

Research Note: Bone ash from immature broilers correlates to bone mineral content calculated from quantitative computed tomography scans

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ABSTRACT Owing to selection for rapid growth and heavy muscle, bone deformities and injuries in broiler chickens are common and present a welfare concern. Recently, clinical quantitative computed tomography scans (QCTs) have been used for laying hens with significant, strong correlations between QCT-generated bone mineral content (BMC), bone ash, and analytical calcium. The objectives of this study were to determine if QCT-generated bone mineral density of tibias and femurs correlated to fat-free ash and if analytical Ca could be correlated to bone ash and digitally calculated density in immature broilers. Male broilers (Ross 708, n = 125) were raised from day 1 after hatching, and at 42 D, right leg quarters were collected from 50 randomly selected birds and frozen at -20°C until analysis. Leg quarters were scanned with muscle

and feathers intact and arranged in rows on plexiglass, and each QCT included a solid Ca hydroxyapatite phantom. Tibias and femurs were removed from leg quarters after autoclaving, ashed, and weighed. Pearson's correlation analysis was conducted to understand the association between analytical bone ash and QCT BMC while paired *t* tests determined the amount of difference between QCT BMC and ash. Ash weight was strongly correlated to QCT BMC in both the femur ($R = 0.86$, $P < 0.001$) and the tibia ($R = 0.91$, $P < 0.001$). The average difference between the amount of actual ash weighed and BMC calculated from the QCT was 0.03 ± 0.22 g ($P = 0.3$) for the femur and 0.04 ± 0.22 g ($P = 0.2$) for the tibia. This study confirms that this technique can supply invaluable skeletal health information without sacrificing birds.

Key words: broiler, bone density, computed tomography

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INTRODUCTION

Owing to selection for rapid growth and heavy muscle, bone deformities and injuries in broiler chickens are common (Julian, 1998). These injuries present both a welfare concern from the pain they cause and usually an economic loss as injured birds typically underperform in bodyweight gains and must be removed from the flock (Bessei, 2006). Many interventions have been proposed to address these issues, ranging from nutritional (Shim et al., 2012) to environmental (Lewis et al., 2009). Unfortunately, these studies have measured bone ash and morphology after processing and have not used methods on living animals. While broilers typically do not have a long lifespan, measuring these variables at certain stages

of growth on representative birds may determine if and when an intervention may be needed.

Recently, clinical quantitative computed tomography scans (QCTs) have been used for laying hens in longitudinal studies (Chargo et al., 2019; Robison and Karcher, 2019). These scans resulted in significant, strong correlations between QCT-generated bone mineral content (BMC), bone ash, and analytical calcium (Robison and Karcher, 2019), providing evidence for reliable use and accuracy in poultry. Researchers preferred clinical QCT over traditional dual-energy x-ray absorptiometry or peripheral QCT because of reduced need for sedation, shortened scan time, and larger number of birds that can be scanned at once (Robison and Karcher, 2019). In addition, three-dimensional image rendering from clinical QCTs is more reliable at detecting keel bone damage than traditional hand palpation in living animals (Chargo et al., 2019). However, none of these previous studies have examined broilers and the potential for clinical QCT to quantify skeletal weakness or abnormalities in these birds.

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Broilers present new challenges for this particular technique. As these birds are still young and growing, their bones have not undergone complete mineralization. Although these birds are also much larger than a typical laying hen, the cartilaginous portion of the tibias and femurs could present problems for QCT analysis which relies more heavily on mineralized bone to analyze morphology and density. However, as this technique provides skeletal health information without sacrificing birds, these issues need to be explored and addressed. The goal of the present study was to validate these previously used methods in laying hens for broilers and provide an additional tool for future research. The objective was to determine if QCT-generated bone mineral density of tibias and femurs correlated to fat-free ash as a measure of total mineral content in immature broilers.

MATERIALS AND METHODS

All procedures were approved by the Michigan State University Animal Care and Use Committee as part of a larger study (PROTO201800040). Male broilers ($n = 125$; Ross 708, Aviagen, Huntsville, AL) were raised from day 1 after hatching. Initially, broilers were divided into 2 pens of 62 and 63 chicks for brooding, but after the first week, chicks were divided randomly into 5 pens at 25 birds/pen. The photoperiod was stepped down from 24 h to 20 h over the course of the first 7 D on study and maintained at 20 h for the remainder of the study in accordance with the Ross Broiler Management Handbook. Chicks had ad libitum access to water and a commercially available starter-grower feed (Kent Nutrition Inc., Muscatine, IA) at all ages. At 42 D, right leg quarters were collected from 50 randomly selected birds and frozen at -20°C until analysis.

Computed Tomography Scans

Clinical QCT scans and analysis were conducted according to the study by Robison and Karcher (2019) with the settings of 120 kV, 320 mAmp, and 0.625 mm slices. Briefly, leg quarters were thawed in a chiller for 24 h before scanning. Legs were scanned with muscle and feathers intact and arranged in rows on plexiglass, and each scan included a solid calcium hydroxyapatite phantom (Image Analysis, Columbia, KY) of 0, 75, and 150 mg/cm^3 Ca. A DICOM of each row of leg quarters with an 11-cm field of view and bone algorithm was generated using Imageworks (General Electric Healthcare, Princeton, NJ) and imported for analysis into Mimics (Materialise, Plymouth, MI). The threshold for Hounsfield units (HU) was determined by applying a range from 200 to 600 HU with differences among thresholds of 25 HU based on thresholds used in the study by Robison and Karcher (2019). Appropriate thresholds were set to 275 HU and 225 HU at the tibia and femur, respectively. After applying thresholds, whole bone volume and average HU were recorded for QCT BMC calculations. To determine QCT BMC,

average HU for each step of the Ca hydroxyapatite phantom was plotted against the known densities of the phantom to generate a standard curve. The following regression equation generated was used to calculate density in $\text{mg Ca hydroxyapatite}/\text{cm}^3$: $y = 0.7589x - 4.646$, $R^2 = 0.99$, where y is density in $\text{mg Ca hydroxyapatite}/\text{cm}^3$ and x is HU. To calculate QCT BMC from CT scans, bone volume was multiplied by the density generated from the regression equation.

Bone Ash

Leg quarters were autoclaved (733HCMC; Gentige, Wayne, NJ) at 121°C for 25 min in a method described by Cloft et al. (2018). After autoclaving, tissue and skin were removed, and tibias and femurs were separated. Each bone was cut into thirds, wrapped in cheesecloth, and placed into a modified soxhlet for ether-extraction for 12 to 24 h, after which they were dried at ambient temperature in a hood for 24 h and weighed. After ether extraction, bones were placed into crucibles and further dried in a DN-81 constant-temperature oven (American Scientific, Portland, OR) at 105°C for 24 h. Dry bone weights were obtained after this period, and crucibles containing bones were placed in an ash oven (Thermolyne 30,400; Barnstead International, Dubuque, IA) overnight at 600°C . Ash was allowed to cool and weighed.

Statistics

Pearson's correlation analysis was conducted to understand the association between analytical bone ash and QCT BMC. Pearson's correlations coefficients (R) between measurements are presented and were calculated using the CORRELATION procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC). Linear regression analysis was completed using the REGRESSION procedure to examine the relationship between analytical bone mineral and QCT BMC. Paired t tests were used to determine the amount of difference between the measurements of bone mineral calculated analytically vs. digitally using the TTEST procedure.

RESULTS AND DISCUSSION

Mean ash weights and percentages were 2.53 ± 0.32 g and $46.7 \pm 0.2\%$ for femurs and 3.62 ± 0.47 g and $49.0 \pm 0.2\%$ for tibias, respectively (Table 1). Ash weights and ash percentages were larger than those reported from both the slow- and fast-growing strains in the study by Shim et al. (2012), likely due to BW differences between the 2 studies. Broilers in the present study weighed 3.37 ± 0.05 kg at 6 wk while Shim et al. (2012) reported BWs of 1.4 and 1.9 kg for different growth-rate populations at 6 wk. Once corrected for BW, femur ash was 0.75 ± 0.01 g/kg BW and tibia ash was 1.08 ± 0.94 g/kg BW which was still larger than the 0.07 ± 0.01 g/kg BW previously reported (Shim et al., 2012). However, ash percentages were similar to those

Table 1. Mean \pm SE of bone mineral content as generated by clinical quantitative computed tomography (QCT BMC), ash, ash percentage, analytical calcium, and percentage of calcium in ash in the femurs and tibias of 42-day-old broilers.

| Bone | Femur | Tibia |
|-----------------------------|-----------------|-----------------|
| N | 48 | 48 |
| QCT BMC (g) | 2.50 \pm 0.39 | 3.58 \pm 0.50 |
| QCT BMC (g/kg BW) | 0.75 \pm 0.01 | 1.07 \pm 0.02 |
| Ash (g) | 2.53 \pm 0.32 | 3.62 \pm 0.47 |
| Ash (g/kg BW) | 0.75 \pm 0.01 | 1.08 \pm 0.01 |
| % Ash | 46.7 \pm 0.2 | 49.0 \pm 0.2 |
| <i>P</i> value ¹ | 0.3 | 0.2 |

¹*P* values were generated from paired *t* tests comparing QCT BMC and ash.

reported for Ross 308 broilers in the study by Lewis et al. (2009), which ranged from 47 to 50% for birds raised in a similar photoperiod. These results likely indicate differences in breeding genetics that influence growth and mineral accrual in Ross broilers as compared to random-bred broilers.

Ash weight was strongly correlated to QCT BMC in both the femur ($R = 0.86$, $P < 0.001$) and the tibia ($R = 0.91$, $P < 0.001$). The average difference between amount of actual ash weighed and BMC calculated from the QCT was 0.03 ± 0.22 g ($P = 0.3$) for the femur and 0.04 ± 0.22 g ($P = 0.2$) for the tibia. These correlations were not as strong as mature laying hens ($R = 0.97$ for femurs and $R = 0.94$ for tibias, Robison and Karcher, 2019) likely due to differences between birds' ages and size. Laying hens used in the previous study were 85 wk old, whereas broilers in the present study were only 42 D old. At this age, broilers' bones are not as mineralized as more mature birds to allow for long bone development, making for less bone mineral overall and estimates generated by QCT BMC slightly less accurate compared to use in laying hens. However, the average ash weights of this study were nearly double the ash weights of the previous study using this method because of the larger tibias in broilers. These large weights and younger birds could have contributed to greater variation, but ultimately, these results follow similar trends as in laying hens and provide support for the use of QCT in broiler research.

Regression equations for QCT BMC to bone ash of femurs and tibias (Table 2) were significant ($P < 0.001$ for all) but did not produce similar R^2 s as in the study by Robison and Karcher (2019). Previous R^2 s ranged from 0.88 to 0.95 for whole bone tibia and femur, respectively,

Table 2. Regression equations of bone mineral content calculated from clinical quantitative computed tomography (QCT BMC) to bone ash in the femurs and tibias of 42-day-old broilers where *y* is amount of bone ash and *x* is QCT BMC.

| Bone | N | Unit | R^2 | Equation |
|-------|----|---------|-------|--------------------|
| Femur | 48 | g | 0.73 | $y = 0.70x + 0.79$ |
| | | g/kg BW | 0.59 | $y = 0.55x + 0.34$ |
| Tibia | 48 | g | 0.80 | $y = 0.83x + 0.63$ |
| | | g/kg BW | 0.69 | $y = 0.64x + 0.39$ |

while R^2 s from the present study for the same bones were 0.80 and 0.73, respectively. Ash and breaking strength can increase based on increases in BW of broilers (Lewis et al., 2009; Shim et al., 2012), so the range of BW in the present study (2.27 to 3.86 kg) could explain these differences in R^2 s. Mean BW in the present study was larger than mean BW of 1.71 ± 0.03 kg used in the previous laying hen study (Robison and Karcher, personal communication), indicating greater potential for variation when examining ash. In addition, fast growth rates will increase porosity and decrease ash content (Williams et al., 2004), and broilers' fast growth to larger sizes could have led to the greater variation in the present study than in the previous study in laying hens. When the linear regressions include body weight as a variable, R^2 s increase to 0.88 and 0.79 for the tibia and femur, respectively ($P < 0.001$ for both). Regression equations based on BW-standardized QCT BMC and ash indicate less accurate predictions by the model, likely due to the birds' age, BW, or growth rate as noted previously.

Clinical QCT provides a noninvasive tool to assess bone quality and problems in longitudinal studies. While useful at assessing laying hen keel damage and BMC, it may not be as accurate in broilers whose bones have not fully mineralized. This study shows that QCT BMC and ash are similar, but predicting ash from QCT BMC in broilers may not be as reliable as in mature laying hens. However, this study validates previous research and confirms that this technique can still supply invaluable skeletal health information without sacrificing birds, as long as results are interpreted with appropriate caution.

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