# Bone Mineral Density and Breaking Strength of White Leghorns Housed in Conventional, Modified, and Commercially Available Colony Battery Cages

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**ABSTRACT** Limited opportunity for movement and load-bearing exercise for conventionally caged laying hens leads to bone loss and increased susceptibility to osteoporosis, bone fractures, and cage layer fatigue, all of which compromise hen welfare and have negative consequences for production. The objective of this study was to compare bone mineral density (BMD) and strength measures of White Leghorns housed in conventional battery cages (CONV), cages modified to incorporate a nest box and perch (MOD), and commercially available, furnished colony cages with (CWDB) or without (CWODB) a raised dust bath. Hens reared on floor litter were randomly allocated to 1 of 4 cage systems at 19 wk of age. Hen-day production and egg quality were measured between 20 and 64 wk. At 65 wk, hens were killed, and right femur, tibia, and humerus were excised. Bone mineral density was assessed using quantitative computed tomography, and breaking strength was measured with an Instron Materials Tester. In the femur and tibia, CONV hens exhibited lower total BMD, bone mass, cortical bone area, cortical bone mass, and bone-breaking strength than CWDB, CWODB, and MOD hens. Density and cross-sectional area of bone in the trabecular space was highest in CONV. In the humerus, total and cortical BMD and mass and breaking strength values were higher for colonyhoused birds than hens in CONV and MOD. The MOD birds did not exhibit increased humeral BMD or strength measures over CONV hens. These findings provide evidence that hens housed in modified and colony cages, furnished systems that promote load-bearing movement, are better able to preserve cortical structural bone than conventionally caged hens and simultaneously have stronger bones. Furthermore, inclusion of raised amenities that encourage wing loading is necessary to reduce humeral cortical bone loss. The overall absence of correlation between egg production or quality and bone quality measures also suggests that improved bone quality in CWDB, CWODB, and MOD furnished cages is not the result of lowered egg production or quality.

Key words: laying hen, battery cage, bone mineral density, quantitative computed tomography, furnished cage

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# INTRODUCTION

It is evident from the high incidence of broken bones observed among hens throughout the production period, and during depopulation, transport, and shackling (Randall and Duff, 1988; Gregory and Wilkins, 1989; Budgell and Silversides, 2004), that osteoporosis has become a widespread condition in laying flocks. Osteoporosis, which is characterized by a progressive loss of fully mineralized structural bone throughout the skeleton, results in bone fragility, thereby increasing susceptibility to fracture (Whitehead and Fleming, 2000; Whitehead, 2004). In the extreme manifestation of structural bone loss, hens may succumb to cage layer fatigue, a condition characterized by spontaneous bone fracture, and vertebral weakening causing exposure of the spinal column and potential paralysis (Urist and Deutsch, 1960; Bell and Siller, 1962; Riddell et al., 1968). Acute and chronic pain, debilitation, and mortality resulting from osteoporotic fractures pose serious animal welfare concerns (Webster, 2004) and incur economic loss during the production period and at processing.

Osteoporosis may result, in part, from prolonged periods of high egg production during which structural bone is mobilized without opportunity for regeneration (Whitehead and Wilson, 1992; Knowles and Wilkins, 1998). At the onset of sexual maturity, cortical and trabecular structural bone formation is ceased in favor of woven, medullary bone deposition (Wilson et al., 1992; Hudson et al., 1993; Whitehead and Fleming, 2000). However, during the period of eggshell construction, mobilization of medullary bone to increase Ca availability (Whitehead and Fleming, 2000; Whitehead, 2004) also results in resorption of exposed structural bone (Dacke et al., 1993).

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Consequently, over the course of the production cycle, the net effect of cortical and trabecular bone resorption without subsequent reconstruction is structural bone loss and skeletal weakening.

As demonstrated in studies comparing bone quality and breaking strength measures of conventionally caged hens with those of birds housed in floor litter, perchery, or aviary systems (Rowland et al., 1968; Rowland and Harms, 1970; Knowles and Broom, 1990; Nørgaard-Nielsen, 1990; Fleming et al., 1994; Abrahamsson and Tauson, 1995; Newman and Leeson, 1998), osteoporosis is also influenced by the extent to which movement and exercise are permitted in a housing system. Flight, wing flapping, walking, and perching, all of which involve load bearing, appear to contribute to the improved bone condition observed in noncage systems (Knowles and Broom, 1990; Abrahamsson and Tauson, 1995). For caged hens, bone loss related to disuse may be minimized by providing birds with increased opportunity for movement, such as exposure to daily periods of exercise (Meyer and Sunde, 1974) or access to perches within the cage (Hughes and Appleby, 1989; Duncan et al., 1992; Hughes and Wilson., 1993; Wilson and Hughes, 1993).

As a result of the behavioral restrictions and limited opportunity for movement in conventional battery cages, many European countries have adopted legislative policies that regulate or prohibit the use of cage systems (SAWO, 1981; SFS, 1998; CEC, 1999; Tauson, 2003; BMELV, 2007). In North America, laying hen husbandry practices are not regulated by legislation, and conventional battery cages remain the predominant housing system. Egg producers are encouraged to adopt minimum space allowances for caged hens (CARC, 2003; UEP, 2006); however, it remains questionable whether the provision of additional floor space is adequate to promote the activity and behavioral repertoires required to maintain structural bone (Lanyon, 1996).

A study was conducted to develop a modified laying hen cage system that would promote activity and behavioral repertoires conducive to bone, and overall hen health and welfare. The modified system, developed from conventional battery cages altered to incorporate a nest box (**NB**) and perch, would potentially provide North American producers with a practical option for promoting hen welfare using existing cage capital. The objective of this paper was to compare bone mineral density and strength measures of laying hens housed in conventional cages, the modified system, and commercially available furnished colony cages to determine if bone health, and therefore hen welfare, could be improved in cage systems.

#### MATERIALS AND METHODS

#### Experimental Design

This research was authorized by the Faculty Animal Policy and Welfare Committee at the University of Alberta and was conducted in accordance with the Guide to the Care and Use of Experimental Animals (CCAC, 1993). Shaver White Leghorn (Pacific Pride Chicks, Abbotsford, British Columbia, Canada) layer chicks were raised in floor pens at a stocking density of 50 birds per pen. Chicks were beak-trimmed with a heated blade trimmer at 1 wk of age. At 19 wk, birds were randomly allocated to 1 of 4 cage treatments housed within the same room. Hens received a standard commercial layer diet in accordance with NRC requirements and primary breeder recommendations and were provided with ad libitum access to food and water throughout the trial. Day length was gradually increased from 10 to 14 h, between 20 and 24 wk. One additional hour of light between midnight and 0100 h was introduced at 30 wk and continued until the end of the trial. Beginning at 32 wk, feed was top-dressed twice weekly with 3 g of oystershell per bird. At 39 wk, this was altered to feeding 6 g of oystershell per bird, once per week.

## Cage Design

**Conventional.** The conventional (CONV) treatment consisted of 3 tiers of 14 six-hen layer cages measuring 60 cm wide, 45 cm deep, and 40 cm high at the rear. Cages in each tier were divided by installation of a vertical bar partition to give 28 three-hen units per tier. A total of 252 hens were housed in the 84 cages, each hen having access to 450 cm<sup>2</sup> of floor space (Figure 1).

# Furnished Cages

Modified. Three tiers of 28 standard 6-hen layer cages were modified by addition of a wooden NB and a softwood perch (MOD). The NB measured 24 cm wide, 45 cm deep, and 35 cm high at the rear and was lined with artificial turf. Access to the NB could be achieved through 1 of 2 entrances located at the front and rear of the cage, each measuring 12 cm wide and 15 cm high and raised 5 cm from the floor. A lightweight door was installed inside each NB and was opened and closed daily 30 min before lights were turned on and off, respectively. The perch, which extended from the NB to the opposite wall of the cage, was 30 cm long, 2.5 cm high, and 5 cm deep and was positioned 12.5 cm from the back of the cage and 32.5 cm from the front of the cage, at a height of 10 cm above the floor. Each of the 84 modified cages housed 3 hens, giving each of the 252 hens permanent access to 450 cm<sup>2</sup> of floor space as well as 360 cm<sup>2</sup> of nest space during the day (Figure 1).

**Colony Cage With Dust Bath and Colony Cage Without Dust Bath.** The furnished colony battery (Parent Stock Cage System, Specht Canada, Stony Plain, Alberta, Canada) consisted of 2 tiers of 12 cages, each measuring 120 cm wide and 110 cm deep. Each unit housed 26 birds and provided 450 cm<sup>2</sup> of floor space per hen. Metal NB integrated as a continuum of the cage measured 60 cm wide and 55 cm deep, providing an additional 126 cm<sup>2</sup> per bird. Access to the artificial turf-lined NB was not restricted and was gained through a single 20-cm-wide entranceway. Softwood perches extended the length of



Figure 1. Housing treatments: A) the conventional cage (CONV), B) the modified cage (MOD), and C) the furnished colony cage with dust bath (CWDB).

the cage on the side opposite the NB. Perches were 5 cm deep and 2.5 cm high. A metal dust bath (**DB**) measuring 60 cm wide and 20 cm deep was present in all cages and was made available for hen use in 12 randomly selected units (colony cage with dust bath, **CWDB**). To deter CWDB hens from nesting in the DB, the facility was opened daily at 1300 h and was closed 1 h before lights were turned off. Dust baths were filled with peat moss at opening, and because birds were inclined to consume this substrate, a small amount of peat moss was also deposited along the edge of the closed DB in the remaining 12 cages (colony cage without dust bath, **CWODB**). A total of 156 hens were housed in each of the CWDB and CWODB treatments (Figure 1).

In the above cage systems, all cage and NB floors were sloped at an angle of 7°. Conventional and colony battery systems were purchased from Specht Canada, and modifications to the conventional units were carried out at the University of Alberta Poultry Research Centre. Although the floor space allowance of 450 cm<sup>2</sup> per bird was consistent between housing conditions, in the instance that 1 or more hens entered a NB or dust-bathing facility in MOD or the colony cages, floor space availability for hens remaining on the cage floor was increased.

# Egg Production

In addition to quantifying total daily egg production per treatment group, per-cage hen-day production and egg quality was assessed on 2 consecutive days every 4 wk, from 20 to 64 wk of age. Eggs were weighed fresh and stored for 4 d at 13°C. All eggs from CWDB and CWODB cages, and eggs from 30 randomly selected CONV and MOD cages, were assessed for specific gravity using the flotation method (Hamilton, 1982). Eggs were then cracked, and shells with intact membranes were rinsed to remove albumen. Shells were dried overnight at room temperature, weighed, and thickness was assessed using an Ames micrometer (Model 25, BC Ames Company, Waltham, MA).

## Bone Quality

At 65 wk, hens were removed from their cages, weighed, and killed via cervical dislocation. Right humerus, tibia, and femur were excised, placed in individual plastic bags, and stored at –20°C. Prior to analysis, bones from 20 randomly selected hens per treatment were thawed overnight and cleaned of all tissue. Bone mineral density and cross-sectional area were assessed using quantitative computed tomography (QCT). Based on differences in bone mineral density, QCT permits distinction between cortical bone and bone in the trabecular space, which includes both trabecular and medullary bone mineral. Quantitative computed tomography therefore provides an indication of structural bone condition (Korver et al., 2004). Using a Stratec XCT scanner (Model 922010, Norland Medical Systems Inc., Fort Atkinson, WI) with XMENU software version 5.40C, bones were longitudinally scanned to set bone midpoints as the cross-sectional x-ray location. Cross-sectional analysis of a 1-mm bone section using threshold density values of 400 and 500 mg/cm<sup>3</sup> for trabecular and cortical bone separation, respectively (Korver et al., 2004), revealed total, cortical, and trabecular bone densities and areas. Density and area measures were then multiplied to calculate the mass (mg QCT) of total and cortical bone, and bone in the trabecular space, for each 1-mm section.

Bone-breaking strength analysis was conducted using an Instron Materials Tester (Model 4411, Instron Corp., Canton, MA) with Automated Materials Test System software version 8.09. Bones were cradled on 2 support points measuring 3 cm apart. Using a 50-kg load cell and a crosshead speed of 100 mm/min, the force of an attached shear plate measuring 8 cm in length and 1 mm wide was applied to the midpoint of the same facial plane of each bone. Breaking strength was recorded.

## Statistical Analysis

Humeral density and strength measures from 1 hen in each of CWODB and CONV treatments were excluded from calculated averages, because the humeral trabecular density values from these hens exceeded average treatment values by more than 2 SD.

Response variables were analyzed for statistical significance using the GLM procedure (SAS Institute, 2002) and average BW difference as a covariate. Average BW difference was calculated using the published 20-wk BW of the breeder as the initial value and the individual hen weight at 65 wk as the final measure. When the effect of treatment was found to be significantly different, means were separated using the least significant means comparison.

Coefficients (r) for correlating bone quality (density, area, and mass) and breaking strength with egg production (hen day) and quality (stored egg mass, specific gravity, eggshell thickness, and eggshell mass) measures were calculated using Pearson correlations (SAS Institute, 2002). Calculations were conducted both with treatments

combined, to examine overall relationships in this strain of hen, as well as for individual housing treatments, to assess treatment effect. Unless otherwise stated, the level of significance for all statistical analyses was assessed at P < 0.05.

#### **RESULTS AND DISCUSSION**

#### Bone Quality and Strength

Femur and Tibia. Femoral and tibial total bone mineral density and total bone mass (mg QCT) were significantly lower for CONV birds than for hens housed in CWDB and CWODB (Table 1). Because total cross-sectional area did not differ between treatments, reduced CONV total density and mass measures were likely not attributable to smaller external bone diameter values. Similar total bone area would be expected, because all birds in the current trial were of the same breed and age and were raised under the same conditions during periosteal bone development. Fleming et al. (1994), who compared humeral radiographs of hens housed in conventional cages, a perchery, aviary, or floor litter system also observed consistent mean bone diameter values across housing conditions. The lower total bone mineral density measure for CONV hens in the current study therefore likely reflects excessive bone mineral loss by birds whose movement was highly restricted. Hens in the furnished systems were able to step onto and roost on a perch, move about the nest, and in the colony cages could also jump up to and potentially bathe in the dust bath. Furthermore, the additional floor space available in the instance that 1 or more hens entered a NB or dust-bathing facility also permitted hens in furnished cages greater freedom of movement within the cage to perform behaviors such as wing and leg stretching, wing flapping, and sham dust bathing. In conventional cages, all of these activities are constrained by both the small surface area of the cage (Moinard et al., 1998) and the absence of a suitable amenity, and movement is likely insufficient to prevent loss of mineralized bone (Leyendecker et al., 2005; Vits et al., 2005).

The nature of this loss is further elucidated by cortical density, area, and bone mass measures. In both the femur and the tibia, cortical bone density did not differ significantly between treatments (Table 1). However, cortical bone area was significantly lower in the femur of CONV hens as compared with CWDB or CWODB hens, and in MOD, the difference approached significance (P = 0.07). The CONV hens also exhibited significantly lower tibial cortical bone area than CWDB hens. In addition, the overall amount (mg QCT) of femoral cortical structural bone was significantly lower in CONV as compared with CWDB, CWODB, and MOD, and in the tibia, CONV hens had significantly lower bone mass than CWDB and CWODB hens (Table 1). These findings suggest that although the density of remaining femoral and tibial cortical bone was similar for birds in the different cage systems, the width of the remaining cortex in these bones

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Table 1. Femur, humerus, and tibia quality of 65-wk-old White Leghorns housed in colony, conventional, and modified cages

Pomo		Density			Area			Mass per 1-mm section <sup>1</sup>		
type and housing <sup>2</sup>	n <sup>3</sup>	Total (mg/cm <sup>3</sup> )	Cortical (mg/cm <sup>3</sup> )	Trabecular (mg/cm <sup>3</sup> )	Total (mm <sup>2</sup> )	Cortical (mm <sup>2</sup> )	Trabecular (mm <sup>2</sup> )	Total (mg QCT)	Cortical (mg QCT)	Trabecular (mg QCT)
Femur										
CWDB	20	785.44 <sup>a</sup>	944.06	260.05	40.39	28.99 <sup>a</sup>	5.47 <sup>b</sup>	31.64 <sup>a</sup>	27.96 <sup>a</sup>	$1.57^{b}$
CWODB	20	763.75 <sup>ab</sup>	945.72	225.45	41.83	29.37 <sup>a</sup>	6.60 <sup>ab</sup>	31.89 <sup>a</sup>	27.97 <sup>a</sup>	1.63 <sup>b</sup>
CONV	20	672.21 <sup>c</sup>	985.93	261.69	40.32	22.87 <sup>b</sup>	11.50 <sup>a</sup>	27.02 <sup>b</sup>	20.75 <sup>b</sup>	3.27 <sup>a</sup>
MOD	20	710.63 <sup>bc</sup>	953.96	226.70	41.69	28.17 <sup>ab</sup>	10.23 <sup>ab</sup>	29.59 <sup>ab</sup>	25.40 <sup>a</sup>	2.57 <sup>ab</sup>
SEM		38.60	33.64	20.17	1.01	2.97	2.84	1.59	2.35	0.78
						Probabilit	ies			
Housing		0.0194	0.5693	0.1011	0.2500	0.1089	0.1149	0.0103	0.0086	0.1002
Humerus										
CWDB	20	216.20 <sup>a</sup>	1,109.05 <sup>a</sup>	4	39.86	10.79 <sup>a</sup>	27.69 <sup>b</sup>	8.53 <sup>a</sup>	12.13 <sup>a</sup>	_
CWODB	19	195.36 <sup>a</sup>	1,095.27 <sup>a</sup>	_	40.51	10.36 <sup>a</sup>	28.76 <sup>b</sup>	7.83 <sup>a</sup>	11.47 <sup>a</sup>	_
CONV	19	153.69 <sup>b</sup>	1,048.90 <sup>b</sup>	_	39.91	9.30 <sup>b</sup>	28.80 <sup>ab</sup>	6.07 <sup>b</sup>	9.59 <sup>b</sup>	_
MOD	20	141.33 <sup>b</sup>	1,042.53 <sup>b</sup>	_	43.08	9.13 <sup>b</sup>	31.84 <sup>a</sup>	5.92 <sup>b</sup>	9.48 <sup>b</sup>	_
SEM		19.01	16.05	_	1.97	0.42	1.77	0.66	0.51	_
						Probabilit	ies			
Housing Tibia		0.0003	< 0.0001	—	0.2686	0.0001	0.0873	0.0003	< 0.0001	_
CWDB	20	832.10 <sup>a</sup>	1.037.38	220.20	32.16	23.02 <sup>a</sup>	6.07	26.72 <sup>a</sup>	$24.40^{a}$	1.36 <sup>b</sup>
CWODB	20	809.36 <sup>b</sup>	1 049 06	235.49	32.32	21 72 <sup>ab</sup>	6.86	26.12 <sup>a</sup>	23.05 <sup>ab</sup>	1.64 <sup>ab</sup>
CONV	20	735.98°	1 057 35	246.04	31.81	19 41 <sup>b</sup>	9.45	23.32 <sup>b</sup>	19.36°	$2.40^{a}$
MOD	20	755.04 <sup>bc</sup>	1 037 48	218.94	32 54	21 44 <sup>ab</sup>	8 84	24.44 <sup>ab</sup>	21 39 <sup>bc</sup>	1 94 <sup>ab</sup>
SEM	20	35.97	28.58	15.27	0.71	1.79	1.82	1.06	1.47	0.45
		Probabilities								
Housing		0.0304	0.8644	0.2100	0.7453	0.2490	0.2103	0.0076	0.0074	0.1343

<sup>a-c</sup>Means within the same column and bone type lacking a common superscript differ significantly (P < 0.05).

<sup>1</sup>QCT = quantitative computed tomography.

 $^{2}$ CWDB = furnished colony cage with dust bath; CWODB = furnished colony cage without dust bath; CONV = conventional cage; MOD = modified cage.

<sup>3</sup>Number of bones assessed.

<sup>4</sup>Note: the average trabecular density for all but 1 humerus in CWODB and 1 humerus in CONV was 0 mg/cm<sup>3</sup>.

was narrowest for conventionally housed birds, and the overall amount of cortical bone was also lowest in CONV. Fleming et al. (1994) attributed increased humeral cortical thinning in conventionally caged hens to excessive bone resorption from endosteal surfaces. Presumably then, in the current study, CWDB, CWODB, and, to some extent, MOD birds, who had increased opportunity for movement and bone loading, were better able to protect femoral and tibial cortical structural bone from endosteal surface erosion than hens in CONV cages.

The trabecular space, as defined for QCT analysis, is comprised of both trabecular and medullary bone mineral (Korver et al., 2004), and changes in trabecular measures are likely representative of changes in medullary bone (Riczu et al., 2004). In the present study, density of bone in the trabecular space was highest for CONV hens (Table 1), with the difference approaching significance in the femur of CWODB (P = 0.07) and MOD birds (P = 0.07) and in the tibia of CWDB (P = 0.09) and MOD (P = 0.07) hens. Cross-sectional area of bone in the trabecular space was also highest in CONV, and the difference was significant for the femoral CWDB value and approached significance for femoral CWODB (P = 0.08) and tibial CWDB (P = 0.07) averages. Taken together with the significantly lower cortical bone area values for CONV birds, these findings support the above suggestion that hens in conventional cages were least successful at preventing cortical structural bone resorption. The CONV birds likely mobilized more cortical bone, but less medullary bone, than hens who had greater opportunity for movement and load-bearing activity, resulting in increased cortical thinning, but a higher density of bone in the trabecular space. Because a greater reduction in the width of the cortex is accompanied by a greater corresponding increase in the diameter of the trabecular or marrow space (Fleming et al., 1994), femoral trabecular area was also higher in CONV than in CWDB cages, as was the overall amount of bone in the trabecular space (mg QCT). In contrast, birds in CWDB and CWODB cages appeared to efficiently mobilize Ca from femoral medullary bone, thereby protecting their structural cortical bone and resulting in higher cortical area and mass values than in CONV, but reduced trabecular area and bone mass (mg QCT).

It is interesting to note that in the femur, density of bone in the trabecular space was higher for CWDB than CWODB or MOD hens, and the difference approached significance (CWODB: P = 0.08; MOD: P = 0.09). This suggests that additional opportunity for bone loading through access to the raised DB may have contributed to

reduced net loss of bone in the trabecular space, as well as having encouraged protection of cortical bone. Because a negative correlation has been determined between medullary and trabecular bone turnover (Rennie et al., 1997), encouraging medullary bone remodeling might therefore minimize trabecular bone loss. In addition, Riczu et al. (2004) proposed that improved bone quality observed in brown egg strain layer hens over white egg strain birds may have resulted from the ability of brown egg hens to both target and replenish medullary Ca reserves, thereby offering increased protection of cortical bone. In the present study, additional movement by CWDB hens may therefore have prevented excessive loss of trabecular and cortical structural bone by encouraging both the mobilization and replenishment of medullary Ca reserves. Passi and Gefen (2005), who demonstrated significant reductions in the mediolateral impact energy required to fracture femurs from which core trabecular tissue had been extracted, suggested that trabecular bone serves an important role in distributing applied impact loads to the cortex and that minimizing trabecular bone loss might therefore be equally important in the prevention of osteoporosis, as is minimizing loss of cortical bone. Allowing caged hens access to a raised dust bath, as well as a nest site and perch, may therefore have important consequences for preventing osteoporosis by protecting both trabecular and cortical structural bone.

The significantly lower cortical and significantly higher trabecular area in CONV also clarifies why CONV hens exhibit significantly lower total bone density, in spite of having comparable cortical and trabecular density values. The total bone diameter of CONV birds is comprised of a large area of lower density bone in the trabecular space and a thin band of higher-density, compact cortical bone. In contrast, colony cage and MOD hens have a thicker band of higher-density cortical bone and a smaller area of lower-density bone in the trabecular space. Total bone density is therefore likely to be higher for birds with a thicker cortex.

Overall, in the femur and tibia of hens from the cage systems examined, conventionally housed birds exhibited the lowest cortical cross-sectional area, suggestive of increased cortical thinning; the highest trabecular density, likely associated with reduced efficiency of medullary bone resorption; and the highest cross-sectional area of bone in the trabecular space, likely resulting from their increased marrow space. Taken together, these results suggest greater loss of structural bone for hens in conventional cages than for birds in furnished systems. Because persistent cortical thinning can lead to osteoporosis (Bell and Siller, 1962) and increased susceptibility to bone fracture (Whitehead and Fleming, 2000), even when medullary stores may be increasing (McCoy et al., 1996), it could therefore be expected that structurally, bones from conventionally housed birds would be weaker. In the current study, breaking strength values were significantly lower in the femur and tibia of birds in conventional cages than for colony birds (Table 2). Breaking strength was highest for CWDB birds, followed by CWODB hens, as might be anticipated, because hens in CWDB cages experienced the greatest freedom of movement and opportunity for bone loading. Significantly enhanced tibial strength has also been previously demonstrated for hens housed in conventional cages containing a perch (Hughes and Appleby, 1989; Duncan et al., 1992); furnished cage systems containing perches, NB, and dust-bathing facilities (Leyendecker et al., 2005); and noncage systems such as aviaries, percheries, and floor litter systems (Rowland et al., 1968; Rowland and Harms, 1970; Knowles and Broom, 1990; Nørgaard-Nielsen, 1990; Fleming et al., 1994; Abrahamsson and Tauson, 1995; Newman and Leeson, 1998; Leyendecker et al., 2005), as compared with conventionally caged hens.

*Humerus.* In the laying hen, the humerus is normally a pneumatized bone, devoid of mineral in the trabecular space. Varying degrees of humeral pneumatization have however been previously reported (Hogg, 1984; Fleming et al., 1996), and the presence of medullary bone appears to increase humeral density and bone strength (Fleming et al., 1996, 1998). In the present study, bone in the trabecular space was detected in the humerus of 1 CWODB hen and 1 CONV hen. Because humeral trabecular density values from both of these hens exceeded average trabecular density values of the respective treatments by more than 2 SD, these values were considered outliers, and humeral density and strength measures from the 2 hens were excluded from calculated averages (Fleming et al., 1994).

Total humeral mineral density and bone mass were significantly higher for CWDB and CWODB hens than for CONV and MOD birds, and, as observed in the femur and tibia, total bone area did not differ between housing conditions (Table 1). In the absence of bone in the trabecular space, total humeral bone measures would be expected to reflect the condition of the cortex. Indeed, cortical density and bone mass (mg QCT) were significantly higher for CWDB and CWODB hens than for birds in CONV or MOD cages. In addition, cortical area values were significantly higher for hens in colony cages than hens in CONV and MOD. Taken together, these findings point to increased humeral cortical thinning for birds with reduced opportunity for wing movement. Fleming et al. (1994) also observed increased humeral cortical thinning for conventionally caged layers as compared with noncaged hens. Furthermore, the significantly lower cortical density values of CONV and MOD hens suggests that in addition to greater cortical bone loss from the endosteal surface, in the pneumatic humerus, bone loss occurring at exposed mineral sites throughout the cortex was advanced when birds had limited wing movement.

Whitehead and Fleming (2000) proposed that decreased humeral density, as measured by radiographic analysis, is indicative of osteoporosis, and Hester et al. (2004) demonstrated a positive correlation between bone radiographic density and humeral breaking strength. Humeral breaking strength values in the current study would therefore be expected to reflect total and cortical density measures. Bone strength measures were in fact significantly higher for hens housed in the colony cages

<b>Fable 2.</b> Femur, I	humerus, and	tibia breaking :	strength of 6	5-wk-old V	White Leghorı	ns housed in	colony,	conven-
ional, and modi	fied cages	0	0		0		-	

	Breaking strength (kgf) <sup>2</sup>					
Cage type <sup>1</sup>	Femur	Humerus	Tibia			
CWDB CWODB CONV MOD SEM	$\begin{array}{c} 29.59^{a} \ (20)^{1} \\ 27.07^{ab} \ (20) \\ 21.92^{c} \ (20) \\ 24.55^{bc} \ (20) \\ 2.42 \end{array}$	$\begin{array}{c} 13.67^{\rm a} \ (20) \\ 11.91^{\rm a} \ (19) \\ 9.73^{\rm b} \ (19) \\ 8.69^{\rm b} \ (20) \\ 0.83 \end{array}$ Probabilities	$\begin{array}{c} 28.62^{a} \ (20) \\ 27.66^{a} \ (20) \\ 21.96^{b} \ (20) \\ 24.48^{b} \ (20) \\ 1.70 \end{array}$			
Housing	0.0158	<0.0001	0.0007			

<sup>a–c</sup>Means within the same column and bone type lacking a common superscript are significantly different (P < 0.05).

<sup>1</sup>CWDB = furnished colony cage with dust bath; CWODB = furnished colony cage without dust bath; CONV = conventional cage; MOD = modified cage.

<sup>2</sup>Means are followed by n values given in parentheses.

than for birds in CONV or MOD cages and were highest for CWDB hens (Table 2). Enabling caged birds to perform activities such as jumping up to the raised DB and dust bathing therefore encouraged humeral cortical bone protection and increased bone strength. In addition, bouts of wing movement including flapping, stretching, and ruffling were less restricted in the colony cages than in CONV and MOD (M. J. Jendral, unpublished data) and likely further contributed to increased humeral strength of CWDB and CWODB hens. Abrahamsson et al. (1996) and Levendecker et al. (2005) also demonstrated significantly higher humeral bone strength for hens housed in furnished cage systems than hens in conventional battery cages, and increased humeral breaking strength has been observed for hens housed on floor litter, in perchery, or in aviary systems, as compared with hens maintained in conventional cages (Knowles and Broom, 1990; Nørgaard-Nielsen, 1990; Fleming et al., 1994; Abrahamsson and Tauson, 1995).

It appears that humeral cortical bone protection was not afforded by perching activity in MOD. Abrahamsson et al. (1996) observed a numerical increase in humeral bone strength when hens in conventional cages were provided with a perch and suggested that the wing movement performed by hens to elevate themselves onto the perch contributed to increased bone strength. Notably, hens in that trial each had access to 600 cm<sup>2</sup> of floor space, considerably more room for wing movement than hens in the current study. Moinard et al. (1998), however, demonstrated that increasing cage height, not area, was necessary to significantly increase humeral strength of conventionally caged hens. The authors attributed this improvement to the higher frequency of comfort wing stretching and flapping displayed by hens in taller cages.

In summary, hens in CWDB cages were best able to protect humeral structural bone. The CWODB hens also exhibited improved humeral condition; however, birds in MOD cages were unable to maintain humeral cortical bone through perching activity. Enabling hens access to a raised amenity and providing hens with the opportunity to dust bathe and increase their wing movement was necessary to minimize cortical structural bone resorption both at the endosteal surface and throughout the cortex and thereby improve humeral bone quality. Because fracture incidence in laying hen bones are highest in the humerus (Gregory and Wilkins, 1989), improving humeral cortical bone quality and reducing fracture rates in caged hens by inclusion of adequate amenities and space has considerable implications for hen welfare and production.

#### Correlation

Treatments Combined: Egg Production or Quality Parameters and Bone Quality or Breaking Strength **Measures.** With the exception of a minimally positive correlation between hen-day production and humeral cortical density (r = 0.37, P = 0.003), overall, no strong correlations were found between egg production and bone quality parameters for the combined treatment values. Because production did not differ significantly between treatments (M. J. Jendral, unpublished data), the absence of correlation suggests that egg production in general was maintained irrespective of bone quality for this highproducing strain of bird. Superior bone quality measures observed for hens housed in furnished cages are therefore not the result of lowered egg production and consequent reduced Ca requirement but rather are attributable to the protective effect of activity on cortical bone. Whitehead et al. (1998) demonstrated that concomitant high egg production and good bone quality are possible at the end of lay, and Rowland et al. (1972) observed no relationship between tibial breaking strength and egg production, suggesting that superior bone strength and egg production measures may be observed simultaneously. Rennie et al. (1997) demonstrated minimal relationships between trabecular bone volume and egg production in both free thoracic vertebrae and proximal metatarsus bones of highly productive Hisex birds, even though the majority of those hens were osteoporotic at the end of lay. The authors, however, attributed the development of osteoporosis to the length of the period of continuous egg production rather than to hen-day production. Inclusion of amenities that provide continuously producing caged laying hens with opportunity for movement and load-bearing exercise may therefore be of utmost importance in deterring the onset of osteoporosis.

Few correlations were observed between measures of egg quality and bone density or breaking strength, further suggesting that, overall, observed treatment differences in bone parameters were not influenced by treatment differences in egg quality.

Individual Treatments: Egg Production or Quality Parameters and Bone Quality or Breaking Strength Measures. In the femur of CWDB and CWODB hens, a significant negative correlation (CWDB: r = -0.60, P =0.04; CWODB: r = -0.60, P = 0.05) was observed between egg production and trabecular density. A reduction in trabecular density that accompanies an increase in production, and hence an increased Ca requirement, is consistent with the suggestion that egg production in active birds is likely maintained by resorption of medullary bone in the trabecular space, rather than at the expense of cortical bone. Indeed, this correlation was not apparent for CONV or MOD hens, who had less opportunity for load-bearing activity.

A positive significant correlation between eggshell weight and total bone density was observed in the femur (r = 0.65, P = 0.02) and between eggshell weight and breaking strength in both the femur (r = 0.70, P = 0.01) and tibia (r = 0.81, P = 0.001) of CWDB birds. In contrast, Riczu et al. (2004) observed negative correlations between eggshell weight and total femoral density, which the authors attributed to Ca mobilization from bone reserves to support eggshell formation. In the present study, the positive relationship between these 2 parameters suggests that for CWDB hens, caged birds with the greatest opportunity for activity, structural bone reserves were protected, and therefore overall bone quality was not compromised by high egg quality. Notably, in the tibia of CWDB hens, a significant negative correlation was found between specific gravity and trabecular area (r = -0.75, P = 0.005), and the relationship approached significance in the femur (r = -0.56, P = 0.06). This would suggest that the quality of the egg increased with decreasing area of bone in the trabecular space or, with reduced endocortical thinning. Taken together, these findings further support the intimation that for caged hens with sufficient opportunity for load-bearing activity, shell formation is maintained by improved mobilization of medullary bone from the trabecular space rather than at the expense of cortical bone.

In contrast to CWDB hens, CWODB birds exhibited a negative significant correlation between eggshell weight and breaking strength in the femur (r = -0.66, P = 0.03), a correlation that approached significance in the tibia (r = -0.54, P = 0.08). Perhaps CWODB hens, who experience less opportunity for mechanical bone loading than CWDB birds but greater opportunity than MOD or CONV hens, are able to minimize structural bone loss but compared with CWDB hens have a lowered capacity to mobilize

medullary Ca reserves for eggshell formation. Bishop et al. (2000) report decreased shell quality in bird lines that are more resistant to osteoporosis. The CWODB hens also demonstrated a significant positive correlation between stored egg weight and trabecular density in the femur (r = 0.74, P = 0.01) and in the tibia (r = 0.81, P = 0.002), as well as between eggshell weight and trabecular density (femur: r = 0.60, P = 0.05; tibia: r = 0.70, P = 0.02), providing additional evidence that to support eggshell formation, CWODB hens source Ca reserves from medullary bone rather than sacrifice structural bone.

A negative significant correlation between trabecular density and eggshell thickness (r = -0.54, P = 0.01), and a positive significant correlation between eggshell weight and cortical area (r = 0.44, P = 0.05) observed in the femur of CONV hens, provides additional evidence that both medullary and structural bone are mobilized to support eggshell formation when hens have little opportunity for load-bearing movement. Significant positive correlations between stored egg weight and total bone area were observed in the tibia of MOD hens (r = 0.53, P = 0.01) and the femur of CONV birds (r = 0.46, P = 0.04), as well as between stored egg weight and trabecular area (r = 0.45, P = 0.04) and eggshell weight and total bone area (r = 0.53, P = 0.02) in the tibia of MOD hens. Stored egg weight and total area were also positively correlated (r = 0.45, P = 0.05) in the humerus of CONV hens. These results likely reflect the tendency for larger hens to lay larger eggs.

The findings from this study provide evidence that movement and load-bearing exercise increase bone strength by enabling caged hens to efficiently mobilize medullary bone and to preserve cortical structural bone. Additional bone preservation in the form of medullary remodeling may also occur in the trabecular space, as noted for CWDB hens. In addition, providing caged hens with a raised amenity and the opportunity to dust bathe and increase their wing movement is necessary to maintain humeral architecture. With the knowledge that exercise has a protective effect on cortical bone, and with the technical means to examine cortical bone in live birds via QCT analysis, management practices, such as inclusion of appropriate amenities in cage systems, should therefore be explored and adopted to encourage structural bone preservation in laying hens. Genetic selection for heritable cortical bone traits associated with bone strength can also be further directed. Bishop et al. (2000), for example, have already demonstrated that bone strength characteristics are moderately to strongly heritable and respond to selection for cancellous and medullary bone traits.

Additional studies will be necessary to quantify and qualify the nature of and extent to which hen structural bone can be protected through mechanical strain. From the present study, however, it is clear that for hens housed in cage systems, structural bone protection is afforded when amenities and space are available to permit sufficient movement and load-bearing exercise. These findings have considerable implications for laying hen welfare and production. ACKNOWLEDGMENTS

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