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The effects of dietary phytase and $1,25(OH)_2D_3$ on growth and strength of bones in broiler chickens at day 21 of life

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ABSTRACT

The investigation was carried out on 90 broiler chickens receiving experimental low-phosphorus starter diets until day 21 of life: C, control; D, with the addition of 750 phytase units/kg; and R, without vitamin D_3 supplemented with 3 μg $1,25(OH)_2D_3$ and 750 PTU/kg. On day 21, 15 chickens from each treatment were killed. Humeri and femora were isolated, weighed and physical and geometrical parameters of bones were determined. Bone mass and length in both experimental groups were similar, but showed greater values in relation to controls. The femora and humeri of control and experimental chickens showed significant differences in physical parameters. The values of geometrical parameters in the experimental group were higher than in the control, but not significantly in either of the experimental groups. Bone breaking strength was significantly affected by the combined addition of $1,25(OH)_2D_3$ and phytase to the diet without vitamin D_3 as compared with the diet with phytase and adequate level of vitamin D_3.

KEY WORDS: chickens, phytase, 1,25-dihydroxycholecalciferol

INTRODUCTION

About two-thirds of phosphorus in plant feed is in the form of phytates. Poultry and pigs do not have enzymes capable of hydrolyzing phytates. The use of microbial phytase can effectively increase digestibility of phosphorus from phytate (Perney et al., 1993; Qian et al., 1996). Phosphorus and calcium are needed for optimal growth and development of chondral and osseous tissues. The control of absorption, transportation and deposition of calcium and phosphorus, important for normal bone mineralization, is the main function of vitamin D_3 and its metabolites. Vitamin D_3 must be provided in the diet for broiler chickens to prevent rickets and other skeletal problems and to optimize growth. Vitamin D_3 must undergo two-step hydroxylation to produce 1,25-dihydroxycholecalciferol, the biologically active hormonal form, in order to carry out its physiological functions. Some conditions may impair absorption or hydroxylation of vitamin D_3. Furthermore, some authors have indicated that 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol are two to four times as active as vitamin D_3 (Aburto et al., 1998). This provides the rationale for the use of $1,25(OH)_2D_3$ in broiler diets.

The aim of the present study was to evaluate the effects of microbial phytase and $1,25(OH)_2D_3$ supplementation of the diet on growth and development of bones in broiler chickens.

MATERIAL AND METHODS

The investigation was carried out on 90 broiler chickens. From the first day of life until day 21, the chickens received an experimental low-phosphorus starter diet (calculated non-phytate P, 0.343%; Ca, 0.718%). The chickens were divided into 3 groups: C, the control group fed on the vitamin D_3 - adequate diet; group D, fed on the diet with the addition of vitamin D_3 on an adequate level plus 750 phytase units (PTU)/kg (Natuphos 5000, BASF, Germany), and group R, fed on the diet without vitamin D_3 but supplemented with 3 μg $1,25(OH)_2D_3$ and 750 PTU/kg. The chickens were kept in standardized zoohygienic conditions. Feed and water were available *ad libitum* throughout the experimental periods.

On day 21, 15 chickens from each treatment were randomly selected and killed by cervical dislocation. Humeri and femora were isolated and frozen for further mechanical and geometrical analysis. The bones were later stripped of all soft tissues. Length and weight of bones were measured. Physical bone characteristics were determined by the three-point bending test using an Instron Testing Machine (Model 4302, Instron Corp., USA) (1kN 10 mm/min). The following parameters were measured: ultimate stress, elastic force, and stiffness. After the mechanical test, structural parameters were measured: second moment of inertia, cross section area, mean relative wall thickness.

The results were analyzed statistically (Anova), using Statistica Software, version 5.

RESULTS

The weight and length of the analyzed bones are presented in Table 1. On day 21 of life, birds fed the diet with $1,25(OH)_2D_3$ and phytase had significantly higher femur and humerus ultimate stress and elastic force than the chickens fed on vita-

TABLE 1

Mean values of weight and length of bones (mean ±SE)

Parameters	Femur			Humerus		
	C[1]	D[2]	R[3]	C	D	R
Weight	$4.32^a \pm 0.121$	$5.32^b \pm 0.158$	$5.26^b \pm 0.148$	$3.27^a \pm 0.076$	$3.67^b \pm 0.075$	$3.84^b \pm 0.104$
Length	$5.31^a \pm 0.043$	$5.74^b \pm 0.045$	$5.74^b \pm 0.042$	$4.91^a \pm 0.03$	$5.26^b \pm 0.045$	$5.24^b \pm 0.043$

[a,b] mean values in rows marked with different letters are significantly different, $P<0.05$

[1] control diet

[2] diet supplemented with vitamine D_3 and phytase

[3] diet supplemented with 1,25-dihydroxycholecalciferol and phytase

TABLE 2

Mean values of stiffness and geometrical parameters of bones (mean ±SE)

Parameters	Femur			Humerus		
	C	D	R	C	D	R
Stiffness	$20.56^a \pm 1.94$	$60.59^b \pm 3.849$	$70.04^b \pm 3.38$	$22.7^a \pm 1.38$	$62.54^b \pm 3.274$	$62.6^b \pm 4.746$
Second moment of inertia, mm^4	$57.14^a \pm 4.24$	$105.92^b \pm 7.86$	$97.32^b \pm 9.82$	$22.22^a \pm 1.49$	$35.11^b \pm 1.861$	$38.12^b \pm 2.95$
Cross section area, mm^2	$20.36^a \pm 0.621$	$27.05^b \pm 1.08$	$27.29^b \pm 1.33$	$13.73^a \pm 0.68$	$17.09^b \pm 0.556$	$17.97^b \pm 0.935$
Mean relative wall thickness	0.88 ± 0.043	0.87 ± 0.053	0.94 ± 0.053	1.01 ± 0.097	1.01 ± 0.035	1.04 ± 0.06

[a,b] – as in Table 1

C, D, R – as in Table 1

min D_3 only or those fed on vitamin D_3 and phytase (Figure 1). This was not accompanied by significantly different values of geometrical parameters for these bones among experimental chickens (Table 2). The geometrical parameters of bones in experimental chickens were greater than in the control group (Table 2). The strength of bones in chickens fed on the diet with phytase and an adequate level of vitamin D_3 were also higher than in the control group. The stiffness of the femora and humeri in both experimental groups did not show significant differences, but was greater than in the control group (Table 2).

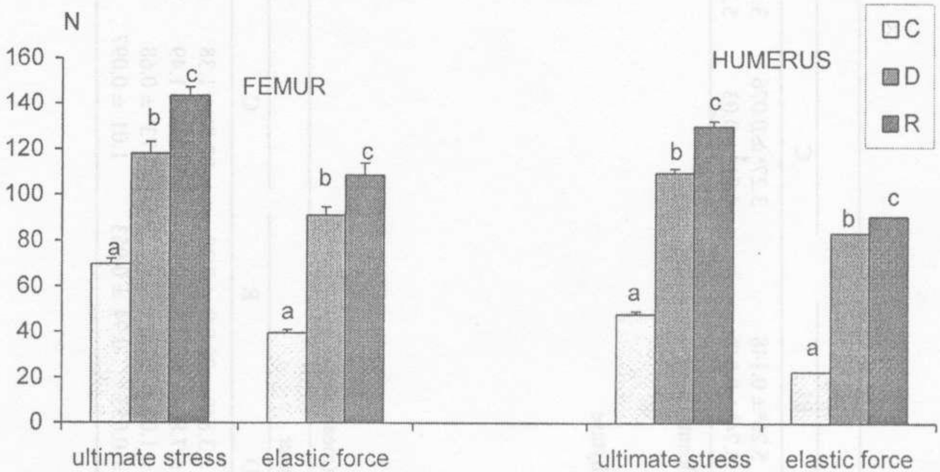

Figure 1. Physical parameters of bones (N-Niuton). Means marked with different letters are significantly different (P<0.05). C, D, R – as in Table 1

DISCUSSION

Bone weight and length were not significantly different in experimental groups, but greater than in the control group. In contrast, Frost et al. (1990) reported that $1,25(OH)_2D_3$ may have an effect on increasing bone mass in laying hens. Values of physical parameters of bones in chickens fed on the diet with the addition of phytase were higher than in the control, which is in agreement with reports of other authors (Perney et al., 1993; Qian et al., 1996; Puzio et al., 2000). In the present study, $1,25(OH)_2D_3$ was more effective on the mechanical properties of bones than vitamin D_3. These results suggest that short term $1,25(OH)_2D_3$ supplementation of a diet without vitamin D_3 will improve the strength of bones. Lack of significant differences among geometrical parameters suggests that these differences in ultimate stress and elasticity among groups may be a result of changes in the mineralization of the bone matrix and probably are not due to differing amounts of bone material. This is not consistent with the reports of other authors (Ferretti et al., 1993; Turner and Burr, 1993; Puzio end Studziński, 2000), who claim that the strength of bones is due to both their mineralization and structural parameters.

CONCLUSION

On day 21 of life, bone breaking strength was significantly affected by combined addition of $1,25(OH)_2D_3$ and phytase to the diet without vitamin D_3 as compared with the diet with phytase and an adequate level of vitamin D_3.

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STRESZCZENIE

Wpływ dodatku fitazy i 1,25$(OH)_2D_3$ na wzrost i wytrzymałość kości kurcząt brojlerów w 21 dniu życia

Badania przeprowadzono na 90 kurczętach brojlerach otrzymujących do 21 dnia życia niskofosforową paszę doświadczalną: grupa C – kontrolna, D – z dodatkiem fitazy w ilości 750 PTU/kg, R – bez witaminy D_3, lecz z dodatkiem 750 PTU/kg i 3 mg 1,25$(OH)_2D_3$. W 21 dniu życia ubito po 15 kurcząt z każdej grupy i izolowano kości udowe i ramienne. Oceniano ich masę i długość oraz parametry fizyczne i geometryczne. Masa i długość kości kończyn kurcząt doświadczalnych były podobne i większe niż kurcząt z grupy kontrolnej. Wartości parametrów fizycznych kości udowych i ramiennych kurcząt kontrolnych i doświadczalnych różniły się istotnie statystycznie. Parametry geometryczne analizowanych kości w grupach doświadczalnych miały większe wartości w porównaniu z kontrolą, lecz nie stwierdzono istotnych różnic pomiędzy grupami doświadczalnymi. Większy wpływ na wytrzymałość mechaniczną kości kurcząt miał dodatek fitazy i 1,25$(OH)_2D_3$ do paszy bez witaminy D_3 niż stosowanie dodatku samej fitazy do paszy o odpowiedniej zawartości witaminy D_3.