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INFLUENCE OF DIETARY CALCIUM LEVELS ON BONE DEVELOPMENT IN BROILER BREEDER HENS

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ABSTRACT: *A study was conducted to determine the effects of three dietary calcium (Ca) levels on bone characteristics of 198 broiler breeder hens during the laying period. The pullets in each experimental diet were randomly divided into three treatment groups with 1.5, 2.5 and 3.5% dietary Ca (66 birds per treatment) fed from 23 to 60 weeks of age. Treatments were arranged in a 2 x 3 factorial block design (effect of 2 ages and 3 Ca levels). Three types of breeder diets containing 1.5, 2.5 and 3.5% Ca were fed from 23 to 60 weeks of age (laying period) and these include: breeder phase 1 (23 to 34 weeks), breeder phase 2 (35 to 46 weeks) and breeder phase 3 (47 to 60 weeks). The diets were isocaloric and isonitrogenous but varied only in Ca and phosphorus (P). Feed was provided in restricted amounts in accordance with the breeders' recommendations. At 35 and 60 weeks of age, 12 birds were randomly selected from each treatment and killed by cervical dislocation and tibiae (left and right) and right humeri from each bird excised. Parameters studied were bone weight, bone length, bone midshaft width, bone breaking strength (BS), bone stress, percent bone, true cortical area and bone ash percentage. These results showed no (P>0.05) beneficial effects of feeding increased Ca levels on all bone parameters except BS. Feed intake and body weight of broiler breeder hens were lower when 1.5% Ca was included in the diet. It seems that 2.5% Ca (4 g Ca/hen/day) is adequate to stimulate feed intake and support growth of broiler breeder hens. Bone stress decreased (P<0.081) with age, indicating that the degree of bone mineralisation was greater at 35 weeks compared to 60 weeks.*

ORIGINAL ARTICLE

Keywords: *Bone dimensions, bone strength, bone stress, calcium, phosphorus*

INTRODUCTION

There are several different types of bones in laying hens. The main types that provide structural integrity are cortical and cancellous (or trabecular) bones, both of which are forms of lamellar bone. These bones are formed during growth, but when a hen reaches sexual maturity, a third type of nonstructural bone, medullary bone, is formed (Whitehead and Fleming, 2000). Medullary bone persists throughout the laying period and its formation is concomitant with maturation of the ovarian follicles (Dacke et al., 1999). It is argued (Whitehead and Fleming, 2000) that the conventional view that medullary bone contributes little to overall bone strength may not be totally correct. Fleming et al. (1998a) demonstrated that the presence of large amounts of medullary bone in the humerus of hens during the laying period improves bone strength. Medullary bone supplies calcium for eggshell formation at periods when dietary supply is not sufficient (Klasing, 1998). The two bones that are rich in medullary bone are femur and tibiotarsus.

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Modern laying hens have a high susceptibility to bone fracture. The high incidence of fractures in live birds, which can occur both during the egg production period and in the course of depopulation and subsequent transport and handling, represents a severe welfare problem (Fleming et al., 1994*).* Although the growth performance of the modern broiler has changed considerably over recent years, their diets have changed little. It has been postulated that probably the porosity observed arises from the occurrence of more rapid bone modelling and remodelling in modern birds, together with an inadequate dietary supply of calcium and phosphorus.

The objective of this experiment was to determine the effects of three levels of calcium on the bone characteristics of broiler breeder hens during the laying period.

MATERIALS AND METHODS

One hundred and ninety eight Ross broiler breeder pullets were reared up to 22 weeks according to body mass guidelines on diets containing 1.0, 1.5 and 2.0% Ca. The pullets in each experimental diet were randomly divided into three treatment groups with 1.5, 2.5 and 3.5% dietary Ca (66 birds per treatment) fed from 23 to 60 weeks of age. A constant Ca : P ratio was maintained in all the diets (Table 2). The pullets were placed in individual cages, which were equipped with individual feed troughs, water nipples and perches. All data were collected on an individual bird basis and each bird was considered as an experimental unit.

Birds were first photostimulated at 154 days (22 weeks) in accordance with recommendation of Ross Breeders (2001). The photoperiod was extended with artificial light by 2 to 3 hours at 22 weeks and thereafter by one hour per week from 24 to 26 weeks of age when the birds received 16 hours of light. This was held constant until birds were depopulated at 60 weeks of age.

Experimental diets are given in Tables 1 and 2. Pullets were fed pre-breeder diet containing 1.0, 1.5 and 2.0% Ca from 19 to 22 weeks of age. Three types of breeder diets containing 1.5, 2.5 and 3.5% Ca were fed from 23 to 60 weeks of age (laying period) and these include: breeder phase 1 (23 to 34 weeks), breeder phase 2 (35 to 46 weeks) and breeder phase 3 (47 to 60 weeks). A diet with 2.5% Ca was obtained by mixing the 1.5% and 3.5% Ca diets. Each dietary treatment of the layer phase was fed to 66 replicates (22 birds per subgroup). Experimental diets were isocaloric and isonitrogenous. Feed was provided in restricted amounts in accordance with the breeders' recommendations, while water was provided *ad libitum*. Feed intake by individual birds was recorded on weekly basis and body weight was determined by weighing each bird at three weekly intervals. Mortality was recorded during the course of the experiment.

At 35 and 60 weeks of age, 12 birds were randomly selected from each treatment and killed by cervical dislocation. The carcasses were stored overnight in a refrigerator at 5 ^{o}C until the following day when the tibiae (left and right) and right humeri from each of the birds were excised and defleshed without boiling. The right tibiae and right humeri were then weighed and total length and bone shaft widths measured by means of a calliper with an accuracy of 0.001 cm (Zhang and Coon, 1997). The tibiae (both left and right) and right humeri were individually sealed in plastic bags to minimise moisture loss, and stored in a freezer at -18 ^{0}C for later analysis (Zhang and Coon, 1997). The bones were then removed from refrigerator for bone ash and breaking strength (BS) determinations. The right tibiae and right humeri were used for BS while left tibiae were used for bone ash determination and histomorphometric analysis. Breaking strength (N) was determined according to procedures described by Fleming et al. (1998b). Bone stress (N/mm^2), which is force per unit area of bone, was calculated by dividing bone strength with true cortical area (mm^2). True cortical area was calculated by multiplying cortical area with mean percent bone and divided by 100. Percent bone which is the reciprocal of porosity was determined from microscopic observations.

Left tibiae were dissected and a 5 mm ring from midshaft taken for histological processing. Two additional samples were taken, 20 mm on either side of the ring, and combined for ash measurements according to the procedures described by Fleming et al. (1998b) and Williams et al. (2000a). The bone cross-section taken for histology was fixed in 10% neutral buffered formalin, decalcified and processed for histomorphometric analysis according to procedures described by (Fleming et al., 1998b).

Data during the laying period (23 to 60 weeks) were analyzed as a 2 x 3 factorial block design (effect of 2 ages and 3 Ca levels) in which data from individual birds served as replicates. Data were subjected to ANOVA using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1996) (version 6.12) to assess the effect of dietary treatment on response variables relating to mechanical properties (bone strength and stress), bone dimensions (length, width and weight), bone chemical composition (ash percentage, Ca and P contents) and Ca intake. The differences between treatment means were separated using Tukey's studentised range test.

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Table 1 - Physical composition of laying diets on air dry basis (%)

	Pre-breeder diet		Breeder Phase 1		Breeder Phase 2		Breeder Phase 3	
	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca
Maize	63.54	63.51	61.92	59.66	63.11	60.81	56.43	62.23
Pollard Glutten	-	-	4.45	2.3	1.8	1.0	-	-
Wheat bran	12.65	6.65	5.15	-	6.55	-	14.90	1.00
Full fat soya	-	-	-	10.0	-	9.95	-	1.70
Soybean oil cake	7.75	11.4	8.6	10.3	8.4	7.55	8.75	9.50
Sunflower oil cake	12.45	11.1	15.0	7.75	15.0	10.00	15.00	15.0
Calcium carbonate (grit)	-	-	2.0	6.15	2.3	6.75	2.25	6.60
Calcium carbonate (fine)	1.15	2.2	0.5	1.5	0.6	1.65	0.6	1.65
Mono calcium phosphate	1.49	4.25	1.29	1.36	1.40	1.50	1.28	1.53
Salt	0.24	0.26	0.41	0.40	0.43	0.44	0.44	0.44
Bicarbonate	0.20	0.15	-	-	-	-	-	-
Choline liquid	0.04	0.04	0.03	0.03	-	0.03	-	-
Lysine	0.10	0.04	0.15	-	0.10		0.03	0.03
Methionine	0.05	0.05	0.005	0.06	0.01	0.05	0.01	0.02
Trace mineral / vitamin premix	0.35	0.35	0.50	0.50	0.30	0.30	0.30	0.30

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Table 2 - Nutrient composition of experimental diets on air dry basis (%)

	Pre-breeder diet		Breeder phase 1		Breeder phase 2		Breeder phase 3	
	1.0% Ca	2.0% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca	1.5% Ca	3.5% Ca
Moisture	11.07	10.37	10.58	9.96	9.77	9.10	9.85	9.19
Metabolisable Energy (MJ/kg)	11.96	11.70	12.09	12.00.	11.94	11.87	11.46	11.43
Protein	15.22	15.50	18.33	17.72	17.03	16.77	16.68	16.06
Crude fat	3.30	3.06	3.00	4.20	2.97	4.07	3.09	2.98
Crude fibre	7.01	5.99	0.00	0.00	6.65	5.08	8.28	6.64
Ash	0.00	0.00	6.21	11.23	6.74	12.05	6.90	11.98
Calcium	1.00	2.01	1.51	3.50	1.52	3.50	1.59	3.46
Phosphorus	0.84	1.37	0.78	0.71	0.80	0.74	0.84	0.78
Available phosphorus	0.45	0.90	0.41	0.40	0.43	0.43	0.43	0.54
Arginine	0.98	1.01	1.11	1.12	1.08	1.09	1.10	1.07
Isoleucine	0.60	0.64	0.74	0.76	0.69	0.71	0.67	0.67
Lysine			0.81	0.83	0.76	0.78	0.73	0.72
Methionine	0.35	0.34	0.38	0.38	0.35	0.36	0.33	0.33
TSAA[1]	0.06	0.64	0.73	0.70	0.68	0.67	0.66	0.64
Threonine	0.55	0.57	0.66	0.66	0.62	0.63	0.61	0.60
Tryptophan	0.17	0.18	0.19	0.20	0.18	0.19	0.19	0.18
TA[2] Arginine	0.91	0.93	1.04	1.04	0.99	1.01	1.01	0.99
TA[2] Isoleucine	0.54	0.57	0.67	0.69	0.62	0.65	0.59	0.60
TA[2] Lysine	0.60	0.60	0.70	0.71	0.64	0.67	0.61	0.61
TA[2] Methionine	0.31	0.31	0.34	0.35	0.31	0.33	0.29	0.30
TA[2] TSAA	0.57	0.57	0.64	0.63	0.59	0.60	0.57	0.56
TA[2] Threonine	0.48	0.50	0.59	0.59	0.55	0.56	0.26	0.53
TA[2] Tryptophan	0.15	0.16	0.17	0.18	0.17	0.17	0.17	0.17
AC:Linoleic acid	1.83	1.68	1.65	2.32	1.65	2.26	1.71	1.64
Xanthophylls	0.00	0.00	23.51	17.68	17.12	14.66	11.29	12.45
Salt	0.24	0.27	0.42	0.41	0.44	0.44	0.45	0.45
Choline	1300.01	1309.56	1205.18	1204.08	1008.79	1003.18	1087.10	993.06
Sodium	0.16	0.16	0.18	0.18	0.19	0.20	0.20	0.20
Chlorine	0.22	0.57	0.33	0.29	0.33	0.31	0.32	0.32
Potassium	0.60	0.60	0.60	0.63	0.63	0.63	0.71	0.61
Magnesium			0.22	0.20	0.23	0.21	0.25	0.23
Manganese			46.82	63.94	50.82	68.71	61.84	71.60

[1] Total sulphur amino acids. [2] Chemically determined

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RESULTS AND DISCUSSION

Feed intake

The hens' feed intake was significantly (P<0.001) different among dietary treatments. Feed intake increased with increasing dietary Ca level with 1.5 and 3.5% Ca diets giving the lowest (989.62±4.72 g) and highest (1059.60±4.80 g) average feed intake values per hen for the total period, respectively. These results are in agreement with those of Clunies et al. (1992a) who fed three levels of Ca (2.5, 3.5 and 4.5%) to white Leghorn hens and found that birds fed 2.5% Ca diet had the lowest feed intake while those fed 3.5% Ca showed the highest. Summers et al. (1976) also reported similar differences by feeding laying hens on 1.5 and 2.96% Ca diets, respectively. Kornegay et al. (1985), Clunies and Leeson (1995) and Ahmad et al. (2003), however, reported no effect of dietary Ca level on feed consumption of hens fed on diets containing Ca levels ranging from 2.5 to 5.0%. Feed intake significantly (P<0.001) increased with age.

Calcium intake

Different intakes of Ca by the hens in each treatment were achieved by feeding the various Ca levels. Keshavarz and Nakajima (1993) and Clunies and Leeson (1995) reported a significant (P<0.05) increase in Ca intake by feeding dietary Ca levels ranging from 2.5 to 5.5%. Kemp and Kenny (2004) suggested that breeders need 4-5 grams of Ca per day from the first egg throughout the laying period. This requirement is satisfied by making the change from pre-breeder (1.5% Ca) to breeder (2.8% Ca) diets immediately prior to the first egg (Ross Breeders, 1998). The 2.5% dietary Ca levels in the current study appeared to provide the recommended requirements (4-5 g). On the other hand, the Ca intake by hens fed 3.5% dietary Ca exceeds the proposed intake.

Daily Ca intake increased with age except for weeks 38 and 58. The variation in Ca intake at especially weeks 38 and 58 could be attributable to high and low temperatures. The maximum and minimum temperatures at week 38 were 35.6 ^{o}C and 17 ^{o}C, respectively. At week 58, maximum and minimum temperatures of 19.4 ^{o}C and 4.0 ^{o}C were recorded.

Body weight

Hen BW increased (P<0.05) as dietary Ca concentration increased from 1.5 to 3.5%. Birds fed 1.5% Ca diet had significantly (P<0.05) lower BW than those fed 2.5 and 3.5% Ca diets, respectively. However, BW for birds fed 2.5% and 3.5% Ca diets was not significantly different, indicating that either of these two levels is sufficient to support growth. The results of this study support those of Clunies and Leeson (1995) and Menge et al. (1977) who reported improved BW in laying Single Comb White Leghorn hens and turkey hens fed increasing dietary Ca levels ranging from 1.16 to 5.5%. A significant (P<0.001) Ca level x age interaction occurred.

Mortality

Twelve birds died (4 from each treatment) during the laying period representing a cumulative mortality rate of 6%. This indicates that treatment did not influence mortality in agreement with Atkinson et al. (1967).

Bone dimensions

As illustrated in Table 3, bone length, width and weight were not significantly (P>.05) influenced by dietary Ca level. The results of the current study are inconsistent with Williams et al. (2000b), who reported that the tibiotarsus width of broilers fed higher levels of Ca decreased linearly with increasing dietary Ca content. These workers suggested that the small dietary Ca effects on body weight and bone ash could have combined to give a stronger, but indirect, effect on bone width. However, the results of the present study do not support these findings. In contrast with the humerus, the length and width of tibia significantly (P<0.001) increased and decreased with age, respectively. No explanation could be given for this.

From Table 3 it is apparent that bone weight did not change significantly (P>.05) with age. The gradual resorption of medullary bone for eggshell formation during the laying period could have contributed to this non-significant bone weight result. Although it is generally thought that medullary bone has non-structural properties, Fleming et al. (1996) have shown that it contributes to overall bone strength. Resorption of medullary bone could result in weaker bones.

Bone mechanical properties

Measurements of breaking strengths of humeri and tibiae are given in Table 3. It seems that bone strength (BS) significantly (P<0.02) increased with increased levels of dietary Ca. Although the BS for birds fed 2.5 and 3.5% Ca diets was not statistically different, birds on 3.5% Ca diet tended to have numerically greater BS values. These results are in agreement with those of Rowland et al. (1968) who reported significantly (P<0.05) higher BS for birds fed 6.8% Ca diets

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compared to 1.0%. In contrast to these results, Moore et al. (1977) observed no statistical differences in the BS of radii of 4 months old commercial layer hens fed 3.78% Ca and 1.0% P and 3.22% Ca and 0.65% P diets.

Table 3 - The effect of calcium level and age on bone dimensions and mechanical properties of bones in broiler breeder hens

		Age (weeks)			Significance of effect (P)			
	Treatment	35	60	Means	Treatment	Age	Interaction	CV
Right tibia								
Length (mm)	1.5% Ca	124.36 ± 1.11	128.87 ± 1.16	126.62 ± 0.80[a]	0.3220	0.0004	0.5903	3.1
	2.5% Ca	124.23 ± 1.11	126.44 ± 1.11	125.33 ± 0.79[a]				
	3.5% Ca	123.19 ± 1.11	126.79 ± 1.11	124.99 ± 0.79[a]				
	Means	123.93 ± 0.64	127.37 ± 0.65					
Width (mm)	1.5% Ca	7.87 ± 1.89	7.24 ± 0.20	7.55 ± 0.14[a]	0.8833	0.0001	0.4287	8.6
	2.5% Ca	7.90 ± 1.89	7.39 ± 0.19	7.65 ± 0.13[a]				
	3.5% Ca	8.10 ± 1.89	7.12 ± 0.19	7.61 ± 0.13[a]				
	Means	7.96 ± 0.11[a]	7.25 ± 0.11[b]					
Weight (g)	1.5% Ca	17.81 ± 0.54	18.60 ± 0.56	18.21 ± 0.39[a]	0.2059	0.9566	0.3813	10.0
	2.5% Ca	18.75 ± 0.54	18.77 ± 0.54	18.76 ± 0.38[a]				
	3.5% Ca	19.55 ± 0.54	18.82 ± 0.54	19.19 ± 0.38[a]				
	Means	18.71 ± 0.31[a]	18.73 ± 0.32[a]					
Bone strength (N)	1.5% Ca	235.00 ± 68.74	252.00 ± 93.43	242.20 ±15.56[a]	0.0001	0.9809	0.7308	23.3
	2.5% Ca	320.42 ± 56.18	315.00 ± 58.54	317.71 ± 14.44[b]				
	3.5% Ca	350.00 ± 54.10	340.00 ± 105.45	343.50 ± 14.79[b]				
	Means	300.93 ± 12.00[a]	301.34 ± 12.38[a]					
Bone stress (N/mm^2)	1.5% Ca	27.90 ± 5.47	19.33 ± 6.86	23.61 ± 4.39[a]	0.5893	0.0081	0.7790	68.5
	2.5% Ca	32.63 ± 5.47	16.50 ± 6.42	24.57 ± 4.22[a]				
	3.5% Ca	37.39 ± 6.05	21.38 ± 5.74	29.38 ± 4.17[a]				
	Means	32.33 ± 3.27[a]	19.07 ± 3.67[b]					
Right humerus								
Length (mm)	1.5% Ca	84.09 ± 1.55	81.99 ± 1.62	83.04 ± 1.22[a]	0.7338	0.7061	0.6245	6.4
	2.5% Ca	82.94 ± 1.55	83.88 ± 1.55	83.41 ± 1.10[a]				
	3.5% Ca	84.39 ± 1.55	84.10 ± 1.55	84.25 ± 1.10[a]				
	Means	83.81 ± 0.90[a]	83.32 ± 0.91[a]					
Width (mm)	1.5% Ca	6.53 ± 0.11	6.50 ± 0.12	6.51 ± 0.80[a]	0.8781	0.4454	0.7765	6.1
	2.5% Ca	6.56 ± 0.11	6.39 ± 0.11	6.48 ± 0.80[a]				
	3.5% Ca	6.47 ± 0.11	6.44 ± 0.11	6.45 ± 0.80[a]				
	Means	6.52 ± 0.07[a]	6.44 ± 0.07[a]					
Weight (g)	1.5% Ca	10.28 ± 0.74	9.83 ± 0.77	10.05 ± 0.53[a]	0.0927	0.5169	0.4503	26.0
	2.5% Ca	8.77 ± 0.74	9.00 ± 0.74	8.89 ± 0.52[a]				
	3.5% Ca	9.76 ± 0.74	11.16 ± 0.74	10.46 ± 0.52[a]				
	Means	9.60 ± 0.42[a]	10.00 ± 0.43[a]					
Bone strength (N)	1.5% Ca	235.00 ± 22.38	252.00 ± 23.47	243.50 ± 16.22[b]	0.0001	0.9768	0.8149	24.4
	2.5% Ca	320.42 ± 21.43	315.00 ± 21.43	317.71 ± 15.15[a]				
	3.5% Ca	350.00 ± 21.43	340.00 ± 22.38	345.00 ± 15.49[a]				
	Means	301.81 ± 12.56[a]	302.33 ± 1.96[a]					

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable

Stress values were not significantly (P>0.05) different for any treatment, suggesting that mineralisation was similar across treatment groups. Although it was apparent from Table 3 that dietary Ca did not influence bone stress, birds fed 3.5% Ca tended to have higher stress values compared to those fed 1.5% and 2.5% Ca diets. The high coefficient of variation could have contributed to these non-significant results. Previous studies (Whitehead and Wilson, 1992) have shown that there is a constant decline in structural bone content of hens throughout the laying period. Whitehead (2004) states that the general net effect of the replacement of structural bone is to weaken the overall strength of the hen's skeleton and thus increase fracture. In the current study, no significant (P=0.98) influence of age on BS occurred. It is, however, evident from Table 3 that bone stress significantly (P<0.0081) decreased with age indicating that the degree of

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mineralisation of bone was greater at 35 weeks compared to 60 weeks. Bone stress decreased by approximately 46% between 35 and 60 weeks of age. This indicates progressive loss of structural bone over the life of the laying hen and its subsequent replacement with medullary bone. Crenshaw et al. (1981) stated that as bone mineralisation increases, maximum stress and bending moment of the bone increase. At a point of optimum mineralisation, stress reaches a maximum. Conversely, the lower stress indicates that the hens had bones that were less mineralised.

Bone chemical composition

Data on bone ash, Ca and P content of bone ash are given in Table 4. There were no significant ($P>0.05$) differences in tibia ash among dietary Ca levels. These results are in disagreement with those of Atteh and Leeson (1983) who reported a significantly ($P<0.05$) higher bone ash content with increasing dietary Ca from 3.0 to 4.2%. In disagreement with Rowland et al. (1968, 1972), tibia ash did not appear to be related to BS in the current study. These workers reported that caged hens that had low BS had significantly lower tibia ash values than floor hens.

No significant ($P<0.05$) differences were observed between the two age periods with respect to tibia ash content. In the present study, average tibia ash content at 35 and 60 weeks was 55.2 and 54.8%, respectively (Table 4). Newman and Leeson (1999) suggested that low ash values probably indicate that medullary bone was being resorbed at a faster rate in order to supply sufficient Ca to maintain shell formation. Whitehead (2004) suggested that the considerable rise in circulating oestrogen at the onset of hen's sexual maturity has a stimulatory effect on osteoblasts, causing them to produce medullary bone instead of structural bone. In the absence of structural bone formation, continued osteoclastic resorption would be expected to give rise to a net depletion of structural bone, leading ultimately to osteoporosis (Fleming et al. 1998b). The decline ($P<0.001$) in Ca content observed from 35 to 60 weeks of age in the current study support the view that the medullary bone was being resorbed to support shell formation resulting in bones with lower bone stress values. These results were, however, not supported by the ash values of bones (Table 4).

Table 4 - The effect of calcium level and age on bone composition of broiler breeder hens

		Age (weeks)			Significance of effect (P)			
	Treatment	35	60	Means	Treatment	Age	Interaction	CV
Left tibia								
Ash content, %	1.5% Ca	56.82 ± 3.24	52.39 ± 7.33	54.61 ± 1.04[a]	0.1080	0.7504	0.0661	9.3
	2.5% Ca	52.74 ± 3.26	54.66 ± 7.11	53.70 ± 1.04[a]				
	3.5% Ca	56.10 ± 4.65	57.47 ± 3.66	56.78 ± 1.04[a]				
	Means	55.22[a]	54.84[a]					
Calcium, %	1.5% Ca	38.22 ± 0.96	14.33 ± 0.92	26.28 ± 0.67[a]	0.7197	0.0001	0.2439	12.2
	2.5% Ca	39.63 ± 0.92	13.73 ± 0.92	26.68± 0.65[a]				
	3.5% Ca	37.33 ± 0.92	14.52 ± 0.92	25.93 ± 0.65[a]				
	Means	38.89 ± 0.54[a]	14.19 ± 0.53[b]					
Phosphorus, %	1.0% Ca	16.87 ± 0.34	6.61 ± 0.33	11.74 ± 0.24[b]	0.0415	0.0001	0.9244	10.2
	1.5% Ca	16.29 ± 0.33	5.92 ± 0.33	11.11 ± 0.23[ab]				
	2.0% Ca	15.98 ± 0.33	5.89 ± 0.33	10.92 ± 0.23[a]				
	Means	16.38 ± 0.19[a]	6.13 ± 0.19[b]					
TCA[1] (mm^2)	1.0% Ca	13.64 ± 1.45	14.14 ± 1.45	13.89 ± 0.99[a]	0.0856	0.0987	0.5925	24.5
	1.5% Ca	15.24 ± 0.99	16.84 ± 1.36	16.04 ± 0.84[a]				
	2.0% Ca	15.25 ± 1.28	18.43 ± 1.21	16.84 ± 0.88[a]				
	Means	14.71 ± 0.72[a]	16.47 ± 0.76[a]					
Percent bone	1.5% Ca	0.82 ± 0.07	0.91 ± 0.06	0.86 ± 0.05[a]	0.9116	0.0209	0.8706	20.6
	2.5% Ca	0.82 ± 0.04	0.92 ± 0.06	0.87 ± 0.04[a]				
	3.5% Ca	0.77 ± 0.06	0.92 ± 0.06	0.85 ± 0.04[a]				
	Means	0.81 ± 0.03[a]	0.92 ± 0.03[a]					

[1]TCA – true cortical area (cortical area multiplied by mean % bone divided by 100). Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable.

The percentage of Ca present in the mineral component of the tibia was not significantly ($P=0.72$) different between Ca levels (Table 4). The mean values for 1.5, 2.5 and 3.5% Ca levels are 26.28, 26.68 and 25.93%, respectively. Clunies et al. (1992b) suggested that perhaps with higher levels of dietary Ca there is a decreased dependence upon medullary bone mineral to supply Ca for shell formation. The results of the current study do not seem to support this view given the low mean Ca content of bone ash for birds on 3.5% Ca diets. According to Hurwitz and Barr (1966), medullary

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bone Ca increases with increasing dietary Ca levels. The results of the present study are consistent with those of Clunies and Leeson (1995) and Keshavarz and Nakajima (1993), who found no beneficial effects of feeding increased dietary Ca levels (2.5 to 5.5% Ca) on bone Ca levels of laying hens.

In the present study, birds fed 3.5% Ca diet had significantly ($P<0.05$) lower P content compared to those fed 1.5% Ca diets. No explanation could be given for this. Phosphorus content of the bone tended to decline with increasing dietary Ca level.

During the test period (35 to 60 weeks) both Ca and P content of bone ash significantly ($P<0.001$) declined by 63.5 and 62.6%, respectively. This represents a monthly decline of 2.1 % for both parameters. The decline in Ca content of bone is expected, as the hen requires Ca for eggshell formation during the laying period. It is well documented that egg weight and size increase with age, indicating that the heavier egg requires more calcium to be deposited as shell than a lighter egg. Most of the calcium required for eggshell formation is obtained from the medullary bone, which is continuously resorbed during the laying period resulting in low Ca in the bones.

True cortical area and percent bone

According to Table 4, true cortical area (TCA) and percent bone were not statistically influenced by dietary Ca level. Percent bone significantly ($P<0.02$) increased with age and TCA did not.

CONCLUSIONS

The present results demonstrated no beneficial effects of feeding increased Ca levels on all bone parameters except BS. The feed intake and body weight of broiler breeder hens were; however, lower when 1.5% Ca was included in the diet. Therefore, it seems that 2.5% Ca (4 g Ca/hen/day) is adequate to stimulate feed intake and support growth of broiler breeder hens. This level will also supply the requirements for bone development and Ca content. Bone stress decreased with age, indicating that the degree of bone mineralisation was greater at 35 weeks compared to 60 weeks.

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