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The nutritive value and phosphorus availability of yellow- and dark-seeded rapeseed cakes and the effects of phytase supplementation in broilers*

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ABSTRACT

Three experiments on 242 Ross 308 broiler females were conducted to evaluate the nutritive value of expeller cakes cold-pressed from 3 yellow-seeded lines (YRC 041, 036, and 022) and black-seeded winter rape (BRC), and the effects of phytase supplementation. The total P content and phytase activity were similar in YRC and BRC and averaged 9.5 g/kg and 411 U/kg, respectively. Total glucosinolate contents in YRC were from 8.7 to 10.2 μmol/g dry matter (DM), in BRC, 12.6 μmol/g DM, in which progoitrin constituted 6% to 15% in YRC, and 35% in BRC. In the first experiment, the apparent metabolizable energy (AME_N) and nutrient digestibility of cakes were determined on 5 groups of 10, three-week-old chickens. Apparent protein digestibility did not differ between YRC and BRC and averaged 79.8%. Apparent crude fat digestibility and AME_N were higher in YRC in comparison with BRC due to the lower fat content in the latter, but AME_N averaged 8.95 MJ/kg of fat-free DM and did not differ between YRC and BRC. In the second experiment, a digestibility trial was conducted on 12 groups of 8 three-week-old chickens fed semisynthetic diets containing 45% cakes unsupplemented or supplemented with phytase (500 or 1000 U/kg). Phosphorus retention from YRC and BRC did not differ and averaged 39%. It increased due to phytase supplementation to 45.5% and 51.7%, on average. The third experiment was conducted on 8 groups of 12 chickens between 8-35 days of age fed low-phosphorus diets containing 30% cakes, unsupplemented or supplemented with 1000 U phytase/kg. Birds fed diets with BRC had a lower feed conversion ratio and higher thyroid weight in comparison with YRC-fed birds. Phytase supplementation increased feed intake ($P<0.01$), body weight gain ($P<0.01$) and tibia

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ultimate strength (P<0.01). In the last two experiments, the concentration of short-chain fatty acids (SCFA) in the ileal contents was not affected by the treatments, but in the caecal contents it increased (P<0.01) due to phytase supplementation.

The results of the digestibility trials and measurements of SCFA in ileal and caecal digesta do not point to great improvement in the utilization of YRC nutrients in comparison with BRC in broilers, however, some results obtained with YRC 041 were better than with the remaining cakes. The low alkenyl glucosinolate contents in YRC cakes favours their use in broiler feeds.

KEY WORDS: yellow-seeded rape, metabolizable energy, phosphorus availability, phytase activity, broiler chickens

INTRODUCTION

Plant selection programmes directed toward development of yellow-seeded *Brassica rapa* and *Brassica napus* rapeseed (canola) as well as *Brassica juncea* (canola type) have been underway for the last years in many countries (Bartkowiak-Broda et al., 2011; Slominski et al., 2011). In Poland the selection programme was directed towards development of yellow-seeded lines of double low winter rapeseed. Lines were derived from crosses of spontaneous mutants of lighter seeds found in breeding material of double-low winter rapeseed with a segregating, spotted-seed-coat spring line from earlier crosses of *B. napus* with *B. rapa* (Bartkowiak-Broda et al., 2011).

It was reported that seeds of the yellow lines/varieties of spring canola had thinner hulls, lower dietary fibre contents (including lignin, polyphenols, crude fibre, neutral detergent fibre) and higher protein, oil, and sucrose contents than dark-seeded varieties (Simbaya et al., 1995; Slominski et al., 1994, 1999, 2011). Myszka et al. (2011) analysed rapeseed meals obtained from 58 yellow-seeded winter lines developed at the Plant Breeding and Acclimatization Institute in Poznań (Poland), from the harvest years of 2006-2008. Rape seeds differed in lightness from 0 (black) to 5 (light yellow). They found that the lighter the seed, the lower the content of dietary fibre and Klason lignin in meals, and the higher the protein, mineral, and sucrose contents.

Published experimental results have pointed to a greater metabolizable energy value and protein digestibility of yellow seeds and meals of the canola type in chickens (Simbaya et al., 1995; Slominski et al., 1994, 1999, 2011). In our previous study (Smulikowska et al., 1998), the nutritional value for chickens and rats of seeds, cakes, and solvent meals from low-glucosinolate yellow-seeded spring rape were better than from dark-seeded winter rape. The greater energy value of yellow than dark rapeseed is ascribed partly to its lower dietary fibre content and higher nutrient digestibility. The content and composition of dietary fibre can also affect bacterial fermentation in the gut, production of short-chain fatty acids (SCFA), and

related energy losses. Moderate SCFA production is important for intestinal health because these acids, mainly butyrate, are the primary energy source for colonocytes (Roediger, 1982) and reduce the concentration of pathogens by maintaining a low luminal pH. A low pH value of digesta can stimulate the absorption of minerals. It can be assumed that the different content and composition of fibre in yellow and black rapeseed may induce differences in the microbial activity and pH of digesta, and may affect the absorption of phosphorus. It was, therefore, interesting to compare the two types of rapeseed not only as sources of energy and nutrients, but also as dietary components affecting the intestinal environment.

Rape seeds are rich in phosphorus, which is present mainly in the form of phytates, bound in the form of phytin that forms globoid phytin crystals. The structure and site of phytin in seeds may determine the extent of interactions with other compounds and determine the utilization of phosphorus by monogastric animals, as well as the efficacy of microbial phytase supplements (Rodehutscord, 2009). No information was found in the available literature concerning the availability of phosphorus and the efficacy of microbial phytase in yellow-seeded winter rapeseed.

The aim of this study was to compare the nutritive value and phosphorus availability of yellow- and dark-seeded rapeseed cakes and effects of phytase supplementation in broiler chickens.

MATERIAL AND METHODS

Material

Rape seeds of 3 yellow-seeded winter 00 lines, 041, 036, and 022, developed at the Plant Breeding and Acclimatization Institute in Poznań (Poland), grown on the same field and under the same climatic conditions, and a black-seeded commercial cultivar, Bojan, were cold pressed with a hydraulic press-shed at a local plant and denoted yellow-seeded lines (YRC) and black-seeded lines (BRC), respectively. Expeller cakes were ground to a fine texture, analysed, and used in the experimental diets. The seed colour of all of the yellow lines was 5 rated on a five-point scale (0-black, 5-light yellow). A commercial enzyme preparation, Phyzyme XP® (Danisco Animal Nutrition) containing 4000 FTU/g phytase according to the producer's declaration, was used.

Animals and experimental procedures

All procedures were approved by the Local Animal Care and Use Committee in Warsaw. In all experiments, one-day-old Ross 308 broiler females were obtained

from a local commercial hatchery and kept in battery cages. Room temperature was maintained at 30°C for the first 3 days and was thereafter gradually reduced according to normal management practices. A light cycle of 18 h light and 6 h darkness was maintained throughout the study, birds had free access to water and feed. In Experiments 1 and 2, the birds were fed *ad libitum* a broiler starter diet based on wheat, maize and soyabean meal. On day 21, the chickens were fasted for 4 h before being individually weighed and were assigned to dietary treatments; the average initial body weight (BW) was similar among treatments. From this time the birds were housed individually in cages (36 x 50 cm floor area) that allowed for total collection of excreta. Each bird was treated as a replication.

Experiment 1

The experiment was designed to measure the digestibility of nutrients and metabolizable energy value of the cakes. Apparent metabolizable energy corrected to zero nitrogen balance (AME_N) and apparent protein and fat digestibilities of reference and test diets were determined using the assay described by Hill and Anderson (1958) with some modifications. A reference diet (Table 1) was prepared and its dry matter (DM) analysed. Four test diets were composed by mixing the reference diet with one of four rapeseed cakes in a 7:3 proportion on a DM basis. Chromium oxide (Cr_2O_2) was added to reference and test diets as an inert marker in the amount of 3 g/kg DM and the diets were pelleted on a CL-2 CPM Laboratory Pellet Mill (CA, USA). Fifty, three-week-old birds,

Table 1. Composition of reference diet[1]. Experiment 1

Components	Air-dry matter, g/kg
Wheat	460
Soyabean meal	280
Maize	200
Rapeseed oil	20
Limestone	11
Monocalcium phosphate	13
NaCl	3
Mineral-vitamin premix[2]	10
DL-methionine	0.5
L-lysine	1.5
Feed enzyme[3]	1

[1] four test diets were prepared by mixing basal diet with evaluated YRC or BRC cakes as 7:3 ratio, on DM basis (see Material and Methods)

[2] provided per kg diet: IU: vit. A 13500, vit. D_3 3500; mg: vit. E 40, vit. K_3 4, vit. B_1 3, vit. B_2 8, vit B_6 5, vit. B_{12} 0.03, biotin 0.1, folic acid 1.75, niacin 60, Ca-panthotenate 15, Mn 80, Zn 70, Fe 70, Cu 15, Co 0.3, J 1, Se 0.2, Mg 100, betaine 133.5, diclazuril 1; g: Ca 1.185

[3] Avizyme 1500 (Danisco Animal Nutrition), containing endo-1,4-β-xylanase 350 U/g and endo-1,3 (4)-β-glucanase 150 U/g, subtilisin 4000 U/g, α-amylase 400 U/g and pectinase 25 U/g according to producers declaration

were assigned to 5 dietary treatments, 10 chickens per treatment. The average body weight in the groups was 950±82 g. The reference diet and test diets were fed for 8 days, the first 3 days were the adjustment period followed by a 5-day-period of total collection of excreta. The excreta of each bird were pooled, homogenized, sampled and kept at -18°C for further analyses.

Experiment 2

The effects of rapeseed cakes and supplementing with two levels of phytase on apparent retention of phosphorus, nitrogen, and organic matter were determined using a balance method; their effects on the ileal and caecal short-chan fatty acd (SCFA) contents and total bacterial counts were analysed post-mortem. Four basal semi-purified diets were prepared, each containing 450 g/kg rapeseed expeller cake (Table 2). Diets met or exceed the requirements of broilers

Table 2. Composition of basal diets[1], g/kg. Experiment 2

Indices	Dietary treatments [1]			
	YRC 041	YRC 036	YRC 022	BRC
Ingredients				
rapeseed cake	450	450	450	450
potato protein concentrate	100	100	100	100
rapeseed oil [2]	7.5	-	1.1	35.8
limestone	7	7	7	7
mineral-vitamin premix [3]	5	5	5	5
NaCl	3	3	3	3
maize starch	427.5	435	433.9	399.2
Analysed[4]				
dry matter, g/kg	89.9	89.9	90.2	90.2
crude protein, g/kg DM	220.0	224.1	228.1	232.7
P total, g/kg DM	4.99	4.68	4.57	4.74
P phytate,g/kg DM	2.85	2.51	2.32	2.00
Calculated				
Ca, g/kg DM [5]	7.15	6.99	6.97	6.94
P phytate/total P, %	57.1	53.6	50.8	42.2

YRC - yellow-seeded cake; BRC - black-seeded cake; [1]each diet was prepared without or with 125 mg/kg (500 FTU) or 250 mg/kg (1000 FTU) phytase preparation substituted by maize starch; [2] oil pressed from the seeds; [3] see Table 1; [4] average from unsupplemented and supplemented diets; [5] calculated based on analysed values of feed ingredients

for nutrients and energy, with the exception of Ca and non-phytic P, which were lower than in NRC (1994). The YRC or BRC were the main P sources in the diets, potato protein concentrate provided only about 8% total P/kg diet. The dietary content of crude fat was equalized to the level of 11.4% of the diet by adding oil obtained by cold pressing of the expeller cake used. The phytase preparation (125 or 250 mg/kg) was added at the expense of maize starch to basal diets to

form 12 dietary treatments, consisting of 4 rapeseed expeller cakes and 3 graded levels of added phytase (0, 500, or 1000 FTU/kg). The diets were prepared as a mash.

A total of 96 three-week-old birds was assigned to 12 dietary treatments, 8 chickens per treatment. The average body weight in the groups was 930±90 g. The experimental diets (Table 2) were fed for 8 days, the first 3 days were the adjustment period, followed by a 5-day period of total collection of excreta. In the last period, feed intake was measured daily and excreta from each bird were quantitatively collected, pooled, weighed, and kept at -18°C for further analyses. After the end of the excreta collection period, the chickens were fed *ad libitum* with the same diets and the following day they were sacrificed by cervical dislocation. The gastrointestinal tract was excised and the luminal content of the posterior ileum (the last 15 cm anterior to the ileocaecal junction) and of the caecum were collected. From each bird, approximately 2 ml of ileal and caecal contents were sampled into sterile Eppendorf tubes for microbiological analysis. The remaining digesta were adjusted to pH 8 with 1 M NaOH and stored at -20°C for SCFA determination.

Experiment 3

The effects of rapeseed cakes and supplementing with one dose of phytase on growth performance were evaluated during a growth test followed by post-mortem measurements of liver and thyroid weights, tibia characteristics, analysis of caecal pH and SCFA contents, and concentration of phosphorus, calcium, and alkaline phosphatase (ALKP) in blood.

Four basal diets were prepared, each with about 300 g of the investigated cakes per kg. Phosphorus from expeller cakes averaged about 40% total P. The calculated available P content in the diets was about 20% lower than the chicken requirements according to NRC (1994). Each diet was prepared as unsupplemented or supplemented with 1000 U phytase/kg (Table 3). The diets were pelleted on a CL-2 CPM Laboratory Pellet Mill (CA, USA).

A total of 144 one-day-old broilers was randomly allocated into 8 groups, maintained in battery cages and provided *ad libitum* the experimental diets (1-3 d in mash, later in pelleted form). On day 8 of age, the birds were weighed and 12 birds per group were placed in individual cages. The average initial body weight within treatments was 142 g. Experimental diets were provided for the following 4 weeks, body weight and feed intake were measured on day 35 of age after 4 h feed deprivation, and body weight gain (BWG) and feed conversion ratio (FCR) were calculated. The chickens were fed with the same diets *ad libitum* and during the following two days they were weighed, slaughtered by cervical dislocation, mixed blood was collected into heparinized tubes, centrifuged at 6000 *g* for 10 min

at 4°C and the plasma was stored at -18°C for further analyses of total protein, P, Ca, and alkaline phosphatase (ALKP). The gastrointestinal tract was excised and ileal and caecal digesta were collected for pH and SCFA determination. The liver, thyroid gland, heart, and abdominal fat were excised and weighed. The right tibias were collected, cleaned of all exterior tissue and frozen at -18°C until analysis.

Table 3. Composition of diets, g/kg. Experiment 3

Indices	Dietary treatments			
	YRC 041	YRC 036	YRC 022	BRC
Components				
rapeseed cake[1]	298.4	304.3	302.0	276.4
maize	200	200	200	200
wheat	305.25	314.2	318.0	303.15
soyabean meal	162.4	153.75	150.55	164.0
rapeseed oil [1]	6.2	-	1.7	28.7
limestone	10	10	10	10
monocalcium phosphate	6	6	6	6
vitamin-mineral premix[2]	5	5	5	5
NaCl	3	3	3	3
DL-Met (98%)	0.5	0.5	0.5	0.5
L-Lys (78%)	2	2	2	2
feed enzyme[3]	1	1	1	1
maize starch or phytase[4]	0.25	0.25	0.25	0.25
Calculated				
crude protein	210	210	210	210
ME, MJ/kg	12.38	12.30	12.21	12.22
crude fat	91.5	91.5	91.5	91.5
Lys	12.0	12.0	12.0	12.2
Met + Cys	8.64	8.65	8.68	8.43
Ca	8.55	8.43	8.40	8.31
P total	6.86	6.74	6.59	6.53
total glucosinolates, mmol/kg	2.35	2.80	3.07	3.10
alkenyl glucosinolates, mmol/kg	0.65	1.00	1.12	1.97

YRC, BRC- see Table 2; [2] oil pressed from the seeds; [2] provided per kg diet: IU vit. A 12000, vit. D 3000; mg: vit. E 45, vit. K 3, vit. B_1 2, vit. B_2 7, vit. B_6 4, vit. B_{12} 0.025, biotin 0.1, folic acid 1.5, niacin 50, choline chloride 325.4, Ca pantothenate 14, betain 125, antioxidant 5, salinomycin 70, Mn 80, Zn 60, Fe 70, Cu 12, Co 0.3, J 1, Se 0.2, Mg 100; g: Ca 1.15; [3] as in Table 1; [4]each diet was prepared without or with 250 mg/kg (1000 FTU) phytase preparation substituted by maize starch

Analytical procedures

In expeller cakes, diets, and excreta the content of DM, N total, crude ash, crude fibre and total P were measured in triplicate according to AOAC (1990). Crude fat was determined by ether extraction preceded by acidification with 4 M hydrochloric acid. In seeds and expeller cakes, glucosinolates were determined according to the ISO 9167-1 method (1992) using an HPLC 1050 apparatus, intrinsic phytase activity was measured according to Eeckhout and De Paepe (1994). One unit of phytase activity is defined as that amount of phytase activity

which liberates inorganic phosphorus from a 0.0015 M Na-phytate solution at a rate of 1 μmol per min at pH 5.5 and 37°C. Phytate P was measured according to Tangkongchitr et al. (1981), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined with a Fibertec System M according to Van Soest (1973). Faecal N was determined according to Ekman et al. (1949). The gross energy of rapeseed cakes, diets, and excreta was measured on a Parr adiabatic oxygen bomb KL-10 calorimeter, chromic oxide was determined spectrophotometrically following wet ashing according to Hinsberg et al. (1953).

The analysis of biochemical indices in blood was performed using an Ectachem DT60 Analyzer (Eastman Kodak, Rochester, NY, USA). The pH of ileal and caecal digesta was measured immediately after collection using a digital pH-meter (WTW pH/340, Germany) and pH standard WTW D-82362 Weilheim (model STP4).

Tibias were weighed and analysed for strength by shear force measurement according to Ferretti et al. (1992) using a Texture Analyzer TA-XT2i (Stable Micro Systems LTD, Godalming, Surrey, UK) with a head of operating range 0-500 kg. Force was applied at a constant rate 10 mm/min. The width of the supports corresponded to 40% of the length of the investigated bone. 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.

The SCFA content in digesta was measured according to the method of Ziołecki and Kwiatkowska (1973) using isocaproic acid as the internal standard and a HP 5890 AII gas chromatograph equipped with a FID and a Supelco Nukol capillary column (30 m x 0.25 mm internal diameter, film 0.25 mm). The initial column temperature was set at 100°C for 2 min, increased to 140 at 10°C/min and held at the final temperature for 20 min.

Ileal and caecal contents sampled for bacterial analysis were mixed with a freshly prepared paraformaldehyde fixative (1:3 w/v) and kept for 3 h at 4°C. The suspensions were centrifuged (approx. 1-2 min, 5000 rpm) and the supernatants were removed. Cells were washed twice in 1×PBS (130 mM sodium chloride, 10 mM sodium phosphate buffer, pH 7.2), resuspended in 50% (v/v) ethanol/PBS and stored at -20°C. Total bacterial counts were obtained by filtering 100 μl samples of diluted caecal content onto black polycarbonate filters (pore size 0.22 μm). The filters were incubated (3 min at room temperature) on a drop of 0.04% acridine orange in 1% formalin. The filters were rinsed gently by floating them on distilled water, air-dried, and mounted on microscope slides using non-fluorescent immersion oil. The slides were stored in the dark at 4ºC until analysis on an Olympus BH-2 epifluorescence microscope (Olympus, Japan).

Calculations and statistical analysis

In Experiments 1 and 2, the total nutrient intake was adjusted for feed refusal. The AME_N value of reference and test diets was calculated according to Hill and Anderson (1958), then AME_N values and apparent digestibility of crude protein (ACPD), crude fat (AFD), and apparent N retention of YRC and BRC were calculated according to Campbell et al. (1983). The AME_N of fat-free matter in cakes was calculated using the equation:

$$AME_N \text{ ffDM (MJ)} = AME_N \text{ (MJ/kg DM)} - (\text{crude fat content (g/kg DM)} \times \text{AFD} \times 0.03883)$$

where: AFD - apparent crude fat digestibility; 0.03883 - AME_N value of 1 g digestible crude fat according to the European Table of Energy Values for Poultry Feedstuffs (1989).

The apparent total tract P retention value was calculated using the equation:

$$AR_P \% = 100 \times (P_I - P_O) / P_I$$

where: AR - the respective apparent P retention expressed as a percentage; P_I - the dietary P intake; P_O - the excreta P output.

Apparent total tract retentions of N and organic matter (OM) were calculated using the same equation with the use of the respective values for N or OM.

The results were subjected to one-way or two-way ANOVA, using the Statgraphics Plus ver. 5.1 programme (Statgraphics 1994-2001). When the ANOVA indicated significant treatment effects, means were separated using Duncan's multiple range test. In the Tables, results are presented as mean values with pooled standard errors. Differences were considered to be significant at $P \leq 0.05$.

RESULTS

The composition of YRC and BRC is shown in Table 4. The YRC contained more crude fat but less crude protein and crude fibre, ADF and NDF than BRC. The total P content in YRC and BRC was comparable, but the ratio of phytate P in P total was lower in BRC than in YRC. The phytase activity in YRC and BRC was in a similar range, from 377 to 433 U/kg. The total glucosinolate content in YRC was from 8.7 to 10.2 μmol/g DM, in BRC 12.6 μmol/g DM, but there was a large difference in the concentration of alkenyl glucosinolates, which was lower in YRC than in BRC. The progoitrin ratio in total glucosinolates was 6%, 13%, and 15% in YRC 041, 036, and 022, respectively, whereas it was 35% in BRC.

Experiment 1

The ACPD determined on 3-week-old broilers averaged 80% and did not differ among rapeseed cakes (Table 5). The AFD and the value of AME_N did not differ among YRC and averaged 76.7% and 14.48 MJ/kg DM, respectively, but was higher in comparison with BRC ($P<0.05$). The AME_N value calculated per kg of fat-free DM was significantly higher for YRC 041 in comparison with YRC 022, but similar to YRC 036 and BRC ($P<0.05$).

Table 4. Composition of yellow-seeded (YRC) and black-seeded (BRC) rapeseed expeller cakes

Indices	YRC 041	YRC 036	YRC 022	BRC
Compound				
dry matter (DM), g/kg	901	907	913	893
crude protein, g/kg DM	306	311	317	334
crude fat, g/kg DM	255	273	269	190
crude ash, g/kg DM	63.2	58.6	58.4	59.3
crude fibre, g/kg DM	71.3	75.8	81.0	102.8
acid detergent fibre, g/kg DM	90.4	96.4	91.8	154.5
neutral detergent fibre, g/kg DM	169	183	173	194
gross energy, MJ/kg DM	24.97	25.57	25.68	24.00
P total, g/kg	10.0	9.47	9.11	9.62
P phytate, g/kg	6.19	5.44	5.01	4.26
P phytate, % P total	61.9	57.4	55.0	44.3
phytase activity[1], U/kg	433	377	428	409
Glucosinolates, µmol/g DM				
progoitrin	0.55	1.32	1.53	4.38
gluconapoleiferin	0.11	0.11	0.11	0.11
gluconapin	1.66	2.09	2.30	2.81
glucobrassicanapin	0.11	0.11	0.11	0.67
total alkenyl glucosinolates	2.43	3.63	4.05	7.97
4-hydroxyglucobrassicin	6.10	6.28	5.91	4.49
other indole glucosinolates	0.22	0.22	0.22	0.11
total glucosinolates	8.75	10.13	10.18	12.57

YRC, BRC - see Table 2; [1] one unit of phytase activity corresponds to the liberation of 1 µmol per min of inorganic P from a solution of 0.0015M sodium phytate at pH 5.5 and 37°C

Table 5. Apparent crude protein digestibility (ACPD), apparent crude fat digestibility (AFD), apparent N retention (ANR) and apparent metabolizable energy (AME_N) of rapeseed cakes. Experiment 1

Rapeseed cake	ACPD % of total	AFD % of total	ANR % of total	AME_N MJ/kg DM	AME_N MJ/kg ffDM
YRC 041	80.2	76.8[a]	43.1	14.59[a]	9.38[a]
YRC 036	80.1	76.8[a]	44.3	14.74[a]	9.08[ab]
YRC 022	79.6	76.5[a]	47.7	14.12[a]	8.38[b]
BRC	79.1	72.9[b]	42.2	12.63[b]	8.95[ab]
SEM	0.81	1.09	2.19	0.28	0.28

YRC, BRC - see Table 2; ffDM - fat-free dry matter; AME_N (MJ/kg ffDM) = AME_N MJ/kg DM - [crude fat content (g/kg DM) x AFD x 0.03883 [AME_N of 1 g of digestible crude fat according to European Table of Energy Values for Poultry Feedstuffs (1989)]

Experiment 2

The total P content in balance diets was from 4.6 to 5 g/kg DM, of which 91%-92% P originated from expeller cakes. The ratio of phytate P to total P was lower in the BRC diet in comparison with YRC diets (Table 2). The P retention (availability) in unsupplemented diets did not differ between YRC and BRC, and averaged 39% (Table 6). P retention increased due to supplementation with 500 FTU phytase by 6.5 percentage points, on average ($P<0.01$). Addition of a further 500 FTU/kg increased P retention in YRC 041 by 1.7, YRC 036 by 3.9, YRC 022 by 8.5, and BRC by 10.5 percentage points, respectively (interaction RC x Phy significant; $P<0.05$; Table 6). Apparent N retention averaged 55.3% and was not affected by dietary treatments. Apparent organic matter retention was higher in groups fed diets with YRC 022 and YRC 041 than in those fed diets with BRC or YRC 036 ($P<0.05$). Phytase supplementation affected neither N nor OM retention.

Table 6. Main effects of dietary treatments on apparent retention of phosphorus (APR), nitrogen (ANR) and organic matter (AOMR) in experimental diets. Experiment 2

Main effects	APR	ANR	AOMR
	% of total		
Rapeseed cake			
YRC 041	44.4^{B}	56.1	76.2^{a}
YRC 036	44.6^{B}	55.4	75.2^{b}
YRC 022	44.2^{B}	55.0	76.0^{a}
BRC	48.3^{A}	54.7	74.7^{b}
SEM	0.93	0.61	0.26
Phytase, FTU/kg diet			
0	39.0^{C}	55.0	75.7
500	45.5^{B}	55.3	75.5
1000	51.7^{A}	55.5	75.3
SEM	0.80	0.53	0.22
RC x Phy	0.017	NS	NS

YRC, BRC - see Table 2; ^{a,b,A,B} within main effects values in columns with different superscripts are different at: ^{a,b} $P<0.05$, ^{A,B} $P<0.01$; NS - not significant

Neither the type of expeller cake nor phytase supplementation affected the total SCFA concentration in the ileal digesta of broilers (average 51.3 μmol/g) or total bacterial counts (average 10.4 log AFU/g digesta). Acetate predominated in ileal SCFA (87%), followed by propionate (5.8%), isobutyrate (3.4%), valerate (2.2%), and butyrate (1.9%) (data not shown).

In caecal digesta (Table 7), the concentration of total SCFA was four times larger than in ileal digesta. It was affected significantly by type of cake used (total SCFA was higher in birds fed the BRC diet in comparison with YRC 036) and increased with phytase supplementation, with the exception of YRC 041,

Table 7. Main effects of dietary treatments on short-chain fatty acids (SCFA) concentration (μmol/g) and on total bacterial counts (log AFU - aggregate forming units/g) in the caecal digesta. Experiment 2

Main effects	Total SCFA	Acetate	Propio-nate	Butyrate	Iso-butyrate	Valerate	Iso-valerate	Total no bacteria
Rapeseed cake								
YRC 041	246^{AB}	230^{ab}	5.41^{AB}	9.58^{AB}	0.92	0.53^{b}	0.22^{AB}	11.55^{B}
YRC 036	223^{B}	208^{b}	4.58^{B}	8.56^{B}	0.85	0.49^{b}	0.21^{AB}	11.57^{B}
YRC 022	264^{AB}	247^{a}	4.88^{B}	9.79^{AB}	0.86	0.64^{ab}	0.18^{B}	11.68^{A}
BRC	268^{A}	248^{a}	6.11^{A}	11.50^{A}	0.90	0.69^{a}	0.30^{A}	11.68^{A}
SEM	11.6	11.0	0.313	0.619	0.057	0.054	0.019	0.023
Phytase, FTU/kg diet								
0	212^{B}	197^{B}	4.62^{B}	8.58^{B}	0.82	0.39^{B}	0.19^{B}	11.58^{b}
500	268^{AB}	250^{AB}	5.49^{AB}	10.41^{AB}	0.92	0.61^{A}	0.22^{AB}	11.65^{a}
1000	270^{A}	252^{A}	5.62^{A}	10.58^{A}	0.91	0.77^{A}	0.27^{A}	11.62^{ab}
SEM	10.0	9.5	0.271	0.536	0.049	0.046	0.017	0.0197
RC x Phy	0.05	0.05	NS	NS	NS	0.01	0.05	0.01

YRC, BRC - see Table 2; ^{a,b,A,B} within main effects values in columns with different superscripts are different at: ^{a,b} $P<0.05$, ^{A,B} $P<0.01$; NS - not significant

in which it was lower at the highest phytase supplementation (interaction RC x Phy significant at $P<0.05$). The major component of SCFA was acetate, 93% on average, and the minor components were butyrate (3.9%) and propionate (2.1%), followed by small amounts of isobutyrate, valerate, and isovalerate. The molar ratios of acetate, propionate and butyrate did not differ significantly among treatments (data not shown). Total bacterial numbers in caecal contents were higher in groups fed YRC 022 and BRC ($P<0.05$) in comparison with YRC 041 and YRC 036. Bacterial counts increased with supplementation of phytase, with the exception of YRC 041 and YRC 036, where they declined with phytase supplementation (interaction RC x Phy significant at $P<0.05$).

Experiment 3

The YRC or BRC cakes supplied from 41% to 43% total dietary P (Table 3). The effects of type of cake and phytase supplementation on performance, relative weight of organs, and tibia parameters are shown in Table 8. Feed intake and BWG was lower in the group fed the diet with YRC 036, in comparison with YRC 041 ($P<0.05$ and $P<0.01$, respectively), but FCR was worse in the group fed the diet with BRC in comparison with YRC ($P<0.05$). Phytase supplementation significantly increased feed intake and BWG ($P<0.01$) with no effect on FCR. Feed intake increased in groups fed YRC and slightly decreased in the group fed BRC after phytase supplementation (interaction RC x Phy significant at $P<0.05$). The FCR was slightly worse in groups fed YRC 041 and YRC 036, but better (1.46 vs 1.48 and 1.48 vs 1.56 in groups fed YRC 022 and BRC, respectively; the interaction RC x Phy was significant at $P<0.05$).

Table 8. Main effects of dietary treatments on performance and on relative weight of liver and thyroids, tibia weight, ash content and tibia ultimate strength in 35-day-old broilers. Experiment 3

Main effects	Body weight kg 8 day	Feed intake kg 8-35 d	BWG kg 8-35 d	FCR kg feed/kg BWG 8-35 d	Liver % LBW	Thyroids mg/100 g LBW	Tibia weight g	Tibia ash %[1]	Tibia ultimate strength N
Rapeseed cake									
YRC 041	0.143^{ab}	2.71^{a}	1.84^{A}	1.469^{a}	2.49^{b}	12.4^{B}	11.3^{a}	38.5	232^{a}
YRC 036	0.138^{b}	2.52^{b}	1.71^{B}	1.470^{a}	2.71^{a}	15.8^{B}	10.5^{ab}	38.7	192^{b}
YRC 022	0.146^{a}	2.55^{ab}	1.73^{AB}	1.474^{ab}	2.57^{ab}	13.5^{B}	10.7^{ab}	39.2	206^{b}
BRC	0.141^{ab}	2.68^{a}	1.77^{AB}	1.518^{b}	2.67^{a}	24.5^{A}	10.2^{b}	39.5	198^{b}
SEM	0.0021	0.054	0.033	0.016	0.066	0.92	0.30	0.64	7.6
Phytase, FTU/kg diet									
0	0.139^{b}	2.53^{B}	1.70^{B}	1.488	2.70^{A}	17.1	10.4	38.7	189^{B}
1000	0.144^{a}	2.70^{A}	1.83^{A}	1.477	2.52^{B}	16.0	11.0	39.3	225^{A}
SEM	0.0015	0.038	0.024	0.011	0.046	0.65	0.22	0.45	5.4
RC x Phy	NS	0.05	NS	0.04	NS	NS	NS	NS	NS

YRC, BRC - see Table 2; $^{a, b, A, B}$ within main effects values in columns with different superscripts are different at: $^{a, b}$P<0.05, ^{AB} P<0.01; BWG - body weight gain; LBW - live body weight; FCR - feed conversion ratio; [1] on fat-free, moisture-free basis; NS - not significant

Birds provided the diet with BRC had enlarged thyroids (P<0.01) in comparison with birds fed diets with YRC, and livers (P<0.05) in comparison with the group fed YRC 041. Phytase supplementation lowered liver weight (P<0.01) and had no significant effect on thyroid weight. Tibia ultimate strength and tibia weight were greatest (P<0.05) in birds fed the diet with YRC 041, however, for the last parameter the difference was significant only with BRC. Tibia ultimate strength increased (P<0.01) due to phytase supplementation.

The effects of RC type and phytase supplementation on blood parameters are shown in Table 9. Total protein and P concentrations and ALKP activity were not

Table 9. Main effects of dietary treatments on the concentration of total protein, P, Ca and alkaline phosphatase (ALKP) in blood of 35-day-old broilers. Experiment 3

Main effects	Total protein, g/l	P, mmol/l	Ca, mmol/l	ALKP, U/l
Rapeseed cake				
YRC 041	31.4	2.09	2.38^{ab}	743
YRC 036	30.8	2.06	2.25^{b}	680
YRC 022	30.2	2.12	2.43^{a}	637
BRC	31.4	2.14	2.37^{ab}	597
SEM	0.85	0.045	0.048	63.5
Phytase, FTU/kg				
0	31.5	2.05^{b}	2.38	759^{a}
1000	30.3	2.15^{a}	2.34	570^{b}
SEM	0.60	0.032	0.034	45.0

YRC, BRC - see Table 2; ^{a,b} within main effects values in columns with different superscripts are different at P<0.05; all interactions were not significant

affected by type of cake, whereas the Ca concentration was higher in birds fed YRC 022, than in birds fed YRC 036. Total protein and Ca concentrations did not differ, while the P concentration in blood was greater ($P<0.05$), and ALKP activity, lower due to phytase supplementation (Table 9).

The SCFA concentration in ileal digesta averaged 77 μmol/g, pH averaged 6.8, and neither was affected by the type of cake or by phytase supplementation (data not shown). In caecal digesta (Table 10), the concentration of total SCFA was three times as large in comparison with ileal digesta. It was higher in birds fed YRC 041 than in birds fed the diet with BRC ($P < 0.01$) and increased with phytase supplementation ($P < 0.05$). The major component of SCFA was acetate, 78% on average, and the minor components were butyrate (15.6%) and propionate (2.8%), while isobutyrate, valerate and isovalerate were detected in measurable amounts in all samples but their sum was less than 4% of total SCFA. Molar ratios of acetate, propionate and butyrate did not differ significantly among treatments (data not shown). The pH of caecal digesta was lower in birds fed YRC 022 than in birds fed the diet with BRC ($P < 0.05$) and increased after phytase supplementation.

Table 10. Main effects of dietary treatments on short-chain fatty acids (SCFA) concentration (μmol/g) and pH of caecal digesta in 35-day-old broilers. Experiment 3

Main effects	Total SCFA	Acetate	Propio-nate	Butyrate	Iso-butyrate	Valerate	Iso-valerate	pH
Rapeseed cake								
YRC 041	259^{A}	200^{A}	5.97	41.2^{A}	2.06^{A}	2.85^{a}	1.42^{a}	6.23^{ab}
YRC 036	236^{AB}	184^{AB}	7.71	37.2^{AB}	1.60^{AB}	2.49^{ab}	1.05^{b}	6.18^{ab}
YRC 022	241^{AB}	189^{AB}	5.98	37.6^{AB}	1.48^{B}	2.21^{b}	0.99^{b}	5.94^{b}
BRC	211^{B}	165^{B}	6.83	32.4^{B}	1.84^{AB}	2.13^{b}	1.05^{b}	6.44^{a}
SEM	9.9	8.4	1.03	2.31	0.128	0.151	0.125	0.110
Phytase, FTU/kg								
0	225^{b}	177	7.68^{A}	34.5^{b}	1.76	2.17^{B}	1.12	6.02^{b}
1000	249^{a}	192	5.57^{B}	39.7^{a}	1.73	2.68^{A}	1.14	6.37^{a}
SEM	7.0	5.9	0.72	1.63	0.09	0.046	0.088	0.078

YRC, BRC - see Table 2; ^{a,b,A,B} within main effects values in columns with different superscripts are different at: $^{a,b}P<0.05$, $^{A,B}P<0.01$; all interactions were not significant

DISCUSSION

Glucosinolates, mainly alkenyl glucosinolates, give rise to products of hydrolysis (isothiocyanates, thiocyanates, and oxazolidinethiones) that negatively affect thyroid and liver morphology and functions and decrease palatability. Alkenyl glucosinolates, especially progoitrin (2-hydroxy-3 butenyl), are considered the most goitrogenic, while the indole glucosinolates are not primarily goitrogenic (Bell, 1995). The total alkenyl glucosinolate (TAG) content in rapeseed cakes

used in our study was low, in YRC from 2.4 to 4.05, in BRC, 8 μmol/g DM, respectively. In YRC 041 it was, on average, 37% lower than in the two remaining YRC, and 76% lower than in the BRC. Moreover, progoitrin constituted only 23% TAG in YRC 041, while in the remaining two YRC, 37%, on average, and 55% TAG in BRC. The respective values reported by Slominski et al. (1999) for yellow canola meals were much higher, from 7.5 to 12.1 μmol TAG/g DM, in which progoitrin constituted from 45% to 65%. Since the alkenyl glucosinolates are the main rapeseed factor negatively involved in performance depression in monogastrics, it seems that cold-pressed cakes from Polish yellow rapeseeds can be safely used in animal diets.

Myszka et al. (2011) analysed rape seeds and rapeseed meals produced from 58 Polish yellow-seeded lines and 7 black-seeded cultivars (including those used in the present study), harvested in 2006–2008. The results showed that in meals from yellow seeds, the content of dietary fibre was by 10, and Klason lignin was by 8.6 percentage points lower than in black meals. Also Simbaya et al. (1995) and Slominski et al. (1994, 1999) reported that the content of Klason lignin in seeds and meals from yellow-seeded canola was lower than from black-seeded ones. All four expeller cakes used in the present study were cold pressed at the same time with the same hydraulic press-shed, however, more crude fat remained in YRC than in BRC. It can be supposed that the lower level of lignin may contribute to worse oil extraction from rape seeds.

In the cold-pressed yellow and black seeds used in the current study, endogenous phytase activity ranged from 360 to 405 U/g (unpublished), in expeller cakes it ranged from 380 to 430 FTU/kg. No data were found in the available literature on the activity of phytase in raw seeds or in expeller cakes of 00 rape/canola. The phytase activity measured in the present study is much higher than values published for most plant feedstuffs used in broiler feed mixtures, with the exception of such cereals as rye, wheat, and by-products of their processing (Eeckhout and De Paepe, 1994; Sauvant et al., 2004). The results of the present study indicate that the endogenous phytase of rapeseed was not inactivated during expelling.

The type of RC did not affect apparent protein digestibility, but crude fat from YRC was better digested than from BRC. Due to the higher content and better digestibility of fat, AME_N values for broilers of YRC averaged 14.48 MJ/kg DM and were higher than BRC. Nonetheless, the AME_N values of fat-free DM of YRC averaged 8.95 MJ/kg and did not differ significantly from BRC. The AME_N values of solvent-extracted canola meals reported recently by Slominski et al. (2011) for broilers and turkeys, respectively, were in a similar range: 9.16 and 9.06 MJ/kg DM in *B. napus* yellow, 7.97 and 8.40 in *B. napus* black.

The availability of P from plant feeds for non-ruminants depends on the content of some minerals and phytic acid and on intrinsic phytase activity. Rape seeds

and rapeseed products are the richest plant source of P in poultry diets, however, information about availability of phosphorus in these sources is scarce. Most of it concerns solvent-extracted rapeseed oil meal which, according to Eeckhout and De Paepe (1994), contains 11.2 g P/kg, of which 64% is non-phytate P, and intrinsic phytase activity averages 16 U/kg. The respective values for solvent-extracted rapeseed meal shown in INRA Tables (Sauvant et al., 2004) are: 11.4 g P/kg, of which 40% is non-phytate P, phytase activity is 10 U/kg, and P availability in broilers is 25%, but there were no data concerning intrinsic phytase activity in raw rape seeds or expellers or their P availability in broilers. The present study shows that despite the lower ratio of phytate P to total P in BRC, the P availability of BRC and YRC in broilers did not differ and averaged 39% and that the added microbial phytase was more active in the former. This may be explained by greater seed cell destruction during pressing in BRC, allowing for closer contact of added phytase with phytate. This assumption is based on the lower content of residual fat in BRC in comparison with YRC. In experiments with black-seeded expeller cakes used as a sole source of dietary phosphorus, the values of total P retention from unsupplemented and phytase-supplemented diets were of similar range: 39% vs 46% (Leske and Coon, 1999), 35% vs 53% (Potkański et al., 1995) or 37% vs 44% (Rutherfurd et al., 2002), respectively.

In the present study, rapeseed cakes (45% diet) were also the main source of non-starch polysaccharides in semisynthetic diets. The type of cake did not affect SCFA concentration in ileal digesta, which indicates that the components of yellow cakes reaching the ileum were not fermented better by ileal microbiota than components of black cake, both in the absence or presence of supplemental phytase. The SCFA concentration in caecal digesta was lower, however, when YRC were fed, in comparison with BRC, and the caecal SCFA concentration increased due to phytase supplementation. Also Zduńczyk et al. (2011) reported that feeding yellow-seeded *B. napus* canola caused a significant decrease in the concentration of caecal SCFA in turkeys, in comparison with its black-seeded counterpart.

In the present study, after 5 weeks of feeding experimental diets containing about 30% rapeseed cakes, the most distinctive difference between experimental birds was found in thyroid weight, which was higher in the group fed the diet with BRC in comparison with YRC. Thyroid weight correlated less with total, but rather with the alkenyl glucosinolate level in the diets. Similarly as in the digestibility study, the type of cake did not affect the SCFA concentration in ileal digesta, which confirms that also in practical-type diets, the components of yellow cakes reaching the ileum were not fermented better by ileal microbiota than components of black cake. The FCR did not differ within groups fed YRC, but it was lower than in the group fed BRC. Slominski et al. (1999) also obtained lower FCR in broilers fed meals from brown in comparison with yellow *B. napus*.

In the present study, phytase supplementation of diets containing about 30% of yellow or black expeller cakes with P levels below chicken requirements, increased feed intake, BWG, P concentration in blood, blood alkaline phosphatase activity, and tibia ultimate strength. All measured parameters, as well as the improvement of FCR in groups fed YRC 022 and BRC cakes proved that reduction of dietary P is achievable in broilers. This feeding strategy can decrease environmental problems connected with excess P in manure. Phytase supplementation increased the SCFA concentration in caecal digesta, decreased liver weight and did not affect thyroid weight. In our former studies we also found an increase of SCFA concentration in caecal digesta after supplementation of a rapeseed cake-containing diet with phytase, but this was accompanied by an increase of thyroid weight in broilers (Smulikowska et al., 2006, 2010). It is worth noting that the glucosinolate levels in press cakes in the former studies were higher.

CONCLUSIONS

The results of the present study showed yellow-seeded *B. napus* winter rapeseed cakes to be superior to commercial *B. napus* black winter rapeseed, mainly with regard to the alkenyl glucosinolate content. Due to this it can be processed without heat treatment, and cakes can be valuable components in monogastric rations supplying both energy and protein. Supplementation of broiler rapeseed cake-based diets with phytase is an effective strategy to reduce the addition of inorganic phosphates to diets, as well as to decrease phosphorus excretion into the environment.

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