GENOTYPE AND NUTRITION INTERACTIONS IN RELATION TO BONE STRENGTH IN LAYING HENS

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Introduction

Osteoporosis in laying hens is defined as a decrease in the amount of fully mineralised structural bone, leading to increased fragility and susceptibility to fracture. A condition involving bone loss characteristic of osteoporosis was first described in caged laying hens by Couch (1955) who reported a problem termed 'cage layer fatigue' involving bone brittleness, paralysis and death. More recently, confirmation of osteoporosis as the main reason for bone loss and subsequent fractures in laying hens has been provided (Randall and Duff, 1988). The consequences of osteoporosis for the laying hen skeleton can be severe. Caged layer fatigue seems to be an extreme consequence of loss of structural bone in the vertebrae which leads to spinal bone collapse and paralysis (Bell and Siller, 1962; Urist and Deutsch, 1960). Generally, however, osteoporosis is not so severe as to result in caged layer fatigue but nevertheless the widespread structural bone loss can lead to high incidences of fractures at various sites throughout the skeleton.

Gregory and Wilkins (1989) reported results of a survey of end-of-lay battery hens in UK in which 29 % of hens had one or more broken bones during their lifetimes. The fractures occurred during their time in their cages or during depopulation, transport to a processing factory and hanging on shackles. Astonishingly, 98 % of carcasses were found to contain broken bones by the time they reached the end of the evisceration line. A subsequent survey of a number of European flocks confirmed these findings, showing that, on average, fractures were received by 10 % of hens during their time in batteries and a further 17 % during depopulation and transport (Gregory et al., 1994). The high fracture incidences show that osteoporosis constitutes a severe welfare problem in hens. Production losses and mortality also arise with caged layer fatigue. These economic losses are compounded by the high fracture incidences during carcass processing which lead to bone splinters in recovered meat and have resulted in processors becoming unwilling to handle spent layers (Brown, 1993).

Some of the characteristics of laying hen osteoporosis have been reviewed by Whitehead and Wilson (1992). Osteoporotic hens show evidence of widespread loss of structural bone throughout the skeleton. This loss has been shown to start when hens reach sexual maturity and to continue throughout the laying period (Wilson et al., 1992) so that osteoporosis is most severe in hens at the end of lay. The observations are consistent with the theory that at the onset of sexual maturity the rise in circulating oestrogen results in a switch in bone formation from structural to medullary bone and that continued resorption of structural bone leads to osteoporosis. Medullary bone does impart some strength to the skeleton (Fleming et al., 1998a) but is not as strong as structural bone. Evidence for a depression in structural bone formation in laying hens has been provided by Hudson et al. (1993) who observed that fluorochrome label was not incorporated into cortical bone. These findings have been confirmed by Fleming (unpublished) who also observed that loss of reproductive condition induced by force moulting resulted in rapid loss of medullary bone and resumption of structural bone formation.

Patterns of bone loss with age have been found to vary between different bones (Fleming et al.,1998b). Striking changes occurred during the first 10 weeks of sexual maturity. There was a marked loss of cancellous bone in both the proximal tarsometatarsus (PTM) and free thoracic vertebra (FTV) suggesting that, in these bones, the major development of osteoporosis occurs within a few weeks of the onset of egg production. Over this period there was also a rapid accumulation of medullary bone in the PTM but at present there is no indication as to whether the loss of cancellous bone is directly linked to the formation of medullary bone. After 25 weeks the further loss of cancellous bone from the FTV was smaller but loss of cancellous bone and the accumulation of medullary bone continue in the PTM, though also at reduced rates. However, there was a continuous net increase in the total amount of bone, measured histomorphometrically, with the result that total bone volume in the PTM can be greatest at 70 weeks.

The origins of osteoporosis are not well defined. It has been suggested that the problem is partly genetic in origin, resulting from the breeding of light weight, energetically efficient birds that maintain a high rate of lay over a prolonged period (Whitehead and Wilson, 1992). Most modern hybrid layer strains seem to be susceptible to osteoporosis, but older unimproved strains, such as the Roslin J-line Brown Leghorn, are relatively resistant (Rennie et al., 1997). However, even in susceptible strains there can be wide individual variation, with some hens retaining good bone quality at end of lay. Confining birds in cages with limited opportunity for exercise has undoubtedly contributed to the problem, resulting in a form of disuse osteoporosis. There appear to be three possible approaches to alleviating osteoporosis, involving nutrition, husbandry and genetics, as discussed in the following sections.

Nutrition

Nutritional criteria for hens have usually been based on optimising responses in egg production and shell quality, with little regard to bone quality. Overt deficiencies of calcium, phosphorus or vitamin D will affect bone quality adversely by causing osteomalacia (Wilson and Duff, 1991) but this is rarely seen under normal nutritional conditions. It has been suggested that the high egg output of hens accelerates osteoporosis by depleting body calcium reserves. Nutritional studies on bone have therefore aimed to establish whether (a) improving the supply of calcium can alleviate osteoporosis and (b) there are any other nutritional factors that can influence bone quality.

A recent study (Rennie et al., 1997) investigated the effects of a number of dietary factors during the laying period on bone composition. The factors included calcium source, phosphorus, protein, fat, fluoride and vitamins K, C and D metabolites. None of the factors, within the dietary concentrations studied, had any beneficial effect on the proportions of cancellous bone in spinal (FTV) or leg (PTM) bones but treatments involving feeding a particulate source of calcium (oystershell) or supplementation with fluoride increased the proportions of medullary bone. Providing calcium in particulate form extends the period of calcium absorption later into the night when shell formation is taking place and has been shown to have beneficial effects on shell quality. The finding that provision of a particulate calcium source can increase the amount of medullary bone without having much impact on the loss of structural bone shows that calcium deficiency is not a primary cause of osteoporosis. Fluoride is known to stimulate bone formation in other species, but the increase in synthesis in hens is evidently confined to medullary bone.

Increases in medullary content may nonetheless have a beneficial effect on bone quality, as indicated earlier. Confirmation of the practical benefits of particulate calcium sources has been provided by Fleming et al. (1998b) who found that feeding particulate limestone resulted in a slower loss of cancellous bone over the early part of the laying period and improved bone strength in older hens, as shown in table 1. Nutrition during rearing is also important in maximising bone content before the onset of sexual maturity. Dietary supplementation during rearing with extra vitamin K, a factor required for the synthesis of osteocalcin, a protein involved in bone formation, may also benefit some aspects of bone quality (Fleming et al., 1998b).

Table 1: Effect of limestone particle size on bone characteristics in end-of-lay hens

Trait	Limestone type	
	Powder	Particles
Proximal tarsometatarsus		
Total bone (%)	23.4	28.6
Cancellous bone (%)	6.6	7.3
Medullary bone (%)	16.8	21.4
Tibia		
Radiographic density (mm Al)	1.96	2.26
Breaking strength (kg)	19.5	23.6
Humerus		
Radiographic density (mm Al)	0.73	0.78
Breaking strength (kg)	11.8	12.1
Keel		
Radiographic density (mm Al)	0.61	0.68

(Fleming et al., 1998b)

These observations are consistent with the hypothesis that osteoporosis in hens arises as a result of cellular processes rather than nutrient supply and that during the laying period there is continued osteoclastic resorption but little formation of structural bone. The balance of cellular activity can be disrupted by feeding bisphosphonate, a drug used in human medicine to combat post-menopausal osteoporosis. This acts by inhibiting the action of osteoclasts and has been shown to slow the loss of cancellous bone in hens (Thorp et al., 1993). However, use of bisphosphonate is unlikely to be a practical solution for laying hen osteoporosis.

Exercice and husbandry system

The effects of loadbearing and biomechanical forces in stimulating bone formation and remodelling are well established (Lanyon, 1992). Induced inactivity has been shown to accelerate osteoporosis in birds (Nightingale et al., 1972) and the relative lack of activity of battery caged hens accounts for the severity of the problem in these birds. Effects of exercise and alternative housing systems have been widely studied as potential means of alleviating osteoporosis.

Effects of exercise as a way of stimulating bone growth during rearing have been studied, but neither housing birds in pens nor giving extra exercise through use of a carousel have improved bone quality at start of lay compared to cage-rearing (Whitehead and Wilson, 1992).

Changes in bone quality during the laying period are influenced by the nature of the exercise involved. Housing hens in pens has resulted in little change in spinal trabecular bone (Wilson et al., 1993). This suggests that merely allowing the birds more opportunity to walk does not result in generalised bone improvements, though the limited nature of the observations did not preclude the possibility of more local benefits, such as in leg bones. Fitting perches to cages resulted in small improvements in PTM trabecular bone volume, but no benefit in tibia strength (Hughes et al., 1993). More vigorous exercise than is obtained by walking or hopping onto low perches is needed to markedly improve bone quality. This was demonstrated by Knowles and Broom (1990) who found superior tibia and humerus breaking strengths in birds housed in terrace or perchery systems rather than in cages. The improvement in humerus strength was particularly apparent in the perchery system that allowed birds freedom to fly.

Confirmation of these findings came from a more detailed study by Fleming et al. (1994) involving comparison of battery cages with three different aviary systems. Considerable improvements in a wide range of bone morphometric and strength characteristics were observed in birds housed in aviaries, as shown in table 2, with the greatest improvement being in the strength of the humerus. The improvements in the strength of this bone was more pronounced in the perchery, that was tiered with many high perches, compared with the litter and wire system that had only relatively low perches. Thus opportunity for flight was an important factor in improving humerus strength. These findings on hens are consistent with findings in other species that biomechanical effects on individual bones are dependent upon the degree of strain experienced by the bone.

Table 2: Bone characteristics in end-of-lay hens housed in different husbandry systems

Trait	Husbandry system		
	Battery cage	Litter and wire	Perchery
Tibia			
Breaking strength (kg)	21.8	25.7	28.6
Radiographic density (mm Al equivalent)	2.95	3.48	3.33
Humerus			
Breaking strength (kg)	13.1	22.6	25.4
Cortical width (mm)	0.51	0.67	0.72
Radiographic density (mm Al equivalent)	0.75	1.11	1.25
FTV			
Cancellous bone volume (%)	11.3	13.6	16.6
PTM			
Cancellous bone volume (%)	14.2	16.7	16.8

FTV: free thoracic vertebra; PTM: proximal tarsometatarsus

(Fleming et al., 1994)

There have been several studies to determine the welfare impact of the improved bone strength of birds kept in alternative housing systems. Lower incidences of new breaks have been found in birds depopulated from aviary or free range systems compared to battery cages (Gregory et al., 1990; Van Niekerk and Reuvekamp, 1994). However, the incidences of old breaks, particularly in the furculum and keel, were higher with aviary and free range systems (Gregory et al., 1990). A more recent comparison of cages with a Boleg aviary system has confirmed that bone breakage was lower during depopulation from the Boleg system but that the incidence of keel deformities was much higher with this system (Van Niekerk, Reuvekamp and Kiezebrink, unpublished). It may be concluded that allowing birds more exercise in alternative systems will improve bone strength, but this does not necessarily improve bird welfare in proportion.

Genetics

The large individual variation observed in the bone characteristics of hens at the end of lay, phenotypically unrelated to egg production in a flock of highly productive hens (Rennie et al., 1997), suggests that the problem of osteoporosis may be alleviated by genetic selection, perhaps without serious consequence for egg productivity.

The possibility of a genetic solution to osteoporosis has been studied by Bishop et al. (1999). The inheritance of characteristics related to osteoporosis was studied over 5 generations in a commercial pure line of White Leghorns previously selected for high egg production. Initially, measurements were made on a range of morphometric, radiological and strength characteristics of different bones in hens at the end of the laying period to determine heritabilities. Morphometric traits involving cancellous and medullary bone volumes were found to be poorly heritable (FTV cancellous bone volume, $h^2 = 0.19$; PTM cancellous bone volume, $h^2 = 0.0$). This was considered surprising in view of the use of cancellous bone to assess severity of human postmenopausal osteoporosis (Khosla et al., 1994) and as a criterion in earlier laying hen studies (Whitehead and Wilson, 1992; Wilson et al., 1993; Rennie et al., 1997). In contrast, heritabilities of other characteristics were higher (tibia strength, $h^2 = 0.45$; humerus strength, $h^2 = 0.30$; keel radiographic density, $h^2 = 0.39$). There was also a positive correlation between body weight and bone strength.

A restricted selection index designed to improve bone characteristics, yet hold body weight (BW) constant, was derived from genetic parameters obtained from these preliminary analyses, using standard selection index theory. Three biologically meaningful and moderately to highly heritable traits that could be measured in a short period of time on a large number of birds were included in the index, namely keel radiographic density (KRD), humerus strength (HSTR) and tibia strength (TSTR). By including characteristics of wing, leg and axial skeleton, this index gave a wide representation of the overall skeleton. The index was: Bone Index = 0.27 x KRD + 0.37 x HSTR + 0.61 x TSTR - 0.25 x BW. The coefficient for bodyweight was increased to 0.35 for selection of the G5 generation to counter a slight divergence in this trait that started to appear between the lines. Selection was performed retrospectively each year, with chickens hatched and raised from all available hens in the experiment. On the basis of the data collected on the hens at the end of their laying period, selection decisions were made with entire full sib families of chickens being kept or rejected. Selection commenced by assigning birds in generation G3 to either the high (G3H) or low (G3L) line on the basis of their dams' (G2) Bone Index.

Consequences of selection

Genetic parameters for the traits in the Bone Index, and the Bone Index itself, showed that all traits were moderately to highly inherited throughout the study, with the heritability of the Bone Index being 0.4. The genetic and phenotypic correlations also show that the three bone measurements in the index were moderately to strongly correlated with each other. Finally, the bone measurements are all positively correlated with body weight, indicating that selection for improved bone strength characteristics alone, without the restriction placed on body weight, would have resulted in considerably heavier birds. Different mean values in the bone strength measurements in different years indicated that these traits were strongly affected by environmental factors, raising the possibility of genotype by environment interactions. However, comparison of full sib flocks reared on different locations gave little evidence for genotype by environment interactions, within the range of environments investigated.

From year three onwards, the lines diverged progressively for KRD, HSTR, TSTR and the Bone Index in the desired direction. Bone characteristics in G5 are shown in table 3. For the hens, the lines differed by 19 % for KRD, 13 % for HSTR and 25 % for TSTR. The differences were highly significant (p<0.01) from year 4 onwards, with the exception of HSTR in year 4 where the difference, although in the desired direction, was not significant. Although selection was based on measurements made on hens, selection was found to also affect bone strength in males as well, with high index males outperforming low index males for all traits. The differences between the lines in G5 were: TSTR 10 % (p<0.01), HSTR 13 % (p<0.05), KRD 15 % (p<0.01) and bodyweight 7 % (p<0.01). The incidences of humeral fractures in hens occurring during the production period and depopulation showed a 6-fold difference between the lines in G5.

Table 3: Bone characteristics and body weights at the end of the laying period in female and male chickens after 3 generations of selection for high (H) or low (L) bone index (G5 generation)

Trait	Females			Males		
	Line H	Line L	Probability	Line H	Line L	Probability
Body weight (kg)	1.67	1.63	<0.001	2.29	2.14	<0.01
Keel radiographic density (mm Al equivalent)	0.58	0.48	<0.001	0.77	0.67	<0.01
Tibia strength (kg)	30.7	23.9	<0.001	68.2	61.5	<0.01
Humerus strength (kg)	15.4	13.5	<0.001	50.0	44.0	<0.05
Humerus fractures (%)	2.8	18.4				

(Bishop et al., 1999)

The responses to selection are encouraging insofar as it appears possible to create sizeable differences in bone strength characteristics of hens in a relatively short period of time, using conventional selection techniques. This response can also be made to be independent of body size, should that be the desire of the breeder, though faster progress could be made if body weight were to be allowed to increase. Moreover, the absence of significant or meaningful genotype by environment interactions implies that selection progress seen in a breeding flock should also be expressed under commercial egg production conditions.

Although the study was aimed mainly at hens, it is interesting that the bone characteristics included in the Bone Index were also changed in males by selection (table 2), suggesting that selection has altered some factors in bone metabolism common to both sexes. Male chickens do not suffer from osteoporosis but this finding raises the possibility that selection procedures aimed at alleviating osteoporosis in hens could also be carried out in male birds.

Changes in bone strength per se are unlikely to be of special interest, however, unless they are accompanied by a change in the incidence of bone fractures or a change in the liability to osteoporosis. Keel and the humerus are two bones observed to be frequently fractured in commercial practice (Gregory and Wilkins, 1989). Although the cage and handling conditions in the present study were not intended to resemble the practices on commercial laying farms, selection has clearly resulted in an altered incidence of fractures in these bones. Few fractures of the tibia, a much stronger bone, were seen in this study. The responses in bone breakage incidence were reflected in the genetic correlations with bone strength. All bone measurements are strongly correlated with the presence/ absence of breakages and the number of sites of broken bones, indicating that genetically altering bone strength will indeed alter the incidence of bone fractures.

Can the selection procedure be simplified or improved to avoid the need for retrospective selection performed on excess birds? Bone strengths in different parts of the skeleton appear to be strongly correlated, indicating that selection to improve the strength of one bone within the skeleton should increase the strength of the skeleton as a whole. This has important implications in terms of reducing the number of measurements required to categorise the strength of the skeleton as a whole; relatively few measurements should be required, making selection for bone strength more feasible. Alternatively, selection could be improved by the use of in vivo predictors of bone strength at an early stage of the laying period, so that eggs are fertilised, collected and hatched only from selected hens. A predictive in vivo method involving digitised fluoroscopy of the humerus under experimental conditions has been described by Fleming et al. (1998c). Other metabolic comparisons now being carried out on the lines may identify alternative approaches.

The genetic and phenotypic relationships between bone strength characteristics and egg production and quality characteristics are key factors to be identified before selection for improved bone quality can be incorporated into commercial breeding programmes. Preliminary evidence suggests that there has been a slight deterioration in shell quality in the high bone index line and this important relationship is currently under further investigation.

Discussion

The three main factors discussed above have all been shown to influence osteoporosis. So what is the best solution to this welfare problem? Nutrition has a part to play, firstly by avoiding deficiencies that can make the problem worse and secondly by promoting good nutrient supply. It is likely that transferring pullets onto a diet of higher calcium content at lighting up rather than at first egg will help to decrease the loss of structural bone during the initial period of formation of medullary bone. Providing a particulate source of calcium during the laying period is also particularly helpful as this seems to benefit bone formation by allowing absorption of calcium from the digestive system into the period of shell formation. This may spare the need to resorb medullary bone during this time. Increasing dietary calcium content above the conventional phase feeding levels has not been thought to be particularly beneficial for bones, but the finding in the genetic study that bone quality appears to be negatively related to shell quality may reopen the question of dietary calcium level. Is it possible in birds with good genetic potential for bone quality that calcium supply becomes limiting for shell formation? The calcium requirement of the osteoporosis resistant line for both bone and shell quality is now an important research priority. Comment is needed on one other nutritional procedure. The practice of feed withdrawal some days before depopulation is to be deplored; this will accelerate loss of bone mass just at a time of severe risk of bone damage. Good nutritional practice is thus important in trying to minimise osteoporosis, but nutrition alone will not prevent it.

Allowing birds more exercise in alternative systems will improve bone strength and this, coupled with careful handling procedures, can result in many fewer fractures during depopulation. However, the greater bone strength does not necessarily improve overall bird welfare. Birds in alternative systems have greater opportunity to experience more damaging accidents than caged birds. For instance, they can crash during flight or fall or be pushed off perches. Thus they can actually suffer higher incidences of bone fractures during the laying period than caged birds, despite having stronger bones. It is likely that the designs and stocking densities of alternative husbandry systems can be established that will minimise welfare problems associated with bone fracture. However, alternative systems are also associated with other welfare problems, such as feather pecking and cannibalism. Abolition of battery cages is thus not a simple panacea for solving welfare problems in general and it is apparent that alternative or additional approaches to minimising bone fracture are needed.

Genetics seems to offer a practical solution. Bone strength characteristics in end-of-lay hens are moderately to strongly inherited and respond readily to selection. A correlated response to this selection has been a change in the incidence of bone fractures, with a lower incidence of fractures occurring in hens selected for increased bone strength. Selection for enhanced bone strength is thus possible and should be implemented as a long-term measure to help alleviate osteoporosis, for two main reasons. Firstly, selection over many years for productive characteristics, including perhaps importantly persistency of lay, has resulted in birds that are highly susceptible to osteoporosis. This trend may continue unless specific steps are taken to reverse it. Secondly, Van Niekerk, Reuvekamp and Kiezebrink (unpublished) have found that the improvement in genetic resistance to osteoporosis is a general effect shown by birds kept either in cages or an aviary. Thus improving bone strength genetically will improve the welfare not only of caged birds but also of birds housed in the alternative systems that the

European egg industry is under increasing pressure to adopt. Procedures are being identified for practical selection for improved bone quality and the incorporation of these into commercial selection programmes is now the challenge presented to the layer breeder industry.

Genetics will thus have an important future role in combating the welfare problem of osteoporosis and bone breakage, but not an exclusive one. Good nutrition will continue to be important, to avoid deficiency and optimise diet composition and supply at important periods. Better cage designs or husbandry systems can also contribute to welfare improvements. And, finally, careful bird handling, particularly at the end of lay, will continue to be needed to minimise fracture incidences.

Summary

Osteoporosis in laying hens is a condition that involves the progressive loss of structural bone during the laying period. This results in increased bone fragility and susceptibility to fracture, with fracture incidences of up to 30 % over the laying period and depopulation not uncommon. A major cause of osteoporosis is the switch in bone formation from structural to medullary bone at the onset of sexual maturity, but structural bone loss is accelerated by the relative inactivity of caged birds. Allowing birds more exercise, as in aviary systems, results in stronger bones, but bone breakage is not always decreased because of the greater risk of accidental damage. Good nutrition can help to minimise osteoporosis, but is unable to prevent it. Best nutritional practice involves transferring birds to a higher calcium diet at lighting up rather than at first egg, providing a source of calcium in particulate form and not withdrawing feed some days before depopulation. Breeding may be an effective way of combating osteoporosis. Some bone strength traits are heritable and divergent selection for resistance or susceptibility to osteoporosis has resulted in lines with a marked difference in bone strength and a 6-fold difference in fracture incidence under commercial breeding conditions. The genetic improvement in bone strength is unaffected by type of housing system and offers a long-term solution to the problem of osteoporosis.

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