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Effects of exogenous phytase in chickens fed diets with differently processed rapeseed expeller cakes*

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ABSTRACT

The effects of phytase supplementation on the nutritional value, P availability and thyroid status of birds fed 4 differently processed rapeseed expeller cakes (RC) were determined. Two experiments were performed: a balance experiment on 10 groups of nine 3-week-old broilers and a growth experiment on 9 groups of one-day-old broiler females, 11 birds per group. In the balance experiment, a basal (B) and 4 test diets (B mixed with RC in a 6:4 proportion with DM) were fed; half of each diet was supplemented with 1000 U phytase/kg. In the growth experiment, the control diet without RC and isonitrogenous diets with RC supplemented or unsupplemented with 1000 U phytase/kg (RCP) were fed. The amount of RC was 100 or 150 g/kg in diets fed between days 1-21 and 22-42 of life, respectively. The levels of Ca and available P in RC diets were as in the control diet, while in RCP diets they were lowered by 7 and 12%, respectively.

Phytase supplementation increased apparent protein digestibility and metabolizable energy values, while it did not affect P availability from RC. BWG was lower in some groups and FCR 5% worse ($P<0.05$) in all RC groups than in the control. The type of RC significantly affected feed intake, BWG, thyroid and kidney weight. Addition of phytase to RCP diets increased thyroid weight ($P<0.01$). Phytase addition did not fully compensate for lower Ca and P levels in RCP diets, as tibia ash content was reduced ($P<0.05$), but this had no effect on tibia weight and ultimate strength.

KEY WORDS: rapeseed cake, phytase, P availability, broiler chickens, performance, thyroids

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INTRODUCTION

Rapeseed expeller cake (RC) is a relatively new commercial product, which remains after de-oiling 00 rape seeds by pressing. Its market share is growing, as expelling technology is more cost-effective and environmentally friendly than prepressig/solvent extraction, yielding rapeseed meal as the final product. The nutritional value of RC is not standardized yet and depends on the chemical composition of the seeds, which is affected by genetic and environmental factors, and on nutrient digestibility and glucosinolate toxicity, which are affected mainly by processing conditions. In Poland, rapeseed is processed on different types of screw press expellers, and the expelling technology may be accompanied by extrusion or hydrothermal treatment called toasting. Both treatments may affect myrosinase (thioglucoside glucohydrolase EC 3.2.3.1) activity, which, under suitable conditions in the gastrointestinal tract, hydrolyzes glucosinolates to thyrotoxic compounds. A sensitive indicator of the dietary glucosinolate level and myrosinase status in pigs and chickens is the weight of the thyroid, liver and kidneys (Schöne et al., 1991, 1997; Bell, 1993).

Rape seeds are rich in phosphorus, which is present mainly in the form of phytates - phytic acid salts (myo-inositol hexakisdihydrogenphosphate-IP_6), which are not fully available to chickens and may have a negative effect on the availability of minerals and on protein digestion. Various phytate-degrading enzyme preparations have been tested on rapeseed meal *in vitro.* Their efficiency depends on enzyme composition and activity, pH and time of incubation. Żyła and Koreleski (1993) found complete dephosphorylation in 4 h incubation with 100 U phytase and 37100 U acid phosphatase/kg at pH 4.5. Rutherfurd et al. (2004) showed that during 4 h incubation with 500 to 2000 phytase U/kg at pH 5.5, from 28 to 54% phytate P was released, while Newkirk and Classen (1998) reported that complete hydrolysis was accomplished with 5000 phytase U/kg at pH 5.0, within 23 h. Broiler feeds, containing wheat as a major component, are commonly supplemented with xylanases. The efficacy of phytase in wheat-based broiler diets can be enhanced by adding xylanase to feeds (Żyła et al., 1999). There is little information about the effect of rapeseed products, which are rich in condensed tannins, on the efficacy of exogenous phytase in the presence of xylanase in the digestive tract of chickens. *In vivo,* Żyła and Koreleski (1993) found a positive effect of phytase derived from *Aspergillus niger* on rapeseed meal phytate phosphorus utilization in broilers, while Rutherfurd et al. (2002) reported no statistically significant effect of added phytase on ileal total and phytate P digestibility in broilers fed a rapeseed meal-based diet.

The aim of the study was to evaluate the effect of phytase supplementation on the digestibility of nutrients from differently processed rapeseed cakes, and on performance, thyroid status and skeletal development in broilers fed diets containing RC.

MATERIAL AND METHODS

Materials

Four rapeseed press cakes (RC), produced from 00 rapeseed (unknown cultivars, harvest year 2002) in 4 different small rural mills were used (Tables 1, 2 and 3). The following processing conditions were applied: RC 1 - seeds were flaked, steam conditioned for 90 min at temperatures increasing from 60 to 105°C, pressed on a De Smet-Rosedowens screw expeller and pelleted (10 × 10 mm); RC 2 - seeds were pressed on a CB-50 screw press expeller (Germany) and pelleted (20 × 5 mm); RC 3 - seeds were heated to 60°C, flaked and pressed on a 02PVO screw press expeller (Bispomasz, Bydgoszcz, Poland); RC 4 - seeds were heated to 50°C, pressed on a 02PVO screw press expeller, dry extruded (125-150°C

Table 1. Chemical composition of rapeseed expeller cakes (RC), in g/kg DM and the total amino acid and available lysine contents (g/16 g N)

Component	RC 1	RC 2	RC 3	RC 4
DM, g/kg	911	909	911	893
Crude protein	331	297	272	337
Crude fat[1]	136	169	222	111
Crude ash	68	60	54	65
Crude fibre	118	126	130	116
N-free extractives	347	348	322	371
Acid detergent fibre (ADF)	203	190	205	200
Neutral detergent fibre (NDF)	293	229	244	241
NDIN (NDF-bound protein),% total	16.3	8.5	9.3	7.4
Total P	11.7	9.63	9.55	10.9
Phytate P	5.6	4.1	4.8	4.0
Amino acids, g/16 g N				
lysine total	6.15	6.15	6.25	5.94
lysine available	5.19	5.10	5.18	5.53
methionine	1.86	1.88	1.82	1.85
cystine	2.22	2.23	2.20	2.21
threonine	4.81	4.68	4.68	4.37
tryptophan	1.24	1.27	1.22	1.25
arginine	6.55	6.45	6.36	6.09
valine	5.58	5.37	5.47	5.13
isoleucine	4.43	4.28	4.40	4.11
leucine	7.67	7.44	7.45	7.12
phenyloalanine	4.37	4.25	4.21	4.07
tyrosine	3.22	3.11	3.11	2.92
histidine	2.87	2.76	2.74	2.66

[1] after acid hydrolysis

Table 2. Lysine availability, ($Lys_{available}/Lys_{total} \times 100$) and solubility of protein from rapeseed expeller cakes (RC)

Item	RC 1	RC 2	RC 3	RC 4	SEM
Lysine availability, %	84.4^{A}	82.9^{A}	82.9^{A}	93.1^{B}	0.72
Protein solubility in 0.5% KOH, %	59.8^{aA}	90.9^{cB}	88.3^{bB}	86.7^{bB}	0.61
Protein solubility in borate, %	39.9^{A}	87.2^{C}	86.8^{C}	58.3^{B}	0.64
Protein dispersibility index (PDI),%	12.5^{A}	24.8^{C}	24.2^{C}	17.7^{B}	0.22

^{a,b,A,B} means in rows with different superscripts statistically significant at $^{ab}P<0.05$; $^{AB}P< 0.001$

Table 3. Glucosinolate content of rape seeds and rapeseed expeller cakes (RC), µmol/g DM

Glucosinolates	Rape seeds				Rapeseed expeller cakes			
	1	2	3	4	RC 1	RC 2	RC 3	RC 4
Alkenyl-glucosinolates								
progoitrin	6.09	4.78	3.62	3.72	6.80	4.73	3.84	5.37
gluconapoleiferin	0.32	0.33	0.21	0.33	0.11	0.44	0.33	0.34
gluconapin	4.70	4.20	3.30	3.08	5.71	2.97	2.74	3.69
glucobrassicanapin	1.28	1.04	0.85	0.53	1.43	0.88	0.77	0.90
Σ alkenyl glucosinolates	12.4	10.3	8.0	7.7	14.0	9.0	7.7	10.3
Indol-glucosinolates								
4-hydroxyglucobrassicin	3.20	3.40	2.45	3.99	2.63	3.85	4.17	5.26
other indol glucosinolates	0.11	0.16	0.11	0.21	0.11	0.11	0.11	0.22
Total glucosinolates (TGL)	15.7	13.9	10.5	11.9	16.8	13.0	12.0	15.8
TGL, µmol/g FFDM	28.4	24.8	19.2	21.2	19.4	17.2	15.4	18.0
TGL in FFDM, relative to seeds, %	100	100	100	100	68	70	80	85

FFDM - fat-free DM

for 20 sec) and pressed again. The RCs were ground to a fine texture prior to incorporation into diets.

Two commercial enzyme preparations were used: Avizyme 1300 (Finnfeeds Int.), which contained xylanase and β-glucanase activity, and Natuphos 5000 G (BASF), which contained 5000 FTU phytase/g, according to the producer's declaration. Actual phytase activity in the feed samples was not measured.

Diets

In Experiment 1 (digestibility trial) 2 basal diets (B and BP) were prepared. The B diet contained (g/kg): wheat 454.3, maize 200, soyabean meal 280.7, limestone 14, dicalcium phosphate 10, NaCl 3, rapeseed oil 30, L-lysine (78%) 1, DL-methionine (0.98%) 1, Avizyme 1300 1 and mineral-vitamin premix 5. The BP diet of the same composition was supplemented with 1000 U phytase per kg

DM. Eight test diets (T) were composed of the B diet mixed with different RC in a proportion of 6:4 on a DM basis. Diets denoted as RC were unsupplemented, while the RCP diets were supplemented with 1000 U phytase per kg. The total P level in the basal diets (B and BP) was 6.4, in the test diets, from 7.6 to 8.1 g/kg air-dry matter. All diets contained 3 g Cr_2O_3 per kg DM as a marker and were cold pelleted on a CL-2 CPM Laboratory Pellet Mill.

In Experiment 2 (growth trial), two sets of diets were prepared (Tables 4 and 5), with nutrient contents covering chicken requirements during the starter (1-21), grower (22-35) and finisher (36-42 d of life) periods. The control diet did not contain RC, while the experimental diets contained 100 (starters) or 150 g/kg respective RC (growers and finishers). Diets denoted as RC were unsupplemented and had P and Ca contents equal to the control diet, while diets denoted as RCP contained 18% less inorganic Ca and P due to a lower amount of added dicalcium phosphate than the respective RC diets and were supplemented with 1000 U phytase /kg. All diets were cold pelleted.

Animal trials

In the digestibility trial, 90 three-week-old broiler females with a mean initial body weight of 677 g were used. The birds, housed individually in balance cages, were allocated to 10 groups, 9 birds per group, and were given 90 g/bird/day of the respective balance diets, in three meals. After two days of preliminary feeding the birds were fasted for 14 h, then given the same diets for 3 days and again fasted for 14 h. Feed intake was recorded daily, while during the last 86 h of the experiment, excreta were quantitatively collected, frozen and kept at -18°C for further analysis.

In the growth trial, 99 one-day-old Cobb 500 broiler females with a mean initial body weight of 43.4 g were randomly allocated into 9 groups, 11 birds per group. For the first week the chickens were kept in pens, on day 8 they were weighed and placed into individual cages. From the first day of life the birds were given the experimental diets *ad libitum*: for weeks 1 to 3, starters (Table 4), for weeks 3 to 6, growers, for week 7 finishers (Table 5). Body weight and feed intake were measured in weekly intervals after 4 h feed deprivation. At the end of the experiment the birds were killed by cervical dislocation, the abdominal cavity was opened and the liver, thyroid glands, kidneys and abdominal fat were excised and weighed. The right tibias were collected, cleaned of all exterior tissue and frozen until analysis.

Both experiments were conducted in compliance with the European Union regulations concerning the protection of experimental animals. The Local Ethics Committee approved the study protocol.

Table 4. Ingredients and nutrient composition (g/kg) of diets unsupplemented (RC) and supplemented (RCP) with phytase fed between 1-21 day in growth experiment

Ingredients	Dietary treatments								
	Control	RC 1	RC 1P	RC 2	RC 2P	RC 3	RC 3P	RC 4	RC 4P
Rapeseed cake (RC)	-	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Wheat	335.7	308.2	308.2	305.3	305.3	305.7	305.7	308.2	308.2
Soyabean meal	377.0	316.9	316.9	322.3	322.3	327.2	327.2	314.6	314.6
Maize	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Limestone	8.1	8.5	8.5	8.5	8.5	8.2	8.2	8.5	8.5
Dicalcium phosphate	18.0	16.5	13.5	16.9	13.9	16.9	13.9	16.7	13.7
Lard	50.0	39.0	39.0	36.0	36.0	31.0	31.0	41.0	41.0
L-lysine (78%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.1
DL-methionine (98%)	1.2	0.7	0.7	0.8	0.8	0.8	0.8	0.7	0.7
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Avizyme 1300	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Premix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Wheat starch	-	0.2	3.0	0.2	3.0	0.2	3.0	0.2	3.0
Natuphos 5000 G	-	-	0.2	-	0.2	-	0.2	-	0.2
Calculated									
Crude protein	220	220	220	220	220	220	220	220	220
Crude fat	69.9	70.0	70.0	70.0	70.0	70.1	70.1	70.1	70.1
Ca	9.40	9.38	8.75	9.41	8.78	9.42	8.79	9.38	8.75
P total	7.21	7.57	7.00	7.48	6.91	7.50	6.93	7.51	6.94
P available[2]	4.40	4.41	3.90	4.41	3.90	4.42	3.91	4.40	3.89

[1] mineral-vitamin premix supplied per kg diet: vit. A 13500 IU; vit. D_3 3500 IU; mg: vit. E 50; vit. B_1 3; vit. B_2 8; biotin 0.1; vit. B_6 5; vit. B_{12} 0.03; vit. K 4; niacine 60; folic acid 1.75; Ca pantothenate 15; choline 500; Mn 80; Zn 70; Co 0.3; Se 0.2; Cu 15; Fe 70; I 1; Mg 100; Salinomycin 60; Avilamycin 10 and Ca 1.075 g; [2]calculated with following availability coefficients: rapeseed cake 0.4; wheat 0.35; soyabean meal 0.4; maize 0.15; dicalcium phosphate 0.9

Table 5. Ingredients and nutrient composition (g/kg) of diets unsupplemented with phytase fed during 22-35 (G) and 35-42 (F) day of growth experiment

Ingredients	Dietary treatments[1]									
	Control		RC 1		RC 2		RC 3		RC 4	
	G	F	G	F	G	F	G	F	G	F
Rapeseed cake (RC)	-	-	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Wheat	383.2	405.7	347.5	370.0	338.3	360.8	338.7	361.1	345.1	370.6
Soyabean meal	330.2	301.0	235.1	205.8	248.2	219.1	255.3	226.3	234.7	205.6
Maize	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Limestone	9.0	8.2	9.4	8.1	9.2	7.9	9.4	8.1	9.4	8.1
Dicalcium phosphate[1]	16.5	16.0	14.1	14.0	14.7	14.5	14.6	14.4	14.4	11.2
Lard	50.0	58.4	33.0	41.6	28.7	37.2	21.1	29.6	35.5	44.0
L-lysine (78%)	0.7	0.7	1.0	1.0	0.9	0.9	0.9	0.9	1.0	1.0
DL-methionine (98%)	1.4	1.0	0.7	0.3	0.8	0.4	0.8	0.4	0.7	0.3
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Avizyme 1300	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Wheat starch[1]	-	-	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Calculated										
crude protein	205	195	205	195	205	195	205	195	205	195
crude fat	70.1	78.5	70.0	78.4	70.0	78.4	70.0	78.4	69.9	78.4
Ca	9.29	9.08	9.34	9.03	9.34	9.01	9.35	9.03	9.35	8.40
P total	6.94	6.68	7.36	7.27	7.22	7.11	7.22	7.12	7.26	7.16
P available[3]	4.15	4.07	4.09	4.03	4.09	4.02	4.08	4.02	4.08	4.02

[1] only RC diets; respective RCP diets contained 0.2 g of phytase (Natuphos 5000 G) and 3 g dicalcium phosphate less, which was substituted by wheat starch, similarly as in Table 4

[2] mineral-vitamin premix added to G diets was the same as in Table 4, while premix added to F diets supplied per kg diet: vit. A 11100 IU; vit. D_3 3000 IU; mg: vit. E 50; vit. B_1 1.5; vit. B_2 6; biotin 0.05; vit. B_6 3; vit. B_{12} 0.02; vit. K 2; niacine 40; folic acid 1.25; Ca pantothenate 10; choline 400; Mn 70; Zn 50; Co 0.3; Se 0.2; Cu 10; Fe 60; I 1; Mg 100 and g: Ca 1.35

[3] calculated with following availability coefficients: rapeseed cake 0.4; wheat 0.35; soyabean meal 0.4; maize 0.15; dicalcium phosphate 0.9

Measurements of protein solubility in rapeseed cakes

Solubility in 0.5% KOH was determined as described by Pastuszewska et al. (1998); solubility in sodium borate was determined according to Lee and Garlich (1992). The protein dispersibility index (PDI), that measures protein solubility in water during mechanical blending at 8500 RPM for 10 min, was determined according to Dudley-Cash (1999).

Measurements of tibia strength and tibia ash content

Tibias were weighed and analysed for strength by shear force measurement using a Texture Analyser TA-XT2i (Stable Micro Systems). After the shear test, the tibias were dried, crushed and defatted in refluxing ethyl ether in a Soxlet apparatus for 48 h. The defatted tibias were oven dried and ashed in ceramic crucibles for 24 h at 600°C. Ash content was expressed as a percentage of the fat-free, moisture-free tibia weight.

Chemical analysis

Prior to analysis the excreta were dried for 12 h at 60°C, kept open for 48 h and ground to pass a 1 mm sieve. The chemical composition of press cakes, diets and excreta was determined according to AOAC (1990), (code no. 930.04; 978.04; 930.05; 930.10; 958.01 for dry matter, crude protein, ash, crude fibre and P total, respectively). Crude fat was determined by ether extraction preceded by acidification with 4M hydrochloric acid. In rapeseed cakes, glucosinolates were determined according to the ISO 9167-1 method (1992), using a HPLC 1050 apparatus, phytate P was measured according to Tangkongchitr et al. (1981), neutral detergent fibre (NDF), acid detergent fibre (ADF) and NDF insoluble nitrogen (NDIN) were determined with a Fibertec System M according to Van Soest and Wine (1967) and Van Soest (1973). Amino acid analyses were performed with a Beckman 6300 high pressure amino acid analyser, using modified procedures described by Buraczewska and Buraczewski (1984), available lysine was analysed according to the Carpenter method modified by Booth (1971). Faecal N was determined according to Ekman et al. (1949). Gross energy of RC, diets and excreta were measured on a Parr adiabatic oxygen bomb calorimeter KL-10, chromic oxide was determined spectrophotometrically following wet ashing according to Hinsberg et al. (1953).

Calculations and statistics

In Experiment 1 the total tract apparent digestibility (CTTAD) of dietary protein, fat, organic matter and phosphorus retention and apparent metabolizable energy

(AME_N) value of diets were calculated relative to the ratio of Cr_2O_3 to the content of the nutrient in question or gross energy in feed and excreta. AME_N was corrected to zero nitrogen balance using 34.96 kJ/g N retained (Hill and Anderson, 1958). Respective values for rapeseed cakes were calculated by assuming additivity of the values of the basal (B) and the test (T) diets, from the formula: AME_N RC = AME_N B - (1-X) AME_N T/X, where X = the level of inclusion of RC in the T diet. The values obtained for the B diet were used for calculation of respective values for phytase unsupplemented RC, while the values obtained for the BP diet for phytase supplemented RC. In experiment 2 the body weight gain (BWG) and feed conversion ratio (FCR) were calculated for 42 days of feeding, the weights of thyroids, livers and kidneys were calculated relative to live body weight before slaughter.

The results were subjected to one-way and two-way analysis of variance (ANOVA) generated by Statgraphics ® ver. 5.1 software (SAS, 1994-2001).

RESULTS

Press cakes contained from 111 to 222 g crude fat/kg, from 272 to 337 g crude protein/kg and from 9.55 to 11.7 total P/kg, of which from 37 to 50% was phytate P. The amino acid composition of RC protein was rather uniform, total lysine ranged from 5.94 to 6.25 g/16 g N, in it from 83 to 93% was available lysine (Tables 1 and 2). The share of ADF in fat-free dry matter (FFDM) was about 23% in all RC, while NDF was less uniform and was highest (29%) in RC 1, it was associated with a substantially higher value of NDIN than in the remaining RC (Table 1). The protein solubility and dispersibility indexes were lower in RC 1 and RC 4, higher in RC 2 and RC 3 (Table 2). The glucosinolate concentrations were from 19 to 28 μmol/g FFDM in rape seeds, and from 15 to 19 μmol/g FFDM in RC (Table 3).

The results of the balance trial (Table 6) showed that the CTTAD of protein was from 75.4% for RC 1 to 78.9% for RC 4 and significantly increased after phytase supplementation in RC 1 and RC 3, while CTTAD of fat was lowest in RC 4, highest in RC 1, and decreased after phytase supplementation in RC 1 and RC 2. Organic matter retention in RC 1 amounted to 36.6%, while in the remaining RC it averaged 46.4%. After phytase supplementation it increased in RC 1 by 6 percentage points, while in the remaining RCs it increased by only 0.7 percentage points.

The AME_N value was the lowest in RC 1, the highest in RC 3, and depended significantly ($P<0.05$) on the fat content in RC according to the equation:

AME_N (MJ/kg DM) = 7.58 + 0.03 x total fat content in g/kg DM ($r = 0.93$).

Phytase supplementation increased the AME_N value of RC 1 by 11%, but did not significantly affect the AME_N value of the remaining cakes (Table 6).

Table 6. Effects of rapeseed cake (RC) and phytase (Phyt) supplementation on results of digestibility trial[1] on 3-week old chickens and growth trial (1-42 day old chickens)

Item	Control		Unsupplemented				Supplemented with phytase					Significance[a]		
	B	BP	RC 1	RC 2	RC 3	RC 4	RC 1P	RC 2P	RC 3P	RC 4P	RMSE[ab]	RC	Phyt	RC x Phyt
Digestibility trial (Experiment 1)														
Crude protein CTTAD[1], %			75.4	76.9	77.1	78.9	79.5	78.2	80.8	77.9	1.4	0.011	0.001	0.001
Crude fat CTTAD[1], %			92.8	83.2	91.5	74.3	85.7	80.2	92.6	75.4	3.2	0.001	0.016	0.001
P retention (diet) %	50.8	54.3	33.7	37.1	38.4	37.9	36.1	37.1	39.7	39.5	nc	nc	nc	nc
P retention (RC)[1] %			21.0	22.8	26.9	27.3	20.1	20.8	30.6	27.5	4.9	0.001	0.854	0.471
OM retention[1],%			36.6	46.5	49.1	43.7	42.8	47.5	51.0	42.8	3.2	0.001	0.010	0.016
AME_N MJ/kg DM[1]	10.4	12.4	14.0	11.2	11.6	12.4	14.4	11.3	0.6	0.001	0.001	0.001	0.001	0.023
Growth trial (Experiment 2)														
BWG,kg	2.40		2.30*	2.33	2.21*	2.36	2.22*	2.22*	2.24*	2.36	0.10	0.001	0.079	0.098
Feed intake, kg	3.90		3.94	3.95	3.88	4.07	3.84	3.78	3.89	4.09	0.23	0.013	0.254	0.477
FCR, kg feed/kg BWG	1.63		1.71*	1.69*	1.75*	1.72*	1.73*	1.70*	1.73*	1.73*	0.08	0.259	0.777	0.829
Thyroids, mg/100 g BW	20.4		19.7	40.2*	28.8	27.4	20.9	63.7*	32.1	45.0*	16.8	0.001	0.003	0.098
Liver, % BW	2.33		2.49	2.31	2.30	2.57	2.51	2.48	2.14	2.28	0.41	0.177	0.475	0.283
Kidneys, % BW	0.60		0.59	0.70*	0.73*	0.60	0.65	0.78*	0.69*	0.60	0.08	0.001	0.146	0.073
Abdominal fat, % BW	1.32		1.21	1.10	1.24	1.14	1.27	1.19	1.06	1.22	0.34	0.814	0.926	0.535
Tibia weight, g	20.1		18.4	19.5	18.3	20.0	18.8	19.5	18.4	19.4	2.00	0.082	0.992	0.874
Tibia ash,%[c]	45.8		46.5	46.2	46.3	45.7	45.2	45.6	45.9	44.7	1.40	0.224	0.012	0.731
Tibia ultimate strength, N	14.8		15.5	14.3	15.3	15.2	14.2	14.9	14.2	12.8	2.66	0.774	0.080	0.331

[1] calculated values by the difference technique that assumes additivity; [a] two-way ANOVA for groups with RC only; [b] root of mean square error; [c] on fat-free, moisture free basis; * groups significantly different from control group (one-way ANOVA; $P<0.05$)

B - basal diet; BP - basal diet supplemented with phytase; CTTAD - total tract apparent digestibility; OM - organic matter; AME_N - apparent metabolizable energy corrected to zero nitrogen balance; nc - not calculated

Phosphorus retention from the BP diet was 3.5 percentage points higher than from diet B, while from unsupplemented test diets containing 40% RC it averaged 36.8%, from phytase-supplemented test diets it averaged 38.1%. Phosphorus retention calculated by a difference technique that assumes additivity was from 21% in RC 1 to 27.3% in RC 4 and did not increase significantly after phytase supplementation. There was no significant interaction between effect of type of RC and phytase supplementation on P retention (Table 6).

During the 42-day growth trial, feed intake in RC groups did not differ from that in the control group (Table 6), but BWG was significantly lower in groups RC 1 and RC 3. This resulted on average in a 5% worse FCR in all RC groups compared with the control. Type of RC significantly affected feed intake ($P<0.05$) and BWG ($P<0.001$), as it was higher in group RC 4 than in the remaining ones, however, neither the type of RC nor phytase supplementation affected FCR significantly (Table 6).

The type of RC and phytase supplementation had no effect on liver and abdominal fat weight, while the kidneys were enlarged ($P<0.05$) in groups fed RC 2 and RC 3, the thyroids were enlarged significantly in group RC 2, and numerically in groups RC 3 and RC 4 compared with the control group. Phytase supplementation significantly increased the weight of thyroids in chickens fed RC 2 and RC 4 cakes compared with unsupplemented ones (Table 6). In the growth trial there were no interactions among any measured parameters between the type of rapeseed cake and phytase supplementation.

The tibia weight, tibia ash and tibia ultimate strength in chickens fed RC diets did not differ compared with the control group. In groups fed RC diets with a lower Ca and P content and supplemented with phytase, the tibia ash content was reduced on average by 2% ($P<0.05$) in comparison with the respective unsupplemented groups, but tibia weight and its ultimate strength were not affected (Table 6).

DISCUSSION

In two batches of the commercial grade rape seeds used in this study the glucosinolate content was below the upper limit, in one batch, at the upper limit

and in one, above the upper limit of 25 mmol/kg FFDM defining 00 rapeseed quality, that was proposed some time ago (Krzymański, 1993). In RC, the glucosinolate content ranged from 15 to 19 mmol/kg FFDM. The differences in RC composition in this report reflect the various combinations of differences in the composition of the seeds that were processed and differences in processing factors. It was not the objective of this study to explain the reasons for differences in RC composition

attributed to cultivar or environmental factors, but rather to assess the variation connected with processing conditions. The range of fat and protein content in RC used in the present study was similar, while glucosinolate content was lower than reported previously (Smulikowska et al., 1997). Differences in glucosinolate content reflect cultivar, environmental and processing factors, while processing strongly affected fat content. Extrusion, which was part of the production process for RC 4, enabled the most effective oil extraction. Reduction of protein dispersibility in water (PDI) and its solubility in borate and 0.5% KOH, as well as a substantially higher proportion of NDIN were observed in RC 1 toasted for 90 min at temperatures increasing from 60 to 105°C, in comparison with other press cakes. Pastuszewska et al. (1998) showed that protein solubility in rapeseed meals decreased due to toasting. Buraczewska et al. (1998) found that with increasing time of toasting, the level of NDF and NDIN in rapeseed meals increased, and that apparent ileal amino acid digestibility was negatively affected in pigs. Newkirk and Classen (2002) reported that due to toasting, the share of NDIN in canola meal protein increased from 11 to 20%, while a dose-response study on broilers fed a non-toasted meal resulted in better performance than on toasted meal. This effect was only seen, however, at above 80% replacement of soyabean meal protein by canola protein.

In the present study, the high proportion of NDIN in RC 1 resulted in significantly lower protein digestibility, organic matter retention and energy value in comparison with the other RC in the balance trial. This was seen when RC protein made up about 50% of total dietary protein, while it had no effect on performance in the growth trial, as only 13-17 and 23-32% soyabean meal protein was substituted by RC protein in the starter and grower/finisher diets.

Protein solubility indexes in RC 4 were lower than in RC 2 and RC 3 but higher than in RC 1, NDIN and protein digestibility were not negatively affected, while lysine availability and protein digestibility were the highest of all compared cakes. It seems that a very short time of heating and limited content of moisture during dry extrusion prevented protein damage in RC 4, while the destruction of cell wall structures made the protein more available to the digestive enzymes. The reduction of glucosinolate content was, however, smaller in this cake than in the other RCs.

In the present study, the dietary glucosinolate level had no effect on feed intake in the growth trial, although the level of total glucosinolates was from 1.2 to 1.7 mmol/kg in the starter diets and from 1.8 to 2.5 mmol per kg in the grower/finisher diets, respectively. In the digestibility trial chickens voluntarily ate the entire daily portions containing from 4.8 to 6.8 mmol of total glucosinolates per kg. Newkirk and Classen (2002) reported that feed intake was not affected up to the level of 4.5 mmol of total glucosinolates per kg of diet, while toasting canola meal, which

lowered the glucosinolate content by 55%, even depressed feed intake in the first weeks of the broilers' life. It seems that the bitter taste of glucosinolates and their volatile bitter degradation products are not noticed by broilers when the feed is provided in the form of pellets. Pigs have more sensitive taste receptors than poultry, but Schöne et al. (1997) reported that up to 6 mmol of glucosinolates per kg diet did not affect feed intake in pigs.

The glucosinolate content in broiler and fattener pig diets should still be limited, as glucosinolate degradation products, such as vinyl-oxazolidinethiones, nitriles and thiocyanate ions, may have adverse effects on the physiological status of the thyroid, liver and kidneys and on growth performance (Bell, 1993). Glucosinolates are degraded by the enzyme myrosinase, present in rape seeds. Myrosinase and glucosinolates in intact rape seeds are located in separated cellular compartments (Maheshwari et al., 1981), and the crushing of the seed brings them into contact, so inactivation of myrosinase by heat treatment prior to processing is commonly applied (Bell, 1993). However, it was established that glucosinolates may also be hydrolysed in the digestive tract of animals by bacterial enzymes which possess myrosinase-like activity (Campbell et al., 1987; Nugon-Baudon et al., 1990; Słominski et al., 1988).

Thyroid weight was shown to be a sensitive indicator of dietary glucosinolate level and myrosinase status in pigs and chickens, and supplementation of the diets with iodine (I) prevented thyroid weight increase (Schöne et al., 1991, 1997). In the present study all diets contained 1 mg supplementary 1/kg, however, thyroids were normal in the group fed toasted RC 1, while they were enlarged in chickens fed untoasted RC, most significantly in group RC 2. This indicates that the inactivation of seed myrosinase prior to expelling was inefficient.

In comparison with the control group, the tibia weight, tibia ash and tibia ultimate strength in chickens fed RC-containing diets did not differ. The performance of the chickens fed diets with lowered Ca and P contents and supplemented with phytase was similar to the unsupplemented groups. This indicates that exogenous phytase supplementation enabled lowering the dietary inorganic phosphate level, which is in agreement with the results obtained with soyabean meal and maize-based diets supplemented with phytase (Schöner et al.,
1993; Żyła et al., 1999). Rutherfurd et al. (2002) reported that amino acid digestibility in different feedstuffs also increased in broilers in the presence of exogenous phytase. In the present study, however, despite the equal protein, amino acid and fat contents in diets, FCR in groups fed unsupplemented and phytase-supplemented RC diets decreased in comparison with the control group.

It was surprising that supplementation of the diets with phytase induced the enlargement of thyroids, except in birds fed properly toasted RC 1. The enlargement of thyroids in the chickens from groups fed untoasted, phytase-

supplemented RC indicates that the exogenous phytase increases hydrolysis of glucosinolates to goitrogenic compounds. This may be due to the enhancement of bacterial activity stimulated by the input of nutrients released by phytase from various complexes in the lower parts of the intestinal tract. The important role of gut microflora in glucosinolate metabolism was demonstrated by Campbell et al. (1995) who found that dietary glucosinolates were excreted intact in germ-free rats, while the majority of these compounds disappeared from the digestive tract in animals with normal microflora. In these rats, enlarged thyroids, liver and kidneys were observed, indicating toxicity of glucosinolate derivatives. Also in poultry, the recovery of glucosinolates in excreta was very low in intact hens, but increased greatly in caecectomised or antibiotic-fed hens (Campbell et al., 1987). In the present growth trial, apart from the last week of life, the diets contained an antibiotic growth promoter and a coccidiostatic, which prevent microflora overgrowth to some extent. It may be expected that the effects of phytase on the activation of goitrogenic compounds from cold pressed RC may be even more important when antibiotic-free diets will be fed. The presented results point to the need of further studies on the interactions among of RC production technology, phytase supplementation, and the use of different pre- and probiotic components instead of antibiotics.

The balance trial in the presented study was not aimed to measure phosphorus retention properly (rapeseed cake was not the sole source of phosphorus), and the obtained results have only a limited value. Total phosphorus retention calculated in the balance trial was from 23% in RC 1 and RC 2, and 27% in RC 3 and RC 4, on average, and was not increased due to phytase supplementation. In experiments with rapeseed meal or cake as the sole source of dietary phosphorus, total P retention from unsupplemented and phytase-supplemented diets was higher: 39 vs 46% (excreta collection, Leske and Coon, 1999), 37 vs 44% (ileal digesta collection, Rutherfurd et al., 2002), or 31 vs 44% (excreta collection; Potkański et al., 1995), but in all the experiments the effect of phytase was not statistically significant. In the growth trial, the tibia ash content in broilers fed RC diets supplemented with phytase was slightly reduced in comparison with respective unsupplemented groups, but tibia weight or its ultimate strength were not affected. It seems that reduction of dietary inorganic Ca and P by 18% in RC-containing broiler diets supplemented with phytase may have no negative consequences for broiler performance.

CONCLUSIONS

It is concluded that cold-pressed rapeseed cakes can be used in broiler diets. Phytase supplementation may beneficially influence the nutritional value of

toasted cakes, but it may also negatively affect the thyroid status of birds fed cold-pressed rapeseed cakes.

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