EFFECTS OF PHYTASE SUPPLEMENTATION ON THE MORPHOMETRIC AND BIOMECHANICS CHARACTERISTICS OF FEMUR AND HUMERUS IN BROILERS¹

D. Vitorović, Vera Radović, I.Rajić, Ivana Adamović²

Abstract: A study was conducted to determine the effect of dietary supplementation of phytase on bones morphometric and biomechanics parameters in broiler chickens. Total of 220 Arbor Acres chickens divided into two groups (110 chickens per group) were reared to 42, days of age and fed the diets of the same composition, with Dicalcium phosphate as only source of inorganic phosphorus. First group was control (without phytase supplementation). Second group was experimental (diets contained 0.1 % phytase and twofold lower level of Dicalcium phosphate). The right femur and humerus were taken at the end of rearing period and analyzed.

Supplementing a low dicalcium phosphate diets with phytase resulted in beneficial effect on bone structure and quality. The values of cross sectional diaphyseal cortical area, of investigated bones, were greater and medullar area were smaller in chickens of experimental group compared to average values of control group, but the differences were not significant (p < 0.05). Breaking force and stress of femur (37.2 kg ; 483.6 kg/mm²) and hummers (49.8 kg; 1108.9 kg/mm^2) were significantly higher (p < 0.05 ; p < 0.01) then those of chickens of the control group (femur - 35.6 kg; 456.2 kg/mm² and humerus - 40.8 kg; 816 kg/mm²). In summary these results indicate that phytase supplementation (0.1 %) in the broilers diets with lower concentration of dicalcium phosphate was, at least, effective in maintaining morphometric parameters and strength of bones.

Key words: broilers, phytase, bones

Introduction

The phosphorus is essential element for maintaining vital functions of animals. The satisfying of phosphorus requirements is necessary for optimal growth, fertility and bone development. About 80 % of the animal's phosphorus is located in the skeleton with calcium in the hidroxy-apattite complex, where it acts not only as support system but also as a reservoir of phosphorus.

Diets for broilers, especially starter and finisher, are based of feedstuffs of plant origin. One half to three quarters of phosphorus in these feedstuffs, is in the form phytate phosphorus, which is poorly utilized by broilers (Matyuka et al. 1990; Voigt 1992; Eechout and De Paepe 1994). The inability of broilers to utilize phytate phosphorus produce it's dietary deficiencies and as consequences, decreasing growth rate and abnormal bone development. One of the ways for preventing these problems is supplementing the diets with inorganic phosphates. The most common used is Dicalcium phosphate. However, because of its high price, the cost of feed increased.

Nelson et al. (1968), Simons et al. (1990), Kornegay et al. (1996) were established that the microbial phytase is effective in releasing of phytate phosphorus present in maize and soybean meal. Radovic et al. (2003) reported that diets supplemented with phytase produced increased growth rate and body weight for about 1.5 - 3.8%. Kies et al. (2001) obtained positive effects in broiler chicks fed 0.1% phytase in the diet with lower level of inorganic phosphates. There are a few reports (Qian et al. 1996; Gordon and Roland 1998; Punna and Roland 2001) of the effects of the supplemental phytase on the skeletal system of poultry.

The objective of the present study was to investigate the effects of phytase supplementation in broiler diets with different levels of Dicalcium phosphate on the morphometric and biomechanics characteristics of femur and humerus.

¹ Original scientific paper - supported by the Ministry of Science and Technology, Project no. TR6885B - Originalni naučni rad je finansiran od strane Ministarstva za nauku i tehnologiju Projektom broj: TR6885B 2 Dr Dusko Vitorovic, professor, Faculty of Agriculture, Belgrade; dr Vera Radovic, assistant, faculty of Agronomy, Cacak; Dr Isidor

Rajic, professor, Faculty of Veterinary Medicine, Belgrade, Ivana Adamovic, assistant, Faculty of Agriculture, Belgrade

Materials and methods

Total of 220 Arbor Acres chickens, divided into two groups (110 chickens per group),were reared to 42. days of age and fed the three diets: 0-21 days of age – starter diet, 22-37 days of age – grower diet and 38-42 days of age – finisher diet. The composition of the diets is shown in the Table 1.

Table 1. Composition of chickens diets, %

Ingredients	Starter		Grower		Finisher	
	Group		Group		Group	
	I	II	I	II	I	II
Maize	54,25	54,55	53,56	53,72	59,58	59,64
Corn gluten meal	4,00	4,00	-	-	-	-
Soybean meal	27,00	27,00	25,44	25,42	22,09	22,08
Soybeans full fat	_	-	10,00	10,00	10,00	10,00
Yiest	-	-	2,00	2,00	0,50	0,50
Fish meal	7,00	7,00	-	-	-	-
Limestone pulverised	1,10	1,70	0,82	1,46	0,83	1,70
Dicalcium phosphate	2,00	1,00	1,69	0,87	1,66	0,66
Phytase	-	0,10	-	0,10	-	0,10
Salt	0,25	0,25	0,40	0,40	0,40	0,40
Methionin	0,15	0,15	0,18	0,18	0,15	0,15
Soy oil	3,00	3,00	4,90	4,84	3,79	3,77
Vitamin.mneral mixture	1,25	1,25	1,00	1,00	1,00	1,00
Composition						
Dry matter	89,09	89,06	89,60	89,59	89,14	89,14
Crude proteins	22,37	22,40	20,45	20,45	18,50	18,50
Calcium	1,29	1,23	0,85	0,85	0,80	0,80
Total Phosphorus	0,81	0,65	0,69	0,56	0,66	0,50
Phosphorus available	0,53	0,37	0,42	0,29	0,40	0,24
ME, MJ/kg	12,59	12,63	13,39	13,39	13,39	13,39

The differences between groups feeding were in the level of Dicalcium phosphate and phytase supplementing. In the diets of chicks group 1 were not phytase added and Dicalcium phosphate were main source of inorganic phosphorus. The diets of the chicks group 2, were supplemented with 0,1 % phytase and the content of Dicalcium phosphate were twofold lower than in the diets of group 1.

At the end of experimental period fourteen chicks in all groups were sacrificed and the right femur and humerus were taken and immediately frozen. After thawing at the room temperature, bones used for determination of morphometric parameters: length, inside and outside diameters of the diaphysis (anterior-posterior and lateral-medial). On the basis of these data following parameters were calculated: cross sectional diaphyseal area, cross sectional cortical area, cross sectional medullar area, cortical percent and medullar area percent. Biomechanics characteristics (breaking strength and stress) of bones were investigated using three-point test on IPNIS apparatus according to methodology described by *Masic and Pavlovski* (1994) and *Vitorovic et al.* (1995).

Data were analyzed by analysis of variance (Statistica for Windows) and differences between groups were tested by LSD test.

Results and discussion

Obtained results of influences of phytase on morphometric and biomechanics parameters of broilers femur are presented in table 2.

Table 2. Morphometric and biomechanics parameters of femur of broiler chickens

Parameters	Group		
	I	II	
Length, cm	$7,1\pm 0,15$	$7,1 \pm 0,12$	
Cross sectional diaphyseal area, mm ²	$74,3 \pm 8,92$	$73,5 \pm 8,82$	
Cross sectional cortical area, mm ²	$45,7 \pm 5,48$	$47,1 \pm 5,21$	
Cross sectional medullary area, mm ²	$28,6 \pm 3,43$	$26,3 \pm 3,16$	
Cortical%	$60,6 \pm 5,67$	$64,1 \pm 6,23$	
% of Medullary area	$38,5 \pm 4,12$	$35,8 \pm 5,76$	
Breaking strength, kg	$35,6 \pm 3,07$	$37,2 \pm 3,02^*$	
Stress, kg/cm ²	$451,2 \pm 48,3$	$483,6 \pm 42,2^*$	

Mean ± Standard deviation

Phytase supplementation (0,1 %) to the diets of chicks group 2, with twofold lower content of Dicalcium phosphate had no effect on femur length. Cross sectional diaphyseal area, cross sectional cortical area, cross sectional medullar area, cortical and medullar area percentage were not significantly influenced by phytase supplementing. However, cortical area (47.1 mm²) and cortical percentage (64,1%) were higher, while in the same time, medullar area (26.3 mm²) and medullar area percentage (35.8 %) were lower in femur of chicks group 2 compared to the group 1 (45.7 mm²; 60.6 %; 28.6 mm²; 38.5 %, respectively), but these differences were not significant.

The biomechanics parameters of femur were influenced (p <0.05) by phytase supplementing. The addition of phytase (0.1 %) to the diets with twofold lower level of Dicalcium phosphate, increased (p <0.05) breaking force (37.2 kg vs. 35.6 kg) and stress (483.6 kg/cm² vs. 451.2 kg/cm²).

Similar results were obtained for humerus (table 3). Morphometric parameters of humerus were not influenced by phytase supplementing. Absolute and relative values of humerus cross sectional cortical area (27.5 mm²; 61.1 %) of broilers group 2 were higher compared to the group (26.6 mm²; 60.6 %) but these differences were not statistically significant.

Table 3. Morphometric and biomechanics parameters of humerus of broiler chickens

Parameters	<u>Group</u>		
	I II		
Length, cm	$6,9\pm0,13$ $7,0\pm0,07$		
Cross sectional diaphyseal area, mm ²	43.9 ± 5.27 45.0 ± 5.40		
Cross sectional cortical area, mm ²	$26,6 \pm 3,19$ $27,5 \pm 3,22$		
Cross sectional medullary area, mm ²	$17,3 \pm 2,08$ $17,5 \pm 2,80$		
Cortical/%	$60,6 \pm 7,05$ $61,1 \pm 5,57$		
% of Medullary area	$39,4 \pm 3,26$ $38,9 \pm 4,46$		
Breaking strength, kg	40.8 ± 5.31 $49.8 \pm 4.02^{**}$		
Stress, kg/cm ²	$816,0 \pm 148,0 1108,9 \pm 165,6^*$		

Mean ± Standard deviation/

Qian et al. (1996),) reported that tibias of broiler fed with supplemental phytase and inorganic phosphorus were longer and wider than those of broilers fed the P-deficient diets (low levels of inorganic phosphate without phytase). Supplementing the diet with phytase markedly improved tibia breaking strength,

Signifficant differences (p < 0.05)

Signifficant differences $(p < 0.05)^{**}$ Signifficant differences (p < 0.01)

which resulted from better mineralization of bone. Similar results obtained *Gordon and Roland (1998), Punna and Roland (2001).*

In our study the biomechanics parameters of humerus were influenced by phytase supplementing. The addition of phytase (0.1 %) to the diets with twofold lower level of Dicalcium phosphate, markedly increased (p < 0.01) breaking force (37.2 kg vs. 35.6 kg) and (p < 0.05) stress (483.6 kg/cm² vs. 451.2 kg/cm²).

Conclusion

Supplementing a low dicalcium phosphate diets with phytase resulted in beneficial effect on bone structure and quality. The values of cross sectional diaphyseal cortical area, of investigated bones, were greater and medullar area were smaller in chickens of experimental group compared to average values of control group, but the differences were not significant (p < 0.05).

The biomechanics parameters of bones were influenced (p <0.05) by phytase supplementing. The addition of phytase (0.1 %) to the broilers diets with twofold lower level of Dicalcium phosphate, increased (p <0.05) breaking force and stress of femur and humerus.

UTICAJ DODAVANJA FITAZE U ISHRANU BROJLERSKIH PILIĆA NA MORFOMETRIJSKE I BIOMEHANIČKE OSOBINE KOSTIJU

D. Vitorović, Vera Radović, I, Rajić, Ivana Adamović

Rezime

Cilj ovog rada je bio da se ispitaju efekti dodavanja enzima fitaze u smeše za ishranu brojlerskih pilića sa različitim nivoom Dikalacijum fosfata, na morfometrijske i biomehaniče osobine butne i ramene kosti. Ogledom je bilo obuhvaćeno 220 pilića provenijence Arbo Acres, podeljenih u dve grupe (po 110 pilića u grupi). Prva grupa je bila kontrolna, bez dodataka enzima fitaze. Druga grupa je bila ogledna koja je u smešama za ishranu dobijala enzim fitazu (0,1 %) uz dvostruko manju zastupljenost Dikalcijum fosfata.

Na kraju tova (42 dana) izvršeno je žrtvovanje po 14 pilića iz svake grupe. Za morfometrijske i biomehančke analize koršćene su butna i ramena kost sa desnog ekstremiteta od svakog pileta

Dobijeni rezultati su pokazali da se dodatakom enzima fitaze (0,1 %) u smeše za ishranu brojlerskih pilića, uz istovremeno dvostruku manju zastupljenost Dikalcijum fosfata, postižu pozitivni efekti u pogledu kvaliteta koštane građe butne i ramene kosti. Površina kortikalnog dela preseka dijafize, ispitivanih kostiju, bila je veća kod pilića ogledne grupe uz manju površinu preseka međularne šupljine. Sila loma i čvrstoća butne i ramene kosti pilića ogledne grupe (sa dodatkom fitaze i dvostruko manjim udelom Diklacijum fosfata u smešama za ishranu) bile su značajno veće u odnosu na ove vrednosti kostiju pilića kontrolne grupe (bez dodatka fitaze).

Ključne reči: brojleri, fitaza, kosti

References

- 1. EECKHOUT W., DE PAEPE M. (1994): Total phosphorus, phytate phosphorus and phytase activity in plant feedstuffs. Animal Feed Science and Technology, 4: 19-29.
- GORDON R., ROLAND D. (1998): Influence of Supplemental phytase on calcium and phosphorus utilization in laying hens. Poult. Sci., 77: 290-294.
- 3. KIES K., HEMERT VAN F., SAUER W. (2001): Effect of phytase on protein and aminoacid digestibility and energy utilisation. Wrld's Poult.Sci., 57:109-126.
- KORNEGAY T., DENBOW M., RAVINDRAN V. (1996): response of broilers to graded levels of microbial phytase added to maize-soybean-meal-based diets containing three levels of non-phytate phosphorus. Br.J.Nutr.,75:839-852.
- MAŠIĆ B., PAVLOVSKI Z. (1994): Mala jata kokoši nosilja u različitim sistemima držanja. Monografija, Naučni institu za stočarstvo, Beograd-Zemun.

- MATYKA S., KOROT W., BOGUSZ G. (1990): The retention of phytin phosphorus from diets with fat supplementats in broiler chics. Animal Feed Science and Technology, 31:223-230.
- NELSON T., SHIEH T., WODZINSKI R., WARE J. (1968): The availability of phytate phosphorus in 7. soy-bean meal before and after treatment with a mould phytase. Poult.Sci., 41: 1842-1848.
- PUNNA S., ROLAND D. (2001.) Influence of dietary phytase supplementation on incidence and severity in broilers divergently selected for tibial dyschondroplasia. Poult. Sci., 80:735-740.
- QIAN H., VEIT P., KORNEGAY T., RAVINDRAN V., DENBOW M. (1996): Effects of supplemetal phytase and phosphorus on histological and othet tibial bone characteristics and performances of broilers fed semi-purified diets. Poult.Sci., 75: 618-626.
- 10. RADOVIĆ V., RAJIĆ I., STANIĆ D., NADAŽDIN M. (2003): Uticaj različitih izvora fosfora na telesnu masu i prirast pilića u tovu. Biotehnologija u stočarstvu, 19:43-47.
- 11. SIMONS C., VERSTEEGH A., JONGBLOED W., KEMME P., SLUMP P., BOS K., WOLTERS G., BEUDEKER R., VERSCHOOR G. (1990): Improvement of phopshorus availability by micronial phytase in broilers and pigs. Br.J.Nutr., 64:525-540.
- VITOROVIĆ D., POPOVOV-RALJIĆ J., MITROVĆ S. (1995): The effect of age and rearing system on bone strength of broiler chicks. Biotehnologija u stočarstvu, 11:21-26.
- VOIGT H. (1992): Einsatz von phytase im broilermastfutter mit unterscheidlichem phosphorgehalt. Arch.Geflugelk., 56:93-98.