BIOLOGIJA. 2006. Nr. 1. P. 69–72

Effect of phytase on P and Ca utilization at different age periods of laying hens fed higher amount of rapeseed meal

V. Šašytė[1*],

A. Racevičiūtė-Stupelienė[1],

R. Gružauskas[1],

R. Mosenthin[2]

[1] *Lithuanian Veterinary Academy, Tilžės 18, 47181 Kaunas, Lithuania*

[2] *Institut für Tierernährung der Universität Hohenheim, Germany*

The aim of the present research was to determine the content of phytate phosphorus in rapeseed meal and to evaluate the effects of exogenous enzyme (phytase) on the utilization of phosphorus (P) and calcium (Ca) by laying hens. The chemical analyses of rapeseed meal used in the further test with laying hens showed it to contain a very significantly high level of phytate-P (8.61 mg/g dry mass, DM) – about 77% DM of the total P in the form of phytate-P. The results of the metabolic test with Lohmann LSL hens between 33 and 42 wk of age fed diets with a different content of available P (0.20–0.40%) and with a higher content of rapeseed meal indicated the phytase (produced from fungus *Pheniophora lycii*) additives to improve digestibility of both P and Ca in particular. Dietary P concentration had a positive influence on the digestibility of Ca ($P < 0.05$). It allows not only reducing a dietary P level, but also significantly saving on feed cost.

Key words: laying hens, rapeseed meal, phytase, phosphorus, calcium, utilization

INTRODUCTION

Defatted rapeseed meal, with up to 40% protein rich in lysine and sulphur-containing amino acids, is considered to have a high potential nutritional value [1]. Using local feedstuffs would enable to replace an imported protein source in the poultry feeds [2]. However, the presence of phytate (myco-inositol hexakiphosphate) in rapeseed meal causes phosphorus (P), calcium (Ca), protein and other nutrient deficiency syndromes in poultry [1, 3]. Poultry lack the enzyme needed to digest efficiently phytate in their feed. The additional supplementation of inorganic feed phosphates to poultry diets results in a substantial excretion of phosphorus, leading to the accumulation of this element in soils and subsequently entering surface and ground waters, and sparking off major environmental concerns [4, 5].

Exogenous enzyme phytase (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) could be one of the solutions to avoid a negative influence of antinutritional factors present in rapeseed and their by-products. It catalyses the release of phosphate from phytate. The efficiency of such enzymes depends on the content of the diets, the concentration of antinutritional factors in the raw materials, poultry age and species, enzyme inclusion level in the diet, etc. [6–8].

Another alternative approach to a decrease of phytic acid content in agricultural products could be the use of chemical methods. However, such methods are generally expensive and also affect the nutritional quality of the products [9].

The objectives of the present studies were: 1) to determine the phytate-P content in rapeseed meal; 2) to evaluate the effects of a new phytase on the digestibility of P and Ca by laying hens of different age fed a higher amount of rapeseed meal.

MATERIALS AND METHODS

The scientific investigations have been made following the provisions of Law of Republic of Lithuania on Protection, Keeping and Use of Animals.

Analyses of rapeseed meal. The amounts of total P, phytate-P in the rapeseed meal used in the experiment with laying hens were investigated at the Institute of Animal Nutrition, Hohenheim University (Germany). The phytate-P content was determined according to the AOAC-method [10] by exchange of anions. The phytate-P was extracted from a double-dried sample diluted with 2.5% hydrochloric acid, mixed with EDTA/NaOH solution and put into an anion-exchange column. Phytate was eluted with 0.7 mol NaCl and broken down by a mixture of concentrated HNO_3 / H_2SO_4 to determine the total P colorimetrically. The phosphorus analyses in rapeseed me-

* Corresponding author. E-mail: sasvil@lva.lt

Table 1. **Composition of the experimental diets (%)**

Ingredients	Treatment1		Treatment 2		Treatment 3	
Calculated values	Phy* –	Phy +	Phy –	Phy +	Phy–	Phy +
Corn	38.00		38.25		38.50	
Wheat	10.00		10.00		10.00	
Soybean meal	15.60		15.60		15.60	
Rapeseed meal	15.00		15.00		15.00	
Sunflower meal	4.00		4.00		4.00	
Sunflower oil	5.40		5.40		5.40	
Limestone	8.60		9.15		9.75	
Dicalcium phosphate	2.10		1.30		0.45	
NaCl	0.30		0.30		0.30	
Premix**	1.00		1.00		1.00	
AME (MJ/kg)	11.39		11.42		11.45	
Crude protein	16.99		17.01		17.03	
Crude fat	7.88		7.89		7.90	
Lysine	0.85		0.85		0.86	
Methionine + cystine	0.65		0.65		0.65	
Total Ca	3.60		3.60		3.60	
Total P	0.84		0.69		0.53	
Available P	0.40		0.30		0.20	
Na	0.13		0.13		0.13	

* Enzyme preparation obtained from *Pheniophora lycii* (DSM, 4070 Basel, Switzerland). Product dosage is 180 g/t (2500 FYT/g).

** Composition (per kg): Vit. A 10000 IU; Vit. D_3 2500 IU; Vit. E 30 mg; Vit. K_3 3 mg; Vit. B_1 1 mg; Vit. B_2 4 mg; Vit. B_3 8 mg; Vit. B_4 400 mg; Vit. B_5 30 mg; Vit. B_6 3 mg; Folic acid 0.5 mg; Vit. B_{12} 0.015 mg; Vit. H 0.025 mg; Zinc 60 mg; Copper 5 mg; Iron 25 mg; Manganese 100 mg; Iodine 0.5 mg; Cobalt 0.1 mg; Selenium 0.2 mg; Butylhydroxytoluene 100 mg.

Table 2. **Digestibility of phosphorus at different age periods by laying hens fed corn-soybean rapeseed meal diets supplemented with phytase enzyme (%)**

Age periods	Treatment					
	1		2		3	
	Phytase supplementation					
	–	+	–	+	–	+
34th week of life (Trial 1)	34.76 ± 3.44	35.77 ± 3.13	34.29 ± 1.83	35.03 ± 3.53	30.89 ± 8.69	34.18 ± 4.32
38th week of life (Trial 2)	34.33 ± 4.00	35.38 ± 3.55	33.13 ± 3.23	37.83 ± 6.36	32.14 ± 5.93	36.54 ± 3.71
42nd week of life (Trial 3)	36.61 ± 2.70	37.18 ± 3.06	30.84 ± 4.09	35.06 ± 1.00	34.57 ± 5.24	36.81 ± 3.74

Results of ANOVA: "Treatment" – P = 0.349; "Phytase" – P = 0.002; "Period" – P = 0. 416; "Treatment*Phytase" – P = 0. 203; "Treatment*Period" – P = 0.448; "Phytase*Period" – P = 0.935; "Treatment*Phytase*Period" – P = 0.371.

Values represent means (n = 5) ± standard errors of the means (SEM).

al were made according to the methods of VDLUFA [11]. The total phosphorus was measured spectrophotometrically at a wavelength of 420 nm after mineralising the sample by breaking down and converting with vanadomolybdate.

Test with laying hens. The metabolic test was carried out with 30 Lohmann LSL (Lohmann Tierzucht GbmH, Germany) hens aged between 33 and 42 wk, divided into 6 groups 5 birds each, kept individually in single cages (0.36 m × 0.44 m) on a wire netted floor with stationary feeding troughs and drinking bowls. The test was conducted in the aviary of the Lithuanian Veterinary Academy. All birds were clinically healthy. The ambient temperature varied from 18 °C to 22 °C, artificial light was on for 14 hours per day, and light intensity was from 10 to 15 Lux.

Table 3. **Digestibility of calcium at different age periods by laying hens fed corn-soybean rapeseed meal diets supplemented with phytase enzyme (%)**

Age periods	Treatment					
	1		2		3	
	Phytase supplementation					
	–	+	–	+	–	+
34th week of life (Trial 1)	45.47 ± 10.14	62.11 ± 6.39	58.47 ± 4.30	59.98 ± 3.88	43.33 ± 9.42	52.15 ± 14.67
38th week of life (Trial 2)	47.63 ± 3.17	58.92 ± 5.78	59.55 ± 4.57	62.87 ± 4.13	50.95 ± 6.13	51.58 ± 6.81
42nd week of life (Trial 3)	52.29 ± 5.31	59.48 ± 6.30	58.63 ± 4.18	60.40 ± 3.89	51.81 ± 3.34	53.07 ± 4.35

Results of ANOVA: "Treatment" – $P < 0.001$; "Phytase" – $P < 0.001$; "Period" – $P = 0.230$; "Treatment*Phytase" – $P = 0.008$; "Treatment*Period" – $P = 0.669$; "Phytase*Period" – $P = 0.462$; "Treatment*Phytase*Period" – $P = 0.890$.
Values represent means ($n = 5$) ± standard errors of the means (SEM).

The hens were fed a corn-soybean rapeseed meal diets (Table 1) containing 0.84, 0.69, 0.53% total phosphorus corresponding to 0.40, 0.30, 0.20% of available phosphorus. Each of the three diets was fed unsupplemented (control) or supplemented with phytase (2500 FYT/g). This phytase is produced by submerged fermentation of *Aspergillus oryzae* carrying a gene from *Peniophora lycii* coding for the phytase (I.U.B. 3.1.3.26). It is a 6-phytase, i.e. attacks the phytate ring at the 6-P position. In this respect it is similar to phytase occurring naturally in plants [12]. The composition of the diet (170 g/kg crude protein and 11.4 MJ ME/kg) was adjusted to the respective requirements of the National Research Council [13]. The experiment was conducted according to the methodical direction of Schieman [14]. The content of nutrients in the diets had been determined before the trial. Activity of phytase had been certified before the experiment. The diet was the only source of P and Ca. Utilization of P and Ca by the birds was determined on the 34th, 38th and 42nd week of life. The periods for adaptation, preparation and collections lasted three, five and again five days, respectively. The excrements were collected individually at 07:00 and 19:00, weighed and stored frozen at –18 °C until analysed. After finishing the collecting period, the excrements were thawed and samples were dried in an oven at 65 °C. Excrements along with diet samples were ground to pass a 1 mm sieve.

The phosphorus and calcium analyses in feed mixtures and excrements were made according to the methods of VDLUFA [11]. Calcium was extracted from matters by ashing a known weight of sample and dissolving the ash in mixed hydrochloric and nitric acids. Calcium was precipitated with ammonium oxalate. Precipitates were dissolved with sulphuric acid, and oxalic acid was titrated against the standard permanganate solution.

Statistical methods. Data were subjected to ANOVA procedures using the MINITAB™ computational package for Windows 13.0. Statements of statistical significance were based on $P < 0.05$ [15].

RESULTS AND DISCUSSION

Analyses of rapeseed meal. One of the objectives of our investigation was to determine phytate-P in rapeseed meal to be used in the experiment with laying hens. Rapeseed meal has a very high level of phytate-P (8.61 mg/g dry matter (DM)), comprising about 77% DM of the total phosphorus in the form of phytate-P. Our results are in the range to those obtained by other authors [16–19]. The different values found in the literature on the content of phytate-P in rapeseed meal may be due to genetic differences, variety, soil, climatic conditions and the analytical method used.

Layer trial. The results of the trial with laying hens fed corn-soybean rapeseed meal diet with or without phytase are presented in Tables 2 and 3. Phytase additives particularly improved the digestibility of both P and Ca (Tables 2 and 3; $P < 0.001$ and $P = 0.002$, respectively). Digestibility of Ca varied also depending on the dietary P concentration (Table 2; $P < 0.001$). The level of available P in the diets did not have a significant influence on the digestibility of P, however, dietary P concentration had a positive influence on digestibility of Ca.

Khan [12] reported an increase in Ca and P digestibility by 35% in broilers after addition of 6-P phytase. Therefore, reducing the available phosphorus (to 0.20%) in the diet of laying hens by the addition of phytase, the digestibility of Ca and P is improved allowing not only to reduce the dietary phosphorus level, but also to lower the feed cost significantly. Phosphorus is the third most expensive

nutrient in feed formulation for laying hens (after energy and protein). It could be a good solution for reducing the problems of potential phosphorus pollution and for a higher level of rapeseed meal in the diets.

Received 5 December 2005
Accepted 22 February 2006

References

1. Zyla K, Koreleski J. J Sci Food Agric 1993; 61: 1–6.
2. Keller TH, Hauschild A, Jeroch H. Übersichten für Tierernährung. Frankfurt am Main 1996. 24(2): 167–98.
3. Kies AK, Selle PH. Proc Aust Poult Sci Sym 1998; 128–31.
4. Kornegay ET, Harper AF. Prof Anim Sci 1997; 13: 99–111.
5. Yu B, Jan YC, Chung TK et al. J Anim F Sci Tech 2004; 117: 295–303.
6. Lim HS, Namkung H, Paik IK. J Poult Sci 2003; 82: 92–9.
7. Scott TA, Kampen R, Silversides FG. Can J Anim Sci 2001; 81: 393–401.
8. Van Der Klis JD, Versteegh HAJ, Simons PCM, Kies AK. J Poult Sci 1997; 76: 1535–42.
9. Pandey A, Szakacs G, Soccol CR, Rodriguez-Leon JA, Soccol VT. B Tech 2001; 77: 203–14.
10. AOAC. J Assoc Off Anal Chem. 15th ed. Arlington 1990; II: 800.
11. Naumann C, Bassler R. (ed.). Methodenbuch, Band III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt 1993.
12. Khan N. In*:* 3rd European Symposium on Feed Enzymes, Noordwijkerhout, the Netherlands 2000; p. 35.
13. National Research Council. Nutrient Requirements of Poultry. 9th ed.; National Academy Press: Washington DC, 1994.
14. Schiemann R. Arch. Tiernährung, Berlin 1981; 31(1): 1–19.
15. MINITAB. MINITAB Reference Mannual, PC Version, Release 13.0, (State College, PA, Minitab Inc) 1996.
16. Eeckhout W, De Paepe M. Anim Feed Sci Technol 1994; 47: 19–29.
17. Ravindran V, Cabahug S, Ravindran G, Bryden WL. Poult Sci 1999; 78: 699–706.
18. Thompson LU. In 4th World Congress of Animal Feeding. Madrid, 1986.
19. Viveros A, Centeno C, Brenes A et al. J Agric Food Chem 2000; 48: 4009–13.

V. Šašytė, A. Racevičiūtė-Stupelienė, R. Gružauskas, R. Mosenthin

FITAZĖS POVEIKIS FOSFORO IR KALCIO PASISAVINIMUI SKIRTINGAME VIŠTŲ AMŽIUJE NAUDOJANT DIDESNĮ RAPSŲ IŠSPAUDŲ KIEKĮ

S a n t r a u k a

Šio darbo tikslas buvo nustatyti fitatų fosforo kiekį rapsų sėklų išspaudose ir įvertinti egzogeninio fermento – fitazės – poveikį fosforo ir kalcio pasisavinimui vištų dedeklių organizme. Rapsų sėklų išspaudų cheminiais tyrimais nustatyta, kad juose yra labai daug fitatų fosforo (8,61 mg/g SM), o tai sudaro 77% SM bendrojo fosforo kiekio. Lohmann LSL vištų dedeklių (33–42 savaičių amžiaus) medžiagų apykaitos tyrimo rezultatai rodo, kad fitazės (pagaminta iš bakterijų kamieno *Pheniophora lycii*) įterpimas į lesalus, esant skirtingam pasisavinamo fosforo kiekiui (0,2–0,4%) ir didesniam rapsų sėklų išspaudų kiekiui, statistiškai patikimai pagerino kalcio ir fosforo pasisavinimą ($p < 0,05$). Tai leidžia sumažinti fosforo įterpimą į vištų dedeklių lesalus kartu sumažinant fosforo išskyrimą į aplinką.