ABSTRACT

Researchers have provided much insight into the various factors that influence the incidence of musculoskeletal problems in the poultry industry. However, a better understanding of the mechanobiology of broiler bone and tendon can have a positive effect on the welfare of the production bird and assist in the development of improved production practices. This study investigated the mechanical adaptability responses due to disuse on the biomechanical properties of the broiler tibia and gastrocnemius tendon. Beginning at 3 wk of age, broilers were placed in a harness system designed to eliminate load bearing of the leg. After 2 wk of this treatment, the average values for body mass and shank length of the birds were 58 and 85% of the values for the controls, respectively. The treatment reduced the mineral content of the tibia by approximately 50%, tibia structural strength by 40%, and tibia material strength by 8%. The structural strength and toughness of the gastrocnemius tendon were reduced by 10 and 30%, respectively, whereas the material strength, material toughness, and material stiffness of the tendon increased by approximately 75, 65, and 70%, respectively.

Key words: broiler, immobilization, tibia, gastrocnemius tendon

INTRODUCTION

The causes of musculoskeletal abnormalities in meat-type birds have been of significant interest to the poultry industry for years. Although researchers have provided much insight into various factors, such as housing practices, that influence the incidence of musculoskeletal problems, more information concerning the basic mechanobiological relationships of broiler bone and tendon is needed. This knowledge can have a positive effect on the welfare of the production bird and assist in the development of improved production practices.

The principles of mechanobiology (van der Meulen and Huiskes, 2002) dictate that the mechanical environment placed on a musculoskeletal system will influence the architecture of bone and tendon. Basically, overloading the musculoskeletal system will stimulate cell activity to add bone, tendon, or both, whereas underloading the musculoskeletal system or disuse will stimulate cell activity to remove bone, tendon, or both. Weeks et al. (2000) indicated that broilers spend 76 to 86% of their time lying down, creating an environment of musculoskeletal disuse. If broiler physiology follows mechanobiology principles, then this type of environment would reduce the structural integrity of the bone and tendons of a broiler.

Avian bone has been shown to follow the principles of mechanobiology, in which increased mechanical loading of the chicken tibia corresponds to increased bone mineralization and stiffness (Judex et al., 1997; Judex and Zernicke, 2000; Foutz et al., 2007a), and mechanical disuse corresponds to decreased mass, strength, and stiffness of the ulna and radius (Foutz et al., 1997). However, studies that have investigated the mechanobiology of avian tendon, in particular the gastrocnemius tendon, are limited and not definitive. A guinea fowl model (Buchanan and Marsh, 2001) indicates that increased loading via running increases the stiffness of the Achilles’ tendon of the birds but does not affect the architecture of the tendon. Using layers, Benevides et al. (2004) found that increased activity via walking increased the strength, stiffness, and size of the superficial digital flexor tendon, whereas Foutz et al. (2007b) found that increased walking had no affect on the strength, stiffness, and size of the broiler gastrocnemius tendon. These studies did not assess the relationship of bird inactivity to avian tendon biomechanics.

The purpose of this work is to provide insight into the effects of extreme decreased mobilization of broiler chicken on the biomechanical performance of both the tibia and the gastrocnemius tendon.

MATERIALS AND METHODS

Birds and Housing

One hundred fifty 1-d-old commercial female Arbor-Ross chickens were used as test subjects and raised in
environmental chambers (2.5 × 2.5 m) located at the Driftmier Engineering Center at the University of Georgia. Chamber temperature was held at 29.4°C (±1°C), and humidity was kept low although not measured. To provide supplemental heat, a brooder was placed in 1 corner of each environmental chamber for the first week of the study. The flooring consisted of 76 mm of wood chips covering a concrete pad. For the first 2 wk, all birds were fed a broiler starter diet, and for the remainder of the study, a broiler grower diet was used (Foutz et al., 2007b). The birds had free access to food and water.

The housing and immobility procedures reported herein were approved by the University of Georgia Institutional Animal Care and Use Committee (A970154).

**Viral Screening**

Each week, each flock was monitored for the presence of reovirus. Blood was drawn from the birds, submitted to the University of Georgia Poultry Diagnostic and Research Center, and examined for neutralizing antibodies to reovirus using an ELISA. Only reovirus-negative flocks were analyzed for this study.

**Experimental Protocol**

The nature of this study made it necessary to select birds that had similar behavior toward physical activity. At 1 d of age, all chicks were placed on a treadmill with the belt speed set to 0.22 m/s. Chicks unwilling or not able to walk on the treadmill at this speed were removed from the study. Each remaining bird was placed on the treadmill each day for the first 3 wk of the study. Daily treadmill sessions lasted for 5 min, using a treadmill speed of 0.22 m/s. During this 3-wk period, any bird deemed unwilling or unable to walk during the session was removed from the study. This treadmill scheme was based on work published by Brackenbury et al. (1990).

At 4 wk of age, the birds were divided into 2 groups, controls and immobilized, and were no longer exposed to the treadmill procedure. Both the control and immobilized groups were housed in the environmental chambers. The control group was raised under the conditions given above. The immobilized group was placed in a whole-body suspension system (Figure 1), in which the birds were suspended in a harness designed to eliminate load bearing on the legs of the birds (Musacchia et al., 1980). The harness was adjustable to accommodate growth of the birds. The birds remained suspended throughout the duration of the study. Food and water were placed so that the immobilized birds could eat and drink freely. The system allowed each bird to face another bird but far enough away to prevent pecking.

**Bird Growth**

Normal growth was monitored by measuring bird body mass, shank length, and shank width. These measurements were made beginning at 3 wk of age and every 7 d thereafter. Shank length was defined as the distance from the footpad to the tibiotarsal joint. Shank width was defined as the largest diameter of the shank at the location equal to one-half of the length of the shank. Each growth parameter was recorded for individual birds.

**Tissue Collection**

Birds were killed with an overdose of CO₂. At the time of death, the legs were disarticulated at the hip joint and dissected from the carcass. The gastrocnemius muscle-tendon-bone complex and the tibia were dissected from the right leg, wrapped separately in towels soaked in isotonic avian saline, placed in a freezer bag, and then stored at −70°C until needed for testing.

**Bone Parameters**

The previously frozen tibias were thawed at room temperature. All cartilage and muscle were removed from the tibia. Bone length was measured with digital calipers from the condyle to the malleolus. The tibia shaft midpoint was determined and marked on the bone with a tissue marker. This location was used when calculating the tibia midshaft cross-sectional area (CSA) as specified by engineering standard ANSI/ASAE S459 (ASAE, 2004).

**Bone Mineral Content and Bone Mineral Density**

Bone mineral content (BMC) and bone mineral density (BMD) were determined using a Hologic QDR 1500 dual X-ray scanner (Adams, 1997). Bone mineral content is defined as the amount of mineralized tissue in the scan area, and BMD is the total amount of mineralized tissue normalized to the length of the scan region. The right tibia was examined just before biomechanical testing.
Figure 2. A control area was used to determine the biomechanical properties of the tendon. This area was the middle third of the length of the tendon, which was defined as the length of the dissected tendon beginning at the bony insertion to the top of the bifurcation. The cross-sectional area of the tendon was calculated at the midpoint and was assumed to be elliptical. Deformation was defined as the movement of the proximal and distal end of the control area away from each other.

**Bone Biomechanical Properties**

The in vitro biomechanical properties of the tibia were determined using engineering standard procedure ANSI/ASAE S459, which was developed for strength testing of animal bone (ASAE, 2004). Briefly, this standard specifies that the 3-point bending test requires the length-to-diameter ratio of a bone specimen to be 10:1. This ratio eliminates fulcrum effects on the biomechanical data. Herein, the tibia specimens did not meet this condition, and therefore the standard specifications for the double shear test method were followed. Each bone was mechanically tested in double shear using an Instron model 4201 material-testing machine (Instron Corp., Canton, MA). The Instron deformed the bone until initial structural failure occurred. The maximum shear load response to the bone deformation was recorded. The rate of deformation was set at 5 mm/min.

**Gastrocnemius Tendon Midregion Cross-Sectional Area**

Before the quasistatic tensile testing, the gastrocnemius muscle-tendon-bone complex was removed from −70°C storage, thawed at room temperature, and placed in a physiologic saline bath (0.85% saline, 38.9°C). For each tendon, the relaxed length of the gastrocnemius tendon was measured. Relaxed length was defined as the length of the dissected tendon beginning at the bony insertion to the top of the bifurcation. Based on the length measurement, the middle third of the tendon was determined, marked using an ink marker, and used as the tendon control area for biomechanical testing (Figure 2). Assuming that the tendon shape was elliptical, the CSA at the middle of the control area was calculated.

**Tendon Biomechanical Properties**

The gastrocnemius muscle-tendon-bone complex was loaded in an Instron 4201 material tester by clamping the gastrocnemius muscle end and the bone end, which left the tendon free of the clamping mechanism. When clamping soft tissue to the Instron, damage will typically occur and thus change the biomechanical behavior of tissue. Matthews et al. (1996) found that cryoclamps can be used to overcome these clamping effects. Cryoclamps described in Foutz et al. (2007b) kept the tissue near the clamps near freezing while keeping the tissue in the control area from 24 to 30°C. Thus, all deformation and failure occurred within the control area.

All quasistatic failure testing was conducted at a strain rate of 1%/s. Before testing, each tendon was preconditioned to a strain of 1% for 50 cycles using a strain rate of 1%/s (Fung, 1993). Following preconditioning, the slack was removed; however, care was taken to ensure that the tendon was not under any load before the start of the test. The tendon was then pulled uniaxially, and a load-deformation curve was generated. Testing was continued until complete rupture occurred. Deformation was defined as the measured movement of the distal and proximal ends of the tendon control area (Figure 2). A video dimension analyzer (Yamamoto et al., 1999) system was used to measure this movement.

The load-deformation curve was used to determine parameters that describe tendon biomechanical behavior. These parameters were strength, toughness, tangent modulus, secant modulus, maximum relaxation load, and maximum relaxation deformation. Each parameter was calculated for the tendon structure and the tendon material. Strength was defined as the maximum load applied. Toughness was defined as the area under the load-defor-
Table 1. Parameters used to monitor the effects of immobilization on bird growth and on tibia structural integrity at 4, 5, and 6 wk of age

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Control</th>
<th>Immobilized</th>
<th>Control</th>
<th>Immobilized</th>
<th>Control</th>
<th>Immobilized</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>988.8d (15.2)</td>
<td>996.7e (12.8)</td>
<td>1,526.2c (23.6)</td>
<td>979.4d (34.8)</td>
<td>2,062.0c (32.2)</td>
<td>1,187.4d (81.9)</td>
</tr>
<tr>
<td>Shank length (mm)</td>
<td>3.04d (0.03)</td>
<td>3.06d (0.02)</td>
<td>3.50d (0.03)</td>
<td>3.19d (0.04)</td>
<td>3.82d (0.04)</td>
<td>3.32d (0.08)</td>
</tr>
<tr>
<td>Shank width (mm)</td>
<td>0.41c (0.01)</td>
<td>0.41c (0.01)</td>
<td>0.47b (0.01)</td>
<td>0.42d (0.01)</td>
<td>0.50b (0.01)</td>
<td>0.44c (0.02)</td>
</tr>
<tr>
<td>Tibia length (cm)</td>
<td>76.9f (0.7)</td>
<td>77.3f (0.9)</td>
<td>88.5c (1.0)</td>
<td>80.9d (0.7)</td>
<td>96.8c (0.9)</td>
<td>85.0f (2.1)</td>
</tr>
<tr>
<td>Tibia bone mineral content (g)</td>
<td>1.48d (0.08)</td>
<td>1.54d (0.15)</td>
<td>2.56e (0.14)</td>
<td>1.22f (0.09)</td>
<td>3.23d (0.26)</td>
<td>1.73c (0.29)</td>
</tr>
<tr>
<td>Mineral density (g/cm³)</td>
<td>0.154c (0.006)</td>
<td>0.150c (0.007)</td>
<td>0.170f (0.008)</td>
<td>0.132d (0.005)</td>
<td>0.188d (0.008)</td>
<td>0.129f (0.007)</td>
</tr>
<tr>
<td>Maximum shear load (N)</td>
<td>285.4d (14.1)</td>
<td>274.6e (15.0)</td>
<td>348.6d (17.8)</td>
<td>226.6c (14.1)</td>
<td>345.3e (29.4)</td>
<td>207.9d (13.3)</td>
</tr>
<tr>
<td>Maximum shear stress (GPa)</td>
<td>6.0f (0.3)</td>
<td>6.2f (0.3)</td>
<td>5.8f (0.3)</td>
<td>5.0f (0.3)</td>
<td>4.8f (0.3)</td>
<td>4.4f (0.3)</td>
</tr>
</tbody>
</table>

* Means in the same row with no common superscript differ significantly (P < 0.05).
1 Standard error of the mean.

RESULTS AND DISCUSSION

Developmental Responses to Immobilization

Before placing the broilers into the suspension system, the values for body mass (Table 1), shank length, and shank width of the control group were the same as those for the immobilized group. Following 1 wk of suspension, significant effects of the immobilization were found for these 3 growth parameters. Average values for body mass of the birds immobilized for 1 and 2 wk were 64 and 58% of the values for the controls, respectively. After 2 wk of immobilization, shank length and width were approximately 85% of those from the control group. Overall, the immobilization treatment dramatically halted normal age-related growth. These results are consistent with published literature (Biewener and Bertram, 1994; Bloomfield et al., 1997; Han et al., 1999; Inman et al., 1999; Almeida-Silveira et al., 2000; Mosekilde et al., 2000; Zarzhevsky et al., 2001).

Skeletal Response to Immobilization

Immobilization significantly affected the tibia length of broilers (Table 1), particularly after 2 wk of suspension, in which the average value was 87% of control value. Comparison of tibial midshaft CSA values indicated that immobilization caused a 24% decrease in CSA after 1 wk of suspension and a 32% decrease after 2 wk of suspension.

Immobilization significantly reduced the tibia BMC and BMD (Table 1). After 2 wk of suspension, the average BMC value for the immobilized birds was approximately 50% less than the average value for the control birds, and the average BMD value was approximately 30% less. Examination of the radius and the ulna produced similar results, indicating that the immobilization produced a significant reduction of BMC and BMD throughout the skeletal system.

Immobilization significantly reduced the maximum shear load that the tibia could withstand (Table 1). Overall, the average shear load, which describes tibia structural strength, decreased by 35% after 1 wk of suspension and 40% after 2 wk of suspension. Normalizing shear load by the bone CSA gives average shear stress, which is a descriptor of material strength. The immobilization decreased the average value of the maximum shear stress by approximately 8%. As expected, age effects were seen for both treatment groups.

The alterations of bone architecture reported herein are similar to those reported in other animal immobilization studies. Biewener and Bertram (1994) and Rubin et al. (1996) reported that disuse increased bone porosity of chicken and turkey tibia with remodeling activity observed in the turkey. Twenty-one days of wing immobilization in layers resulted in a decrease in the bone apposition rate on the posterior side of the ulna and radius, a reduction...
### Parameters used to monitor the effects of immobilization on the biomechanical properties of broiler gastrocnemius tendon at 4, 5, and 6 wk of age

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Control</th>
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<th>Control</th>
<th>Immobilized</th>
<th>Control</th>
<th>Immobilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midpoint cross-sectional area (mm²)</td>
<td>8.48① (0.35)</td>
<td>8.76⑥ (0.39)</td>
<td>10.57④ (0.51)</td>
<td>6.19④ (0.56)</td>
<td>11.57⑥ (0.55)</td>
<td>6.44④ (0.69)</td>
</tr>
<tr>
<td>Structural strength (N)</td>
<td>357⑦ (12)</td>
<td>352⑦ (16)</td>
<td>46③ (15)</td>
<td>46③ (23)</td>
<td>57③ (20)</td>
<td>52① (37)</td>
</tr>
<tr>
<td>Structural toughness (Nm)</td>
<td>193① (16)</td>
<td>20③ (21)</td>
<td>345⑤ (28)</td>
<td>23③ (25)</td>
<td>44⑤ (50)</td>
<td>30① (38)</td>
</tr>
<tr>
<td>Structural secant modulus (Nm)</td>
<td>425⑤ (74)</td>
<td>390⑤ (20)</td>
<td>450⑤ (25)</td>
<td>44③ (41)</td>
<td>48⑤ (41)</td>
<td>49⑤ (41)</td>
</tr>
<tr>
<td>Material strength (GPa)</td>
<td>44① (2)</td>
<td>40① (3)</td>
<td>46① (2)</td>
<td>84⑤ (9)</td>
<td>53⑤ (3)</td>
<td>50① (12)</td>
</tr>
<tr>
<td>Material toughness (GPa)</td>
<td>2.2① (0.2)</td>
<td>2.3⑤ (0.3)</td>
<td>2.9⑤ (0.2)</td>
<td>3.8⑤ (0.4)</td>
<td>3.0① (0.2)</td>
<td>5.0① (1.0)</td>
</tr>
<tr>
<td>Material secant modulus (GPa)</td>
<td>518① (61)</td>
<td>41⑤ (31)</td>
<td>48① (26)</td>
<td>80① (125)</td>
<td>54① (55)</td>
<td>93① (84)</td>
</tr>
<tr>
<td>Maximal relaxation strain (%)</td>
<td>0.23④ (0.003)</td>
<td>0.19④ (0.003)</td>
<td>0.02④ (0.003)</td>
<td>0.02④ (0.003)</td>
<td>0.02④ (0.003)</td>
<td>0.02④ (0.003)</td>
</tr>
</tbody>
</table>

①Means in the same row with no common superscript differ significantly (P < 0.05).
②Standard error of the mean.

in the cortical thickness, and a reduction in bone stiffness but did not affect the growth rate and the width of these bones (Foutz et al., 1997). Rat hind limb suspension studies report an approximately 15% reduction in femur CSA and significant reduction in tibia BMD (Bloomfield et al., 1997; Inman et al., 1999).

These outcomes correlate well with results that would be predicted by the minimum effective strain (MES) concept. Martin et al. (1998) provided details of the MES concept, but a brief description is given here. Two MES limits bound the physiological normal deformation range of bone. These limits are called MES set points. When loading deformed bone such that the deformation exceeds an upper MES set point, more bone is produced, making the skeletal structure stiffer, and this continues until deformation returns to the physiological normal MES range. When bone is underloaded and does not deform enough to reach a lower MES set point, bone is removed, making the skeletal structure more compliant. This continues until deformation returns to the physiological normal MES range. The reduction in tibia shear strength reported herein is consistent with the MES concept and shows that loading of the tibia of a broiler is needed to maintain a structurally sound bone.

### Tendon Response to Immobilization

Two weeks of immobilization caused the average value of tendon structural strength to decrease by approximately 10%, and structural toughness decreased by approximately 30% (Table 2). The material properties of the tendon were found by normalizing structural properties by the CSA of the tendon. After 2-wk of immobilization, the average values of the material strength, material toughness, and material stiffness (Table 2) increased by approximately 75, 65, and 70%, respectively. After 1 wk in the suspension system, the average tendon CSA values from the immobilized birds were approximately 40% less than the values from the control birds (Table 2), and thus the increase in material properties is due to less material being available to carry the applied load.

The immobilization treatment had no effect on structural parameters of stiffness (Table 2), as defined by the secant modulus and by tangent modulus; on maximum relaxation load; on maximum relaxation deformation; or on the material parameters of maximum relaxation stress and maximum relaxation strain (Table 2).

These results indicate that the chicken gastrocnemius tendon responds to mechanical disuse as predicted by the mechanobiology process and is comparable to those reported for other animal tendons. Using cages to limit motion, Benevides et al. (2004) reported that structural strength of the gastrocnemius tendon was not affected by inactivity when caged layers were compared with activity of penned layers, but tendon material strength did increase by 50% due to 60 d of the caged treatment. Tendon material stiffness, defined as the secant modulus to the maximum stress, from the penned birds was approximately 40% greater than the material stiffness of the caged birds. Mammalian studies (Fujie et al., 2000; Yasuda et al., 2000; Palmes et al., 2002; Matsumoto et al., 2003) indicate similar responses, in which immobilization reduced structural strength and stiffness of tendons while it increased the material strength and stiffness of the tissue.

Analysis of the treatment tendons for immunohistochemistry was not conducted; however, data found in the literature (Josza and Kannus, 1997; Benevides et al., 2004) allows some conjecture of what would occur. The immobilization via leg suspension would have decreased the concentration of proteoglycans and glycosaminoglycans and would have decreased both the organization and diameter of the collagen fibers. Proteoglycans and glycosaminoglycans influence the energy absorption capabilities of tendon, and this decrease in concentration could explain the dramatic reduction in structural toughness of the gastrocnemius tendon reported herein. Collagen organization influences the stiffness of tissues, and a reduction of this organization would help explain the increase in gastrocnemius tendon material stiffness reported herein; although structural stiffness was not affected. Also, the anticipated reduction in collagen size would result in a reduction in tendon strength and again explain the dramatic drop in tendon rupture strength as found in this study. Further investigation would be needed to confirm these conjectures; such relationships would be consistent with the mechanobiology processes found with mammalian tendons.
**Study Limitations**

Overall, the use of the suspension apparatus provided mixed results in that the systemic health of the subjects could not always be maintained. It is conceivable that the immobilization protocol used here was too extensive for the birds to handle. A graduated suspension protocol may prove more effective and consistent in bringing about the desired results. Alterations to the time spent in the suspension apparatus may have great effect on the overall health and welfare of birds.

In future investigation of the effect of disuse on the musculoskeletal system of bipeds, comparing the response to the suspension apparatus to a proven model for birds (i.e., forced sitting) is suggested. Although not providing the same loading conditions, immobilization generated a no-load situation, whereas forced sitting causes compression in the area of interest; a comparison between the 2 models would give a standard to measure bird health and welfare.

**REFERENCES**


