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Nutritional factors affecting poultry bone health

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Outlined are two main current research concerns relating to skeletal disorders in poultry: (a) osteoporosis in egg-laying hens; (b) leg problems caused by rapid bone growth in broiler chickens. Surveys indicate that 30% of caged laying hens suffer at least one lifetime fracture (a severe welfare issue). Modern hybrids produce one egg per d for 50 weeks. For this period ‘normal’ bone turnover ceases; only medullary bone (MB) is formed, a woven bone type of limited structural value. MB is resorbed for eggshell formation alongside structural bone, leading to increased fracture risk. Avian osteoporosis is reduced by activity and genetic selection but nutrition is also important. Fluoride and vitamin K are beneficial but the timing of nutritional intervention is important. Ca, inorganic P and vitamin D must be adequate and the form of Ca is critical. Limestone fed as particulates benefits skeletal and eggshell quality. In hens fed particulate limestone compared with flour-fed hens the tibiotarsus breaking strength and radiographic density are increased at 56 weeks of age ($P<0.01$ and $P<0.001$ respectively) and the number of tartrate-resistant acid phosphatase-positive stained active osteoclasts (mean number per microscopic field) is decreased ($P<0.001$). In broiler (meat) chickens selection for rapid growth from approximately 50 g to 3 kg in 42 d has inadvertently produced skeletal disorders such as tibial dyschondroplasia, rickets and associated valgus–varus deformities leading to lameness. The beneficial skeletal effects during growth of increased dietary *n*-3 PUFA:*n*-6 PUFA (utilising salmon oil) have been demonstrated. Experiments simulating daylight UVB levels have produced beneficial skeletal effects in Ca- and vitamin D-deficient chicks.

Bone health: Poultry: Skeletal disorders: Nutritional factors

Optimal levels for poultry nutrition are achieved mainly with the productive performance of the bird in mind. Nutrient levels are set to maximise the production of eggs or poultry meat and requirements are set and reviewed according to National Research Council guidelines⁽¹⁾. Although these guidelines also indicate requirements for skeletal health, there are some suggestions that they are no longer optimal and may be out of date in relation to cholecalciferol and Ca^(2,3).

Currently, there are two main research concerns relating to skeletal disorders in poultry: (a) osteoporosis (OP) in egg-laying hens; (b) leg disorders caused by rapid bone growth in broiler (meat-type) chickens. Both these disorders cause concern for animal welfare, but OP in hens in particular also impacts on food safety as broken bones and bone splinters find their way into the food chain

through lower-grade poultry products manufactured from so-called ‘spent’ end-of-lay hens.

Osteoporosis in egg-laying hens

Background

In the laying hen approximately 2.4 g Ca is required in approximately 20 h to produce a shelled egg of 60 g. Only 60–75% of the eggshell Ca can be provided by the feed, the remainder must be found from body stores^(4,5). Follicular maturation (16–18 weeks of age) coincides with large increases in circulating plasma oestradiol that appears to drive the formation of a labile woven bone type deposited mainly in the marrow cavities of most long bones in the hen. This phenomenon is widespread in avian species but

Abbreviations: MB, medullary bone; OP, osteoporosis.

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modern hybrid laying hens have been selected for maximum egg production and so this medullary bone (MB) is present from sexual maturity and rapidly turned over on a diurnal basis until the end of the laying cycle. Hens produce one egg per d for a 50-week period and the Ca demands of the shell gland are partly met by these MB stores. During the laying period formation of normal structural lamellar bone ceases; only MB is formed. Unfortunately, large numbers of active osteoclasts are recruited to resorb and mobilise Ca from MB, and cortical and trabecular bone types are also resorbed. The net effect is a gradual loss of structural bone, and MB is of limited structural value except when present in particularly high levels⁽⁶⁾. In older hens in particular (>40 weeks of age) osteopenia and the risk of osteoporotic fractures increase.

Bone fractures caused by OP in laying hens are a continuing welfare issue⁽⁷⁾. Although OP in hens has strong genetic and environmental components^(8,9), nutritional approaches can also alleviate the condition^(10,11). The timing of nutritional intervention is critical, however. If dietary changes are left until sexual maturity, the effects will mainly be on MB formation⁽¹²⁾. Adequate inclusion rates of Ca, vitamin D and P are particularly important during the rearing period to maximise peak bone quality before the excess resorption encountered during the laying period.

Calcium

During early rearing (0–14 weeks of age) recommended Ca levels are approximately 9 g/kg diet. This level should be increased to 35 g/kg diet from 14 weeks onwards to benefit early eggshell quality. There are certainly no detrimental effects of feeding this level of Ca at this stage in terms of skeletal integrity⁽¹³⁾ and it may serve to minimise structural bone loss whilst MB is initially forming.

Vitamin D

It is generally thought that vitamin D is not limiting in slow-growing layer pullets but deficiencies should be avoided where possible⁽¹⁴⁾. The active metabolite 25-hydroxyvitamin D is commercially available and studies have shown that some skeletal characteristics in laying hens can be improved but results are inconsistent^(15,16). It may be possible to utilise less-toxic and more-pharmacologically-effective analogues of vitamin D, such as ED-71 (2 β -(3-hydroxypropoxy)-1,25-dihydroxycholecalciferol), which has been demonstrated to have beneficial effects on bone strength and density in growing layer pullets⁽¹⁷⁾. It should be noted, however, that in the same study this analogue was also found to cause a slight depression in live-weight gain at an inclusion rate of 4 μ g/kg.

Phosphorus

P deficiency should also be avoided in poultry diets; there can be considerable variability in P content in practice. It has been suggested that 5–15% of poultry flocks could have marginal to inadequate P intake because of variations in P content and availability in diets⁽¹⁸⁾. There is

considerable pressure to reduce P levels in poultry diets for environmental reasons and the role of microbial phytase in poultry diets to improve P availability has been widely investigated (for review, see Selle & Ravindran⁽¹⁹⁾). The balance between Ca and P in poultry diets is of the utmost importance, particularly in starter diets; Ca:P should be approximately 2:1⁽¹⁴⁾.

Vitamin K

In the skeleton vitamin K is required for the post-translational modification of osteocalcin, a protein associated with bone growth⁽²⁰⁾. In growing hens there are some beneficial effects for trabecular bone of an additional 10 mg as menadione/kg^(10,11), but these effects do not continue throughout the laying period. Interestingly, carboxylated osteocalcin levels, whilst easily detectable during growth, become too low to detect during the laying period even though woven MB is being rapidly turned over⁽¹¹⁾. This finding suggests that osteocalcin may not be an important protein in MB formation.

Calcium in particulate form

It has been known for some time, even anecdotally, that provision of Ca as 'grit' (normally particulate limestone or oystershell fragments) can improve eggshell quality, particularly towards the end of the laying period. This improvement is a result of the ability of the bird to store the particulate material in the gizzard, releasing the Ca slowly overnight when it cannot feed⁽²¹⁾. Peak osteoclastic activity, as evidenced by an increase in ruffled borders in avian osteoclasts, occurs partly during darkness⁽²²⁾. Thus, when the hens are not feeding it seems logical to provide slow-release dietary Ca in the form of particulates at this stage.

To investigate the effects of feeding limestone in particulate form, combined with genetic effects in avian OP, hens from several generations of divergent selection for skeletal traits⁽⁸⁾ (currently forming two distinct OP-resistant and OP-susceptible lines with a two-fold difference in bone strength) have been studied. During the laying period, equal numbers of OP-resistant and OP-susceptible hens were fed a standard layer ration containing Ca as either limestone flour or entirely in particulate form (ARCAL L105; 2.5–4.0 mm diameter range; Hanson Aggregates, Cheddar, Somerset, UK). Both grades of limestone were sourced from the same quarry. Bones from 100 hens in total were examined at 25 and 56 weeks of age (twenty-five OP-resistant hens and twenty-five OP-susceptible hens receiving flour and particulate diets at each age) from a total flock of 260 hens. Right tibiotarsi were removed for measurements of radiographic density by digitisation of post-mortem X-rays of whole bones. A section of mid-diaphysis of the left tibiotarsus was also removed and processed for histomorphometrical measurements of cross-sectional area, defined as:

$$(\text{tibiotarsus external area} - \text{marrow area}) \times (1 - p),$$

where p is a factor for cortical porosity, derived from measurements of twelve microscopic fields per hen).

Table 1. Measured bone and eggshell† variables at 25 and 56 weeks of age in laying hens (twenty-five hens per dietary treatment for each line at each age) divergently selected for osteoporosis (OP) resistance (RES) or OP susceptibility (SUS) and fed from 15 weeks onwards a diet containing calcium as limestone flour or entirely in particulate form‡

	OP line	Limestone		SED	Significance of effect of:		
		Flour	Particles		Line	Diet	Line × diet
25 weeks of age:							
Body wt (g)	RES	1595	1617	47.3			
	SUS	1631	1618		NS	NS	NS
Tibia BS(N)	RES	327	355	18.4			
	SUS	258	241		***	NS	NS
Tibia X-area (mm ²)	RES	6.56	6.81	0.27			
	SUS	6.22	6.05		**	NS	NS
Tibia RD (mmAe)	RES	2.07	2.11	0.05			
	SUS	1.89	1.90		***	NS	NS
Mean no. of osteoclasts per microscopic field	RES	11.6	10.5	0.70			
	SUS	13.6	11.8		***	**	NS
56 weeks of age:							
Body wt (g)	RES	1787	1770	33.7			
	SUS	1848	1839		NS	NS	NS
Tibia BS(N)	RES	390	432	17.7			
	SUS	217	248		***	*	NS
Tibia X-area (mm ²)	RES	6.17	6.42	0.18			
	SUS	5.40	5.34		***	NS	NS
Tibia RD (mmAe)	RES	2.50	2.64	0.06			
	SUS	1.94	2.10		***	***	NS
Mean no. of osteoclasts per microscopic field	RES	9.23	5.41	0.74			
	SUS	10.2	8.17		***	**	NS
Eggshell thickness (µm)†	RES	413	422	1.50			
	SUS	413	423		NS	***	NS
Eggshell strength (N)†	RES	33.7	34.7	0.29			
	SUS	33.4	34.9		NS	***	NS

BS, breaking strength; X-area, cross-sectional area; RD, radiographic density; mmAe, mm Al equivalent.

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

†Mean of entire egg-production period.

‡For details of experimental procedures, see p. 178.

At 25 and 56 weeks the number of active osteoclasts per microscopic field (mean of nine fields per hen) was also recorded by histochemical reaction with tartrate-resistant acid phosphatase. Following radiography right tibiotarsi and humeri were subjected to destructive three-point bending tests for estimation of peak breaking strength. Eggshell thickness and shell breaking strengths were also measured over the entire production period.

The differences between lines for bone variables were found to be highly significant (two-way ANOVA; Table 1). These genetic differences are well established^(8,9,23). However, clear benefits for bone strength and structure (and for eggshell thickness and strength) of the provision of particulate limestone in the diet were also demonstrated. Numbers of active osteoclasts were found to be reduced in the group fed particulate limestone, reducing overall bone resorption. This finding suggests that dietary intake is improved with slow overnight release of Ca, producing a protective effect for bone reserves, and the practice of feeding particulate sources of Ca is therefore to be encouraged. These benefits are simply additive to genetic improvements; there is no evidence of genetic–nutritional interaction.

Rapid bone growth in broiler chickens

Background

Leg and gait disorders have been a considerable problem for the broiler industry, and although recent genetic, management and nutritional approaches have improved the situation, there remains scope for further improvement. There are numerous causes of leg-bone abnormality, from specific lesions associated with tibial dyschondroplasia to unspecific distortions of longitudinal growth. Severe defects greatly impair the walking ability of birds, leading to mortality from starvation and dehydration, but even mild deformities have been shown to cause discomfort or pain. There is evidence that bone defects leading to eventual lameness can be induced at an early age in chicks, and recent physiological studies have shown the importance of early nutrition on chick development.

Calcium, vitamin D and phosphorus

The balance between Ca and P is of utmost importance for leg health in broilers⁽²⁴⁾; the normal content of starter diets is about 10 g Ca and 4.5 g available P/kg diet. The broiler

Table 2. Performance and tibia characteristics in broilers at 2 weeks of age fed diets containing different *n*-3 PUFA:*n*-6 PUFA resulting from substitution of maize oil (MO) with salmon oil (SO) (from McCormack *et al.*⁽³²⁾)†

Diet content (g/kg)			Live wt at 14 d (g)	Feed:gain	Tibia characteristics					
MO	SO	<i>n</i> -3: <i>n</i> -6			Breaking strength (N)		Ash (%)	True bone cortical area (mm ²)	Bending stress (N/mm ²)	
					1‡	2§				
Expt 1										
50	0	0.05	327	1.74	87.0 ^a	90.2 ^a	37.3			
25	25	0.27	341	1.63	95.4 ^b	92.8 ^{a,b}	37.9			
0	50	0.78	336	1.62	96.6 ^b	96.0 ^b	38.2			
SED			9.2	0.10	4.2	1.1	0.5			
Significance			NS	NS	*	**	NS			
Expt 2										
50	0	0.04	319 ^a	1.83	89.3 ^a	106	37.4 ^a	5.69		122
40	10	0.15	354 ^b	1.81	106 ^b	102	38.5 ^b	6.42		120
30	20	0.28	355 ^b	1.75	109 ^b	104	38.6 ^{b,c}	6.50		120
0	50	1.26	359 ^b	1.63	114 ^b	106	39.5 ^c	6.64		124
SED			10.2	0.09	5.2	4.3	0.5	0.44		5.06
Significance			*	NS	***	NS	***	NS		NS
Expt 3										
50	0	0.05	316 ^a	1.83	63.1 ^a	65.8 ^a	36.3 ^a			
25	25	0.20	336 ^b	1.78	72.6 ^b	69.9 ^b	37.4 ^b			
SED			5.9	0.06	1.9	1.1	0.3			
Significance			**	NS	***	**	**			

^{a,b,c} Within an experiment and column values with unlike superscript letters were significantly different.

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

†Expts 1 and 2 contained four replicate pens of each treatment group of twelve birds (i.e. forty birds per group). Expt 3 was a part repeat of Expt 1 but without the total substitution of salmon oil for maize oil and contained sixteen replicate pens of twelve birds each (192 birds per treatment group). The absolute values of breaking strength differed between Expts 1 and 3 because of some environmental and genetic differences between chicks (e.g. hatchery source, maternal age).

‡For this reason birds from Expts 1 and 3 were not combined, although the magnitude of significant differences in bone strength was broadly similar.

§Values for three-point breaking strength.

¶Value for three-point breaking strength after covariate analysis for live weight.

requirement for cholecalciferol is usually based on measurements of bone quality such as bone ash content or incidence of rickets, which have generally been found to be more sensitive indicators of requirement than growth rate. Cholecalciferol metabolites have been shown to be very effective in the prevention of tibial dyschondroplasia^(25–27). Since broilers have been increasingly selected for rapid growth, there are some suggestions that current National Research Council guidelines for Ca, P and vitamin D⁽¹⁾ should be reviewed^(3,28). Recent findings suggest that inducing a high vitamin D status in young chicks by feeding high concentrations of vitamin D can alleviate or prevent tibial dyschondroplasia^(3,29).

n-3 PUFA:*n*-6 PUFA

PUFA occur as two principal families distinguished by the position of the first double bond in relation to the terminal methyl group; the *n*-6 family is derived from linoleic acid and the *n*-3 family is derived from α -linolenic acid. PG derived from *n*-6 series fatty acids, and in particular PGE₂, have been shown to have some inhibitory effects on bone development, while PG derived from the *n*-3 series can have more beneficial effects of stimulating osteoblast function and bone formation, as demonstrated in cell-culture studies⁽³⁰⁾. PUFA from fish oil can improve tibial strength in quail (*Coturnix japonica*)⁽³¹⁾. It has been

demonstrated that substitution of maize oil with increasing amounts of salmon oil in 14-d-old broilers improves bone characteristics (Table 2), although effects on growth-plate disorders are inconclusive because of low incidences overall in the study⁽³²⁾.

There are also possible benefits for human health of the provision of *n*-3 PUFA-rich fish oils in poultry diets⁽³³⁾. Additionally, the inclusion of this type of *n*-3 PUFA-rich salmon oil additive (currently a waste product of the fish farming industry) to poultry diets may reduce the requirement for fishmeal and is therefore of considerable environmental importance.

Simulation of natural daylight with low levels of UV-B illumination

In contrast to all other vitamins, cholecalciferol is produced endogenously in the skin when exposed to sunlight. During exposure to sunlight, UVB (290–315 nm) photons photolyse pro-cholecalciferol (7-dehydrocholesterol) to pre-cholecalciferol, which in turn is converted to cholecalciferol. The purpose of the following research was first to establish the optimum doses and methods of safe (for birds and human subjects) application of UV illumination to chicks to enhance their vitamin D status under laboratory conditions. If safe and effective procedures for enhancing early chick vitamin D status can be identified

Table 3. Body weight at 1 and 14 d of age and tibia breaking strength, radiographic density (RD) and ash content at 14 d of age for birds exposed to UV light†

	Control (<i>n</i> 61)	Treatment (<i>n</i> 61)	SED	Significance of effect
Weight (g) at 14 d	285	303	8.15	*
Tibia breaking strength (N)	41.2	55.3	2.39	***
Tibia breaking strength (wt at 14 d as covariate (cov))	43.2	53.3	1.61	*** (cov ***)
Whole tibia RD (mm Al equivalent)	0.82	0.95	0.02	***
Whole tibia ash (%; fat-free bone)	32.5	37.5	0.44	***

†For details of experimental procedures, see p. 181.

Table 4. Contingency table for growth-plate pathology in UV-treated chicks†

Group	Growth-plate pathology						Significance (χ^2)
	Normal		Hypocalcaemic rickets		TD		
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Control	26	43	24	39	11	18	***
UV-treated	57	98	0	0	1	2	

TD, tibial dyschondroplasia.

†For details of experimental procedures, see p. 181.

using UV radiation, then this approach could represent an alternative to feeding higher levels of vitamin D in starter diets, and may provide a safeguard against dietary imbalances that can occur in commercial practice.

Following initial studies to determine safe exposures of UVB (for both chicks and human subjects), self-ballasted compact UVB reptile lamps (ZooMed ReptiSun 10.0 UVB Desert lamps; ZooMed Laboratories Inc., San Luis Obispo, CA, USA; energy efficient (26 W) with 10% UVB output) were installed in one of two environmentally-controlled rooms. Both rooms were conventionally lit for 23 h light–1 h dark and the environmental conditions were controlled to provide the same temperature and overall luminance in both rooms. One room was fitted with an additional four overhead compact UVB lamps suspended approximately 1.4 m above the floor of the pen. The four lamps were distributed across the room to provide as even an illumination pattern as possible at floor level. UV intensity was measured directly at various points within the room and the average irradiance calculated using a radiometer (Macam UV203; Macam Photometrics Ltd, Livingston, West Lothian, UK) fitted with two broad-band filters with cosine correction: UVAB (λ peak 352 ± 5 nm; band width 35 ± 2 nm) and UVB (λ peak 311 ± 2 nm; band width full width at half maximum 19 ± 2 nm). The total exposure time was then calculated such that the total irradiance received did not exceed 6 KJ UVB/m²; this level equated to 12 h exposures beginning when the chicks were placed in the rooms. Two groups of 30-d-old broiler chicks were placed in each room. An imbalanced Ca (7.5 g/kg), P (7.6 g/kg) and low vitamin D (5 μ g/kg) content diet was fed to all chicks to determine whether supplemental UVB illumination could prevent any skeletal pathology caused by

these imbalances. Birds were killed at 14 d and bone characteristics measured by similar methodologies to those used in the bone assessments for layer birds described earlier. Growth-plate abnormalities were assessed both by gross pathology and by histological examination. Compositional analysis of whole bones was determined by standard fat extraction and ashing procedures. Experiments were replicated with the rooms reversed and the results were combined (*n* 61 for controls, *n* 58 for UVB treatment).

No differences were found in body weight at 1-d-old, but UV-treated birds were heavier at 14 d (Table 3). Bone strengths were found to be >30% improved in the UV-treated group, with this difference being maintained even when body weight was analysed as a covariate. Significant improvements were also found in tibia radiographic density and tibia ash values, suggesting that UV-treated birds had better mineralised bones. Although the control group were fed an imbalanced diet, the results suggest that early exposure to UVB illumination in chicks can partly restore bone characteristics towards the level previously recorded in birds fed Ca, vitamin D and P in balance and sufficiency, particularly in terms of bone ash values (see Table 3). Table 4 demonstrates that UVB illumination can also virtually eliminate growth-plate abnormalities caused by deficiency of Ca and vitamin D. The levels of UVB illumination were approximately 10% of a mid-summer's day at 55° latitude and no evidence of keratitis was found in UV-treated chicks compared with controls. No evidence of pathological changes was found on histological examination of corneal tissue from UV-treated chicks.

These results suggest that there may be benefits in commercial situations (in which dietary imbalances can

occur) from the use of early UV illumination in broiler chicks. There may also be some potential to investigate the use of UV illumination in layer chicks to maximise skeletal mineralisation during growth and reduce the effects of excessive resorption during the laying period.

Conclusions

Osteoporosis in laying hens

Levels of Ca, vitamin D and P must be adequate and Ca dietary inclusion rates in particular should be increased to layer-diet levels well before sexual maturity (approximately 14 weeks of age). Most dietary interventions are ineffective if hens are already in lay, since effects will only be on non-structural woven medullary bone. Genetic selection and some nutritional factors (especially Ca in particulate form) are effective in maintaining and improving bone strength, and therefore resistance to fractures. Genetic selection is relatively more effective than nutrition in increasing bone strength but the effects are additive.

Rapid bone growth in broiler chickens

Leg problems will be less evident if slower-growing strains are used. However, problems in faster-growing strains can be mitigated by ensuring Ca, vitamin D and P are adequate and balanced correctly. A salmon oil additive, rich in *n*-3 PUFA, has been found to improve bone characteristics in broiler chickens. This additive is currently a waste product of the fish-farming industry and could be added easily to poultry diets. There may also be some benefit for human health in this approach, by increasing the *n*-3 PUFA content of poultry meat. Salmon oil does not taint chicken meat to the same extent as other fish oils (A Gibson, unpublished results) and would be an environmentally-sound product compared with fishmeal.

It has also been found that a short early period of UVB illumination has substantial benefits for bone characteristics in broiler chickens when dietary factors become imbalanced. Further work is required to determine whether this effect is evident in the commercial situation, in which the illumination system used experimentally could be applied easily.

These factors (*n*-3 PUFA and UVB illumination) may also be effective in young layer chicks in improving peak bone mass and therefore increasing their resistance to fracture for a longer period.

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References

1. National Research Council (1994) *Nutrient Requirements of Poultry*, 9th revised ed. Washington, DC: National Academy Press.
2. Kasim AB & Edwards HM (2000) Evaluation of cholecalciferol sources using broiler chick bioassays. *Poult Sci* **79**, 1617–1622.
3. Whitehead CC, McCormack HA, Mcteir L & Fleming RH (2004) The maximum legal limit for vitamin D3 in broiler diets may need to be increased. *Br Poult Sci* **45**, Suppl. 1, S24–S26.
4. Driggers JC & Comar CL (1949) The secretion of radioactive calcium (⁴⁵Ca) in the hen's egg. *Poult Sci* **28**, 420–424.
5. Mueller WJ, Schraer R & Schraer H (1964) Calcium metabolism and skeletal dynamics of laying pullets. *J Nutr* **84**, 20–26.
6. Fleming RH, McCormack HA, Mcteir L & Whitehead CC (1998) Medullary bone and humeral breaking strength in laying hens. *Res Vet Sci* **64**, 63–67.
7. Sandilands V, Sparks N, Wilson S & Nevison I (2005) Laying hens at depopulation: the impact of the production system on bird welfare. *Br Poult Abstr* **1**, 23–24.
8. Bishop SC, Fleming RH, McCormack HA, Flock DK & Whitehead CC (2000) Inheritance of bone characteristics affecting osteoporosis in laying hens. *Br Poult Sci* **41**, 33–40.
9. Fleming RH, McCormack HA, Mcteir L & Whitehead CC (2005) Environmental and genetic interactions in avian osteoporosis. *Br Poult Abstr* **1**, 26–27.
10. Fleming RH, McCormack HA, Mcteir L & Whitehead CC (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.
11. Fleming RH, McCormack HA, Mcteir L & Whitehead CC (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.
12. Rennie JS, Fleming RH, McCormack HA, McCorquodale CC & Whitehead CC (1997) Studies on effects of nutritional factors on bone structure and osteoporosis in laying hens. *Br Poult Sci* **38**, 417–424.
13. Leeson S, Julian RJ & Summers JD (1986) Influence of pre-lay and early lay dietary calcium concentration on performance and bone integrity of Leghorn pullets. *Can J Anim Sci* **66**, 1087–1095.
14. Whitehead CC (2001) Nutrition and poultry welfare. *World's Poult Sci J* **58**, 349–356.
15. Fleming RH, Korver D, McCormack HA & Whitehead CC (2003) Assessing bone mineral density in vivo: digitized fluoroscopy and ultrasound. *Poult Sci* **82**, Suppl. 1, 55.
16. Nadeau KL, Korver DR, Zuidhof MJ, Robinson FE & Renema RA (2003) Strain and vitamin D source effects on egg production and bone mineral density of laying hens. *Poult Sci* **82**, Suppl. 1, 47.
17. Fleming RH, McCormack HA & Whitehead CC (1997) The effect of ED-71 (A vitamin D3 analogue) on skeletal growth and development in the maturing domestic hen. In *Vitamin D Chemistry Biology and Clinical Applications of the Steroid Hormone – Tenth Workshop on Vitamin D – Strasbourg France May 24–29 1997*, pp. 693–694 [AW Norman, R Bouillon and M Thomasset, editors]. Riverside, CA: Vitamin D Workshop, Inc.

18. Rao SK, Roland DA & Hoerr FJ (1992) Response of early-maturing and late-maturing commercial leghorn pullets to low-levels of dietary phosphorus. *Poult Sci* **71**, 691–699.
19. Selle PH & Ravindran V (2007) Microbial phytase in poultry nutrition. *Anim Feed Sci Tech* **135**, 1–41.
20. Hauschka PV, Lian JB, Cole DEC & Gundberg CM (1989) Osteocalcin and matrix GLA protein – vitamin K-dependent proteins *Bone Physiol Rev* **69**, 990–1047.
21. Rao KS, Roland DA Sr, Adams JL & Durboraw WM (1992) Improved limestone retention in the gizzard of commercial leghorn hens. *J Appl Poult Res* **1**, 6–10.
22. Miller SC (1977) Osteoclast cell-surface changes during the egg-laying cycle in Japanese quail. *J Cell Biol* **75**, 104–118.
23. Fleming RH, Bishop SC, McCormack HA, Flock DK & Whitehead CC (2000) Continued genetic selection for skeletal traits has produced laying hens with reduced likelihood of bone fracture. *Proceedings of the 21st World Poultry Congress (Montreal Canada)*, CD-ROM, Abstr W9.03. Montreal, Canada: World's Poultry Science Association.
24. Williams B, Waddington D, Solomon S & Farquharson C (2000) Dietary effects on bone quality and turnover, and Ca and P metabolism in chickens. *Res Vet Sci* **69**, 81–87.
25. Edwards HM Jr (1990) Efficacy of several vitamin D compounds in the prevention of tibial dyschondroplasia in broiler chickens. *J Nutr* **120**, 1054–1061.
26. Rennie JS, Whitehead CC & Thorp BH (1993) The effect of dietary 1,25-dihydroxycholecalciferol in preventing tibial dyschondroplasia in broilers fed on diets imbalanced in calcium and phosphorus. *Br J Nutr* **69**, 809–816.
27. Rennie JS & Whitehead CC (1996) The effectiveness of dietary 25- and 1-hydroxycholecalciferol in preventing tibial dyschondroplasia in broiler chickens. *Br Poult Sci* **37**, 413–421.
28. Leeson S (2007) Vitamin requirements: is there basis for re-evaluating dietary specifications? *World's Poult Sci J* **63**, 255–266.
29. Whitehead CC, McCormack HA, McTeir L & Fleming RH (2004) High vitamin D-3 requirements in broilers for bone quality and prevention of tibial dyschondroplasia and interactions with dietary calcium, available phosphorus and vitamin A. *Br Poult Sci* **45**, 425–436.
30. Chang DJ, Ji C, Kim KK, Casinghino S, McCarthy TL & Centrella M (1998). Reduction in transforming growth factor beta receptor 1 expression and transcription factor CBFa1 on bone cells by glucocorticoid. *J Biol Chem* **273**, 4892–4896.
31. Liu D, Veit HP, Wilson JH & Denbow DM (2003) Long-term supplementation of various dietary lipids alters bone mineral content, mechanical properties and histological characteristics of Japanese quail. *Poult Sci* **82**, 463–473.
32. McCormack HA, Fleming RH, McTier L & Whitehead CC (2006) Influence of dietary fatty acid n-3/n-6 balance on bone characteristics in young broilers. *Br Poult Abstr* **3**, 25–26.
33. Rymer C & Givens DI (2005) n-3 Fatty acid enrichment of edible tissue of poultry: A review. *Lipids* **40**, 121–130.