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Effect of Phytase on Digestibility and Performance of Growing and Finishing Pigs Fed Diets with Lupins and Rapeseed Meal

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Abstract: Lupin seeds and rapeseed meal (RSM) contain relatively high amounts of poorly digestible phytate. Phytase additive can help in the utilization of nutrients from the diet. The aim of this study was to determine total tract digestibility coefficients of nutrients and performance results of finishing pigs fed diets containing yellow lupin or narrow-leafed lupin seeds and/or RSM with similar or increasing levels of phytase. Three experiments were conducted. In Experiment I the effect of RONOZYME®HiPhos (100 g/t) in diets containing narrow-leafed or yellow lupin seeds and RSM on production parameters of fatteners was analyzed. In Experiment II the effect of phytase RONOZYME®HiPhos (1,000 FTU/t) in similar diets was analyzed but calcium (Ca) and phosphorus (P) levels in diets were reduced. In Experiment III the effect of increasing levels of Quantum Bluephytase (0, 5,000, 10,000 and 15,000 FTU/t) in diets with yellow lupin seeds with reduced Ca and P level on total tract digestibility coefficients of selected nutrients and performance of pigs was analyzed. In none of the experiments the enzyme additives included in the diet affected pig performance ($p > 0.05$). There were no significant differences ($p > 0.05$) in apparent total tract digestibility coefficients of dry matter (DM) and crude protein (CP). The phytase additives significantly improved P and Ca digestibility coefficients ($p < 0.05$) in comparison with the control diet, but this improvement was not linearly related with phytase dosage. By improving digestibility phytase allows to reduce mineral contents in diets, thus reducing the cost of pig nutrition.

Key words: Phytase, lupin, rapeseed meal, weaners, fatteners, digestibility.

1. Introduction

Feed components for pigs contain different amounts of phosphorus (P) (e.g., maize—0.26%, wheat—0.32%, soybean meal (SBM)—0.62% and rapeseed meal (RSM)—1.16%), but more than 30% of total P may be found in an indigestible form such as phytic acid or phytate [1]. They are the main storage forms of P in plants. Generally, only about 27% of phytate P is digestible for pigs, whereas the rest is excreted in the feces [2]. Phytic P is poorly available to monogastric animals due to the lack of phytase production. Therefore, it is recognized as an anti-nutrient due to its effect on mineral absorption. Phytic acid impairs the absorption of iron (Fe), zinc (Zn) and calcium (Ca) and may promote mineral deficiencies [3]. Phytate can also interfere with other important nutrients such as proteins and starch by forming insoluble complexes, as well as reduce digestibility and utilization of individual nutrients. In many studies investigating diets including faba bean, pea, whole flour, protein concentrate, protein isolates and other supplements, it was found that interactions of phytic acid and protein negatively affect protein digestibility *in vitro*. Phytate, however, maybe degraded by phytases. Phytases are enzymes that catalyze the hydrolysis of phytic acid to inorganic phosphate and myo-inositol phosphate derivatives [4]. Most phytases can cleave five of the six phosphate groups from phytic acid [5]. They have been grouped based on the first phosphate position of phytic acid that is hydrolyzed as 3-phytases (EC-3.1.38) and

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6-phytases (EC 3.1.3.26). The 3-phytases are generally found in some bacteria and fungi, especially *Aspergillus niger*, but they may not always completely dephosphorylate a phytic acid molecule. The 6-phytase is of microbial origin, derived from *Escherichia coli* or *Peniophora lycii*. The microbial phytases are relatively more effective than fungal ones [6]. Commercial phytases differ in optimal pH, resistance to endogenous proteases and affinity to phytate substrate, which may be the main factors influencing theirs *in vivo* efficacy. The effect of microbial phytase depends also on the dietary phytate concentration, the source of phytate, species and age of animals, mineral concentrations in the diet, phytase sources and phytase dosage [7-9]. Phytase can reduce the anti-nutritional effect of phytate and improve the digestibility of P, Ca, amino acids and energy, as well as reduce the negative impact of inorganic P excretion to the environment [2, 10]. Moreover, in some studies phytase has been shown to improve performance of pigs [11, 12]. In some cases, however, performance effects could not be attributed to enhanced P digestibility, but rather increased protein and amino acid digestibility [9]. Some authors [13-16] found a positive correlation between percentage improvements in feed efficiency in response to phytase and dietary phytate contents. It is especially evident in the diet based on legume seeds, SBM, RSM, sesame meal, sunflower meal and sorgo [17, 18]. In view of the above, the hypothesis of this study is that the use of phytase in the diet containing lupin seeds and RSM, being rich in phytate, will improve nutrient digestibility and performance parameters of growing pigs.

In order to verify this hypothesis three experiments were conducted on growing and fattening pigs. The aim of this study was to determine total tract digestibility coefficients of protein and minerals and investigate performance results of growing and finishing pigs fed diets containing yellow lupin seeds (YL) or narrow-leafed lupin seeds (NL) and/or RSM with similar or increasing levels of phytase.

2. Materials and Methods

2.1 Enzymes

In the experiments two different phytases were used. In Experiments I and II, it was RONOZYME®HiPhos (DSM). According to the producer’s information, it is phytase thermostable up to 95 °C, exhibiting excellent flow ability enabling accurate dosing of 500-4,000 FTU/kg, with many particles, ensuring good homogeneity, virtually dust-free and safe to use. It is produced by *A. oryzae*. In Experiment III it was Quantum Blue phytase (AB Vista) produced by *E. coli* with the activity of 40,000 FTU/kg.

2.2 Ethical Statement

All the experimental procedures complied with the guidelines of the Local Ethical Committee for Experiments on Animals in Poznan regarding animal experimentation and the animal care under study (EU Directive 2010/63/EU for animal experiments). The pigs received all the necessary veterinary vaccinations and had unlimited access to water.

2.3 Animals, Diets and Experimental Design

2.3.1 Experiment I: The Effect of RONOZYME®HiPhoson Production Parameters of Fatteners Fed Diets with NL and RSM

The experiment was conducted on 216 (♀, ♂) crossbreed pigs (P76 × Naima) with an initial body weight of about 30 kg. Animals were housed in groups (10 pigs) on straw and were divided into two dietary treatments with 108 individuals within each group. The experiment lasted 80 d, and was divided into two stages: Finisher I lasting 45 d, and Finisher II lasting 35 d. Both the control and experimental diets contained NL and RSM as the main protein feed component, while in the experimental diet RONOZYME®HiPhos (E) was added in the amount of 100 g/t (Table 1). All the diets were provided in the mash form and prepared according to the *Recommended Allowances and Nutritive Value of Feedstuffs for Swine* [19]. Data on weight gain (WG) and feed intake (FI) were collected on the last day of each stage. Based on the possessed data, feed conversion ratio (FCR) was calculated.

2.3.2 Experiment II: The Effect of RONOZYME®HiPhos Phytase on Production Parameters of Fatteners Fed Diets with SBM, Lupin Seeds and RSM

The experiment was conducted on 60 crossbreed pigs (P76 × Naima) with an initial body weight of about 33 kg. Animals were housed on straw in single pens and were divided into six dietary treatments with 10 individuals within each group (5♀ and 5♂). The experiment lasted 80 d, and was divided into two stages: Finisher I lasting 45 d, and Finisher II lasting 35 d. The diets were prepared according to the design presented in Table 2. In diets 1 and 2, pigs were offered SBM as the main protein feed, whereas in diets 3 and 4, SBM was totally replaced by YL and in diets 5 and 6 by NL with RSM. In addition, in diets 2, 4 and 6, RONOZYME®HiPhos (E) was added at 100 g/t, whereas in Finisher I, the Ca and P levels were

Table 1 Composition and nutritional value of diets of Finishers I and II—Experiment I.

Components (%)	Finisher I		Finisher II	
	Control	Experiment	Control	Experiment
NL meal	22.38	22.38	13.47	13.47
RSM (35% CP)	6.83	6.82	11.43	11.42
Triticale	65.00	65.00	70.00	70.00
Soya oil	1.75	1.75	1.50	1.50
Monocalcium phosphate	1.05	1.05	0.63	0.63
Limestone	1.16	1.16	1.50	1.50
NaCl	0.31	0.31	0.30	0.30
Premix*/**	0.50	0.50	0.50	0.50
L-lysine (98.5%)	0.60	0.60	0.42	0.42
D,L-methionine (99%)	0.21	0.21	0.12	0.12
L-threonine (99%)	0.18	0.18	0.12	0.12
Phytase	-	0.01	-	0.01
Calculated nutritional value of diets				
Metabolizable energy (MJ/kg)	13.1		13.0	
CP (g/kg)	17.6		15.5	
Lysine (g/kg)	0.98		0.86	
Methionine (g/kg)	0.31		0.26	
Tryptophan (g/kg)	0.19		0.17	
Threonine (g/kg)	0.65		0.53	
Ca (g/kg)	0.86		0.72	
P (g/kg)	0.58		0.46	
Na (g/kg)	0.13		0.10	

* mineral and vitamin premix content (per kilogram). Choline chloride 40,000 mg, Fe 15,000 mg, Cu 4,000 mg, Co 60 mg, Mn 6,000 mg, Zn 15,000 mg, I 120 mg, Se 30 mg, antioxidants (butylated hydroxyanisole, butylated hydroxytoluene); vit. A 1,500,000 IU, vit. D3 300,000 IU, vit. E 10,500 mg, vit. K3 220 mg, vit. B1 220 mg, vit. B2 600 mg, vit. B6 450 mg, pantothenic acid 1,500 mg, nicotinic acid 3,000 mg, folic acid 300 mg, vit. B12 3,700 µg, biotin 15,000 µg, Ca 260 g.

** mineral and vitamin premix content (per kilogram). Choline chloride 20,000 mg, Fe 10,000 mg, Cu 4,000 mg, Co 40 mg, Mn 4,000 mg, Zn 8,000 mg, I 80 mg, Se 20 mg, antioxidants (butylated hydroxyanisole, butylated hydroxytoluene); vit. A 1,000,000 IU, vit. D3 200,000 IU, vit. E 7,000 mg, vit. K3 150 mg, vit. B1 215 mg, vit. B2 400 mg, vit. B6 300 mg, pantothenic acid 1,000 mg, nicotinic acid 2,000 mg, folic acid 200 mg, vit. B12 2,500 µg, biotin 10,000 µg, Ca 280 g.

NL: narrow-leafed lupin seeds; RSM: rapeseed meal; CP: crude protein.

Table 2 Experimental design—Experiment I.

Group	1	2	3	4	5	6
Finisher I	SBM	SBM + E	YL	YL + E	NL + RSM	NL + RSM + E
Finisher II	SBM	SBM + E	YL	YL + E	NL	NL + E

SBM: soybean meal; E: enzyme; YL: yellow lupin seeds; NL: narrow-leafed lupin seeds; RSM: rapeseed meal.

Table 3 Composition and nutritional value of diets of Finisher I—Experiment II.

Components (%)	1	2	3	4	5	6
SBM	17.01	17.01	-	-	-	-
YL	-	-	21.99	21.99	-	-
NL	-	-	-	-	9.98	9.98
RSM	-	-	-	-	15.00	15.00
Triticale	79.88	80.77	73.73	74.62	70.00	70.90
Limestone	1.29	1.10	1.29	1.10	1.20	1.00
Monocalcium phosphate	0.73	-	0.73	-	0.73	-
Salt	0.29	0.29	0.29	0.29	0.29	0.29
Soya oil	-	-	0.99	0.99	1.98	1.98
Premix*	0.51	0.51	0.51	0.51	0.51	0.51
L-lysine (99.5%)	0.20	0.20	0.25	0.25	0.25	0.25
D,L-methionine (99%)	0.02	0.02	0.09	0.09	0.03	0.03
D,L-threonine (99%)	0.07	0.07	0.09	0.09	0.02	0.02
D,L-tryptophan (99%)	-	-	0.04	0.04	0.01	0.01
Phytase	-	0.01	-	0.01	-	0.01
Calculated nutritional value of diets						
Metabolizable energy (MJ/kg)	13.1	13.1	13.1	13.1	13.1	13.1
CP (g/kg)	17.4	17.5	17.4	17.5	17.3	17.4
Lysine (g/kg)	0.98	0.98	0.98	0.98	0.98	0.98
Methionine (g/kg)	0.32	0.32	0.31	0.31	0.31	0.31
Tryptophan (g/kg)	0.19	0.19	0.20	0.20	0.19	0.19
Threonine (g/kg)	0.66	0.66	0.65	0.65	0.65	0.65
Ca (g/kg)	0.82	0.59**	0.81	0.58**	0.84	0.65**
P (g/kg)	0.55	0.39**	0.56	0.40**	0.58	0.45**
Na (g/kg)	0.13	0.13	0.12	0.12	0.13	0.13

*premix composition identical as in Experiment I; ** reduced value.
SBM: soybean meal; YL: yellow lupin seeds; NL: narrow-leafed lupin seeds; RSM: rapeseed meal; CP: crude protein.

reduced to 70% of pig dietary requirements and in Finisher II the Ca level was reduced to 70% and the P level to 85% of pig dietary requirement (Tables 3 and 4). All the diets were provided in the mash form in this same amount for each group and prepared according to the *Recommended Allowances and Nutritive Value of Feedstuffs for Swine* [19]. Data on WG and FI were collected on the last day of each stage. Based on the possessed data the FCR was calculated.

2.3.3 Experiment III: The Effect of Increasing the Level of Quantum Blue Phytase Added to Diets with YL on Total Tract Digestibility Coefficients of Selected Nutrients and on Growth Parameters of Pigs

The experiment was conducted on 50 (♀, ♂) crossbreed pigs (P76 × Naima) with an initial body weight of about 20 kg. Animals were housed on straw in individual pens and were divided into five dietary treatments with 10 individuals within each group. The experiment lasted 28 d. Pigs were offered feed mixtures according to the following experimental design:

PC: Positive control, without the enzyme;

NC: Negative control, without the enzyme, Ca level reduced by 30%, P level reduced by 20%;

E5000: Reduced Ca and P levels + phytase 5,000 units/t;

E10000: Reduced Ca and P levels + phytase 10,000 units/t;

E15000: Reduced Ca and P levels + phytase 15,000 units/t.

All the diets (Table 5) were provided *ad libitum* in the mash form and prepared according to the *Recommended Allowances and Nutritive Value of Feedstuffs for Swine* [19]. To facilitate determination of digestibility, titanium dioxide was included as a non-absorbable marker at 3 g/kg. Data on WG and FI were collected on the last day of each stage. Based on the recorded data the FCR was calculated. At the end of the experiment excreta collection was performed for 3 d. Excreta was individually collected twice per day and immediately frozen and lyophilized before analysis. The coefficients of apparent total tract digestibility (ATTD) for the nutrients contained in the diets were calculated according to the following equation:

$$\text{ATTD (\%)} = 100 - 100 \times (\text{\% of marker in feed/\% of marker in feces}) \times (\text{\% nutrient in feces/\% of nutrient in feed}) \quad (1)$$

Table 4 Composition and nutritional value of diets of Finisher II—Experiment II.

Components (%)	1	2	3	4	5	6
SBM	11.00	11.00	-	-	-	-
YL	-	-	15.00	15.00	-	-
NL	-	-	-	-	20.00	20.00
Triticale	86.44	87.13	82.38	83.07	76.32	77.01
Limestone	1.30	0.09	1.30	0.90	1.30	0.90
Monocalcium phosphate	0.23	0.23	0.23	0.23	0.23	0.23
Salt	0.30	-	0.3	-	0.03	-
Soya oil	0.50	0.50	0.5	0.50	0.50	0.50
Premix*	-	-	-	-	1.00	1.00
L-lysine (99.5%)	0.20	0.20	0.20	0.20	0.25	0.25
D,L-methionine (99%)	-	-	0.04	0.04	0.05	0.05
D,L-threonine (99%)	0.01	0.01	0.03	0.03	0.03	0.03
D,L-tryptophan (99%)	0.02	0.02	0.02	0.02	0.02	0.02
Phytase	-	0.01	-	0.01	-	0.01
Calculated nutritional value of diets						
Metabolizable energy (MJ/kg)	13.1	13.1	13.1	13.1	13.1	13.1
CP (g/kg)	15.4	15.5	15.5	15.6	15.4	15.5
Lysine (g/kg)	0.84	0.84	0.84	0.84	0.85	0.85
Methionine (g/kg)	0.27	0.27	0.26	0.26	0.26	0.26
Tryptophan (g/kg)	0.17	0.17	0.17	0.17	0.17	0.17
Threonine (g/kg)	0.54	0.54	0.53	0.53	0.53	0.53
Ca (g/kg)	0.70	0.49**	0.70	0.49**	0.72	0.49**
P (g/kg)	0.45	0.38**	0.46	0.39**	0.46	0.36**
Na (g/kg)	0.10	0.10	0.10	0.10	0.10	0.10

*premix composition identical as in Experiment I; ** reduced value.

SBM: soybean meal; YL: yellow lupin seeds; NL: narrow-leafed lupin seeds; CP: crude protein.

Table 5 Composition of diets—Experiment III.

Components (%)	PC	NC	E5000	E10000	E15000
YL	30.00	30.00	30.00	30.00	30.00
Corn	20.00	20.00	20.00	20.00	20.00
Triticale	44.46	45.16	45.1475	45.1350	45.1225
Rapeseed oil	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate	0.35	-	-	-	-
Limestone	0.95	0.60	0.60	0.60	0.60
NaCl	0.23	0.23	0.23	0.23	0.23
TiO_2	0.30	0.30	0.30	0.30	0.30
Premix*	0.50	0.50	0.50	0.50	0.50
L-lysine (98.5%)	0.15	0.15	0.15	0.15	0.15
L-threonine (95%)	0.04	0.04	0.04	0.04	0.04
L-tryptophan (99%)	0.02	0.02	0.02	0.02	0.02
Phytase	-	-	0.0125	0.0250	0.0375
Nutritional value					
Metabolizable energy (MJ/kg)	13.58			13.67	
CP (g/kg)	183.0			183.8	
Lysine digestible (g/kg)	7.75			7.77	
Methionine + cysteine digestible (g/kg)	4.75			4.78	
Threonine digestible (g/kg)	4.79			4.80	
Tryptophan digestible (g/kg)	1.33			1.34	
Ca (g/kg)	6.11			4.02	
P (g/kg)	4.84			4.06	
P available (g/kg)	2.46			1.67	
Na (g/kg)	1.02			1.02	
Ca/P	2.4:1			2.4:1	

*mineral and vitamin premix content (per kilogram). Choline chloride 80,000 mg, Fe 20,000 mg, Cu 32,000 mg, Co 80 mg, Mn 8,000 mg, Zn 28,000 mg, I 160 mg, Se 40 mg, antioxidants (butylated hydroxyanisole, butylated hydroxytoluene); vit. A 2,400,000 IU, vit. D3 300,000 IU, vit. E 14,000 mg, vit. K3 300 mg, vit. B1 300 mg, vit. B2 800 mg, vit. B6 600 mg, vit. B12 5,000 mg, pantothenic acid 2,000 mg, nicotinic acid 4,000 mg, folic acid 400 mg, vit. B12 2,000 µg, biotin 20,000 µg, Ca 180 g.

PC: positive control group, without the enzyme; NC: negative control group, without the enzyme, Ca level reduced by 30%, P level reduced by 20%; E5000: group where reduced Ca and P levels + phytase 5,000 units/t; E10000: group where reduced Ca and P levels + phytase 10,000 units/t; E15000: group where reduced Ca and P levels + phytase 15,000 units/t.

YL: yellow lupin seeds; CP: crude protein.

2.4 Chemical Analysis

For chemical analyses all the samples were ground to pass through a 1.0- or 0.5-mm sieve. The seeds were analyzed in duplicate for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), crude ash (CA), acid detergent fiber (ADF), neutral detergent fiber (NDF) and total P using methods 934.01, 976.05, 920.39, 978.10, 942.05, 973.18, 984.27 and 965.17, respectively, according to AOAC [20]. The level of titanium dioxide (POCH, Gliwice, Poland) was determined according to Short *et al*. [21]; the samples were prepared in accordance with the procedure proposed by Myers *et al*. [22].

2.5 Statistical Analysis

In Experiments I and II, the results were compared in pairs using Student's *t*-test, with alpha level of $p < 0.05$ used to assess the significance between means. In Experiment III the significance of differences between the groups was calculated using two-way analysis of variance (ANOVA) with Duncan's post-hoc test, with alpha level of $p < 0.05$ used to assess the significance between means. The statistical analysis was performed using SAS, ver. 5.0. (Iowa, USA).

3. Results

3.1 Experiment I

Phytase additive had no significant effect ($p > 0.05$) on pigs' performance (Table 6). There were no differences in the studied parameters between the groups in phases T1 and T2 and in the whole experiment, except for FI in T1, where the experimental pigs consumed more feed ($p = 0.023$). In phase I a trend towards higher FCR ($p = 0.064$) was found in the experimental group. In the whole period similar gains in both groups were recorded, although in the experimental group pigs consumed about 7% more feed and had higher FCR ($p > 0.05$).

3.2 Experiment II

Differences between feed variants were observed in the case of all the parameters ($p > 0.05$) (Table 7). No enzyme effect on performance parameters was found ($p > 0.05$). A significant effect of protein source in feed on daily WG was recorded in T1, T2 and T1 + T2 ($p < 0.05$), on FCR in T2 and T1 + T2 and on final body weight ($p < 0.05$) among the groups. Pigs offered YL as the main protein source in the diet had significantly lower gains and higher FCR in comparison with pigs offered SBM in their diets. There were no differences between the animals offered SBM and NL + RSM in their diets.

3.3 Experiment III

There were no significant differences ($p > 0.05$) in terms of ATTD coefficients of DM and CP between the groups (Table 8). ATTD coefficients of fat, P and Ca differed significantly between the groups ($p < 0.05$). Reduction of Ca and P contents in the NC diet improved ($p < 0.05$) Ca digestibility, but did not affect ($p > 0.05$) P and fat digestibility in comparison with the PC diet. Phytase additive significantly improved P and Ca digestibility coefficients ($p < 0.05$) in comparison with the PC diet, but this improvement was not linearly related with phytase dosage ($p > 0.05$). Fat digestibility coefficient was reduced in group E5000 ($p < 0.05$) in comparison with the PC group. Phytase additive had no significant effect on body mass, WG of pigs and feed utilization ($p > 0.05$) in comparison with the PC and NC diets (Table 9). Moreover, pigs from the NC group, with reduced Ca and P levels in their diet, achieved similar results as pigs with normal Ca and P contents in the diet, and as pigs offered diets with a phytase additive.

Table 6 Performance results of fatteners—Experiment I.

Parameters	Control	Experiment	SEM	*p*
Initial body mass (kg)	30.9	30.6	0.2	0.540
Final body mass T1 (kg)	75.7	75.7	0.6	0.933
Total gains T1 (kg)	44.8	45.0	0.5	0.851
Daily gains T1 (kg)	0.786	0.789	0.008	0.851
Feed intake T1/group (kg)	$1{,}180^{b}$	$1{,}283^{a}$	20	0.023
FCR T1 (kg/kg)	2.57	2.80	0.06	0.064
Final body mass T2 (kg)	99.1	98.7	0.7	0.801
Total gains T2 (kg)	23.3	23.1	0.3	0.681
Daily gains T2 (kg)	0.863	0.854	0.011	0.681
Feed intake T2/group (kg)	836	875	30	0.540
FCR T2 (kg/kg)	3.46	3.61	0.11	0.488
Total gains T1 + T2 (kg)	68.1	68.1	0.6	0.957
Daily gains T1 + T2 (kg)	0.811	0.810	0.007	0.957
Total feed intake T1 + T2/group (kg)	2,016	2,158	48	0.165
FCR T1 + T2 (kg/kg)	3.01	3.21	0.07	0.168

$^{a, b}$ values in the same rows with different letters differ significantly at $p < 0.05$.

FCR: feed conversion ratio; SEM: standard error mean.

Table 7 Performance results of fatteners—Experiment II.

Groups	IBW (kg)	FBW (kg)	T1				T2				T1 + T2			
			BWG (kg)	DWG (kg)	FI (kg)	FCR (kg/kg)	BWG (kg)	DWG (kg)	FI (kg)	FCR (kg/kg)	BWG (kg)	DWG (kg)	FI (kg)	FCR (kg/kg)
SBM	33.1	94.6	36.8	0.836	90.7	2.48	24.8	0.707	90.6	3.68	61.6	0.779	181.3	2.95
SBM + E	32.7	94.3	36.4	0.827	90.8	2.50	25.2	0.718	90.5	3.66	61.6	0.780	181.3	2.97
YL	32.9	90.2	34.8	0.791	90.6	2.61	23.6	0.639	90.4	4.08	57.2	0.722	181.0	3.18
YL + E	33.1	91.0	34.3	0.778	90.6	2.68	24.1	0.673	90.4	3.87	57.9	0.734	181.0	3.15
NL + RSM	33.2	92.1	34.9	0.792	91.0	2.62	24.1	0.688	90.9	3.79	58.9	0.746	181.9	3.09
NL + RSM + E	32.9	92.6	35.3	0.805	90.3	2.57	24.5	0.700	90.3	3.72	59.7	0.755	181.6	3.03
PS: SBM	32.9	94.4^{a}	36.6	0.832^{a}	90.8	2.49	24.9^{a}	0.712^{a}	90.6	3.67^{b}	61.5^{a}	0.779^{a}	181.3	2.96^{b}
PS: YL	33.0	90.5^{b}	34.5	0.784^{b}	90.6	2.65	23.0^{b}	0.657^{b}	90.4	3.97^{a}	57.5^{b}	0.728^{b}	181.0	3.16^{a}
PS: NL + RSM	33.1	92.3^{ab}	35.1	0.796^{ab}	90.6	2.60	24.2^{ab}	0.694^{ab}	90.6	3.75^{ab}	59.3^{ab}	0.750^{ab}	181.2	3.06^{ab}
E (-)	33.1	92.4	35.5	0.807	90.8	2.57	23.8	0.679	90.6	3.84	59.3	0.750	181.4	3.07
E (+)	32.9	92.6	35.3	0.802	90.6	2.59	24.4	0.697	90.4	3.75	59.7	0.763	181.0	3.05
p: PS	0.966	0.011	0.051	0.045	0.899	0.056	0.033	0.031	0.806	0.038	0.008	0.007	0.874	0.011
p: E	0.782	0.758	0.783	0.788	0.490	0.736	0.265	0.272	0.422	0.304	0.638	0.570	0.443	0.641
p: PS × E	0.930	0.891	0.831	0.812	0.541	0.681	0.817	0.827	0.716	0.710	0.943	0.938	0.600	0.852

$^{a, b}$ values in the same rows with different letters differ significantly at $p < 0.05$.

SBM: soybean meal; YL: yellow lupin seeds; NL: narrow-leafed lupin seeds; RSM: rapeseed meal; IBW: initial bogy weight; FBW: final body weight; BWG: body weight gain; DWG: daily weight gain; FI: feed intake; FCR: feed conversion ratio; PS: protein source; E: enzyme; E (-): without enzyme; E (+): with enzyme; *p*: *p*-value.

Table 8 Apparent total tract digestibility coefficients of nutrients—Experiment III.

Digestibility (%)	PC	NC	E5000	E10000	E15000	SEM	*p*
DM	78.97	77.60	78.86	78.58	79.51	0.55	0.848
CP	74.69	73.11	75.09	75.79	76.67	0.67	0.527
Crude fat	82.51^{a}	79.96^{ab}	77.16^{b}	81.70^{a}	83.74^{a}	0.59	0.009
P	40.42^{b}	42.54^{b}	59.32^{a}	56.22^{a}	58.02^{a}	1.14	0.001
Ca	53.78^{b}	63.71^{a}	67.72^{a}	71.62^{a}	71.79^{a}	1.38	0.001

$^{a, b}$ values in the same rows with different letters differ significantly at $p < 0.05$.

DM: dry matter; CP: crude protein; SEM: standard error mean; PC: positive control group, without the enzyme; NC: negative control group, without the enzyme, Ca level reduced by 30%, P level reduced by 20%; E5000: group where reduced Ca and P levels + phytase 5,000 units/t; E10000: group where reduced Ca and P levels + phytase 10,000 units/t; E15000: group where reduced Ca and P levels + phytase 15,000 units/t.

Table 9 Performance results of pigs—Experiment III.

Performance	PC	NC	E5000	E10000	E15000	SEM	P
Initial body mass (kg)	25.9	25.9	26.1	25.8	26.1	0.33	0.995
Final body mass (kg)	41.9	41.7	42.4	41.3	42.6	0.36	0.782
Total gains (kg)	16.1	15.8	16.2	15.5	16.5	0.25	0.755
Daily gains (kg)	0.575	0.564	0.578	0.552	0.588	0.90	0.755
Feed intake (kg)	35.0	34.9	35.2	35.1	35.6	0.20	0.749
FCR (kg/kg)	2.22	2.20	2.20	2.30	2.19	0.07	0.823

FCR: feed conversion ratio; SEM: standard error mean; PC: positive control group, without the enzyme; NC: negative control group, without the enzyme, Ca level reduced by 30%, P level reduced by 20%; E5000: group where reduced Ca and P levels + phytase 5,000 units/t; E10000: group where reduced Ca and P levels + phytase 10,000 units/t; E15000: group where reduced Ca and P levels + phytase 15,000 units/t.

4. Discussion

The application of phytase is generally useful when the level of phytate in the diet is high [2]. It is especially true in pig diets based on legume seeds, SBM, RSM, sunflower meal and grains [4, 17]. In the first experiment phytase was added to the diet containing NL and RSM. The enzyme was added in the doses suggested by the producer, while the levels of minerals (Ca and P) in the diet met pig dietary requirements [19]. No effect of phytase on pigs' performance was observed in this experiment, for this reason in the second trial, identical enzyme doses were added to this as well as the other diets based on SBM and YL, which are rich in phytate. However, in the experimental groups the mineral contents in diets were reduced (Ca and P levels by 30% in the first phase, the Ca level by 30% and the P level by 15% in the second phase). This facilitates total exclusion of monocalcium phosphate from the diets and partial reduction of limestone level in the diets, thus also reducing the cost of these diets, because minerals are some of the costliest feed additives [10, 23]. In no group the enzyme additive improved the pigs' parameter results, but animals from both groups offered NL with RSM achieved similar results as those offered only SBM in the diets. The results from Experiment II show that phytase effectively acts in the diet, because in these groups reduction of mineral levels in the diets had no negative effect on the pigs' health and performance. On the other hand, the values of Ca and P recommended in the Nutritional Standards for Pigs [19] maybe too high. It is also proved by the results of Experiment III, wherein the NC group with reduced Ca and P levels in the diets (Ca level reduced by 30%, P level reduced by 20%), performance results of the animals did not differ significantly to those in the control group, with recommended Ca and P levels in the diet. In the NC group ATTD coefficients of Ca and P were also significantly higher than in the control (PC). Nevertheless, if inorganic sources were not routinely added to the most non-ruminant diets, the P deficiency would exist [6], whereas the excess to the requirement is excreted [10]. The application of phytase allows the animals to access much of the plant phytate P, and thus reduce reliance on inorganic phosphate sources. This was also found in Experiment III. Total tract digestibility coefficients of fat, Ca and P were improved depending on the phytase dosage, with the lowest coefficients recorded for the dose of 5,000 units/t (suggested by the producer), whereas there were no differences between 10,000 units/t and 15,000 units/t. Thus, it seems that the medium dose of 10,000 units/t is optimal, yet although in this experiment the phytase dosage did not affect pigs' performance, some quantitatively lower production results in this group were found ($p > 0.05$), which may be important for pig producers. In Experiment III pigs from the group with the highest phytase level had the lowest FCR and the highest gains during the experimental period ($p > 0.05$). Grela *et al.* [23], influenced by dietary treatment, with phytase at the dose of 500 FTU/kg to 1,000 FTU/kg, found significant daily gain and FCR while FI was not affected. Madrid *et al.* [12] also found significantly improved digestibility coefficients of P and Ca. Those authors, similarly as in this study observed no effect of phytase additive on apparent total DM and CP digestibility. Similar to the current study, Grela *et al.* [24] found no effect of phytase (Nauphoss 5,000 FTU/kg) on FI and feed utilization. Those authors also reported improved digestibility coefficients (total and ileal) for P, Ca and CP from the diets containing phytase. Fario *et al.* [25] found that the applied phytase additive to the SBM/corn/rice diet improved digestibility of Ca and P, but not protein. These authors also found no effect of phytase on intake and utilization of feed and growth parameters of pigs. Many works show that phytase enhances the utilization of some nutrients, but it is not always connected with improved performance [26]. In contrast, Beers and Jongbloed [27] reported that 1,450

FTU/kg phytase increased growth rate by 12.8%, FI by 8.5% and feed efficiency by 4.4% in diets for weaner pigs containing 2.9 g/kg non-phytate P. Campbell *et al*. [28] found that 500 FTU/kg phytase improved WG of weaner pigs by 14.4% and feed efficiency by 9.2%, when the animals were offered diets containing either 3.5 g/kg or 4.5 g/kg available P. In contrast, but similar to the current study, Barnett *et al.* [29] observed no enhanced growth performance in weaner pigs at the addition of 1,000 FTU/kg phytase to the diet. Moreover, in the presented Experiment III, monocalcium phosphate was totally excluded, while limestone was partially reduced from the experimental diets. Similar performance results of the control and experimental diets also showed efficacy of the enzyme additive. It is important that better mineral digestibility and lower phosphate and limestone additions to the diet also reduced P excretion to the environment [10, 12, 25].

These results also show that dietary substrate levels are an important determinant for the magnitude of phytase responses, so the phytase form and dosage should be specially prepared according to the diet composition. The phytate concentration in the diet can significantly impact growth performance of animals. For example, increasing the dietary phytate concentration by only 0.16% can reduce daily gains by 3% and feed efficiency by 10% points, particularly in piglets 7-14 d post-weaning. This slight increase in dietary phytate level could be the result of a 2%-5% increase in the inclusion of high phytate grains, such as full fat rice bran or SBM, or the result of natural variation in the phytate concentration in grains. In this study (Experiment III) the diet contained different phytate sources such as lupin seeds, corn and triticale. According to the literature data [2, 4, 5], these compounds contain different quantities of phytate: lupin seeds 0.6%-2.4%, maize corn 0.7%-2.2% and grains about 0.4%-1.4% per kilogram. Thus, in the case of feed mixtures used in the experiment, the maximum level of phytate was about 1.79% and that of P-phytate was about 4.2 g/kg. It means that about 70%-80% of total P was in the phytic form. Selle *et al*. [13] found a positive correlation between percentage improvements in feed efficiency in response to phytase and dietary phytate contents ($r = 0.923$; $p < 0.005$). It is especially evident in the pig diet based on legume seeds, SBM and RSM [4].

5. Conclusions

In summary, both phytases, i.e., RONOZYME®HiPhos and Quantum Blue, were effective feed additives in the diets containing lupin seeds and RSM, although they did not affect directly pigs' performance. By improving digestibility coefficients of Ca and P from the diet, phytase allows reducing minerals in diets, consequently reducing the cost of nutrition, but the dose of phytase must be adequate to the phytate level in diets. Thus, using phytase is effective in high-phytate diets when substrates for phytase degradation are present in the diet and when Ca and P levels are decreased in diets by reduction of dietary phosphate and limestone contents.

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