Nutrition and Skeletal Problems in Poultry

H. M. Edwards, Jr.

Department of Poultry Science, The University of Georgia, Athens, Georgia 30602-2772

ABSTRACT

Several excellent reviews regarding nutrition and skeletal disorders have appeared in the last 20 yr. This review will cover several areas of vitamin D research, the area of feed deprivation, and bone abnormalities, because there has been considerable interest in these areas during the past 10 yr. Studies indicate that the quantitative requirement for cholecalciferol (D3) for broiler chickens is much greater than previously thought. Ascorbic acid may play a role in stimulating 1-hydroxylation of 25-hydroxycholecalciferol [25-(OH)D3], but the evidence is not clear under exactly what conditions this relationship is important in practical prevention of tibial dyschondroplasia. Studies indicate that dietary supplementation with 1,25-dihydroxycholecalciferol [1,25-(OH)2D3] will reduce the incidence of tibial dyschondroplasia in three different strains of broilers bred to develop a high incidence of the disease. But it did not prevent the disease totally in the strains, unless high enough levels of 1,25-(OH)2D3 were fed to reduce growth rate. These studies indicate that these high tibial dyschondroplasia strains have a defect(s) in vitamin D metabolism. Studies continue to elucidate the role of ultraviolet light in preventing leg abnormalities. Only a few studies have been conducted on the efficacy of various vitamin D3 derivatives to prevent tibial dyschondroplasia. Feed deprivation continues to be an intriguing method of preventing tibial dyschondroplasia, and examination of exactly how this prevents the bone abnormality could open avenues for explaining the disease.

(Key words: skeletal disorders, ascorbic acid, cholecalciferol, 25-hydroxycholecalciferol, 1,25-dihydroxycholecalciferol)

INTRODUCTION

During the last two decades, reviews have summarized information regarding nutrition and skeletal disorders (Pierson and Hester, 1982; Sauveur, 1984, 1986; Leeson and Summers, 1988; DeGroote, 1989; Whitehead, 1989; Edwards, 1991; Leach and Lilburn, 1992; Orth and Cook, 1994). The scientific literature contains evidence that dietary levels of 8 vitamins, 13 elements, and 6 amino acids and protein and energy may be directly involved in leg disorders or skeletal problems in poultry. Several other nonnutrient compounds and elements that may be found in the diet may cause skeletal problems by direct or indirect action in the gastrointestinal tract or in the animal. During the last two to three decades, interest has focused on vitamin D, Ca, P, Cl, Zn, Cu, cystine, cysteine, homocysteine, fatty acids, and total diet intake. During the past 5 to 6 yr, work on vitamin D and its metabolites has been by far the major area of investigation of nutrition-skeletal abnormality interactions. Therefore, this review will cover several of the areas of vitamin D research and the interesting area of food deprivation and bone abnormalities.

VITAMIN D3

Quantitative Requirement for D3

The quantitative requirement for cholecalciferol by young growing chickens is different, depending upon the criteria under evaluation (Edwards et al., 1994). The cholecalciferol requirement was 275 ICU (6.9 µg)/kg for growth, 503 ICU (10.1 µg)/kg for bone ash, 552 ICU (13.8 µg)/kg for blood plasma Ca and 904 ICU (22.6 µg)/kg for rickets prevention. The chickens received a corn-soybean meal diet containing 1.1% Ca and 0.72% total P, and precautions were taken to minimize their exposure to ultraviolet light. In more recent studies to evaluate commercial vitamin D3 products, 1,000 ICU (25 µg) or 1,250 ICU (31.3 µg)/kg was not sufficient vitamin D3 to prevent rickets or produce maximum bone ash (Edwards et al., 1996). When broiler chicks are fed a corn-soybean diet deficient...
in P, a very high level of cholecalciferol (1250 µg/kg) was required to produce maximum growth and bone ash (Baker et al., 1998). However, in their studies when the diet was slightly deficient in Ca or was adequate in both Ca and P, there was very little response to adding more than 30 to 40 µg cholecalciferol per kg of feed. In contrast to this work with diets containing corn and soybean oil meal, studies with diets of purified animal proteins as the only protein sources indicated D₃ requirements for maximum growth (100 ICU/kg), bone ash (200 ICU/kg), and for complete prevention of rickets as less than 400 ICU/kg (Kasim et al., 1996). However, in all of their studies, the birds received a diet containing 400 ICU (10 µg)/kg of diet from 0 to 7 or 8 d before being fed the experimental diets. Cholecalciferol stores in these experiments would be even more important than the amounts present in the day-old chicken. The day-old chicken stores of vitamin D₃-active compounds appears to be very important. For instance, when the dam’s diet contains only 500 ICU (12.5 µg) D₃/kg of diet, the chick cannot reach maximum growth or bone ash when the chick’s diet contains 2,000 ICU (50 µg) D₃/kg (Edwards, 1995).

Very high dietary levels of vitamin A (45,000 IU/kg) and E (10,000 IU/kg) may increase the requirement for cholecalciferol (Aburto and Britton, 1998; Aburto et al., 1998); however, moderate levels of vitamin E (150 IU/kg) do not exacerbate cholecalciferol deficiency in young broiler chickens (Bartov, 1997).

The quality of soybean meal and the amounts and form of cholecalciferol stored in hatching eggs deserve further attention in regards to their possible effects on the quantitative requirements for cholecalciferol by fast-growing broilers.

**Effect of Dietary Ascorbic Acid on Efficacy of D₃ Derivatives**

Ascorbic acid supplementation has been reported to prevent an abnormal cartilage condition resembling tibial dyschondroplasia in willow ptarmigans (Hanssen et al., 1979). However, dietary supplementation with ascorbic acid does not prevent tibial dyschondroplasia in broilers (Leach and Burdette, 1985; Edwards, 1989b). Weiser et al. (1988) reported a synergistic effect of ascorbic acid with 24R,25-dihydroxycholecalciferol [24R,25-(OH)₂D₃] and cholecalciferol on various vitamin D-dependent variables in broiler chickens. Addition of ascorbic acid to the diet resulted in increased duodenal Ca-binding protein and plasma 1,25-dihydroxycholecalciferol [1,25-(OH)₂D₃] that was concluded to be an indication of increased 1-hydroxylase activity. However, Edwards (1989b) observed no ascorbic acid x cholecalciferol interaction on growth, bone ash, or tibial dyschondroplasia in broilers.

Ascorbic acid supplementation of a low-Ca, tibial dyschondroplasia-inducing diet that contained 2 µg/kg of 1,25-(OH)₂D₃ either eliminated tibial dyschondroplasia or further reduced the incidence from the reduction that was obtained from the low level of 1,25-(OH)₂D₃ supplementation (Whitehead et al., 1994). However, in subsequent experiments, ascorbic acid supplementation seemed to play no role in enhancing antibibial dyschondroplasia effects of 1,25-(OH)₂D₃ or 25-hydroxycholecalciferol [25-(OH)D₃] (Rennie and Whitehead, 1996a). Feeding ascorbic acid at 250, 500, or 1,000 mg/kg of diet was not effective in reducing the incidence of tibial dyschondroplasia in the absence or presence of 10 µg/kg of 1,25-(OH)₂D₃ in the diet in several studies with broiler chickens fed a low-Ca, high-P, tibial dyschondroplasia-inducing diet. In these studies, the high level of 1,25-(OH)₂D₃ supplementation did not reduce the incidence of tibial dyschondroplasia to zero so the ascorbic acid could have had an effect.

Cell culture results indicate that ascorbic acid-induced chondrocyte differentiation may be influenced by increased 1,25-(OH)₂D₃ synthesis and receptor numbers (Farquharson et al., 1998). Because chondrocyte receptor numbers are reduced in tibial dyschondroplasia lesions (Berry et al., 1996), it appears likely that ascorbic acid plays a role with 1,25-(OH)₂D₃ at the cellular level, influencing the possible development of tibial dyschondroplasia.

Because dietary supplementation with ascorbic acid results in significant effects in development of tibial dyschondroplasia in some animal studies but not others, ideas must be developed and explored as to which factors are determining when ascorbic acid is effective.

**Efficacy of D₃ Derivatives in Preventing Tibial Dyschondroplasia in Genetic Lines Bred for High and Low Incidence of Tibial Dyschondroplasia**

In their initial description of tibial dyschondroplasia, Leach and Nesheim (1965) reported success in genetically selecting for birds with a high incidence of the anomaly. Other workers (Riddell, 1976; Sheridan et al., 1978) have subsequently confirmed this genetic predisposition to develop tibial dyschondroplasia. Besides the strains developed by Leach and Nesheim, two other research groups have selected strains for a high and low predisposition to develop tibial dyschondroplasia (Sorensen, 1991; Wong-Valle et al., 1993). Both of the lines were recently developed through the use of the Lixiscope to select for high and low incidence of tibial dyschondroplasia in populations (Bartels et al., 1989).

By using lines developed in Denmark by Sorensen (1991), it was observed that supplementation of the diet with 5 or 10 µg/kg of 1,25-(OH)₂D₃ reduced the incidence of tibial dyschondroplasia in the high line to 21 and 0%, respectively, from an incidence of 63% in the high-line chickens fed the basal diet with no supplemental 1,25-(OH)₂D₃ (Thorpe et al., 1993). The supplementation of the diets with 10 µg/kg of 1,25-(OH)₂D₃ did result in a slightly reduced growth rate of chickens receiving the 1,25-(OH)₂D₃. With the Pennsylvania State University line of birds predisposed to develop tibial dyschondroplasia, supplementation of the diet with 5 or 10 µg/kg of 1,25-(OH)₂D₃ has been shown to reduce the incidence of tibial
dyschondroplasia in the high-line birds (Xu et al., 1997). However, 15 µg/kg of 1,25-(OH)2D3 supplementation was required to completely prevent the development of tibial dyschondroplasia, which caused slower growth of the birds. These changes in tibial dyschondroplasia incidence occurred even though there was no effect on serum 1,25-(OH)2D3 levels when 5 µg/kg of 1,25-(OH)2D3 was fed. Studies conducted at the University of Georgia with high tibial dyschondroplasia (HTD) and low tibial dyschondroplasia (LTD) lines developed at Auburn University (Wong-Valle et al., 1993) confirm the overall conclusion of the two above studies. This conclusion was, mainly, that tibial dyschondroplasia is a result of incomplete differentiation of the transitional chondrocytes and is associated with a subnormal ability to metabolize vitamin D in chicks selected for a predisposition to develop tibial dyschondroplasia. However, the studies with the Auburn lines (Mitchell et al., 1997a,b) indicate that neither vitamin D metabolites nor ultraviolet light is effective in preventing tibial dyschondroplasia in HTD chickens but that altered vitamin D metabolism does exist between HTD and LTD chickens. Low-level supplementation (5 µg/kg) with 1,25-(OH)2D3 was effective in reducing the incidence of tibial dyschondroplasia in the HTD line, but the incidence was still very high (Mitchell et al., 1997b). When graded levels of 1,25-(OH)2D3 were fed at 0, 5, 10, and 15 µg/kg of diet, the incidence of tibial dyschondroplasia in the HTD line birds decreased, but the growth rate also decreased, and there was a significant positive linear relationship (r = 0.82) between the incidence of tibial dyschondroplasia and body weight. Plasma 1,25-(OH)2D3 and intestinal vitamin D receptors were not affected by dietary 1,25-(OH)2D3; however, plasma 25-(OH)D3 was decreased by supplementation with 1,25-(OH)2D3. When the two lines were fed diets containing 0.75% Ca and 1,100 IU (27.5 µg) cholecalciferol/kg and then exposed to ultraviolet light, the incidence of severe tibial dyschondroplasia was reduced in the HTD line from 64 to 50% incidence and in the LTD line from 33 to 6% incidence (Mitchell et al., 1997a). In another experiment, increasing levels of cholecalciferol at 0, 200, 400, 800, and 1,600 IU (0, 5, 10, 20, and 40 µg/kg) of diet produced a significant linear response in the incidence of severe tibial dyschondroplasia in both lines. With the LTD line, this experiment produced a high incidence of severe tibial dyschondroplasia associated with very good bone calcification (40.1% bone ash). A similar study with the same graded levels but with the supplement being 25-(OH)D3 added to the diet in addition to 1,100 IU (27.5 µg) cholecalciferol/kg resulted in completely preventing severe tibial dyschondroplasia in the LTD line but had no effect on the birds of the HTD line. Of four vitamin D derivatives tested for efficacy to prevent tibial dyschondroplasia, 25-(OH)D3, 1,25-(OH)2D3, 24R,25-(OH)2D3, and 1α-hydroxycholecalciferol (1α,25-(OH)2D3), all except the 24R,25-(OH)2D3 were effective in preventing the anomaly in the LTD line birds, but none of them was very effective in reducing the incidence of tibial dyschondroplasia in the HTD line birds. There are certain differences in the HTD and LTD of the Auburn strains; the LTD birds have higher bone ash and higher plasma P levels, and the plasma levels of 25-(OH)D3 and 1,25-(OH)2D3 are equal to or lower than the levels in the HTD birds.

Even though the supplementation of the diet of the Auburn HTD line with 1,25-(OH)2D3 did not completely prevent occurrence of tibial dyschondroplasia as it did with the lines developed by Sorensen and the Pennsylvania State line, the data indicate that these HTD line birds have a defect in vitamin D metabolism. Whereas part of the effect of 1,25-(OH)2D3 supplementation may result in failure to absorb and transport Ca and P, there appears to be a defect in chondrocyte differentiation. The high incidences of tibial dyschondroplasia in the Auburn LTD line in some of the experiments but not others indicates some uncontrolled variable that is of great importance in the expression of this disorder. From these studies, using these genetic lines, it is clear that it is not inherited.

**Ultraviolet Light**

Experiments were conducted to determine the effect of removing all sources of ultraviolet light while feeding different levels of cholecalciferol on the incidence and severity of tibial dyschondroplasia in broilers fed either a low (0.65%) or adequate (0.95%) level of dietary Ca (Edwards et al., 1992). The results demonstrated that ultraviolet light decreased the incidence and severity of tibial dyschondroplasia when diets contained 0.95% Ca and 200 or 2,000 IU (5 or 50 µg) D3/kg. These results could indicate that cholecalciferol synthesized in the animal is more active than that given orally in the feed. Later studies indicated that the ultraviolet irradiation provided the equivalent of 20 to 40 µg/kg of D3 in the feed (Edwards et al., 1994). Further studies showed that when young, rapidly growing broilers are fed diets adequate in Ca and P and are not exposed to fluorescent lights, they will have a high incidence of tibial dyschondroplasia that cannot be reduced by feeding 10 times the National Research Council’s (1994) recommended level of cholecalciferol. However, exposure to ultraviolet light or feeding 1,25-(OH)2D3 completely prevented the development of tibial dyschondroplasia (Elliot and Edwards, 1997). When broiler chickens were receiving a diet containing 1,100 IU (27.5 µg) cholecalciferol/kg, 1.0% Ca, and 0.7% total P, exposure to ultraviolet light significantly decreased the incidence of tibial dyschondroplasia in chicks selected for a low incidence of tibial dyschondroplasia but had no effect on the chicks selected for a high incidence of tibial dyschondroplasia (Mitchell and Edwards, 1997b).

These studies raise more questions than they answer. The amounts of D3 synthesized by specific ultraviolet treatments needs to be determined, as well as basic studies on the transportation and storage of D3 synthesized from 7-dehydrocholesterol vs. the D3 absorbed from the diet.
Efficacy of Various Derivatives for Preventing Rickets and Tibial Dyschondroplasia 1,25-Dihydroxycholecalciferol


The effectiveness of the 1,25-(OH)2D3 compound in preventing the development of tibial dyschondroplasia in broilers has been confirmed (Edwards et al., 1992; Rennie et al., 1993, 1995, Thorpe et al., 1993; Roberson and Edwards, 1994). Studies have indicated that broilers are tolerant to 1,25-(OH)2D3 supplementation with diets containing low dietary Ca concentrations, but that they become much more sensitive to 1,25-(OH)2D3 as Ca concentrations approach 1% in the diet (Edwards et al., 1992; Rennie et al., 1995). Studies from this same laboratory (Farquharson et al., 1993) present results that suggest that 1,25-(OH)2D3 supplementation does not increase the rate of chondrocyte proliferation but accelerates the onset of maturation. Studies also indicate that supplementation with 1,25-(OH)2D3 may decrease the incidence of rickets and decrease the necrotic dyschondroplastic lesion in turkeys (Sanders and Edwards, 1991). Several studies have been conducted that demonstrate that broilers reared on litter to market age (5 to 6 wk) have reduced leg problems and superior performance when the diets were supplemented with 3, 5, or 6 µg/kg of 1,25-(OH)2D3 (Elliot et al., 1995; Roberson and Edwards, 1996). In most of the trials, supplementation with 1,25-(OH)2D3 decreased the incidence and severity of tibial dyschondroplasia and increased the bone ash. The results of studying six broiler flocks in Australia indicate that flocks with a high incidence of endochondral ossification defects have significantly lowered bone ash and plasma 1,25-(OH)2D3 concentrations compared with mildly affected flocks. Their results indicated an impaired ability of the young broilers to produce 1,25-(OH)2D3 (Vaino et al., 1994).

25-Hydroxycholecalciferol

Supplementation of the diet with 25-(OH)D3 caused a significant reduction in the incidence and severity of tibial dyschondroplasia, but the effects were not of the magnitude as those effects caused by 1,25-(OH)2D3 supplementation (Edwards, 1989a). The effects of 25-(OH)D3 supplementation were not as consistent among experiments as the effects observed from 1,25-(OH)2D3.

Two papers appeared in 1995 (Yarger et al., 1995 a,b) and indicated that 25-(OH)D3 could be safely used in place of cholecalciferol in commercial broiler rations. These studies used rather high levels of 25-(OH)D3 in the diets (69, 207 and 690 µg/kg). They concluded that 25-(OH)D3-supplemented birds grew faster and had improved feed efficiency. Only when fed at 10 to 20 times the level fed the basal group (69 µg/kg) did birds exhibit some signs of toxicity.

Several studies have indicated that 25-(OH)D3 supplementation of the diet may alleviate the incidence and severity of tibial dyschondroplasia (Rennie and Whitehead, 1996a,b; Mitchell et al., 1997a; Zhang et al., 1997). However, one report appeared indicating that 25-(OH)D3 supplementation does not prevent tibial dyschondroplasia in broiler chickens (Roberson, 1999). In the studies where 25-(OH)D3 supplementation prevents tibial dyschondroplasia, it was usually added to the diet at fairly high levels, 75 and 250 µg/kg in the studies in the United Kingdom (Rennie and Whitehead, 1996a) and 68.9 and 344.5 µg/kg in the studies in the U.S.A. (Zhang et al., 1997). However, significant decreases in the incidence of tibial dyschondroplasia was obtained with as little as 5 µg/kg of 25-(OH)D3 in the other U.S.A. studies (Mitchell et al., 1997a).

1,25-Dihydroxy-16-ene-23yne cholecalciferol (RO 23-7553)

This D3 analog has been shown to increase chondrocyte differentiation and antiproliferative effects in certain cell cultures (Zhou et al., 1989). When it or 1,25-(OH)2D3 was given to chicks as an oral dose (300, 400, or 500 mg/d for Week 1, 2, or 3, respectively), only the 1,25-(OH)2D3 reduced the percentage of birds with tibial dyschondroplasia (Farquharson et al., 1996). This compound has tremendous activity in inhibiting proliferation and inducing terminal differentiation of cultured human keratinocytes, while having minimal activity in enhancing intestinal absorption or bone mobilization (Chen et al., 1993).

FEED DEPRIVATION

The length of time each day that feed is available to young broiler-type chickens significantly influences the development of skeletal abnormalities (Edwards and Sorensen, 1987; Elliot and Edwards, 1994; Su et al., 1999). The studies by Edwards and Sorensen were stimulated by unpublished studies in which they observed that light restriction and feeder space restrictions were really imposing a feed deprivation of the broiler, and so they conducted experiments to test this hypothesis. Two- or four-hour feed deprivation did not significantly reduce the development of tibial dyschondroplasia, whereas 8- or 10-h deprivation during the night or day was effective in reducing tibial dyschondroplasia. Feed deprivation for 8 h/d, every other day or every fourth day was effective in reducing the incidence of tibial dyschondroplasia. This effect of feed deprivation on development of tibial dyschondroplasia was shown to be true in three different commercial broiler strains (Elliot and Edwards, 1994). Eight h feed deprivation decreased the incidence and severity of tibial dyschondroplasia when diets contained both adequate and high levels of Ca. The higher dietary
Ca levels also decreased tibial dyschondroplasia, but there was no interaction between feed deprivation and Ca levels (Roberson et al., 1993). The effects of meal feeding (2, 3, or 4 times per d) were studied by Su et al. (1999). All of the meal feeding treatments reduced tibial dyschondroplasia and gait scores as compared to birds fed ad libitum. Because all of the meal-fed birds had at least one 8-h restriction between two meals, the results and development of tibial dyschondroplasia were predictable from the previous studies of Edwards and Sorensen (1987). In the studies of Su et al. (1999), all of the feeding treatments caused a significant decrease in growth; however, when the data were adjusted for body weight, the meal feeding produced less tibial dyschondroplasia in the birds.

In the study of Edwards and Sorensen (1987), feed deprivation did not affect plasma total Ca, ultrafilterable Ca, dialyzable P, or growth hormone levels. A physiological basis for the dramatic effect of feed deprivation on the development of tibial dyschondroplasia needs to be discovered and elucidated.

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