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THE EFFECTIVENESS OF MICROBIAL PHYTASE ON PERFORMANCE AND BONE TISSUE CHARACTERISTICS OF BROILERS

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The possibilities to use microbial phytase in maize/soybean meal diets for broilers with different dietary total and available phosphorus (TP and AP) levels were investigated. A 21-day experiment was carried out on 300 Arbor Acres broilers of both sexes divided into six groups. The broilers were fed diets with 0.46%, 0.25% or 0.10% AP, with or without phytase supplementation. During the experiment, health status and mortality, performance and physical, chemical and morphological bone tissue characteristics were investigated. Total exclusion of a 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. Reduction of dietary TP and AP level induced lower body mass and daily gain and feed consumption, as well as higher feed conversion. However, by introducing phytase into diets negative effects of P reduction were, to some extent, alleviated. Physical, chemical and histological 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

In poultry diets, dietary phosphorus originates from plant and animal feedstuffs, as well as from inorganic supplements. The phosphorus that is present in plant sources can be: 1) organically bound (phytate phosphorus) and 2) phosphorus in non-phytate form. Between 60% and 75% of the phosphorus in vegetable feedstuffs is present as phytate, which is poorly available for monogastric animals that lack the enzyme necessary to hydrolyze phytate (Kies at al., 2001). Consequently, under conventional feeding conditions and in order to meet the requirements for available phosphorus (AP), supplementation of inorganic phosphates in poultry diets proved to be necessary. The high P concentration in broiler excreta, however, creates serious environmental problems. The supplementation of phytase to the diet enables phytate hydrolysis, resulting in decrease of phosphorus content in the diets; thus reducing its output in the manure. Phytase is a much studied enzyme, and it's ability to hydrolyze phytates is well established.

Phytase activity (myo-inositol hexaphosphate phosphohydrolase) is detected in numerous plant seeds, including rice, wheat, barley, maize, rye, soybeans, as well as oil plants. Good results in digestibility of nutritive matter and improved productivity in animal production is confirmed by abundant scientific research were the main motive for a wider use of phytase. Modern feedstuffs production necessarily implies application of enzymes if different forms (Annison et Choct, 1993; Broz, 1993; Edens et al., 1999; Miles and Sistani, 2002**.**).

Considering the vital role of phosphorus in energy metabolism, its balanced content and appropriate form within the feedstuff product is of outmost importance. Providing adequate phosphorus content is particularly important in young broilers due to their reduced ability to utilize poorly available phosphorus, i.e. phytate phosphorus. According to investigation of Lacey and Huffer (1982), Long et al. (1984) and Ewing et al. (1995) pathological changes in bones of young chicks (up to 4 weeks of age) were primarily determined by the phosphorus/calcium ratio in the feed and by the rate of their resorption in the digestive tract. Phosphorus requirement of broiler chicks have still not been fully determined. Genetic selection results in rapid changes in genetic characteristic of animals, and consequently in different recommendations on mineral contents in poultry diets. Defining the concentration of dietary P that is optimal for body weight gain, as well as demands for adequate skeletal development is of particular concern (Williams et al., 1999). Application of phytase and newly developed feedstuffs characterized by high contents of AP offers a promise in the field of poultry nutrition (Pen et al., 1993). Researchers addressing issues of nutrition and environment protection strongly emphasize the need of redefining recommendations on concentrations of non-phytate or AP in poultry feed mixes (Waldroup et al., 2000; Yan et al., 2000, 2003; Harter-Dennis et al., 2001; Gill, 2002). In the future, an important issue for the nutritionists is to provide a diet with a minimal amount of P to meet nutritional requirements of poultry with the aim of providing maximal performance and good health and welfare of the birds, as well as to produce adequate manure applicable as fertilizer in agricultural production.

The objective of this study was to evaluate the effectiveness of microbial phytase on the performance, health status of broilers and bone tissue characteristics.

MATERIAL AND METHODS

Animals. The experiment included 300 one-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 technological conditions were in accordance with the normatives prescribed for this provenience (Arbor Acres Farm, 1997).

Diets. The broilers were distributed in 6 groups. Ratio and quantitative share of the feedstuff in feed mixes for experimental broilers was formulated in accordance with feeding standards (Table 1). The nutritive matter content in the diets was adjusted to the needs of broilers aged 1-21 day (NRC, 1994; AEC Tables, 1993). The control group (K) was fed standard a diet, age-adjusted feed mixes - Preformulated broiler starter diet I (1 - 21 day) (Table 1). Broilers in the K+ group were fed a standard diet supplemented with phytase. The content of TP and AP in the diet for the experimental group O-I was regulated by reducing the concentration of dicalcium phosphate, while the diet for O-I+ group was of the same composition, but supplemented with phytase. Diets for O-II and O-II+ groups were formulated without addition of dicalcium phosphate and with phytase addition for the O-II+ group. Maize–soybean diets were compounded to contain respectively 0.46%, 0.25% and 0.10% AP (Table 1). A constant level of calcium, phytate phosphorus metabolizable energy, crude protein and essential amino acids was maintained in all diets. The used enzyme complex "*Allzyme Phytase*" (supplier: "Alltech", USA) is of microbial origin, obtained as fermentation extract of genetically unmodified strain of *Aspergillus niger*.

Experimental procedure. Broilers had access to water and diet *ad libitum*. Preventive measures were applied, and all investigated broilers were subjected to permanent veterinary control. All changes of health status were monitored and recorded. Control measurements of body mass of broilers were performed at the beginning and at the end of the experiment (1 and 21 day) using technical scales with an accuracy rate 10^{-3} kg. Throughout the investigation period precise measurements of the feed mix amounts fed to each particular group were performed. At the end of the experiment the total food consumption was determined by summarizing the daily amounts fed to the broilers. From the obtained results on food consumption and weight gain the food conversion was calculated.

Feedstuff samples for microbiological and chemical assaying were collected immediately before the onset of the experiment, as well as at regular seven-day intervals throughout the investigation period. At the end of the experiment (day 21) ten broilers from each group were sacrificed 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 up to mechanical and chemical analysis.

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 (JUS ISO 6869/1994). 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 SpectrAA10 (JUS ISO 6869/1994).

After defrosting samples of left tibia all soft tissues were completely removed. Subsequently, the bone strength was determined applying modified method of mechanical breakage using IPNIS-apparatus (Mašić et al., 1985). Prior to determination of the contents of ash, Ca and P the bones were cleaned and defatted by cooking and mechanical removal of muscular tissue 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**.** Decalcificated 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 the 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 length calcification zone 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 the well-balanced P content in the feed mixes fed to K groups (K and K+), being 0.80% for the total and 0.46% for AP in starter diets (Table 1). The basic chemical composition of diets fed to O groups (O-I, O-I+, O-II, O-II+) did not differ from the K groups, except for P concentration. Decrease of the TP and AP in feed mixes to the level of 0.46% and 0.10%, respectively, was accomplished by reducing or excluding the dicalcium phosphate. In comparison to the K and K+ groups, the concentrations of TP and AP were lower for 28.75-42.50% and 45.65-78.26%, respectively. Relative proportion of phytate phosphorus in the TP content ranged from 25% in diets fed to K groups to 65.90% in diets fed to O groups. The obtained results support the available literature data that more than 60% of P contained in poultry diets is present as phytate-phosphorus (Kornegay, 1999; Jongbloed et al., 2000). Feed mixes 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).

Table 1. Composition of broiler starter diets (%) 1-21 days

Feedstuffs, %	Groups					
	K	K_+	O-I	O-I_+	O-II	O-II_+
Maize	51.8	51.8	52.3	52.3	52.5	52.5
Soybean meal	33.2	33.2	33.2	33.2	33.2	33.2
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	4.6	4.6	4.6	4.6	4.6	4.6
DL Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	0.7	0.7	1.6	1.6	2.2	2.2

Cont. Table 1						
Feedstuffs, %	Groups					
	K	K+	O-I	O-I+	O-II	O-II+
Dicalcium phosphate	2.2	2.2	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
Chemical composition						
Crude protein**, %**	22.50	22.75	23.05	22.95	22.95	23.00
Crude fat, %	6.59	6.37	6.45	6.42	6.52	6.39
Crude fiber, %	4.33	4.76	4.43	4.33	4.66	4.66
ME, MJ/kg	13.00	13.00	13.06	13.06	13.09	13.09
Lysine, %	1.28	1.27	1.25	1.27	1.28	1.28
Methionine+cystine, %	0.90	0.91	0.92	0.93	0.91	0.91
Triptophan, %	0.29	0.28	0.27	0.29	0.28	0.29
Calcium, %	1.05	1.01	1.06	1.02	1.01	1.10
Phosphorus, total, %	0.80	0.80	0.57	0.57	0.46	0.47
Phosphorus, available, %	0.46	0.46	0.25	0.25	0.10	0.10
Phosphorus, phytine, %	0.25	0.26	0.26	0.26	0.25	0.26
Manganese, mg/kg	107.90	101.53	105.23	106.03	103.67	104.80
Zinc,mg/kg	77.57	74.80	77.77	75.13	77.97	78.00
Copper, mg/kg	16.20	19.48	13.10	13.70	14.10	14.22
Iron, mg/kg	133.97	130.00	146.00	154.03	131.70	123.00

Broilers from K, K+, O-I and O-I+ were of well-built physical constitution, properly developed bone and muscle tissue, lively and in good health throughout all the period of investigation. Their ability of active movement and locomotion coordination were normal, muscle tonus marked and in perfect condition. Mortality rates observed in these groups were within the range of technologic normatives (Table 2). The most frequent cause of mortality was contusions and injuries of vital organs due to mechanical pressure (feeders, drinking troughs). Mortality rates observed in O-II and O-II+ groups were significantly higher than in other groups and the technologic normative (Table 2). The highest mortality rates in the O-II and O-II+ group were recorded in the second and third week, respectively. The obtained results correspond with the results of Denbow et al. (1995) reporting on mortality rates ranging 35-45% in broilers fed diets containing 0.20 and 0.27% non-phytate phosphorus throughout the investigation period (day 1 to day 21). This phenomenon is related to the complete depletion of body reserves of calcium and phosphorus associated with a weak activity of intestinal phytase. Broilers from the O-II group exhibited signs of acute phosphorus deficiency in the first week of life, thus most frequent cause of death in this group was exhaustion that was due to impeded movement and poor consumption of feed and water. Post mortem examination revealed characteristic "rubber" legs, bills and cranial bones, deformations on sternal bone and ribs, as well as general runtiness. Mortality rate in group O-II+ was substantially lower than in the group O-II. These positive effects of phytase were manifest in the first week, when the mortality rate in group O-II+ was within the margins of technologic normatives for this provenience. Harter-Dennis (2000) reported that reducing the content of AP from 0.45% to 0.25% in starter diets (broilers 0-20 days of age) was associated with increased mortality rate (12.5%). However, supplementation of phytase in the diets resulted in absolute decrease in mortality rate.

Table 2. Broiler mortality rate throughout the investigation period, (%)

Time of trial (week)	Group					
	K	K+	O-I	O-I+	O-II	O-II+
1.	1.92	1.92	7.70	1.92	12.00	4.00
2.	1.92	0	0	-	20.45	2.00
3.	0	0	2.17	-	16.67	4.25
1-3.	3.85	1.92	10.00	1.92	49.12	10.25

Body mass range, average daily weight gain and food conversion values are summarized in Table 3. Body mass of broilers was within the margins of technologic normatives at the beginning of the investigation period, and differences in body mass among groups were not statistically significant ($p>0.05$). Body mass, average daily weight gain and feed conversion values in group K were within the margins of technologic normatives for Arbor Acres provenience. It is to be emphasized that values for the cited parameters were somewhat higher in broilers fed diets containing recommended phosphorus content and phytase supplement. Reduction of phosphorus content in diets for O groups resulted in corresponding reduction of body mass, daily weight gain and food conversion rate, and differences among groups were highly significant ($p<0.01$). Supplementation of phytase to low-phosphorus diets substantially increased body mass, daily weight gain and food conversion rates in O-I+ and O-II+ groups ($p<0.01$); however, the effect was not sufficient to reach the corresponding parameter values in the K and K+ groups. Similar values for body mass in broilers fed diets containing approximately same calcium and phosphorus concentrations and phytase supplement are reported by Perney et al. (1993), Simons and Versteegh (1993), Rutkowski et al. (1997) and Ahmad et al. (2000). The results of our study demonstrate that effect of phytase on body mass increase was not sufficient to provide the same body mass increase rate as in the groups feed a standard diet (K and K+). This strongly supports the thesis that phytase-related phosphorus content in broiler diets is determined not only by phytase content, but also by the total phosphorus and calcium concentration in the diet (Rama Rao et al., 1999). Correspondingly, Perney et al. (1993) reported on better production output obtained by increasing available phosphorus content and dicalcium phosphate ratio or by releasing of phytine salts through phytase activity. The obtained results are in accordance with the reports of Rutkowski et al., (1997). The authors suggested that a decrease of phosphorus content by 33% resulted in significantly lower weight gain rate in broilers. Cabahug et al. (1999) and Ahmad et al. (2000) reported on better production output associated with phytase supplementation, whereas effects were more pronounced at lower concentrations of available phosphorus. The results of our investigation correspond to some extent with results of Harter-Dennis (2000), who reports on significant decrease in weight gain (37.5%) that results from decrease of phosphorus content from 0.45% to 0.25% in broiler diets based on maize/soybean pellets. Phytase supplementation (Allzyme Phytase) positively affects weight gain. The obtained results are in accordance with reports of Ravindran et al. (2001), who observed that phytase supplementation in the starter phase (1-21 day) positively affected appetite, i.e. food consumption. Rutkowski et al. (1997) observed that phytase supplementation (250 or 750 PU/kg) in diets with phosphorus content decreased by 33% was associated with an increase of food conversion rate, being 2% higher than in broilers fed standard feed mixes.

Table 3. Body mass, average daily weight gain and food conversion * throughout the investigation period

	Body mass, g		Daily weight gain, g	Food conversion, kg	
	Time of trial (days)				
Group	1.	21.	1-21.	1-21.	index
K	37.6±2.8	732.7±80.1^{x}	32.60±4.99^{x}	1.41	100.00
K+	37.9±3.4	748.9±100.5^{x}	33.29±5.54^{x}	1.56	110.64
O-I	37.9±3.6	554.5±75.6^{y}	24.34±3.95^{y}	1.68	119.15
O-I+	37.2±2.9	593.8±85.2^{z}	26.51±4.06^{z}	1.55	109.93
O-II	36.7±2.9	299.6±47.5^{w}	9.39±4.14^{w}	2.16	153.19
O-II+	36.2±3.3	439.7±85.6^{q}	18.41±4.93^{q}	1.23	87.23

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

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.). Clearly pronounced negative effect on the investigated parameters was recorded in diets with the decreased level of TP and AP. Supplementation of phytase to feed mixes with decreased concentration of the TP and AP exhibited some positive effects; however, differences among groups were not statistically significant (p>0.05). Determination of ash content in the tibia revealed statistically significant differences among investigated groups.

Table 4. Tibia breaking force and content of ash, calcium and phosphorus in tibia of experimental broilers

Group	Tibia breaking force, kg	Content of ash, %	Content of calcium, %	Content of phosphorus, %
K	20.03 ± 3.01^{x}	49.50 ± 6.27^{x}	15.58 ± 2.30^{x}	8.52 ± 1.02^{x}
K+	19.42 ± 3.90^{x}	$48.71 \pm 5.87^{a.x}$	17.68 ± 3.85^{x}	8.29 ± 0.93^{x}
O-I	10.33 ± 2.52^{y}	$52.97 \pm 6.70^{a.x}$	17.10 ± 1.72^{x}	8.49 ± 0.79^{x}
O-I+	$9.01 \pm 1.50^{a.z}$	$53.99 \pm 2.67^{b.x}$	16.15 ± 1.90^{b}	8.32 ± 0.65^{x}
O-II	$3.16 \pm 0.87^{b.z}$	$41.34 \pm 5.04^{b.y}$	$13.56 \pm 2.01^{a.y}$	$7.03 \pm 1.04^{a.y}$
O-II+	$5.19 \pm 1.78^{b.z}$	$47.25 \pm 4.36^{a.y}$	13.01 ± 1.94^{y}	7.97 ± 0.60^{b}

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

Histological findings in the proximal tibia of broilers did not differ among K and K+ group, revealing normal histologic morphology (Figures 1a, 1b, 1c, 1d, 1e and 1f). Differences in the length of DH and MPS zones were not statistically significant, also (Table 5).

In broilers from groups K and K+ the proliferated prehypertrophic zone (PPHZ) revealed the presence of chondrocyte series arranged parallel to the vertical bone axis and separated by the matrix (Fig. 1a and 1b). Epiphyseal blood vessels are located in this zone. Hypertrophic zone (HZ) is formed by hypertrophic chondrocytes. The hypertrophic chondrocytes of the degenerated hypertrophic zone (DHZ) are arranged in series mutually separated by the metaphyseal blood vessels. Chondrocytes predominate in the region of

Figure 1a. K group

Figure 1b. K+ group

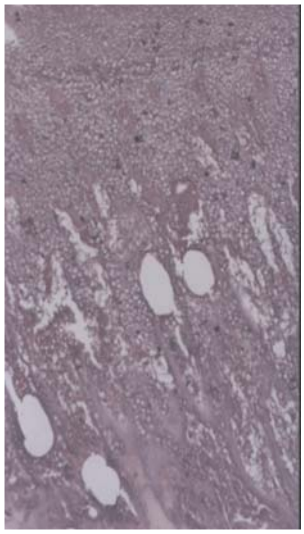

Figure 1c. O-I group

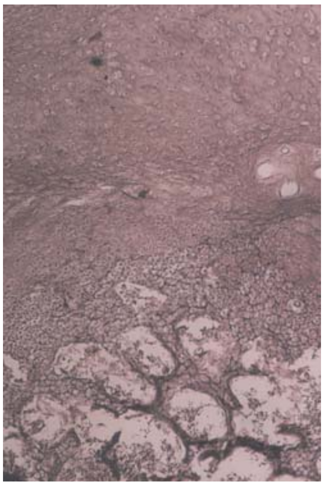

Figure 1d. O-I+ group

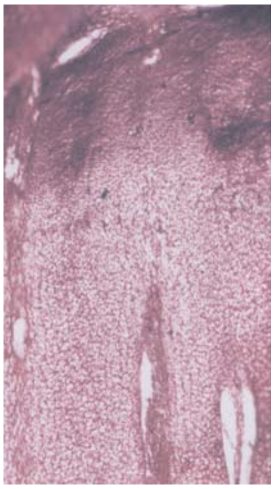

Figure 1e. O-II group

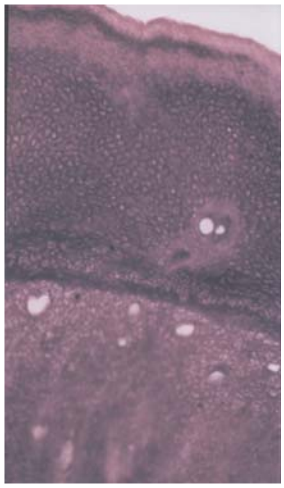

Figure 1f. O-II+ group

Figures 1a, 1b, 1c, 1d, 1e and 1f. Microphotographs of proximal part of the tibia of 21 day old broilers. H&E 100x

metaphyseal primary spongiosis (MPS), whereas the metaphyseal secondary spongiosis (MSS) consists mostly of osteoid tissue. In comparison with the K and K+ group, broilers from groups O-I and O-II reveal increased length of the DHZ. Furthermore, the cartilaginous zone is wider, which is due to a moderate lateral resorption and substitution by the bone tissue. PPHZ and HZ are slightly shorter in comparison with the K group.

Table 5. Length of degenerated hypertrophic zone of tibia (DHZ), μm

Group	Length of degenerated hypertrophic zone (DHZ), μm
K	47.35 ± 3.52 ^{x}
K+	43.54 ± 2.60 ^{x}
O-I	81.22 ± 9.26 $^{b,\ y}$
O-I+	63.10 ± 6.73 ^{b}
O-II	324.79 ± 29.96 $^{c,\ z}$
O-II+	156.38 ± 8.55 $^{d,\ e}$

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

Differences in DH zone lengths are highly statistically significant (Table 5). Length of degenerated hypertrophic zone of the tibia observed in broilers from group O-II+ are significantly decreased compared to group O-II. 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 hystological picture of rickets induced by phosphorus deficiency in the body.

Moreover, it is obvious that dimensions and severity of lesions strongly correlate with the rate of phosphorus deficit in a diet, as well as with the supplementation or lack of phytase. The most pronounced in our experiment changes were observed in length of DHZ of tibia of the broilers from group O-II revealing 6.86-fold increase of DHZ length in relation to the K group. The length of this zone in group O-I was 1.7 times higher than in the K group. Changes in length of degenerated hypertrophic zone of tibia in broilers from group O-I+ are less distinct and associated with normal histological morphology, indicating that 30% phosphorus deficiency is compensated by phytase supplementation.

The results suggest that phytase can improve growth performance and phosphorus availability of broilers fed with low phosphorus diets. One of the greatest benefits of phytase supplementation appeared to be maintaining livability at lower dietary levels of TP and AP. Reduction of dietary TP and AP level in experimental broilers induced lower body mass nad daily gain, as well as higher feed conversion bringing a lower production index. However, by introducing phytase into diets negative effects of P reduction were, to some extent, alleviated.

Supplementation of phytase in diets for broilers aged 1-21 days 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 extent and significance of changes depend on P deficiency and phytase addition. Phytase efficacy was greater in diets with reduced level of dicalcium phosphate.

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EFIKASNOST MIKROBIJALNE FITAZE U POBOLJŠANJU PROIZVODNIH PERFORMANSI I KARAKTERISTIKA KOSTIJU BROJLERA

ŽIVKOV-BALOŠ MILICA, MIHALJEV Ž, ORLIĆ D, KOVAČEVIĆ MIRA, LEVIĆ JOVANKA, i GLEDIĆ D

SADRŽAJ

Izučavane su mogućnosti korišćenja fitaze mikrobijalnog porekla u smešama za brojlere na bazi kukuruza i sojine sačme, sa različitim količinama ukupnog i iskoristivog fosfora. Ogled koji je trajao 21 dan, izveden je na 300 brojlera Arbor Acres provenijence, oba pola, podeljenih u šest jednakih grupa. Brojleri su hranjeni smešama u kojima je količina iskoristivog fosfora bila formulisana na 0,46%, 0,25% 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. Potpuno isključivanje mineralnog izvora fosfora izazvalo je pogoršanje zdravstvenog stanja i povećanje mortaliteta brojlera, a dodatak fitaze u obrok je značajno smanjio mortalitet brojlera i ublažio negativne efekte isključivanja dikalcijum fosfata na zdravstveno stanje. Smanjivanje sadržaja ukupnog i iskoristivog fosfora u obrocima oglednih grupa izazvalo je posledično niže telesne mase, slabiji prirast, nižu konzumaciju, veću konverziju i niži proizvodni broj, a dodatak fitaze oglednim smešama je ublažio negativne efekte redukcije fosfora. Histološke 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.