

## THE INFLUENCE OF SYNTHETIC ENZYME PHYTASE (*RONOZYME P*) ON UTILIZATION OF PHOSPHORUS AND CALCIUM IN BROILER CHICKENS FED DIETS WITH INCREASED AMOUNT OF RAPE CAKE

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**Summary.** The present study was designed to assess the role of synthetic enzyme phytase (*Ronozyme P*) on feed conversion ratio, phosphorus and calcium utilization, and *tibia* bone mineralization in broiler chickens fed diets with increased amount of rape cake. The results from this study indicate that marked phosphorus reduction in the diet highly increased the feed conversion rate and decreased the growth of chickens. However, the mentioned enzyme supplement improved calcium and phosphorus utilization. Our results showed that addition of not dehulled rape cake to the diet increased utilization of total phosphorus and calcium on 2.8% and 3.0% ( $p>0.05$ ) and addition of dehulled rape cake on 18.3% and 9.6% ( $p>0.05$ ) compared to the controls, respectively. Further, addition of synthetic phytase to the diet along with a low amounts of nonphytate phosphorus in not dehulled rape cake increased amount of phosphorus in the *tibia* on 0.8% ( $p>0.05$ ) and in group with dehulled rape cake on 1.1% ( $p>0.05$ ), respectively.

**Keywords:** Phytase enzyme preparation, dehulled and not dehulled rape cake, broiler chickens, productivity.

## FOSFORO IR KALCIO PASISAVINIMAS VIŠČIUKŲ BROILERIŲ ORGANIZME VEIKIANT FITAZĖS FERMENTINIAM PREPARATUI *RONOZYME P* IR LESINANT DIDESNIU RAPSO IŠSPAUDŲ KIEKIUI

**Santrauka.** Buvo ištirta fitazės fermentinio preparato *RONOZYME P* įtaka viščiukų broilerių produktyvumui, lesalų sąnaudoms, kaulų *tibia* mineralizacijai, taip pat kalcio ir fosforo pasisavinimui, lesaluose naudojant didesnę rapso rupinių kiekį. Lesinimo bandymo tyrimai parodė, kad per mažas fitazės ir fosforo kiekis apie 3,0% padidino lesalų sąnaudas 1 kg priesvorio gauti ir sumažino viščiukų augimą. Tačiau minėto fermentinio preparato priedas pagerino lesalų maistinių medžiagų pasisavinimą. Lesalų maisto medžiagų virškinamumas bandymo metu naudojant rapso rupinius, pagamintus iš nelukštentų rapso sėklų, bendrojo kalcio ir fosforo pasisavinimas pagerėjo atitinkamai 2,8% ir 3,0% ( $p>0,05$ ), o bandyme su rapso rupiniais, pagamintais iš lukštentų rapso sėklų, fermentinio preparato *RONOZYME P* priedas pagerino bendrojo fosforo ir kalcio pasisavinimą palyginti su kontroline grupe atitinkamai 18,3% bei 9,6% ( $p>0,05$ ). Fitazės priedas lesaluose su mažesniu fosforo kiekiu bandyme su nelukštentais rapso sėklų rupiniais fosforo susikaupimą paukščių šlaunikaulyje (*tibia*) padidino 0,8% ( $p>0,05$ ), o bandyme su lukštentų rapso sėklų rupiniais – 1,1%.

**Raktažodžiai:** fitozė, lukštentų ir nelukštentų rapsų sėklų rupiniai, viščiukai broileriai, produktyvumas.

**Introduction.** In our country the major protein supplements in poultry feeds are soya bean and rapeseed cakes. Defatted rapeseed meal, with up to 40% protein in lysine and sulphur – containing amino acids, is considered to have a high potential nutritional value (Zyla, 1992). Several anti-nutritional factors prevent this ingredient from being extensively used in poultry nutrition (Koreleski et al., 1989). Although during the last decade varieties of low glucosinolate and erucic acid content ("double low rapeseed") have been developed by plant breeding programmes, the presence of phytates in rapeseed causes phosphorus, zinc, calcium and magnesium deficiency syndromes in chickens (Nwokolo et al., 1977).

According to various literature, phytate phosphorus amounts in cereal grains can be as high as 50–80 %, in legumes – 50–68 % in oil-producing plant seeds and their by-products – 51–76 % (Eeckhout et al., 1994; Jeroch et al., 2000; Oloffs et al., 2000). A large part of phosphorus in phytic acid is indigestible by birds because their gastrointestinal tracts lack sufficient amounts of endogenous enzyme to catalyse phytate reduction (Iragavarapu et al., 1999). So phosphorus supplements are

necessary (nonorganic or animal origin) in feed to meet the needs of the bird organism.

It is well documented that microbial phytase could be the solution to avoid negative influence of phytate on the digestibility of phosphorus, other minerals and nitrogen (Knap, 1995; Leeson, Summers, 1997). The dietary supplementation with phytase reduces the necessity to include mineral phosphates and reduces P-excretion of broiler chickens (Kornegay et al., 1996; Simons et al., 1990). Recently, a new phytase (*RONOZYME P*) has been developed for the feed industry. This phytase is produced by submerged fermentation of *Aspergillus oryzae* carrying a gene from *Peniophora lycii* coding for the phytase (Klünter et al., 2001).

The aim of this investigation was to determine the influence of the synthetic phytase preparation *RONOZYME P* on broiler chickens productivity, feed conversion ratio, bone mineralisation, calcium and phosphorus utilization, using higher amount of variously processed rape cake.

**Materials and methods.** The scientific investigation has been made following the provisions of Law of Republic of Lithuania N<sup>o</sup> 8-500 on Protection, Keeping

and Use of Animals of November 6, 1997 (“Valstybės žinios”, N<sup>o</sup> 108, 1997. 11. 28) and of the by-laws, i.e. orders of State Veterinary Service of Republic of Lithuania: On Breeding, Care, Transportation of Laboratory Animals (N<sup>o</sup> 4-361, 1998. 12. 31) and Use of Laboratory Animals for Scientific tests (N<sup>o</sup> 4-16, 1999. 01. 18).

*The feeding experiment.* In order to analyse the influence of a synthetic phytase preparation (Ronozyme P) feed supplement on broiler chickens growth and feed conversion ratio, the feeding experiment was conducted using 600 Hybro G breed broiler chickens 1–35-days-old. The chicks were divided into 2 groups and contained 3 replicates of 100 birds each. The both groups were given

*ad libitum* access to crumbled feed mixtures (with 20 % not dehulled rape cake). The feed mixture of the experimental group contained no monocalcium phosphate additive (0.53% total P, 0.28% nP), though was supplemented with phytase preparation (300 g/t feed). The birds were maintained on deep litter and watered from stationary watering containers. The feed composition and sustenance is showed in Table 1.

The body weight gain of birds was measured fortnightly and feed conversion ratio was calculated after the 35 day of age. During the feeding experiment, individual chicken body weight, feed conversion ratio and mortality were analysed.

Table 1. Feed composition and sustenance of feeding experiment (%)

Components	Control group	Experimental group
Wheat	14.00	14.00
Corn	35.00	35.30
Soya bean meal 48	19.00	19.00
Rape cake	20.00	20.00
Fish meal 66	3.00	3.00
Sunflower oil	6.00	6.00
Monocalcium phosphate	0.80	–
Limestone	1.00	1.30
NaCl	0.30	0.30
Methionine	0.10	0.10
Premix	1.00	1.00
Enzyme preparation*	–	0.03
Calculated values %: ME (MJ/kg)	12.63	12.67
Crude protein	22.00	22.02
Crude fat	10.46	10.47
Crude fiber	7.46	7.48
Lysine	1.25	1.25
Methionine/Cystine	0.89	0.89
Methionine	0.52	0.52
Threonine	0.91	0.91
Tryptophane	0.27	0.27
Ca	0.83	0.80
P (total)	0.72	0.53
Nonphytate P (nP)	0.47	0.28
Na	0.16	0.16

\* Enzyme preparation of phytase (*Ronozyme P*). Product activity is 2500 FYT/g

*The experiments of feed nutrient digestibility.* The phytase supplement influence on Ca and P utilization in the birds was tested using two feed nutrient digestibility experiments.

The first experiment of feed nutrient digestibility was performed with 10 broiler chickens using not dehulled rape cake. The experiment began with 21-d-old broiler chickens. The chickens were divided into 2 groups of 5 birds each, identically housed (individually) and fed. The feed ration was the same as in the feeding experiment.

Analogously, another experiment of feed nutrient digestibility was performed. The composition of the ration containing 20% of dehulled rape cake is shown in Table 2.

During the experiments of feed nutrient digestibility the birds were housed identically, individually, each having their own feeding trough and stationary watering containers. In the preparatory period all animals received daily amount of feed measured exactly. One particular aim was to minimize the left-overs of feed. By the help of the amount of feed which was taken in, one could determine the necessary amount of feed for the main period. The birds were fed twice a day during the main period, always before collecting the excrements.

After 3-4 days of settling in and 5 days of preparatory period the feed intake was completely quantitatively measured for 5 days. During the main period the excrements were completely collected twice a day and frozen. Feed particles, feathers and scalls had to

be removed with the help of pair of tweezers to avoid their getting into the excrements samplings.

After finishing the collecting period the excrements were thawed and representative samples were dried in an oven at 65 °C. Excrements, along with diet samples, were ground to pass 1 mm sieve.

Phosphorus and calcium in the feeds and excrements

were made according to the methods of VDLUFA (Naumann and Bassler, 1976/1993).

The accumulation rate of phosphorus in the bones of *tibia* was determined according to the Huyghebaert method (1996) (Huyghebaert, 1996).

The data were analysed with computer program “Statistica for Windows” (StatSoft Inc., 1995).

Table 2. The experiment of feed nutrient digestibility using dehulled rape cake, feed ration composition and sustenance (%)

Components	Control group	Experimental group
Wheat	14.40	14.40
Corn	35.00	35.50
Soya bean meal 46	20.00	20.00
Rape cake	20.00	20.00
Fish meal 70	3.00	3.00
Sunflower oil	4.50	4.50
Monocalcium phosphate	0.80	–
Limestone	1.00	1.30
NaCl	0.30	0.30
Lysine	0.30	0.30
Methionine	0.25	0.25
Premix	1.00	1.00
Enzyme preparation*	–	0.03
Calculated values %: ME (MJ/kg)	12.84	12.90
Crude protein	22.19	22.23
Crude fat	7.46	7.48
Crude fiber	4.76	4.00
Lysine	1.19	1.19
Methionine/Cystine	0.87	0.87
Methionine	0.57	0.57
Threonine	0.68	0.68
Tryptophane	0.20	0.20
Ca	0.86	0.84
P (total)	0.73	0.54
Nonphytate P (nP)	0.47	0.28
Na	0.16	0.16

\*Enzyme preparation of phytase (*Ronozyme P*). Product activity is 2500 FYT/g.

Footnote: the methionine, lysine and enzyme preparation was mixed in with the premix.

**Results.** The feeding experiment (using not dehulled rape cake) data showed that the weight of 21–35-day-old broilers was by 3.0% ( $P>0.05$ ) less as compared to the control group (Fig 1).

Feed conversion ratio using the phytase supplement was by 3–4% ( $P>0.05$ ) larger than in the control group (Fig. 2).

The feed nutrient digestibility experiment using not dehulled rape cake showed that in comparison to control group, in the experimental group the digestibility of calcium and total phosphorus increased by 2.8% and 3.0% respectively ( $P>0.05$ ) (Table 3).

During this investigation, phosphorus mineralisation amounts in the *tibia* were also analysed. It was determined that the phosphorus content (%) in the control group (*tibia*) was  $39.3\pm 1.3$  and in the experimental group –  $40.1\pm 1.8$  ( $P>0.05$ ).

The results of experiment of feed nutrient digestibility showed that phytase enzyme preparation in feeds containing 20% of dehulled rape cake and no

additional phosphorus, total calcium and total phosphorus digestibility were higher –18.3% and 9.6% respectively ( $P>0.05$ ) (Table 4).

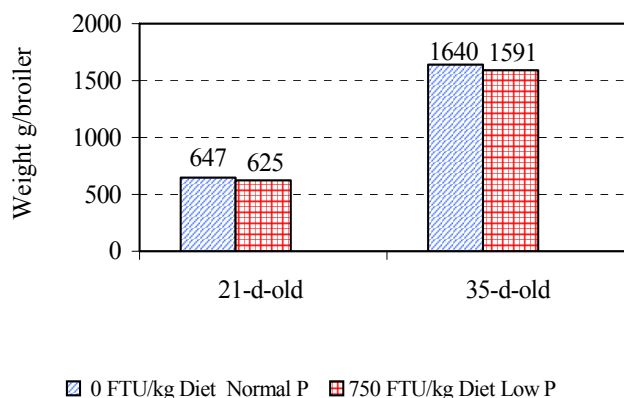


Fig 1. The effect of microbial phytase on the growth of broiler chickens.

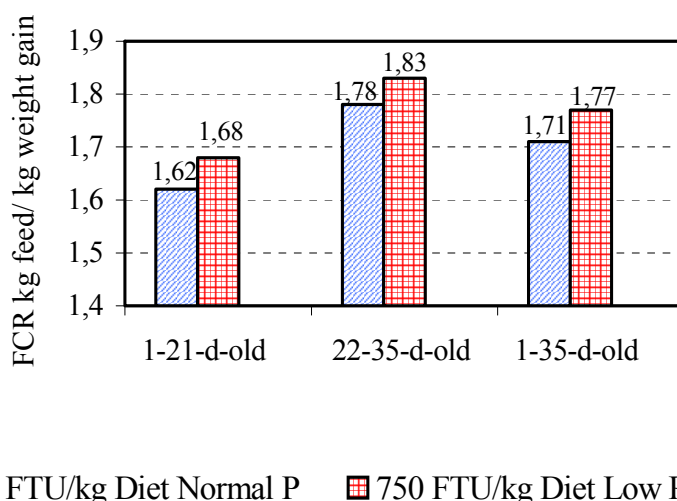


Fig. 2. The effect of microbial phytase on the FCR

Table 3. The results of feed nutrient digestibility experiment using not dehulled rape cake

Utilization, %	Control group	Experimental group
Ca	33.9±1.9	36.7±4.0
P	45.2±2.0	48.2±2.7

Table 4. The results of feed nutrient digestibility experiment using dehulled rape cake

Utilization %	Control group	Experimental group
Ca	32.1±3.6	50.4±9.7
P	38.3±5.4	47.9±5.8

Accumulation of phosphorus in the *tibia* was also analysed, showing that the content of this element in control group was 40.4±2.6%, in the experimental group – 41.5±2.3 % (P>0.05).

**Discussion.** The objective of the feeding and feed nutrient digestibility trials on chicken broilers was to determine opportunities for using a new enzyme preparation containing phytase as an active ingredient, produced by submersion fermentation of *Aspergillus oryzae*. The new phytase, derived from *Peniophora lycii* is a 6-phytate i.e. it attacks the phytate ring at the 6-P position. In this respect it is similar to natural phytases in plants (Khan, 2000). According to the literature (Korsbak, 2001) by using this phytase enzyme preparation at a dose of 300 g/t of feed, it is possible to reduce the amount of digestible phosphorus to 891 g. In this case, according to the recommendations, the addition of phytase could reduce the amount of nP to 0.38 %, but it was reduced to 0.28 %. So, the results presented here demonstrate that the

growth and FCR of broiler chickens of control group was worse than in the experimental group. This finding is supported by observations in other experiments (Zyla, 1992). Three diets (A, B, C) were formulated for the broiler chickens. The control diet A was formulated to provide sufficient level of nP (0.47 %) and the level of nP in the diets B and C was insufficient for chickens (0.27 %) but the enzyme preparation in diet C was added. The results have demonstrated that the body weight gain and *tibia* mineralization of chicks in group A and C did not differ significantly, but the body weight gain in group B was significantly lower (12 %).

The results of feed nutrient digestibility experiments show that supplementation of phytase improves the utilization of total Ca, total P and accumulation of P in the bone of *tibia*. The better indices in the feed nutrient digestibility experiment using dehulled rape cake might be explained by a bigger amount of nP (6.23 g/kg DM) in the rape cake produced from dehulled rapeseed (Table 5).

Table 5. Phytate-P and total P amount in the various processed rape cake

	Phytat-P g/kg DM <sup>1)</sup>	CV <sup>2)</sup> %	Total P g/kg DM	CV %
The rape cake from not dehulled rapeseed	7.96	0.58	10.34	0.28
The rape cake from dehulled rapeseed	6.14	2.02	12.37	0.43

<sup>1)</sup> DM – dry matter

<sup>2)</sup> CV – coefficient of variation

### Conclusions.

1. The feeding experiment using not dehulled rape cake without monocalcium phosphate additive, though supplemented with phytase preparation showed a 3.0% decrease in body weight when compared to the control group ( $P < 0.05$ ), and the feed conversion ratio was by 3.0% better than in the control group ( $P > 0.05$ ).

2. The above-mentioned enzyme preparation improved the digestibility of total calcium by 2.8% and total phosphorus by 3.0% ( $P > 0.05$ ) in feed containing not dehulled rape cake.

3. The feed nutrient digestibility investigation with dehulled rape cake showed that the enzyme addition improved the digestibility of total calcium and total phosphorus in comparison with the control group by 18.3% and 9.6% respectively ( $P > 0.05$ ).

4. Phytase supplement in feed with a decreased level of phosphorus in the ration that includes 20% not dehulled rape cake component increases accumulation of phosphorus in the *tibia* bone by 0.8% ( $P > 0.05$ ) and in the experiment using dehulled rape cake – by 1.1 % ( $P > 0.05$ ).

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