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Dyschondroplasia in poultry

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A number of different skeletal disorders cause considerable welfare and production problems in commercial poultry production. Laying hens suffer from a generalized loss of structural bone mass characteristic of osteoporosis. In growing birds of meat-type strains, which have been selected over the past 50 years for fast growth, the most common skeletal defects occur in leg bones and joints. The welfare problem of broiler leg deformities was highlighted in a report of the Farm Animal Welfare Council (1992).

Leg-bone deformities, frequently categorized under the heading of 'leg weakness', have been a cause for concern in the broiler industry for very many years. Many pathologies have been described but, despite much research, problems are still widespread. Research has been successful in identifying and eliminating some causes, such as chondrodystrophies caused by simple nutrient deficiencies. However, the types of problem that remain have grown in importance to the point that they are now seen as major causes of poor welfare for birds, as well as lost production for producers.

Developmental disorders are characterized by angular and rotational deformities of the leg bones and are seen in both broilers and turkeys. They are typified by hock joint distortions commonly known as varus or valgus deformity. These distortions of the long bones and the joints may, in turn, lead to secondary soft tissue pathologies. Dyschondroplasia is the most commonly observed lesion in the bones of broilers showing these defects. It is usually referred to as tibial dyschondroplasia (TD) because it most severely affects the proximal tibia, which is the fastest-growing bone in young broilers, and has been shown to be a direct cause of clinical deformity and lameness (Lynch *et al.* 1992).

CHARACTERISTICS OF TIBIAL DYSCHONDROPLASIA

The normal process of endochondral bone formation in the long bones of chickens involves growth plate chondrocytes passing through an orderly series of well-defined stages. Chondrocytes from the resting or reserve zone first commence proliferation to form a columnar zone of flattened cells. They then alter their phenotype as they start to differentiate into hypertrophic chondrocytes. In doing so, they first start to enlarge to form a zone of prehypertrophic chondrocytes which then secrete a matrix containing type X collagen as they mature to become fully hypertrophic. Mineralization starts within the matrix, capillary invasion occurs and bone formation takes place.

The TD lesion takes the form of a plug of avascular cartilage underlying the bone growth plate. It is made up of an accumulation of prehypertrophic chondrocytes and can vary considerably in size from a small focal accumulation in one part of the growth plate to a large mass occupying the full width of the growth plate and extending to a depth of 10 mm or more. The lesion thus differs from both hypocalcaemic rickets, in which the thickening of the growth plate is due to an accumulation of proliferating chondrocytes, and hypophosphataemic rickets where hypertrophic chondrocytes accumulate.

The lesion first becomes apparent at about 2 weeks of age and can be visualized by X-rays. Radiography can show the lesion typically increasing in size up to about 4 or 5 weeks of age and then regressing. Gross examination of the tibia in older birds can often reveal

little indication of the previous presence of a TD lesion, although histology will show remnants of dyschondroplastic chondrocytes within physeal bone. The lesion may thus be transient, but it can have permanent effects on the bone. Fractures may occur through the weakened area of cartilage. Alternatively, the orientation of longitudinal growth of the proximal tibia may be distorted. This can be demonstrated by measurements of the tibial plateau angle from radiographs. The presence of a lesion can increase the angle from a normal value of below 20° to over 35° (Lynch *et al.* 1992). The resultant curvature of the bone can be a direct cause of lameness or can result in abnormal biomechanical forces, especially in the hock joint, which can lead to secondary pathologies and causes of lameness.

The characteristics of TD lesions have been studied extensively by histology and immunohistochemistry. The lesion does not result from change in the rate of proliferation of chondrocytes, but instead arises from a failure of the normal process of differentiation which leads to a build up of the prehypertrophic chondrocytes (Farquharson *et al.* 1992). This enlarged transitional zone lacks other characteristics of more-fully-differentiated cells. There is a lack of formation of matrix based on type X collagen. Immunolocalization shows the presence of intracellular type X collagen, indicating that there is a failure in matrix secretion. Lesion chondrocytes show other abnormalities compared with differentiated chondrocytes, including a lack of transforming growth factor (TGF)-β and c-*myc* protein (Loveridge *et al.* 1993). These two factors are associated with the process of differentiation and, indeed, they become restored in cells that start to repair, mainly around the margins of epiphyseal blood vessels. However, interpretation of these findings is complicated by the 'chicken and egg' situation. Did lack of these factors inhibit differentiation or are they absent because of a lack of differentiation?

At present, the cellular causes of TD remain obscure. That they are not attributable to an inherent cellular defect has been shown by studies involving harvesting growth plate chondrocytes and culturing them in a high-cell-density pellet system. Starting with normal proliferating chondrocytes, this sytem allows the cells to go through normal stages of differentiation, with production of type X collagen matrix, TGF-β and c-*myc* protein, and finally foci of mineralization under physiologically normal concentrations of phosphate in the culture medium (Farquharson & Whitehead, 1995). Significantly, cells cultured from within the TD lesion can resume their maturation process and go through the same stages. This suggests that some systemic factor, lacking in the circulation but present in the medium, has some profound effect on the induction of TD.

NUTRITION AND TIBIAL DYSCHONDROPLASIA

Many nutritional factors have been shown to influence the occurrence or severity of TD. Changes in the contents of monovalent ions (Na^+, K^+, Cl^-), nature of the protein source, content of amino acids such as cysteine and homocysteine, trace minerals such as Mo, the presence of mycotoxins (especially from species of *Fusarium*) and supplementing diets with cholecalciferol metabolites have all been shown to affect TD. However, most of these factors induce TD; cholecalciferol metabolites are the only factors found so far to prevent TD.

Calcium and phosphorus

The dietary Ca : P value has been shown to have a major effect on the occurrence of TD (Edwards & Veltmann, 1983). The incidence can be increased by decreasing the dietary Ca

content at constant P content, or by increasing the P content at adequate Ca content. Although optimizing the dietary Ca and P contents can minimize TD, this procedure cannot eliminate TD. Diets with relatively low Ca and high P contents have been particularly effective in enhancing the occurrence of TD, with incidences of 40–50 % being regularly obtained with diets containing 7·5 g of each element/kg. This finding has been of great value, because being able to reproduce high incidences has been of considerable help in the design of subsequent experiments to study the causes and means of prevention of TD.

Riddell & Pass (1987) observed that before typical TD developed, chickens fed on a diet of high P content had uniformly thickened growth plates at 2 weeks of age. The majority of this thickening was due to an increase in the zone of proliferation, identical to that seen in hypocalcaemic rickets. This has raised the question as to whether TD is caused by Ca deficiency and, if so, how TD is related to rickets, the classical symptom of Ca deficiency. This has also caused experimental problems, because of difficulties over the differential diagnosis of TD and rickets. These conditions are sometimes not easy to distinguish visually and histology is needed for accurate diagnosis.

Ionic balance

An effect of dietary ionic balance (expressed as meq $(Na + K - Cl)$/kg) on TD was first proposed by Mongin & Sauveur (1977). A subsequent series of studies (Hulan *et al.* 1986, 1987*a*,*b*; Simons *et al.* 1987) suggested that a value based on this calculation was an inadequate measure in relation to TD, but confirmed the principle that metabolic acidosis (an increase in Cl) was associated with an increase in incidence of TD, and alkalosis with a decrease.

Protein and amino acids

A curious effect of protein source on the occurrence of TD was reported by Edwards (1985) who observed that soyabean meal from two sources repeatedly gave a greater incidence than soyabean meal from another source. The most striking difference between the meals was the high anti-trypsin (*EC* 3.4.21.4) and urease (*EC* 3.5.1.5) activities of the meals giving high TD incidence. A mechanism involving effects on energy, Ca or P metabolism was discounted. Could the effect be related to altered amino acid balance? This possibility is raised by subsequent findings on cysteine and homocysteine.

Orth *et al.* (1992) reported that supplementing diets of normal amino acid content and balance with cysteine (2·5–20 g/kg) or homocysteine (1·5–6 g/kg) resulted in a dose-dependent increase in TD incidence. This procedure seems to be a powerful way of inducing TD, because Bai & Cook (1994) used it to produce severe TD-like lesions by 10 d of age in lightweight Leghorn-type chicks, the type of chicken not normally associated with TD. The complex nature of interactions involved was shown in a subsequent finding (Bai *et al.* 1994) that dietary supplementation with Mo could prevent cysteine-induced TD but not spontaneous TD in broilers, and the induction of TD by cysteine was not related to Mo deficiency.

Thiocarbonates

A family of thiocarbonates used as fungicides (thiuram) or alcohol deterrents (disulfiram) have been found to induce TD when fed to broiler (Vargas *et al.* 1983; Edwards, 1987) and Leghorn chicks (Veltmann *et al.* 1985). There is little information on metabolic factors

involved, although Edwards (1987) reported that both compounds lowered intestinal Ca absorption. Wu *et al.* (1993) subsequently reported that excess dietary Cu could decrease the incidence of thiuram-induced TD.

Mycotoxins

Mycotoxins were first shown to have a role in TD by Walser *et al.* (1982) who reported that a *Fusarium* toxin could induce TD. A compound subsequently identified as fusarochromanone was found to be responsible for the effect. Wu *et al.* (1993) reported that both excess Zn and Cu could decrease the incidence of fusarochromanone-induced TD. The ability to induce TD is not shared by all mycotoxins. Feeding aflatoxin has been reported to decrease the incidence of TD (Huff, 1980), but this effect is more likely to have been related to the accompanying growth depression rather than to a specific property of aflatoxin.

Feed management

It has been generally assumed that rapid weight gain has been a major cause of TD. Despite evidence that there is no genetic correlation between TD and body weight (Kuhlers & McDaniel, 1996), nutritional evidence suggests that dietary regimens that depress growth rate decrease the incidence of TD (Poulus *et al.* 1978; Lilburn *et al.* 1989). The retardation in growth rate can be achieved by either qualitative or quantitative food restriction. Fasting has also been shown to decrease the incidence of TD, without causing growth depression, provided the fasts are of about 8 h duration (Edwards & Sorensen, 1987).

Cholecalciferol metabolites

The most important nutritional findings on TD have been the observations on the effects of cholecalciferol metabolites. Edwards (1989, 1990) reported that dietary supplementation with 1,25-dihydroxycholecalciferol (1,25-D) could reduce the incidence of TD. More recently, Rennie *et al.* (1993) showed, using histological techniques to accurately diagnose TD, that 1,25-D could completely prevent TD. In contrast, increasing dietary cholecalciferol above normal amounts had no effect on the incidence of TD.

There have been several studies on responses to different doses of 1,25-D. In experiments with low dietary Ca : P values, it was found that a dietary supplement of 5 μg 1,25-D/kg was effective in preventing TD, and that 10 μg/kg could be fed without adverse effect on growth rate. However, in a subsequent experiment involving a diet of more normal Ca content, a supplemental amount of 5 μg/kg was found to result in a growth depression (Rennie *et al.* 1993). This finding prompted a study of the interaction between dietary Ca and 1,25-D (Rennie *et al.* 1995). A factorial experiment involving dietary concentrations of 7·5, 10 and 12·5 g Ca/kg and 0, 2, 3·5 and 5 μg 1,25-D/kg confirmed a strong interaction between these dietary factors. The results are summarized in Table 1. They show that combinations of the higher dietary concentrations of both factors caused growth depression. However, with 7·5 g Ca/kg, dietary amounts of up to 5 μg 1,25-D did not affect growth adversely. The cause of this interaction was explained by plasma ionized Ca measurements, which showed that the depression in growth was associated with hypercalcaemia. One of the biological roles of 1,25-D is regulation of Ca absorption via its effect on Ca-binding protein. Thus, high dietary amounts of Ca, combined with the stimulatory effects on Ca absorption of large 1,25-D supplements leads to excessive Ca absorption, hypercalcaemia and consequent growth depression.

Table 1. *Effects of different dietary levels of calcium and 1,25-dihydroxycholecalciferol (1,25-D) on body weight, incidence of tibial dyschondroplasia (TD) and plasma ionized calcium at 3 weeks of age*

Diet content				
Ca (g/kg)	1,25-D (μg/kg)	TD (%)	Wt (g)	Ionized Ca (mmol/l)
7·5	0	50	741	1·50
	2	15	748	
	3·5	5	731	
	5	0	738	1·56
10	0	10	739	
	2	20	733	
	3·5	5	712	
	5	5	699*	
12·5	0	15	710	1·55
	2	15	730	
	3·5	0	677*	
	5	0	617*	1·89

Mean values indicated a significant growth depression: * $P < 0·05$.

TD incidence was higher at the lower dietary Ca levels, 1,25-D was effective in preventing TD at all dietary Ca concentrations, although there was a tendency for more to be needed for complete prevention at the lower dietary Ca concentrations. Over all the dietary Ca concentrations, the highest amount of 1,25-D not causing a growth depression was 2 μg/kg, whereas 3·5 μg/kg was needed for effective prevention of TD. However, it is likely that the lower dose would be effective in preventing clinical abnormality, because the severity of the lesion is generally related to incidence, and small TD lesions are less likely to result in a major deformity of bone growth. Nevertheless, it is apparent that the safety margin between effective TD prevention and deleterious growth-depressing doses of 1,25-D is small, and a means of enhancing the effectiveness of small doses of 1,25-D in preventing TD would be desirable.

Studies on a range of other cholecalciferol metabolites suggest that the most effective derivatives are those that are 1-hydroxylated. Thus, 1-hydroxycholecalciferol and 1,24,25-trihydroxycholecalciferol are also effective in doses up to 5 μg/kg in preventing TD. However, it has also been found that 25-hydroxycholecalciferol (25-D) can decrease substantially the incidence and severity of TD lesions, as shown in Table 2 (Rennie & Whitehead, 1996), although at somewhat higher dose rates (75 μg/kg). However,

Table 2. *Effects of dietary cholecalciferol and 25-hydroxycholecalciferol (25-D) on body weight and incidence and severity of tibial dyschondroplasia (TD) in broiler chickens at 3 weeks of age*

Diet content (μg/kg)		Body wt	TD	TD
Cholecalciferol	25-D	(g)	incidence (%)	severity
75		634	64	2·2
	75	677*	10	1·7

Mean value was significantly different from the control value: * $P < 0·05$.

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responses have not been observed in all experiments, leading to the possibility that some other factor may interact with 25-D.

Ascorbic acid

Ascorbic acid has also been studied as a factor that may influence TD. Dietary supplementation with ascorbic acid alone has not been found to affect TD (Leach & Burdette, 1985). However, adding ascorbic acid to diets containing insufficient 1,25-D to completely prevent TD has resulted in elimination of residual TD (Whitehead *et al.* 1994). This suggests that ascorbic acid may act in an additive or synergistic manner along with 1,25-D. If these findings can be validated, an effective means of combating TD might involve feeding a combination of a small dose of 1,25-D along with ascorbic acid as synergist. Possible synergistic effects of ascorbic acid with other cholecalciferol metabolites have not been identified.

AETIOLOGY OF TIBIAL DYSCHONDROPLASIA

Despite extensive research, it has to be admitted that the aetiology of TD remains obscure. The induction of TD by the wide range of apparently disparate factors listed previously leaves open the possibility that there may be several aetiologies, each of which results in the inhibition of chondrocyte differentiation that leads to the appearance of the lesion. There is at present no unifying theory that links Ca, cysteine, thiocarbonate, Mo or 1,25-D in the inhibition of differentiation.

There have been a number of studies that give some insight into possible mechanisms of action of cholecalciferol metabolites in TD. 1,25-D is the most biologically active metabolite and regulates several genes. The need for dietary supplementation with 1,25-D to normalize growth plate development suggests that the normal mechanism for endogenous 1,25-D synthesis may be inadequate in young broilers. Low plasma concentrations of 1,25-D have not been associated with occurrence of TD within genotypes and dietary supplementation has not been observed to result in increased plasma concentrations (Rennie *et al.* 1993). However, a strain difference has been reported in which the strain with the greater predisposition to TD also showed lower plasma 1,25-D concentration (Parkinson *et al.* 1996). In contrast, plasma concentrations of 24,25-dihydroxycholecalciferol (24,25-D) can be elevated by supplementation with 1,25-D. This effect can be explained by the supply of dietary 1,25-D down-regulating endogenous synthesis of 1,25-D and diverting 25-D down an alternative hydroxylation pathway; there is no other evidence to suggest that prevention of TD involves an effect of 24,25-D.

The vitamin D receptor (VDR) is found in many tissues, but VDR numbers and affinities for 1,25-D have been shown to be decreased in chondrocytes within the TD lesion (Berry *et al.* 1996). 1,25-D acts via VDR to regulate a number of genes involved in the developmental pathway and can also regulate its own receptor. It is thus possible that, if the aetiology of TD involves defective VDR, the adverse consequences of this are overcome by the provision of extra 1,25-D.

The previously described mechanism would imply a local effect of 1,25-D in chondrocytes. Cellular studies *in vivo* have suggested that TD is caused by a slowing of the normal process of chondrocyte differentiation and that 1,25-D acts by stimulating this process (Farquharson *et al.* 1993). However, *in vitro* studies have failed to demonstrate direct effects of vitamin D metabolites in stimulating rate of chondrocyte differentiation, although some effects on TGF-β production have been reported (Farquharson *et al.* 1996*a*).

Vitamin D analogues that have enhanced differentiation and decreased calcaemic properties have been found to be ineffective in preventing TD (Farquharson *et al.* 1996*b*). At this stage, therefore, it is not possible to say whether 1,25-D prevents TD by a direct effect on chondrocytes themselves or by a more systemic mechanism, perhaps involving Ca metabolism.

The mechanism of ascorbic acid in its possibly synergistic action with 1,25-D is still uncertain. There have been suggestions that ascorbic acid has a stimulatory effect on the 1-hydroxylase (*EC* 1.14.15.3) enzyme that converts 25-D to 1,25-D (Weiser *et al.* 1988). However, in other *in vitro* and *in vivo* experiments, ascorbic acid has been found to stimulate the production of 24,25-D but not 1,25-D (Berry *et al.* 1994). These findings raise the possibility that 24,25-D might have a role in TD, but the lack of response to feeding this metabolite suggests that this is not so. It is of interest to note in this context that ascorbic acid stimulates chondrocyte differentiation *in vitro*. It has been thought that it exerts this effect through cross-linking of the collagen that forms an essential part of the intercellular matrix. Interactions between chondrocytes and the surrounding matrix are vitally important in the overall process of chondrocyte differentiation. This, therefore, raises the possibility that ascorbic acid exerts its effect on TD by a mechanism involving the cellular matrix that is independent of 1,25-D yet complements the cellular effects of this metabolite.

The metabolic role of 25-D in preventing TD is uncertain. The molecule is a naturally-occurring metabolite that binds strongly to chick serum vitamin D-binding protein (Edelstein *et al.* 1972, 1973). It is also known to bind to VDR, although with much lower affinity than 1,25-D (Skowronski *et al.* 1995). However, the presence of higher physiological concentrations of 25-D may result in sufficient binding to VDR to activate vitamin D-responsive genes, such as the gene for TGF-β which may be involved in the progression of TD (Loveridge *et al.* 1993; Farquharson *et al.* 1994). Alternatively, high physiological concentrations of 25-D may induce endogenous synthesis of 1-hydroxylated metabolites that have been shown to be relatively more potent in alleviating TD. If this synthesis occurs, it would have to be at a systemic level because it has been shown that chick growth plate chondrocytes metabolize 25-D to 24,25-D but not to 1,25-D (Farquharson *et al.* 1995).

PRACTICAL SITUATION

There is still much developmental work needed to establish the efficacy of the appropriate nutritional compounds or mixtures for preventing TD and to produce suitable commercial products. However, the increasing amount of information on the roles and effects of different factors affecting TD suggests that practical nutritional means of combating TD are likely to become available in time. Of the vitamin D metabolites, it is probable that 25-D will be more suitable than 1,25-D because of its lower toxicity, but more developmental work needs to be carried out to determine quantitative effects and interactions involving this metabolite. Of course, it will also be important to establish the overall consequences for broiler leg health that arise from the prevention of TD. It is hoped that these will be significant, although it is unlikely that leg problems will be banished overnight. There are undoubtedly many factors contributing to the wide range of pathologies seen in broilers, but a solution to the problem of TD should play an important part in improving health, welfare and productivity in broilers. A comparison of the relative effectiveness of feed restriction, meal feeding and use of 1,25-D in improving broiler leg health (shown in Fig. 1) suggests that the last method is potentially the most effective.

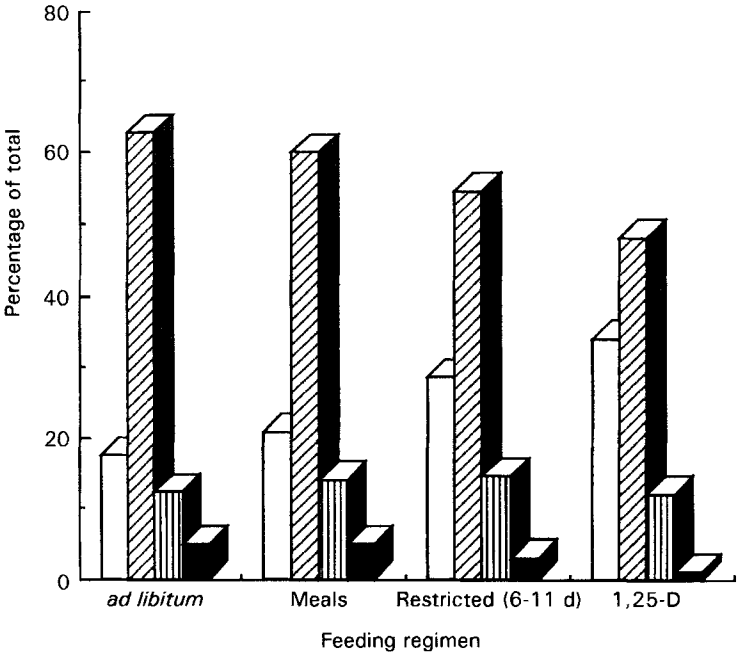

Fig. 1. Distributions of leg abnormality scores, ranging from 0 (normal) to 3 (severe), in broilers at 8 weeks of age fed *ad libitum*, given food in meals, restricted in intake between 6 and 11 d of age or fed on a diet containing 5 μg 1,25-dihydroxycholecalciferol (1,25-D)/kg for the first 3 weeks. (□), score 0; (▨), score 1; (▥), score 2; (■), score 3.

The insights being obtained into the treatment of bone growth disorders in broilers may also have implications for other farm animal species. Turkeys are an obvious possibility, because they are affected with problems that have many similarities to those seen in broilers. Turkeys are susceptible to TD, for instance, although at a later stage in their growth period than broilers. Pigs and sheep also suffer from lameness associated with bone growth abnormalities. The bone growth disorders in broilers have an important genetic component and, in particular, appear to be strongly associated with selection for fast growth. Broilers may thus be further down this selection path than pigs or sheep, so the understanding of the causes and means of prevention of broiler skeletal problems may provide some lessons that can be used to prevent such severe problems arising in other species.

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