

THE EFFECTIVENESS OF PHYTASE IN BROILER DIETS IN IMPROVING PRODUCTION PERFORMANCES AND BONE FEATURES

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The possibilities of use of microbial phytase in maize/soybean meal diets for broilers from 21 to 42 days of age with different dietary total and available phosphorus (TP and AP) levels were investigated. A 22-day experiment was carried out on 240 Arbor Acres broilers of both sexes divided into six groups. The broilers were given diets with 0.43%, 0.23% or 0.10% AP, with or without phytase supplementation. During the experiment, health status and mortality, physical, chemical and morphological bone characteristics were investigated. Reduction of the mineral source of P from the diet increased mortality and health problems. Addition of phytase decreased mortality and alleviated negative effects of dicalcium phosphate exclusion. Histological, physical and chemical analysis of broiler tibia indicates that the extent and significance of changes depend on P deficiency and phytase addition. Phytase efficacy was greater in diets with a reduced level of dicalcium phosphate.

Key words: broilers, phytase, production performances, tibia

INTRODUCTION

The needs of phosphorus (P) in poultry diet presents a tremendous problem. A considerable number of studies has been carried out in the field of poultry nutrition in order to specify these needs. Several factors affect P needs in broilers. The most important is the composition of the feed mix, the environment, production management, and climate (Rama Rao et al., 1999). The level of phosphorus in the diet, higher or lower than the optimal, can effects the health and production performance of broilers.

The optimal concentration of phosphorus in poultry diet primarily depends on the management conditions and ways of regulating the environmental pollution problem. Excess phosphorus in the diet reduces the availability of the other divalent cation of phytase phosphorus, so the waste from poultry farms is a rich source of nitrogen and phosphorus, and therefore presents a significant pollutant of the environment (Toor et al., 2009). The problem is even more complicated by the increasing trend of using vegetable proteins, that contain

large amounts of phytate phosphorus (Rama Rao *et al.*, 1999). Given the role in energy metabolism (ATP, ADP), phosphorus deficiency in metabolically active tissues causes reduction of intermediary metabolism and synthesis of nucleic acids, or metabolic activity of cells (Kuznetsov *et al.*, 1987). Pronounced long-term deficit causes irregular ossification, structural deformities of bones and occurrence of soft and brittle bones in young (rickets) and adult animals (osteomyelitis), especially in intensive production. The process of bone demineralization (osteoporosis) may be associated with the replacement of bone fibrous tissue (fibrous osteodystrophy). In birds, mineralization disorders caused by phosphorus deficiency relates to the change in the length of DHZ or metaphyseal primary spongiosa (Jandá and Dickson, 1980, Long *et al.*, 1984, Ewing *et al.*, 1995) and low penetration of metaphyseal blood vessels (Wise, 1975).

For young broilers it is more important to provide an adequate amount of phosphorus, because more than the adult birds they use phosphorus of low availability, or phosphorus phytine salt, which is due to lower endogenous activity of intestinal phytase. The level of endogenous phytase activity highly correlates to the body mass of chickens (Shakouri *et al.*, 2009). Intestinal phytase activity on the surface of small-bowel mucosa villi in laying hens and broilers is 5.2, and 3.8 mmol P/min, respectively (Maenz and Classen, 1998). Edens *et al.* (1999) point on the ability of older broilers to use phytine phosphorus by adapting the digestive tract and increasing the activity of intestinal phytase. Similar views have other authors (Edwards, 1993; Van der Klis *et al.*, 1997) who report that the digestibility of phosphorus phytase increases from 31% at the age of 14 days to 38.2% at the age of 25 days.

In commercial poultry production, target animals are broilers from 21 days until they reach slaughter weight. This is the period when modern hybrid broilers consume about 70-80% of the anticipated amount of fattening feed, hence at this period the most positive effects of phytase can be observed and registered.

Nelson (1967) and Nelson *et al.* (1968) were among the first who reported positive effects of adding microbial phytase to the meal. Body weight gain in broilers fed the diet without mineral premix, but given a phytase supplement, was as good as the group of broilers whose diets used dicalcium phosphate as a source of phosphorus. Farrell *et al.* (1993) examined the effect of microbial phytase on P release from mixtures composed of sorghum and soybean meal and achieved satisfactory performance related to the standard diet with dicalcium phosphate.

In trials carried out on broilers 24, i.e. 28 days old, Simons *et al.* (1990) confirmed a positive effect of phytase on growth performance, feed conversion, availability and reduced amount of phosphorus excreted. Harter-Dennis *et al.* (2001) confirmed that reducing the available phosphorus from 0.35% to 0.15% in broiler diet based on corn/soybean meal caused a significant decrease in body weight gain and poor feed conversion, while the use of phytase improved body weight gain and reduced conversion. The authors report that the use of mixtures with different concentrations of AP (0.15 to 0.35% without phytase; 0.15% with phytase) in the diet for broilers aged 21-42 days, the tibia breaking force and ash

content increased linearly in proportion to the content of AP from 24.21 kg to 40.53 kg, and from 45.46% to 53.51%. Supplementing phytase increases the breaking force to 31.17 kg, and tibia ash content to 49.18%. Based on these results the authors believe that the enzyme Allzyme Phytase "saves" AP from 0.05 to 0.075%.

The aim of this paper is to point out the possibilities and benefits of phytase supplement in broiler diets, with a special emphasis on the degree of mineralization.

MATERIAL AND METHODS

Animals. The experiment was performed on 240, 21 day old, Arbor Acres chickens of both sexes, originating from the same parental flock. The broilers were individually labeled by wing-marks. Preventive measures, housing, management, feeding and watering conditions during the experiment were adapted to the ground floor breeding system. Throughout the investigation period the technological conditions were in accordance with the normatives prescribed for this provenience (Arbor Acres Farm, 1997).

Diets. The broilers were divided into 6 groups. Ratio and quantitative share of the feedstuff in feed mixes for experimental broilers was formulated in accordance with different stages of fattening (NCR, 1994). The nutritive matter content in the diets was adjusted to the needs of broilers – starter feed II (from day 21 to 35), i.e. feed mix for the final fattening feed II (from day 35 to 42). The control group (C and C+) of broilers was fed the standard diet.

The content of TP and AP in the diet for the experimental groups was regulated by reducing the concentration of dicalcium phosphate (E-I and E-I+), except the diet for the broilers in the second experimental group (E-II and E-II+) where the total amount of phosphorus comes from raw materials. The diet for the experimental groups (C+, E-I+ and E-II+) was supplemented with "Allzyme Phytase" (supplier: "Alltech", USA). The enzyme is of microbial origin, obtained as fermentation extract of a genetically unmodified strain of *Aspergillus niger*.

Experimental procedure. The experimental broilers had free access to water and diet (*ad libitum*). Preventive measures were applied, and all investigated broilers were subjected to permanent veterinary control, and all health status changes were monitored and recorded. Control measurements of body mass of broilers were performed at the beginning and at the end of the experiment using a technical scale with accuracy rate 10^{-3} kg.

Feedstuff samples for microbiological and chemical assaying were collected immediately before the onset of the experiment, as well as throughout the investigation period. At the 35th day of the experiment, as well as at the end of the experiment (day 42) ten broilers from each group of both sexes were sacrificed (with equal sex ratio) and bone samples collected (left and right tibia) for further investigation. Immediately after sacrificing the right tibia samples were prepared for histological analysis. Left tibia samples were frozen in plastic bags for mechanical and chemical analysis.

Table 1. Composition (%) of broiler grower diets; 21-35 days of age

Feedstuffs, %	Group					
	C	C+	E-I	E-I+	E-II	E-II+
Maize	58.3	58.3	58.8	58.8	59.0	59.0
Soybean meal	26.2	26.2	26.2	26.2	26.2	26.2
Sunflower meal	4.0	4.0	4.0	4.0	4.0	4.0
Yeast	2.0	2.0	2.0	2.0	2.0	2.0
Vegetable oil	5.2	5.2	5.2	5.2	5.2	5.2
DL Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	0.7	0.7	1.5	1.5	2.1	2.1
Dicalcium phosphate	2.1	2.1	0.8	0.8	-	-
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Mineral-vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0
Phytase, 250 PU/kg	-	+	-	+	-	+
Σ	100	100	100	100	100	100

Table 2. Composition (%) broiler finisher diets; 35-42 days of age

Feedstuffs, %	Group					
	C	C+	E-I	E-I+	E-II	E-II+
Maize	62.2	62.2	62.7	62.7	62.9	62.9
Soybean meal	21.8	21.8	21.8	21.8	21.8	21.8
Sunflower meal	5.0	5.0	5.0	5.0	5.0	5.0
Yeast	2.0	2.0	2.0	2.0	2.0	2.0
Vegetable oil	4.8	4.8	4.8	4.8	4.8	4.8
DL Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	0.6	0.6	1.4	1.4	2.0	2.0
Dicalcium phosphate	2.1	2.1	0.8	0.8	-	-
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Mineral-vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0
Phytase, 250 PU/kg	-	+	-	+	-	+
Σ	100	100	100	100	100	100

Method of analysis. The basic chemical composition of the feedstuff was determined using standard methods (Pravilnik, 1987). Feed and bone samples were dried and examined for mineral matter content (SRPS EN ISO 6869:2008). All samples were analyzed for phosphorus content by the use of

spectrophotometry, whereas the phytate-P content was determined by the method of Haug and Lantzsch (1983). Calcium concentration was analyzed using flame emission spectrophotometry on Varian SpectraAA10.

After thawing the left tibia samples, soft tissues were completely removed. Subsequently, the bone strength was determined by applying the modified method of mechanical breakage using IPNIS-apparatus (Mašić *et al.*, 1985). For ash determination, Ca and P content the bones were cleaned and defatted by cooking and mechanical removal of muscular tissues and ligaments, and then dried to a constant weight at 105°C.

Samples of the growth plate of the right tibia, about 5 mm in length, were carefully taken by costotomy and decalcified with 10% EDTA. Decalcified samples were fixed in 10% neutral formalin and absolute alcohol and embedded in paraffin using standard techniques. Tissue sections, 5-8 µm thick, were stained by standard HE method (Scheuer and Chalk, 1988). Microphotographs of histology preparations were performed on OLYMPUS BX 40. The same microscope was used for morphometric examination, i.e. determination of the calcification zone length in the tibia using a linear ocular micrometer.

Statistical analysis. The obtained results were grouped into corresponding statistical series and subjected to standard ANOVA procedure using the software package SPSS 8.0 for Windows.

RESULTS AND DISCUSSION

Chemical analyses confirmed that the well-balanced P content in the feed mixes for the control groups at 21-35 days were 0.80% for the total and 0.46% for AP. Chemical composition of diets for the control group at 35-42 days was 0.70% for TP and 0.43% for AP. Basic chemical composition of broiler feed did not differ from the control mix, except the concentration of phosphorus. Decrease of the TP and AP in feed mixes to the level of 0.42% (0.44%) and 0.09% (0.10%) respectively, was accomplished by reducing or excluding dicalcium phosphate. The concentration of phytate-phosphorus, as well as calcium content was the same in all mixes. In comparison to the control group, the concentrations of TP was lower by 30.38-45.57%, and the concentration of AP was lower by 46.51-79.08%. Relative proportion of phytate phosphorus in the TP content ranged from 31.6% in diets fed to control groups to 61.90% in diets fed to experimental groups. The obtained results support the available literature data that more than 60% of P contained in poultry diets is present as phytate-phosphorus (Harland and Morris, 1995; Kornegay, 1999; Jognbloed *et al.*, 2000). Feed mixtures based on maize and soybean pellets mostly contain 8.0 to 9.0 g/kg phytic acid, i.e. 2.2 to 2.5 g/kg phytate (Cabahug *et al.*, 1999). Phytate content, degraded under phytase, reduces the need for phosphorus by 1.0 to 1.2 g/kg in broiler mix (Yi *et al.*, 1996). Ravindran *et al.* (2001) detected that the digestibility of phytate in meals, not supplemented with phytase was 28%, while by supplementing with phytase digestibility increased for more than 50%. Using phytase in broiler mix increased AP for more than 60% (Simons *et al.*, 1990; Schwarz and Hoppe, 1992; Huyghebaert, 1997). Based on the aforementioned results it can be concluded

that the applied phytase content is adequate and corresponds to the mixes based on wheat and soybean meal with a low concentration of P of mineral origin.

Most producers recommend supplementation with 250-100 PU/kg in broiler diet, where P concentration may be reduced, depending on the level of phytase, TP and AP in the meal. Adding phytase in the recommended concentration increases AP, and 800 PU phytase/kg can be replaced with 0.1% phosphorus, without no consequences on production results (Vogt, 1992).

Table 3. Chemical composition (%) of broiler grower diets; 21 to 35 days of age

Analysed values	Group					
	C	C+	E-I	E-I+	E-II	E-II+
Crude protein, %	20.40	20.25	20.50	19.95	20.10	20.00
Crude fat, %	6.55	6.33	6.41	6.42	6.50	6.12
Crude fiber, %	4.32	4.43	4.41	4.53	4.66	4.08
ME, MJ/kg	13.39	13.39	13.45	13.40	13.37	13.32
Lysine, %	1.08	1.07	1.12	1.07	1.10	1.09
Methionine+cystine, %	0.82	0.83	0.89	0.84	0.82	0.81
Tryptophan, %	0.25	0.27	0.25	0.25	0.23	0.28
Calcium, %	1.15	1.09	1.10	1.05	1.01	1.10
Phosphorus, total, %	0.79	0.78	0.56	0.55	0.42	0.43
Phosphorus, available, %	0.43	0.43	0.23	0.22	0.09	0.10
Phosphorus, phytine, %	0.26	0.25	0.24	0.24	0.25	0.26
Manganese, mg/kg	104.30	111.33	102.50	100.07	98.43	108.87
Zinc, mg/kg	74.77	71.43	70.97	64.73	69.90	78.23
Copper, mg/kg	15.48	12.60	12.58	18.12	14.28	18.22
Iron, mg/kg	148.38	152.55	147.32	160.00	142.02	150.04

Body mass range and average daily weight gain of broilers in the experiment are shown in Table 3. Body mass of broilers and average daily gain of the control group (C) were within the margins of technological normatives for Arbor Acres provenience. It is to be emphasized that values of the cited parameters were somewhat higher in the broilers fed with diets containing the recommended dietary allowance for phosphorus. Body mass of the broilers in C+ group on the 35th day was significantly higher compared to body mass of broilers in group C ($p < 0.05$). Reduction of phosphorus content in diets for experimental groups resulted in corresponding reduction of body mass and daily weight gain. Differences among groups were highly significant ($p < 0.01$). Supplementation of phytase to low-phosphorus diets substantially increased body mass and daily weight gain in the corresponding group ($p < 0.01$). However, the effect was not sufficient to reach the corresponding parameter values in the control group.

Similar values for body mass in broilers fed diets containing approximately the same calcium and phosphorus concentrations and phytase supplement are reported by Simons and Versteegh (1993), Cabahug *et al.* (1999), Ahmad *et al.* (2000), Ravindran *et al.* (2001), Mulyantini *et al.* (2004), Vohra *et al.* (2006), Timmons *et al.* (2008) and Shakouri *et al.* (2009).

Table 4. Chemical composition (%) of broiler finisher diets; 35 to 42 days of age

Analysed values	Group					
	C	C+	E-I	E-I+	E-II	E-II+
Crude protein, %	18.50	18.70	18.50	18.20	18.25	18.55
Crude fat, %	6.67	6.57	6.28	6.16	6.09	6.64
Crude fiber, %	4.51	4.48	4.57	4.33	4.51	4.74
ME, MJ/kg	13.36	13.30	13.12	13.25	13.36	13.36
Lysine, %	0.96	0.96	0.97	0.97	0.97	0.96
Methionine+cystine, %	0.77	0.77	0.78	0.77	0.77	0.79
Tryptophan, %	0.22	0.23	0.22	0.24	0.23	0.23
Calcium, %	0.88	0.91	0.89	0.92	0.88	0.90
Phosphorus, total, %	0.70	0.74	0.56	0.57	0.46	0.44
Phosphorus, available, %	0.43	0.44	0.22	0.25	0.10	0.12
Phosphorus, phytine, %	0.25	0.27	0.27	0.26	0.26	0.26
Manganese, mg/kg	102.50	108.57	99.97	104.57	100.03	100.97
Zinc, mg/kg	68.90	75.92	77.76	66.56	71.45	78.55
Copper, mg/kg	13.77	16.53	15.68	16.60	16.33	18.65
Iron, mg/kg	174.00	170.50	156.33	154.67	144.33	131.33

Examination of the mineralization rate indicators, such as bone strength, ash content and calcium and phosphorus concentration in the tibia of experimental broilers revealed substantial differences among investigated groups (Table 4). Bone density in the control broilers corresponds to age and increases with time. A clearly pronounced negative effect on tibia strength was recorded in diets with decreased level of TP and AP. Similarly to previous groups, tibia strength increased with time, but in comparison to the control group it was significantly reduced. Supplementation of phytase to the diet with decreased concentration of the TP and AP exhibited some positive effects. Denbow *et al.* (1998) reported similar results which points that the increase of AP concentration, as well as the presence of enzyme, has positive effects on tibia breaking force. Similar conclusions were reached by Richter (1993), Denbow *et al.* (1995), Qian *et al.* (1996), Pillai *et al.* (2006), Venäläinen *et al.* (2006), as well as Jiang *et al.* (2009).

Table 5. Body mass and average daily weight gain (*) throughout the investigation period

Group	Body mass, g		Daily weight gain, g		
	Day		Investigation period (days)		
	35.	42.	21-35.	35-42.	1-42.
C	1624.0±173.6 ^{a,x}	2216.1±268.6 ^x	63.68±8.94 ^{a,x}	82.47±17.17 ^x	51.11±6.42 ^x
C+	1725.9±249.9 ^{b,x}	2259.5±313.0 ^x	70.26±11.75 ^x	83.95±21.13 ^x	54.10±7.33 ^x
E-I	1324.8±166.4 ^y	1865.0±234.0 ^y	55.42±8.40 ^{b,y}	78.56±10.15 ^x	43.51±5.56 ^y
E-I+	1444.6±174.6 ^z	1973.7±246.8 ^y	59.94±8.56 ^{c,y}	76.45±15.69 ^x	46.11±5.87 ^y
E-II	827.5±204.3 ^w	1282.2±259.2 ^z	35.00±11.03 ^w	51.45±20.75 ^y	29.65±6.20 ^z
E-II+	964.4±209.3 ^q	1388.6±295.0 ^w	28.90±14.67 ^z	48.40±15.99 ^y	32.18±7.01 ^z

*Values expressed as $\bar{X} \pm SD$; ^{a,b} $p < 0.05$; ^{x,y,z,w,q} $p < 0.01$

Tibia strength in the broiler group fed a diet without inorganic phosphorus were highly statistically significant to significantly lower compared to both previous groups. Harter-Denis *et al.* (2000, 2001) reported that reduction of the content of AP from 0.45% to 0.25% in the starter diet significantly reduced the bone breaking force up to 72.2% (11.5 to 3.20 kg). Phytase supplementation increased the bone breaking force from 3.2 to 4.9 kg. Using the diet with different concentrations of AP in the diet for broilers at 21-42 days of age, bone breaking forces linearly increases from 24.21 kg to 40.53 kg, proportional to the AP content.

Ash content, phosphorus and calcium concentration in the tibia of experimental broilers increase proportionally with the age, which is in accordance to the reports of Dukes (1975) and Lukić (2001). Phytase efficacy, in the sense of increased content of ash, phosphorus and calcium in the tibia is increased in diets with reduced concentration of AP, and is slightly lower when lacking a mineral source of phosphorus. At the end of the investigation period (day 42) the highest ash, phosphorus and calcium content in the tibia was noticed in group C+ (0.74% of TP and 0.44% of AP), although the differences were not statistically significant. Ash content, phosphorus and calcium concentration in the tibia of broilers fed the diet with lower P concentration (0.57% TP and 0.25% AP) was not statistically different from group C (0.74% TP and 0.44% AP), which is in accordance to the results of Ahmad *et al.* (2000), Ravindran *et al.* (2001) and Harter-Dennis *et al.* (2000, 2001). Besides explicit positive effects, it may be concluded that phytase supplementation was not able to compensate the lack of TP and AP in diets.

Histological findings in the proximal tibia of broilers in the control group (C and C+) did not differ revealing normal histological morphology (Figures 1a, 1b, 2a and 2b). Differences in the length of DH and MPS zones (Table 5) were not statistically significant.

Table 6. Breaking force of tibia and the content of ash, calcium and phosphorus in the tibia of experimental broilers

Group	Breaking force, kg	Content of ash, %	Content of calcium, %	Content of phosphorus, %
C	28.36±4.65 ^{a,x}	56.46±4.70 ^x	21.18±2.96 ^x	9.47±0.71 ^x
C+	33.38±6.05 ^{b,x}	55.40±3.24 ^x	21.37±4.35 ^x	9.67±1.43 ^{a,x}
E-I	22.41±4.31 ^{c,y}	45.30±4.16 ^{a,y}	14.86±4.00 ^y	7.56±0.81 ^{b,x}
E-I+	27.47±6.62 ^{a,y}	50.08± 3.73 ^{b,y}	19.12±2.20 ^z	8.02±0.49 ^{b,x}
E-II	9.36±1.90 ^z	37.36±6.61 ^{b,z}	12.64±1.78 ^y	6.14±0.85 ^y
E-II+	11.50±3.06 ^z	41.10±2.56 ^{b,z}	13.01±1.94 ^y	6.76±0.51 ^y
42. day				
C	35.71±7.66 ^{a,x}	59.90±4.35 ^x	21.01±2.38 ^x	10.14±0.69 ^x
C+	38.25±8.13 ^x	60.88±2.86 ^x	22.15±1.71 ^{a,x}	10.44±0.49 ^{a,x}
E-I	38.87±8.10 ^x	58.52±5.30 ^x	19.86±1.81 ^{b,x}	9.71±1.35 ^{b,x}
E-I+	42.94±11.97 ^{b,x}	61.18±7.32 ^x	20.92±2.87 ^x	9.50±0.95 ^{b,x}
E-II	17.60±3.55 ^y	47.93±3.29 ^y	13.04±0.46 ^y	7.78±0.45 ^y
E-II+	19.22±4.94 ^y	45.20±3.79 ^y	14.06±2.61 ^y	7.59±0.61 ^y

*Values expressed as $\bar{X} \pm SD$; ^{a,b} p<0.05; ^{x,y,z,w} p<0.01

Table 7. Length of degenerated hypertrophic zone of tibia (DHZ), [μ m]

Group	Length of degenerated hypertrophic zone (DHZ), [μ m]	
	35. day	42. day
C	41.86 ± 2.74 ^a	38.49 ± 1.92 ^{a,x}
C+	37.79 ± 2.61 ^{a,x}	36.19 ± 1.98 ^x
E-I	93.10 ± 6.24 ^{b,y}	71.86 ± 5.96 ^{b,y}
E-I+	52.74 ± 3.76 ^c	51.23 ± 2.52 ^{c,z}
E-II	139.09 ± 7.37 ^{d,w}	79.14 ± 7.06 ^y
E-II+	121.86 ± 14.10 ^{e,q}	75.32 ± 5.19 ^y

*Values expressed as $\bar{X} \pm SD$; ^{a,b} p<0.05; ^{x,y,z,w,q} p<0.01

In the 35-days-old broilers in groups C and C+ (Fig. 1a and 1b) a decrease in DH zone and increase in length of secondary spongiosa (MSS) was noticed, while in the same groups in 42-day-old broilers an increase of secondary spongiosa was observed. The differences in DH zone length were also highly statistically significant (Table 5) on days 35 and 42 of the experiment. In broilers aged 35 days from groups O-I and O-II cartilage columns in DHZ were longer comparing to the control group (Fig. 1c and 1e). Besides, in the group O-II

epiphyseal blood vessels were located in the hypertrophic zone (HZ) and cause deviation in this part of the bone. The differences in the length of DHZ are statistically significant.

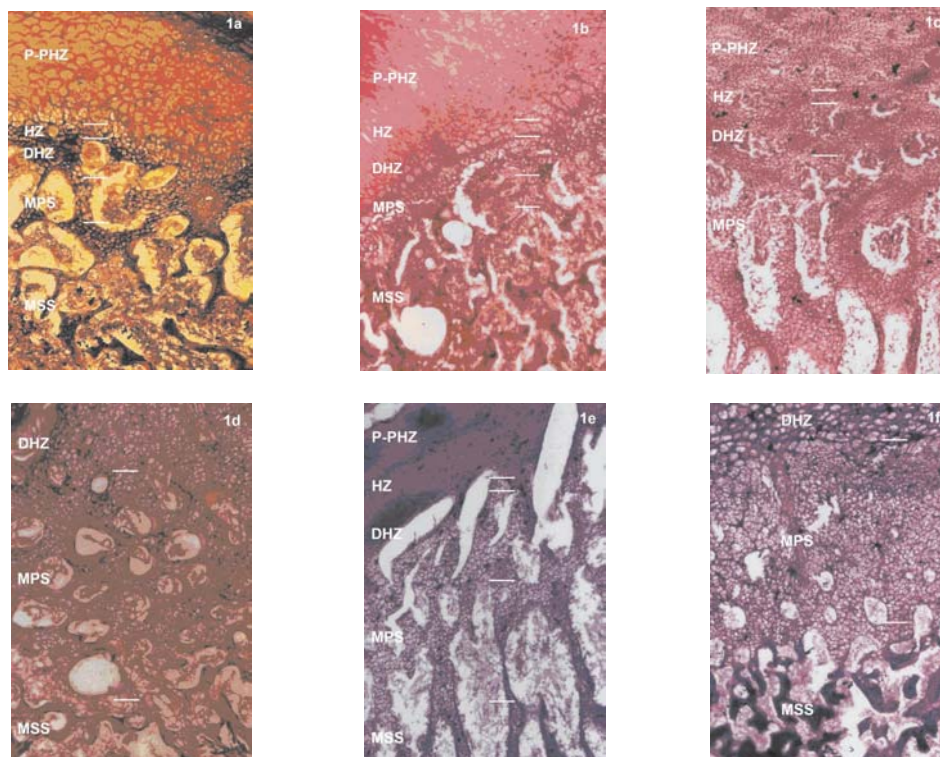


Fig. 1a-f. Microphotographs of proximal part of tibia of 35 days old broilers. H&E 100x
 P-PHZ – proliferating-prehypertrophied zone, HZ – hypertrophied zone, DHZ –
 degenerating hypertrophied zone, MPS – metaphyseal primary spongiosa, MSS –
 metaphyseal secondary spongiosa
 1a-b) C and C+ group, revealed normal histological morphology, 1c-d) E-I and E-I+
 group, note lengthened DHZ and MPS, 1e-f) E-II and E-II+ group, there is a
 lengthening of MPS and MSS

Histological findings in the proximal tibia of broilers from the experimental group E-I at the age of 42 days was featured by shorter MSS and longer MPS zone compared to the control group. The junction of the metaphyseal primary spongiosa (MPS) to metaphyseal secondary spongiosa (MSS) in these experimental groups was stepped and discontinuous (Fig. 2c and 2e), but osteoblasts and osteocytes were smaller and less numerous. In both age groups of broilers in E-I+ a reduced DHZ and MPS was observed (Fig 1d and 2d) and statistically significant differences in the length of DHZ. Changes on the bones in

broilers from group E-II+ are distinct and the changes correspond to changes observed in group E-II.

The most recognized histological changes in the bone growth zone of the experimental group that was fed with a low phosphorus diet (E-I) or was totally withheld (E-II) was the increase of the length of DHZ and metaphyseal primary spongiosa (MPS), whereas the metaphyseal secondary spongiosa (MSS) consisted mostly of osteoid tissue. These findings conform to reports of other authors (Lacey and Huffer, 1982; Long *et al.*, 1984; Quian *et al.*, 1996) acknowledging that specified changes are typical for the histological picture of rickets induced by phosphorus deficiency in the body. Moreover, it is obvious that the size and severity of lesions strongly correlate with the rate of phosphorus deficit in the diet, as well as with the supplementation or lack of phytase.

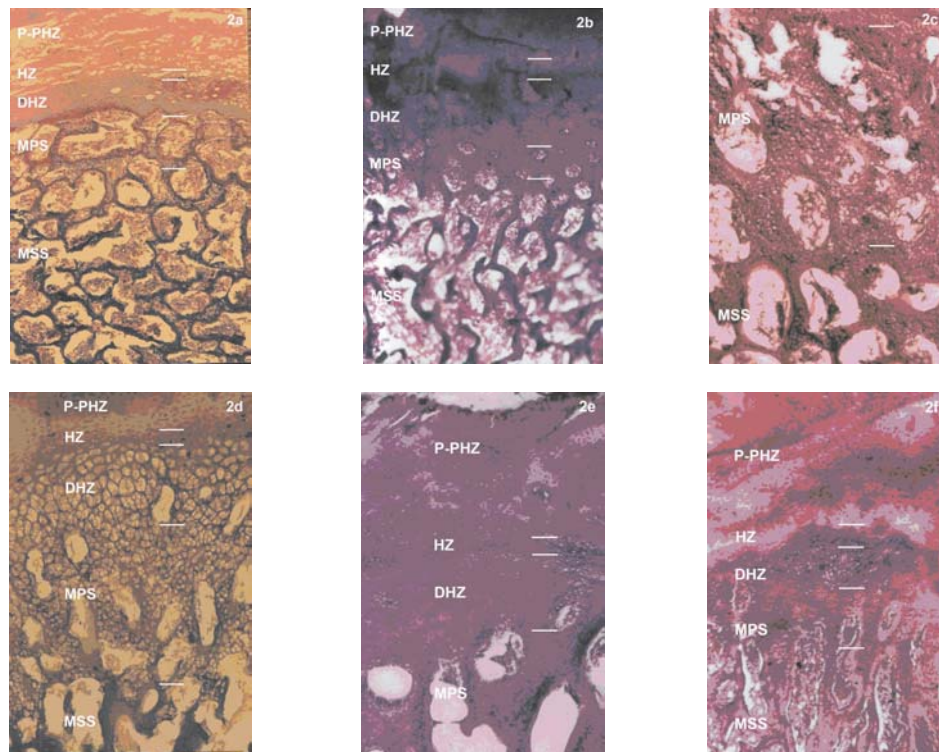


Fig. 2a-f. Microphotographs of proximal part of tibia of 42 days old broilers. H&E 100x
P-PHZ- proliferating-prehypertrophied zone, HZ – hypertrophied zone, DHZ – degenerating hypertrophied zone, MPS – metaphyseal primary spongiosa, MSS – metaphyseal secondary spongiosa
2a-b) C and C+ group, revealed normal histological morphology, 2c-d) E-I and E-I+ group, note lengthened MPS and shortened MSS, 2e-f) E-II and E-II+ group, there is a shortening of MPS and MSS

The results suggest that phytase can improve growth performance and phosphorus availability of broilers fed with low phosphorus diets. This is in agreement with the results of the experiment carried out on broilers aged 0-21 days (Živkov-Baloš *et al.*, 2008). Reduction of dietary TP and AP level in experimental broilers resulted in lower body mass and daily gain. However, by introducing phytase into diets negative effects of P reduction were, to some extent, alleviated. Supplementation of phytase in diets lacking mineral source of phosphorus could not prevent significant changes in bone composition and mineralization impairments. Histological, physical and chemical analysis of broiler tibia indicates that the extent and significance of changes depend on P concentration in a diet, as well on phytase addition. Phytase efficacy was greater in diets with reduced level of dicalcium phosphate.

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**EFIKASNOST FITAZE U SMEŠAMA ZA BROJLERE U POBOLJŠANJU
PROIZVODNIH PERFORMANSI I KARAKTERISTIKA KOSTIJU**

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STOJANOVIĆ DRAGICA I PETROVIĆ JELENA

SADRŽAJ

Izučavane su mogućnosti korišćenja fitaze mikrobijalnog porekla u smešama za brojlere od 21-42. dana, na bazi kukuruza i sojine sačme, sa različitim količinama ukupnog i iskoristivog fosfora. Ogled koji je trajao 22 dana, izveden je na 240 brojlera Arbor Acres provenijence, oba pola, podeljenih u šest jednakih grupa. Brojleri su hranjeni smešama u kojima je količina iskoristivog fosfora bila formulisana na 0,43%, 0,23% i 0,10%, sa ili bez dodatka fitaze. Tokom ogleda praćeni su proizvodni rezultati, zdravstveno stanje i mortalitet, fizičko-hemijske osobine i morfološke karakteristike tibije brojlera. Smanjivanje sadržaja ukupnog i iskoristivog fosfora u obrocima oglednih grupa izazvalo je posledično niže telesne mase i slabije priraste, a dodatak fitaze oglednim smešama je ublažio negativne efekte redukcije fosfora. Histološke, fizičke i hemijske analize tibije brojlera ukazuju na to, da stepen i značajnost promena na kostima zavisi od nivoa fosfora u obroku, kao i prisustva fitaze. Efikasnost fitaze bila je veća u kod ishrane brojlera smešama sa nižim učešćem dikalcijum fosfata.

