EFFICACY OF *ESCHERICHIA COLI*-DERIVED PHYTASE ON PERFORMANCE, BONE MINERALIZATION AND NUTRIENT DIGESTIBILITY IN MEAT-TYPE TURKEYS

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Summary. A total of 600 male BUT Big 6 turkeys were randomly allocated to 60 pens, 10 birds in each. Each of six treatment groups consisted of 10 pens (replicates), 100 birds in each treatment, at a stocking density of 2.5 birds per m^2. The turkeys were reared until 112 days (16 weeks) of age, and they were fed diets based on corn, soybean meal and wheat. Group I (positive control - PC) received a diet with a standard phosphorus (P) and calcium (Ca) content at all feeding stages, and group II (negative control - NC) was fed a diet with a lower P and Ca content. Groups III – VI received the same diet as group II, but with graded levels of *Escherichia coli* phytase, i.e. 125, 250, 500 and 1000 FTU/kg, respectively. The performance parameters of turkeys (body weight, weight gain, feed intake and feed conversion), tibia mineralization parameters (ash, Ca, P, specific gravity, breaking strength) and the apparent ileal digestibility of dry matter (DM), P and Ca were determined in the study. A slaughter analysis (carcass yield, proportions of muscles and abdominal fat) was also carried out. It can be concluded that the supplementation of *E. coli* phytase at a level of 500–1000 FTU/kg diet provided the best effects, with performance results very close to those noted in the PC group. All phytase-supplemented groups were characterized by better tibia bone mineralization as reflected by a higher ash content, and a higher (significantly higher in group VI) phosphorus content of tibia ash compared with the NC group (II). Graded levels of *E. coli* phytase had variable effects on ileal DM digestibility. A curvilinear dose-response was observed for P digestibility, with significant effects of phytase at a minimum dose of 250 FTU/kg. Ca digestibility was also improved by phytase, and the noted increase was significant in all groups. There were no statistical differences in carcass quality parameters between the dietary treatments.
Keywords: turkeys, phytase, performance, slaughter yield, P retention, tibia mineralization.

FITAZĖS, GAUTOS IŠ *ESCHERICHIA COLI,* POVEIKIS MĖSINIŲ KALAKUTŲ PRODUKTYVUMUI, KAULŲ MINERALIZACIJAI IR LESALŲ VIRŠKINAMUMUI

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Santrauka. Atlikti tyrimai su 600 „BUT Big 6“ kalakutais, analogų principu suskirstytais į 60 gardų, po 10 paukščių kiekviename. Kiekvieną iš šešių poveikio tyrimo grupių sudarė 10 gardų (kartotiniai bandymai), po 100 paukščių kiekvieno tyrimo metu. Talpinimo tankis – 2,5 paukščio 1 kv. metre. Kalakutai auginti iki 112 amžiaus dienų (16 savaičių) ir lesinti lesalais, kurių pagrindinės sudedamosios dalys buvo kukurūzai, sojų rupiniai ir kviečiai. I grupė (teigiamos kontrolės – PC) gavo lesalus, kurių sudėtyje buvo standartinis fosforo (P) ir kalcio (Ca) kiekis visais lesinimo etapais, o II grupė (neigiamos kontrolės – NC) lesinta sumažinto P ir Ca kiekio lesalais. III–VI grupės kalakutai gavo tokius pačius lesalus, kaip ir II grupė, bet su palaipsniui didinama *Escherichia coli* fitazės doze, t. y. atitinkamai 125, 250, 500 ir 1000 FTU/ 1 kilogramui lesalo. Tyrimo metu nustatyti kalakutų produktyvumo parametrai (kūno masė, masės prieaugis, lesalų suvartojimas ir lesalų konversija), blauzdikaulio mineralizacijos parametrai (pelenai, Ca, P, lyginamasis svoris, atsparumas trūkimui) ir tikrasis SM, P ir Ca virškinamumas klubinėje žarnoje. Atlikta ir skerdenų analizė (nustatyta skerdenos išeiga, raumenų ir pilvo riebalų santykis). Tyrimų duomenimis, veiksmingiausi buvo lesalai, papildyti *E. coli* fitazės 500–1000 FTU/ 1 kilogramui, produktyvumo rodikliai buvo beveik tapatūs nustatytiems PC grupėje. Visoms fitaze papildytus pašarus gavusioms paukščių grupėms buvo būdinga geresnė blauzdikaulio mineralizacija, pasireiškusi didesniu pelenų ir didesniu (reikšmingai didesniu VI grupėje) fosforo kiekiu blauzdikaulio pelenuose palyginti su NC grupe (II). Skirtingas *Escherichia coli* fitazės kiekis nevienodai veikė SM virškinamumą klubinėje žarnoje. Gautas kreivinis dozės-atsako P virškinamumui pobūdis, kai reikšmingas fitazės poveikis nustatytas esant minimaliai dozei – 250 FTU/ 1 kilogramui. Fitazė taip pat reikšmingai pagerino Ca virškinamumą visų grupių kalakutams. Tarp skirtingų lesinimo bandymų nebuvo statistinio skerdenos kokybės parametrų skirtumo.
Raktažodžiai: kalakutai, fitazė, produktyvumas, skerdenos išeiga, P kiekis, blauzdikaulio mineralizacija.

Introduction. Numerous experiments have been conducted since the publication of the first positive results of studies investigating microbial phytases as supplements to feed mixtures for poultry (Nelson et al., 1968). Reviews of research data on the effects of microbial phytases have also been published (Jeroch, 1994; Selle and Ravindran, 2007; Singh, 2008). However, relatively few experiments with microbial phytases have been performed on turkeys.

Turkey meat production has increased significantly over the last two decades in different countries, both in Europe and on the other continents. In Poland, current production levels are 1.460 kt per year, i.e. approximately 25% of the total poultry meat production. Therefore, the aims of the present study were as follows:

- to examine the influence of *Escherichia coli*-derived 6-phytase expressed in *Pichia pastoris* (trade name Optiphos, company HUVEPHARMA NV, Belgium) on diets formulation,
- to significantly decrease the levels of total and available phosphorus and to moderately decrease the calcium content of turkey diets, based on an evaluation of phytase efficacy,
- to calculate the optimal supplementation levels of phytase per kg feed, based on the performance results of turkeys, bone mineralization parameters and nutrient digestibility.

Materials and Methods. The experiment was conducted on a poultry experimental farm in Baldy (near Olsztyn) owned by the University of Warmia and Mazury (UWM) in Olsztyn, Poland. All experimental procedures were approved by the local Ethics Commission for Animal Experiments (decision no. 66/2008). The trial was carried out over 112 days and it involved day-old male BUT Big 6 turkeys from the commercial hatchery, ZWD Grelavi in Kętrzyn (NE Poland). The birds were divided into the following groups:

Group I - positive control (PC) – a standard P content of diets

Group II - negative control (NC) – a lower P content of diets (0.2% on available P (aP) - basis)

Group III - aP content of diets as in group II + 125 FTU *E. coli* phytase per kg feed

Group IV - aP content of diets as in group II + 250 FTU *E. coli* phytase per kg feed

Group V - aP content of diets as in group II + 500 FTU *E. coli* phytase per kg feed

Group VI - aP content of diets as in group II + 1000 FTU *E. coli* phytase per kg feed

Each treatment consisted of 10 replicates with 10 birds per replicate (i.e. 100 male turkey poults per treatment, 600 in total). The birds were kept on straw litter in 60 pens (4 m^2 each) in an experimental house for growing poultry with a stocking density of 2.5 birds per m^2. The house was equipped with an air-conditioning system, forced ventilation and an electric heating system. The temperature, lighting and ventilation program was consistent with the breeder's recommendations (BUT, 2008). A four-stage feeding program was applied, and the diets were formulated so as to meet the nutrient and energy requirements of growing turkeys (BUT, 2008). Tables 1 and 2 show the composition of diets fed to the PC (I) and NC (II) groups. Groups III-VI (phytase-supplemented groups) received the same diets as group II, but supplemented with graded levels of *E. coli* phytase. All rations had a low content of plant phytase from wheat.

Feed mixtures for four stages of experimental feeding were prepared in the "Agrocentrum" Feed Mill in Kałęczyn. Diets for the first four weeks were in crumble form, and diets for the following weeks were fed as pellets. *E. coli* phytase was administered as an ingredient of feed premixes. Pelleting temperature was below 80ºC.

Feed and water were offered *ad libitum*. Phytase activity in all experimental diets was analyzed in triplicate according to the assay outlined in Han et al. (1999). The trial began after the confirmation that the actual concentrations of the experimental diets were in compliance with the target levels for each treatment group. Experimental basal diets for the PC and NC groups were assayed for the content of DM, crude protein, crude fat, crude fiber, Ca and P. At the beginning of the experiment and at the end of the 4th, 6th, 12th and 16th week, the birds were weighed on a pen basis. Feed intake was determined on a pen basis for a 4-week-period on day 28, 56, 84 and 112. Based on the above results, the feed conversion ratio (FCR) was calculated (kg feed intake per kg body weight gain).

At the end of the experiment, a slaughter analysis was performed. In each treatment, birds representing an average body weight of each group were selected for carcass quality assessment (8 birds per treatment, 48 birds in total). Carcass weight (after 12 hours of chilling), the proportions of breast meat without skin, thigh and drumstick meat, the proportion of abdominal fat and carcass yield were determined, according to the method recommended by WPSA (Hahn and Spindler, 2002). Tibia samples, taken from the above birds, were separated from muscles and cartilage, and they were freeze-stored (-25ºC) until analysis. The bones were assayed for the maximum bone breaking strength, specific gravity, the average content of ash, Ca and P. Tibia samples were weighed, and their volume was measured in distilled water. Bone weight (w) and volume (v) provided a basis for calculating bone specific gravity (w:v). Tibia breaking strength was measured with the use of an INSTRON 4302 (USA) tensile testing machine connected to a computer. The mechanical properties of tibial bones were determined in a three-point bending test (Feretti et al., 1993). Feed and bone samples were analyzed by the following methods: samples were mineralized in a mixture of nitric acid and perchloric acid (3:1) (Merck, Germany). Mineralization was carried out in an aluminum heating block with temperature control (VELP DK 20, VELP Scientifica, Italy). Reference samples were prepared together with test samples. The Ca content of mineralizates was determined by flame atomic absorption spectrometry (acetylene-air flame), with the use of a Unicam 939 atomic absorption spectrometer (Solar, UK), equipped with an Optimus data station, a background correction source (deuterium lamp) and cathode lamps (Whiteside and Miner, 1984). In order to determine Ca content, a 10% aqueous solution of lanthanum chloride was added to all experimental solutions, in the amount ensuring the final La^{+3} concentration of 1%. Phosphorus concentration was determined in mineralizates by colorimetry with ammonium molybdate and with sodium sulfate and hydroquinone. Absorbance was measured using a VIS 6000 spectrophotometer (KRÜSS–OPTRONIC, Germany), at a wavelength of λ = 610 nm (Fiske and Subbarow, 1925). Ca and P content was determined using standards at a concentration of 1 mg/cm^3, diluted with a 0.1 M solution of HNO_3 (BDH, Germany).

During the last feeding period, birds from all groups received feed with an indigestible dietary marker (Celite - HCl insoluble ash, inclusion level - 2%). On day 112, eight birds from each group (48 birds in total) were killed and their small intestines were immediately exposed. The contents of the lower ileum (last two-thirds) were expressed by gentle flushing with distilled water into plastic containers. The ileum was defined as the segment of the small intestine extending from the Meckel's diverticulum to the ileo-cecal junction. Digesta samples were frozen immediately after collection, and subsequently lyophilized. The digestibility of dry matter, Ca and P was determined in the ileal digesta. Ileal nutrient digestibility was calculated by the following formula:

$$\text{il dig (\%)} = 100 - 100 * [(AIA_{feed} * NT_{digesta})/(AIA_{digesta} * NT_{feed})]$$

where:

AIA_{feed} and $AIA_{digesta}$ = acid-insoluble ash (Celite) content of feed and digesta, respectively,

NT_{feed} and $NT_{digesta}$ = nutrient content of feed and digesta, respectively.

The experimental results were verified statistically by one-way analysis of variance (ANOVA) in orthogonal and non-orthogonal designs. The significance of differences was estimated by Duncan's test. The calculations were performed using the STATISTICA software package ver. 9.0 (StatSoft Inc., 2009). Data in tables are given as means and standard deviations.

Results. A proximate feed analysis showed that the actual nutrient concentrations were consistent with the calculated values listed in Table 1. The results of an analysis of phytase activity in the diets are shown in Table 3. They were satisfactory and corresponded with the target values.

The birds of all experimental groups were characterized by good livability. In view of the long rearing period, mortality rates were low, ranging from 1.0% (group IV) to 4.0% (groups I and V), and they were not affected by experimental treatments. Feed intake data are summarized in Table 4. The reduced aP content of the ration for group II had only a marginal effect on feed consumption, compared with the PC group (P>0.05). The graded levels of phytase supplementation had no influence on feed intake.

Table 1. **Composition and nutritive value of the PC diets** (group I)

Components (%)	Feeding phase (weeks)			
	I (0-4)	II (5-8)	III (9-12)	IV (13-16)
Wheat	15.00	15.00	15.00	15.00
Corn	30.12	35.36	39.41	46.35
Soybean meal	43.34	40.19	37.63	28.23
Potato protein	5.00	3.00	-	-
Soybean oil	1.44	1.10	2.00	-
Animal fat	-	1.10	2.13	5.01
$NaHCO_3$	0.15	0.10	0.10	0.10
Salt	0.21	0.22	0.20	0.15
Limestone	1.68	1.38	1.23	1.12
MCP	2.00	1.55	1.23	1.05
DL-Methionine	0.31	0.34	0.34	0.33
L-Lysine HCl	0.32	0.29	0.29	0.30
L-Threonine	0.07	0.05	0.13	0.08
Premix (vitamins + trace minerals)	0.25	0.25	0.25	0.22
Choline chloride	0.11	0.08	0.07	0.06
Celite	-	-	-	2.00
Energy and nutrient content				
AME_N (MJ/kg)	11.61[1]	12.01[1]	12.62[1]	13.22[1]
Crude protein (g/kg)	280[1]/284[2]	255[1]/257[2]	225[1]/231[2]	190[1]/202[2]
Methionine (g/kg)	7.4[1]	7.2[1]	6.6[1]	6.1[1]
Methionine + Cystin (g/kg)	11.8[1]	11.3[1]	10.4[1]	9.4[1]
Lysine (g/kg)	18.2[1]	16.1[1]	13.9[1]	11.7[1]
Calcium (g/kg)	12.0[1]/12.8[2]	10.0[1]/10.0[2]	8.5[1]/8.4[2]	7.5[1]/7.8[2]
Total phosphorus (g/kg)	8.8[1]/8.7[2]	7.7[1]/7.4[2]	7.0[1]/6.8[2]	6.0[1]/5.9[2]
Available (Av) phosphorus (g/kg)	6.0[1]	5.0[1]	4.3[1]	3.8[1]

[1]Calculated; [2] Analyzed (Naumann and Bassler, 1993)

Growth data are contained in Table 5. The growth rate of male turkeys in the PC group was in agreement with the recommendations of the turkey breeder company (BUT, 2008). The feeding of rations with a reduced P and Ca content (NC group II) significantly decreased the growth rate of birds throughout the entire experiment in comparison with group I (PC) fed diets with standard P and Ca levels. At the end of the trial, group II turkeys had 6% lower body weight than group I toms. Phytase supplementation significantly improved the growth performance of birds. At the end of the experiment (day 112), there were no differences in body weight between group I (PC) and the groups whose diets were supplemented with 500 and 1000 FTU phytase/kg feed. Feed conversion efficiency is presented in Table 6. The feed conversion ratio reflected P supply to the animals, and the least desirable FCR was noted in the NC group. Yet the differences between groups I and II were only numerical. Birds of group V and VI (500 and 1000 FTU/kg, respectively) were characterized by the best feed conversion ratio, which was significantly better than in the NC group (II) during the entire experiment.

Table 2. **Composition and nutritive value of NC diets** (group II) **and phytase-supplemented diets** (groups III-VI)

Components (%)	Feeding phase (weeks)			
	I (0-4)	II (5-8)	III (9-12)	IV (13-16)
Wheat	15.00	15.00	15.00	15.00
Corn	31.84	36.86	41.64	48.43
Soybean meal	43.03	39.82	37.20	27.83
Potato protein	5.00	3.00	-	-
Soybean oil	0.93	1.00	2.00	-
Animal fat	-	1.00	1.38	4.31
$NaHCO_3$	0.15	0.10	0.10	0.10
Salt	0.21	0.22	0.20	0.15
Limestone	1.70	1.37	1.18	1.01
MCP	1.09	0.63	0.23	0.18
DL-Methionine	0.31	0.33	0.34	0.32
L-Lysine HCl	0.33	0.29	0.29	0.30
L-Threonine	0.07	0.06	0.13	0.08
Premix (vitamins + trace minerals)	0.25	0.25	0.25	0.22
Choline chloride	0.11	0.08	0.07	0.06
Celite	-	-	-	2.00
Energy and nutrient contents				
AME_N (MJ/kg)	11.62[1]	12.11[1]	12.62[1]	13.22[1]
Crude protein (g/kg)	280[1]/283[2]	255[1]/263[2]	225[1]/238[2]	190[1]/207[2]
Methionine (g/kg)	7.4[1]	7.2[1]	6.6[1]	6.1[1]
Met + Cys (g/kg)	11.8[1]	11.3[1]	10.4[1]	9.4[1]
Lysine (g/kg)	18.2[1]	16.1[1]	13.9[1]	11.7[1]
Calcium (g/kg)	10.1[1]/10.5[2]	8.0[1]/7.9[2]	6.5[1]/6.3[2]	5.5[1]/5.4[2]
Total phosphorus (g/kg)	6.8[1]/7.0[2]	5.7[1]/5.8[2]	4.8[1]/4.9[2]	4.1[1]/4.1[2]
Available (Av) phosphorus (g/kg)	4.0[1]	3.0[1]	2.1[1]	1.9[1]

[1]Calculated; [2]Analyzed (Naumann and Bassler, 1993)

Table 3. **Activity of *E. coli* phytase in experimental diets** (FTU/kg feed)

Period, days	Groups					
	I (PC)	II (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
	0[1]	0[1]	125[1]	250[1]	500[1]	1000[1]
0–28	86[2]	51[2]	142[2]	265[2]	565[2]	1095[2]
29–56	33[2]	31[2]	134[2]	288[2]	561[2]	1088[2]
57–84	16[2]	11[2]	143[2]	261[2]	518[2]	1111[2]
85–112	46[2]	60[2]	142[2]	280[2]	559[2]	1407[2]

[1]Expected; [2]Analyzed

Carcass characteristics are presented in Table 7. Due to the lower final body weights of turkeys in group II (lower P content) also their carcass weights were lower in comparison with the other groups, yet the noted differences were only numerical. The reduced P content (group II) and different levels of supplemental phytase had no effect on dressing percentage (%) and carcass composition.

Table 4. **Average feed intake** (kg/bird/day) **of experimental turkeys at different age**

Period, days	Groups					
	I (PC)	II (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
0–28	0.079 ± 0.002	0.075 ± 0.002	0.079 ± 0.003	0.079 ± 0.004	0.078 ± 0.003	0.079 ± 0.005
29–56	0.256 ± 0.009	0.246 ± 0.010	0.249 ± 0.009	0.254 ± 0.006	0.249 ± 0.009	0.250 ± 0.007
57–84	0.403 ± 0.007	0.391 ± 0.016	0.398 ± 0.019	0.401 ± 0.013	0.383 ± 0.039	0.404 ± 0.023
85–112	0.593^{a} ± 0.038	0.560^{ab} ± 0.033	0.588^{a} ± 0.057	0.568^{ab} ± 0.032	0.585^{a} ± 0.026	0.545^{b} ± 0.014
0–112	0.343 ± 0.022	0.336 ± 0.021	0.355 ± 0.032	0.336 ± 0.020	0.342 ± 0.034	0.347 ± 0.024

Mean values within a row with the different letters (abc) differ significantly; (P≤ 0.05)

Table 5. **Average body weight[1] of experimental turkeys at different age, kg**

Age of birds, day	Groups					
	I (PC)	II (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
28th	1.353^{a} ± 0.041	1.277^{c} ± 0.038	1.347^{ab} ± 0.050	1.378^{a} ± 0.073	1.299^{b} ± 0.056	1.340^{ab} ± 0.043
56th	5.080^{a} ± 0.119	4.791^{b} ± 0.156	5.047^{a} ± 0.079	5.029^{a} ± 0.182	5.044^{a} ± 0.175	5.120^{a} ± 0.187
84th	9.467^{ab} ± 0.137	8.957^{c} ± 0.287	9.331^{b} ± 0.162	9.331^{b} ± 0.395	9.410^{b} ± 0.318	9.686^{a} ± 0.276
112th	14.420^{a} ± 0.404	13.549^{c} ± 0.329	13.946^{b} ± 0.532	13.943^{b} ± 0.320	14.210^{ab} ± 0.505	14.380^{a} ± 0.286

[1]Mean weight of turkey poults at the beginning of experiment 56.0 ± 1.0 g
Mean values within a row with the different letters (abc) differ significantly; (P≤ 0.05)

Table 6. **Average feed conversion ratio** (FCR) **of experimental turkeys, kg/kg**

Period, days	Groups					
	I (PC)	II (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
0–28	1.709 ± 0.067	1.750 ± 0.177	1.706 ± 0.034	1.690 ± 0.054	1.764 ± 0.069	1.710 ± 0.059
29–56	1.931 ± 0.056	1.967 ± 0.093	1.915 ± 0.133	1.943 ± 0.054	1.891 ± 0.051	1.858 ± 0.062
57–84	2.606^{b} ± 0.094	2.659^{b} ± 0.080	2.606^{b} ± 0.161	2.616^{b} ± 0.132	2.458^{a} ± 0.216	2.474^{a} ± 0.096
85–112	3.432 ± 0.435	3.581 ± 0.269	3.686 ± 0.161	3.452 ± 0.207	3.521 ± 0.376	3.533 ± 0.228
0–112	2.608^{abc} ± 0.112	2.684^{c} ± 0.061	2.662^{bc} ± 0.106	2.620^{abc} ± 0.064	2.580^{ab} ± 0.102	2.552^{a} ± 0.065

Mean values within a row with the different letters (abc) differ significantly; (P≤ 0.05)

Table 7. **Carcass characteristics of experimental turkeys**

Specification	Groups					
	I (PC)	II (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
Body weight before slaughter (kg)	14.463^{a} ± 0.680	13.525^{b} ± 0.755	13.988^{ab} ± 0.461	14.054^{ab} ± 0.595	14.201^{a} ± 0.460	14.380^{a} ± 0.563
Carcass weight (kg)	11.654 ± 0.628	10.879 ± 0.590	11.378 ± 0.482	11.384 ± 0.539	11.486 ± 0.364	11.623 ± 0.445
Dressing percentage (%)[1]	80.56 ± 1.24	80.45 ± 1.31	81.32 ± 1.21	80.99 ± 1.23	80.89 ± 0.92	80.83 ± 0.53
Breast muscles (%)[2]	20.94 ± 0.97	21.30 ± 1.16	20.95 ± 0.86	21.75 ± 0.91	21.72 ± 1.62	21.59 ± 0.88
Thigh muscles (%)[2]	11.03 ± 0.53	11.43 ± 0.90	11.66 ± 0.29	11.39 ± 0.57	11.39 ± 0.55	11.19 ± 0.72
Drumstick muscles (%)[2]	7.99 ± 0.71	7.88 ± 0.63	8.12 ± 0.40	8.06 ± 0.57	8.19 ± 0.49	8.07 ± 0.48
Abdominal fat (%)[2]	0.63 ± 0.13	0.54 ± 0.24	0.64 ± 0.32	0.55 ± 0.16	0.54 ± 0.20	0.56 ± 0.07

[1] % carcass weight to body weight before slaughter; [2] body weight before slaughter = 100%
Mean values within a row with the different letters (abc) differ significantly; (P≤ 0.05)

Table 8 shows the results of an analysis of the tibia for ash, Ca and P content. The feeding of rations with reduced levels of aP and Ca (II - NC group) resulted in reduced levels of ash, calcium and phosphorus in the tibia, compared with the group fed a diet with a standard P and Ca content (I - PC group). The differences were only partly significant. By phytase supplementation to the diets mostly higher P and Ca concentrations were observed in phytase-supplemented groups. All phytase-supplemented groups were characterized by better tibia bone mineralization as reflected by a higher ash content, and a higher (significantly higher in group VI) phosphorus content of tibia ash compared with the NC group (II).

Table 8. **Tibia mineralization parameters of experimental turkeys on day 112**

Specification	Groups					
	I (PC)	II (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
Tibia ash, %	54.46 ± 4.01	51.27 ± 4.07	54.25 ± 3.99	53.18 ± 3.59	55.39 ± 3.81	52.65 ± 4.10
P content in tibia ash, %	$17.42^{a} \pm 0.97$	$16.61^{b} \pm 0.62$	$16.96^{ab} \pm 0.34$	$17.08^{ab} \pm 0.24$	$17.03^{ab} \pm 0.44$	$17.44^{a} \pm 0.65$
Ca content in tibia ash, %	37.28 ± 1.13	36.43 ± 1.91	36.01 ± 1.22	36.46 ± 0.68	36.57 ± 1.35	37.03 ± 1.98
Specific gravity, g/cm^3	0.952 ± 0.057	0.903 ± 0.063	0.887 ± 0.059	0.907 ± 0.042	0.941 ± 0.028	0.922 ± 0.044
Maximum bone breaking force, N	616.4 ± 147.3	553.4 ± 116.7	593.5 ± 91.8	609.1 ± 103.2	600.4 ± 128.5	592.7 ± 93.9

Mean values within a row with the different letters (abc) differ significantly; ($P \leq 0.05$)

Ileal digestibility values are summarized in Table 9. Dry matter digestibility decreased significantly in group II, in comparison with group I. Phytase supplementation improved DM digestibility. Phytase activity was not dose-dependent, and the differences noted in this respect were only partly significant (in groups with 125, 500 and 1000 FTU). The significantly higher share of phytate-P in the total P content of the diet fed to group II (NC), compared with group I (PC), had a considerable negative effect on the ileal digestibility of P and Ca. Supplemental phytase improved the ileal digestibility of P, but no clear dose-response effect was observed, and the noted influence was only partly significant. In the group with the highest phytase addition (VI – 1000 FTU), P digestibility values fell within the range reported in group I (PC). Ca digestibility was significantly improved by phytase in all groups, but the levels of the PC group had not been achieved.

Table 9. **Apparent ileal digestibility of dry matter, phosphorus and calcium on day 112**

Specification	Groups					
	I (PC)	II (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
DM	$79.0^{a} \pm 2.2$	$71.7^{c} \pm 2.2$	$75.5^{b} \pm 1.5$	$73.9^{bc} \pm 2.6$	$72.9^{cd} \pm 2.6$	$74.9^{bd} \pm 1.6$
P	$61.1^{a} \pm 7.6$	$44.2^{c} \pm 8.0$	$50.3^{bc} \pm 10.3$	$56.0^{ab} \pm 6.5$	$52.1^{bc} \pm 7.5$	$61.9^{a} \pm 9.1$
Ca	$44.8^{a} \pm 4.9$	$31.2^{c} \pm 3.7$	$37.3^{b} \pm 6.2$	$40.0^{ab} \pm 3.8$	$41.1^{ab} \pm 4.0$	$42.0^{ab} \pm 4.1$

Mean values within a row with the different letters (abc) differ significantly; ($P \leq 0.05$)

Table 10. **Improvement in ileal P digestibility following phytase addition**

Specification	Groups				
	Reduced P (NC)	III (NC+125 FTU)	IV (NC+250 FTU)	V (NC+500 FTU)	VI (NC+1000 FTU)
P content in diets, g/kg	4.07	4.07	4.07	4.07	4.07
Ileal digestibility, %	44.21	50.27	55.96	52.06	61.91
Ileal digested P, g/kg	1.80	2.05	2.28	2.12	2.52
Additional ileal digested P, g/kg	0	0.25	0.48	0.32	0.72

An improvement in ileal P digestibility resulting from phytase supplementation is presented in Table 10. The addition of 125 - 1000 FTU phytase to experimental diets increased the amount of ileal digested phosphorus by 0.25 - 0.72 g/kg feed.

Discussion. The levels of aP in the PC group were any lower than the recommendations of Smulikowska and Rutkowski (2005) as well as Leeson and Summers

(2005). The latter authors recommend 7.5 g aP/kg feed for the period of 0-4 weeks of age and 4.8 g aP/kg feed from the 17^{th} week. According to Smulikowska and Rutkowski (2005), the corresponding values are 7.0 - 7.2 g aP/kg and 4.9 - 5.4 g aP/kg, respectively. Although the aP content of diets for the NC group was significantly lower than in the PC group, the growth performance of birds did not decrease as much as it could be expected. It can be concluded that a review of aP recommendations is urgently needed. As reported previously (GfE, 2004; Rodehutscord et al., 2003), there is a considerable potential for P sparing in turkeys.

The effectiveness of supplementary phytase seems undoubted, and it was found to increase with increasing supplementation levels. Phytase had a particularly beneficial influence on body weight gains. The decrease in the aP content of experimental diets by approximately one third was compensated for by the highest level of phytase supplementation. In Table 11 is summarized the effect of microbial phytase on some performance and quality parameters of male turkeys fed corn-soybean meal diets. Our results are mostly compared with listed there the findings of other authors.

Table 11. **Effect of microbial phytase on growth performance and P retention in male turkeys fed corn-soybean meal diets, %**

Researchers	Origin	Age of birds	FTU/kg	Percent improvement							
				BW	BWG	FI	FCR	Tibia ash	Toe ash	APR	BBF
Atia et al., 2000	*Aspergillus*	14 wk[1]	500	4.22*			-1.16	3.77			
		16 wk	500	3.45			2.63	3.53			
Applegate et al., 2003	*Aspergillus*	10-20 d	500		9.41	3.23	8.52	3.23*	5.29*	5.50	
	Peniophora	10-20 d	500		5.83	3.38	8.18	4.03*	6.44*	3.68	
	E.coli	10-20 d	250		10.50	-0.83	5.66	7.76*	10.44*	11.42*	
		10-20 d	500		14.58	-5.08	6.41	9.65*	10.30*	15.74*	
		10-20 d	750		4.86	1.83	10.63	10.39*	13.66*	20.24*	
		10-20 d	1000		4.11	3.59	7.23	10.67*	12.73*	18.10*	-3.10
Esteve-Garcia et al., 2005	*H. polymorpha*	32 d[1]	500	17.60*	19.78*	-15.57	3.16*	14.93*	28.32*	33.25*	
		32 d[1]	1000	20.72*	23.08*	-14.97	5.99*	21.39*	29.20*	36.39*	
		15 wk[1]	1000		1.42	-1.97	-0.66		0.71		
Ledoux et al., 1995	*Aspergillus*	21 d	500	2.63*	14.77*	-0.30	15.17				
Pirgozliev et al., 2007	*E.coli*			0.71			2.30	2.00*			
Ravindran et al., 1995	*Aspergillus*	8 wk	900	1.94*			1.55				
Roberson et al., 2005	*Aspergillus*	11 wk	900	0.87			1.17				
		14 wk	600	1.21			0.38				14.89*
		17 wk	600	2.63*			1.03	2.15			
		16 wk	1000	0.88			0.41	2.00*			
Yan et al., 2003	*Aspergillus*	20 wk	1000				0.99	1.32			

BW-body weight, BWG-body weight gain, FI-feed intake, FCR-feed conversion ratio,
APR-apparent P retention, BBF-bone breaking force
*significant effect ($P \le 0.05$); [1] turkey hens

Conclusion. The present study showed that the supplementation of *E. coli* phytase at a level of 500 - 1000 FTU/kg diet provided the best effects, with performance results very close to those noted in the PC group. All phytase-supplemented groups were characterized by better tibia bone mineralization as reflected by a higher ash content, and a higher (significantly higher in group VI) phosphorus content of tibia ash compared with the NC group (II). Graded levels of *E. coli* phytase had variable effects on ileal DM digestibility. A curvilinear dose-response was observed for P digestibility, with significant effects of phytase at a minimum dose of 250 FTU/kg. Ca digestibility was also improved by phytase, and the noted increase was significant in all groups. There were no statistical differences in carcass quality parameters between the dietary treatments. By the addition of phytase (125 - 1000 FTU/kg feed) the amount of aP in experimental diets can be reduced about 0.25 - 0.72 g/kg feed.

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