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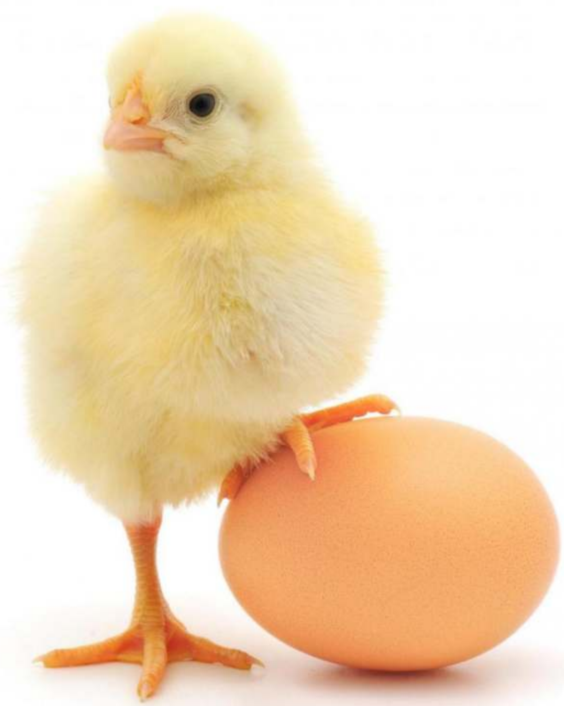

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Effect of Different Calcium Sources and Calcium Intake on Shell Quality and Bone Characteristics of Laying Hens at Sexual Maturity and End of Lay

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Abstract: The influence of supplemental calcium given in flour or granular form and calcium intake on bone properties and egg characteristics of brown-egg laying hens was investigated at sexual maturity and at end of lay. Physical and mechanical bone characteristics were determined using 3-point and torsional tests. There was no effect of calcium source on the measured bone characteristics at sexual maturity. Bone breaking strength was positively correlated with both stiffness and total calcium intake. At 72 weeks, birds which had been offered additional calcium of either source exhibited considerably higher bone breaking strength than did control birds. Bone stiffness of birds offered limestone granules was significantly higher than control birds. Regression analysis showed strong relationships between calcium intake, bone stiffness and breaking strength. Shell quality was significantly better for birds consuming limestone in a granular form than for control birds. Shell quality for birds given limestone flour was intermediate between the other two groups. It is concluded that calcium supplementation increases egg quality, mechanical properties of the bone and as a consequence, may be able to reduce the risk of broken bones at the end of laying period. These effects are independent of the form of the supplemented calcium.

Key words: Osteopenia, calcium, shell quality, bone strength

INTRODUCTION

Osteopenia is a loss of bone mass due to osteomalacia and/or osteoporosis resulting in bone fragility and possible adverse effects on the performance, health and welfare of birds (Webster, 2004; Whitehead and Fleming, 2000). The immediate and predisposing causes which can lead to a bone mass loss have been investigated; the effects of exercise (Knowles and Broom, 1990), different genotypes (Knowles *et al*., 1993), sexual maturity (Koutoulis *et al*., 1997b), nutritional deficiencies (Roland and Rao, 1992), use of perches (Hughes *et al*., 1993), cage height (Moinard *et al*., 1998) and different husbandry systems (Jendral *et al*., 2008; Fleming *et al*., 1994) on bone strength have been examined at various stages of the hen's life, but with current systems for caged layers it is unlikely that radical changes could be introduced. Further improvement in bone strength is likely to come from genetic selection and dietary supplementation. Although nutritional approaches have been the most commonly adopted, the involvement of nutrition is still being questioned (Fleming *et al*., 2006; Whitehead, 1996). Deficiencies and imbalances of calcium, phosphorus or cholecalciferol have been shown to result in osteomalacia (Wilson and Duff, 1991). Conversely, increased calcium concentration in the diet (Cheng and Coon, 1990a; Frost and Roland, 1991) and supplementation of calcium in various forms and particle sizes (Fleming *et al*., 2003; Fleming *et al*., 1998; Cheng and Coon, 1990b; Hellwig and Waldroup, 1985) have been shown to improve most bone characteristics. The beneficial effect of additional calcium from different sources has also been well documented (e.g. Proudfoot and Hulan, 1987; Cheng and Coon, 1990b). It has been demonstrated that a progressive loss of structural bone occurs throughout the laying year and results in increased incidences of bone fragility and broken bones (Whitehead and Wilson, 1992). Structural bone mass at the end of the laying year depends on peak bone mass obtained during the growing period and on the rate of bone loss during the laying period. There have been few studies of the effect of pubertal calcium supply on structural bone characteristics at sexual maturity (Hurwitz and Bar, 1971). There is also a dearth of information on bone characteristics related to feeding calcium in a particular form throughout the laying period. This experiment was conducted to test the hypotheses that (a) an improvement on bone properties, physical or mechanical, could be achieved by supplying the birds with a different source of calcium during the rearing period and (b) shell quality and bone characteristics are closely correlated with higher calcium intake and its provision in a particular form.

MATERIALS AND METHODS

This study was conducted at the Division of Animal Health and Husbandry, School of Veterinary Science of Bristol University.

Two hundred forty ISABROWN chicks were reared together from 1-56 d of age on the littered floor of a lightproof room (3.2 mx2.9 m). All chicks were given continuous light on day 1 and transferred to an 8-h photoperiod on day 2, with a mean light intensity of approximately 10 lux at bird height. All birds were fed a commercial chick starter crumb from 1-56 d, a commercial grower mash from 56 d to sexual maturity and transferred to a layer mash when each lighting treatment group reached a 50% rate of lay (Table 1). Food intake and sample body weights were recorded weekly during the growing period, but the data are not reported here. At 49 d, all birds were wingtagged and at 56 d, 60 birds were transferred to 2-bird cages arranged on three tiers in 5 blocks of 6 cages, located in each of four controlled environment rooms. An 8L:16D, 12L:12D, 16L:8D or 4 (3L:3D) regimen was provided at transfer to produce a range of ages at sexual maturity. All groups were fed *ad libitum* and given free access to water. Commencing at 70 d, 20 birds in each room were offered supplementary limestone flour ($CaCO_3$ 93.63%), 20 birds received supplementary limestone granules ($CaCO_3$ 93 % and size >0.4 mm) and 20 birds acted as non-supplemented controls. Both food (± 1 g) and limestone (± 0.1 g) intakes were recorded at weekly intervals to 72 weeks. The birds had free access to the additional limestone, which was offered in a pot (8.5 cm height and 9.0 cm diameter) located in a corner of the trough, throughout the laying year.

Birds were weighed individually (± 1 g) at 8 weeks, at weekly intervals to 42 weeks, at 14 d intervals from 42-72 weeks and on the day of their first oviposition.

Within each cage, alternate birds were euthanased by cervical dislocation on the day after they laid their first egg and stored at -15°C for future examination. The first egg from each bird was identified by bird number and date, weighed, broken out and the shell ovendried at 100 °C for 25 min and an index of shell thickness was calculated using the equation $y = \text{shell wt}*1000/(4.67*\text{egg wt}^{0.6667})$ (Lewis, 1987). The remaining bird from each pair was kept to 72 weeks when the birds were weighed, euthanased and stored in the freezer for future examination.

The carcasses were thawed at room temperature (20°C) overnight; left and right tibiae were removed and defleshed before testing. The fresh weight, length and midpoint width of the tibiae were measured and breaking strength of the left tibia determined using a 3-point jig (Gregory *et al.*, 1993). The stiffness of the right tibia at sexual maturity and stiffness and breaking strength at 72 weeks were determined by torsional testing according to the technique of Latham *et al.* (1992).

Table 1: Calculated analyses of the diets

	Starter crumb	Grower mash	Layer mash
Oil (%)	3.80	3.46	6.02
Crude Protein (g/kg)	195	153	178
Ash (%)	6.53	6.72	13.90
Calcium (%)	1.20	1.20	4.19
Total P (%)	0.67	0.69	0.59

Egg production, mean egg weight and mean shell weight were recorded throughout lay and thickness index determined at 25, 33, 38, 42, 46, 50, 56, 60, 64 and 70 weeks using three eggs from the week's production. Data were subjected to an analysis of variance using a General Linear Model and nutritional treatment, room and their interaction as variables. Significant differences between means identified using a pooled Standard Error of Mean and student's *t* test. Nutritional data are presented as means for the four rooms, because no interactions were identified between nutritional treatments and rooms. Breaking strength data at first egg and at 72 weeks were classified according to and regressed on total calcium intake or bone stiffness. Data from birds which died during the trial were excluded from the analysis.

RESULTS

At first egg: The supply of additional calcium had no significant effect upon age at first egg, egg weight, shell weight or mean shell thickness of first egg, whether single or double yolk. Birds offered additional limestone flour had significantly lighter body weight at first egg than control birds, with birds offered limestone granules being intermediate and not significantly different from the other two treatments (Table 2).

Birds given access to limestone flour consumed significantly less food but significantly more total calcium than controls. Whilst the food intake of birds with access to limestone granules was intermediate between the other two groups, they consumed 0.17 g/d more supplemental calcium before first egg than those supplied with limestone flour, although the difference was not statistically significant and as a consequence consumed significantly more calcium in total than either of the other two groups (Table 3).

There were no significant effects of calcium source on any of the bone characteristics at first egg (Table 4). When the data were classified according to stiffness, there was a strong positive relationship between stiffness and breaking strength of the tibiae (Fig. 1). Total calcium intake (g) had a positive correlation with

Table 2: The effect of Limestone Flour (LF), Limestone Granules (LG) and no Calcium Supplementation (C) on mean age and body weight at first egg and egg production data throughout the laying period of hens. Shell quality means are averages of ten collections (three eggs each) from all the birds throughout the laying year and pooled across rooms

	Nutritional Treatments			
	C	LF	LG	Pooled SEM (n)
AFE (d)	115.4	113.6	115.2	1.13 (120)
Body weight at first egg (g)	1650[b]	1564[a]	1606[ab]	20.3 (120)
Total egg production	341	330	337	6.7 (112)
Mean egg wt (g)	62.7	63.4	62.5	0.70 (112)
Mean shell wt (g)	6.07	6.22	6.21	0.085 (112)
Mean shell thickness (mg/cm^2)	82.37[a]	83.70[ab]	84.56[b]	0.774 (112)

Within rows, means with the same superscripts are not significantly different at $p<0.05$

breaking strength (Fig. 2) and bone stiffness, but the latter correlation failed to reach significance (p=0.090).

First egg to 72 weeks: The absence of significant differences in feed intake resulted in intakes of dietary calcium being similar in all groups. However, birds given access to supplementary calcium, irrespective of source, consumed significantly more calcium per day than did control birds (Table 3).

Although calcium supplementation significantly increased breaking strength at 72 weeks, regardless of the method of assessment, the physical properties of the bones were unaffected. Whilst birds offered limestone granules had significantly stiffer bones than the control group, birds offered limestone flour were intermediate between, but not significantly different from, the other two groups (Table 4). At 72 weeks, breaking strength was positively correlated with both bone stiffness and total calcium intake (Fig. 3, 4), whereas the latter did not affect bone stiffness.

Both groups offered supplemented calcium significantly increased their tibial breaking strength between sexual maturity and end of lay (18.4% and 20.1% respectively); whereas the breaking strength of control birds increased only marginally (2.0%) in the same period. The same data comparison showed that birds given access to limestone granules and limestone flour during the laying year increased their stiffness by 56.4% and 49.8% respectively, whilst the stiffness of control birds increased by 34.8%. Only the difference between limestone granules and control group was statistically significant (Table 4).

Calcium supplementation did not significantly affect egg production, mean egg weight or mean shell weight. However, birds offered limestone granules had a significantly higher mean shell thickness than the control group, with birds offered limestone flour intermediate between, but not significantly different from, the other two groups (Table 2).

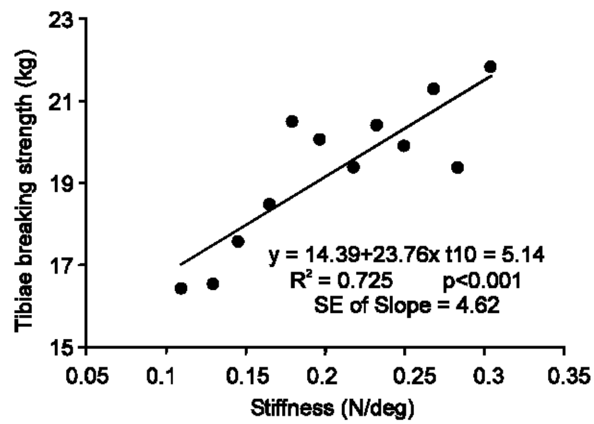

Fig. 1: Relationship between stiffness and left tibiae breaking strength of laying hens at first egg

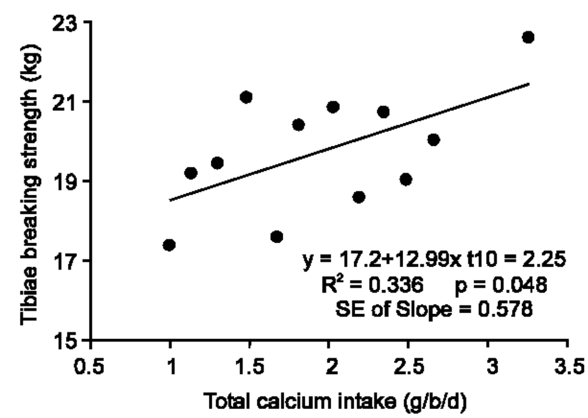

Fig. 2: Relationship between low and high calcium intake and left tibiae breaking strength of laying hens at first egg

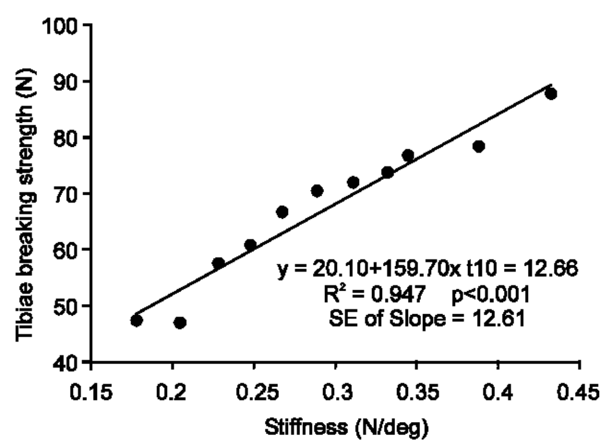

Fig. 3: Relationship between stiffness and breaking strength of right tibiae of laying hens at 72 weeks of age

Table 3: The effect of limestone flour (LF), limestone granules (LG) and no calcium supplementation (C) on mean daily feed, supplemental and total calcium intake from 70 d to first egg and from first egg to 72 weeks of hens, pooled across rooms

	Nutritional Treatments			
	C	LF	LG	Pooled SEM (n)
From 70 d to first egg				
Feed intake (g/b/d)	90.9[b]	86.5[a]	88.4[ab]	1.04 (120)
Calcium from feed (g/b/d)	1.28[b]	1.16[a]	1.22[ab]	0.032 (120)
Calcium from limestone (g/b/d)	-	0.34	0.51	0.078 (80)
Total calcium intake (g/b/d)	1.28[a]	1.50[b]	1.73[c]	0.077 (120)
From first egg to 72 weeks				
Feed intake (g/b/d)	115.5	111.2	111.3	3.30 (112)
Calcium from feed (g/b/d)	4.84	4.65	4.67	0.138 (112)
Calcium from limestone (g/b/d)	-	1.81	2.17	0.259 (74)
Total calcium intake (g/b/d)	4.84[a]	6.46[b]	6.84[b]	0.255 (112)

Within rows, means with the same superscripts are not significantly different at $p<0.05$.

Table 4: The effect of limestone flour (LF), limestone granules (LG) and no calcium supplementation (C) on tibiae physical properties, stiffness and breaking strength at first egg and end of lay of birds, pooled across rooms

	Nutritional Treatments			
	C	LF	LG	Pooled SEM (n)
At first egg				
Length of right tibiae (cm)	11.95	11.84	11.96	0.063 (119)
Stiffness of right tibiae (N/deg)	0.2087	0.2002	0.2014	0.00743 (119)
Width of left tibiae (mm)	6.66	6.68	6.73	0.063 (120)
Weight of left tibiae (g)	11.26	11.01	11.16	0.158 (120)
Breaking strength of left tibiae (g)	19265	19338	19448	463.2 (119)
At 72 weeks of age				
Length of right tibiae (cm)	12.04	12.07	12.00	0.055 (112)
Stiffness of right tibiae (N/deg)	0.2812[a]	0.2999[ab]	0.3150[b]	0.00903 (112)
Breaking strength of right tibiae (N)	62.20[a]	71.82[b]	73.15[b]	1.873 (112)
Width of left tibiae (mm)	6.71	6.80	6.77	0.063 (112)
Weight of left tibiae (g)	11.61	11.93	11.90	0.170 (112)
Breaking strength of left tibiae (g)	19645[a]	22891[b]	23350[b]	950.1 (112)

Within rows, means with the same superscripts are not significantly different at $p<0.05$.

DISCUSSION

This experiment investigated the effects of calcium supplementation during rearing and laying on egg shell quality and characteristics and whether these are affected by the provision of calcium in a particular form. Calcium supplementation had few effects on both birds at point of lay and their first eggs. The lack of influence by calcium supplementation during the growing period on age at first egg is not surprising and is in agreement with the findings of Hamilton *et al.* (1985) and Classen and Scott (1982). What is surprising, however, is that the provision of additional calcium prior to onset of sexual maturity influenced neither shell weight nor shell quality of first eggs. Our findings are consistent with those of Leeson *et al.* (1986), who found that various levels of pre-lay dietary calcium had no effect on shell quality at first egg.

The significantly heavier body weight at point of lay of the control birds compared with supplemented with limestone flour was probably a reflection of the difference in feed intake between the treatment groups. Control birds consumed more feed in an attempt to receive more calcium. It is well established that birds have the ability to regulate their feed intake to meet their

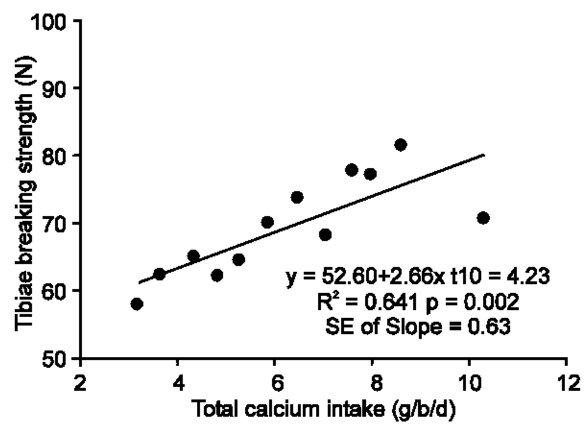

Fig. 4: Relationship between low and high calcium intake and right tibiae breaking strength of laying hens at 72 weeks of age

own needs for calcium (e.g. Classen and Scott, 1982) and to adjust to low dietary calcium levels. By attempting to eat for the deficient nutrient (calcium) birds would over consume the other nutrients and energy. The over consumption of energy has been suggested to be responsible for the increase in body weight observed (Roland *et al.*, 1985). The fact that birds supplemented with limestone flour slightly decreased feed consumption up to the point of lay, whereas birds consuming limestone granules did not, may be the outcome of differences in palatability between calcium sources. The dustiness of limestone flour and the ease of picking up the limestone granules due to their larger size are probable the reasons why birds prefer to consume more from the latter. There were no differences in feed intake achieved by the three groups between point of lay and up to 72 weeks of age although there were differences in the achieved calcium intake between the treatment groups. This probably suggest that the laying diet was not deficient in calcium.

The fact that calcium supplementation affected neither the mechanical properties of the tibia at point of lay nor egg shell quality further suggests that the control diet contained sufficient calcium to satisfy the needs of pullets for maximum bone stiffness, breaking strength and egg shell formation. The poor usage of extra calcium, offered as supplementary limestone granules or flour, indicates that the benefits of calcium supplementation during the pre-laying period may be limited. Indeed, Hurwitz and Bar (1971) and Meyer *et al.* (1971) suggested that the dietary calcium concentration during the growing period for maximum bone strength, need not exceed 1.2%.

The physical properties of the tibia were assumed to be indicators of skeletal growth and structural changes. The results at point of lay in terms of tibia length and width and the marginal increases during the laying year provide evidence that bone growth is minimal after the onset of sexual maturity and is relatively unaffected by long term calcium supplementation. These results are consistent with the findings of Meyer *et al.* (1971), who found that the dietary calcium intake during rearing did not appear to have an effect upon metatarsus length and assumed that epiphysial growth is not influenced by dietary calcium.

Torsional testing has been used (Koutoulis *et al.*, 1997a,b) to measure the effects of sexual maturity and different forms of calcium on bone stiffness and breaking strength. The strong relationship observed between bone stiffness and breaking strength at point and at end of lay allows an accurate estimation of bone breaking strength from the measurements of stiffness without testing it to destruction.

Bone stiffness is the internal bending resistance of the molecular structure of the bone to external rotational forces and may be an indicator of the degree of mineralization. Crenshaw *et al.* (1981) suggested that mechanical properties of bones in pigs respond differently to nutritional treatments; as bone mineralization increases, so bending moment increases. The beneficial effect of calcium intake on tibia breaking strength, but its lack of an effect on bone stiffness at sexual maturity and at 72 weeks may point towards the importance of the contribution of medullary bone to the structural function of bone. Medullary bone, which is formed during the last 14 d before first egg, is not believed to have any structural function (Bell and Freeman, 1971). However, the higher breaking strength observed in this investigation in birds given supplementary calcium, despite similar degrees of stiffness and mineralization, may have been a result of a better medullary bone formation. Evidence to support this hypothesis has been presented by Fleming *et al.* (1998), who found that large amounts of medullary bone in the humerus improved bone breaking strength and McCoy *et al.* (1996), whose findings suggested that medullary bone may contribute to the thickness of the cortex and in turn, to an increase in the strength of the bone, especially in severe cases of osteoporosis.

This possibility is also complemented by our findings in terms of bone stiffness and breaking strength differences among the groups. Birds supplemented with limestone granules had stronger and stiffer bones than controls, probably due to slow, constant and uniform release of extra calcium which not only benefited egg quality, but might as well have contributed to increased bone mineralization and MB formation. Rennie *et al.* (1997) found that MB volumes were increased significantly by feeding oyster shell. In contrast, bones from birds supplemented with limestone flour were not mineralised to the degree that bones from birds supplemented with limestone granules were, probably because limestone flour was used to form the egg shell and stored in MB. Thus, little limestone flour left to be used for mineralization purposes. The marginal increase of breaking strength in the control birds at the end of lay suggests that bone structure might have undergone changes throughout the laying period which can be reversed by long term supplementation of extra limestone in any form. Whitehead and Wilson (1992) recorded a progressive loss of bone strength, associated with a marked decline in the volume of trabecular bone, during the laying period. This contradiction may be due to differences in the nutritional treatments and/or genotype, as Knowles *et al.* (1993) found differences in bone breaking strength assessments among four different genotypes of birds.

The increase in absolute values of bone stiffness which was found in all groups at end of lay, compared with the data at sexual maturity, might be a result of better mineralization due to the higher calcium concentration in layer diets compared with grower diets. Possibly, bones at point of lay were mineralised to the maximum degree consuming a grower diet, but when switched to a layer diet, bone mineralization increased further. The reason for this result might be the hen's greater need for calcium to sustain egg production during the laying year than to lay their first egg.

The lack of effect of calcium supplementation on egg production, egg weight, shell weight and feed intake is in agreement with the findings of Watkins *et al.* (1977) and Brister *et al.* (1981). The only significant improvement noted due to calcium supplementation was in the egg shell thickness of birds given limestone granules; it is suggested that this was a response to the slower dissolution of the granules in the upper digestive tract and the corresponding more uniform and sustained release of calcium. Similar findings have been reported by Proudfoot and Hulan (1987) and Cheng and Coon (1990b).

Conclusion: In conclusion, supplementation of calcium from various sources during the pre-laying period might not have increased bone quality to the maximum degree, but there is strong evidence that, in the long term, it helps to increase egg quality, mechanical properties of the bone and, as a consequence, to reduce the risk of broken bones at the end of laying period.

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REFERENCES

Bell, D.J. and B.M. Freeman, 1971. In: Freeman, B.M. (Ed.) Physiology and Biochemistry of the domestic fowl, London, 2: 269.

Brister, R.D., Jr., S.S. Linton and C.R. Creger, 1981. Effects of dietary calcium sources and particle size on laying hen performance. Poult. Sci., 60: 2648-2654.

Cheng, T.K. and C.N. Coon, 1990a. Sensitivity of various bone parameters of laying hens to different daily calcium intakes. Poult. Sci., 69: 2209-2213.

Cheng, T.K. and Coon, C.N., 1990b. Effect of calcium source, particle size, limestone solubility *in vitro* and calcium intake level on layer bone status and performance. Poult. Sci., 69: 2214-2219.

Classen, H.L. and Scott, T.A., 1982. Self-selection of calcium during the rearing and early laying periods of White Leghorn pullets. Poult. Sci., 61: 2065-2074.

Crenshaw, T.D., Peo, E.R., Jr., Lewis, A.J. and Moser, B.D., 1981. Bone strength as a trait for assessing mineralisation in swine: A critical review of techniques involved. J. Anim. Sci., 53: 827-835.

Fleming, R.H., C.C. Whitehead, D. Alvey, N.G. Gregory and Wilkins, L.J., 1994. Bone structure and breaking strength in laying hens housed in different husbandry systems. Br. Poult. Sci., 35: 651-662.

Fleming, R.H., H.A. McCormack and C.C. Whitehead, 1998. 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.

Fleming, R.H., H.A. McCormack, L. McTeir and C.C. Whitehead, 1998. Medullary bone and humeral breaking strength in laying hens. Res. Vet. Sci., 64: 63-67.

Fleming, R.H., H.A. McCormack, L. McTeir and C.C. Whitehead, 2006. Relationships between genetic, environmental and nutritional factors influencing osteoporosis in laying hens. Br. Poult. Sci., 47: 742-755.

Fleming, R.H., H.A. McCormack, L. McTeir and C.C. Whitehead, 2003. Effects of dietary particulate limestone, vitamin K3 and fluoride and photostimulation on skeletal morphology and osteoporosis in laying hens. Br. Poult. Sci., 44: 683-689.

Frost, T.J. and D.A. Roland, SR., 1991. The influence of various calcium and phosphorus levels on tibia strength and eggshell quality of pullets during peak production. Poult. Sci., 70: 963-969.

Gregory, N.G., L.J. Wilkins, S.A. Tucker, D.M. Alvey and Belyavin, C.G., 1993. Effect of lighting pattern during lay on bone strength and the prevalence of broken bones in end of lay hens. J. Appl. Poult., Res., 2: 103-106.

Hamilton, R.M.G., R.W. Fairful and R.S. Gowe, 1985. Use of particulate limestone or oyster shell in the dietary regimen of White Leghorn hens. Poult. Sci., 64: 1750-1762.

Hellwig, H.M. and P.W. Waldroup, 1985. Nutritional effects on processing-induced bone breakage in spent hens. Poult. Sci., 64: 23 (abs).

Hughes, B.O., S. Wilson, M.C. Appleby and S.F. Smith, 1993. Comparison of bone volume and strength as measures of skeletal integrity in caged laying hens with access to perches. Res. Vet. Sci., 54: 202-206.

Hurwitz, S. and A. Bar, 1971. The effect of pre-laying mineral nutrition on the development, performance and mineral metabolism of pullets. Poult. Sci., 50: 1044-1055.

Jendral, M.J., D.R. Korver, J.S. Church and J.J. Feddes, 2008. Bone mineral density and breaking strength of white leghorns housed in conventional, modified and ommercially available colony battery cages. Poult. Sci., 87: 828-837.

Knowles, T.G. and D.M. Broom, 1990. Limb bone strength and movement in laying hens from different housing systems. Vet. Rec., 126: 354-356.

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.

Koutoulis, K.C., G.C. Perry and P.D. Lewis, 1997a. The influence of calcium form and intake on bone characteristics and shell quality in laying hens. Proc. XIth Intern. Cong. World Vet. Poult. Assoc., Budapest, pp: 143.

Koutoulis, K.C., G.C. Perry and P.D. Lewis, 1997b. Effect of lighting regimen, calcium supply and age at first egg on tibia stiffness and breaking strength and shell quality in laying hens. Br. Poult. Sci., 38: S9-S10.

Latham, N., A.E. Goodship, P.D. Lewis and G.C. Perry, 1992. The use of torsional testing to determine bone strength in end of lay hens. In: Whitehead, C.C. (Ed.), Bone Biology and Skeletal Disorders in Poultry, Abingdon, England, pp: 367-368.

Leeson, S., R.J. Julian and J.D. Summers, 1986. Influence of prelay and early-lay dietary calcium concentration on performance and bone integrity of Leghorn pullets. Can. J. Anim. Sci., 66: 1087-1095.

Lewis, P.D., 1987. Responses of laying hens to interrupted lighting regimes. Ph.D. Thesis, Univ. of Bristol.

McCoy, M.A., G.A.C. Reilly and D.J. Kilpatrick, 1996. Density and breaking strength of bones of mortalities among caged layers. Res. Vet. Sci., 60: 185-186.

Meyer, G.B., S.W. Babcock and M.L. Sunde, 1971. Effect of various prelaying levels of dietary calcium upon subsequent performance in chickens. Poult. Sci., 50: 536-547.

Moinard, C., J.P. Morisse and J.M. Faure, 1998. Effect of cage area, cage height and perches on feather condition, bone breakage and mortality of laying hens. Br. Poult. Sci., 39: 198-202.

Proudfoot, F.G. and H.W. Hulan, 1987. Effect on shell strength of feeding supplemental sources of calcium to adult laying hens given insoluble grit during the rearing period. Br. Poult. Sci., 28: 381-386.

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.

Roland, D.A., Sr. and S.K. Rao, 1992. Nutritional and management factors related to Osteopenia in laying hens. In: Whitehead, C.C. (Ed.), Bone Biology and Skeletal Disorders in Poultry, Abingdon, England, pp: 281-295.

Roland, D.A., M. Farmer and D. Marple, 1985. Calcium and its relationship to excess feed consumption, body weight, egg size, fat deposition, shell quality and fatty liver hemorrhagic syndrome. Poult. Sci., 64: 2341-2350.

Watkins, R.M., B.C. Dilworth and E.J. Day, 1977. Effect of calcium supplement particle size and source on the performance of laying chickens. Poult. Sci., 56: 1641-1647.

Webster, A.B., 2004. Welfare implications of avian osteoporosis. Poult. Sci., 83: 184-192.

Whitehead, C.C. and R.H. Fleming, 2000. Osteoporosis in cage layers. Poult. Sci., 79: 1033-1041.

Whitehead, C.C., 1996. Nutrition and bone disorders in poultry. Proc. XXth World's Poult. Cong., New Delhi, Vol. II, pp: 161-171.

Whitehead, C.C. and S. Wilson, 1992. Characteristics of Osteopenia in hens. In: Whitehead, C.C. (Ed.), Bone Biology and Skeletal Disorders in Poultry, Abingdon, England, pp: 265-280.

Wilson, S. and S.R.I. Duff, 1991. Effects of vitamin or mineral deficiency on the morphology of medullary bone in laying hens. Res. Vet. Sci., 50: 216-221.