Nutritional and genetic interactions in avian osteoporosis

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Abstract
The effects upon bone quality of feeding calcium in particulate form to hens divergently selected for skeletal traits were investigated. As in previous generations, highly significant genotypic differences were observed in all measured bone traits at peak egg production (25weeks) and towards the end of production (56weeks). At 25 weeks there were no significant effects on bone parameters of feeding particulate calcium although a significant reduction in bone-resorbing osteoclast recruitment was observed at this age. By 56 weeks osteoclast numbers were further reduced in hens fed calcium as particulates and significant beneficial effects on some bone parameters were observed in this treatment group. The genotypic and dietary improvements upon bone quality were independent and additive at both ages. There were no detrimental effects of genotypic improvement on egg production; in fact, hens selected for better bones had significantly higher egg production by 56 weeks. Egg numbers were unaffected by diet.

Introduction
Bone fractures caused by osteoporosis (OP) in laying hens are a continuing welfare issue (Sandilands et al, 2005). OP in hens has strong genetic (Bishop et al, 2000) and environmental components (Fleming et al, 2005) and, to a lesser extent, a nutritional component in which the physical form of dietary calcium (flour or particulate) is primarily important (Fleming et al, 1998, 2003).

The mechanisms underlying the genetic improvements are unclear and therefore the aim of this experiment was to investigate the effects of genotype, calcium source and possible interactions on the skeletal integrity of laying hens.

Materials and Methods
Hens from several generations of divergent selection for skeletal traits (now forming 2 distinct OP-resistant [OP-RES] and OP-susceptible [OP-SUS] lines with a 2-fold difference in bone strength) were floor-reared communally to 15 weeks then caged individually from 15 weeks until 56 weeks. During the laying period, equal numbers of OP-RES and OP-SUS hens were fed a standard layer ration containing calcium as either limestone flour, or entirely in particulate form (ARCAL L105, 2.5 to 4mm diameter range, Hanson Aggregates, Cheddar, UK). Both grades of limestone were sourced from the same quarry. 100 hens were randomly culled at 25 and 56 weeks (25 OP-RES, 25 OP-SUS from flour and particulate diets at each age) from a total flock of 260 hens. Blood samples were taken for plasma ionised Ca^{2+} levels at 32 and 53 weeks from all hens.

Following culling at both ages, right tibiotarsi and the proximal 4cm of the *carina sterni* from the keel were removed for measurements of radiographic density (RD) by digitisation of *post mortem* x-rays of whole bones. A section of mid-diaphysis of left tibiotarsus was also removed and processed for histomorphometrical measurements of cross-sectional area (TIB X-AREA), defined as:
(tibiotarsus external area – marrow area) x (1-*p*)

(where *p* is a factor for cortical porosity, derived from measurements of 12 microscopic fields/hen)

At 25 and 56 weeks, the number of active osteoclasts per microscopic field (mean of 9 fields/hen) were also recorded by histochemical reaction with tartrate-resistant acid phosphatase (TRAP). Following radiography, right tibiotarsi and humeri were subjected to destructive 3-point bending tests for estimation of peak breaking strength (BS). Tibiotarsus bending stress (BendStress, [N/mm^2]) was also derived from tibia BS and TIB X-AREA measurements and removes effects caused by dimensional differences alone.

Results and Discussion

No genotypic or dietary differences were observed in plasma Ca^{2+} levels. All other results are shown in the Table. Substantial significant improvements for all measured skeletal traits were observed in OP-RES hens compared to OP-SUS hens at 25 and 56 weeks. As in previous generations, there were no differences in body weight between OP-RES and OP-SUS hens, since a subtractive coefficient for body weight had been applied to the selection process (Bishop et al, 2000). The provision of calcium entirely in particulate form afforded significant improvements over a limestone flour source in some measured bone parameters at 56 weeks (Tibia BS, Tibia BendStress, Tibia RD) but not at 25 weeks. At the cellular level however, OP-RES hens had significantly fewer osteoclasts than OP-SUS hens, and hens on the particulate calcium diet had significantly fewer osteoclasts than those fed the calcium flour diet at both 25 and 56 weeks. This is a key finding, as substitution of limestone flour with limestone in particulate form appears to allow slow overnight digestion following crop storage, reducing osteoclast recruitment and ultimately leading to decreased resorption of structural bone. This dietary effect appears to be simply additive to genotypic differences. Egg production appears to be slightly increased in the OP-RES hens compared to OP-SUS hens by 56 weeks, but no dietary effects were observed.

We conclude that there is a clear, beneficial, skeletal effect of feeding calcium in particulate form. Dietary and genotypic improvements are associated with a reduction in recruitment of active bone-resorbing osteoclasts (evident from 25 weeks of age). The dietary effect occurs independently of genotypic improvements in bone quality.

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Table: *Measured bone parameters at 25 and 56 weeks in a random sub-sample of hens divergently selected for OP resistance (OP-RES) or OP susceptibility (OP-SUS), housed in individual cages and fed from 15 weeks onwards a diet containing calcium as limestone flour, or entirely in particulate form*.

AGE	PARAMETER	OP-LINE	Ca FORM FLOUR	PARTICLE	SED	*Sign. Of Effect* LINE	DIET	LxD
25 WKS:	Body Wt. (g)	RES SUS	1595 1631	1617 1618	47.3	NS	NS	NS
	Tibia BS(N)	RES SUS	327.4 258.0	355.4 240.5	18.4	***	NS	NS
	TIB X-AREA (mm^2)	RES SUS	6.56 6.22	6.81 6.05	0.27	**	NS	NS
	Tibia BendStress (N/mm^2)	RES SUS	50.5 42.0	52.8 39.6	3.1	***	NS	NS
	Tibia RD (mmAe)	RES SUS	2.07 1.89	2.11 1.90	0.05	***	NS	NS
	Keel RD (mm Ae)	RES SUS	0.465 0.431	0.454 0.414	0.02	***	NS	NS
	Mean No. Osteoclasts /Field	RES SUS	11.60 13.63	10.45 11.75	0.70	***	**	NS
56 WKS:	Body Wt. (g)	RES SUS	1787 1848	1770 1839	33.7	NS	NS	NS
	Tibia BS(N)	RES SUS	390.4 216.9	431.6 247.6	17.7	***	*	NS
	TIB X-AREA (mm^2)	RES SUS	6.17 5.40	6.42 5.34	0.18	***	NS	NS
	Tibia BendStress (N/mm^2)	RES SUS	63.2 39.7	68.1 46.4	2.7	***	*	NS
	Tibia RD (mmAe)	RES SUS	2.50 1.94	2.64 2.10	0.06	***	***	NS
	Keel RD (mmAe)	RES SUS	0.443 0.316	0.441 0.352	0.013	***	NS	NS
	Mean No. Osteoclasts /Field	RES SUS	9.23 10.15	5.41 8.17	0.74	***	**	NS
	Mean Total No. Eggs	RES SUS	218.5 209.6	221.5 216.0	3.5	*	NS	NS

mmAe = mm of aluminium equivalent.