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Agricultural Science and Technology
Faculty of Agriculture, Trakia University
Student's campus, 6000 Stara Zagora
Bulgaria
Telephone.: +359 42 699330
+359 42 699446
http://www.uni-sz.bg/ascitech/index.html

Technical Assistance:

Nely Tzvetanova
Telephone.: +359 42 699446
E-mail: ascitech@uni-sz.bg

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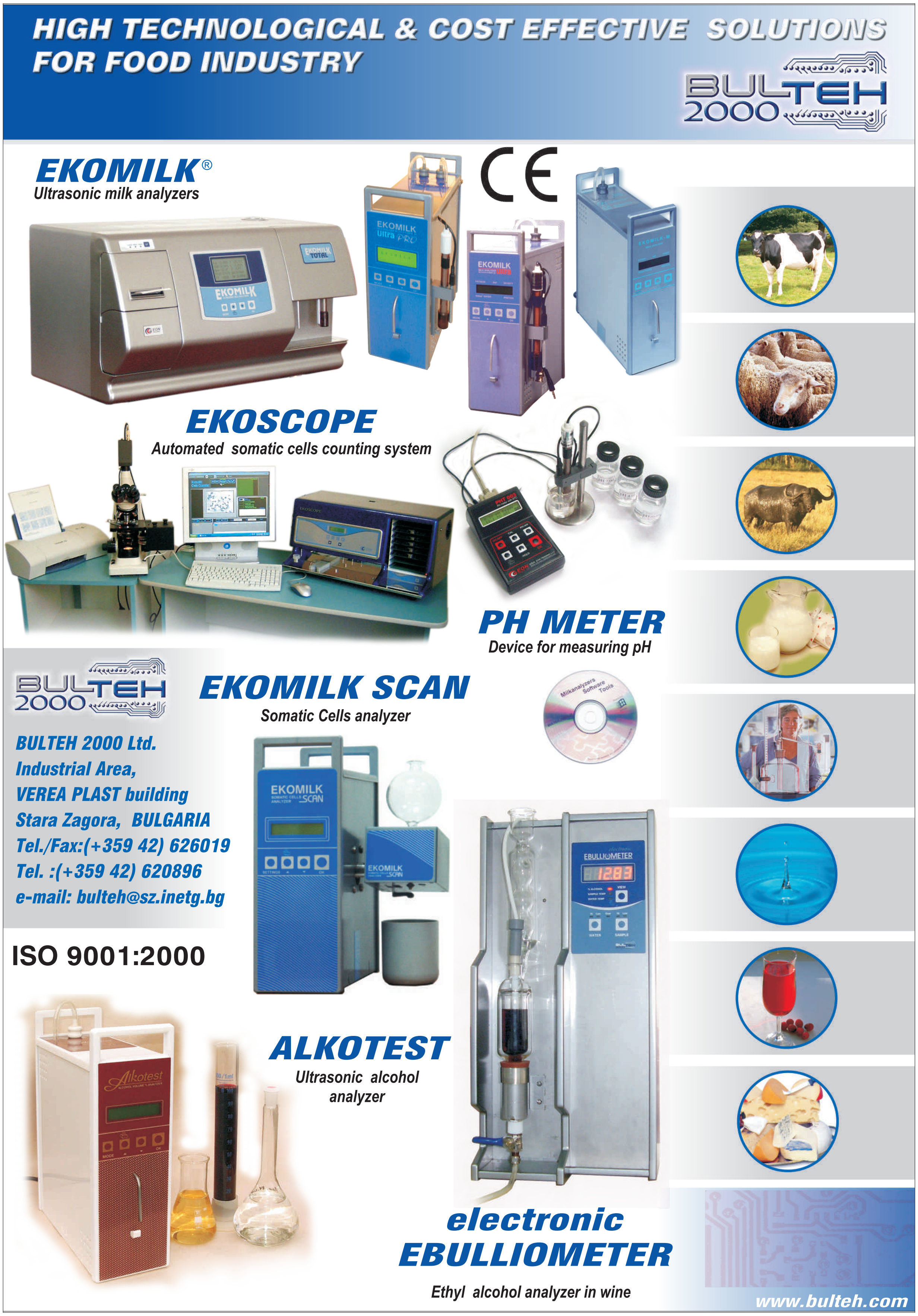

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Comparison of the effects of Natuphos® 5 000 G and Optiphos® phytases utilization in broiler chicken feeding with maize-soybean diets

P. Valkova*

Department of Physiology, Morphology and Nutrition of Animals, Faculty of Agriculture, Trakia University, 6000 Stara Zagora , Bulgaria.

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Abstract. *An experiment with 96 male broiler chickens (hybrid ROSS 308), distributed in 3 groups, 32 chickens each, was conducted. Each group consisted of 4 subgroups, 8 chickens each. Live weight, the retention of nitrogen, calcium and phosphorus in the chickens' organisms were monitored at the age of 1, 11, 29 u 42 days. The birds received 3 types of compound feed. The control group birds were fed a maize-soybean diet without phytase and the birds from the experimental groups were fed a diet, supplemented by Natuphos 5000G or Optiphos phytase. Diets for all growing periods were balanced as to CP, Ca and available phosphorus. In the phytase groups the total phosphorus quantity was decreased from 0.81% to 0.66% for the starter period, from 0.76 to 0.61% for the grower one and from 0.72% to 0.56% for the finisher period. The obtained results showed that phytase supplementation to the maize-soybean diet improved the utilization of nitrogen, calcium and phosphorus and the tibia ash quantity in broiler chickens during the 0-42–day-period. For the whole fattening period broilers fed maize-soybean diets supplemented by Natuphos or Feedophyt phytase had a reliably higher live weight by 10.95% and 11.93%, respectively, in comparison with the group without phytase. The forage consumption for 1 kg weight gain in chickens decreased by 17.02% ($p \le 0.01$) on average as a result of the use of exogenous phytase. By supplementing phytase to a maize-soybean diet the quantity of dicalcium phosphate decreased by 34% (0-42 days) thus reducing the price of compound forage.*

Keywords: phytase, broilers, nutrient, mineral, retention, tibia bone

Abbreviations: CP–crude protein, aP– available phosphorus, Ca–calcium, P–phosphorus, tP–total phosphorus, PP–phytin phosphorus, Hcl–hydrochloric acid, N–nitrogen, PU–phytase units

Introduction

The pollution of the environment by the phosphorus of the poultry feces is one of the main problems in contemporary poultry breeding. The unassimilated phosphorus is excreted along with the feces and is accumulated in the soil, and then it passes into the underground waters and causes eutrophication of the water basins. Phosphorus (P) quantity in the diets, its availability and absorption from the plant sources are disputable in the feeding of monogastric animals. About 60-70% of the total P in cereals and oleaginous seeds is found under the form of phytates (Cheryan, 1980).These compounds can be hydrolyzed only under the influence of the enzyme phytase. The insufficient quantity of phytase in the avian digestive tract makes the phytin less available for them (Ravindran et al, 1995).

It has been established that the phytin P availability for birds varies between 0 and 50% (Edwards1993). Other factors influencing the availability of the phytin P are the Ca level in the diet, the Ca-total P correlation in the diets, nutrient type, the level of inorganic phosphorus, the age, sex, genotype of animals and vitamin ”D_3” level (Edwards 1993). The phytase phosphorus content in the forage components is different. It is 72% in the maize, 69% in the wheat, and in the soybean meal it is 60% out of the total phosphorus (Eeckhout and De Paepe, 1994).

The wheat contains endogenous phytase activity which has a positive influence on the hydrolysis of the phytin phosphorus thus increasing the phosphorus availability for the animals. The different forages contain different endogenous phytase activity - wheat (915-1581 PU kg^{-1}), maize (0-46 PU kg^{-1}), barley (408-882PU kg-1), soybean meal (44%СП), (0-120 PU kg^{-1}), (Eeckhout and De Paepe, 1994). A maize-soybean diet contains 6 U endogenous phytase/kg whereas the wheat-soybean - 410 U endogenous phytase /kg (Perez et al., 2006).

A bigger percentage of available phosphorus has been established in the wheat and triticale - 50% и 46%, respectively, compared to maize and the soybean meal which contain less than 15% available phosphorus (Cromwell and Coffey, 1991). In his investigations with broiler chickens in our country Tanin (1987) has also established a significantly higher biological availability of phosphorus from wheat (76.81%) compared to maize (67%). According to the same author the soybean meal has 79% biological availability of phosphorus, and the sunflower meal – 49%. Most forages have a low content of endogenous phytase activity and because of this the phytin phosphorus hydrolysis is low. That imposes the use of exogenous phytases in the compound feed for monogastric animals.

The contemporary thorough researches of Zyla et al. (2000); Snow et al. (2003) show that by using exogenous phytase in the poultry diets the utilization of nutrients is improved and the phosphorus excretion is decreased. A lot of microorganisms such as bacteria and molds produce exogenous phytase. On the basis of the phosphate group which is hydrolyzed, the first two phytase types are known: 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26), (Kies et al., 2001). Microorganisms produce 3-phytase which is mainly used

* e-mail: pvalkova72@gmail.com

in the monogastric animal feeding, whereas 6-phytases are usually found in plants (Reddy et al., 1982). The microbial phytase optimum activity is manifested from pH 2 to pH 6 whereas the plant phytase optimum activity is about pH 5 (Selle et al., 2000). The advantages of microbial phytases such as optimal pH, thermostability, resistance to the digestive proteolitique enzymes make them more suitable as a supplement to the food for monogastric animals in order to increase the phytin P availability in comparison to plant phytases (Wyss et al., 1998).

The aim of this study is to establish and compare the effects from the supplementation of two types of exogenous phytases - Natuphos® 5000G and Optiphos® in the feeding of broiler chickens with maize-soybean based diets.

Material and methods

Birds and Feeding Treatments

An experiment with 96 male broiler chickens (ROSS 308) distributed in 3 groups, 32 birds each, was conducted. Each group included 4 subgroups of 8 chickens. The experiment plan was as follows:

- Group I - maize-soybean diet
- Group II - maize-soybean diet supplemented by Natuphos phytase
- Group III - maize-soybean diet supplemented Optiphos phytase

The chicks were raised in 4-floor cell batteries from 0 to 42 days. All chicks were given water and feed ad libitum. A 3-phase feeding plan was accomplished - starter (day 1-10), grower (day 11-28), finisher (day 29-42). The heat and light programs complied with the ROSS 308 hybrid requirements. The exogenous phytase was included in the experimental groups' diet in quantity of 0.01% for the three experimental periods – starter, grower and finisher.

Natuphos 5000®G is a pellet phytase enzyme, produced by BASF, Germany. It is an *Aspergillus niger* 3-phytase containing the active substance myo-Inositol hexaphosphat-Phosphohydrolase and its activity is 5000 PU/g. The microbial phytase Natuphos® has a wide pH optimum – between 2 and 5.

Optiphos® is a ferment preparation, produced by Biovet, Peshtera town. It contains a Feedophyt 5000 3-phytase, made of the Penicillium canescens strain, maize, starch and magnesium sulfate as rarefying and stabilizing substances. Its color ranges from beige to brown and its phytase activity is 5000 PU/g. According to the company-producer the Optiphos® maximum activity reaches 85°C.

During the experiment the feed consumption and the weight development of chicks were controlled by individual weighing on day 1, 11, 29 and 42. The health status and the mortality rate of the chicks were monitored every day. All experiments conducted comply with the requirements of the European convention for the protection of vertebrate animals, used for experimental and other purposes dated 16.05.1986 – Strasbourg; the Law for the protection of animals in the Republic of Bulgaria effective as of 31.01.2008, promulgated in the State Gazette issue 13 dated 08.02.2008 and the Law for veterinary medical activities, chapter VII, section II „Experimental Animals" effective as of 02.05.2006, with the latest amendments, published in the State Gazette issues 13 and 36 from 2008.

Analysis

In order to determine bone mineralization 5 chickens from each group were randomly chosen on days 21 and 42. Their left tibia bones (tibiotarsus) were removed post mortem. The tibias were cleaned from the soft tissues and cartilages, dried at 105°C for 24h to constant weight and ashed in a muffle furnace at 535°C for 18h. The ash was dissolved in concentrated HCl to determine the mineral substances content. Calcium was determined by the permanganate method (Sandev, 1964), and phosphorus by the colorimetric method (Gerike and Kyrmis, 1965). The total chemical content of forages and of autoclaved chickens was established according the Veende method (BDS-ISO, 1997, 1999, 2001, 2002, 2007). Data from chemical analyses were statistically processed by a program package „Statistica 6" of Windows.

Determination of nitrogen, calcium and total phosphorus retention

Five chicks from each group aged 1, 29 and 42 days were autoclaved following the method, described by Kotarbinska and Keilanovski, (1967). The chemical content and balance of nitrogen, phosphorus, calcium and total phosphorus in the autoclaved samples were determined.

Results and discussion

Data about the composition and nutrient value of compound forages used for the chicks from day 0 to 42 are given in Table 1. Diets were balanced according to the hybrid requirements. The protein level in the starter, grower and compound feeds was 22, 21 and 19%, respectively. Calcium content during the three growing periods was 1.00; 0.90 и 0.85%, respectively. The only difference regarding the combined forage composition was in the total phosphorus quantity. By including phytases into the compound feeds for the experimental groups the quantity of dicalcium phosphate, added during the starter period, decreased from 2.28% to 1.70%, by which the total phosphorus fell from 0.81% to 0.66% and the available phosphorus quantity remained unchanged. The changes during the grower and finisher periods were analogical.

Weight development of chicks

The effect of the phytase supplementation to the maize-soybean based diets was manifested by the increase in the chicks'live weight (Table 2). The phytase effect was established even as early as during the first ten days. On day 11 the chicks that were fed compound feed supplemented by Natuphos or Feedophyt phytases increased their live weight by 19.28% and 31.67%, respectively, compared to the control group. The Feedophyt phytase had a better effect - by 10.39% - on the live weight of the chicks until their 11th day of age in comparison with the Natuphos phytase ($p \leq 0.01$). The experimental groups during the grower period also had a higher weight gain compared with the group fed without phytase. For the period 11-28 day the chicks from the group fed Natuphos had live weight by 12% higher live weight and those fed Feedophyt by 13.52% higher live weight than the chicks from group I. A statistically significant difference between the groups fed a different phytase source in relation to the broiler chickens live weight during the grower period was not established. During the 29-42-day period the supplementation of Natuphos phytase to the maize-soybean diet increased the chickens' live weight by 10.75% ($p \leq 0.01$) and the supplementation of Feedophyt phytase - by 11.71% ($p \leq 0.001$) in comparison with the control group without phytase. A statistically significant difference was not established in comparison with the effect of both phytases on the live weight of chickens from groups II and III during the finisher period.

The phytase effect on the chickens' weight gain is the strongest during the starter period when the growth is the most intensive

Table 1. Composition and nutritive value of maize-soybean diets, %

Components, %	Starter			Grower			Finisher		
	I -phy tase	II +Natu phos	III +Opti phos	I -phy tase	II +Natu phos	III +Opti phos	I -phy tase	II +Natu phos	III +Opti phos
Maize	59.18	58.99	58.99	52.89	55.92	55.92	60.91	62.76	62.76
Soybean oil meal	29.90	32.80	32.80	36.30	35.30	35.30	60.91	29.60	29.60
Fish meal	5.00	2.80	2.80	-	-	-	-	-	-
Sunflower oil	2.30	1.90	1.90	6.50	5.00	5.00	4.60	4.20	4.20
VMP	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.35	0.35	0.35	0.30	0.30	0.30
Limestone	0.30	0.70	0.70	0.47	0.75	0.75	0.47	0.77	0.77
Dicalcium phosphate	2.28	1.70	1.70	2.65	1.80	1.80	2.50	1.60	1.60
DL- Methionin	0.19	0.21	0.21	0.25	0.25	0.25	0.20	0.20	0.20
L- Lysine	0.05	0.08	0.08	0.06	0.08	0.08	0.01	0.04	0.04
Treonine	-	0.01	0.01	0.03	0.04	0.04	0.01	0.02	0.02
Natuphos 5000 phytase	-	0.01	-	-	0.01	-	-	0.01	-
Feedophyt phytase	-	-	0.01	-	-	0.01	-	-	0.01
Total:	100	100	100	100	100	100	100	100	100
Compound feed content									
Metabolic energy MJ/kg	11.90	11.90	11.70	12.50	12.50	12.60	12.80	12.80	12.80
Crude protein %	22.00	22.00	22.10	21.00	21.00	21.10	19.00	19.00	19.10
Digestible lysine, %	1.16	1.16	1.17	21.00	1.09	1.10	0.92	0.92	0.93
Digestible methionin+cystin, %	0.81	0.81	0.82	0.81	0.81	0.82	0.72	0.72	0.73
Calcium, %	1.00	1.00	1.01	0.90	0.90	0.94	0.85	0.85	0.89
Phosphorus, total, %	0.81	0.66	0.66	0.76	0.61	0.61	0.72	0.56	0.56
Phosphorus, available, %	0.50	0.50	0.50	0.45	0.45	0.45	0.42	0.42	0.41

Table 2. Effect of phytase on body weight of broiler chickens.

Body weight, (g).								
Groups	n	1day	n	11day	n	28 day	n	42 day
		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$
I - phytase	32	40.63 ± 0.54	32	156.41 ± 2.95 $^{a\,b}$	20	1119.50 ± 27.58 $^{c\,d}$	15	2083.67 ± 59.50 $^{e\,f}$
II + Natuphos	32	40.63 ± 0.54	32	186.56 ± 3.89 $^{a\,n}$	22	1253.86 ± 22.21^{c}	17	2307.65 ± 35.36 ^{e}
III + Optiphos	32	40.63 ± 0.54	32	205.94 ± 5.49 $^{b\,n}$	22	1270.91 ± 25.08 ^{d}	17	2327.65 ± 71.32 ^{f}

The differences among the groups are statistically proven at the same letters. a, b, c, d, f - $p \leq 0{,}001$, e, n - $p \leq 0{,}01$

compared to the grower and the finisher periods. The phytase effect decreases with the chickens' age. Zyla et al., (2000); Augspurger et al., (2003) have established a similar dependence. For the whole growing period the Natuphos or Feedophyt phytase supplementation led to a significant increase in the chickens' live weight by 10.95% and 11.93%, respectively.The results of our investigation are analogical to those, established by Dilger et al., (2004) who have established a significant increase in the weight gain of chickens aged 1 - 42 d fed maize-soybean diets with microbial phytase.

The phosphorus reduction in maize-soybean diets without supplemented phytase had a negative influence on the weight gain of chickens aged 0-6 wk (Qian et al., 1996a; Punna and Roland, 1999). The weight gain of chickens aged 0-6 wk fed low phosphorus diets without phytase, was by 18.7% lower and the feed consumption was decreased to 5.9% in comparison with birds, fed diets with normal phosphorus level (Viveros et al., 2002). The same authors (Viveros et al., 2002) have established that the supplementation of 500 U phytase/kg forage improves the weight gain of chickens aged 0-3 w by 6.7% and of those aged 0-6 w – by 6.1%, whereas the feed consumption has increased only in the period 0-3 w by 5.3%.

Feed utilization.

During the starter period the phytase fed groups had lower forage consumption – by 7.20% and 13.60 %, respectively, compared to the control group (Table 3). A statistically significant difference on the food conversion during 0-10-day period had only the Feedophyt phytase in comparison with the group without

Table 3. Effect of phytase on feed convertion ratio of broiler chickens.

	Feed efficiency, kg / kg LW			
Groups	0-10 day	11-28 day	29-42 day	0-42 day
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
I - phytase	1.25 ± 0.03 [a]	1.61 ± 0.10 [b c]	2.24 ± 0.27	1.88 ± 0.16 [d e]
II + Natuphos	1.16 ± 0.08	1.36 ± 0.03 [b]	1.82 ± 0.08	1.56 ± 0.06 [d]
III + Optiphos	1.08 ± 0.03 [a]	1.39 ± 0.03 [c]	1.82 ± 0.10	1.56 ± 0.05 [e]

The differences among the groups are statistically proven at the same letters. b, c - $p \leq 0.001$ ** a, d, e - $p \leq 0.05$ *

phytase at a significance level $p \leq 0.05$.

For the grower and finisher periods the trend of lower forage consumption in chickens fed phytase, compared to those of the control group was kept. With chickens aged 11-28 d the differences between the control and experimental groups in relation to the forage conversion were statistically significant at $p \leq 0.01$. In spite of the fact that the groups fed phytase had a lower forage consumption for 1 kg of weight gain (by 18.75%) than the group without phytase, the established differences were not statistically proven. For the whole fattening period forage consumption for 1 kg weight gain was on average by 17.02% lower in the groups fed phytase than that of the control group ($p \leq 0.05$). For the 0-42-day period statistically significant differences of the forage consumption between the groups fed different phytase types were not established. A better effect from the exogenous phytase supplementation on the utilization of the maize-soybean diets has also been established in research made by Cowieson et al. (2006).

Nitrogen utilization.

In Table 4 we can see that in the 1-28-day period the control group ingested by 3.23% more nitrogen than group II and by 0.53% more nitrogen than group III. The obtained results are explained by the bigger forage consumption of the control group during this period. The use of phytase led to bigger nitrogen retention in the experimental groups. In comparison with the control group the chickens fed Natuphos or Feedophyt phytase retained more nitrogen – by 20.62% and 13.55%, respectively. Statistically significant differences were not established in comparing the effect of both phytases on nitrogen utilization by chicks aged 0-28 d. For the finisher period statistically significant differences were not established in the quality of the deposited nitrogen in the chickens from the experimental groups in comparison with the control one - 33.38g (group II) and 32.67g nitrogen (group III), against 29.54g (group I). Data about nitrogen retention for this period, which ranges between 57.70% and 55.97% for the phytase groups against 46.90% for the group without phytase, were analogical.

For the whole growth period the chickens deposited in their bodies 69.07g and 66.28g nitrogen, respectively, whereas those from the control group deposited 59.13g nitrogen. Phytase supplementation to the maize-soybean diets improved nitrogen utilization by 24.10% ($p \leq 0.01$) in chickens fed Natuphos phytase, and in the group fed Feedophyt - by 16.62% in comparison with the control group. For the period mentioned the Natuphos phytase effect on the nitrogen utilization by the chickens was by 7.48% higher than that of the Feedophyt phytase.

Ravindran et al. (1998a); Cowieson et al. (2004) have experimentally proven that in the phytate hydrolysis through phytase more protein and amino acids are released thus increasing their digestibility and absorption in monogastric animals. Sebastian et al. (1997) have established sex differences in protein utilization by the use of phytase in maize-soybean diets. According to the authors the

Table 4. Effect of phytase on utilization of nitrogen of broiler chickens.

Indices/Groups	I - phytase	II + Natuphos	III + Optiphos
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
0-28 day			
N ingested, g.	56.51 ± 1.87	54.45 ± 0.39	56.21 ± 1.12
N deposited in carcass, g.	29.59 ± 1.03 [a b]	35.69 ± 0.89 [a]	33.60 ± 0.21 [b]
N retention, %	52.70 ± 3.59 [c d]	65.55 ± 1.66 [c]	59.84 ± 1.09 [d]
29-42 day			
N ingested, g.	64.71 ± 5.51	58.24 ± 2.64	58.64 ± 2.66
N deposited in carcass, g.	29.54 ± 1.07	33.38 ± 0.25	32.67 ± 1.95
N retention, %	46.90 ± 4.97	57.70 ± 2.94	55.97 ± 3.58
0-42 day			
N ingested, g.	121.22 ± 7.25	112.69 ± 2.77	114.85 ± 2.64
N deposited in carcass, g.	59.13 ± 1.58 [e h]	69.07 ± 1.03 [e]	66.28 ± 2.12 [h]
N retention, %	49.51 ± 4.17 [g]	61.44 ± 2.23 [g]	57.74 ± 1.63

The differences among the groups are statistically proven at the same letters. a,c,e-$p \leq 0.001$ b,h,g-$p \leq 0.01$ d-$p \leq 0.05$

phytase effect is present only in female individuals.

Calcium utilization.

Regardless of the fact that the quantity of calcium ingested by the different groups was the same, the quantity of calcium deposited in the chickens' bodies depended on the phytase content in the compound feed (Table 5). During 0-28-day period in the Natuphos fed group the deposited calcium (6.23g) was by 22.16% more than that of the control group ($p \leq 0.001$). Broiler chickens fed Feedophyt phytase deposited 7.11g calcium which was by 39.41% more than that in the group without phytase. For the same period the Feedophyt phytase had a better effect on calcium retention in chickens' organisms in comparison with the Natuphos, the differences being statistically proven at $p \leq 0.001$. Data about calcium retention showed that from day 0-28 II group chicks utilized 42.45% from the calcium ingested with the food, those from group III - 45.35%, and the control group - 33.77%. Differences between the experimental groups and the control one were statistically significant at $p \leq 0.001$.

During the finisher period the Natuphos phytase had a better effect on calcium utilization by 9.50%, and the Feedophyt phytase - by 29.57% in comparison with the group without phytase.A better effect on the calcium deposition in chickens aged 29-42 days was established in the group fed Feedophyt phytase - they had by 18.33% more body calcium in comparison with the group fed Natuphos phytase. The III group broiler chickens utilized the calcium by 33.77% ($p \leq 0.05$) better than those from group I, and the II group broiler chickens did the same by 19.11%. In comparing the results from both phytases the Feedophyt phytase had by 12.31% better effect on calcium retention in comparison with the Natuphos phytase in chickens during the finisher period.

The supplementation of phytase to the maize-soybean diet made the quantity of the deposited calcium increase for the whole fattening period - 10.69g (group I), 12.34g (groupII) and 14.34g (group III). In comparing the effect of both phytases regarding calcium deposition in the chickens'bodies during 0-42-day-period the Feedophyt phytase had by 16.21% better effect than the Natuphos phytase ($p \leq 0.001$). During the whole fattening period chickens from the groups fed compound feed with phytase had a higher calcium utilization in comparison with the control group. Group II had higher calcium utilization by 22.62% and group III – by 34.44%, in comparison with the group without phytase. Statistically significant differences on the calcium retention between the phytases used during the whole fattening period (0-42d) were not established. The phytase positive effect on the absorption of mineral substances from maize-soybean diets for monogastric animals has been reported from numerous experiments of Pallauf et al., (1992). According to Viveros et al., (2002) the digestibility and retention of phosphorus and calcium were increased in chickens, fed low phosphorus diet supplemented by phytase, in comparison with adequate phosphorus diets without phytase.

Table 5. Effect of phytase on utilization of calcium of broiler chickens.

Indices/Groups	I - phytase	II + Natuphos	III + Optiphos
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
0-28 day			
Ca ingested. g	15.21 ± 0.50	14.68 ± 0.11	15.70 ± 0.31
Ca deposited in carcass. g	5.10 ± 0.18 $^{a\,b}$	6.23 ± 0.15 $^{a\,c}$	7.11 ± 0.04 $^{b\,c}$
Ca retention. %	33.77 ± 2.28 $^{d\,e}$	42.45 ± 1.07 ^{d}	45.35 ± 0.83 ^{e}
29-42 day			
Ca ingested. g	18.09 ± 1.54	16.28 ± 0.74	17.08 ± 0.77
Ca deposited in carcass. g	5.58 ± 0.21^{h}	6.11 ± 0.05 ^{g}	7.23 ± 0.44 $^{h\,g}$
Ca retention. %	31.71 ± 3.36 ^{j}	37.77 ± 1.92	42.42 ± 2.10 ^{j}
0-42 day			
Ca ingested. g	33.31 ± 2.01	30.96 ± 0.77	32.77 ± 0.77
Ca deposited in carcass. g	10.69 ± 0.29 $^{n\,m}$	12.34 ± 0.18 $^{n\,k}$	14.34 ± 0.46 $^{m\,k}$
Ca retention. %	32.58 ± 2.75 $^{l\,o}$	39.95 ± 1.45 ^{l}	43.80 ± 1.36 ^{o}

The differences among the groups are statistically proven at the same letters. a, b, c, d, e, h, m, k, p - $p \leq 0.001$
n, o - $p \leq 0.01$ j, g, l - $p \leq 0.05$

Phosphorus utilization.

For all growing periods the quantity of the total phosphorus ingested was reliably less in the groups fed phytase in comparison with the control group regardless of the fact that all the diets were balanced as to the available phosphorus content. This is due to the smaller quantity of dicalcium phosphate in the compound feed for the experimental groups as a result of the phytase use. The results from the phosphorus balance (Table 6) show that by supplementing 0.01% phytase to the diets of groups II and III the quantity of the deposited phosphorus in the chickens' organisms increased to 5.60g (group II) and 5.40g (group III), against 4.55g phosphorus for group I. This was by 23.08% and by 18.68% more in comparison with the control group. The quantity of the deposited phosphorus compared to the ingested one for the period mentioned was also reliably higher in the groups, fed phytase, and for groups II and III it was 56.44% and 52.99%, respectively, against 35.78% for group I ($p \leq 0.001$).

During the finisher period a statistically significant difference on the phosphorus deposition in chickens' organisms was not established - 4.95g (group I); 5.01g (group II) and 5.05g (group III) phosphorus in the body. However, the percentage of the deposited phosphorus compared to that ingested with food was essentially

Table 6. Effect of phytase on utilization of phosphorus of broiler chicke

Indices/Groups	I - phytase	II + Natuphos	III + Optiphos
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
0-28 day			
P ingested. g	12.80 ± 0.42 $^{a\,b}$	9.92 ± 0.07 ^{a}	10.19 ± 0.20 ^{b}
P deposited in carcass. g	4.55 ± 0.15 $^{c\,d}$	5.60 ± 0.14 ^{c}	5.40 ± 0.03 ^{d}
P retention. %	35.78 ± 2.41 $^{e\,i}$	56.44 ± 1.41 ^{e}	52.99 ± 0.96 ^{i}
29-42 day			
P ingested. g	15.33 ± 1.30 $^{h\,j}$	10.73 ± 0.49 ^{h}	10.75 ± 0.49 ^{j}
P deposited in carcass. g	4.95 ± 0.18	5.01 ± 0.04	5.05 ± 0.31
P retention. %	33.18 ± 3.51 $^{g\,k}$	46.98 ± 2.39 ^{g}	47.12 ± 2.33 ^{k}
0-42 day			
P ingested. g	28.12 ± 1.70 $^{m\,n}$	20.65 ± 0.51 ^{m}	20.94 ± 0.48 ^{n}
P deposited in carcass. g	9.50 ± 0.25 $^{o\,p}$	10.60 ± 0.16 ^{o}	10.45 ± 0.32 ^{p}
P retention.%	34.29 ± 2.89 $^{q\,s}$	51.48 ± 1.87 ^{q}	49.95 ± 1.52 ^{s}

The differences among the groups are statistically proven at the same letters a, b, c, d, e, i, m, n, q, s - $p \leq 0.001$
h, j, g, k, o - $p \leq 0.01$ p - $p \leq 0.05$

different from the group without phytase – 46.98% and 47.12% against 33.18%, which, on average, was by 41% higher in the phytase fed groups in comparison with the control group.

For the whole fattening period broiler chickens from the phytase fed groups ingested by 26.56% (group II) and by 25.53% (group III) less phosphorus with the forage but deposited by 11.58% and 10.00% more phosphorus in the body compared to group I broilers. The increased phosphorus deposition in chickens' organisms was due to the phytase supplementation which probably influences the metal-phytate complexes and released more phosphorus from the maize-soybean feeds. Thus the phosphorus became available for chickens' organisms. As a result from the phytase use in compound forages a significant improvement in phosphorus utilization by chicks aged 0-42 days was observed too. In the Natuphos group this increase was by 50.13%, and in the Feedophyt group - by 45.67% in comparison with the control group. In comparing the effect of both phytases on phosphorus utilization from the maize-soybean diets for the whole fattening period of chickens (0-42 days) no statistically significant differences were established. Perez et al., (2006) have obtained similar results. According to the same researchers the phytase supplementation to a maize-soybean diet reliably improves the availability of the total phosphorus, phytate phosphorus and calcium.

Mineral analysis of left tibia.

Phytase use allows the reduction of total phosphorus in compound forages without having a negative effect on the bone mineralization in chickens (Sebastian et al., 1996; Leeson et al., 2000). One of the indices, used in the control of the sufficient quantity of minerals, is the strength of tibia bone. Literature data about the tibia mineral content are contradictory. Some authors think that the quantity of mineral substances in the whole tibia varies (Sohail and Roland, 1999), whereas according to some others this quantity does not change and only the mineral content in the tibial head is influenced by the phytase use (Sebastian et al., 1996).

In our experiment the phytase supplementation to maize-soybean compound feed allowed the decrease in total phosphorus by 18.52% for the starter period, by 19.74% for the grower one and by 22.22% for the finisher period without interfering with the diet balance regarding the available phosphorus. In 21-day-old chicks the tibia ash quantity increased by 8.63% after the Natuphos phytase use and by 6.16 % - in the Feedophyt phytase fed group in comparison with the group without phytase (Table 7). Dilger et al. (2004); Onyango et al. (2005) have obtained results similar to ours. Some researchers such as Rama Rao et al. (1999), have not established an increase in the tibia ash quantity as a result of phytase supplementation. Phytase supplementation had a considerable influence regarding calcium quantity in the tibia ash. In group II calcium ash quantity was 31.07%, and in group III - 31.57%. These values were reliably higher in comparison with those of the control group ($p \leq 0.001$). At the age of 21 days the broilers fed maize-corn diets containing 0.66% total phosphorus, supplemented by phytase, deposited by 10-11% on average more calcium in tibia bones. In 0-3 -week-old chickens the phytase use in maize-corn diets increased the phoshorus content as percentage of the tibia ash by 6.99% in the Feedophyt ($p \leq 0.05$) fed group and by 2.81% - in the Natuphos fed one in comparison with the group without phytase. Higher levels of tibia ash in chickens aged 42 days from the groups fed compound feed with phytase were not established. The tibia ash quantity in group I was 49.35%, in group II - 49.21%, and in group III - 51.11% (Table 8). In broilers aged 42 days an increased quantity of calcium and phosphorus in tibia ash was observed as a result of phytase supplementation to the compound feed. The Natuphos fed group had by 16.63%, and the Feedophyt fed group - by 11% more Ca in the tibia ash in comparison with the control group. The increase in phosphorus quantity in the tibia ash of broilers of the same age was analogical. The chickens fed Natuphos supplemented food had by 13.29% ($p \leq 0.01$), and those fed Feedophyt supplemented food - by 14.82% ($p \leq 0.05$) higher phosphorus content in tibia ash, compared to the group without phytase.

Sebastian et al. (1996); Qian et al. (1996); Sohail and Roland (1999),Viveros et al. (2002) have established that phytase supplementation to maize-corn diets has a positive effect on the tibia ash quantity, the mineral content, density and strength of the tibia bone in broilers, which supports our results. Perney et al. (1993)

Table 7. Effect of phytase on tibia bone ash. Ca and P contents in the ash of 21-day-old broiler chickens.

Groups		21 day				
		Ash	Ca	P	Ca	P
		% Ash of DM	% of DM		Ash Ca (%)	Ash P (%)
	n	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
I - phytase	5	$55.64 \pm 0.26^{a\,b}$	$15.69 \pm 0.45^{c\,d}$	$8.12 \pm 0.11^{e\,f}$	9.21 ± 0.13^{f}	14.59 ± 0.27^{m}
II + Natuphos	5	$55.64 \pm 0.26^{a\,b}$	18.77 ± 0.07^{c}	9.06 ± 0.13^{e}	31.07 ± 0.16^{g}	15.00 ± 0.31
III + Optiphos	5	59.07 ± 0.92^{b}	18.62 ± 0.23^{d}	9.21 ± 0.13^{f}	31.57 ± 0.66^{h}	15.61 ± 0.26^{m}

The differences among the groups are statistically proven at the same letters. c, d, e, f, g, h - $p \leq 0.001$ a - $p \leq 0.01$
b, m - $p \leq 0.05$

Table 8. Effect of phytase on tibia bone ash. Ca and P contents in the ash of 42-day-old broiler chickens.

Groups		42 day				
		Ash	Ca	P	Ca	P
		% Ash of DM	% of DM		Ash Ca (%)	Ash P (%)
	n	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
I - phytase	5	49.35 ± 2.19	$14.38 \pm 0.31^{a\,b}$	$5.79 \pm 0.06^{c\,d}$	$29.16 \pm 0.64^{f\,g}$	$11.74 \pm 0.13^{h\,m}$
II + Natuphos	5	49.21 ± 1.09	16.74 ± 0.40^{a}	6.55 ± 0.30^{c}	34.01 ± 0.72^{f}	13.30 ± 0.58^{h}
III + Optiphos	5	51.11 ± 1.66	16.53 ± 0.42^{b}	6.89 ± 0.12^{d}	32.37 ± 0.90^{g}	13.48 ± 0.26^{m}

The differences among the groups are statistically proven at the same letters. a,b,d,f-$p \leq 0.001$ c,m-$p \leq 0.01$ g,h-$p \leq 0.05$

maintain the opposite thesis stating that tibia ash quantity in chickens fed phytase supplemented maize-corn diets does not increase.

Conclusions

The total phosphorus decrease in the diets by 20.16% on average for the whole rearing period did not have any negative effect on chickens' health status, the tibia ash quantity and the content of calcium and phosphorus in it as well as on the quantities of nitrogen, calcium and phosphorus deposited in the organism.

The lower total phosphorus content in the phytase supplemented diets decreased the quantity of dicalcium phosphate used by 34 % on average. This decreases the price of the compound feeds.

The phytase influence on the chickens' weight gain and the utilization of nitrogen, calcium and phosphorus were the strongest during the starter rearing period. During the grower and the finisher periods the phytase effect gradually decreased.

For the whole rearing period (0-42 days), broilers fed maize-soybean diets supplemented by Natuphos or Feedophyt phytase had a reliably higher live weight by 10.95 % and 11.93 %, respectively, compared to the group without phytase .

The forage consumption for 1 kg weight gain in chickens, fed maize-soybean diets supplemented by phytase until the age of 42 days, decreased by 17.02 % ($p \leq 0.05$) on average.

For the whole rearing period of chickens the phytase use increased nitrogen retention by 24.10% for the Natuphos phytase ($p \leq 0.01$) and by 16.62% for the Feedophyt phytase, compared to the control group. A statistically significant difference between both phytases regarding nitrogen retention was not established.

In comparing the effects of both phytases during all the rearing periods of chickens the Feedophyt phytase had a reliably better effect on calcium deposition in chickens' organisms in comparison with the Natuphos phytase.

For the 0-42-day period broilers fed maize-soybean diets supplemented by Natuphos phytase had by 50.13%, and those, fed Feedophyt phytase – by 45.67% better phosphorus utilization, than the group without phytase. A statistically significant difference between both phytases regarding this index was not established.

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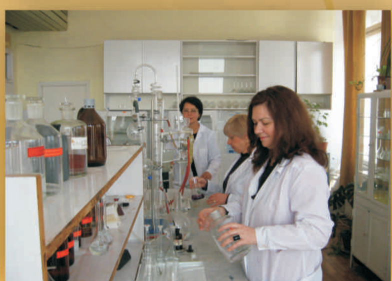

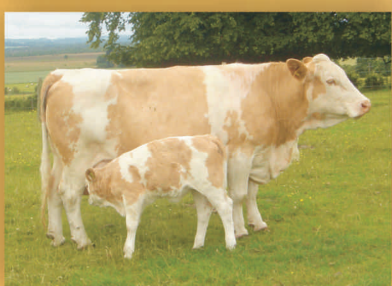

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