Research Note

Effects of Different Bone Preparation Methods (Fresh, Dry, and Fat-Free Dry) on Bone Parameters and the Correlations Between Bone Breaking Strength and the Other Bone Parameters

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ABSTRACT A study was conducted to evaluate effects of different bone preparation methods on bone parameters and the correlations between bone breaking strength and the other bone parameters. Bone breaking strength is dramatically changed depending on bone preparation methods, whereas other bone parameters such as ash content and ash concentration are not significantly influenced by bone preparation methods. Thus, the objective of this study was to evaluate the effects of 3 bone preparations (fresh, dry, and fat-free dry) on bone parameters and the relationship between bone breaking strength and bone parameters. Sixty Single Comb White Leghorn hens were used for this study. Hens were euthanized by CO_2 gas, and the right tibia and femur were collected. The bones were divided into 3 treatment groups: fresh, dry, and fat-free dry. There were no significant differences in fresh weight, bone volume, dried weight, ash weight, and ash concentration of tibia and femur among the treatments. However, fresh tibia (24.13 kg) exhibited more bone breaking strength compared with the dried (9.90 kg) and fat-free dried bones (7.41 kg) ($P < 0.05$). The bone breaking strength (20.97 kg) of fresh femur was also significantly higher than the dried (9.22 kg) and fat-free dried femurs (6.94 kg). The bone breaking strength of the fresh bone was highly correlated with dried weight, ash weight, and ash concentration, whereas that of the fat-free dried bone was poorly correlated with the other bone parameters. The results indicate that fresh bone gives better bone breaking strength correlated to the other bone parameters than dry or fat-free dry preparation.

(*Key words*: bone breaking strength, bone preparation, ash weight, ash concentration)

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INTRODUCTION

Bone weakness problems in laying hens are important welfare and economic issues for the poultry industry. Widespread structural bone loss in laying hens can cause high incidences of fractures at various sites of the skeleton (Whitehead and Fleming, 2000). In particular, the structural bone loss related to osteoporosis can enhance the skeletal fragility and contribute to the high fracture incidence at the end of the laying cycle in old laying hens (Gregory and Wilkins, 1989). Thus, accurate measurements for bone status in laying hens are critical to develop nutritional and management strategies that can reduce structural bone loss in laying hens. There are several bone measurements to evaluate bone status: bone ash (Garlich et al., 1982; Al-Batshan et al., 1994; Kim et al., 2003; Park et al., 2003), bone ash concentration (Garlich et al., 1982; Cheng and Coon, 1990; Kim et al., 2003), bone mineral content (Kim et al., 2003; Schreiweis et al., 2003), bone density (Watkins and Southern, 1992; Orban et al., 1993; Kim et al., 2003; Schreiweis et al., 2003), and bone breaking strength (Crenshaw et al., 1981; Ruff and Hughes, 1985; Fleming et al., 1998; Park et al., 2003; Schreiweis et al., 2003). Among the measurements, bone breaking strength is one of the most accurate parameters to evaluate direct bone fracture resistance. One of the common traits of bone breaking strength is bending moment, which is a measure of the amount of force withstood by the bone (Crenshaw et al., 1981). However, values of bone breaking strength are different depending on bone preparation methods (Lott et al., 1980; Park et al., 2003). Crenshaw et al. (1981) indicated that wet bones bend more than dry bones and even short exposure to air can change in the mechanical properties of wet bones. Lott et al. (1980) reported that the breaking strength of dry tibia (11.24 kg) was significantly lower than that of fresh tibia (27.02 kg). Park et al. (2003) indicated that breaking strength was significantly higher in refrigerated tibias (2.48 kg/g) than in frozen bones (1.75 kg/g),

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TABLE 1. Effects of different bone preparations (fresh, dry, and fat-free dry) on various bone parameters of tibia

Bone parameters	Fresh	Dry	Fat-free dry	Pooled SE
Fresh weight (g)	8.584	8.729	8.628	0.184
Bone volume (cm^3)	6.386	6.310	6.320	0.130
Dried weight (g)	5.777	5.801	5.778	0.149
Ash weight (g)	2.774	2.948	2.967	0.112
Ash concentration[1] (g/cm^3)	0.437	0.467	0.470	0.014
Bone breaking strength (kg)	24.13[a]	9.90[b]	7.41[c]	0.760

[a–c]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Ash concentration = ash weight/bone volume.

TABLE 2. Effects of different bone preparations (fresh, dry, and fat-free dry) on various bone parameters of femur

Bone parameter	Fresh	Dry	Fat-free dry	Pooled SE
Fresh weight (g)	7.538	7.679	7.676	0.173
Bone volume (cm^3)	5.543	5.589	5.584	0.120
Dried weight (g)	4.287	4.463	4.432	0.132
Ash weight (g)	2.288	2.483	2.468	0.099
Ash concentration[1] (g/cm^3)	0.414	0.447	0.444	0.015
Bone breaking strength (kg)	20.97[a]	9.22[b]	6.94[b]	0.811

[a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Ash concentration = ash weight/bone volume.

whereas there was no difference for bone ash between refrigerated storage and frozen storage. Therefore, the objectives of the study were 1) to evaluate various bone parameters from different bone preparation methods (fresh, dry, and fat-free dry) and 2) to evaluate correlation between bone breaking strength and the other bone parameters to determine which preparation methods yield better bone breaking strength values.

MATERIALS AND METHODS

Samples

Sixty Single Comb White Leghorn hens were used for this study. Hens were euthanized by CO_2 gas, and right tibia and femur were obtained from each hen. The bones were cleaned of attached tissue and divided into 3 groups: fresh, dry, and fat-free dry.

Bone parameters were measured according to the methods described by Zhang and Coon (1997) and Park et al. (2003). All bones were first weighed in the presence of air, then reweighed while suspended in water at room temperature. Bone volume was calculated with the assumption that the specific gravity of water is 1 g/cm^3 at room temperature. For the fresh bone preparation, breaking strength was first measured, and bones were then dried at 100°C for 24 h and weighed again. The bones were then ashed at 600°C for 24 h, cooled in a desiccator, and weighed. For the dry bone preparation, the bones were first dried at 100°C for 24 h and weighed again, and bone breaking strength was then measured. After breaking strength measurement, the bones were ashed at 600°C for 24 h, cooled in a desiccator, and weighed. For the fat-free dry preparation, the bones were dried at 100°C for 24 h. To determine their fat-free dry matter, the dried bones were refluxed in a Soxhlet apparatus for 48 h in 333 mL of 95% ethanol and 667 mL of benzene at 70°C. The fat-free bones were dried in an oven at 100°C for 24 h and weighed. After breaking strength measurement, the bones were subsequently ashed at 600°C overnight, cooled in a desiccator, and weighed. Bone ash concentrations were calculated by dividing the ash weight of each bone by its volume. This measurement has been reported to best reflect the bone status of laying hens (Zhang and Coon, 1997). Bone breaking strength was measured using an Instron universal testing machine[2] with a 50-kg load cell at 50-kg load range with a crosshead speed of 50 mm/min with bone supported on a 3.00-cm span (Park et al., 2003). Animal care procedures described herein were approved by Texas A&M University Institutional Animal Care and Use Committee.

Statistical Analysis

All data were subjected to 1-way ANOVA as a completely randomized design using the GLM procedure of SAS (SAS Institute, 2001). Significant differences among the means were determined using Duncan's multiple-range test at $P < 0.05$. Correlations of bone parameters were evaluated by Pearson correlation procedures.

RESULTS AND DISCUSSION

Various bone parameters of tibia using different bone preparations are shown in Table 1. There were no significant differences in fresh weight, bone volume, dried weight, ash weight, or ash concentration among the different bone preparations ($P > 0.05$). However, the bone breaking strength of the dry and fat-free dry preparations (9.90 and 7.41 kg, respectively) were significantly lower compared with that of the tibia from the fresh preparation (24.13 kg) ($P < 0.05$). The fat-free dry preparation reduced the bone breaking strength compared with the dry preparation ($P < 0.05$). The various bone parameters of femur using different bone preparations are shown in Table 2. The results of femur parameters using different bone preparations showed similar trends to those of tibia parameters. The bone breaking strength of the fresh preparation (20.97 kg) was significantly greater than that of the dry (9.22 kg) or fat-free preparation (6.94 kg) ($P < 0.05$). However, there were no significant differences in fresh weight, bone volume, dried weight, ash weight, and ash concentration ($P > 0.05$). These results indicated that the bone breaking strength was greatly influenced by bone preparation methods.

[2]Model 1011, Instron Corp., Canton, MA.

TABLE 3. The effect of tibia preparations (fresh, dry, and fat-free dry) on the correlations between bone breaking strength and the other bone parameters

	Bone breaking strength		
Bone parameters	Fresh	Dry	Fat-free dry
Fresh weight	0.526*	0.502*	–0.012
Dried weight	0.484*	0.620**	0.216
Ash weight	0.796***	0.547*	0.158
Ash concentration	0.735***	0.462*	0.383

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

The present results are in agreement with the study of Lott et al. (1980). They reported that the bone breaking strength of dried tibia (11.24 kg) was significantly lower than that of fresh tibia (27.02 kg). Huyghebaert et al. (1988) evaluated the effects of fluoride treatments on bone breaking strength in broilers. They indicated that dry (10.4 kg) and fat-free bone (10.9 kg) preparations greatly reduced breaking strength compared with fresh bones (31.3 kg). Furthermore, they reported that the effect of dietary fluoride treatment on the bone breaking strength of the dried or defatted bones was completely different from that of fresh bone. When dietary fluoride levels increased from 0 to 400 ppm, tibia breaking strength of fresh bone decreased, whereas that of dried or defatted tibia gradually increased.

The effects of tibia preparations on the correlations between bone breaking strength and the other bone parameters are shown in Table 3. The bone breaking strength of the fresh tibia was highly correlated with fresh weight (0.526; $P < 0.05$), dried weight (0.484; $P < 0.05$), ash weight (0.796; $P < 0.001$), and ash concentration (0.735; $P < 0.001$). The bone breaking strength of the dry preparation also yielded high correlations with fresh weight (0.502; $P < 0.05$), dried weight (0.620; $P < 0.01$), ash weight (0.547; $P < 0.05$), and ash concentration (0.462; $P < 0.05$). However, the bone breaking strength of the fat-free preparation did not have any significant correlations with the other bone parameters ($P > 0.05$). The correlations between bone breaking strength and the other bone parameters of femur had similar trends to the results from the tibia preparations (Table 4). The bone breaking strength of the fresh femur had highly positive correlations with fresh weight (0.515; $P < 0.05$), dried weight (0.705; $P < 0.001$), ash weight (0.788; $P < 0.001$), and ash concentration (0.717; $P < 0.001$). However, the bone breaking strength from the dry or fat-free dry preparation did not have any correlations with the other bone parameters ($P > 0.05$). Schreiweis et al. (2003) indicated that the breaking strength of fresh prepared bone was positively correlated with bone ash weight (0.58; $P < 0.001$). The present study showed that a more accurate estimate of bone strength is obtained from fresh bone compared with dry or fat-free dry preparations. A heat or chemical treatment for drying or fat extraction appears to have a detrimental impact on bone integrity and strength. Therefore, the present study suggests that fresh bone preparation is beneficial for evaluating bone breaking strength reflecting intact bone integrity and strength.

TABLE 4. The effect of femur preparations (fresh, dry, and fat-free dry) on the correlations between bone breaking strength and the other bone parameters

	Bone breaking strength		
Bone parameters	Fresh	Dry	Fat-free dry
Fresh weight	0.515*	–0.302	–0.328
Dried weight	0.705***	–0.245	–0.386
Ash weight	0.788***	–0.323	–0.402
Ash concentration	0.717***	–0.228	–0.278

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

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