (J) moments of inertia (I) were found with respect to the centroid with the following equations:

$$I_{xx} = I_{lat-med} = \sum [\text{pixel area} \times (\text{pixel } y)^2] - \text{area} \times y_c^2; \quad [1]$$

$$I_{yy} = I_{post-ant} = \sum [\text{pixel area} \times (\text{pixel } x)^2] - \text{area} \times x_c^2; \quad [2]$$

$$J = I_{xx} + I_{yy}, \quad [3]$$

where x and y are the respective coordinate points of the pixel in the image, x_c and y_c are the x and y centroids, respectively; pixel x and pixel y correspond to the physical x -length and y -length of the pixel sides, and pixel area corresponds to the physical area of the pixel. Physical dimensions were determined by using the microCT tube as a reference because its dimensions were known and each sample was scanned using the same tube (Figure 2).

Mechanical Testing

Mechanical testing was conducted in 3-point bending of beams cut from the femoral diaphyses (Figure 1B). The diaphyses were first cut into quadrants (anterior, lateral, posterior, and medial) with a low-speed saw (Buehler Isomet 1000) and at least 2 beam-shaped samples per quadrant were obtained by cutting along the longitudinal axis of the femur. Samples used for the 3-point bending test were approximately 25 mm in length, 2 mm in width, and 1 mm in height at the mid-point. Two samples from each turkey-line, age, and quadrant ($n = 128$) were loaded into a 3-point bending setup (TestResources 800L, TestResources Inc., Shakopee, MN) with a span length of 20 mm. Force data were obtained by a 10-lb load cell (Omega Engineering Inc.,

Stamford, CT). The force-displacement reading was obtained through Test Resources software until failure. The displacement data were recorded at the mid-span of the sample by monitoring the displacement of the mid loading point using an LVDT built in the materials testing machine. The force-displacement reading was converted into stress and strain as follows:

$$\sigma = \frac{3FL}{2bd^2}, \quad [4]$$

$$\varepsilon = \frac{6Dd}{L^2}, \quad [5]$$

where σ = stress, ε = strain, F = force, L = span length, b = width of sample, d = thickness of sample, and D = displacement.

Young's modulus was obtained as the slope of the stress-strain curve spanning the origin and half the maximum stress. The slope was obtained by linear regression. Yield stress and strain were obtained by finding the intersection of the stress-strain data and a 0.2% strain offset of the Young's modulus. Maximum stress and strain were obtained by finding the maximum stress, then obtaining the corresponding strain value. Failure stress and strain were obtained by determining the point at which a steep drop in stress occurred, and obtaining the corresponding strain. Toughness was obtained by finding the area under the stress-strain curve prior to the failure stress and strain.

Statistical Analysis

The data for each age and line were pooled, and outliers were identified and removed with the Grubb's method (ASTM, 2008). Differences in morphological and mechanical properties due to genetic line, age, and quadrant were analyzed with a general linear model multivariate ANOVA (GLM ANOVA, Minitab 16, Minitab Inc., State College, PA). Tukey's post hoc comparison was used to determine differences between the 3 turkey lines, 4 ages, and 4 quadrants. Covariance was examined with GLM ANOVA (Minitab 16) with weight as the covariant. Statistically significant differences were noted at $P < 0.05$. All values are expressed in the form mean \pm SD. Linear regression analysis was done to analyze the dependency of geometric and material level properties on BW and age. The rate of changes of geometric and mechanical properties with age and BW were determined as the slopes of the regression lines. Differences between these time rate of change in properties as represented by the slopes were compared using the linear test method (GraphPad Prism 5, GraphPad Software Inc., La Jolla, CA; Mitchell et al., 2004). In these calculations, RBC2 and R-EQ were regarded as a single group using the actual weights and ages of the R-EQ birds. Results are reported as the β coefficient, which is the ratio of the change in the dependent variable to the change in the independent variable.

RESULTS

In accordance with results reported by Updike et al. (2005), BW increased significantly with age in all of the 3 lines. The commercial and F-line toms were significantly heavier than the RBC2, whereas no significant difference was present between the commercial and F-line toms (Figure 3). The RBC2 toms were estimated to gain approximately 0.59 kg per week compared with 1.17 and 1.33 kg for the commercial and F-line toms, respectively (Figure 3; Table 1). This resulted in the commercial and F-line toms being approximately 2-fold heavier than the RBC2 at equivalent ages.

Geometric Properties

At 8 wk of age, the RBC2 femora had only 40 to 50% of the cross-sectional area observed in the commercial and F-line femora, respectively (Table 2; Figure 4A). The moments of inertia (anterior-posterior, lateral-medial, and polar) of the commercial and F-line femora were approximately 3-fold greater than the RBC2 (Table 2; Figure 4B–D). At 12 wk of age, the cross-sectional area of the RBC2 increased to 66 to 68% of the commercial and F-line femora, respectively, and these lines maintained 2.5- to 3-fold greater moments of inertia than the RBC2. The cross-sectional area of RBC2 femora stayed relatively constant at approximately 67% of commercial and F-line femora at 16 and 20 wk, whereas the commercial and F-line maintained 2-fold greater moments of inertia (Table 2; Figure 4A). When weight was regarded as the covariant, the moments of inertia were no longer significantly different between strains. Cross-sectional area was still significantly different between commercial and F-line at 8 wk,

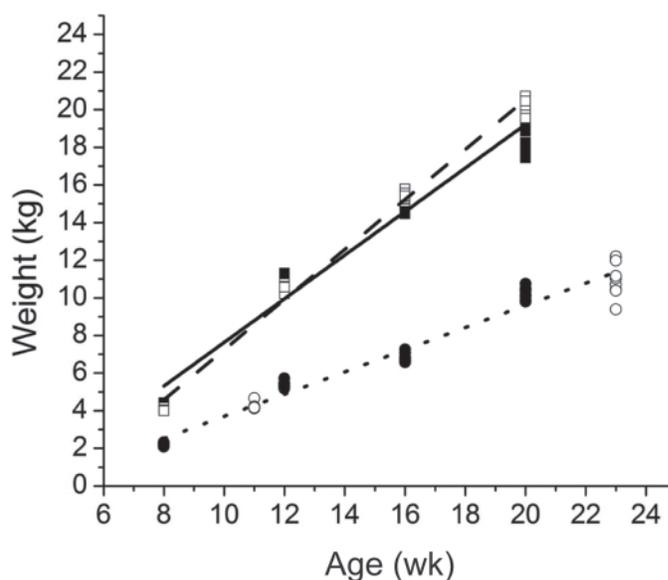


Figure 3. Trends in weight of the 3 lines over time. Body weight changes for commercial (solid square, solid line), F-line (empty square, dashed line), randombred control line 2 (filled circle, dotted line), and R-EQ (open circle) turkey lines from 8 to 20 wk of age.

Table 1. The β -coefficient of the regression analysis with age or weight as independent variables, and mechanical, morphological, and compositional properties of 3 genetic lines of turkeys¹

Dependent variable	Age (per wk)			Weight (per kg)		
	Comm	F-Line	RBC2	Comm	F-Line	RBC2
Weight (kg)	1.167 ^{bc}	1.325 ^{ac}	0.587 ^{ab}	—	—	—
Area (mm ²)	2.34 ^c	2.75 ^c	1.06 ^{ab}	2.00	2.14	2.07
Lateral-medial moment of inertia (mm ⁴)	168 ^c	210 ^c	70 ^{ab}	148	161	128
Posterior-anterior moment of inertia (mm ⁴)	202 ^c	230 ^c	79 ^{ab}	172	177	148
Polar moment of inertia (mm ⁴)	369 ^c	439 ^c	149 ^{ab}	320	338	276
Young's modulus (MPa)	196 ^b	277 ^{ac}	175 ^b	165 ^f	204 ^f	284 ^{de}
Yield stress (MPa)	2.73 ^b	4.58 ^{ac}	2.95 ^b	2.30 ^{ef}	3.40 ^{df}	5.02 ^{de}
Maximum stress (MPa)	3.79 ^b	5.55 ^{ac}	4.02 ^b	3.21 ^f	4.12 ^f	6.81 ^{de}
Failure stress (MPa)	3.17 ^b	5.64 ^{ac}	4.01 ^b	2.73 ^{ef}	4.15 ^{df}	6.83 ^{de}
Toughness (MPa-mm/mm)	0.103	0.117	0.117	0.089 ^f	0.089 ^f	0.199 ^{de}
Ash (%)	0.35	0.31	0.53	0.27 ^f	0.22 ^f	0.88 ^{de}

^aDenotes statistical difference from Comm line in rate of change per week of age ($P < 0.05$).

^bDenotes statistical difference from F-line in rate of change per week of age ($P < 0.05$).

^cDenotes statistical difference from RBC2 line in rate of change per week of age ($P < 0.05$).

^dDenotes statistical difference from Comm line in rate of change per kilogram of BW ($P < 0.05$).

^eDenotes statistical difference from F-line in rate of change per kilogram of BW ($P < 0.05$).

^fDenotes statistical difference from RBC2 line in rate of change per kilogram of BW ($P < 0.05$).

¹Values are reported in the form of rate of change per week, or rate of change per kilogram of BW. Comm = commercial; RBC2 = a randombred control line; F-line = sub-line of the RBC2 that has been continuously selected since 1969 for a single trait, BW at 16 wk.

²Units for the β -coefficient are in terms of dependent variable per independent variable.

and between RBC2 and both commercial and F-line at 16 wk.

The cross-sectional areas of the femora in all of the 3 lines started to plateau after approximately 12 wk of age (Figure 4A). The moments of inertia (Figure 4B–D) also increased during the first 12 wk of growth. Unlike the trend observed in cross-sectional area, moment of inertia values kept increasing following 12 wk of age, but at a lower rate than the increase observed before 12 wk.

The cross-sectional areas of commercial and F-line femora increased at rates of 2.3 to 2.8 mm²/wk of age, which was significantly higher than the RBC2 (1.1 mm²/wk; Table 1 and Figure 4E). The moments of inertia trends were similar, with those of the commercial and F-line femora increasing approximately 200 mm⁴/wk, a rate significantly greater than that of the RBC2 (75 mm⁴/wk; Table 1). The increases observed in the polar moments of inertia were also significantly greater in the commercial and F-line femora (400 mm⁴/wk) compared with the RBC2 (149 mm⁴/wk).

When the morphological properties were correlated with BW, the increases in the geometric properties of the femora per kilogram increase in BW were not significantly different between the genetic lines (Table 1). The cross-sectional areas of all lines increased at approximately 2 mm²/kg (Table 1 and Figure 4E). The femora from all 3 lines also had statistically similar increases in the second moment of inertia per kg BW (150 mm⁴ per kg, Table 1). The polar moment of inertia increases were about 300 mm⁴ per kg of BW and were not statistically different between lines (Table 1).

Mechanical Properties

The femora from the commercial turkeys at 8 wk of age had some superior mechanical properties com-

pared with the other 2 lines (Table 2; Figure 6A). Yield stresses of the commercial femora were significantly higher (33%) than the other lines. However, the toughness of commercial femora was only greater than that of the F-line, and there were no differences in Young's modulus between the lines. At 12 wk, the mechanical properties were similar between all 3 lines, with no significant differences present. At 16 wk, the commercial turkey femora maintained greater (20%) mechanical properties in all categories than the RBC2 femora. Only in yield stress did the commercial turkey femora have greater values than the F-line. However, at 20 wk of age, the Young's modulus and yield stress of the femora from the F-line turkeys exhibited significantly (27%) greater mechanical properties than RBC2 (Table 2; Figure 6D). Toughness was comparable among all lines of turkeys by 20 wk (Table 2). When weight was regarded as the covariant, the Young's modulus and toughness were no longer significantly different between strains. Yield stress was still significantly different between commercial and F-line at 8 wk, and between all 3 strains at 16 wk.

At equal BW, the Young's modulus, yield stress, and toughness in R-EQ turkey femora were significantly greater (15%) than the F-line (Table 2; Figure 6A-B) at 8 wk while having similar values with the commercial femora. The R-EQ femora had 15 to 25% greater mechanical properties at 8 wk of age when compared with the commercial and F-line. At 12 wk of age, R-EQ femora had significantly greater (35 to 50%) mechanical properties than both commercial and F-line femora. A graphical summary of the changes and line comparisons is shown in Figure 6.

With the exception of inertia where it was different only from RBC2, the F-line toms exhibited a significantly greater increase in the rate of change in mechan-

Table 2. Mean \pm SD of mechanical and morphological properties of the 3 turkey lines at various time points¹

Item	Line			
	Comm	F-line	RBC2	R-EQ
8 wk				
Geom				
Area (mm ²)	64.7 \pm 4.0 ^{bcd}	53.4 \pm 4.0 ^{ac}	27.0 \pm 1.9 ^{abd}	51.6 \pm 4.7 ^{ac}
LM MOI (mm ⁴)	998 \pm 213 ^{cd}	861 \pm 220 ^c	222 \pm 30 ^{abd}	733 \pm 130 ^{ac}
AP MOI (mm ⁴)	865 \pm 177 ^c	788 \pm 140 ^c	196 \pm 20 ^{abd}	754 \pm 115 ^c
PMOI (mm ⁴)	1,864 \pm 384 ^c	1,650 \pm 357 ^c	419 \pm 47 ^{abd}	1,487 \pm 228 ^c
Mech				
YM (MPa)	4,001 \pm 450	3,475 \pm 389 ^d	3,673 \pm 581 ^d	4,748 \pm 452 ^{bc}
Ysts (MPa)	59.3 \pm 6.82 ^{bc}	44.4 \pm 4.53 ^{ad}	44.4 \pm 6.6 ^{ad}	68.2 \pm 5.0 ^{bc}
Tghns (MPa-mm/mm)	1.61 \pm 0.21 ^b	1.25 \pm 0.24 ^{ad}	1.45 \pm 0.33 ^d	2.01 \pm 0.16 ^{bc}
Ash (%)	44.8 \pm 1.1 ^{bd}	42.4 \pm 1.5 ^a	43.9 \pm 0.9 ^d	41.4 \pm 1.1 ^{ac}
12 wk				
Geom				
Area (mm ²)	82.5 \pm 10.9 ^{cd}	85.2 \pm 9.53 ^{cd}	56.4 \pm 8.2 ^{ab}	65.8 \pm 8.4 ^{ab}
LM MOI (mm ⁴)	2,452 \pm 621 ^{cd}	2,674 \pm 569 ^{cd}	942 \pm 212 ^{abd}	1,591 \pm 354 ^{abc}
AP MOI (mm ⁴)	2,163 \pm 325 ^{bc}	2,738 \pm 515 ^{acd}	1,017 \pm 282 ^{abd}	1,716 \pm 278 ^{bc}
PMOI (mm ⁴)	4,614 \pm 890 ^{cd}	5,412 \pm 1,072 ^{cd}	1,959 \pm 486 ^{abd}	3,307 \pm 626 ^{abc}
Mech				
YM (MPa)	4,648 \pm 324 ^d	4,465 \pm 498 ^d	4,192 \pm 392 ^d	6,670 \pm 589 ^{abc}
Ysts (MPa)	70.7 \pm 5.06 ^d	63.2 \pm 7.8 ^d	65.1 \pm 6.2 ^d	96.3 \pm 10.0 ^{abc}
Tghns (MPa-mm/mm)	2.18 \pm 0.28 ^d	2.03 \pm 0.44 ^d	2.03 \pm 0.19 ^d	3.27 \pm 0.28 ^{abc}
Ash (%)	46.9 \pm 0.4	43.9 \pm 1.9 ^d	44.9 \pm 3.1 ^d	49.8 \pm 2.2 ^{bc}
16 wk				
Geom				
Area (mm ²)	83.0 \pm 6.4 ^c	78.7 \pm 5.8 ^c	55.6 \pm 4.8 ^{ab}	
LM MOI (mm ⁴)	2,627 \pm 467 ^c	2,840 \pm 281 ^c	1,245 \pm 226 ^{ab}	
AP MOI (mm ⁴)	2,649 \pm 577 ^c	2,833 \pm 298 ^c	1,225 \pm 129 ^{ab}	
PMOI (mm ⁴)	5,275 \pm 1,016 ^c	5,673 \pm 500 ^c	2,469 \pm 326 ^{ab}	
Mech				
YM (MPa)	5,613 \pm 574 ^c	5,164 \pm 421	4,748 \pm 365 ^a	
Ysts (MPa)	83.7 \pm 7.2 ^{bc}	72.8 \pm 3.7 ^a	63.0 \pm 11.8 ^a	
Tghns (MPa-mm/mm)	2.65 \pm 0.27 ^c	2.30 \pm 0.27	2.17 \pm 0.52 ^a	
Ash (%)	42.9 \pm 1.4 ^{bc}	39.3 \pm 2.7 ^{ac}	45.8 \pm 1.2 ^{ab}	
20 wk				
Geom				
Area (mm ²)	95.7 \pm 5.9 ^c	92.2 \pm 9.2 ^c	62.8 \pm 3.7 ^{ab}	
LM MOI (mm ⁴)	3,174 \pm 338 ^{bc}	3,602 \pm 566 ^{ac}	1,504 \pm 151 ^{ab}	
AP MOI (mm ⁴)	3,390 \pm 524 ^c	3,817 \pm 644 ^c	1,687 \pm 188 ^{ab}	
PMOI (mm ⁴)	6,565 \pm 755 ^{bc}	7,419 \pm 1,055 ^{ac}	3,191 \pm 323 ^{ab}	
Mech				
YM (MPa)	6,291 \pm 675 ^{bc}	6,970 \pm 435 ^{ac}	5,316 \pm 226 ^{ab}	
Ysts (MPa)	91.3 \pm 10.0 ^b	102.9 \pm 9.6 ^{ac}	81.5 \pm 4.0 ^b	
Tghns (MPa-mm/mm)	2.85 \pm 0.59	2.72 \pm 0.45	2.94 \pm 0.30	
Ash (%)	50.8 \pm 2.1	48.0 \pm 2.1	49.3 \pm 2.2	

^aDenotes statistical difference from Comm ($P < 0.05$).

^bDenotes statistical difference from F-line ($P < 0.05$).

^cDenotes statistical difference from RBC2 ($P < 0.05$).

^dDenotes statistical difference from R-EQ ($P < 0.05$).

¹Data are reported in the form of mean \pm SD ($n = 8$ for the groups). Geom = geometric properties; Mech = mechanical properties; Area = cross-sectional area; LM MOI = lateral-medial moment of inertia; AP MOI = anterior-posterior moment of inertia; PMOI = polar moment of inertia; YM = Young's modulus; Ysts = yield stress; Tghns = toughness. Comm = commercial; RBC2 = a randombred control line; F-line = sub-line of the RBC2 that has been continuously selected since 1969 for a single trait, BW at 16 wk.

ical properties with age compared with both the commercial and RBC2 toms (Table 1). Young's modulus increased at 277 MPa per week in F-line femora, compared with 196 and 175 MPa in the commercial and RBC2 femora, respectively (Table 1 and Figure 5A). The yield stress, maximum stress, and failure stress increased at a significantly greater percentage rate (50%) per week of age in the F-line femora compared with both the commercial and RBC2. Toughness, however, increased at statistically similar rates in all of the 3 turkey lines (Table 1).

With the exception of moments of inertia, when the increases in mechanical properties were correlated with

BW, the mechanical properties of the RBC2 femora increased at a significantly greater rate than in the commercial or F-line femora. Further, Young's modulus in the RBC2 femora also increased at a 42 and 28% faster rate per kilogram of BW compared with the commercial and F-line femora, respectively (Table 1 and Figure 5B). Moreover, the RBC2 femora had significantly greater (35 to 50%) increases in yield, maximum, and failure stress per kilogram of BW compared with the other 2 lines (Table 1). The increases in yield and failure stress per kilogram of BW were significantly greater in F-line than the commercial line. The increases in toughness of the RBC2 femora were significantly great-

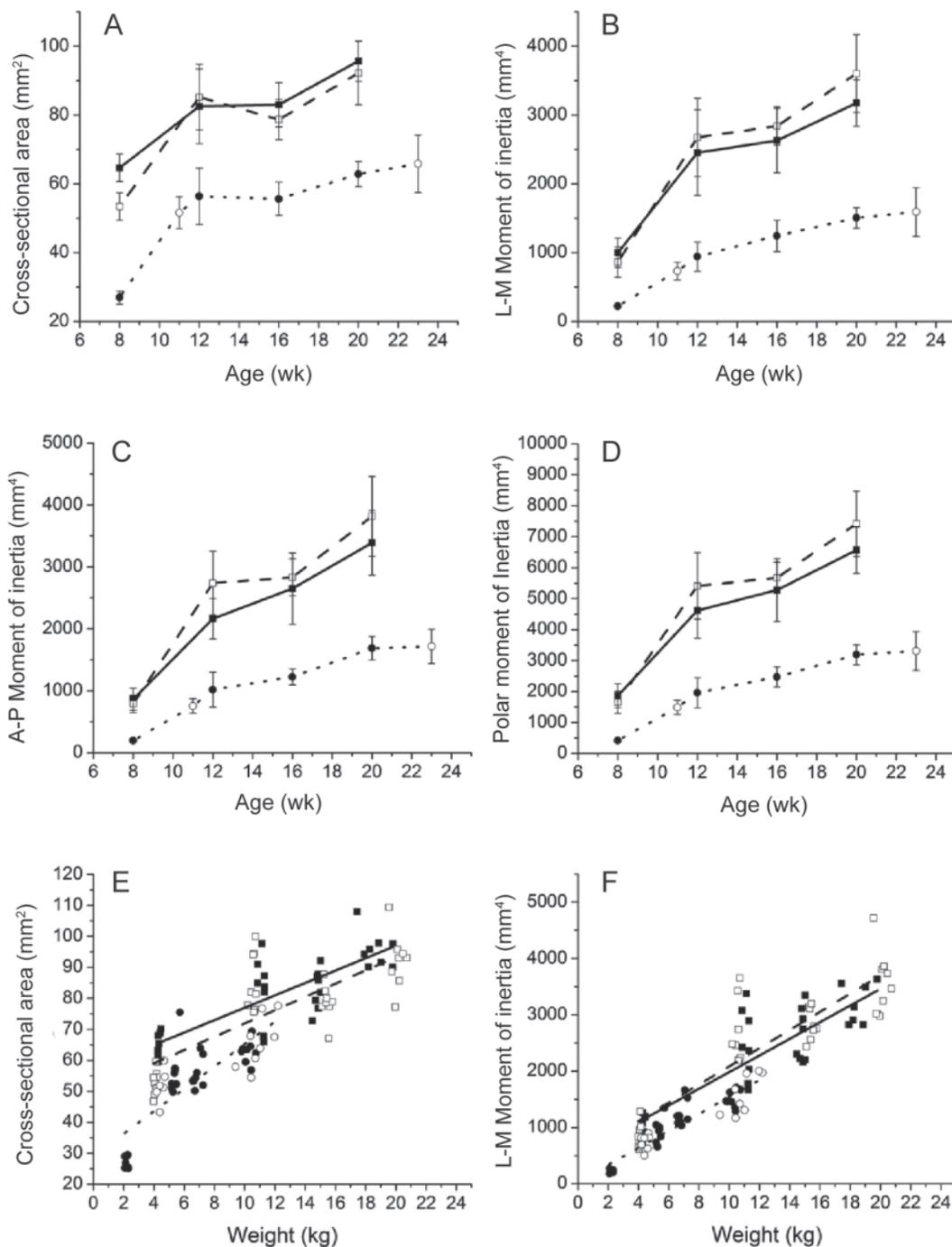


Figure 4. Trends in morphological properties over time. Cross-sectional area and moment of inertia changes for commercial (solid square, solid line), F-line (empty square, dashed line), randombred control line 2 (filled circle, dotted line), and R-EQ (open circle) turkey lines from 8 to 20 wk of age (A to D) and the corresponding weights (E, F). A-P = anterior-posterior; L-M = lateral-medial.

er per kilogram of BW compared with F-line or commercial femora (Table 1).

Bone Ash

Bone ash showed a general positive correlation with age at 8, 12, and 20 wk with the exception of values at 16 wk, which showed a decrease in ash among all strains with the exception of RBC2 (Table 2; Figure 7). Across all 3 lines, the highest values were observed at 20 wk. The R-EQ ash values at 12 wk were higher than

the commercial and F line. The RBC2 demonstrated an almost 4-fold increase in ash content per kilogram of BW (Table 1; Figure 7B). However, no differences were observed in the rate at which ash content increased per week of age (Table 1).

DISCUSSION

Studying the material level mechanical properties of bone is a useful means to assess how the bone will perform in day-to-day loading conditions (Currey, 1970;

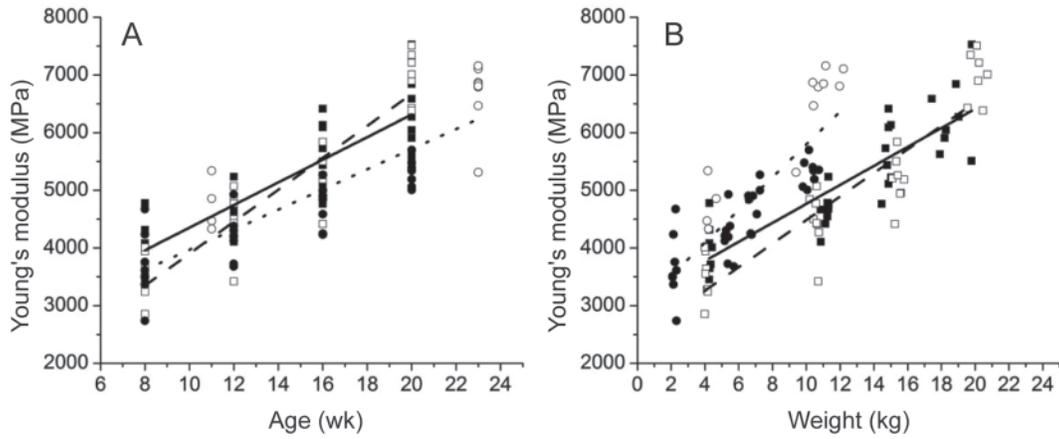


Figure 5. Trends in modulus across time and BW. Young’s modulus changes for commercial (solid square, solid line), F-line (empty square, dashed line), RBC2 (filled circle, dotted line), and R-EQ (open circle) turkey lines from 8 to 20 wk of age.

Rho et al., 1998). Quantities such as elastic modulus give an indication of the bone’s stiffness. Yield stress quantifies how much stress the bone can take before breaking. Yield strain can inform how much the bone may bend. Measurements of material-level properties, which are presented here, give a better indication of bone quality, whereas whole bone properties measure bone quality in addition to other factors such as shape

and size, which are changing throughout development (Currey, 1970).

Consistent with our hypothesis, the faster growing commercial and F-line turkeys modulated their cross-sectional geometry to compensate for increased rate of gain when comparisons are made with the RBC2 toms at similar ages. In accordance with several studies, one adaptive response to improving whole bone bending

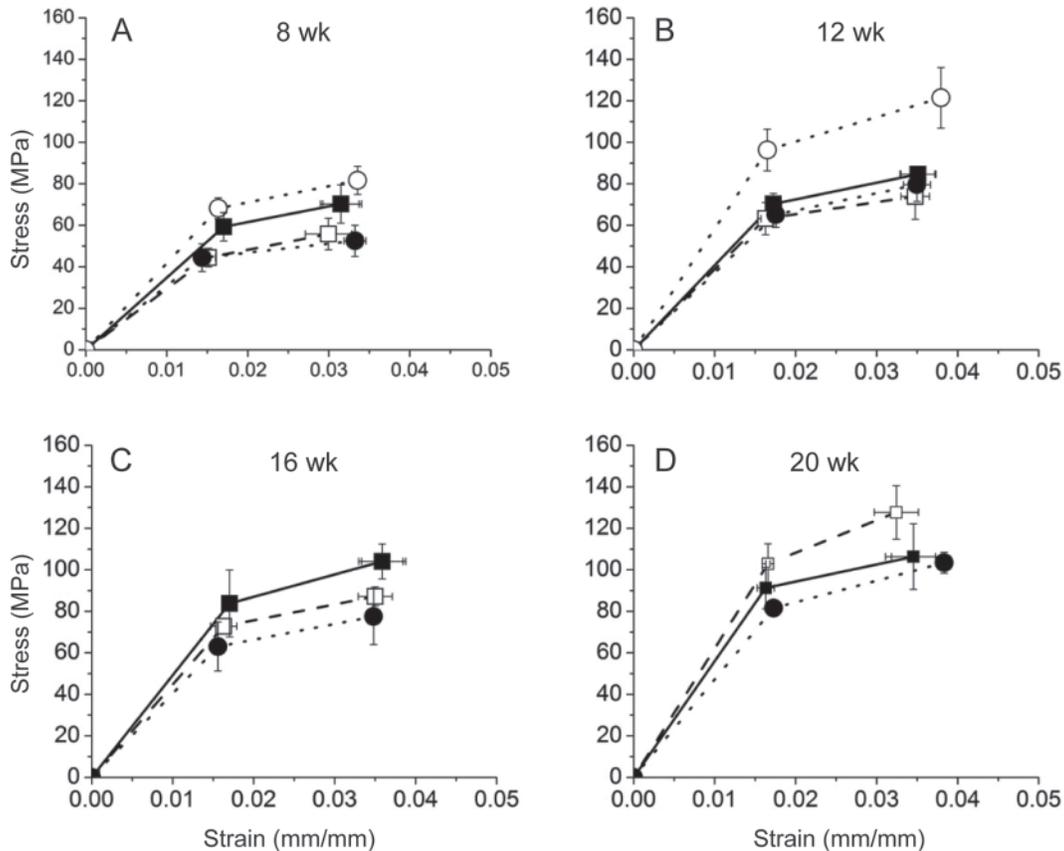


Figure 6. Summary plots of stress-strain variables for the 3 lines at 4 ages. Yield and failure stresses for commercial (solid square, solid line), F-line (empty square, dashed line), randombred control line 2 (filled circle, dotted line), and R-EQ (open circle, dotted line) turkey lines from 8 to 20 wk of age. Markers denote mean and error bars denote SD (n = 8 for groups).

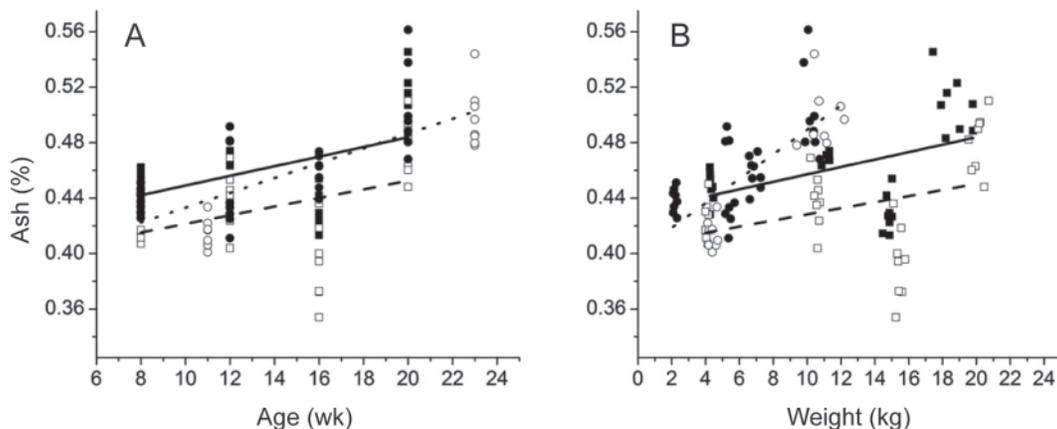


Figure 7. Trends in ash content over time and BW. Ash content changes for commercial (solid square, solid line), F-line (empty square, dashed line), randombred control line 2 (filled circle, dotted line), and R-EQ (open circle) turkey lines from 8 to 20 wk of age.

strength is to increase cross-sectional area (Akhter et al., 1998), as was observed in the significantly larger cross-sectional areas in the commercial and F-line toms at equivalent ages (Crespo et al., 2000). Whereas the variations of geometric properties at a given age were substantial between different strains, this variation between genetic strains diminished when data were observed at a similar BW. This suggests that the size of bone is primarily influenced by BW. The physiological forces, a direct function of BW, are the primary factors associated with the changes in the geometry of the turkey femur. The sizes of femurs from the 2 heaviest turkey lines were much larger than the RBC2 control at equivalent ages. This can be explained by Frost's mechanostat theory, which foresees increased bone formation with increasing strain. The femurs of the heavier toms would be more likely to surpass the typical peak strain threshold to initiate bone modeling (Frost, 2003). It is also possible that turkeys of the commercial and F-line variety may have surpassed the operational microdamage threshold noted by Martin (2000) and Frost (2003), yet this assertion remains to be confirmed with microdamage analysis.

Unlike the geometric properties, which differed substantially between different strains at any given age, Young's modulus did not differ as notably between different strains at any given age (e.g., compare Figure 4A with Figure 5A). This observation implies that matrix modulus is a parameter that is dependent on time. The overall maturation of the matrix involves gradual accretion of mineralization in the collagen framework (as well as maturation of collagen crosslinks), and it is apparent that the time rate at which the matrix matures does not differ between the turkey strains included in this study. When the variation of modulus between different strains at a given BW is observed (Figure 5B), it is seen that the modulus does not seem to vary. The only exception to this is RBC2 12-wk BW-equivalent group, which had a significantly greater modulus than the other 2 strains. However, it took 11 and 23 wk for the RBC strain to reach a BW that is equivalent to 8 and 12 wk BW of F-line or commercial strains,

respectively. The greater mechanical properties for the femora of the R-EQ group of 23 wk of age, as compared with the RBC2 line at 12 wk of age, demonstrates the important role of time-dependent processes of matrix maturation. Therefore, at least in these turkey strains, time-dependent matrix maturation determines material-level mechanical properties as opposed to genetic factors.

When comparisons of mechanical properties are made at similar BW, the R-EQ femurs had greater mechanical strength than other lines. It was also observed that R-EQ had greater ash content, which may explain enhancement of strength. As indicated, weight-matched R-EQ birds were older than their counterparts; therefore, the bone matrix in R-EQ was more mature. This extra time may have resulted in increase in the mineral amount and matrix maturation, resulting in greater ash content. This is in accordance with a study done by Tommasini et al. (2008), who indicated that bone adaptation may also occur by variation of bone compositional properties. Similarly, these data suggest that mineralization differences contribute to bone strength; however, accretion of mineralization takes time. Therefore, modulation of mineralization may not meet acute demands associated with an expedited increase in BW. It is also important to note that the modeling in RBC2 birds occurs at a lower rate (as reflected by amount of increase in geometric properties per week measured in Table 1), implying that formation and resorption is occurring at a lower pace than the other strains. Therefore, the rate of modeling in response to the rate of change in BW is potentially determining the matrix mechanics by way of affecting matrix mineralization. Our observations suggest that insufficient time provided for matrix maturation may be playing a role in the reported fractures of fast-growing turkeys by others (Crespo et al., 1999) such that the stresses placed on the femur in commercial toms exceeds the yield strength of the bone (Frost, 2003).

One observation in all 3 lines was the dichotomy between the age-related changes in geometrical and mechanical properties. The change in geometric prop-

erties appears to be bilinear, with the rate of change decreasing at 12 wk of age such that geometric properties begin to plateau or increase at a lesser rate (Figure 4a to d). This corresponds to the data of Turner and Lilburn (1992), who showed that linear skeletal growth begins to plateau at approximately 12 to 14 wk. However, the observed mechanical properties increase at a constant, linear rate across the entire time frame of the study, similar to the monotonous increase in the BW, with these trends apparently independent of genetic line. This suggests that even after the skeletal maturation is attained, modulation of mechanical strength is possible. This is consistent with previous studies that analyze ontogenetic patterns of limb loading and its relation to geometry changes (Main and Biewener, 2004; Main and Biewener, 2007).

From a biomechanical perspective, the axial loads, the bending loads, and the torsional loads are born by the cross-sectional area, moment of inertia, and polar moment of inertia, respectively. It was observed that moment of inertia and polar moment of inertia kept increasing monotonously over time, whereas the cross-sectional area remained stagnant after 12 wk (Figure 4). The differences in trend may imply that when compared with axial loading, the bending and torsional loads become more dominant over age and as the bones grow larger in size.

While the results imply bone modeling dynamics (i.e., bone resorption and bone formation) as a factor that determines the geometry and matrix mechanics, we have not measured bone resorption or bone formation in this study. However, the increasing BW are strongly associated with geometric variation and mechanical properties of the bone matrix. Further examination of the interplay of these underlying mechanisms in animal models and how they affect formation and resorption processes may lead to a more thorough understanding of how mechanical competence of bones develop or deteriorate in the skeletal envelope.

ACKNOWLEDGMENTS

This project was supported by the Midwest Poultry Research Program (grant #103648, St. Paul, MN).

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