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EFFECT OF HIGH RAPE CAKE CONTENT SUPPLEMENTED IN ENZYMES ON THE NUTRITIONAL VALUE OF A BROILER DIET AND INTESTINAL LACTIC ACID BACTERIA NUMBER

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The studies were designed to determine an effect of partial replacement of soybean meal with rape cake supplemented with enzymes on the nutritional value of diets and the number of lactic acid bacteria (LAB) in the ileum and caecum of broilers. The experiment 1 (growth trial) was carried out on 120 one-day-old broiler chickens Ross 308 which were randomly divided into four homogenous groups, 30 birds per group (15 males and 15 females). Each treatment consisted of six replicates of 5 birds. A control diet contained soybean meal whereas in the experimental, a part of the soybean meal was replaced by 15% rape cake from the Kaszub cultivar and supplemented with enzyme preparations containing xylanase or phytase added individually or in combination. On the 21st day of experiment six birds from each group were scarified and the ileum and caeca were isolated for lactic acid bacteria (LAB) determination.

The experiment 2 (digestibility trial) was carried out on 60 seven-day-old chickens divided into four treatments of 20 birds (4 replications of 5 birds) to determine nutrient digestibility of diets used in the growth trial. The digestibility test was carried out by the total collection method.

The inclusion of 15 % rape cake instead of soybean meal and the addition of enzyme preparations did not decrease body weight gain and feed intake. The feed conversion ratio (FCR) was higher in the group fed the diet in which phytase was added separately. A simultaneous application of xylanase and phytase statistically ($P<0.05$) increased the digestibility of crude fibre and N-free extracts. The lactic acid bacteria number was the highest in the caecum and ileum of birds fed the diet containing the xylanase preparation, whereas xylanase and phytase preparations added in combination had no effect on lactic acid bacteria number.

Key words: broilers, digestibility, enzymes, lactic acid bacteria, rape cake

INTRODUCTION

Rapeseed is characterized by high protein, fat, total phosphorus and gross energy contents. Amino acid composition of rapeseed protein is beneficial (6 g Lys, 2 g Met, 4.5 g Thr and 1.2 g/16 g N of Trp). Digestibility of rapeseed and rape cake proteins for poultry is 70% and 76%, respectively (European Table of Energy Values, 1986) and of rapeseed meal protein – 80% (Pastuszewska *et al.,*1987). Fat digestibility amounts to 60-70% (Banaszkiewicz, 1995). About 75% of total phosphorus is in the phytate form. Rapeseed contains about 8% crude fibre and 20% N-free extracts. The non-starch polysaccharides in rapeseed oil meal contain up to 35% dry matter. Digestibility of N-free extracts ranges from 37 to 68% (Smulikowska *et al.,*1997). Rape products are characterized by low available energy and phytate phosphorus of about 25% (Nwokolo and Bragg, 1980). Low glucosinolate rape cakes are appropriate alternatives to soybean meal as a vegetable protein source in a broiler diet. A study by Lesson *et al.* (1987) showed that canola meal could replace up to 100% of soybean meal without major negative effects on performance when lysine was added, but according Bell (1993) the level of indigestible carbohydrates increased compared with soybean meal. The nutritive value of these feeds may be improved by enzyme addition (Lesson and Caston, 1996; Kocher *et al.*, 2001). Supplementation with enzyme preparations can degrade dietary fibre and improve digestibility of crude fat and protein (Mikulski *et al*., 2000), phosphorus and other nutrients (Janocha *et al.*, 2000). Addition of carbohydrates to the basal wheat-rapeseed diet, may influence the nutritive value of rapeseed meal, but an inclusion of rapeseed meal to replace SBM, and an addition of Roxazyme G or Ronozyme VP to canola meal diets did not significantly affect broiler performance (Kocher *et al.,* 2000). Further improvement of nutritive value of diets can be obtained by the combined introduction of enzymes. Wu *et al.* (2004) reported that the combined addition of phytase and xylanase to wheat-based diets significantly increased the value of AME.

Changes in the composition of the diets and use of feed enzymes can affect the intestinal microbial populations in chickens (Brenes *et al.*, 1993; Simon, 1998; Danicke *et al.*, 1999; Apajalahti, 2004). Xylanase supplementation led to significantly lower CFU values for *enterobacteria* and gram positive cocci in the first 3 weeks of broiler chickens life which, however, were higher for the *Lactobacillus* sp. colony (Vahjen *et al.,* 1998). Most experiments conducted to study enzyme addition regarded wheat-soybean and maize-soybean diets.

The present studies were designed to determine the effect of partial replacement of soybean meal with rape cakes from the Kaszub cultivar in wheat broiler diets supplemented with two commercial enzyme preparations (individually or in combination) on the nutritional value of diets and the number of lactic acid bacteria (LAB) in the ileum and caecum of broiler chickens.

MATERIAL AND METHODS

In experiment 1 (growth trial), 120 one-day-old female Ross 308 broilers were randomly divided into four groups, 30 birds per group (15 males and 15 females). Each treatment consisted of six replicates of 5 birds. The birds were housed in metabolic cages. For the first 5 days the temperature was set at 31°C and then gradually decreased according to the recommendations of the supplier of broiler chickens. A control diet (SBM) contained soybean meal whereas in the experimental diet, part of the soybean meal was replaced by 15% rape cake from the Kaszub cultivar and supplementation with enzyme preparations containing xylanase (RC XY) or phytase (RC FYT) was added individually or in combination (RC XY+FYT). Rape cakes were obtained in an oil mill by pressing to 400 kG/cm^2 of Kaszub rapeseed cultivar. According to the supplier's information, the enzyme preparation XY was derived from *Aspergilus oryzae* and contained endo-1,4-β xylanase (min. 1000 FXU(W)/g), the enzyme preparation FYT was a preparation of phytase from *Peniophora lycii* derived from the fermentation of *Aspergilus oryzae* (min. 2500 FYT/g).The composition of experimental diets is shown in Table 1. Experimental diets (in a mashed form) were given *ad libitum* from the 1st to the 21st day of life. The diets contained similar amounts of protein and energy. Introduction of 15% Kaszub rape cakes instead of part of the soybean meal increased crude fibre and crude fat contents (by about 1% and 1.5%) in the experimental diets. Feed consumption was recorded during the experiment. On the 1st and 21st day of age the birds were weighed. On the 21st day of the experiment six birds from each group were scarified and the ileum and caeca were isolated for lactic acid bacteria (LAB) determination. Particular segments of the alimentary tract (1 g) were sliced and transferred to 100 mL 0.85% NaCl and precisely smeared in the solution. Next, they were shaken out for 30 minutes and than cultures of 1 mL suspension were made from the subsequent decimal dilution and spread on Petri dishes with 10 mL of modified Blickfeldt medium. The cultures were incubated at 30°C for 48 h and then at room temperature (at 20°C) for 24 h. After incubation, the total number of colonies was counted and the results were expressed as the number of log of unit-forming colonies (log cfu).

Experiment 2 (digestibility trial) was carried out on 60 seven-day-old chickens divided into four treatments of 20 birds (4 replications of 5 birds) to determine the nutrient digestibility of diets used in the growth trial. The digestibility test was carried out by the total collection method. The test included an adaptation period (six days), followed by a collection period of 3 days, during which all excreta were quantitatively collected and feed consumption was recorded. All the excreta were dried at 60°C and ground.

The chemical composition (dry matter, crude protein, crude fat, crude ash, crude fibre, crude phosphorus) of feedstuffs, diets and excreta samples from both experiments were analyzed by the procedure of the AOAC (1990). Faecal nitrogen content in the excreta was determined by Terpsta and De Hart (1974) procedure. Glucosinolates in rapeseed were determined by HPLC method. For fatty acid analyses rapeseed samples were extracted and the extracted fat was hydrolyzed by means of 0.5 M KOH in methanol and esterified with 5% HCL in methanol

(Matyka, 1976). Next, 0.2 mL of hexane was added and fatty acid methyl esters were separated and quantified using the gas chromatography apparatus CHROM 5 equipped with a column of 2.5 m flame ionization detector and integrator. The identification of fatty acid peaks was carried out by comparison with the retention times of known authentic fatty acid standards purchased from Fluka. Fatty acids were expressed as weight percents.

Table 1. Composition (%) and nutritional value of the diets

Components	Groups			
	SBM	RC XY	RC FYT	RC XY+FYT
Wheat meal	62.0	56.2	56.2	56.2
Soybean meal	32.1	25.8	25.8	25.8
Rape cake of Kaszub cultivar	–	15	15	15
Rapeseed oil	2.6	-	-	-
L-lysine (99%)	0.1	0.1	0.1	0.1
DL-methionine (99%)	0.15	0.1	0.1	0.1
Dicalcium-phosphate	1.4	1.35	1.35	1.35
Limestone	0.8	0.6	0.6	0.6
Salt	0.35	0.35	0.35	0.35
Premix DKA*	0.5	0.5	0.5	0.5
Calculated nutritive value of 1kg:				
– metabolizable energy,MJ	11.7	11.6	11.6	11.6
– crude protein,%	20.50	20.80	20.80	20.80
– crude fibre,%	4.3	5.3	5.3	5.3
– lysine,%	1.09	1.1	1.1	1.1
– methionine,%	0.48	0.45	0.45	0.45
– total Ca ,%	0.88	0.87	0.87	0.87
– available P,%	0.36	0.37	0.37	0.37
– Na,%	0.16	0.16	0.16	0.16
Enzyme preparation	-	XY	FYT	XY+FYT

Enzyme preparation XY was added at a quantity of 0.4 $g \cdot kg^{-1}$ of diet, enzyme preparation FYT – 0.35$g \cdot kg^{-1}$

*-per 1kg – Vit. A (4 000 000 j.m.); D_3 (600 000 j.m.; E (16 g); K_3 (0.6 g); B_1 (0.5 g); B_2 (1.75 g); B_6 (1.0 g); B_{12} (0.0048 g); Biotin (0.04 g); Nicotinic acid (10 g); Folic acid (0.3 g); Choline (100 g); Fe (16 g); Cu (1.8 g); Mn (16 g); Zn (14 g); Co (0.06 g); I (0.25 g); Se (0.055 g); Ca (192 g); Maxus Elanco (1.8 g); Salinomycin (12 g); BHT+Ethoxyquin (1.0 g)

Coefficients of apparent digestibility for dry matter, crude protein, crude ash, crude fat, crude fibre and nitrogen-free extracts were calculated following the total collection method:

$$AD_{nutrient} = 100 (Q_{feed} \times N_{feed} - Q_{faeces} \times N_{faeces}) / Q_{feed} \times N_{feed}$$

where:
$AD_{nutrient}$ = apparent digestibility coefficient of the nutrient (%),
Q_{feed} = quantity of ingested feed (g),
N_{feed} = dietary concentration of the nutrient (%),
Q_{faeces} = quantity of faeces produced (g),
N_{feacal} = concentration of the nutrient (%).

The data obtained was statistically analyzed by analysis of variance. Differences between means were tested by Duncan's multiple range test.

RESULTS AND DISCUSSION

The chemical composition of Kaszub rapeseeds is shown in Table 2. Protein and crude fibre contents were lower compared with other double-zero cultivars (Banaszkiewicz, 1999), however, crude fat content was higher and approached the content of the traditional Górczanski cultivar (Kinal and Króliczek, 1981). The content of saturated (5.05%) and unsaturated (94.78%) fatty acids in the lipid fraction of this cultivar was higher than in other double zero cultivars (Banaszkiewicz, 2000). Korol *et al.* (1994) recorded some differences, especially in palmitic, oleic, eicosenoic and erucic acid contents in rapeseeds. The total glucosinolate content (14.5 μmol g^{-1}) did not exceed 25 μmol·g^{-1}, which confirmed that this cultivar was a double-zero one. The chemical composition of the rape cakes from Kaszub cultivar was as follows: dry matter (95.42%); crude ash (5.36%); crude protein (22.18%); crude fat (27.59%); crude fibre (12.27%) and crude phosphorus (0.984%). The content of basic nutrients (except crude fat) was similar to the content specified by the Poultry Feeding Standards (2005). Podkówka *et al.* (2006) reported the variability of nutrients in rapeseed cakes, crude fat in particular.

Results of body gain and feed efficiency of broilers are summarized in Table 3. Body weight and body weight gain results of chickens fed rape diet at 21 day were not lower than for the control group. Body weight values at 21 days of age ranged from 672 g to 692 g whereas body weight gain values over the period from day 1 to 21 ranged from 628 g (chickens fed the diet containing rape cake with xylanase and phytase preparations) to 649 g (group fed the diet containing rape cake with xylanase preparation) and were not significantly different between treatments. Weight gain per chick was not significantly reduced up to the level of 15% rapeseed meal in the diet in the experiment conducted by Zeb *et al.* (1999), whereas feed to gain ratio was lowest for 5% rapeseed meal.

Simultaneous application of phytase and xylanase in broiler wheat-rape-soybean diet had no significant effect on body weight gains compared with the group where phytase and xylanase were applied separately.

The feed conversion ratio (FCR) ranged from 1.55 kg/kg body weight gain (soybean group) to 1.67 kg/kg (group fed the diet containing rape cake supplemented in the phytase preparation) and were statistically different. The FCR in group RC XY (xylanase) and RC XY+FYT (xylanase + phytase) was similar to the control group whereas for group RC FYT (phytase) it was statistically higher.

Kocher *et al.* (2001) showed that canola meal can be included in broiler diets to replace soybean meal without loss in bird performance. Moreover, an addition of commercial feed enzymes A (Roxazyme G) or B (Ronozyme VP) in the respective amounts of 0.15 g kg^{-1} and 0.4 g kg^{-1} to sorghum-canola meal diets had no significant effect on growth, feed intake or FCR. Rassmussen and Petterson (1997) and Guenter *et al.* (1998) showed that supplementation of canola-soybean diets with carbohydrase preparations significantly improved weight gain. However, the above studies used an energy and protein-deficient control diet. According to Sliwinski *et al.* (2000), the combined application of 0.01% xylanase and 0.001% glucanase in triticale mixtures was effective and improved chicken body weight gain, but the result depended on the enzyme and substrate.

Table 2. Chemical composition of Kaszub rapeseed

Specification	Content in rapeseed
Dry matter, $g \cdot kg^{-1}$	937.4
Crude ash, $g \cdot kg^{-1}$	41.5
Crude protein, $g \cdot kg^{-1}$	161.9
Crude fat, $g \cdot kg^{-1}$	441.7
Crude fibre, $g \cdot kg^{-1}$	53.3
N-free extracts, $g \cdot kg^{-1}$	231.9
Crude phosphorus, $g \cdot kg^{-1}$	77.4
Gross energy, $MJ \cdot kg^{-1}$	28.04
Fatty acids (% of total acids) $C_{16:0}$ $C_{18:1}$ $C_{18:2}$ $C_{18:3}$ $C_{22:1}$ – saturated – unsaturated – monounsaturated	 3.48 69.84 16.97 6.86 0.15 5.05 94.78 70.95
Glucosinolates, $\mu mol \cdot g^{-1}$ progoitryn gluconapin glucobrassicanapin napoleiferyn 4-OH-glucobrassicin total glucosinolates alkenyl glucosinolates	 6.6 3.8 0.8 0.2 3.0 14.5 11.4

Table 3. Body weight gain and feed efficiency of broilers (growth trial)

Specification	Groups				SEM
	(SBM)	(RC XY)	(RC FYT)	(RCXY+FYT)	
Body weight at 1 day, g	40	40	41	40.5	1.92
Body weight at 21 day, g	672	692	679	672	9.92
Body weight gain 1-21days, g	631	649	633	628	11.27
Feed/gain ratio, kg/kg 1-21 day	1.55^{a}	1.59^{a}	1.67^{b}	1.62^{ab}	0.02

^{a,b} – means in rows followed by different letters are significantly different ($p \leq 0.05$)
SEM – pooled standard error of mean

Numbers of lactic acid bacteria in the ileum and caecum of 21-day-old broiler chickens are shown in Table 4.

Table 4. Colony forming units of *Lactobacillus* (log CFU·g^{-1}) of intestinal content in broiler chickens

Specification	Groups				SEM
	(SBM)	(RC XY)	(RC FYT)	(RC XY+FYT)	
Ileum	6.43^{b}	7.44^{a}	6.58^{b}	6.60^{b}	0.13
Caecum	8.44^{b}	9.74^{a}	8.19^{b}	8.27^{b}	0.07

^{a,b} – means in rows followed by different letters are significantly different ($p \leq 0.05$)
SEM – pooled standard error of mean

Addition of phytase to diets containing glucanase and xylanase was as effective as xylanase in improving the performance of broiler chickens fed wheat-based diets containing adequate P levels (Wu *et al.,* 2003). Addition of phytase or xylanase, or simultaneous application of these enzymes in low P wheat-soybean broiler diets from day 21 to 25 had no significant effect on the analyzed parameters, except for total phosphorus retention. However, no negative interaction between phytase and glycosidase enzymes were found, which indicates that both enzymes may be used together in wheat-soybean-based diets (Juanpere *et al.,* 2005).

The number of lactic acid bacteria in the caecum was from 8.19 to 9.74 log cfu x g^{-1}. There was a statistical difference for lactic acid bacteria number in the caecum between group RC XY and the remaining groups. The highest ($p<0.01$) total lactic acid bacteria number was observed in the caecum of broilers fed wheat-soybean-rape diets supplemented with the xylanase preparation (group RC XY). The number of lactic acid bacteria in the caecum of chickens of the group fed diets supplemented with both xylanase and phytase (RC XY+FYT) was similar to the control and the remaining rapeseed groups.

The number of lactic acid bacteria in the ileum was from 6.43 to 7.44 log cfu x g^{-1}. The dependence referring to the amount of *Lactobacilli* in the ileum was similar to the number in the caecum. Introduction of phytase to the diet containing xylanase gave similar results in terms of performance and number of lactic acid bacteria. Mathlouti *et al*. (2002) did not ascertain any significant differences in the number of *Lactobacilli* when xylanase and β-glucanase were added to wheat and barley-based diets containing soybean meal.

The digestibility of protein in the control diet SBM (Table 5) was 88.2%, and in rape cake diets it ranged from 85.82 (phytase group) to 87.92% (xylanase group). The differences obtained between groups were significant. The fat digestibility was from 65.89% to 87.43% and significant differences between groups were found. The highest digestibility was found in the control group whereas the lowest was the digestibility in the groups fed rape cake diets supplemented with phytase preparation. Simultaneous application of phytase and xylanase to broiler wheat-rape diets only slightly enhanced fat and protein digestibility compared with the group where only phytase was applied (Table 5). Partial replacement of soybean meal with rape cake and enzyme supplementation had a significant effect on the digestibility of crude fibre and N-free extracts (Table 5). Crude fibre digestibility in the diets containing rape cake and supplemented with enzyme preparations was higher compared with the control diet, however the digestibility of N-free-extracts in the wheat-soybean diet was the highest. Results of the study conducted by Kocher *et al*. (2000) indicated that commercial enzyme products had some effects when applied in diets containing high concentrations of canola meal, however these effects could only be seen after detailed analyses and did not result in significant improvement of broiler growth performance. Partial replacement of soybean meal with rape cake supplemented with an enzyme preparation individually or in combination had not statistical effects on crude phosphorus digestibility.

Table 5. Apparent digestibility of nutrients in experimental diets (digestibility trial)

Groups	Digestibility, %				
	Crude protein	Crude fat	Crude fibre	N-free extracts	Crude phosphorus
(SBM)	88.20^{a}	87.43^{a}	18.06^{c}	82.4^{a}	57.93^{a}
(RC XY)	87.92^{ab}	71.24^{b}	42.76^{a}	79.15^{b}	54.18^{a}
(RC FYT)	85.82^{c}	65.89^{c}	37.67^{b}	76.48^{c}	50.10^{a}
(RC XY+FYT)	86.30^{bc}	67.24^{c}	46.58^{a}	79.49^{b}	55.91^{a}
SEM	0.37	2.28	2.90	0.63	1.25

^{a,b,c} – means in columns followed by different letters are significantly different ($p \leq 0.05$)
SEM – pooled standard error of mean

Smulikowska *et al.* (2006) showed there was not significant interaction between an effect of rape cakes and phytase supplementation. Similarly,

Juanpere *et al.* (2005) did not observe a phytase effect on apparent metabolizable energy (AME), dry matter, lipid and starch digestibility in wheat-based diets whereas xylanase improved dry matter and starch digestibility.

The results of the present study showed that rape cakes obtained from Kaszub cultivar supplemented with enzyme products could partially replace soybean meal in wheat-based diets without any detrimental effects on body weight gain in the first 3 weeks of life. The number of Lactic acid bacteria in the caecum of 3-week-old broiler chickens was higher than in the ileum and depended on type of enzyme preparations which were added. Simultaneous application of xylanase and phytase to a wheat-soybean-rape diet increased digestibility of crude protein, fat, crude fibre and N-free extractives in comparison with a diet in which phytase was added separately.

The data obtained in this study showed that a part of soybean meal could be replaced by 15% rape cakes of Kaszub cultivars supplemented with xylanase or phytase preparations individually or in combination in broiler starter diets. However, the best results (compared with the control group) were obtained for the diet supplemented with xylanase when added separately.

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UTICAJ VEĆIH KOLIČINA REPINE POGAČE SUPLEMENTIRANE ENZIMIMA NA HRANLJIVU VREDNOST OBROKA ZA BROJLERE I BROJ MLEČNO-KISELINSKIH BAKTERIJA U CREVIMA

BANASZKIEWICZ TERESA, BORKOWSKA KAROLINA i KOT BARBARA

SADRŽAJ

Ova ispitivanja su sprovedena sa ciljem da se utvrdi uticaj delimične zamene sojinog brašna repinom pogačom sa dodatkom enzima, na hranljivu vrednost obroka i broj mlečno-kiselinskih bakterija u ileumu i cekumu brojlera. Prvi ogled (ispitivanje prirasta) je izveden na 120 jednodnevnih brojlera hibrida Ross 308 koji su bili podeljeni u četiri jednake grupe od po 30 ptica (15 kokica I 15 petlića). Svaki tretman se sastojao od šest ponavljanja sa po 5 ptica. Kontrolni obrok je sadržavao sojino brašno dok je u oglednim grupama ovaj sastojak bio zamenjen sa 15% repine pogače uz dodatak ksilanaze ili fitaze (pojedinačno ili u kombinaciji). Nakon tri nedelje (21. dan), žrtvovano je po šest ptica iz svake grupe radi uzorkovanja sadržaja ileuma i cekuma I određivanja broja mlečno-kiselinskih bakterija.

Drugi ogled (ispitivanje svarljivosti) je izveden na 60 brojlera u uzrastu od sedam dana, podeljenih u četiri grupe od po 20 ptica. U svakoj grupi je bilo četriri ponavljanja sa po pet ptica i test svarljivosti je izvođen metodom totalne kolekcije.

Uključivanje 15 % repine pogače sa dodatkom enzima u cilju zamene sojinog brašna nije smanjilo prirast i unos hrane. Konverzija hrane je bila najveća u grupi kojoj je dodavana samo fitaza dok je kod jedinki hranjenih obrocima sa ksilanazom i fitazom bila značajno povećana svarljivost sirovih vlakana ($p<0,05$) i bezazotnih materija. Broj mlečno-kiselinskih bakterija je bio najveći u cekumima i ileumu brojlera hranjenih repinom pogačom sa dodatkom ksilanaze, dok kombinacija ksilanaze sa fitazom nije uticala na ovaj parametar.