

End-of-Cycle Bone Quality in White- and Brown-Egg Laying Hens

C. M. Riczu, J. L. Saunders-Blades, Å. K. Yngvesson, F. E. Robinson, and D. R. Korver¹

Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Canada T6G 2P5

ABSTRACT Broken and weak bones of laying hens are major welfare concerns in the table egg industry. Bone quality at the end of lay of brown- (Shaver 579) and white- (Shaver 2000) egg strains were compared. Prior to the start of the experiment, the hens had been housed in laying hen cages (2/cage). At 423 d of age (60 wk + 3 d), 24 hens of each strain were selected and individually caged, and egg production records were kept until 462 d of age (the end of 65 wk) for a total of 39 d. Egg quality analysis was undertaken during wk 65 of age. Hens were killed at 66 wk of age (463 d), and carcass and reproductive morphology traits were measured. Femur and humerus mineral density were measured using quantitative computed tomography, and breaking strength was measured by an Instron Materials Tester. The white-egg strain

produced 3.7% more marketable eggs during the experiment due to a 0.3 d shorter mean pause length in egg production. Eggs from the brown strain were 3.4% heavier, had 4.0% more eggshell, and had a higher specific gravity than the white strain eggs (1.077 and 1.072, respectively). Final BW was 330 g greater in the brown-egg strain. Total bone density of the femur was not different between the 2 strains but was greater in the humerus of the brown-egg layers. Total femur and humerus bone areas were greater in the brown strain than the white-egg strain. Bone breaking strengths of the brown-egg strain were greater by 22% (femur) and 18% (humerus) than in the white-egg strain hens. These results indicate that this brown-egg strain may be more resistant to weak and broken bones at the end of production than the white-egg strain.

(Key words: bone breaking strength, bone mineral density, egg quality, laying hen, quantitative computed tomography)

2004 Poultry Science 83:375–383

INTRODUCTION

The incidence of broken and weak bones at the end of lay is a serious problem in the table egg industry. In a United Kingdom study, 29% of laying hens were found to have broken bones by the time flocks were dispensed at the end of their laying period (Gregory and Wilkins, 1989). The increasing number of broken bones has also led to processing problems. Processing plants may refuse to accept these hens due to increased risk of bone fragments in the final product (Gregory and Wilkins, 1989). Until recently, this problem has not received much attention from the poultry industry or the research community (Mench and Duncan, 1998).

The most common occurrence of broken bones in a commercial laying hen is during removal from the cage at the end of lay. Handling (Gregory and Wilkins, 1989), cage structure, and flight behavior may result in trauma to the hen from contact with the cage or with other birds, thereby causing breaks (Duncan, 2001). However, the un-

derlying cause of bone breakage in laying hens is due to weak bones as a result of Ca depletion (Knowles and Wilkins, 1998). A total Ca output of 28 to 30 times the hen's total body Ca reserves is required for egg production throughout the entire production cycle (Elaroussi et al., 1994). The high level of egg production over prolonged periods of time results in aging hens having a skeletal system that is highly susceptible to breaks (Fleming et al., 1998a).

A laying hen has 3 types of bone tissue: cortical, trabecular, and medullary bone, all of which have important roles in bone strength and Ca metabolism (Whitehead and Fleming, 2000). Cortical and trabecular bone provide most of the structural strength to the bone (Whitehead and Fleming, 2000), whereas medullary bone acts as a source of available Ca for eggshell formation (Fleming et al., 1998b). Medullary bone is readily turned over during times of insufficient dietary Ca levels and replaced when dietary Ca levels are in excess of the hen's requirement (Fleming et al., 1998b). This cycle can occur on a daily basis. If insufficient medullary bone is available, trabecular and cortical bone can be utilized to meet the hen's Ca

©2004 Poultry Science Association, Inc.

Received for publication August 15, 2003.

Accepted for publication November 13, 2003.

¹To whom correspondence should be addressed: doug.korver@ualberta.ca.

Abbreviation Key: BMD = bone mineral density; SG = specific gravity.

requirement. The loss of cortical bone is critical, as the hen has no method by which to replace it during the laying cycle (Taylor and Dacke, 1984). An abnormally high amount of cortical bone mobilized for egg production predisposes the skeletal system to breaks and other disorders.

Several studies have been conducted regarding bone strength in layers. These studies have focused on the effects of housing (Norgaard-Nielsen, 1990; Appleby et al., 2002) and nutrition (Rennie et al., 1997; Rath et al., 2000; Cransberg et al., 2001) as factors affecting bone quality. To date, little reported research has examined the differences in bone quality between white and brown-egg strains at the end of lay.

Potential differences in bone quality of various white and brown-egg strains may be due to reported differences in age at sexual maturity, egg production (Renema et al., 2001), egg size (Scott and Silversides, 2000; Renema et al., 2001; Silversides and Scott, 2001), egg quality (Hunton, 1982; Scott and Silversides, 2000; Bell et al., 2001; Bar et al., 2002), and BW (Scott and Silversides, 2000; Renema and Robinson, 2001).

There is increased interest from the poultry industry and scientific community regarding bone quality and welfare of egg laying hens. The main objective of this study was to determine if there were any differences in bone density, bone strength, egg production, and eggshell quality between brown- and white-egg laying hens. It was hypothesized that the brown-egg laying strain would have an increased bone quality [measured as bone mineral density (BMD) and breaking strength] due to greater body mass, without a reduction in egg production or eggshell quality.

MATERIALS AND METHODS

Experimental Design

This study was approved by the University of Alberta's Faculty Animal Policy and Welfare Committee according to the *Guide to the Care and Use of Experimental Animals* (Canadian Council on Animal Care, 1984). Hens in the present study had been housed from 18 wk of age at a density of 2 birds per cage as part of a larger population. The hens in the present study were randomly selected from the birds that were in lay, based on egg production records. At 423 d (60 wk + 3 d) of age, Shaver 2000² (white-egg strain, n = 24) and Shaver 579² (brown-egg strain, n = 24) hens were placed individually in the top tier of a 2-tier battery cage system, allowing for 1,239 cm²/hen. All birds were fed according to the same layer nutritional program throughout the prestudy and study periods. All hens received water ad libitum and a standard layer ration fed in mash form (Table 1), which was top-dressed once weekly with 5 g of oyster shell per hen.

TABLE 1. Ingredient and calculated nutrient composition of standard layer ration

Ingredient	Amount of ingredient (g/kg of diet)
Wheat, hard red spring	633.91
Soybean meal, 48% CP	199.0
Corn gluten meal	20.0
Canola oil	27.4
Limestone	95.2
Dicalcium phosphate	10.75
Choline Cl premix ¹	5.0
Layer premix ²	5.0
NaCl	2.5
DL-Methionine	1.24
Calculated nutrient composition	
ME (kcal/kg)	2,833
CP (%)	20.4
Ca (%)	3.89
Available P (%)	0.35
Lys (%)	0.83
Met (%)	0.41
Met + Cys (%)	0.74
Linoleic acid, %	1.49

¹Choline chloride premix provided 100 mg of choline/kg of diet.

²Layer premix provided the following (/kg of diet): vitamin A, 10,000 IU; vitamin D₃, 2,500 IU; vitamin E, 35 IU; vitamin K, 2.0 mg; pantothenic acid, 14.0 mg; riboflavin, 5.0 mg; folacin, 0.8 mg; niacin, 65.0 mg; thiamine, 2.0 mg; pyridoxine, 4.0 mg; vitamin B₁₂, 0.015 mg; biotin, 0.18 mg; iodine, 0.5 mg; manganese, 70.0 mg; copper, 8.5 mg; zinc, 80.0 mg; selenium, 0.1 mg; iron, 100.0 mg.

Hens were kept on a 14L:10D photoperiod throughout the entire production cycle in a light-tight poultry house.

Egg Production and Eggshell Quality

Beginning at 423 d (60 wk + 3 d) of age, egg production was recorded daily on a per-hen basis for 39 d, to 462 d (the end of 65 wk) of age. Egg laying sequence length was determined using the methods described by Robinson et al. (2001) for the 39 d of recorded egg production.

From 455 to 462 d of age (wk 65), all eggs laid were collected for eggshell quality analysis. After each egg was stored for 1 wk at 13°C, eggs were weighed, and eggshell quality was determined by specific gravity (SG) with the floatation method as described by Hamilton (1982); a series of saline solutions with SG ranging from 1.064 to 1.110 in increments of 0.002 were used. Eggs were broken, and shells were washed of adhering albumen and membranes, left to dry at room temperature overnight, and weighed.

Carcass and Reproductive Measurements

At 463 d of age, all hens were carefully removed from their cages and immediately killed by cervical dislocation. The hens were then weighed, and right shank length was measured by a vernier caliper as the length of the tibiotarsus from the footpad to the hock joint. Birds were dissected, and weights of the liver, abdominal fat pad, breast muscle, oviduct, total ovary, total large (greater than 1 cm) yellow follicles, and stroma were recorded. Birds were examined for the incidence of internal abnor-

²Hubbard ISA, Duluth, Georgia.

malities such as internal ovulation, internal laying, follicular atresia, and cystic right oviducts.

Bone Quality

The right humerus and femur of each hen were removed and stored at -25°C until further bone quality measurements were conducted. In cases where the right femur or humerus was fractured, the left bone was collected. Bones were thawed and then cleaned of soft tissue prior to bone quality analysis. Bone density analysis was performed using quantitative computed tomography. A Stratec XCT³ scanner with XMENU software version 5.40C was used in the present study. A longitudinal scan of the bone was taken to set the location for the cross-sectional x-ray picture, set at the midpoint of each bone. The resulting cross-section was analyzed by the Stratec XCT software, which calculated total, cortical, and trabecular bone densities and areas. Bone in the trabecular space was assumed to include medullary bone.

After BMD measurements were completed, bone breaking strength was measured using a modified version of the method described in Fleming et al. (1998c). An Instron Materials Tester⁴ with Automated Materials Test System software version 8.09, a standard 50-kg load cell, and a modified shear plate (8 cm in length and 1 mm in width) were used. A 3-cm distance between the 2 fixed points supporting the bone and a crosshead speed of 100 mm/min were held constant throughout each measurement. The force was applied to the mid point of the same facial plane of each bone, and the breaking strength was recorded.

Statistical Analysis

One hen from the white-egg strain was omitted from analysis as this bird stopped laying after being selected for the study. The femur from an additional white-egg strain hen was eliminated from analysis of bone weight, length, and breaking strength measurements due to bone damage incurred during dissection. The humerus of one white-egg strain hen was eliminated from all bone analysis as it had been broken and subsequently healed prior to the dissection. Three humeri of white-egg strain hens were eliminated from bone weight, length, and breaking strength measurements due to damage during the dissection. All femurs from the brown-egg strain were used in all bone analysis procedures; however, the humeri of 2 brown-egg strain hens were omitted, because during the removal of the soft tissues, these bones were found to have been previously broken and healed.

The response variables were analyzed as a one-way ANOVA with strain as the main effect using the GLM procedure of SAS software (SAS Institute, 1999). When the effect of strain was significantly different, means were

separated using least squares means comparisons. Correlation coefficients (r) of bone quality measurements with production and body composition parameters were calculated using Pearson correlations in SAS software (SAS Institute, 1999). The level of significance, unless otherwise stated, was assessed at $P < 0.05$.

RESULTS AND DISCUSSION

Egg Production, Sequence Length, and Quality

Throughout the final 39 d of production, the strains had similar total ($P = 0.0531$) and percentage hen-day ($P = 0.0531$) egg production; these differences approached statistical significance (Table 2). The white-egg strain hens produced, on average, 36.8 marketable eggs per hen; the brown-egg hens produced 34.5 marketable eggs throughout the experimental period (Table 2). Because only the final 39 d of egg production was recorded, the differences may not accurately reflect egg numbers throughout the entire production cycle. It does, however, give an indication of egg production at the end of lay and can be compared with early egg production by the same strains as reported by Renema et al. (2001). These authors reported a 5.8% higher percentage of hen-day egg production for the brown-egg strain from 21 to 45 wk of age. The results from the current study show a 6.9% higher egg production for the white-egg strain during the latter stages of egg production. This finding indicates that the brown-egg strain had a greater decrease in egg production near the end of lay. Because the brown-egg strain has previously been shown to have a higher level of egg production during the early stages (Renema et al., 2001), the overall egg production may not be different between these 2 strains.

The mean egg sequence length was similar between the 2 egg strains with an average length of 14.8 and 18.9 d for the brown and white-egg strains, respectively (Table 2). However, the length of the pause between sequences was longer for the brown strain than the white-egg strain, with pauses of 1.1 and 0.8 d, respectively (Table 2). Renema et al. (2001) reported a 13 d greater average sequence length for the brown-egg strain as compared with the white-egg strain from 21 to 45 wk of age. This finding would account for the higher level of egg production in the brown-egg strain at this younger age. The brown-egg strain laid eggs that were more than 4 g heavier, had more eggshell (both on a weight and percentage of egg weight basis), and had a higher SG (1.077 and 1.072, respectively) than the eggs of the white-egg strain during wk 65 (Table 3).

Other researchers have found brown-egg strains to produce heavier eggs (Curtis et al., 1986; Scott and Silversides, 2000; Renema et al., 2001), have a greater eggshell weight (Scott and Silversides, 2000; Renema et al., 2001), and a greater percentage of eggshell (Curtis et al., 1986; Scott and Silversides, 2000). However, Renema et al. (2001) reported that percentage of eggshell and SG of

³Norland Medical Systems, Inc., Fort Atkinson, WI.

⁴Model 4411, Instron Corp., Canton, MA.

TABLE 2. Egg production of brown- and white-egg strains from 60 to 65 wk of age

Strain	n	Total egg production ¹ (n)	HD ² (%)	Marketable eggs (n)	Mean sequence length (d)	Mean pause length (d)
Brown	24	34.5	88.4	34.1 ^b	14.8	1.1 ^a
White	23	36.8	94.3	36.7 ^a	18.9	0.8 ^b
SEM		0.8	2.1	0.9	2.7	0.1
ANOVA		Probabilities				
Strain	47	0.0531	0.0531	0.0470	0.2822	0.0411

^{a,b}Means within the same column with no common superscript are significantly different ($P < 0.05$).

¹Total egg production during final 39 d of lay per hen.

²Hen-day egg production.

eggs were not different between the brown and white-egg strains from 21 to 45 wk of age. The differences in results between the previous report and the present study may be attributed to the age and breed of the hens studied.

Eggshell quality normally deteriorates with increasing age (Flock, 1994). Silversides and Scott (2001) found a relative decrease in the amount of shell as a percentage of egg weight as the hen ages. The evidence of greater eggshell quality and greater amount of eggshell by the brown-egg strain at the end of lay from the current study suggest that as the hens age, the white-egg strain had a greater decrease in eggshell quality than the brown-egg strain. Silversides and Scott (2001) also found a larger decrease in eggshell quality in a white-egg strain, although the white and brown-egg strains compared in that study were different than those in the current study. Poor shell quality will increase the incidence of cracked eggs. Holder and Bradford (1979) found an almost 2-fold greater incidence of cracks in eggs that had a SG of 1.070 than in eggs that had a SG of 1.075. The results from the current study suggest that more of the white eggs would be downgraded as a result of the lower egg SG and the thinner eggshells.

Carcass and Reproductive Morphology at 66 wk of Age

The brown-egg strain hens were on average 330 g larger than the white-egg hens (Table 4). This weight difference was associated with a greater fat pad weight of the brown-egg strain relative to the white-egg strain (5.68 and 4.30% of BW, respectively; Table 4). Renema and

Robinson (2001) also showed that BW, at sexual maturity and at 45 wk of age, was greater in the brown than the white-egg strain when comparing the same strains as used in the current study.

The white-egg strain hens had a greater percentage of breast muscle compared with hens of the brown-egg strain (Table 4), in contrast to results presented by Renema and Robinson (2001). This difference may be attributed to the changes that occur as the hen ages; hens in the current study were 66 wk old as compared with 45 wk of age in their study by Renema and Robinson (2001).

The white-egg strain had a greater percentage of liver weight (Table 4), which may be directly related to the increased egg production of the white-egg strain as the liver would need to process more lipids for the production of egg yolk. There were no differences in regard to weight for any of the reproductive tissues measured (Table 4). This finding agrees with the results reported by Renema and Robinson (2001) who also did not report any differences in reproductive system component weights between the same 2 strains.

Femur Characteristics and Correlations

Femur BMD was not different between the 2 strains; however, total cross-sectional area of the femur was 8% greater in the brown than in the white-egg strain (Table 5). The same pattern was also observed for the femur cortical bone, in which the cortical density was not different between the 2 strains, but the brown-egg strain had an 11% greater cortical bone area (Table 5). The BMD results show that bone strength for the brown-egg strain

TABLE 3. Egg quality of brown- and white-egg strains 60 to 65 wk of age

Strain	n ¹	Egg weight (g)	SG ²	Eggshell weight (g)	Eggshell weight (% of egg weight)
Brown	24	66.01 ^a	1.077 ^a	6.27 ^a	9.54 ^a
White	23	61.71 ^b	1.072 ^b	5.43 ^b	8.80 ^b
SEM		0.99	0.00	0.12	0.17
ANOVA		Probabilities			
Strain	47	0.0033	0.0009	<0.0001	0.0030

^{a,b}Means within the same column with no common superscript are significantly different ($P < 0.05$).

¹Total egg production during final 39 d of lay per hen.

²Specific gravity.

TABLE 4. Body morphology of brown- and white-egg strains at 66 wk of age

Strain	n ¹	BW (kg)	Shank length (mm)	Breast weight (% of BW)	Liver weight (% of BW)	Fat pad weight (% of BW)	Oviduct weight (g)	Ovary weight (g)	LYF ³ (n)	LYF weight (g)	Stroma weight (g)	
Brown	24	2.12 ^a	97.29	10.31 ^b	2.05 ^b	5.68 ^a	77.3	48.6	5.33	39.63	8.96	
White	23	1.79 ^b	97.09	11.33 ^a	2.34 ^a	4.30 ^b	73.0	48.0	5.09	39.49	8.55	
SEM		0.04	0.65	0.30	0.10	0.36	1.6	1.8	0.15	1.68	0.40	
ANOVA		Probabilities										
Strain	47	<0.0001	0.8271	0.0175	0.0419	0.0066	0.0571	0.8245	0.2454	0.9521	0.4661	

^{a,b}Means within the same column with no common superscript are significantly different ($P < 0.05$).

¹Total egg production during final 39 d of lay per hen.

²Percentage = tissue weight/BW × 100.

³Large yellow follicles (>10 mm in diameter).

would be expected to be greater than for the white-egg strain; this interpretation is supported by the greater femur breaking strength of the brown-egg strain (Table 5). The brown-egg hens had a greater total bone area and, more importantly, cortical bone while maintaining the same bone density as the white-egg strain, which had a smaller bone area. The femur has been identified as one of the primary bones contributing minerals for eggshell calcification (Taylor and Moore, 1954). BMD is a very important measure of bone quality and is positively related to bone breaking strength (McCoy et al., 1996). Rowland et al. (1972) found bone breaking strength to vary among several different strains of laying hens, indicating a genetic potential for some strains to have better bone quality than others. More recently, Bishop et al. (2000) reported success in selecting laying hens for increased bone quality.

Bone strength has been shown to have a positive relationship with BW (Bishop et al., 2000). Knowles et al. (1993) found heavier birds to have stronger bones but also to have an increased incidence of bone breakage. These authors hypothesized that the increased bone strength was not sufficient to offset the stress of support-

ing the extra weight placed on the hens skeletal system. Knowles et al. (1993) compared hens with a greater difference in BW than the current study. The 330 g of greater BW of the brown-egg hens used in the current study might have resulted in a significant amount of increased stress on the bones of those hens as compared with the white-egg hens, resulting in an increased susceptibility to bone breakage.

Femur length was not different between the 2 strains, although the bone weight was about 9% greater in the brown-egg strain hens (Table 5). This result also supports the finding that the overall thickness of the femur is greater for the brown-egg strain and suggests there is more bone tissue in an equivalent length of bone.

Medullary bone is found within the same space as the trabecular bone and contributes somewhat to bone strength, although to a lesser extent than cortical bone (Fleming et al., 1996). Medullary bone is mainly important for the mobilization of Ca for eggshell formation (Whitehead and Fleming, 2000). The quantitative computed tomography method used in the present study could not separate avian trabecular and medullary bones. Therefore, changes in trabecular bone measurements are

TABLE 5. Femur and humerus quality of end of lay for brown- and white-egg strains at 66 wk of age

Bone type and strain	Density			Area			BBS ¹ (kg)	Bone weight (g)	Bone length (cm)
	Total (mg/cm ³)	Cortical (mg/cm ³)	Trabecular (mg/cm ³)	Total (mm)	Cortical (mm ²)	Trabecular (mm)			
Femur									
Brown	545.82 (24) ²	934.80 (24)	177.44 ^b (24)	48.41 ^a (24)	22.18 ^a (24)	21.14 (24)	30.68 ^a (24)	9.46 ^a (24)	8.1 (24)
White	550.66 (23)	917.48 (23)	248.30 ^a (23)	41.16 ^b (23)	17.94 ^b (23)	18.72 (23)	19.54 ^b (22)	7.87 ^b (22)	7.9 (22)
SEM	18.09	12.20	9.04	0.97	1.06	1.49	1.46	0.20	0.06
ANOVA	Probabilities								
Strain	0.8491	0.3155	<0.0001	<0.0001	0.0064	0.2520	<0.0001	<0.0001	0.1361
Humerus									
Brown	213.33 (22)	1,001.33 ^a (22)	0.00 (22)	40.60 (22)	11.24 ^a (22)	28.49 (22)	15.20 ^a (22)	5.18 ^a (22)	7.8 ^a (22)
White	158.86 (22)	963.30 ^b (22)	4.21 (22) ³	39.61 (22)	9.29 ^b (22)	28.69 (22)	10.57 ^b (20)	3.83 ^b (20)	7.4 ^b (20)
SEM	19.45	9.49	2.98	0.93	0.44	1.04	0.83	0.21	0.08
ANOVA	Probabilities								
Strain	0.0543	0.0070	0.3230	0.4575	0.0031	0.8911	0.0004	<0.0001	0.0003

^{a,b}Means within the same column and bone type with no common superscript are significantly different ($P < 0.05$).

¹Bone breaking strength.

²Means are followed by n values given in parentheses.

³Note: the average trabecular density for all but one humerus of the white was 0 mg/cm³.

TABLE 6. Correlation coefficients (r) of production and body measurements with bone parameters of the brown-egg strain

Bone and measurement	SG ¹	Eggshell weight	Hen-day egg production	BW
Femur				
Total density	-0.5857**	-0.6822***	-0.1242	0.0670
Total area	0.2928	0.5365**	-0.1062	0.4824*
Trabecular density	-0.0992	-0.0964	-0.2658	0.0709
Trabecular area	0.5921**	0.7552***	0.0621	0.1538
Cortical density	0.0072	0.0416	0.4606*	-0.0726
Cortical area	-0.4532*	-0.4518*	-0.2733	0.2758
Bone weight	0.0396	0.1463	0.0607	0.3915
Bone length	-0.0566	0.0322	0.0005	0.3317
BBS ²	-0.4227	-0.4797*	-0.1229	0.2286
Humerus				
Total density	-0.2034	-0.1269	0.4024	-0.1583
Total area	0.2284	0.3482	-0.2268	0.6149**
Trabecular area	0.2632	0.2421	-0.2937	0.4208
Cortical density	-0.1550	-0.2844	0.4761*	-0.0200
Cortical area	-0.2061	0.0647	0.3751	0.1917
Bone weight	-0.0900	-0.1484	0.2450	-0.0889
Bone length	0.0012	-0.2639	-0.1258	0.2367
BBS ²	-0.2589	-0.2422	0.4040	0.1620

¹Specific gravity.

²Bone breaking strength.

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

assumed to reflect, although not necessarily measure, changes in medullary bone stores.

The white-egg strain had 16% greater density in the femur trabecular bone but a similar femur trabecular area to the brown-egg strain (Table 5). Medullary bone can be formed at the cost of cortical bone (Taylor and Moore, 1954) if dietary Ca or Ca absorption is insufficient. The results of the current study suggest the brown-egg strain was able to preserve cortical bone by mobilizing a greater amount of Ca from medullary bone, thus reducing density of the trabecular region. The improved bone quality in the brown-egg strain may be due to a capacity not only to mobilize sufficient amounts of Ca from their medullary bone but also to replenish those reserves. Subsequently, a minimal amount of structural bone might have been lost from the brown-egg strain hens during the egg production cycle.

The difference in Ca mobilization from the medullary bone could also be related to the length of pause between successive egg sequences in the 2 strains studied (Renema et al., 2001). The longer pauses of the brown-egg strain, although slightly reducing egg production, may be enough to allow medullary reserves to be replenished to a greater extent. However, Renema et al. (2001) reported a much greater sequence length for the brown than the white-egg strain from 21 to 45 wk of age. Taken together, these results indicate that the brown-egg strain has a more significant reduction in egg production with age. This reduction may allow the brown-egg strain hens to build up Ca reserves, thereby reducing reliance on structural bone Ca to support egg production.

Hen-day egg production during the final 39 d of lay (60 wk + 3 d to 65 wk of age) was positively correlated with femur cortical BMD ($r = 0.46$) for the brown-egg strain (Table 6) but not for the white-egg strain (Table 7).

Egg production was only recorded for the final 39 d of the production cycle; thus, actual total production may be a better indicator to use when determining the relationship between bone strength and egg production.

Egg production is rapidly approaching the biological limit of one egg per day (De Ketelaere et al., 2002), which puts a great metabolic demand on the hen. Research has shown a relationship between the occurrence of osteoporosis with egg production and BW (Cransberg et al., 2001). When hens do not consume sufficient nutrients, especially during peak production, BW will decrease (Cransberg et al., 2001). Hens that lost weight during peak production had reduced egg production and were more prone to osteoporosis later in the production cycle (Cransberg et al., 2001). Therefore, a negative correlation between bone quality and egg production may be expected. However, a positive correlation found in this study may be indicative of the brown-egg hens' ability to replenish bone Ca reserves. Rowland et al. (1972) found no relationship between tibia breaking strength and egg production among different strains of laying hens. Previous research has shown that some hens are capable of high egg production and good bone quality at the end of lay (Whitehead et al., 1998). Therefore, depending on the breed and strain, bone quality may not be negatively affected by high rates of egg production.

Specific gravity ($r = -0.58$ and $r = -0.53$) and eggshell weight ($r = -0.68$ and $r = -0.46$) for the brown and white-egg strains, respectively, were found to be negatively correlated with total femur density (Tables 6 and 7). Shell weight was also negatively correlated with femur cortical area ($r = -0.45$) and femur breaking strength ($r = -0.48$) for the brown-egg strain (Table 6). Similar relationships were observed for the white-egg strain, in which total femur density was negatively correlated with SG ($r =$

TABLE 7. Correlation coefficients (r) of production and body measurements with bone parameters of the white-egg strain

Bone and measurement	SG ¹	Eggshell weight	Hen-day egg production	BW
Femur				
Total density	-0.5260**	-0.4572*	0.3554	0.4605*
Total area	0.1188	0.2388	-0.2013	0.2042
Trabecular density	-0.1801	-0.3065	0.0323	0.1379
Trabecular area	0.5854**	0.4539*	-0.3126	-0.2843
Cortical density	0.3169	0.1154	0.0062	0.0272
Cortical area	-0.5405**	-0.3242	0.3315	0.4648*
Bone weight	-0.0027	0.1595	-0.2351	0.3586
Bone length	0.1204	0.1712	-0.0052	0.2626
BBS ²	-0.1479	-0.0726	0.1261	0.3980
Humerus				
Total density	-0.1511	-0.0496	0.1800	0.0622
Total area	-0.1925	-0.1768	0.1909	0.3968
Trabecular area	-0.0332	-0.0506	-0.0095	0.2307
Cortical density	-0.0397	0.0632	-0.0379	0.2118
Cortical area	-0.2490	-0.1306	0.2542	0.3085
Bone weight	0.2323	0.0305	0.2799	-0.2176
Bone length	0.3314	-0.0144	0.3538	-0.1073
BBS ²	-0.0300	-0.0884	0.1151	0.2316

¹Specific gravity.

²Bone breaking strength.

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

-0.53) and eggshell weight ($r = -0.46$), whereas femur cortical area was negatively correlated with SG ($r = -0.54$, Table 7). These results are explained by the fact that some of the Ca needed for eggshell formation is drawn from bone reserves. In this study, the brown-egg strain laid larger eggs with more shell and still showed higher bone quality. There are 2 possible explanations for these results. One possible explanation is that the brown-egg strain hens are able to maintain bone quality and still produce high quality eggs. The brown-egg strain hens may be able to more efficiently mobilize medullary bone, thereby making more Ca available for eggshell formation, as demonstrated by a greater femur cortical area but a less dense trabecular bone. It also could be possible that the brown-egg strain lost the same amount of bone mineral as the white-egg strain but had more bone mass upon which to draw.

Body weight of the brown-egg strain was positively correlated with femur area ($r = 0.48$, Table 6). In the white-egg strain, BW was not correlated with cross-sectional area but was positively correlated with total bone density ($r = 0.46$, Table 7). This finding suggests that the larger birds may have greater bone strength to support the extra weight. The 2 strains show different physiological changes in response to increased BW, which may be related to the difference in bone strength. The brown-egg strain maintained the same density, independent of BW, but increased the bone area. The white-egg strain maintained the same cross-sectional area, but density increased as BW increased. Knowles et al. (1993) found bone strength and BW to be positively correlated; however, this was not the case with either of the strains in the current study.

Humerus Characteristics and Correlations

In the hen, the humerus has one of the highest fracture rates of all bones (Fleming et al., 1998c). It is normally a pneumatized bone of the hen's wing but has been found to contain variable amounts of trabecular bone (Fleming et al., 1996). The humerus is especially susceptible to breaks as it does not usually have the added support of the trabecular bone. This, combined with the high susceptibility to hysteria of laying hens, increases the chance for injury if the bird flaps its wings against the cage.

The mean total humerus density of the brown-egg strain was found to be marginally greater than the white-egg strain, although this difference was not significant ($P = 0.0543$; Table 5). The humerus of one white-egg laying hen had a measurable amount of trabecular bone, resulting in a higher average total BMD. This single observation, in the context of the small sample size in the study, resulted in a relatively high degree of variance. Therefore, the actual mean total density value for the white-egg layers might be skewed. No other humeri from the brown or the white-egg strains were found to have measurable trabecular bone. The presence of medullary bone within the humerus has been found to vary among strains of laying hens, but bone density and strength have been shown to increase in its presence (Fleming et al., 1998b).

The humerus cortical density was 2% greater in the brown-egg laying strain than in the white-egg laying strain (Table 5). These results are supported by the greater bone breaking strength in the brown-egg strain (Table 5). The brown-egg strain showed an 18% higher humerus breaking strength than the white-egg strain. However, unlike the femur, the humerus of the brown-egg strain

was longer than the white-egg strain (Table 5). The longer humerus and the increased humerus cortical density and area of the brown-egg layers resulted in an overall heavier humerus and, thus, more bone Ca in the brown-egg strain (Table 5). Knowles et al. (1993) found that humerus breaking strength did not differ among the 4 breeds of hens they examined.

Humerus trabecular density and area were found to be similar between the brown and white-egg strains (Table 5). As previously mentioned, only one humerus had trabecular bone within the bone cavity, which may make humeri more susceptible to breaks as they do not contain trabecular bone for internal strength.

Similar to total femur area, the total humerus area was correlated ($r = 0.61$) with BW in the brown-egg strain. However, BW was not correlated with any of the bone quality measurements observed for the white-egg strain. Hen-day egg production was correlated with cortical bone density in the brown-egg strain but not in the white-egg strain. Humerus quality measurements were not correlated with SG or eggshell weight for either strain. This finding indicates that the differences observed within the density and breaking strength of the brown and white-egg strains are most likely attributed to the differences in their genetic makeup.

The improved femur and humerus bone quality of the brown-egg strain in the current study may indicate that these hens are less susceptible to conditions such as osteomalacia, osteoporosis, and caged layer fatigue. These conditions, although related in terms of bone fragility, have separate causes. Osteomalacia is generally caused by poor bone mineralization as a result of nutritional deficiencies (Whitehead and Fleming, 2000), whereas osteoporosis has been related to many underlying causes such as nutritional deficiencies (Ca, P, and vitamin D), housing, genetics, and high egg production (Newman and Leeson, 1997). Osteoporosis is a gradual loss in structural bone mass over time (Bishop et al., 2000) and is defined by a uniform loss of cortical bone throughout entire body (Whitehead and Fleming, 2000). Cage layer fatigue is a syndrome observed in extreme cases of osteoporosis that mainly affects hens with a high rate of egg output, such as White Leghorns, or hens housed in cages (Leeson et al., 1995).

The present results indicate that there may be a potential for improved welfare of egg laying hens through selection of certain strains. Although this study involved 2 strains of laying hens, it has laid the groundwork for larger-scale study to be conducted with different strains and types of egg-laying birds of other brown and white-egg breeds. The brown-egg strains currently used in the egg laying industry have production rates similar to the white-egg strains. Although the larger body size of the brown hens results in a greater feed intake, this cost may be offset by the loss of white strain hens due to poor bone quality. If the results from this study can be applied to other strains of white- and brown-egg hens, they may eventually alter the choice of laying hen strains to optimize bird health as well as egg production.

ACKNOWLEDGMENTS

The authors thank K. Nadeau, M. J. Zuidhof, R. Fleming, and the staff and students at the Alberta Poultry Research Centre, for their technical assistance.

REFERENCES

- Appleby, M. C., A. W. Walker, C. J. Nicol, A. C. Lindberg, R. Freire, B. O. Hughes, and H. A. Elson. 2002. Development of furnished cages for laying hens. *Br. Poult. Sci.* 43:489–500.
- Bar, A., V. Razaphkovsky, and E. Vax. 2002. Re-evaluation of calcium and phosphorus requirements in aged laying hens. *Br. Poult. Sci.* 43:261–269.
- Bell, D. D., P. H. Patterson, K. W. Koelkebeck, K. E. Anderson, M. J. Darre, J. B. Carey, D. R. Kuney, and G. Zeidler. 2001. Egg marketing in national supermarkets: Egg quality—Part 1. *Poult. Sci.* 80:383–389.
- Bishop, S. C., R. H. Fleming, H. A. McCormack, D. K. Flock, and C. C. Whitehead. 2000. Inheritance of bone characteristics affecting osteoporosis in laying hens. *Br. Poult. Sci.* 41:33–40.
- Canadian Council on Animal Care. 1984. Guide to the Care and Use of Experimental Animals. Vol. 2. Canadian Council on Animal Care, Ottawa, Ontario, Canada.
- Cransberg, P. H., G. B. Parkinson, S. Wilson, and B. H. Thorp. 2001. Sequential studies of skeletal calcium reserves and structural bone volume in a commercial layer flock. *Br. Poult. Sci.* 42:260–265.
- Curtis, P. A., F. A. Gardner, D. B. Mellor. 1986. A comparison of selected quality and compositional characteristics of brown and white shell eggs. III. Composition and nutritional characteristics. *Poult. Sci.* 65:501–507.
- De Ketelaere, B., T. Govaerts, P. Coucke, E. Dewil, J. Visscher, E. Decuyper, and J. De Baerdemaeker. 2002. Measuring the eggshell strength of 6 different strains of laying hens: techniques and comparisons. *Br. Poult. Sci.* 43:238–244.
- Duncan, I. J. H. 2001. Animal welfare issues in the poultry industry: Is there a lesson to be learned? *Appl. Anim. Welf. Sci.* 4:207–221.
- Elaroussi, M. A., L. R. Forte, S. L. Eber, and H. V. Biellier. 1994. Calcium homeostasis in the laying hen. 1. Age and dietary calcium effects. *Poult. Sci.* 73:1581–1589.
- Fleming, R. H., H. A. McCormack, L. McTeir, and C. C. Whitehead. 1996. Influence of medullary bone on humeral breaking strength. *Br. Poult. Sci.* 37:S30–32.
- Fleming, R. H., H. A. McCormack, L. McTeir, and C. C. Whitehead. 1998a. Digitised fluoroscopy (DF) predicts breaking strength in osteoporotic avian bone in vivo. *Br. Poult. Sci.* 39:S49–SS51.
- Fleming, R. H., H. A. McCormack, L. McTeir, and C. C. Whitehead. 1998b. Medullary bone and humeral breaking strength in laying hens. *Res. Vet. Sci.* 64:63–67.
- Fleming, R. H., H. A. McCormack, and C. C. Whitehead. 1998c. Bone structure and strength at different ages in laying hens and effects of dietary particulate limestone, vitamin K and ascorbic acid. *Br. Poult. Sci.* 39:434–440.
- Flock, D. K. 1994. Targets for selection, limits to performance and market requirements: Eggs. Pages 27–32 in *Proc. 9th European Poultry Conference*, Glasgow, UK. Walker and Connell Ltd., Darvel, UK.
- Gregory, N. G., and L. J. Wilkins. 1989. Broken bones in domestic fowl: Handling and processing damage in end-of-lay battery hens. *Br. Poult. Sci.* 30:555–562.
- Hamilton, R. M. G. 1982. Methods and factors that affect the measurement of egg shell quality. *Poult. Sci.* 61:2022–2039.
- Holder, D. P., and M. V. Bradford. 1979. Relationship of specific gravity of chicken eggs to number of cracked eggs observed and percent shell. *Poult. Sci.* 58:250–251.
- Hunton, P. 1982. Genetic factors affecting egg shell quality. *World's Poult. Sci. J.* 38:75–84.

- Knowles, T. G., D. M. Broom, N. G. Gregory, and L. J. Wilkins. 1993. Effect of bone strength on the frequency of broken bones in hens. *Res. Vet. Sci.* 54:15–19.
- Knowles, T. G., and L. J. Wilkins. 1998. The problem of broken bones during the handling of laying hens—A review. *Poult. Sci.* 77:1798–1802.
- Leeson, S., G. Diaz, and J. D. Summers. 1995. Pages 149–153 in *Poultry Metabolic Disorders and Mycotoxins*. University Books, Guelph, Ontario, Canada.
- McCoy, M. A., G. A. C. Reilly, D. J. Kilpatrick. 1996. Density and breaking strength of bones of mortalities among caged layers. *Res. Vet. Sci.* 60:185–186.
- Mench, J. A., and I. J. H. Duncan. 1998. Poultry welfare in North America: opportunities and challenges. *Poult. Sci.* 77:1763–1765.
- Newman, S., and S. Leeson. 1997. Skeletal integrity in layers at the completion of egg production. *World's Poult. Sci. J.* 53:265–277.
- Nørgaard-Nielsen, G. 1990. Bone strength of laying hens kept in an alternative system, compared with hens in cages and on deep-litter. *Br. Poult. Sci.* 31:81–89.
- Rath, N. C., G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. *Poult. Sci.* 79:1024–1032.
- Renema, R. A., and F. E. Robinson. 2001. Effects of light intensity from photostimulation in four strains of commercial egg layers: 1. Ovarian morphology and carcass parameters. *Poult. Sci.* 80:1112–1120.
- Renema, R. A., F. E. Robinson, J. J. R. Feddes, G. M. Fasenko, and M. J. Zuidhof. 2001. Effects of light intensity from photostimulation in four strains of commercial egg layers: 2. Egg production parameters. *Poult. Sci.* 80:1121–1131.
- Rennie, J. S., R. H. Fleming, H. A. McCormack, C. C. McCorquodale, and C. C. Whitehead. 1997. Studies on effects of nutritional factors on bone structure and osteoporosis in laying hens. *Br. Poult. Sci.* 38:417–424.
- Robinson, F. E., R. A. Renema, H. H. Oosterhoff, M. J. Zuidhof, and J. L. Wilson. 2001. Carcass traits, ovarian morphology, and egg laying characteristics in early versus late maturing strains of commercial egg-type hens. *Poult. Sci.* 80:37–46.
- Rowland, L. O., Jr., J. L. Fry, R. B. Christmas, A. W. O'Steen, and R. H. Harms. 1972. Differences in tibia strength and bone ash among strains of layers. *Poult. Sci.* 51:1612–1615.
- SAS Institute Inc. 1999. *The SAS System for Windows, NT Version 4.0.1381*. SAS Institute Inc., Cary, NC.
- Scott, T. A., and F. G. Silversides. 2000. The effect of storage and strain of hen on egg quality. *Poult. Sci.* 79:1725–1729.
- Silversides, F. G., and T. A. Scott. 2001. Effect of storage and layer age on quality of eggs from two lines of hens. *Poult. Sci.* 80:1240–1245.
- Taylor, T. G., and C. G. Dacke. 1984. Calcium metabolism and its regulation Pages 149–165 in *Physiology and Biochemistry of the Domestic Fowl*. Vol. 5. B. W. Freeman, ed. Academic Press, Montreal.
- Taylor, T. G., and J. H. Moore. 1954. Skeletal depletion in hens laying on a low calcium diet. *Br. J. Nutr.* 8:112–124.
- Whitehead, C. C., and R. H. Fleming. 2000. Osteoporosis in cage layers. *Poult. Sci.* 79:1033–1041.
- Whitehead, C. C., B. Fleming, and S. Bishop. 1998. Towards a genetic solution to osteoporosis in laying hens. Pages 51–54 in *Roslin Institute 1997/98 Annual Report*. H. Griffin, ed. Roslin Institute, Edinburgh.