# The effects of particulate limestone and 25-hydroxy-cholecalciferol in broiler chicken diets on the mechanical properties of bones

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#### ABSTRACT

In an experiment carried out on 560 broiler chickens, the effect of supplementing diets with particulate limestone (grit, diameter 1.2-4 mm) and 25-hydroxycholecalciferol (25-OH-D<sub>3</sub>) on selected biomechanical parameters of tibia bones was studied. Replacement of 30% of pulverized limestone by grit increased ultimate load, yielding load and stiffness of tibias at 43 days of age. Partial or complete substitution of 25-OH-D<sub>3</sub> for cholecalciferol positively affected yielding load and stiffness of bones. The best quality of tibia bones was found in chickens fed a diet with simultaneous addition of grit and replacement of 90 or 100% of cholecalciferol by 25-OH-D<sub>3</sub>.

KEY WORDS: chickens, grit, 25-hydroxycholecalciferol, bone parameter

# INTRODUCTION

Bone abnormalities in heavy, rapidly growing broilers are becoming a serious problem, causing economic losses and impairing the welfare of birds. Nutrition is one of the main factors affecting bone quality. Calcium, phosphorus and vitamin  $D_3$  are nutrients necessary for normal bone growth and development. In studies on broiler chickens, the particle size of calcium carbonate significantly affected performance, Ca and P retention and bone characteristics (Guinotte et al., 1991; Zahravi, 2002). Vitamin  $D_3$  is generally added to diets in the form of cholecalciferol, but in order to carry out its physiological function, it must be hydroxylated in a two-step process: in the liver (to 25-OH- $D_3$ ) and in the kidney (to 1, 25-OH- $D_3$ ). The high incidence of tibial dyschondroplasia (TD) reported in commercial broilers (Whitehead, 1995) may suggest that this process is not

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sufficient. Birds fed diets supplemented with 25-OH-D<sub>3</sub> had a greater body weight and bone ash at 21 and 42 days of age and significantly lower incidence and severity of TD (Yarger et al., 1995; Frits and Waldroup, 2003).

The aim of this study was to evaluate the effect of particulate limestone and 25-OH-D<sub>3</sub> supplementation of the diet for broiler chickens on selected biomechanical characteristics of tibia bones.

## MATERIAL AND METHODS

The experiment was carried out on 560 Cobb chickens, kept in cages on a mesh floor, from 1 to 43 days of age. The broilers were divided to 14 groups with 5 replicates, 8 birds each (4 male and 4 female). The experiment consisted of a  $5 \times 2$  factorial arrangement with two forms of limestone and seven vitamin D<sub>3</sub> and 25-OH-D<sub>3</sub> ratios in the diet. Maize and soyabean meal based starter (day 1-21) and grower-finisher (day 22-43) diets with a standard level of all nutrients contained pulverized limestone (0.9% of the diet) or a mixture of 70% of pulverized and 30% of particulate (1.4-4 mm diameter) limestone as a source of calcium. Both forms of limestone were mined from Lower Carbon layers of compact limestone of organic origin in Czatkowice near Kraków. Both types of diets were supplemented with 2000 (starter) or 1500 (grower-finisher) I.U. of vitamin D<sub>3</sub> (cholecalciferol) and 25-OH-D<sub>3</sub> (Hy-D Beadlet, DSM) was substituted for cholecalciferol in amounts equivalent to 0, 20, 40, 60, 80 and 100% this component.

At the end of the experiment (43 days of age), 8 birds (4 male and 4 female) from each group were sacrificed by decapitation. The tibias from both legs were prepared, cleaned of soft tissues, weighed and stored frozen (-25°C) until analysis. The biomechanical properties of bones (maximum load, yielding load and stiffness) in the three-point bending test were determined using an Instron 5542 Testing Machine (a constant speed of head, 10 N/min, distance between supports, 50 mm). The obtained data were subjected to statistical analysis using two-way factorial analysis of variance. The significance of differences between means was determined by Duncan's multiple range test (Statistica 5.0 PL).

#### RESULTS

Addition of grit to the diet had a beneficial effect on tibia bone quality (Table 1). Replacement of 30% of pulverized limestone by grit increased the ultimate load by 6.1% (P $\leq$ 0.05), yielding load by 10.7% (P $\leq$ 0.05), and stiffness by 6.3% (P $\leq$ 0.01).

Substitution of 25-OH-D<sub>3</sub> for cholecalciferol positively affected bone yielding load and stiffness. The yielding load in chickens fed the diets with 90 or 100% substitution of vitamin D<sub>3</sub> by 25-OH-D<sub>3</sub> was 18.2 and 15.4% higher, respectively,

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than in chickens fed the diet without 25-OH-D<sub>3</sub> (P $\leq$ 0.05). In the case of stiffness, all levels of vitamin D<sub>3</sub> replacement by 25-OH-D<sub>3</sub> significantly increased value of this parameter (P $\leq$ 0.05), on average by 14.6%.

In general, the best quality of tibia bones (the highest ultimate load, yielding load and stiffness) was obtained in birds fed the diet simultaneously supplemented with grit and 25-OH-D<sub>3</sub> (80 or 100% substitution of vitamin D<sub>3</sub>).

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% of 25- OH-D, in	Ultimate N load			Yielding load N			Stiffness N·mm <sup>-1</sup>		
total added	% of grit in total added calcium carbonate								
vitamin D <sub>3</sub>	0	30	mean	0	30	mean	0	30	mean
0	384	390	387	247	247	247 <sup>x</sup>	127	136	131 <sup>x</sup>
20	394	427	409	260	288	274 <sup>xy</sup>	140	157	148 <sup>y</sup>
40	410	390	401	269	270	269 <sup>xy</sup>	148	154	151 <sup>y</sup>
50	389	429	409	269	295	282 <sup>xy</sup>	147	155	151 <sup>y</sup>
60	410	417	414	267	285	276 <sup>xy</sup>	150	143	147 <sup>y</sup>
80	385	438	411	261	324	292 <sup>y</sup>	146	160	153 <sup>y</sup>
100	386	436	411	251	318	285 <sup>y</sup>	144	158	151 <sup>y</sup>
Mean	394ª	418 <sup>b</sup>		261ª	289 <sup>b</sup>		143ª	152 <sup>b</sup>	
SEM	4.37			4.70			1.90		
Effect of:									
Ca source	**			**			*		
vitamin D <sub>3</sub>	NS			*			*		
interaction	NS			NS			NS		

Table1. Effect of grit and 25-OH-D<sub>3</sub> on the mechanical properties of tibia bones

<sup>a,b</sup> - values in the rows with different letters differ significantly ( $P \le 0.05$ ), <sup>x,y</sup> - values in the columns with different letters differ significantly ( $P \le 0.05$ ), NS - P > 0.05; \* -  $P \le 0.01$ 

#### DISCUSSION

In this experiment, partial replacement of finely ground limestone by grit positively affected the biomechanical parameters of bones. Similar results were obtained by Zahravi (1991), who noted that use of medium (0.6-1.18 mm) or coarse (1.18-4.75 mm) limestone significantly improved tibia ossification characteristics (as compared with finely ground calcium). Our findings are, however, in contrast to the study of Guinotte et al. (1991), who found a negative effect of coarse particle limestone (>1.18 mm) on ash percent and mechanical parameters of tibias. The probable reason for these differences was that they used a higher substitution level of ground limestone by grit (100%) than in our experiment (30%).

The beneficial effect of adding 25-OH-D<sub>3</sub> to the diet on bone quality observed in this experiment indicates that hydroxylation of cholecalciferol to 25-OH-D<sub>3</sub> in the liver may be not effective enough for bone development in fast-growing young chickens. Yarger et al. (1995) suggested that absorption or liver hydroxylation of vitamin D<sub>3</sub> may

be impaired in broilers raised under stressful conditions, such as high bird density, heat stress, some diseases or immune disorders. In a study on chickens and turkeys, Bar et al. (1980) found that absorption of 25-OH-D<sub>3</sub> is greater than vitamin D<sub>3</sub>. Similarly as in our study, a positive influence of 25-OH-D<sub>3</sub> on bone quality and prevention of TD was observed by Rennie and Whitehead (1996) and Fritts and Waldroup (2003).

#### CONCLUSIONS

Replacement of 30% of pulverized limestone in the diet by grit had a beneficial effect on the biomechanical parameters of tibia bones in chickens. Partial or complete substitution of 25-OH-D<sub>3</sub> for cholecalciferol increased the yielding load and stiffness of tibias. The results of the experiment indicated that simultaneous addition of particulate limestone and 25-OH-D<sub>3</sub> to the diet could improve bone quality in broiler chickens.

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#### STRESZCZENIE

## Wpływ żwiru wapiennego i 25-OH-D $_{\rm 3}$ w paszy na jakość kości kurcząt rzeźnych

W doświadczeniu przeprowadzonym na 560 kurczętach rzeźnych Cobb badano wpływ wprowadzenia do diety węglanu wapnia o dużej średnicy cząsteczek (żwir, 1.2-4 mm) i aktywnej formy witaminy  $D_3$  (25-OH- $D_3$ ) na wybrane parametry biomechaniczne kości piszczelowych. Zastąpienie 30% miałkiej kredy paszowej przez żwir istotnie zwiększyło wytrzymałość, elastyczność i sztywność kości w 43 dniu życia kurcząt. Częściowe lub całkowite zastąpienie w diecie cholekalcyferolu przez 25-OH- $D_3$  miało korzystny wpływ na elastyczność i sztywność kości. Najlepszą jakość kości piszczelowych stwierdzono w przypadku jednoczesnego dodania do paszy żwiru i zastąpienia witaminy  $D_3$  w 90 lub 100% przez 25-OH- $D_3$ .

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