Associations among computed tomographic measures of bone and muscle quality and biomechanical measures of tibiotarsal bone quality in laying hens

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OBJECTIVE
The objectives of the current study were to quantify laying hen sternal carina (keel) and tibiotarsal bone and muscle quality using clinical CT, tissue level, and biomechanical measures; test associations among muscle transverse sectional area, bone mineral density, and biomechanical measures of bone quality; and determine whether CT measures of bone and muscle quality would be predictive of biomechanical measures of tibiotarsal bone quality.

ANIMALS
60 40-week-old Hy-Line brown laying hens were used.

METHODS
Associations among CT imaging, tissue level, and biomechanical measures of tibiotarsal and keel bone and muscle quality were tested using multivariate correlational analyses. Bivariate and generalized regressions were performed to determine whether CT measures were predictive of biomechanical measures of tibiotarsal bone quality.

RESULTS
Low positive correlations were identified between tibiotarsal muscle transverse-sectional area (cross-sectional area [CSA]) and bone mineral density (BMD) in the proximal location of the bone (r = −0.11 to 0.31). Tibiotarsal muscle CSA was also low to moderately correlated with biomechanical measures of bone quality (r = 0.20 to 0.41). Keel muscle CSA values were not correlated with keel BMD values, but they were correlated with biomechanical measures of tibiotarsal bone quality (r = 0.18 to 0.40). Keel CT measures of bone quality were not correlated with tibiotarsal CT measures of bone quality. At the proximal location, muscle CSA and tibiotarsal BMD were predictive of biomechanical failure load (F = 9.68, P = .0003[keel muscle CSA]; F = 9.13, P = .004[tibiotarsal BMD]).

CLINICAL RELEVANCE
Findings supported using noninvasive CT measures of muscle and bone quality in longitudinal research studies evaluating the effects of interventions on laying hen welfare.

Keywords: osteoporosis, keel bone damage, computed tomography, poultry, biomechanics

Laying hens are increasingly being kept in backyard flocks, and there is also a growing consumer demand for improved welfare practices in commercial operations.1,2 Poor bone quality is a major welfare concern because it places laying hens at an increased risk for sustaining bone damage, with the sternal carina (keel) and tibiotarsal bones being common sites.3 Fractures and bone margin deviations secondary to low bone mineral density (BMD; osteoporosis) can cause pain, stress, increased susceptibility to disease, reduced activity, and impaired respiratory function in affected birds.4 Bone quality is therefore an important measure used by researchers, veterinarians, commercial producers, and backyard flock owners for assessing laying hen welfare.5

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Computed tomography is a noninvasive technique that is commonly used to quantitatively assess bone quality in poultry. Previously reported CT measures of bone quality include BMD of the sternum,14 transverse sectional area (cross-sectional area [CSA]) of the keel and tibiotarsus, cortical thickness, and angulation (deviation) of the ventral margin of the keel.2 Keel and tibiotarsal BMD values have been reported for total, medullary, and cortical bone locations.9–11,17–19 Values have also been reported for the following anatomic locations: proximal, middle, and distal tibiotarsal diaphysis and the distal epiphysis.15,17,38,20

Biomechanical assessments of the tibiotarsus are another measure of bone quality in poultry research.21 The 3-point bending test has been commonly used to quantify breaking strength and other values for the tibiotarsus. This test involves applying a uniaxial force to a specimen until it breaks to assess the force required to induce structural failure.22 Experimental parameters such as the crosshead speed and support width have varied among previous reports; however, most have described the application of the load in a cranial-caudal direction with the load being applied at the midpoint of the bone.17–19 At –20 °F for further testing. Based on our review of the literature, no published studies have reported associations between CT and biomechanical measures of tibiotarsal bone quality in laying hens.

In humans, measures of both bone and muscle quality are of interest because osteoporosis and decreased muscle size (sarcopenia) can occur concurrently (collectively termed osteosarcopenia).24 Associations between bone and muscle quality have been reported, with Rantalainen et al25 demonstrating a positive correlation between tibial bone traits and the mid tibial muscle area in older men ($r = 0.35$ to 0.37). Computed tomography was used in this previous report to quantify mid tibial BMD and CSA of the mid tibial muscle mass. Published assessments of muscle quality in poultry have generally been done from a consumer standpoint, evaluating variables, whole bird CT scans were acquired using a head 0- to 10-kg, 0.5-mm protocol, CT scan had an applied FC31 reconstruction filter, and all relevant anatomical structures were visible in the CT images.

Computed tomography scans not adhering to the inclusion criteria were excluded. Final decisions for inclusion and exclusion were made based on a consensus among a graduate student (CH), an American College of Veterinary Radiology-certified veterinarian radiologist (JJ), and a veterinarian specializing in laying hen behavior and welfare (AA).

**Methods**

**Selection and description of subjects**

This study was a prospective, descriptive, observational design. Sixty, 40-week-old Hy-Line brown laying hens used for another study were used with investigator permission. All research protocols for the prospective study had been approved by Clemson University’s Institutional Animal Care and Use Committee (protocol No. AUP2021-0068) before the start of data collection. Birds for the current study were randomly selected, euthanized using carbon dioxide ($CO_2$), placed on ice, and immediately transported for CT scanning. Inclusion criteria for the current study were as follows: laying hens were 40 weeks of age and had unique identifiers, whole bird CT scans were acquired using a head 0- to 10-kg, 0.5-mm protocol, CT scan had an applied FC31 reconstruction filter, and all relevant anatomical structures were visible in the CT images.

Computed tomography scans not adhering to the inclusion criteria were excluded. Final decisions for inclusion and exclusion were made based on a consensus among a graduate student (CH), an American College of Veterinary Radiology-certified veterinarian radiologist (JJ), and a veterinarian specializing in laying hen behavior and welfare (AA).

**CT acquisition and image analysis protocols**

Computed tomography scanning was performed by staff at the Clemson University Godley-Snell Animal Research Center, in consultation with the veterinary radiologist (JJ). Images were acquired using a 16-slice clinical CT scanner (Toshiba Aquilion TSX-101A; GE Healthcare). The birds were positioned in a dorsal recumbent position inside of a cradle atop a hydroxyapatite (HA) calibration phantom (QRM Quality Assurance in Radiology and Medicine). The head and legs were simultaneously extended in opposite directions, and the birds were taped to the phantom to maintain this position during image acquisition (Figure 1). Computed tomographic images were acquired using a helical, head 0- to 10-kg, 0.5-mm protocol, and an FC31 reconstruction filter was applied after image acquisition. After scanning, the birds were then dissected and frozen at –20 °F for further testing.

All CT image analyses were performed by the graduate student in consultation with the veterinary radiologist, using a standardized protocol and open-source image analysis software (Horos v3.3.6, https://horosproject.org/; Mac OS High Sierra, 10.13.6, MacPro Quad Core, Apple, Inc). The standardized protocol for recording CT measures of muscle and bone quality, biomechanical measures of bone quality, and tissue measures of cortical thickness, muscle volume, and muscle weight. Additionally, we hypothesized that CT measures of bone and muscle quality could be used as predictors of biomechanical measures of tibiotarsal bone quality.
The protocol for recording measures of keel bone and muscle quality included the same steps listed for the tibiotarsus. A more detailed description of the image analysis protocol is provided elsewhere (Supplementary Material S1), and summaries of the protocols for each category are provided below.

**Tibiotarsal image analysis protocol**

In the sagittal view of the multiplanar reformatting (MPR) feature of Horos, the tibiotarsus was divided into 4 equal segments using the length tool. Four lines of equal length were placed along the length of the bone from the proximal to distal epiphyseal heads. Lines perpendicular to those previously drawn were placed at the intersections of the original 4 lines. These perpendicular lines marked the proximal, middle, and distal tomographic slices to be evaluated. In the transverse MPR window, the slices corresponding with the proximal, middle, and distal locations specified in the sagittal MPR window were assessed. With the use of the pencil tool, regions of interest (ROIs) were drawn around the cortical and medullary bone margins using a CT bone window level/window width (WL/WW) combination of 300/1,500. The applied reconstruction filter resulted in black margins appearing around the cortical and medullary bone. Regions of interest were placed around the outer cortical margin and the outer margin of the medullary bone. The WL/WW setting was adjusted to the Abdominal preset of 40/350, and an ROI was drawn around the muscle group surrounding the tibiotarsus in the corresponding slice. Density, area, and standard deviation values generated by the software were recorded in spreadsheet software (Excel; Microsoft Corp). Three known values of HA in a phantom were recorded using the oval tool, and a calibration curve was generated, plotting the average HU against the known HA concentration (0, 100, and 200 g X cm²). A linear trendline was displayed, and using the line equation in slope intercept form, the density values for total and medullary bone were converted to BMD. In the equation: $y = mx + b$, the software-generated density value is the “$y$” value. Additionally, in the sagittal view, the density of the distal epiphyseal head was recorded using the pencil tool. This measure was adapted from the methods of Shipov et al.20

**Keel bone and muscle image analysis protocol**

As performed in the tibiotarsal image analysis protocol, the keel bone was divided into 4 sections of equal size with the length tool in which the intersection of each line denoted the proximal, middle, and distal locations to be evaluated in the transverse MPR view. ROIs were drawn around the cortical and medullary keel bone using the bone WL/WW preset, and an ROI was drawn around the muscle mass surrounding the keel using the abdominal WL/WW preset. Hydroxyapatite phantom values were recorded, and a calibration curve was used to convert HU values to BMD values.

**Biomechanical testing**

Mechanical properties of the right tibiotarsi were assessed using a 3-point bending test.22 The testing was performed using an Instron materials tester (Model 5944; Instron Corp) equipped with a 500-N load cell. Before testing, the frozen legs were thawed at room temperature. Then, CH carefully dissected the muscles surrounding the tibiotarsus, ensuring the dissection was performed in the same manner for each leg in separating the muscles of the tibiotarsus from the muscles of the femur and avoiding damaging the bone. Muscles were preserved for volumetry measurements (details below). Rounded support pins and breaking blades were manufactured based on the American National Standards Institute/American Society of Agricultural Engineers S459 MAR1992 (R2017) standards for the application of 3-point bending on animal bones.22 A furculum width of 4 cm was used. This width did not adhere to the American National Standards Institute standards but was decided on based on a consensus among graduate students CH and GA, JJ, and AA. Due to the anatomy of the laying hen tibiotarsus, a 4-cm width ensured that the tibiotarsus was able to rest on the furculum in a way the load would be applied to the midpoint of the bone evenly in the craniocaudal plane. More support pins and breaking blades were manufactured to avoid damaging the bone. The crosshead speed used was 3 mm/min, and the test was carried out to failure. Load and displacement data were collected and were used to obtain the ultimate load (N), failure load (N), yield point (N), stiffness (N/mm), maximum bending moment (Nm), and stress (N/mm²). The cortical thickness of the tibiotarsus was recorded after the
bone was successfully fractured at the location of the fracture using digital calipers. This measurement was made at the cranial surface of the bone.

**Tissue level muscle and bone quality measures**

The muscles surrounding the tibiotarsus were weighted, and volumetry was performed to determine the muscle volume. To determine the volume of the muscle mass, water was added to a graduated cylinder, and the starting volume was recorded. After, the muscles were placed into the graduated cylinder. The resulting volume was recorded, and the difference between the starting and final volume was recorded. The tibiotarsal length and diameter at the midpoint were recorded, and the bones were then wrapped in saline-soaked paper towels until testing.

**Statistical analysis**

Statistical analyses were performed by the graduate student (CH), in collaboration with a statistician (WB), and all statistical calculations were performed using JMP Pro 17 (SAS Institute Inc). The first stage of the statistical analysis to establish associations among the bone, muscle, tissue, and biomechanical measures was a correlation analysis. The correlations were grouped by the location (proximal/middle/distal). The magnitude of the estimated correlation coefficients and the $P$ values were used to determine the significance of the associations between the measures. Correlation estimates (denoted $r$) were classified as weak, moderate, or strong based on the following levels: weak was $r < .39$, moderate was $0.40 < r < 0.79$, and strong was $r > 0.80$; and $P < .05$ was considered evidence of statistical significance.

The second stage of the statistical analysis involved a series of simple- and multiple-regression analyses to assess the ability of individual and combined imaging-based measures of bone and muscle quality to predict biomechanical measures. The regression models were estimated, and then ANOVA was used to calculate $F$ statistics and corresponding $P$ values for the bone and muscle measures in the models. Once again, $P < .05$ was considered evidence of statistical significance, and the bone and muscle measures were useful in predicting biomechanical measures.

**Results**

Of the 60 birds scanned, 54 met the inclusion criteria. Six were excluded because a computer malfunction occurred during image reconstruction and images meeting the required technical parameters were not available. On average, the time required to complete all CT image analyses was 1 hour/bird.

**Correlational analyses**

**Muscle CSA X imaging, tissue level, and biomechanical measures of quality**—Significant positive correlations between proximal tibiotarsal muscle CSA, and the following variables were identified: total tibiotarsal BMD ($r = 0.29, P = .04$), cortical tibiotarsus BMD ($r = 0.3132, P = .02$), tibiotarsal muscle group weight ($r = 0.71, P < .0005$), muscle group volume ($r = 0.39, P = .005$), ultimate load ($r = 0.41, P = .003$), failure load ($r = 0.41, P = .003$), yield load ($r = 0.39, P = .006$), and stiffness ($r = 0.42, P = .003$; Supplementary Table S1). No other significant correlations were found at the proximal tibiotarsal level.

Significant, low, negative correlations between distal tibiotarsal muscle CSA and the following variables were identified: muscle weight ($r = -0.15, P < .0005$), ultimate load ($r = -0.15, P = .005$), failure load ($r = -0.15, P = .005$), yield load ($r = -0.08, P = .05$), stiffness ($r = -0.15, P = .004$), and maximum bending moment ($r = -0.02, P = .01$). No significant correlations were observed at the middle tibiotarsal level.

Significant positive correlations were present between the proximal cortico-medullary bone density ratio and the sagittal cancellous BMD ($r = 0.33, P = .01$). In the middle section of the tibiotarsus, significant positive correlations were identified between the cortico-medullary bone density and area ratios and the following measures: total tibiotarsal BMD ($r = 0.30, P = .05$) area, medullary keel BMD ($r = 0.34, P = .02$) area, sagittal cancellous BMD ($r = 0.39, P = .004$) density, cortical thickness at location of fracture ($r = 0.36, P = .01$) density, ultimate load ($r = 0.41, P = .003$) density, failure load ($r = 0.41, P = .004$) density stiffness ($r = 0.41, P = .004$) density, and stress ($r = 0.37, P = .01$) density. In the distal section of the tibiotarsus, significant positive correlations were identified between the cortico-medullary bone density ratio and the following measures: medullary keel BMD ($r = 0.28, P = .04$), ultimate load ($r = 0.30, P = .04$), failure load ($r = 0.30, P = .04$), and yield load ($r = 0.30, P = .04$).

**Tibiotarsal BMD X keel BMD**—At the distal level, significant correlations exist between total tibiotarsal...
BMD and total keel BMD \((r = 0.29, P = .03)\) and medullary tibiotarsal BMD and medullary keel BMD \((r = 0.27, P = .05)\). At the middle level, significant correlations exist between total tibiotarsal BMD and the measures of medullary keel BMD \((r = -0.27, P = .05)\) and cortical keel BMD \((r = 0.29, P = .03)\). No significant correlations were observed between the imaging measures of keel and tibiotarsal BMD at the proximal location.

**Regression analyses**

**Muscle CSA X biomechanical measures**—At the proximal location, tibiotarsal muscle CSA was predictive of the biomechanical measures of ultimate

**Table 1**—Results of keel bone X tibiotarsus bone mineral density correlation analyses.

<table>
<thead>
<tr>
<th>Proximal</th>
<th>Total tibiotarsal BMD</th>
<th>Medullary tibiotarsal BMD</th>
<th>Cortical tibiotarsal BMD</th>
<th>Middle</th>
<th>Total tibiotarsal BMD</th>
<th>Medullary tibiotarsal BMD</th>
<th>Cortical tibiotarsal BMD</th>
<th>Distal</th>
<th>Total tibiotarsal BMD</th>
<th>Medullary tibiotarsal BMD</th>
<th>Cortical tibiotarsal BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total keel BMD</td>
<td>0.2214</td>
<td>0.1316</td>
<td>0.1365</td>
<td>0.152</td>
<td>0.0893</td>
<td>0.0079</td>
<td>0.2887*</td>
<td>0.1378</td>
<td>-0.0073</td>
<td></td>
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</tr>
<tr>
<td>Medullary keel BMD</td>
<td>-0.0572</td>
<td>-0.0139</td>
<td>-0.045</td>
<td>-0.2731*</td>
<td>-0.0016</td>
<td>-0.14</td>
<td>0.2217</td>
<td>0.2701*</td>
<td>-0.1878</td>
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<td></td>
</tr>
<tr>
<td>Cortical keel BMD</td>
<td>0.1683</td>
<td>0.0832</td>
<td>0.1119</td>
<td>0.2891*</td>
<td>0.0734</td>
<td>0.0913</td>
<td>0.1586</td>
<td>-0.0214</td>
<td>0.1036</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation coefficient \((r)\) values are reported, and significant correlations are bolded.

BMD = Bone mineral density.

*\(P = .05\) to .02.

**Table 2**—Regression analyses of computed tomographic measures of bone and muscle quality versus biomechanical measures of tibiotarsal bone quality.

<table>
<thead>
<tr>
<th>Paired measures</th>
<th>Total tibiotarsal CT area</th>
<th>Total tibiotarsal CT BMD</th>
<th>Medullary tibiotarsal CT area</th>
<th>Medullary tibiotarsal CT BMD</th>
<th>Cortical tibiotarsal CT area</th>
<th>Cortical tibiotarsal CT BMD</th>
<th>Tibiotarsal muscle CT CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibial area/muscle area X ultimate load</td>
<td>(P &lt; .0001)</td>
<td>-</td>
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<td>(P &lt; .0001)</td>
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<tr>
<td>Tibial area/muscle area X failure load</td>
<td>(P &lt; .0001)</td>
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<td>-</td>
<td>(P &lt; .0001)</td>
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<tr>
<td>Area/area X maximum displacement</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>Area/area X yield load</td>
<td>(P = .0043)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(P &lt; .0001)</td>
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<tr>
<td>Area/area X stiffness</td>
<td>(P &lt; .0001)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(P &lt; .0001)</td>
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<tr>
<td>Area/area X bending moment</td>
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<td>-</td>
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<tr>
<td>Area/area X stress</td>
<td>(P &lt; .0001)</td>
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<td>(P &lt; .0009)</td>
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<td>Tibial BMD/muscle CSA density X ultimate load</td>
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<td>Tibial BMD/muscle CSA density X failure load</td>
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<tr>
<td>Tibial BMD/muscle CSA density X maximum displacement</td>
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<td>(P = .0294)</td>
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<td>Tibial BMD/muscle CSA density X yield load</td>
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<tr>
<td>Tibial BMD/muscle CSA density X stiffness</td>
<td>-</td>
<td>-</td>
<td>(P = .0022)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(P = .0369)</td>
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<td>Tibial BMD/muscle CSA density X bending moment</td>
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<td>Tibial BMD/muscle CSA X ultimate load</td>
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<td>Tibial BMD/muscle CSA X maximum displacement</td>
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<td>Tibial BMD/muscle CSA X stiffness</td>
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<td>(P = .0023)</td>
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<td>(P = .0369)</td>
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<td>Tibial BMD/muscle CSA X stress</td>
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Significant pairings are indicated by their respective \(P\) values.

CSA = Cross-sectional area.

- = Not significant.
load (F = 9.61, P = .003), failure load (F = 9.68, P = .003), yield load (F = 8.47, P = .006), and stiffness (F = 9.80, P = .003). At the middle and distal locations, muscle CSA was not a predictor of biomechanical measures of tibiotarsal bone quality (Supplementary Table S2).

At the proximal location, total and cortical tibiotarsal BMDs of the proximal right leg were significant predictors of ultimate load (F = 12.83, P = .0008), total bone CSA (F = 5.37, P = .02), and max. displacement (F = 11.19, P = .002). The distal location showed significant correlations among measures of bone quality and muscle quality, and determining whether biomechanical measures of bone quality could be predicted based on CT measurements.

For the tibiotarsus, significant positive correlations between muscle CSA and tibiotarsal BMD were present at the proximal location. This supported the first hypothesis that there would be associations between tibiotarsal muscle CSA and imaging, tissue level, and biomechanical measures of bone and muscle quality of the tibiotarsus. The total and cortical bone correlations were low (0.29 and 0.31, respectively), but similarly low correlations were observed in the human literature. Rantalainen et al. identified low positive correlations of r = 0.35 to 0.37 between tibial bone traits and tibial muscle CSA in older men. A nonsignificant correlation between medullary BMD and tibiotarsal muscle group CSA was the lowest at -0.11 and could possibly be explained by the nature of medullary bone, a labile bone type that has a rapid rate of turnover as it functions in egg production. Potential differences in medullary bone deposition/resorption between individual birds could have a dampening effect on the correlation as it has the potential to be present in the tibiotarsus in varying amounts. The differential deposition/resorption of medullary bone could also explain the associations observed for total and cortical bone. The presence/absence of medullary bone could influence the total BMD and subsequently the cortical BMD as it was the calculated difference between total and medullary BMD. Additional significant, positive correlations were observed between the tissue level measures of muscle weight and volume and the biomechanical measures of ultimate load, failure load, yield load, and stiffness, but no other current studies evaluate the correlations between imaging measures of bone and muscle quality and tissue level and biomechanical measures of tibiotarsal bone quality. Results that did not support hypothesis 1 were the lack of correlations observed at the middle tibiotarsus and the proximal, middle, and distal keel bones. A possible explanation for the lack of associations at the tibiotarsal locations could be due to the tibiotarsal musculature. The tibiotarsal muscle CSA exhibits a decreasing size between the proximal, middle, and distal locations, with the proximal location having the largest muscle CSA. Additional studies should be done to investigate the lack of significant correlations observed and identify if muscle accretion occurs similarly at all 3 assessed locations or if there are site differences that could possibly influence the associations of measures.

For the tibiotarsal BMD X keel BMD correlational analysis, significant correlations were observed in the middle and distal sections of the bone, but not
the proximal region. These findings supported the second hypothesis, which stated there would be an association between the measures of BMD for the 2 bones. There is currently no explanation for the differences in the association of the measures at each location, but it could be partly due to the differing ratios of medullary and cortical bone at each location assessed that, in turn, influenced the total density of the bone. Additional investigation is needed to confirm the findings of this analysis. Our findings are comparable to those of Gehbardt-Henrich et al in that bone mineral content measures of the total tibiotarsus and keel bone were positively correlated with one another ($r = 0.25$). The findings of Toscano et al also corroborate our findings in that, they found a correlation positive correlation between tibiotarsal and keel BMD. Results that did not support this hypothesis were the lack of observed correlations at the proximal location.

The findings of the regression analyses supported the third hypothesis that imaging measures of bone and muscle quality are predictors of biomechanical measures of bone quality. At the proximal location, tibiotarsal muscle CSA was a significant predictor of the ultimate load, failure load, yield load, and stiffness. Failure load, a commonly reported measure of bone strength, being predicted by muscle CSA in this location indicates the potential of utilizing muscle CSA measurements as an indirect measure of assessing the resistance of the bone to fracture in laying hens. This could potentially reduce the need for implementing 3-point bending tests and would allow for longitudinal studies that assess the effects of interventions on laying hen bone quality. Additionally, the proximal location being the only predictor of these biomechanical measures could potentially reduce the need to perform image analysis measures for multiple locations of a single bone or muscle as was done in this study. This paired with the reduced need for destructive biomechanical testing would greatly reduce the amount of time required to collect data and would therefore make the protocol better suited for research studies with large data sets.

There is a lack of studies in the literature assessing the ability of muscle CSA to predict biomechanical measures of bone quality in laying hens. Additionally, the human literature, which routinely utilizes the measurement of muscle CSA to assess muscle quality, does not report the implementation of destructive testing to assess the efficacy of CSA in predicting biomechanical measures of the quality of long bones. With this considered, our measure of muscle CSA should be implemented in future studies to compare findings with those of this study.

For the tibiotarsal bone CSA X biomechanical measures analysis, the third hypothesis was not supported as bone CSA was not able to predict relevant biomechanical measures of bone quality. At the proximal, middle, and distal locations, stress, a measure of internal resistance of an object to an applied load, was the only predicted measure. However, the BMD of the tibiotarsus at the proximal location was a predictor of ultimate load, failure load, stiffness, and stress. Therefore, while tibiotarsal bone CSA should not be used as an indirect measure of bone quality based on our findings, the BMD at the proximal location can be used. The findings of this analysis can be explained by the bone properties that are reported to contribute to bone strength and its subsequent resistance to fracture. Cortical thickness is reported to be a major contributor to bone strength rather than the CSA of the bone itself. Birds that experience cortical bone resorption as a result of having insufficient calcium stores at the time of eggshell formation would exhibit thinning cortices while having an unchanging bone CSA and would therefore have bones that are structurally weaker compared to a bone of the same CSA with a thicker cortex.

Cortico-medullary bone density and area ratios were calculated to account for differences in cross-sectional bone geometry between birds. Findings supported the third hypothesis in that the middle and distal locations were successful in predicting the ultimate load, failure load, maximum displacement, stiffness, and stress. These findings contrast the findings of the tibiotarsal BMD X biomechanical measures regression in that the proximal location was the only predictor of the biomechanical measures. The findings of Donkó et al support our findings in that their regression analysis demonstrated that CT BMD was a predictor of the breaking strength of the tibiotarsal of laying hens ($r = 0.78$ to $0.91$). Collectively, the findings of these 2 analyses indicate the need for further testing before solely utilizing either method of assessment for the purpose of indirectly assessing bone strength.

Finally, the regressions run pairing imaging measures of bone and muscle supported the third hypothesis in that bone and muscle CSA successfully predicted the measures of ultimate load, failure load, yield load, stiffness, and stress. The paired regressions did not support the third hypothesis in that BMD X muscle density and BMD X muscle CSA only predicted the measures of stiffness and bending moment, respectively. It can be concluded that these paired measures are not optimal at predicting biomechanical measures of bone quality as the most important measure, the failure load, was not predicted. As stated before, the lack of implementation of the measure of muscle CSA prevents comparisons between other studies.

One limitation of the current study was the time required to perform the imaging measurements. At an hour per bird, the adapted methodologies are not suited for large-scale studies and are better suited for smaller scale research applications. Another limitation of this study was the reconstruction algorithm applied to the CT scans. The scans had an FC31 reconstruction filter applied after image acquisition that altered the interpretation of the margins of the cortical and medullary bone. A decision was made to place an ROI around the outer margin of the cortical and medullary bone, but this likely resulted in an overestimation of the cortical bone area. Further image acquisition protocol refinement to choose a
reconstruction filter that will lessen the appearance of the black banding around bone margins would further improve the accuracy of measuring the cortical bone area. Additionally, the inclusion of only 1 age group in this study was a limitation. Different developmental stages such as the pullet phase and the onset of lay could bear different correlations as the bone composition is different during these stages. Another limitation was our choice to freeze and thaw muscles before performing weight and volume measurements. While this is a common procedure reported in other research studies, authors acknowledge that this could have introduced an outside source of variation for our tests of association between CT muscle measures and tissue-level muscle measures.

In conclusion, findings from the current study supported using noninvasive CT measures of muscle and bone quality in longitudinal research studies evaluating the effects of interventions on laying hen welfare. Standardized, step-by-step image analysis protocols were provided. We also introduced evidence that muscle quality is associated with bone quality in laying hens. We additionally demonstrated that select imaging-based measures of both bone and muscle quality were predictors of meaningful biomechanical measures of bone quality. To help reduce image analysis time, findings from our study also supported focusing on the proximal tibiotarsal region in future studies.

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Disclosures

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References


Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.