Validation of Dual-Energy X-Ray Absorptiometry in Live White Leghorns

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ABSTRACT Dual energy x-ray absorptiometry (DEXA) was evaluated for use as a noninvasive tool to monitor skeletal integrity in live laying hens. The objectives of the current study were 1) to validate the use of DEXA in evaluating bone integrity in live birds as compared with excised bones under a normal nutritional regimen as well as in hens fed varying levels of dietary Ca and 2) to correlate densitometric scans with other bone strength criteria and egg traits. Densitometric scans were conducted on the tibia and humerus of live hens at 10-wk intervals from 17 to 67 wk of age. After each scan, bones were excised from euthanized hens to measure breaking strength characteristics and bone ash (experiment 1). Similar measurements were collected at 38, 48, and 58 wk of age from hens fed hypercalcemic (5.4%), control (3.6%), and hypocalcemic (1.8%) diets from 32 to 58 wk of age (experiment 2). The bone mineral density (BMD) and bone mineral content (BMC) between live and excised bone scans were highly correlated (r = 0.85 and 0.92, respectively, P < 0.0001, experiment 1). Densitometric scans of live birds were positively correlated with bone breaking force and bone ash (r = 0.68 and 0.73, respectively, P < 0.001) with little to no correlation with shell traits. In experiment 2, the excised tibial scan had lower BMD and BMC than the live bird (P < 0.01), whereas no difference was detected in densitometric scans of the humerus. The live and excised BMD and BMC of the tibia (r = 0.87 and 0.82, respectively, P < 0.001) and humerus (r = 0.94 and 0.93, respectively, P < 0.001) were highly correlated. Due to the high correlations between live and excised bone scans and the significant correlations of live scans to more traditional invasive bone measurement tests such as bone breaking force and bone ash, we concluded that DEXA is a useful noninvasive tool for evaluating skeletal integrity in live birds.

(Key words: bone mineral density, bone mineral content, White Leghorn, dual-energy X-ray absorptiometry, dietary calcium)

INTRODUCTION Noninvasive measurement of bone is an important and developing field of interest in the study of metabolic bone diseases, including osteoporosis. In laying hen flocks housed in cages, osteoporosis has been found to be a widespread disease (Whitehead and Wilson, 1992) contributing to approximately 35% of the mortalities during the egg production cycle (McCoy et al., 1996). Osteoporosis, or cage layer fatigue, is a progressive loss in structural bone (Whitehead and Fleming, 2000). Bone fragility in laying hens due to age-related osteoporosis has created welfare issues as well as economic problems for the poultry industry (Webster, 2004).

Bone fractures in egg laying strains of birds due to continuous egg production, handling, and transportation have been reported in 29% of the birds that reach the processing facilities (Gregory and Wilkins, 1989) and 98% of the birds by the end of the processing line (Belyavin, 1995). These fractures create bone splinters in the meat product causing food safety concerns. Therefore, food companies are reluctant to use spent hens for meat products and have turned to the broiler industry for their meat (Wilson and Harner, 1988; Whitehead and Wilson, 1992; Bhat, 1993; Brown, 1993). This limited market for spent hen meat imposes economic and environmental burdens on producers (Roland and Rao, 1992; McCoy et al., 1996; Newberry et al., 1999).

Possible factors that contribute to the onset of osteoporosis in laying hens include the high demand for Ca during eggshell formation, limited exercise of birds due to the cage system, and genetic selection for production traits.

Abbreviation Key: BMD = bone mineral density; BMC = bone mineral content; DEXA = dual-energy x-ray absorptiometry; SPA = single photon absorptiometry.
(Rowland et al., 1968; Rowland and Harms, 1970; Rowland and Harms, 1972; Hughes and Appleby, 1989; Knowles, 1990; Knowles and Broom, 1990; Fleming et al., 1994). The disorder was identified shortly after the introduction of battery cages and has become more prevalent with the increasing use of battery cages in commercial operations (Couch, 1955). Birds housed in battery systems had decreased cortical thickness in the humerus, decreased trabecular bone volume in the proximal tarsometatarsus and free thoracic vertebrae, and decreased breaking strength in the tibia and humerus as compared with birds housed in perchy cages and floor pens (Fleming et al., 1994). Additionally, age-related decline in bone integrity is accelerated by limited exercise by chickens (Rowland and Harms, 1972). Therefore, the limited ability of caged birds to exercise is recognized as a major cause of the disease (Rowland et al., 1968; Rowland and Harms, 1970; Hughes and Appleby, 1989; Knowles, 1990; Knowles and Broom, 1990; Fleming et al., 1994). In addition to lack of exercise, genetic selection for earlier sexual maturity, low BW to improve feed efficiency, and a high rate of lay may also contribute to osteoporosis in laying hens (Urist and Deutsch, 1960; Whitehead and Wilson, 1992; Gregory and Wilkins, 1996; Newman and Leeson, 1997).

The impact of osteoporotic bone fractures on the poultry industry has created a growing need for techniques to monitor bone quality in chickens. Many invasive techniques are currently used in chickens for measuring bone quality, including bone breaking traits, bone ash weight, and histomorphometric measurements such as trabecular and cortical bone volume, number of trabeculae, and characterization of the trabecular network (Frost et al., 1990; Frost and Roland, 1991; Clunies et al., 1992; Whitehead and Wilson, 1992; Orban et al., 1993a; Cranseberg et al., 2001; Hall et al., 2003). These techniques do not allow sequential observations of bone quality and thus require different groups of animals over time to evaluate changes in bone, introducing variation. Meyer et al. (1968) adapted a noninvasive technique for measuring bone mineral content using a monoenergetic photon beam, or single photon absorptiometry (SPA), developed by Cameron and Sorenson (1963) for use in chickens. This technique was used to detect changes in bone mineral content (BMC) of the tibia in turkeys when fed varying levels of dietary phosphorus. The BMC determined using SPA compared well with tibial bone ash (r = 0.958) and an increase in dietary phosphorus levels resulted in an increase in BMC of the tibia (Akpe et al., 1987). Thus, photon absorptiometry has been shown to be an effective tool in detecting differences in bone mass in poultry in vivo (Meyer et al., 1968, 1971; Beljan et al., 1971; Miller and Sunde, 1975a,b; Akpe et al., 1987; Rao et al., 1993) as well as with excised bones (Cantor et al., 1980; Frost and Roland, 1991; Orban et al., 1993a,b; Rao et al., 1993; Orban et al., 1999).

Dual-energy x-ray absorptiometry (DEXA) has been recently developed and is commonly used to predict bone quality in humans, dogs, and rats (Johnston et al., 1991; Ammann et al., 1992; Markel et al., 1994; Beamer et al., 2001; Libouban et al., 2002). The densitometric readings obtained using DEXA are lower than the BMC due to the thickness of bone (Faulkner et al., 1991; Genant et al., 1991; Markel et al., 1991, 1994; Libouban et al., 2002).

The dependence of the densitometric readings on bone geometry and thickness when using DEXA introduces potential error if there is inconsistent positioning of the bone. Bones of birds that are to be scanned must be placed in the same position to minimize variation due to orientation. Our laboratory has adapted the use of DEXA to chickens (Schreiweis et al., 2003) and tested the repeatability of the densitometer. The tibia and humerus of 10 White Leghorns were scanned twice to obtain duplicate readings. The birds were removed from the restraint device and then restrapped between scans to account for variation in orientation. The mean CV of the duplicate readings for the tibia and humerus were 3.3 and 2.2%, respectively (Hester et al., 2004). This repeatability has also been observed in using DEXA of live rats to measure the BMD of lumbar spine, proximal tail vertebrae, and tibia. The mean CV for 4 to 6 measurements per location in 7 rats ranged from 0.66 to 1.36% (Ammann et al., 1992).

The first objective of the current study was to validate the use of DEXA in chickens by comparing densitometric scans in vivo with excised bone scans. Additionally, densitometric scans were compared with traditional invasive measurements of bone strength including bone breaking strength measurements and bone ash weight. The validation of DEXA in chickens may contribute to selection for increased bone strength as well as genetic studies to identify regulatory factors in bone metabolism.

**MATERIALS AND METHODS**

**Experiment 1**

A 67-wk trial was conducted with 30 pedigreed Hy-Line White Leghorn female breeders. One-day-old chicks were housed in wire cages with 8 chicks per cage, providing 465 cm²/bird. Chicks were fed a starter diet from 0 to 5 wk, a grower diet from 6 to 7 wk, a developer diet from 8 to 14 wk, and a prelay diet from 15 to 17 wk of age (Table 1). At 17 wk of age, each bird was transferred...
TABLE 1. Composition of the control diets (experiment 1).

<table>
<thead>
<tr>
<th>Ingredient, %</th>
<th>Starter 0 to 5 wk</th>
<th>Grower 6 to 7 wk</th>
<th>Developer 8 to 14 wk</th>
<th>Prelay 15 to 17 wk</th>
<th>Breeder 18 to 65 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn (6.12% CP), %</td>
<td>68.33</td>
<td>66.38</td>
<td>64.66</td>
<td>59.66</td>
<td>50.81</td>
</tr>
<tr>
<td>Soybean meal (47.50% CP), %</td>
<td>27.97</td>
<td>20.46</td>
<td>12.53</td>
<td>12.53</td>
<td>29.17</td>
</tr>
<tr>
<td>Wheat middlings, %</td>
<td>—</td>
<td>10.00</td>
<td>20.00</td>
<td>20.00</td>
<td>—</td>
</tr>
<tr>
<td>Oyster shell, %</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.00</td>
<td>—</td>
</tr>
<tr>
<td>Limestone, ground, %</td>
<td>1.00</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
<td>8.07</td>
</tr>
<tr>
<td>Dicalcium phosphate, %</td>
<td>1.85</td>
<td>1.50</td>
<td>1.30</td>
<td>1.30</td>
<td>1.84</td>
</tr>
<tr>
<td>Soybean oil, %</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.47</td>
</tr>
<tr>
<td>Salt, %</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin-mineral premix1, %</td>
<td>0.28</td>
<td>0.25</td>
<td>0.23</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Mold inhibitor2, %</td>
<td>0.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.05</td>
</tr>
<tr>
<td>L-Lys-HCL, %</td>
<td>0.10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>dl-Met, %</td>
<td>0.08</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.15</td>
</tr>
<tr>
<td>Antioxidant3, %</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.02</td>
</tr>
<tr>
<td>Sand, %</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Total, %</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Calcd nutrient composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>2,976</td>
<td>2,910</td>
<td>2,843</td>
<td>2,675</td>
<td>2,822</td>
</tr>
<tr>
<td>CP, %</td>
<td>17.60</td>
<td>15.39</td>
<td>13.07</td>
<td>12.77</td>
<td>17.05</td>
</tr>
<tr>
<td>Met, %</td>
<td>0.36</td>
<td>0.31</td>
<td>0.26</td>
<td>0.26</td>
<td>0.42</td>
</tr>
<tr>
<td>Met + Cys, %</td>
<td>0.68</td>
<td>0.60</td>
<td>0.53</td>
<td>0.53</td>
<td>0.73</td>
</tr>
<tr>
<td>Lys, %</td>
<td>1.04</td>
<td>0.80</td>
<td>0.63</td>
<td>0.62</td>
<td>0.99</td>
</tr>
<tr>
<td>Total P, %</td>
<td>0.74</td>
<td>0.71</td>
<td>0.70</td>
<td>0.69</td>
<td>0.65</td>
</tr>
<tr>
<td>Nonphytate P, %</td>
<td>0.48</td>
<td>0.42</td>
<td>0.38</td>
<td>0.38</td>
<td>0.44</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.90</td>
<td>0.81</td>
<td>0.72</td>
<td>0.62</td>
<td>3.60</td>
</tr>
</tbody>
</table>

1Provided per kilogram of diet: vitamin A, 8,800 IU; vitamin D3, 2,695 IU; vitamin E, 33 IU; vitamin K, 1.2 mg; riboflavin, 6.6 mg; pantothenic acid, 11 mg; niacin, 33 mg; choline, 385 mg; vitamin B12, 0.011 mg; pyridoxine, 3.63 mg; thiamine, 0.11 mg; folic acid, 0.88 mg; biotin, 0.11 mg; Mn, 82.5 mg; Zn, 66 mg; Fe, 44 mg; Cu, 4.4 mg; I, 1.1 mg; and Se, 0.297 mg.

2Myco-Curb Dry, Kemin Industries, Inc., Des Moines, IA.

3Ethoxyquin (66%), Prime Quality Feeds, North Little Rock, AR.

to an individual laying cage (1,084 cm²/bird) and at 18 wk of age was fed a breeder diet (Table 1).

Five birds per age were randomly selected and the BMD and BMC of the left leg (tibia and fibula) and humerus were measured once in the morning (0700 to 1100 h) at 17, 27, 37, 47, 57, and 67-wk of age. The live, unanesthetized birds were restrained on their back in a foam holding device and secured with Velcro straps (Schreiweis et al., 2003). Using a pDEXA x-ray bone densitometer (model 476D014), scanning began at the proximal end of the bone and took approximately 10 min for each bone. The orientation of the respective bone was the same for each scan. Individual BW was recorded following each scan. After the live scan, birds were euthanized by cervical dislocation, and the left humerus and tibia with its fibula were excised. The bones were cleaned of all tissue and rescanned in the same orientation as the live scan. After the scan, bones were wrapped in 0.85% saline-soaked gauze, placed in a plastic bag, and frozen at −7 to −10°C for later analysis of bone strength characteristics and ash weight as described by Schreiweis et al. (2003).

With the exception of birds at 17 wk of age, 2 eggs were collected from each bird immediately prior to each scan. Individual egg weight was measured, the yolk and albumen were siphoned from the egg with a syringe, the shell with intact membranes was rinsed and dried, and shell weight was recorded. Shell thickness and percentage of shell were determined as described by Klingensmith and Hester (1985). Each egg trait was measured on 2 eggs collected from each hen at each age; the measurements per hen per age were averaged prior to statistical analysis.

**Experiment 2**

A 26-wk trial was conducted with 45 pedigree Hy-Line White Leghorn female breeders. Birds were housed in individual laying cages (1,084 cm²/bird) and provided feed and water ad libitum as described in experiment 1. Varying Ca diets (5.4, 3.6, and 1.8% Ca) were fed from 32 to 58 wk of age with 15 birds each receiving 1 of the 3 Ca diets (see Schreiweis et al., 2003, for composition of diets).

The BMD and BMC of the left leg (tibia and fibula) and humerus were measured at 38, 48, and 58 wk of age. Individual BW was recorded following each scan. After the live scan, birds were euthanized by cervical dislocation, and the left humerus and tibia with its fibula were excised. The bones were cleaned of all tissue and rescanned in the same orientation as the live scan.

**Statistical Analysis**

For experiment 1, the BMD and BMC were analyzed by an analysis of covariance with BW as a covariate. The
TABLE 2. Bone, egg traits, and body weight of White Leghorns between the ages of 17 and 67 wk (experiment 1)

<table>
<thead>
<tr>
<th>Age</th>
<th>Bone mineral density</th>
<th>Bone mineral content</th>
<th>Bone ash weight</th>
<th>Bone breaking force</th>
<th>Stress</th>
<th>Modulus of elasticity</th>
<th>Egg weight</th>
<th>Shell weight</th>
<th>Shell thickness</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>0.161</td>
<td>1.89</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1,166</td>
</tr>
<tr>
<td>27</td>
<td>0.151</td>
<td>1.64</td>
<td>1.8</td>
<td>14.4</td>
<td>9</td>
<td>424</td>
<td>57</td>
<td>5.5</td>
<td>9.6</td>
<td>0.36</td>
</tr>
<tr>
<td>37</td>
<td>0.146</td>
<td>1.62</td>
<td>1.5</td>
<td>12.8</td>
<td>10</td>
<td>368</td>
<td>61</td>
<td>5.6</td>
<td>9.2</td>
<td>0.37</td>
</tr>
<tr>
<td>47</td>
<td>0.156</td>
<td>1.74</td>
<td>1.8</td>
<td>14.1</td>
<td>9</td>
<td>431</td>
<td>62</td>
<td>6.0</td>
<td>9.7</td>
<td>0.45</td>
</tr>
<tr>
<td>57</td>
<td>0.146</td>
<td>1.60</td>
<td>1.6</td>
<td>12.7</td>
<td>11</td>
<td>608</td>
<td>67</td>
<td>6.0</td>
<td>8.9</td>
<td>0.44</td>
</tr>
<tr>
<td>67</td>
<td>0.196</td>
<td>2.13</td>
<td>2.1</td>
<td>15.2</td>
<td>11</td>
<td>442</td>
<td>67</td>
<td>6.2</td>
<td>9.1</td>
<td>0.37</td>
</tr>
<tr>
<td>SEM</td>
<td>0.005</td>
<td>0.07</td>
<td>0.1</td>
<td>0.9</td>
<td>2</td>
<td>98</td>
<td>2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Orthogonal contrast

Significance level

Linear  ** NS * NS NS NS *** NS NS NS ***

Quadratic *** *** * NS NS NS NS NS NS NS NS NS

1Values represent the mean of 5 hens averaged over 2 bones (tibia and humerus), or n = 10 observations per age.
2Values represent the mean of traits of eggs laid by 5 hens (1 to 2 eggs per hen), or n = 5 observations per age.
3Values represent the mean of 5 hens per age.

**P < 0.05; ***P < 0.01; ****P < 0.001.

fixed effects included age (17, 27, 37, 47, 57, and 67 wk), type of bone (tibia and humerus), and status of bone (live and excised bone scans). Bone strength traits were analyzed using an ANOVA with the fixed effects of type of bone and age. Egg traits were analyzed using an ANOVA with the fixed effect of age. Orthogonal polynomial comparisons were used to examine age effects. A Pearson's correlation analysis was performed on BW, egg traits, and bone traits as well as live bird vs. excised bone scans (Steel et al., 1997).

For experiment 2, the BMD and BMC were analyzed by an analysis of covariance using BW as the covariate. Fixed effects were as described for experiment 1 including age (38, 48, and 58 wk) with the addition of diet (5.4, 3.6, and 1.8% Ca). Differences of least square means were used to partition means for significant interactions. A Pearson's correlation analysis was performed on live bird vs. excised bone scans (Steel et al., 1997).

For both experiments, the individual bird was the experimental unit for all parameters measured in the study. The mixed model procedure of the SAS system was used (SAS Institute, 1988; Littell et al., 1996).

TABLE 3. Correlation values for bone traits, egg traits, and body weight of White Leghorns between the ages of 17 and 67 wk (experiment 1)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Bone mineral density</th>
<th>Bone mineral content</th>
<th>Bone breaking force</th>
<th>Bone ash weight</th>
<th>Percentage shell</th>
<th>Shell thickness</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral density†</td>
<td>0.68***</td>
<td>0.73***</td>
<td>–0.04</td>
<td>–0.09</td>
<td>0.48***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral content†</td>
<td>0.58***</td>
<td>0.92***</td>
<td>0.03</td>
<td>–0.01</td>
<td>0.57**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone breaking force</td>
<td>0.62***</td>
<td>–0.01</td>
<td>–0.08</td>
<td>0.33*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Includes live bird scans only.

**The r values are significant at P < 0.05.

**The r values are significant at P < 0.01.

***The r values are significant at P < 0.001.

RESULTS

Experiment 1

Age effects showed an increase in BMD and BMC at 17 and 67 wk of age relative to the other ages observed (linear and quadratic effect for BMD, quadratic effect for BMC, P < 0.01). Between 27 and 57 wk of age, values for BMD and BMC were unchanged (Table 2). Likewise, bone ash weight increased at 67 wk of age (linear and quadratic effect, P < 0.05). No age effects occurred with bone breaking force, stress, or modulus of elasticity between 27 and 67 wk of age. Egg weight and BW increased linearly with age (P < 0.001) with no age effects observed in the remaining egg traits (Table 2).

A high correlation existed between BMD and BMC (r = 0.85, P < 0.001, Table 3). In addition, BMD and BMC were positively correlated with other bone quality traits. The BMD was correlated with bone breaking force (r = 0.68, P < 0.001) and bone ash weight (r = 0.73, P < 0.001). Similarly, the BMC correlated with bone breaking force (r = 0.58, P < 0.001) and bone ash weight (r = 0.92, P < 0.001).
FIGURE 1. Comparison of the bone mineral density and content of live bird scans with excised bone scans of the tibia and humerus (experiment 1). Least square means ± SEM with no common letter are significantly different \( (P < 0.05) \). Each value represents 5 hens averaged over 2 bones (tibia and humerus) and 6 ages (17, 27, 37, 47, 57, and 67 wk) or \( n = 60 \) observations.

Densitometric scans were not correlated with modulus of elasticity or bone stress. Neither the densitometric scans nor the traditional bone quality measurements were correlated with eggshell traits (percentage of shell and shell thickness). The bone traits were moderately and positively correlated with BW, whereas the egg traits showed no correlation with BW (Table 3).

The BMD and BMC of excised bone scans responded in the same manner as the live bird scans to age for the humerus and tibia (nonsignificant interactions between age and status of bone as well as type of bone and status of bone). Therefore, results presented in Figure 1 are averaged across type of bone and age. There was no difference in the BMD of live bird scans as compared with the BMD of the excised bone scans resulting in a high correlation between the 2 types of scans \( (r = 0.75 \text{ for the tibia and } 0.91 \text{ for the humerus}, \ P < 0.0001, \text{Figures 1 and 2}) \). The BMC of the excised bone scan was lower than that of the live bird scan \( (P < 0.05, \text{Figure 1}) \); however, the live bird scans and excised bone scans were highly and positively correlated \( (r = 0.73 \text{ for the tibia and } 0.89 \text{ for the humerus}, \ P < 0.0001, \text{Figure 3}) \). Additionally, bone ash weight was highly correlated with BMD and BMC of live bird scans \( (r = 0.73 \text{ and } 0.92, \text{respectively}, \ P < 0.001; \text{Table 3}) \) as well as with the BMD and BMC of excised bone scans \( (r = 0.73 \text{ and } 0.97, \text{respectively}, \ P < 0.001; \text{data not presented}) \).

Experiment 2

The effects of manipulating Ca in the diet on BMD, BMC, and bone breaking traits as well as correlations between bone and egg traits have been reported previously (Schreiweis et al., 2003). As hens consumed diets with less Ca, the BMD and BMC of the tibia and humerus from live bird scans as well as bone breaking traits of excised bones decreased linearly \( (P < 0.01) \). The BMD and BMC of excised bone scans responded in the same manner as the live bird scans to dietary Ca (nonsignificant interactions between dietary Ca and status of bone). Therefore, results presented in Figures 4 and 5 are averaged across diets. The BMD and BMC of the excised tibia were lower than that of the tibial scans of live birds. However, the BMD and BMC of the humerus of live bird scans did not differ from the scans of the excised humerus, resulting

FIGURE 2. Comparison of bone density scans of live and excised bones (experiment 1). Live scans of the tibia and humerus are correlated with excised bone scans \( (r = 0.73 \text{ and } 0.91, \text{respectively}, \ P < 0.0001) \).

FIGURE 3. Comparison of bone content scans of live and excised bones (experiment 1). Live scans of the tibia and humerus are correlated with scans of excised bones \( (r = 0.73 \text{ and } 0.89, \text{respectively}, \ P < 0.0001) \).
Comparison of the bone mineral density of live bird scans and excised bone scans of the tibia and humerus (experiment 2). Within a bone, least square means ± SEM without a common letter are significantly different (type of bone × status of bone interaction, \( P < 0.01 \)). No significant interactions between dietary Ca and status of bone were observed; therefore, results are averaged across diets. Each value represents 5 hens averaged over 3 diets (high, control, and low Ca diets) and 3 ages (38, 48, and 58 wk) or \( n = 45 \) observations.

in a type of bone by status of bone interaction \((P < 0.01)\). High correlations occurred between the BMD and BMC of the live bird and excised bone scans for the tibia \((r = 0.87 \text{ and } 0.82, \text{ respectively}, \ P < 0.001)\) and humerus \((r = 0.94 \text{ and } 0.93, \text{ respectively}, \ P < 0.001)\). Additionally, bone ash weight was highly correlated with BMD and BMC of live bird scans \((r = 0.77 \text{ and } 0.94, \text{ respectively}, \ P < 0.001; \text{ data not presented})\) as well as with the BMD and BMC of excised bone scans \((r = 0.74 \text{ and } 0.99, \text{ respectively}, \ P < 0.001; \text{ data not presented})\).

**DISCUSSION**

The results of this study as well as the report of Schreiweis et al. (2003) suggest that DEXA is a useful tool for the noninvasive evaluation of skeletal integrity in live hens. This conclusion is based on the positive correlations between live bird and excised bone scans \((r = 0.73 \text{ to } 0.94, \ P < 0.001)\) as well as between live scans and more traditional invasive bone measurement tests such as bone breaking force \((r = 0.58 \text{ to } 0.68, \ P < 0.001)\) and bone ash weight \((r = 0.73 \text{ to } 0.99, \ P < 0.001)\). In addition, live bird scans of hens consuming diets of 5.4, 3.6, or 1.8% Ca showed a linear decrease \((P < 0.01)\) in BMD and BMC of the tibia and humerus as Ca levels decreased in the diet (Schreiweis et al., 2003). The only discrepancy to date with regard to validating the accuracy of DEXA in live birds for the determination of bone mineralization is that in some instances, live scans had higher values than excised bone scans (BMC, Figure 1). Although the BMD and BMC of the humerus in experiment 2 were similar between the live bird and excised bone scans, the live bird scans of the tibia showed higher BMD (Figure 4) and BMC (Figure 5) than the excised bone scans. Soft tissue causes an overestimation of the BMD when using DEXA (Lochmiller et al., 2001). The larger amount of soft muscle tissue surrounding the tibia as compared with the humerus might have contributed to the higher readings during the live scans. Another possibility is that the bone positioning during scans between the live and excised bone scans were dissimilar. The positioning of the bone is critical in determining BMD using DEXA due to its 2-dimensional display (Markel et al., 1994). Any rotation of the bone causes a change in the area of the bone that is observed, altering the BMD and BMC. Therefore, the removal of soft tissue prior to the excised bone scan might have caused positioning errors in that the placement of the cleaned tibia did not mimic the in vivo orientation exactly. These complications were not observed in the densitometric scans of the humerus due to the smaller amount of soft muscle tissue surrounding the bone.

The BMD was correlated with bone breaking force and bone ash weight \((r = 0.68 \text{ and } 0.73, \text{ respectively}, \ P < 0.001, \text{ Table 3, experiment 1})\). This significantly positive correlation between measurements was also observed in the rat with the correlation of BMD of the tibia as determined by DEXA to bone ash weight reported at \( r = 0.88 \) (Libouban et al., 2002). Similar results were reported in chickens when excised bones were measured with densitometry (Meyer et al., 1968; Cantor et al. 1980; and Akpe et al. 1987). Meyer and Sunde (1974) reported bone breaking strength in chicken tibia and humerus to be positively correlated with bone mass as determined by SPA \((r = 0.96)\). Similar results have been reported in the medical field using excised femur and vertebrae (Faulkner et al., 1987). Previous work conducted in our laboratory reported that BMD and BMC were positively correlated with bone breaking force \((r = 0.65 \text{ and } 0.49, \text{ respectively})\) and bone ash weight \((r = 0.77 \text{ and } 0.94, \text{ respectively})\) in laying hens fed varying levels of dietary Ca (Schreiweis et al., 2003).

The significantly positive correlations between densitometric scans and bone breaking force \((r = 0.58 \text{ to } 0.68, \ P < 0.001)\) as well as bone ash weight \((0.73 \text{ to } 0.99, \ P < 0.001)\)
0.001) suggest that a low BMD may indicate increased susceptibility to fracture and thus may be useful in selecting chickens for improved skeletal integrity. Mandour et al. (1989) was able to increase humerus strength of broilers after 3 generations of selection. Similarly, Bishop et al. (2000) created a selection program in which skeletal integrity of hens was improved in a high index line as compared with a low bone index line after 7 generations of divergent selection. Birds of the high bone index line as compared with the low bone index line had greater titubal and humeral breaking strengths and higher radiographic densities of the keel as measured by a Faxitron 405 soft x-ray apparatus (Fleming et al., 1994, Bishop et al., 2000). All of these measurements conducted by Bishop et al. (2000) were done in old hens at the end of lay and were invasive. Because BMD of the current study was significantly correlated to bone breaking force (r = 0.58 to 0.68, P < 0.001), which is similar to the invasive measurement of breaking strength used by Bishop et al. (2000), potential exists for use of DEXA in selecting live birds for improved bone mineralization, perhaps early in their life cycle, without having to use full sibs or progeny of the euthanized birds as was done by Mandour et al. (1989) and Bishop et al. (2000).

An increase in BMD and BMC occurred at 67 wk of age (P < 0.001, Table 2). A similar increase in BMD with age was observed in a previous study conducted in our laboratory (Schreweis et al., 2004) and was likely due to the accumulation of medullary bone throughout the laying cycle. Medullary bone is as radiographically dense as structural bone (Whitehead, 2004), resulting in an increase in BMD with accumulation of medullary bone. Medullary bone develops in the marrow space of the long bones, such as the tibia, and small amounts of medullary bone have been found to develop in the pneumatic bones including the humerus. The presence of medullary bone in the humerus increases its breaking strength (Fleming et al., 1996), thus reflecting the positive correlation between BMD and bone breaking force. The BMD of 17-wk-old pullets was higher than the BMD from hens aged 27 and 37 wk because they had not yet initiated egg laying and, therefore, were not using bone Ca for shell formation. In addition, it is likely that a decrease in egg production as the hens aged also contributed to the increase in BMD and BMC at 67 wk of age because of a lesser need to mobilize Ca from the bone for shell formation.

Linear increases in egg weight and BW were observed as the birds aged; however, no differences were observed in the shell traits measured (Table 2). This finding contradicts a previous study conducted in our laboratory in which we found increased shell weight and decreased thickness and percentage of shell with age (Schreweis et al., 2004). The smaller number of observations in the current study as compared with the earlier study might have affected the statistical power to determine significant results. In the current study, different birds were observed at each age, whereas in the previous study, the same birds were used and repeated measurements were collected, resulting in lower variation within bird at different ages as compared with between birds. There was little correlation between bone traits and shell traits in this study as well as in a previous study (Schreweis et al., 2003). Rennie et al. (1997) found that the correlation between egg production and the trabecular bone volume of the proximal tarso-metatarsus and free thoracic vertebrae of 68-wk-old hens in a commercial population to be 0.00 and −0.16, respectively. Similarly, Bishop et al. (2000) reported no difference in egg production between the high and low bone index lines over a laying year; however, a decrease in shell thickness was observed in the high bone index line (Whitehead, 2004).

In conclusion, we have demonstrated that densitometric bone scans of the tibia and humerus of live birds are positively and significantly correlated to densitometric scans of excised bones. The traits of BMD and BMC correlated well with invasive bone measurements such as bone breaking strength and bone ash weight. These results suggest that DEXA is a useful tool for the noninvasive evaluation of skeletal integrity in live hens.

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REFERENCES


