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INFLUENCE OF DIETARY CALCIUM LEVELS ON BONE DEVELOPMENT IN BROILER BREEDER PULLETS UP TO 18 WEEKS OF AGE

J.C. MOREKI[1]*, H.J. VAN DER MERWE[2] and J.P. HAYES[3]

[1]*Department of Animal Science and Production, Botswana College of Agriculture, Private Bag 0027, Gaborone, Botswana.*
[2]*Department of Animal Science, Wildlife and Grassland Sciences, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa*
[3]*Department of Animal Sciences, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa*

*Email: jcmoreki@gmail.com

ABSTRACT: *The effects of three levels of dietary calcium on bone development in broiler breeder pullets up 18 weeks of age were investigated. A total of 640 one-day-old Ross broiler breeder pullets were used and were randomly assigned to four treatment groups, each having four replicates. The experimental design was split plot with four dietary treatments being the main plots and age as split plots. The four treatments were 1.0% Ca (0.45% Pi), 1.5% Ca (0.7% Pi), 2.0% Ca (0.9% Pi) and 1.0% Ca (0.45% Pi). The first 3 treatments were feed restricted according to Ross Breeders recommendations while the latter was ad libitum fed (served as control). Pullets were fed threeisocaloric and isonitrogenous diets: pre-starter (0 to 2 weeks); starter (2 to 4 weeks) and grower (4 to 18 weeks). At 6, 12 and 18 weeks of age, 5 pullets from each replicate were randomly selected and sacrificed by cervical dislocation and tibiae (left and right) and right humeri from each bird excised. Parameters studied were bone weight, bone length, bone width, bone ash, percent bone, true cortical area, bone strength and stress. These results showed that dietary Ca hadn't statistical (P>.05) influence on bone formation of broiler breeder pullets on restricted feeding, except bone strength. Ad libitum feeding of broiler breeder pullets resulted in a significant (P<.05) increase in bone dimensions and bone breaking strength. However, ad libitum birds had significantly (P<0.0001) lower bone stress values than restricted groups, indicating less mineralization. However, stress required to break bones from ad libitum birds was significantly (P<0.0001) lower than that required to break bones from restricted group. These results showed that dietary Ca level had no significant effect on bone formation in broiler breeder pullets on restricted feeding up to 18 weeks of age, except bone breaking strength.*

ORIGINAL ARTICLE

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

INTRODUCTION

Broiler breeders are continuously selected for higher growth rate. Therefore, feed restriction during the growing and laying period is a common practice to prevent excess body weight (Ingram et al., 2001). Williams et al. (2000*a*) suggested that bone characteristics might be changing in modern fast growing broilers, with increases in cortical bone porosity and changes in composition that could affect mechanical properties of bone. Feed restriction in broiler breeders results in changes in the relative growth of different body components, but there is little information on the impact of this on bone structure and composition (McCormack et al. 2001).

In broiler breeders, Calcium (Ca) is associated with eggshell formation, but has many important functions in the body, of which the structuring of bones is the most important. About 99% of Ca is contained in the skeleton (Hurwitz et al., 1987). Several researchers (Shafey, 1993; Roberson et al., 2004) reported an increase in bone

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breaking strength (BS) because of increased levels of dietary Ca and phosphorus (P) in commercial growing and laying chickens as well as growing turkeys. Accordingly, higher bone ash percentages were recorded as the Ca level in over one year-old laying hen dietsincreased. Hulan et al. (1986) found that biological performance (body weight gain, final live weight and feed conversion) declines as the total Ca+avP and Ca: avP ratio increase in the diets.

Further research on higher Ca levels in broiler breeder pullets' diets is of utmost importance, as most of the studies regarding dietary Ca levels on bone development involved laying hens. Therefore, a study was undertaken to investigate the effects of dietary Ca levels and feed restriction on bone development of broiler breeders up to 18 weeks of age.

MATERIAL AND METHODS

Six hundred and forty one-day-old Ross broiler breeder pullets were obtained from a commercial hatchery and were randomly assigned to 4 treatment groups, each having 4 replicates. The 4 treatments were 1.0% Ca (0.45% Pi), 1.5% Ca (0.7% Pi), 2.0% Ca (0.9% Pi) and 1.0% Ca (0.45% Pi). The first 3 treatments were feed restricted according to Ross Breeders (2001) recommendations while the last treatment was *ad libitum* fed (served as control) throughout the rearing period (18 weeks). Feed restriction started at 2 weeks of age. Individual feeds were analysed for Ca and P to ensure accurate diet formulation. Water was provided *ad libitum* for all treatments. The initial weight of the birds was determined by weighing 5% of the birds prior to allocation to 4 dietary treatments.

Pullets were fed different diets during the 3 feeding phases of the experimental period namely, pre-starter (0 to 2 weeks); starter (2 to 4 weeks) and grower (4 to 18 weeks). The physical and nutrient compositions of diets are shown in Tables 1 and 2, respectively. The diets for each feeding phase were isocaloric and isonitrogenous. Calcium and feeding levels were the only differences during each specific phase. A diet with 1.5% Ca was obtained by mixing the 1.0% and 2.0% Ca diets.

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

	Pre-starter diet		Starter		Grower	
	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca
Maize	58.62	58.15	58.15	58.37	67.12	64.82
Maize glutten	1.85	-	-	-	-	-
Wheat bran	6.50	12.00	12.00	5.45	12.0	9.30
Full fat soya	-	-	-	1.30	-	-
Soybean oil cake	17.85	17.85	17.85	18.95	6.70	11.60
Sunflower oil cake	8.00	8.00	8.00	8.00	10.0	6.40
Fishmeal	1.00	-	-	-	-	-
Calcium carbonate	1.30	1.45	1.45	3.00	1.70	2.95
Calcium monophosphate	1.27	1.31	1.31	3.37	1.47	4.08
Salt	0.17	0.23	0.23	0.24	0.23	0.26
Sodium bicarbonate	0.30	0.28	0.28	0.25	0.26	0.14
Choline liquid	0.03	0.021	0.02	0.03	0.052	0.05
Lysine	0.33	0.18	0.18	0.15	0.91	0.01
Threonine	0.33	-	-	-	-	-
Methionine	0.24	0.18	0.18	0.18	0.32	0.03
Trace mineral / vitamin premix	0.35	0.35	0.35	0.35	0.35	0.35

Day-old chicks were reared in 16 pens with 40 birds per pen and 4 pens (replicates) per treatment in a closed house with windows for ventilation. Each replicate was housed in floor pens, measuring 4 m^2 with wood shavings as litter material. The stocking density at 12 and 18 weeks of age was 0.11 and 0.13 per m^2, respectively as numbers decreased due to birds that were sacrificed for bone samples. Each pen was equipped with an electric brooder, 2 tube-type feeders and 2 automatic drinkers.

Pullets were reared at a time when day length was decreasing (May to July). Chicks received continuous light for the first 2 days of life and thereafter it was reduced to natural day length pattern of a decreasing and increasing photoperiod throughout the rearing period. Pullets were vaccinated in accordance with a vaccination programme obtained from a local parent stock company.

Feed consumption was measured by giving pre-weighed feed allocations to each replicate group throughout the week and then weighing back all of the unconsumed feed at the end of the week. Pen body weights (BW) were also recorded on weekly basis.

At 6, 12 and 18 weeks of age, 5 pullets were randomly selected from each replicate (i.e. 20 birds per treatment at each age) and killed by cervical dislocation and their carcasses stored in the refrigerator overnight and the bones removed the following day. 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).

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

	Pre-starter diet		Starter		Grower	
	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca
Moisture	11.19	11.31	11.31	10.93	11.20	10.96
ME (MJ/Kg)	12.10	11.80	11.80	11.60	12.10	11.70
Protein	20.26	17.99	17.99	17.99	14.00	14.32
Crude fat	3.01	3.05	3.05	3.05	3.26	3.12
Crude fibre	5.53	6.13	6.13	6.13	6.45	5.41
Calcium	0.99	1.01	1.01	2.00	1.10	2.01
Phosphorus	0.79	0.81	0.81	1.28	0.82	1.36
Available phosphorus	0.45	0.90	0.45	0.90	0.45	0.90
Arginine	1.25	1.15	1.15	1.16	0.88	0.90
Isoleucine	0.84	0.74	0.74	0.76	0.55	0.58
Methionine	0.59	0.49	0.48	0.48	0.30	0.29
TSAA[1]	0.95	0.81	0.81	0.81	0.58	0.57
Threonine	0.78	0.66	0.66	0.67	0.51	0.53
Tryptophan	0.23	0.21	0.21	0.21	0.16	0.16
TA[2] arginine	1.16	1.07	1.06	1.07	0.81	0.83
TA[2] isoleucine	0.76	0.67	0.67	0.69	0.49	0.55
TA[2] lysine	1.05	0.85	0.85	0.85	0.55	0.55
TA[2] methionine	0.56	0.45	0.45	0.45	0.27	0.51
TA[2] TSAA	0.87	0.73	0.73	0.73	0.51	0.46
TA[2] Threonine	0.69	0.58	0.58	0.59	0.45	0.46
TA[2] Tryptophan	0.21	0.19	0.19	0.19	0.14	0.15
Linoleic acid	1.59	1.68	1.68	1.65	1.82	1.72
Salt	0.21	0.23	0.23	0.25	0.24	0.27
Choline (mg/kg)	1410.68	1288.83	1288.83	1308.81	1311.38	1307.09
Sodium	0.18	0.18	0.18	0.18	0.18	0.16
Chlorine	0.24	0.22	0.66	0.66	0.22	0.22
Potassium	0.71	0.70	0.70	0.70	0.57	0.59

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

The tibiae and 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 for bone ash and 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. (1998). Bone stress (N/mm^2), was calculated by dividing BS with true cortical area (mm^2). True cortical area (TCA) 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. (1998) and Williams et al. (2000*a*, 2000*b*). The bone cross-section taken for histology was fixed in 10% neutral buffered formalin, decalcified and processed for histomorphometric analysis according to the procedures described by (Fleming et al., 1998).

Bone data obtained at each sampling period were regressed on average Ca intake and/or levels per chicken during the particular period, namely from day-old to 6 weeks of age, from day-old to 12 weeks of age and for the entire period of day-old to 18 weeks of age. Calcium intakes were calculated from average feed intake values of the birds on a particular dietary Ca level. The Minitab Statistical Software package (Release 8.2) (Minitab Inc., 1991) was employed to analyse data sets.

In a second analysis of the data the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1996) was used to estimate differences between treatment means for the different levels of Ca intake within and between age periods. In this analysis, data were regarded as a split plot design with 4 dietary treatments being the main plots and age as split plots. The differences between treatment means were separated using the Tukey test.

RESULTS AND DISCUSION

Feed intake

Dietary Ca levels did not appear to influence feed intake. This is in line with the findings of Smith et al. (2003) who found that increasing dietary Ca level from 0.9 to 1.5% had no effect on feed consumption of broilers. Similar results were reported by Ahmad et al. (2003) in Bovans hens. On the other hand, Shafey and McDonald (1991) found that high dietary Ca (2.43 *vs.* 0.89%) reduced feed intake in broiler chicks reared up to 17 days of age. It seems that the influence of Ca levels on feed intake differs between *ad libitum* and feed restricted birds. In restricted birds, Ca levels within limits had no pronounced effect on feed intake.

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Feed intake increased significantly ($P<0.05$) with age and the *ad libitum* group consumed significantly ($P<0.05$) more feed than restricted groups. The average feed intake of restricted birds (1.0% Ca diet) was 34.2% of that of the *ad libitum* fed birds (0-18 weeks). Yu et al. (1992a) reported average feed intake of restricted birds to be 37.2% of the full-fed (*ad libitum*) birds during rearing (4-18 weeks).

Calcium intake

The daily Ca intake of the birds significantly ($P<0.05$) increased with increasing dietary Ca level. The average daily Ca intake per bird during the rearing period for birds fed 1.0%, 1.5%, 2.0% Ca diets and *ad libitum*-fed birds was 0.66 g, 0.81 g, 1.0 g and 1.6 g, respectively. Accordingly, Yu et al. (1992b) reported daily average Ca intakes of 0.6 g for feed restricted and 1.67 g for *ad libitum* fed Indian River breeder hens from 4 to 18 weeks of age. In accordance with feed intake, Ca intake of the restricted birds in the present was significantly ($P<0.05$) lower than that of the *ad libitum* group.

Body weight

Dietary Ca levels had no statistical significant ($P<0.05$) influence on the growth rate of birds fed a restricted isonitrogenous and isocaloric diet. These results are consistent with Rosa et al. (2010). In disagreement with these results, Shafey and McDonald (1991) found that increased dietary levels of Ca alone or Ca (2.56%) and P (.49% available P) significantly ($P<0.01$) reduced body weight gain in broiler chickens. The results of the current study suggest that the NRC (1994) recommendations for broiler breeders (1.0% Ca) may be sufficient to support the required growth. As expected, the *ad libitum* group was significantly ($P<0.05$) heavier than restricted groups. In agreement with these results, Yu et al. (1992b) reported that restricted birds not only weighed less, but also had a significantly shorter length than *ad libitum*-fed birds, an indication of stunted growth.

Mortality

Overall mortality for the entire rearing period was 7.2% (46 birds), which is higher than the Ross Breeders standard mortality of 5.1% at 18 weeks of age. The mortality rate was 6.9%, 8.1%, 7.5% and 6.3% for birds on 1.0%, 1.5%, 2.0% Ca and *ad libitum* group, respectively. The high mortality observed in the restricted groups was mainly due to cannibalism as the birds were not debeaked. Atkinson et al. (1967) found that Ca levels *per se* had no significant influence on bird mortality during rearing.

Bone dimensions

Bone length

The mean values for tibia and humerus length are given in Table 3. It is evident that bone length increased non-significantly with increased dietary Ca intake. Restricted birds had shorter tibiae and humeri than *ad libitum* birds, an indication of stunted growth. Yu et al. (1992b) found that restricted birds had significantly shorter tibiae than *ad libitum* fed birds at 18 weeks of age.

A regression analysis of the tibia and humerus length data (Table 4) at all ages showed a highly significant response to Ca intake when data of the *ad libitum* group was included in the calculations. However, no significant response in tibia lengths due to Ca intake could be demonstrated for the restricted groups.

As shown in Table 3 the length of tibia increased significantly ($P<.0001$) with age up to 12 weeks of age while that of humerus increased with age throughout the rearing period. Tibia length increased by 46% and 13% between 6 and 12 weeks and 12 and 18 weeks, respectively. On the other hand,increases in humerus length of 37 and 10% were noted at 6 and 12 weeks and 12 and 18 weeks, respectively. These values show that bone development and growth in broiler breeder pullet is rapid during the first 12 weeks of age.

Bone width and weight

No significant influence of dietary Ca intake on bone width of restricted birds could be detected (Table 3).However, bone width showed a constant significant increase, as the birds got heavier because of age and *ad libitum* feeding. Accordingly Williams et al. (2000a) reported that heavier birds had longer and wider tibiotarsi.

The results of the regression analysis accordingly did not result in any response of bone width to increasing dietary Ca levels (Table 4). However, when the bones from the *ad libitum* group were included in the data set a significant response in bone dimensions to increasing Ca intake occurred (Table 4). The explanation for the significant responses in bone dimensions when data from the *ad libitum* group was included in the data set lies most probably in the larger bone mass that was due to *ad libitum* feeding.A significant ($P<0.0001$) Ca level x age interaction for bone weight occurred, indicating that the influence of dietary Ca on bone weight varied during different periods. Therefore, the effect of dietary Ca levels on bone weight was compared statistically within each age and the effect of age within Ca levels (Table 3). Dietary Ca levels did not significantly ($P>.05$) influence bone weight of the restricted birds (Table 4). However, a tendency for tibia weight to decrease with increased dietary concentration was observed among the restricted group (Table 3). This result is consistent with Williams et al. (2000*b*) who reported a tendency for bone weight to decrease with increasing dietary Ca concentration.

Ad libitum feeding resulted in a heavier bone weight at all ages in agreement with McCormack et al. (2001). The study of Yu et al. (1992b) reported tibia weights of the restricted groups at 18 weeks to be 85% of the *ad libitum* group, whereas it was 88% of the *ad libitum* group in the present study.

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Table 3 - Effect of calcium levels on bone dimensions of broiler breeder pullets during rearing

		Age (weeks)				Significance of effect (P)			
	Treatment	6	12	18	Means	Treatment	Age	Interaction	CV
Right tibia									
Length (mm)	1% Ca	71.84 ± 5.43	100.00 ± 22.65	118.47 ± 11.31	96.76^{b}	0.0064	0.0001	0.2692	70.71
	1.5% Ca	72.11 ± 7.01	109.54 ± 21.72	118.35 ± 8.09	98.50^{b}				
	2% Ca	71.71 ± 5.58	105.54 ± 6.46	118.24 ± 9.00	100.00^{ab}				
	1% Ca &*ad lib.*	92.63 ± 5.54	190.14 ± 26.09	134.52 ± 4.69	139.09^{a}				
	Means	77.07^{a}	122.39^{b}	126.30^{b}					
Width (mm)	1% Ca	4.90 ± 0.66	6.81 ± 0.38	7.10 ± 0.67	6.27^{b}	0.0001	0.0001	0.6769	9.40
	1.5% Ca	4.78 ± 0.48	6.62 ± 0.48	7.23 ± 0.67	6.21^{b}				
	2% Ca	4.98 ± 0.71	6.62 ± 0.62	7.30 ± 0.82	6.30^{b}				
	1% Ca &*ad lib.*	7.03 ± 0.55	8.92 ± 0.64	9.63 ± 0.82	8.53^{a}				
	Means	5.42^{a}	7.24^{b}	7.81^{c}					
Weight (g)	1% Ca	4.05 ± 1.03^{a}	11.08 ± 2.53^{a}	15.22 ± 4.29^{a}		0.0001	0.0001	0.0001	19.64
	1.5% Ca	4.35 ± 1.13^{a}	11.19 ± 3.12^{a}	14.57± 2.66^{a}					
	2% Ca	4.07 ± 1.12^{a}	11.00 ± 1.61^{a}	14.70 ± 2.78^{a}					
	1% Ca &*ad lib.*	10.82±1.50^{b}	20.48^{b}	26.65^{b}					
Right humerus									
Length (mm)	1% Ca	52.77 ± 3.80	70.44 ± 16	80.26 ± 5.60	67.82^{b}	0.0001	0.0001	0.0644	8.59
	1.5% Ca	53.38 ± 3.80	74.32 ± 4.1	80.60 ± 5.90	69.44^{a}				
	2% Ca	53.40 ± 4.80	73.60 ± 3.6	80.20 ± 3.80	69.07^{b}				
	1% Ca &*ad lib.*	65.80 ± 3.70	83.80 ± 4.4	86.30 ± 3.80	78.65^{a}				
	Means	56.33^{a}	75.46^{b}	81.86^{c}					
Width (mm)	1% Ca	4.40 ± 0.40	6.31 ± 0.40	6.61 ± 0.40	6.27^{b}	0.0001	0.0001	0.4970	9.27
	1.5% Ca	4.36 ± 0.40	6.23 ± 0.50	6.49 ± 0.60	6.21^{b}				
	2% Ca	4.45 ± 0.50	6.12 ± 0.30	6.59 ± 0.70	6.30^{b}				
	1% Ca &*ad lib.*	6.28 ± 0.40	7.91 ± 1.10	8.69 ± 0.60	8.53^{a}				
	Means	4.88^{a}	6.64^{b}	7.09^{c}					
Weight (g)	1% Ca	2.27 ± 0.77^{b}	5.95 ± 1.81^{a}	7.51 ± 2.86^{a}		0.0001	0.0001	0.0001	27.19
	1.5% Ca	2.50 ± 0.81^{b}	5.71 ± 1.82^{a}	6.53 ± 1.70^{a}					
	2% Ca	2.33 ± 0.64^{b}	5.56 ± 1.47^{a}	6.98 ± 1.83^{a}					
	1% Ca &*ad lib.*	5.69 ± 1.09^{c}	11.46 ± 2.29^{b}	15.09 ± 2.92^{a}					

*Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred. Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

The weight of the right tibia increased significantly (P<0.0001) for each 6 weeks increment up to 18 weeks. On the other hand, the weight of the right humerus increased significantly (P<0.0001) up to 12 weeks and thereafter flattened off. This finding is consistent with Fisher (1998) and Ross Breeders (2001) who stated that skeletal size in broiler breeder pullets is fixed at 12 weeks.

Bone mechanical properties

Breaking strength (BS) data for humeri and tibiae are shown in Table 5. A significant (P<0.0036) Ca level x age interaction for BS occurred. Although different Ca levels did not significantly (P>0.05) influence BS, birds fed 2.0% Ca diet tended to have greater BS than those fed 1.0% and 1.5% Ca diets. According to regression analyses (Table 6), however, tibia BS in the restricted groups responded significantly to increasing intakes of Ca at 6 weeks, as well as, at 12 weeks of age. The response in humerus BS was only significant for the data collected at 6 weeks of age. These findings are different from what was found for bone dimensions for pullets on restricted feeding. Only when data of the *ad libitum* group was included in the data sets that significant responses in bone characteristics were noted (Table 5). In agreement with Frost (1997) and Rath et al. (1999, 2000), tibia BS significantly (P<.0001) increased with age. From Table 5, dietary Ca levels did not have a significant effect on tibia stress in the restricted groups. This is in agreement with McCormack et al. (2001) who found no significant influence of Ca levels on the bone stress of 6 weeks old Cobb broiler breeder pullets on restricted feeding.

Stress required to break bones from *ad libitum* birds was significantly (P<0.0001) lower than that required to break bones from restricted group (Table 5). Crenshaw et al. (1981a) states that as bone mineralisation increases, maximum stress of the bone increases. According to these results the degree of bone mineralisation was greater for restricted groups than for the *ad libitum* group. Such finding is to be expected, as rapid growth does not allow enough time for the production of strong tissue, remodelling, and alignment of bone resulting in less mineralisation. These results suggest that less mineralisation occurred in birds on *ad libitum* feeding, an observation that agrees with that of Nimmo et al. (1980).

Bone chemical composition

Different dietary Ca levels resulted in no significant differences in the ash, Ca and P content of the restricted groups (Table 7).The result on bone Ca is consistent with Hocking et al. (2002) and Smith et al. (2003) who reported no benefits of feeding growing turkeys and broilers diets containing Ca levels ranging from 0.6 to 1.5%. Although 2.0% Ca level in the current study did not significantly influence Ca content of bone of restricted birds, this level showed a slightly lower Ca value. It seems from the present results that too high levels of Ca (2.0% and more) could influence the Ca content of the bone detrimentally. The mechanism of suppression of bone calcification by high

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dietary levels of Ca remains unclear. The feeding of high levels of Ca during the rearing period of broiler breeder pullets could probably result in the body's mechanism for Ca mobilisation to malfunction and gear the body for high levels of excretion. The result on bone ash is consistent with Fard et al. (2010).

There was a significant (P<.0001) increase in bone ash and Ca with age (Table 7). However, bone P declined (P<.0007) with age. According to Table 8, these variables did not increase with age. Hocking et al. (2002) reported a decline in Ca, P and bone ash due to age in growing turkeys up to 13 weeks of age. Only the result on the P reported in the present study confirms the work of Hocking et al. (2002). The finding on bone ash in the current study is in agreement with Rath et al. (1999) who reported an increase in tibia bone ash of female broiler breeder chickens aged 7 and 72 weeks. The differences in results with regards to bone ash and Ca may be attributable to length of experiment and differences in animal species but not Ca levels, as levels of Ca used in the present study and that of Hocking et al. (2002) were similar. The decline in bone ash values from 33.7 to 30% between 6 and 12 weeks of age could be associated with the birds increased demand for nutrients, notably Ca due to rapid growth rates. Accordingly, skeletal size is fixed at 12 weeks of age (Fisher, 1998; Ross Breeders, 2001). This pattern in bone development probably explains the high bone ash content at 18 weeks of age (Table 7).

Bone ash values obtained at 6 weeks age for the restricted groups in this study are consistent with the results of McCormack et al. (2001) who reported ash value of 33.63% after feeding Cobb broiler breeder pullets' diets containing 0.9 g Ca and 5.8 g total P during a 6-week period. In the current study, the average bone ash value for the birds on restricted feeding was 33.97% at 6 weeks. However, McCormack et al. (2001)) found a higher bone ash value (40.65%) for *ad libitum* group compared to 33.0% in the current study. The differences in the results relating to bone ash values for the *ad libitum* group could be attributable to differences in housing (*e.g.*, open-sided *vs.* climate controlled systems) and strain of birds (Ross *vs.* Cobb).

According to Ruff and Hughes (1985), bone ash content is correlated with its BS. However, this does not appear to be supported by the results of the current study. In this study, Ca and P contents did not increase significantly with increasing Ca level (Tables 7 and 8) while BS did (Table 6). Bone stress values in this study suggested that less mineralisation occurred in birds on *ad libitum* feeding.

True cortical area and percent bone

The means for true cortical area (TCA) and percent bone are presented in Table 7. A significant (P<.05) Ca level x age interaction for TCA and percent bone occurred, indicating that not all bones responded similarly at each age period or Ca level. A regression analysis of the two parameters at all ages (Table 8) showed a significant (P<.001) response to Ca intake when data of the *ad lib* group was included in the calculations. However, dietary Ca did not have significant (P>.05) influence on TCA and percent bone of the restricted birds (Tables 8).

Percent bone and TCA of restricted birds was not significantly (P>.05) different at 12 and 18 weeks, perhaps indicating that the bone is fully developed at 12 weeks (Table 7), thus confirming the theory that bone in broiler breeder pullets is fixed at 12 weeks of age.

Ad libitum birds had significantly (P<.05) higher TCA values than restricted birds (Table 7) probably due to faster growth rate. At 6 weeks, percent bone for *ad libitum* group was lower than that of restricted treatments but appeared to be similar to that of restricted birds at 12 and 18 weeks of age. The lower percent bone of the *ad libitum*birds may be attributable to rapid growth rate due to higher feed consumption rates. This probably indicates that the rate of mineralisation could not keep pace with rapid growth rate of the *ad libitum* group.

The TCA and percent bone were significantly (P<.0001) influenced by age (Table 7). The two parameters increased significantly from 6 to 12 weeks and then significantly flattened off. Between 6 and 12 weeks TCA and percent bone increased by 59.3% and 9.9%, respectively.

CONCLUSION

These results showed that dietary Ca level had no significant effect on bone formation in broiler breeder pullets on restricted feeding up to 18 weeks of age, except bone breaking strength. The results found for breaking strength were, however, not supported by stress required to break bones, percent bone ash, Ca and P contents as well as percent bone. Thus, it seems that dietary Ca levels higher than 1.0% will not influence bone development advantageously in feed restricted breeder pullets during the rearing period. Therefore, it is concluded that increasing the levels of dietary Ca alone cannot optimise bone formation in restricted broiler breeders during rearing. The levels of other nutrients like protein in the diet should probably be increased as well.

Ad libitum feeding of broiler breeder pullets in the present study resulted in a significant increase in bone dimensions and breaking strength. Bone variables studied such as ash content (percentage), percent bone, true cortical area, Ca and P contents were, however, not significantly influenced by *ad libitum* feeding of broiler breeder pullets. In fact stress required to break bones from *ad libitum* birds was significantly lower than that of restricted groups. The detrimental effect of a rapid growth rate and resulting over-fatness on reproductive performance and health should also be borne in mind when considering increasing other nutrients in the diet such as protein. According to bone length and breaking strength values, bone development of restricted broiler breeder pullets is fixed at 12 weeks of age.

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Table 4 - Multiple regression analysis of bone dimensions on calcium intake of pullets during rearing

		Data from restricted groups only			Data of *ad lib* group included		
Age	**Variable**	**Equations**	**Adj-R^2**	**P value**	**Equation**	**P value**	**Adj-R^2**
6 weeks	Tibia length	Tiblength6 = 72.50 - 0.83 Caint6		0.893	Tiblength6 = 52.2 + 26.2 Cain6	0.000	0.753
	Humerus length	Humlength6 = 52.10 + 1.33 Caint6	0.000	0.755	Humlength6 = 41.0 + 16.2 Caint6	0.000	0.583
	Tibia width	Tibwidth6 = 4.54 + 0.446 Caint6		0.486	Tibwidth6 = 2.79 + 2.77 Caint6	0.000	0.645
	Humerus width	Humwidth6 = 4.30 + 0.152 Caint6	0.000	0.732	Humwidth6= 2.62 + 2.38 Caint6	0.000	0.711
	Tibia weight, g	Tibweight6 = 4.23 - 0.01 Caint6		0.995	Tibweight6 = 1.89 + 8.14 Caint6	0.000	0.699
	Humerus weight	Humweight6 = 2.45 – 0.103 Caint6	0.000	0.892	Humweight6 = -0.789 + 4.21 Caint6	0.000	0.655
12 weeks	Tibia length, mm	Tiblength12 = 107.00 - 0.39 Caint12		0.956	Tiblength12 = 92.3 + 15.2 Caint12	0.000	0.639
	Humerus length	Humlength12 = 68.30 + 4.53 Caint12	0.000	0.355	Humlength12 = 65.5 + 7.41 Caint12	0.000	0.229
	Tibia width	Tibwidth12 = 6.96 - 0.278 Caint12		0.272	Tinwidth12 = 5.39 + 1.37 Caint12	0.000	0.639
	Humerus width	Humwidth12 = 6.52 – 0.297 Caint12	0.000	0.148	Humwidth12 = 5.26 + 1.02 Caint12	0.000	0.449
	Tibia weight	Tibweight12 = 10.80 + 0.30 Caint12		0.769	Tibweight12 = 5.40 + 5.94 Caint12	0.000	0.699
	Humerus weight	Humweight12 = 6.34 – 0.598 Caint12	0.000	0.482	Humweight12 = 2.41 + 3.51 Caint12	0.000	0.542
18 weeks	Tibia length	Tiblength18 = 119.00 - 0.24 Caint18		0.939	Tiblength18 = 107 + 9.91 Caint18	0.000	0.230
	Humerus length	Humlength18 = 80.50 - 0.07 Caint18	0.000	0.966	Humlength18 = 76.0 + 3.67 Caint18	0.000	0.127
	Tibia width	Tibwidth18 = 6.93 + 0.203 Caint18		0.389	Tibwidth18 = 5.30 + 1.58 Caint18	0.000	0.436
	Humerus width	Humwidth = 6.57 – 0.009 Caint18	0.000	0.962	Humwidth18 = 5.01 + 1.31 Caint18	0.000	0.424
	Tibia weight	Tibweight18 = 15.70 - 0.61 Caint18		0.583	Tibweight18 = 6.67 + 7.00 Caint18	0.000	0.376
	Humerus weight	Humweight18 = 7.68 – 0.502 Caint18	0.000	0.487	Humweight8 = 1.39 + 4.80 Caint18	0.000	0.368

Tib = tibia; Hum = humerus; Caint = calcium intake

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Table 5 - Effect of calcium levels on bone mechanical properties of broiler breeder pullets during rearing

	Treatment	Age				Significance of effect (P)			
		6	12	18	Means	Treatment	Age	Interaction	CV
Right tibia									
Bone strength (N)	1% Ca	97.65 ± 22.23^{c}	213.90 ± 22.23^{b}	269.78 ± 22.23^{a}		0.0001	0.0001	0.0030	17.43
	1.5% Ca	93.30 ± 22.23^{b}	221.00 ± 22.23^{a}	249.28 ± 22.23^{a}					
	2% Ca	108.55 ± 22.23^{b}	253.50 ± 22.23^{a}	268.15 ± 22.23^{a}					
	1% Ca & *ad lib*	298.30±22.23^{c}	387.50±22.23^{b}	599.5± 22.23^{a}					
Bone stress (N/mm^2)	1% Ca	23.52 ± 1.51	19.0 ± 1.51	23.09 ± 1.51	21.87^{b}	0.0001	0.0003	0.1951	14.80
	1.5% Ca	24.86 ± 1.51	20.25 ± 1.51	26.84 ± 1.51	23.98^{b}				
	2% Ca	21.16 ±1.51	19.23 ± 1.51	26.89 ± 1.51	22.43^{b}				
	1% Ca & *ad lib*	11.23 ± 1.51	12.89 ± 1.51	15.22 ± 1.51	13.11^{a}				
	Means	20.19^{ab}	17.84^{a}	23.01^{b}					
Right humerus									
Bone strength (N)	1% Ca	93.65 ± 29.38^{b}	236.00 ± 29.38^{a}	273.85 ± 29.38^{a}		0.0001	0.0001	0.0036	20.70
	1.5% Ca	90.20 ± 29.38^{b}	258.50 ± 29.38^{a}	262.63 ± 29.38^{a}					
	2% Ca	120.70 ± 29.38^{b}	276.50 ± 29.38^{a}	294.43 ± 29.38^{a}					
	1% Ca & *ad lib*	294.60 ± 29.38^{c}	501.00 ± 29.38^{b}	704.38 ± 29.38^{a}					

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant ($P>0.05$) interaction occurred. Means with the same letter within a row (age) are not significantly different for the same variable, where a significant ($P<0.05$) interaction occurred.

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Table 6 - Multiple regression analysis of bone mechanical properties data on calcium intake of boiler breeder pullets during rearing

		Data from restricted groups only			*Ad libitum* group data included		
Sampling age	**Variables**	**Equations**	**P value**	**Adj-R^2**	**Equations**	**P value**	**Adj-R^2**
6 weeks	Tibia breaking strength	RTib6 = 53.4 + 57.8 Caint6	0.029		RTib6 = -98.70 + 261 Caint6	0.000	0.793
	Humerus breaking strength	RHum6 = 21.1 + 104 Caint6	0.021		Rhum6 = -93.7 + 257 Caint6	0.000	0.740
	Bone stress	Stress6 = 27.2 – 6.6 Caint6	0.545		Stress6 = 30.5 – 10.9 Caint6	0.006	0.000
12 weeks	Tibia breaking strength	Rtib12 = 165.0 + 64.7 Caint12	0.008		RTib12 = 124 + 107 Caint12	0.000	0.608
	Humerus breaking strength	RHum12 = 201.0 + 55.8 Caint12	0.055		Rhum12 = 102 + 160 Caint12	0.000	0.730
	Bone stress	Stress12 = 19.4 + 0.13 Caint12	0.960		Stress12 = 23.2 – 3.92 Caint12	0.001	0.000
18 weeks	Tibia breaking strength	Rtib18 = 252.0 + 4.4 Caint18	0.855		RTib18 = 4.3 + 213 Caint 18	0.000	0.366
	Tibia breaking strength	Rhum18 = 251.0 + 21.2 Caint18	0.549		Rhum18 = -36.8 + 264 Caint18	0.000	0.415
	Bone stress	Stress18 = 20.5 + 3.79 Caint18	0.232		Stress18 = 31.3 – 5.31 Caint18	0.061	0.000

Rtib = right tibia; RH = right humerus; Caint = calcium intake

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Table 7 - Bone chemical composition of broiler breeder pullets reared on different calcium levels in feed

	Treatment	Age (weeks) 6	12	18	Means	Significance of effect (P) Treatment	Age	Interaction	CV
Left tibia									
Ash content (%)	1.0% Ca	34.43 ± 1.70	29.31 ± 1.70	58.40 ± 1.70	40.71± 0.91[a]	0.8606	0.0001	0.4952	8.32
	1.5% Ca	33.91 ± 1.70	29.53 ± 1.70	60.93 ± 1.70	41.46 ± 0.91[a]				
	2.0% Ca	33.57 ± 1.70	32.24 ± 1.70	56.37 ± 1.70	40.73 ± 0.91[a]				
	1.0% Ca & *ad lib*	33.00 ± 1.70	29.07 ± 1.70	58.76 ± 1.70	40.27 ± 0.91[a]				
	Means	33.73 ± 0.85[a]	30.04 ± 0.85[b]	58.62 ± 0.84[c]					
Calcium, %	1.0% Ca	26.77 ± 3.62	29.19 ± 3.62	35.28 ± 3.62	30.41 ± 0.79[a]	0.8228	0.05	0.9199	24.62
	1.5% Ca	27.57 ± 3.62	30.74 ± 3.62	32.82 ± 3.62	30.37 ± 0.79[a]				
	2.0% Ca	26.70 ± 3.62	29.00 ± 3.62	29.21 ± 3.62	28.30 ± 0.79[a]				
	1.0% Ca & *ad lib*	24.13 ± 3.62	26.75 ± 3.62	34.64 ± 3.62	28.51 ± 0.79[a]				
	Means	26.29 ± 1.81[a]	28.92 ± 1.81[a]	33.00 ± 1.81[b]					
Phosphorus, %	1.0% Ca	17.71 ± 0.87	16.63 ± 0.87	15.95 ± 0.87	16.76 ± 47[a]	0.3785	0.0007	0.7347	10.64
	1.5% Ca	18.26 ± 0.87	17.03 ± 0.87	14.86 ± 0.87	16.72 ± 47[a]				
	2.0% Ca	17.05 ± 0.87	17.67 ± 0.87	14.24 ± 0.87	16.32 ± 47[a]				
	1.0% Ca & *ad lib*	16.95 ± 0.87	15.68 ± 0.87	14.28 ± 0.87	15.64 ± 47[a]				
	Means	17.49 ± 0.43[a]	16.75 ± 0.43[a]	14.83 ± 0.43[b]					
TCA[1] (mm^2)	1.0% Ca	6.08 ± 0.71[b]	12.25 ± 0.71[a]	13.35 ± 0.71[a]		0.0001	0.0001	0.0458	10.66
	1.5% Ca	6.44 ± 0.71[b]	12.65 ± 0.71[a]	13.15 ± 0.71[a]					
	2.0% Ca	6.72 ± 0.71[b]	13.52 ± 0.71[a]	13.40 ± 0.71[a]					
	1.0% Ca & *ad lib*	17.70 ± 0.71[b]	20.25 ± 0.71[b]	24.60 ± 0.71[a]					
Percent bone	1.0% Ca	83.52 ± 0.81[b]	89.86 ± 0.81[a]	92.46 ± 0.81[a]		0.0017	0.0001	0.0047	1.85
	1.5% Ca	83.13 ± 0.81[b]	90.46 ± 0.81[a]	91.10 ± 0.81[a]					
	2.0% Ca	82.50 ± 0.81[b]	91.43 ± 0.81[a]	91.93 ± 0.81[a]					
	1.0% Ca & *ad lib*	77.08 ± 0.81[b]	90.32 ± 0.81[a]	90.79 ± 0.81[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, where no significant (P>0.05) interaction occurred. Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

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Table 8 - Multiple regression analysis of bone chemical composition on calcium intake of pullets during rearing

		Data from restricted groups only			Data of *ad lib* group included		
Age	**Variable**	**Equations**	**P value**	**Adj-R^2**	**Equation**	**P value**	**Adj-R^2**
6 weeks	Ash, %	%Ash6 = 26.20 + 10.1 Cain6	0.090	0.032	%Ash6 = 35.4 – 2.2 Caint6	0.312	0.000
	Percent bone	%bone6 = 86.6 – 3.25 Cain6	0.204	0.000	%bone = 89.1 – 7.95 Caint6	0.000	0.780
	True cortical area, mm^2	TCA6 = 5.13 + 1.57 Cain6	0.360	0.000	TCA6 = -4.53 + 14.5 Caint6	0.000	0.873
12 weeks	Ash, %	%Ash12 = 27.7 + 1.77 Cain12	0.228	0.008	%Ash12 = 29.5 – 0.117 Caint12	0.823	0.000
	Percent bone	%bone12 = 88.1+ 2.48 Cain12	0.609	0.081	%bone = 90.3 + 0.133 Caint12	0.854	0.000
	True cortical area, mm^2	TCA12 = 10.7 + 2.00 Cain12	0.052	0.140	TCA12 = 8.03 + 4.90 Caint12	0.000	0.854
18 weeks	Ash, %	%Ash18 = 62.4 - 1.83 Cain18	0.326	0.000	%Ash18 = 66.3 – 5.14 Caint18	0.000	0.000
	Percent bone, %	%bone18 = 92.5- 0.45 Cain18	0.305	0.000	%bone318 = 92.9 – 0.837 Caint18	0.103	0.120
	True cortical area, mm^2	TCA18 = 13.3 + 0.013 Cain 18	0.988	0.000	TCA18 = 4.96 + 7.02 Caint18	0.001	0.516

TCA = true cortical area

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