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TIBIA MINERALIZATION OF CHICKENS DETERMINED TO MEAT PRODUCTION USING A MICROBIAL PHYTASE

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ABSTRACT

The target of the research was 6-phytase of microbial origin. It was used in feed mixtures for chickens determined to meat production. Its effect has been studied in relation to the tibia mineralization by calcium, phosphorus and magnesium. 6-phytase is a product of *Aspergillus oryzae*. That was obtained by means of biotechnological processes of production of commercially available enzymes. It was incorporated in the feed mixtures 0.1%. In a 38-day feeding trial, 300 one-day-old, as hatched, Cobb 500 chickens determined to meat production (100 birds per group) were fed on one concentrations of dietary non-phytate phosphorus (2.32, 2.31 $g.kg^{-1}$, respectively and supplemental microbial phytase (0 and 500 $FTU.kg^{-1}$ feed mixtures). Control group was used to compare the results and control feed mixtures contained 4.5 $g.kg^{-1}$ without microbial phytase. At days 40 it was selected 6 birds in every group, which were slaughter in accordance with the principles of welfare. Left tibias of every bird were used to determination of calcium, phosphorus and magnesium contents. According to *in vivo*, it was found that the addition of microbial phytase to reduced dietary non-phytate phosphorus increased concentrations of calcium (Ca), phosphorus (P) and magnesium (Mg) in tibia. The differences among groups were statistically significant ($p < 0.05$). It was concluded that reducing of dietary non-phytate phosphorus on the 2.32, 2.31 $g.kg^{-1}$, respectively, by monocalcium phosphate and microbial phytase supplementation in feed mixtures facilitated tibia mineralization at chicken determined to meat production.

Keywords: broiler; microbial phytase; additive; phosphorus; tibia; mineralization

INTRODUCTION

The availability of phosphorus in feedstuffs of plant origin is generally very low, ranging from 30 to 40% **(Nelson et al., 1968a)**. To increase phosphorus bioavailability, the most commonly used method is supplementing high dosage of inorganic phosphorus in feed, which leads to the excretion of large amounts of phosphorus in animal manure. Consequently, the cost of feed and the environmental adverse impact are increased. Moreover, phytate limits the availability of several other essential nutrients, such as minerals, protein and amino acids **(Biehl and Baker, 1996)**. Among the strategies designed to reduce excessive drainage of phosphorus but also calcium into the environment from the poultry industry, the two most important are (1) determining the exact phosphorus and calcium requirements for present modern chickens determined to meat production and formulating to meet those requirements exactly and (2) using additives such as microbial phytase to increase phytate phosphorus utilization and decrease excretion of these nutrients into environment **(Angel et al., 2005)**.

As phytase is increasingly used worldwide, science and technology related to the enzyme have evolved to a new exciting field at a fast pace. Clearly, supplemental phytases improve dietary phytate-phosphorus utilization by food-producing animals, and reduce environmental pollution of phosphorus from animal waste in areas of intensive animal production **(Kliment et al., 2010)**.

Phytases are a group of enzymes that catalyze the release of orthophosphate from inositol hexakisphosphate **(Mullaney and Ullah, 2003)**. The phytases are divided into four classes: histidine acid phosphatases, β-propeller phytases, cysteine phosphatases and purple acid phosphatases **(Puhl et al., 2007; Huang et al., 2011)**. These include the fungal phytases from Aspergillus ficuum **(Kostrewa et al., 1997)** A. fumigatus **(Xiang et al., 2004)** A. niger **(Oakley, 2010)** and Debaryomyces castellii **(Ragon et al., 2009)** and the bacterial phytases from Escherichia coli **(Lim et al., 2000)** and Klebsiella pneumoniae **(Böhm et al., 2010)**. The enzymes are additionally classified into 3-, 5- or 6-phytases (EC 3.1.3.8, EC 3.1.3.72 and EC 3.1.3.26, respectively) based on the carbon position on the inositol ring at which they initiate phosphate hydrolysis. Thus 3-phytases first remove the phosphate group at the C3 or C1 position (1L- vs. 1D-convention) while 6-phytases do so at the C6 position (or

C4 in the 1L convention) **(Lei and Porres, 2003)**. Very few 5-phytases have been identified (for example that from from Selenomonas ruminantium **(Chu et al., 2004)**.

The efficacy and safety of a microbial 6-phytase expressed via the use of synthetic genes in Aspergillus oryzae was investigated from days 8 to 22 of age using 480 Ross PM3 broiler chickens. Five treatments were tested. A diet containing 5.6 $g.kg^{-1}$ of phosphorus was fed to the control treatment. Another diet containing 4.1 $g.kg^{-1}$ phosphorus was fed to another treatment as negative control. This diet was fed in 3 other treatments with the addition of phytase (500, 1000, or 2000 $U.kg^{-1}$). Lower feed intake and higher weight gain was obtained with the treatment containing 2000 $U.kg^{-1}$ phytase compared to the two control treatments and the treatment containing 500 $U.kg^{-1}$ phytase, leading to a significant improvement in feed conversion ratio with the 2000 $U.kg^{-1}$ phytase. Tibia strength and ash were improved with the latter and were dose-dependent described by an exponential function. Safety test using a concentrated preparation of the novel 6-phytase enzyme did not reveal any toxicological significant findings. Tested 6-phytase improved broiler performance and reduced the need for phosphate **(Aureli et al., 2011)**.

Recently study demonstrated that diets containing non-phytate phosphorous closer to the requirements along with the use of supplemental phytase decreased the total phosphorus concentrations in litters without affecting phosphorus solubility in litters and amended soils **(Maguire et al., 2004)**.

Phytate phosphorus digestibility can be measured at either ileal or fecal levels. Total tract phosphorus digestibility can be used to determine phosphorus retention as it has fewer practical limitations. However, determination of phytate phosphorus release should be measured at the ileal level because in the hindgut phytate is degraded by the intestinal microflora, but the released phosphorus is not absorbed. Direct measurement of the hydrolysis of phytate phosphorus at the ileal level will give a good indication of the effectiveness of a phytase feed additive. In addition, tibia ash, bone strength and performance data are useful parameters for validation of matrix values (e.g. inorganic phosphorus, calcium, energy and digestible amino acids) for phytase **(Dersjant-Li et al., 2015)**.

Phosphorus supply may indeed play a controlling role for the total numbers of bacteria as suggested by the significant calcium/phosphorus and phytase effects which were independent of one another, and tended to be additive. Phytase seems to have an important role to play in modulating the gut flora but their effects are clearly framed by the background levels of phosphorus and calcium in the diet. The changes in the microbiome as a result of feeding a phytase at high inclusion levels 5000 FTU are novel, although it must be noted that although the changes were significant, the scale of the changes were not extraordinary. The fact that phytase, rather than calcium and phosphorus levels played a significant role in promoting better animal performance suggests that the linkage between microbiome structure and performance is not inextricable **(Ptak et al., 2015)**.

Benefits of phytase supplementation likely occur due to release of phosphorus and extraphosphoric effects simultaneously over a wide range of phytase concentrations. In contrast to the aforementioned knowledge **Watson et al. (2006)** noted that significant extra-phosphoric increases in body weight gain have been reported at concentrations ranging from phytase as low as 300 to 800 FT $U.kg^{-1}$.

Experimentally the low non-phytate phosphorus diet, medium and high non-phytate phosphorus feed mixtures were compared. They found the increasing ash percentage, phosphorus content and breaking strength of tibia on day 21 and 42 ($p < 0.001$). Supplementation of 6-phytase but also 3-phytase significantly improved the ash percentage on day 21 and phosphorus content of tibia at 21 and 42 days of age ($p < 0.001$). Dietary 6-phytase enhanced ash percentage (linear contrast, $P = 0.039$) and tended to increase breaking strength (linear contrast, $P = 0.094$) in tibia of chickens at 42 days of age compared to control diet. There was a significant interaction between non-phytate phosphorus levels and phytase sources on ash percentage at 42 days of age ($p < 0.01$). The ash percentage and phosphorus contents in ashes of bone are the main parameters for mineral deposition in animal bones **(Jiang et al., 2013)**.

A decrease in dietary phosphorus, especially in finishing chickens determined to meat production (21 to 38 days old) is a crucial issue in poultry production from an environmental and economic point of view. Nevertheless, phosphorus must be considered together with other dietary components such as calcium and microbial phytase. Phosphorus level is possible to decrease in finishing chickens, if the calcium content is appropriate. Nevertheless, decreasing the dietary phosphorus and calcium cannot allow a maximization of bone mineralization, but the optimal threshold remains to be determined **(Rousseau et al., 2012)**.

In chickens determined to meat production it has extensively been reported that phytase supplementation to maize-soybean meal feed mixtures permits total phosphorus concentration to be reduced without impairing bone mineralization **(Brož et al., 1994; Qian et al., 1996; Sebastian et al., 1996a; Yan et al., 2001**; **Viveros et al., 2002**; **Brenes et al., 2003**; **Dilger et al., 2004**; **Onyango et al., 2004, 2005; Payne et al., 2005**; **Catalá-Gregori et al., 2006)**.

Scientific hypothesis

The purpose of this study was to present the in vivo results of 6-phytase effect on tibia mineralization in chickens kept for meat production. In the experiment were used the feed mixtures of soy-cereal type with reduced of non-phytate phosphorus content.

MATERIAL AND METHODOLOGY

Carry out of experiment

In vivo experiment was realized on Zámostie Company poultry experimental station with deep litter breeding system. The experiment included 300 pcs of one-day-old hybrid chickens Cobb 500 divided into 3 groups (in each group n = 100) according to scheme in Table 1. Spaced deep litter box allowed chickens to an unlimited access to feed and water as well as to perform their natural activities. The bottom layer of the litter consisted of 8 cm

Table 1 Scheme of feed individual experiment.

Broiler chicks n = 6	Group	Experimental phase, feed mixture	Phosphorus, content per kg of feed mixture and phytase supplement
Cobb 500	Control (CG)	Starter (day 1 to 18)	non-phytate P 4.50 $g.kg^{-1}$
		Grower (day 19 to 32)	non-phytate P 4.50 $g.kg^{-1}$
		Finisher (day 33 to 38)	non-phytate P 4.50 $g.kg^{-1}$
	Experimental 1 (G-RP)	Starter (day 1 to 18)	non-phytate P 2.32 $g.kg^{-1}$
		Grower (day 19 to 32)	non-phytate P 2.32 $g.kg^{-1}$
		Finisher (day 33 to 38)	non-phytate P 2.32 $g.kg^{-1}$
	Experimental 2 (G-RP +MPH)	Starter (day 1 to 18)	non-phytate P 2.32 $g.kg^{-1}$ +0.1% (500 $FTU.kg^{-1}$) 6-phytase
		Grower (day 19 to 32)	non-phytate P 2.32 $g.kg^{-1}$ +0.1% (500 $FTU.kg^{-1}$) 6-phytase
		Finisher (day 33 to 38)	non-phytate P 2.31 $g.kg^{-1}$ +0.1% (500 $FTU.kg^{-1}$) 6-phytase

Note: n = multiplicity; P = phosphorus; CG = control group; G-RP = experimental group 1 with reduced non-phytate phosphorus content to 2.32 $g.kg^{-1}$ feed mixtures; G-RP +MPH experimental group 2 with reduced non-phytate phosphorus content to 2.32 $g.kg^{-1}$ and with addition 6-phytase microbial origin 0.1% (500 $FTU.kg^{-1}$) in feed mixtures.

Table 2. Nutrient and energy contents per kg of feed mixtures.

	Feed mixture					
	Starter		Grower		Finisher	
	experimental	control	experimental	control	experimental	control
$ME_{N,}$ MJ	12.076	12.00	12.081	12.035	12.449	12.421
Crude protein, g	212.95	212.32	192.64	192.68	173.17	172.92
Lysine, g	11.60	11.58	9.97	10.00	10.07	10.00
Methionine, g	4.68	4.67	5.19	5.18	5.40	5.39
Methionine + cystine, g	7.85	7.83	8.04	8.03	7.96	7.94
Threonine, g	8.20	8.18	7.34	7.34	6.53	6.53
Calcium, g	8.18	8.14	7.22	7.19	7.20	7.24
P total, g	5.37	7.96	5.33	7.92	5.24	7.83
P non-phytate, g	2.32	4.50	2.32	4.50	2.31	4.50
6-phytase, FTU	0*, 500**	0*	0*, 500**	0*	0*, 500**	0*

Note: ME_N = metabolizable energy; P = phosphorus; * = Control group; experimental group 1 (reduced phosphorus content); ** = experimental group 2 (reduced phosphorus content +6-phytase).

of wood sawdust's and the top layer consisted of 5 cm of adjusted wheat straws. The chickens to the age of 14 days consumed a feed from plate feeders and water from the hat drinkers located on the floor. Older chickens consumed feed from the tube feeders and drank water from bucket drinkers till the end of the experiment. Microclimatic conditions were equal for all groups in accordance with the recommendations for the final type chickens Cobb 500. The temperature in the hall was ensured by the air conditioner as well as heating lamp to the age 18 days. Ventilation in the hall on the farm was ensured by the ventilation system and ventilation windows. Light regime was automatically adjusted according to the requirements for this type of chickens.

The experiment lasted 38 days and was divided into three phases:

- starter, from day 1 to day 18,
- grower, from day 19 to day 32,
- finisher, from day 33 to day 38 day

The feed mixtures of soybean-cereal type starter, grower and finisher were fed during the different stages. The experiment consisted of two experimental groups G-RP (reduced non-phytate phosphorus content to 2.32, 2.31 $g.kg^{-1}$ feed mixture, respectively without add-6-phytase) and G-RP + MP (non-phytate phosphorus content to 2.32, 2.31 $g.kg^{-1}$ feed mixture, respectively with the addition of compound 6-phytase 0.1% (500 $FTU.kg^{-1}$) in feed mixtures. The reduction of non-phytate phosphorus content was carried out by reducing of share feed ingredient monocalcium phosphate from 1.7% to 0.55% in the starter feed mixture, from 1.75% to 0.60% in the grower feed mixture and from 1.80 to 0.65% in the finisher feed mixture. The control group was used with commonly used feed mixtures to compare of results. In the control feed mixtures was the non-phytate phosphorus content 4.5 $g.kg^{-1}$ and without microbial phytase.

The procedure was chosen for the balance of phosphorus using feed phosphatase by **Angelovičová (1999)**, **Rada and Havlík (2010)**. They predict a phosphorus digestibility of plant feeds 30% in broiler chickens. A share of plant feeds is in our feed mixture starter 89.25%, in grower 93.00% and finisher 92.60%. In experimental feed mixtures was reduced phosphorus content through feed supplement *monocalcium phosphate* (MCP F Belgium) to 2.32 $g.kg^{-1}$ in feed mixtures starter and grower and to 2.31 $g.kg^{-1}$ in feed mixture finisher from need a standard for broiler chickens of non-phytate phosphorus 4.5 $g.kg^{-1}$ (control group). Nutrient and energy content per kg of feed mixtures is shown in Table 2.

In general, creating formulas of the feed mixtures for broiler chickens is predicted on average phosphorus digestibility 30% of plant feeds. The required amount of phosphorus is added in mineral form. The remaining 70% of phosphorus is therefore unavailable from plant feed, which is basically the inaccurate estimate. The percentage of digestible phytate is in the range from 0 to 60% **(Rada and Havlík, 2010)**.

The characteristics of the additive in the experimental feed mixtures

6-phytase of microbial origin used in experiment is commercial feed additive RONOZYME® P5000 (CT).

Gene encoding phytase in microscopic fungus *Peniophora lycii* was transferred to a host of microscopic fungus *Aspergillus oryzae* (DSM 14223) **(EFSA, 2010)**. *Aspergillus oryzae* vektora Phytase encoding cDNA inserts were introduced into *Aspergillus oryzae* vector pHD414. Plazmid DNA was isolated and transferred to *Aspergillus oryzae* with amdS$^+$ plasmid. Clones amd S$^+$ were detected in the medium, the phytase activity and isolated clones producing phytase. Phytases were then purified from Aspergillus oryzae supernetantov clones producing 6-phytase. cDNA nucleotide sequences *Peniophora lycii* phyA are deposited in the European Molecular Biology Laboratory database **(Classen et al., 1995)**.

6-phytáza is a product of *Aspergillus oryzae*. The safety of genetically modified *Aspergillus oryzae* was confirmed by the European Food Safety Authority. Authorization of product was characterized as a supplement improving a digestive physiology and as environmental enzyme.

Preparation of the sample for chemical analysis

At the end of the experiment the chickens were selected from each group of 6 pieces about the same body weight 1800 g. Left tibia of every chicken was removed from the carcasses immediately after the birds had been slaughtered via exsanguination of the *jugular veins* and *carotid arteries* on both sides. The tibiae were stripped of muscle and stored at +4 °C.

Calcium, phosphor and magnesium determine in tibia

The tibiae were boiled in distilled water for 5 min to facilitate removal of any remaining muscle and connective tissue, oven-dried at 105 °C for 24 h, cooled in a desiccator, weighed, and incinerated in a muffle furnace at 550 to 600 °C for 6 h in porcelain crucible. The ash was cooled in a desiccator, and weighed. The obtained ash was used to preparation of solution mineralized. In the solution of mineralized tibia was calcium and magnesium measured by atomic absorption spectrophotometer GBC Avanta at a wavelength of 422 and 285 nm and phosphorus content by JENWAY 6400 spectrophotometer at a wavelength of 666 nm.

Statistical analysis

The results are presented as average values, standard deviation and coefficient of variation. Scheffe's test was used at the significance level of $\alpha = 0.05$ to compare a difference between groups. SAS statistical package (5) was used to perform statistical analyses, verion 8.2.

RESULTS AND DISCUSSION

Approximately 50% of phytine phosphorus from feeds can be released by use of phytase **(Christensen et al., 1997)** but this value can be significantly lower since efficiency of the use of phytase depends on series of factors. In general, moderate reduction of mineral source of phosphorus, with addition of phytase in feed mixtures for broiler chickens is optimal, whereas greater reduction or complete exclusion of mineral phosphorus from diet even with increased concentrations of added enzyme phytase causes lower growth and lower consumption of food in broilers **(Lukić et al., 2005)**.

Calcium content in the tibia of chickens

Results of calcium content in the tibia of chickens are shown in Figure 1 and Table 3.

The highest calcium content in tibia of chickens was 84.86 g.kg^{-1}, which fed the feed mixtures with the reduced non-phytate phosphorus content and with the share of 6-phytase. It represents 43.46% of tibia ash. The lowest calcium content in tibia of chickens was 69.33 g.kg^{-1}, i.e. 33.09% of tibia ash, which were fed with the feed mixtures with the reduced non-phytate phosphorus content and without 6-phytase. In the control group was tibia calcium content of chickens 78.13 g.kg^{-1} (39.39%). The assessment of basic statistical characteristics shows that the lowest values of fluctuation of calcium content in tibia were at chickens fed by feed mixtures with the reduced non-phytate phosphorus content and with the share of 6-phytase. Maximum variation of calcium content in the tibia was in the control group. The differences of calcium content in tibia were statistically significant (p <0.05) between chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the share 6-phytase and control group and also chickens fed by feed mixtures with reduced non-phytate phosphorus content without 6-phytase, between chickens of control group and chickens which fed by feed mixtures with reduced non-phytate phosphorus content and without 6-phytase. Based on the investigation *in vivo* effects of dietary phytase were clarified some relationships between dietary phytate and calcium. Our findings are consistent with the current literature knowledge **(Plumstead et al., 2008)**. A problem was the absence of any clear effect of dietary phytate concentration on calcium digestibility and retention. In contrast to earlier observations, in other experiment **(Nelson et al., 1968b)** the calcium requirement of 3-wk-old chicks was increased by at least 50% when dietary phytate levels were increased from 0.0 to 25.1%. The increased calcium requirement demonstrated in their study was hypothesized to be caused by increased binding of calcium in the intestine, because 1% phytate was found to be able to bind 0.36% calcium. It has been documented that microbial phytase improves the availability of calcium in corn and soybean feed mixture **(Schöner et al., 1991; Schöner et al., 1993; Kornegay et al., 1996). Sebastian et al. (1996a)** reported that phytase supplementation of a low-phosphorus feed mixture increased the relative retention of calcium by 12.2% in chickens. This conclusion was confirmed by their later work **(Sebastian et al., 1996b)** in which microbial phytase supplementation

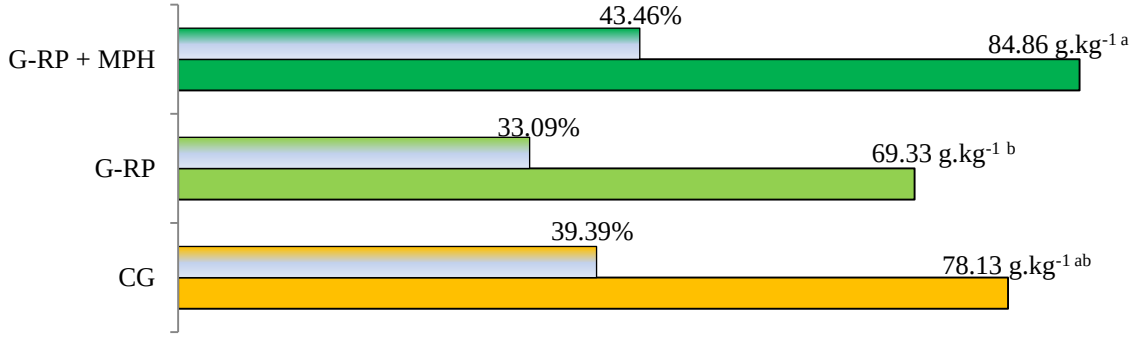

Figure 1. Mean calcium content in the tibia.
Note: CG = control group; G-RP = experimental group 1 with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP + MPH experimental group 2 with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures. Means bearing different superscripts differ statistically significant at (p <0.05).

Table 3 Basic statistical characteristics of calcium content in the tibia.

Group	n	SD (g.kg^{-1})	CV (%)
CG	6	3.16	4.05
G-RP	6	2.97	4.28
G-RP +MPH	6	1.51	1.79

Note: n = multiplicity; SD = standard deviation; CV = coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU kg^{-1}) in feed mixtures.

of a low-phosphorus feed mixture increased growth and relative retention of total phosphorus, calcium, and improved bone mineralization in chickens determined to meat production.

Phosphorus content in the tibia of chickens

Results of phosphorus content in the tibia of chickens are shown in Figure 2 and Table 4.

The highest phosphorus content in tibia of chickens was 24.09 g.kg^{-1}, which were fed by feed mixtures with the reduced non-phytate phosphorus content and with the share of 6-phytase, i.e. 12.55% of tibia ash. The lowest phosphorus content in tibia of chickens was 19.26 g.kg^{-1}, which fed the feed mixtures with the reduced non-phytate phosphorus content without 6-phytase, i.e. 9.21% of tibia ash. In the control group was tibia phosphorus content of chickens 21.88 g.kg^{-1} (11.02%).

The assessment of basic statistical characteristics shows that the lowest values of fluctuation of phosphorus content in tibia were at chickens, which were fed by feed mixtures with reduced non-phytate phosphorus content and with the share of 6-phytase. Maximum variation of phosphorus content in the tibia was in the control group. The differences of phosphorus content in tibia were statistically significant (p <0.05) between chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the share 6-phytase and control group and between chickens, which were fed by feed mixtures with reduced non-phytate phosphorus content without 6-phytase and control group. Similar the results were confirmed by **Singh et al. (2013)** They investigated the influence of different levels (%) of dietary non-phytate phosphorus fed from 0 to 20 day (0.45, 0.40, 0.35, 0.30, 0.25, compared with feeding 0.20 non-phytate phosphorus with and without 500 FTU of phytase per kg of feed mixture) and from days 21 to 36 of age (0.414, 0.364, 0.314, 0.264, 0.214, compared with 0.164 non-phytate phosphorus with and without 500 FTU of phytase per kg of feed mixture) were evaluate using a total of 588 day-old commercial broiler chicks. Each treatment was replicated four times in a completely randomized design. In conclusion, the results showed that the combination of a lower level of non-phytate phosphorus and phytase may be used to increase dietary phosphorus utilization, without severe changes in performance and bone quality. The same conclusion also have found **Karimi et al. (2011)** where results experiment showed that the combination of a lower level of non-phytate phosphorus and phytase may be used to increase dietary phosphorus utilization, without severe changes in performance and bone quality. In fact, the ash content is closely related to phosphorus concentration. Enhancement of ash percentage and phosphorus content of tibia with application of either source of phytase suggests that phytase can increase mineral deposition in phosphorus deficient diet, which is in agreement with the results described in the scientific literature by **Viveros et al., 2002; Sebastian et al., 1996b**. **Ahmad et al. (2000)** observed that phytase in a low-phosphorus feed mixture increased the phosphorus retention by 20.4% and 12.73% as compared to a negative control and normal phosphorus diet supplemented with phytase, respectively.

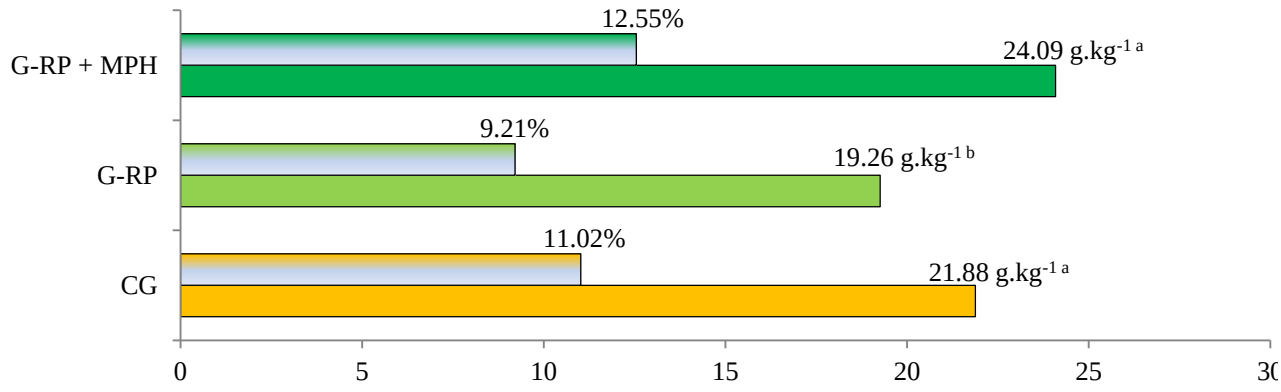

Figure 3. Mean phosphorus content in the tibia.
Note: CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures. Means bearing different superscripts differ statistically significant at p <0.05.

Table 4 Basic statistical characteristics of phosphorus content in the tibia.

Group	n	SD (g.kg^{-1})	CV (%)
CG	6	2.16	5.91
G-RP	6	1.14	4.05
G-RP +MPH	6	0.49	2.03

Note: n = multiplicity; SD = standard deviation; CV = coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures.

Magnesium content in the tibia of chickens

Results of calcium content in the tibia of chickens are shown in Figure 3 and Table 5.

The highest magnesium content in tibia of chickens was 1.27 g.kg^{-1}, which fed the feed mixtures with reduced non-phytate magnesium content and with a share of 6-phytase, i.e. 0,609% of tibia ash. The lowest magnesium content in tibia of chickens was 1.01 g.kg^{-1}, which fed the feed mixtures with reduced non-phytate magnesium content without 6-phytase, i.e. 0.47% of tibia ash. In the control group was tibia magnesium content of chickens 1.21 g.kg^{-1} (0.608%). The assessment of basic statistical characteristics shows that the lowest values of fluctuation of magnesium content in tibia were at chickens feeding the feed mixtures with reduced non-phytate magnesium content. Maximum variation of magnesium content in the tibia was at chickens feeding of feed mixtures with reduced non-phytate phosphorus and with a share 6-phytase. The differences of magnesium content in tibia were statistically significant (p *<0.05*) between chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the share 6-phytase and control group and between chickens, which were fed by feed mixtures with reduced non-phytate phosphorus content without 6-phytase and control group.

Our findings are in accordance with the literature data that shows that supplemental phytase increases the mineral content in tibia **(Sohail and Roland, 1999)**.

However, we were unable to find any literature data on the phytase influence on magnesium content in broiler tibia. It is interesting to note that contents of magnesium was statistically higher in broilers fed by feed mixtures with reduced phosphorus content and 6-phytase addition in comparison with the feed mixtures without 6-phytase or with the commercial feed mixtures. In contribution to the application of phytase are results of the latest studies indicating the positive effect on digestibility and availability not only of calcium, phosphorus, but also the magnesium and other minerals and organic matters with the use of phytase.

Also, because of almost universal presence of phytate in grain cereals and oil meals, phytase represents enzyme with the highest potential among other enzymes which can be used in poultry production **(Lukić et al., 2009)**.

Research of the issues relating minerals and another substances and application of phytase in broiler production are equally important and are mutually complementary **(Huyghebaert et al., 2005)**.

These researches are especially important for the purpose of solving issues and problems which are very frequent and induced by rapid growth of broilers (bone deformations, predisposition to breaking and infections, etc.), as well as poor quality of the product, i.e. bone firmness, which is considered as serious disadvantage in the processing industry, because of frequent breaking of bones during handling, transportation and automatic broiler processing procedures.

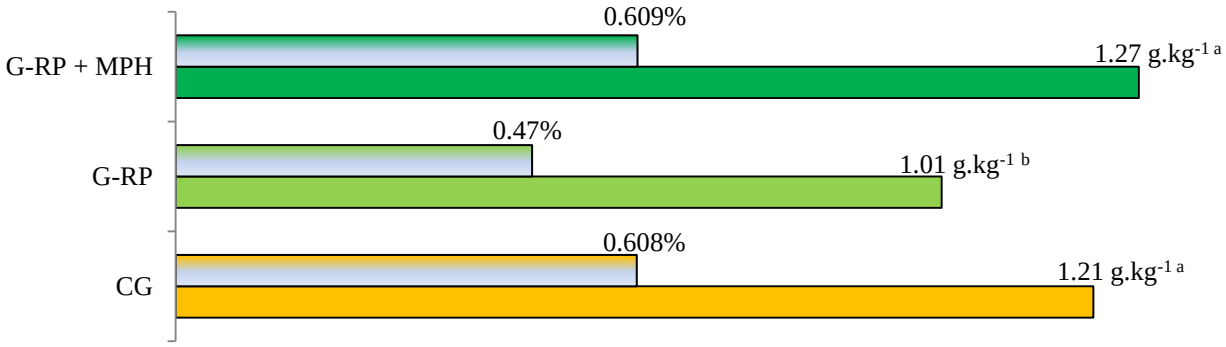

Figure 4. Mean magnesium content in the tibia.
Note: n = multiplicity; SD = standard deviation; CV= coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixture; Means bearing different superscripts differ statistically significant at p <0.05.

Table 5 Basic statistical characteristics of the magnesium content in the tibia.

Group	n	SD (g.kg^{-1})	CV (%)
CG	6	0.06	4.57
G-RP	6	0.02	2.41
G-RP+MP	6	0.08	6.61

Note: n = multiplicity; SD = standard deviation; CV = coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures.

CONCLUSION

The target of our research was 6-phytase of microbial origin and its effectiveness in relation to the tibia mineralization of chickens kept for meat production. In the experiment, we used a 6-phytase of microbial origin, which is the product of a microscopic fungus *Aspergillus oryzae*. We used the final type of hybrid combination Cobb 500. The experimental feed mixtures had reduced non-phytate phosphorus content 2.32 g.kg^{-1} versus 4.50 g.kg^{-1} control feed mixtures. We used the feed mixtures of soybean-cereal type (starter, grower and finisher). Based on the experiment, we came to the following conclusions that statistically significant (p <0.05) the highest content of calcium, phosphorus and magnesium was in the tibia of chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the 6-phytase. Based on the experimental results, we can conclude that it has been confirmed scientific hypothesis, which we have established. Application of 6-phytase of microbial origin had a positive effect on the utilization of phytate phosphorus in feed, which resulted tibia mineralization.

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