Effect of Xylanase Application to Rapeseed Cake Diet on Digestibility and Deposition of Nutrients and Energy in Young Broiler Chickens

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Abstract: Two experiments were conducted to study the effect of rapeseed cake and xylanase application on the digestibility and deposition of nutrients and energy in young broiler chickens. Experiment 1 was carried out on broiler chickens between the ages of 1 to 21 days, which were divided into three groups. The control diet contained soybean meal, whereas in the experimental diets a part of the soybean meal was replaced by 15% of rape cakes. Furthermore, an exogenous enzyme preparation containing xylanase in the amount of 0.2g kg^{-1} was added to one of the two rapeseed cake diets. Seven 1-day-old chickens at the beginning of the experiment and 6 chickens on day 21 of the experiment were slaughtered in each group and the chemical composition of their bodies was analyzed. In experiment 2, a digestibility test of the diets from experiment 1 was carried out on 2-week-old broiler chickens. The application of 15 % rapeseed cakes and the addition of the enzyme preparation did not significantly influence body weight, feed intake, the chemical composition or the energy content in the chickens' bodies. Differences in the digestibility of the dry matter , ether extract , and crude phosphorus of the diets were statistically significant. The highest digestibility of dry matter, ether extract, and crude phosphorus($P<0.05$) was found in the control group. Application of xylanase into the diet containing rapeseed cake increased the digestibility of nutrients, but the differences obtained between the groups were not significant.

Keywords: Rapeseed cakes, enzyme preparation, broilers, apparent digestibility, carcass retention.

INTRODUCTION

Soybean meal (SBM) is the major source of protein in poultry feeding. As soybeans are only grown in North and South America, most countries have to import it. One alternative to soybean might be rapeseed. Banaszkiewicz stated that rapeseed was characterized by a high amount of protein (from 21 to 22%), fat (above 40%), total phosphorus (from 6 to 8 $g \cdot kg^{-1}$) and gross energy content (from 26 to 27 $MJ \cdot kg^{-1}$) [1]. The amino acid composition of rapeseed protein is beneficial, with 6 g Lys, 2 g Met, 4.6 g Thr and 1.2 g per 16g N of tryptophan [1], but the digestibility of some amino acids in rapeseed meal was lower than that of SBM [2]. The protein digestibility of rapeseed and rapeseed cake for poultry amounted from 60 to 70% [3], whereas the fat digestibility of rapeseed and rapeseed cake amounted from 60 to 70% and 80% respectively [4]. Rapeseed and rapeseed cake contain oil that is rich in oleic acid. A study by Kocher *et al*. showed that rapeseed meal could replace SBM in broiler diets even at high levels without any loss in animal performance [5]. According to Bell, replacing SBM with rapeseed meal increased the level of indigestible carbohydrates compared to SBM [6]. Rapeseed products are limited in their usability by having nutritionally unfavourable substances such as glucosinolates, sinapine, tannin and phytate [7], as well as by having a high dietary fiber and non starch polysaccharide (NSP) content [5]. Rapeseed products contain about 200g of total NSP [8] and have a negative effect on nutrient digestibility, mainly on crude fat and amino acids. The NSPs, which include cellulose, β –glucans, arabinoxylans and pectins may increase digesta viscosity and decrease of nutrient digestibility [8]. The presence of NSP may adversely affect the performance of broiler chickens fed diets with high levels of rapeseed oil meal [9]. The nutritive value of rapeseed oil meal may be improved by the application of enzymes [10]. Supplementation of diets with enzyme preparations can degrade dietary fiber and improve digestibility of crude fat and protein [11]. The addition of xylanase to broiler and pig diets caused decomposition of arabinoxylans. The nutritive value of broiler diets containing Chinese rapeseed meal was improved by an appropriate level of xylanase used as a basic enzyme [12], and the addition of xylanase at the level of 0.70 g kg^{-1} to diets containing high proportions of Chinese double-low rapeseed meal significantly increased the daily gain of the pigs and the dry matter (DM) and crude protein (CP) digestibility [13]. The production technology of rapeseed cakes is more environmentally friendly than that of rapeseed meal, which is often used in animal production. The enzyme preparations were mainly added to wheat- soybean and maize- soybean diets. There are only a few studies in which enzyme preparations were added to wheat-

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rapeseed cake diets. In those studies, the effect of the partial substitution of the soybean meal by high levels of rapeseed cake supplemented with xylanase on the digestibility and carcass deposition of nutrients and energy in young broiler chickens were investigated.

MATERIALS AND METHODS

At the beginning of the experiment 115 day old Ross 308 female broiler chickens were weighed and 7 chickens of average body weight (BW) were slaughtered by cervical dislocation and dried. This group was labeled “0” and the bodies of these chickens were analyzed for gross energy and basic nutrient content. The remaining 108 one-day-old broiler chickens were randomly divided into 3 groups, of 36 birds per group. Each group consisted of 6 replicates of 6 birds. The birds were housed in metabolic cages. For the first 5 days the temperature in the poultry house was 31^{o}C and then it was gradually lowered according to the recommendations of the supplier. The control diet (C) contained SBM. For the 2 remaining experimental diets (RC), rapeseed cakes of the Kana cultivar were introduced at a level of 15% (Table **1**), and for 1 of these 2 experimental rapeseed cake diets (RC+A), an exogenous enzyme preparation containing xylanase was added at the level of 0.2 g kg^{-1}. The rapeseed cakes used in this study were obtained in an oil mill by pressing Kana cultivar rapeseed to 400 kg cm^{-2}. The enzyme preparation was derived from *Aspergillus oryzae* and contained endo- 1,4-β-xylanase (min.1000 FXU(W) g^{-1}). The birds were fed the isonitrogenic experimental diets from day 1 to day 21 of life. The diets were in a mash form and they were given *ad libitum*. The diets were analyzed for basic nutrients and gross energy contents.

At the age of 21days, the birds were weighed individually, and six birds from each group, with a BW similar to the average in the group were slaughtered and dried. The chicken' bodies were analyzed for basic nutrients and gross energy contents. During the experiment, the feed intake was recorded in each replication.

Experiment 2 (the digestibility test) was carried out on 60 Ross 308 broiler chickens, 2 weeks old, which were randomly divided into 3 groups, each with 4 replications of 5 birds. The aim of the digestibility test

Table 1: Composition of Experimental Diets, %

Ingredients	Experimental diets (groups)	
	C	RC
Wheat meal (11.90%)	64.0	61.0
Soybean meal (38.5%)	31.1	20.75
Rape cake from Kana cv.	-	15.0
Rapeseed oil	1.15	-
L-lysine (99%)	0.15	0.17
DL-methionine(99%)	0.15	0.13
NaCl	0.35	0.35
Limestone	0.9	0.6
Calcium phosphate	1.7	1.5
Premix*	0.5	0.5
Calculated nutritive value of 1 kg:		
-metabolizable energy, MJ·kg^{-1}	11.43	11.60
-crude protein,% (analyzed)	19.68	19.58
-lysine,%	1.12	1.11
-methionine,%	0.44	0.44
- total Ca,%	1.16	1.05
- available P,%	0.39	0.37
- Na,%	0.16	0.16

*-per 1kg-Vit.A(4.000.000j.m.); D_3(600.000j.m.; E (16 g); K_3(0,6g); B_1(0,5g); B_2(1,75g); B_6(1,0g); B_{12}(0,0048g); Biotin (0,04g); Nicotinic acid (10g); Folic acid (0,3g); Choline (100g); Fe (16g); Cu(1,8g); Mn(16g); Zn(14g); Co (0,06g); I(0,25g); Se(0,055g); Ca(192g); Maxus Elanco (1,8g);Salinomycin (12g); BHT+Ethoxyquin(1,0g).

was to estimate the digestibility of the nutrients in the diets used in experiment 1. The digestibility was evaluated using the total collection method. The digestibility test included an adaptation period of 4 days and a collection period of 3 days. Excreta were collected separately from each pen and the feed intake was measured on a per-pen basis. The excreta were dried in an oven at 60^{o}C, weighed, ground, and stores for analysis [14].

The rapeseed cakes, chicken' bodies, and excreta samples were analyzed for basic nutrient and gross energy content using an AOAC procedure [15], and N-free extracts were also calculated. The crude phosphorus content was determined colorimetrically with eikonogen as a reductive indicator. The glucosinolates in the rapeseed were measured using high- performance liquid chromatography according to the ISO-9167 standard [16]. The gross energy content was determined using an adiabatic bomb calorimeter (KL-10, Precyzja, Bydgoszcz, Poland). The urine nitrogen content in the excreta was determined using the uranyl acetate method [17]. Rapeseed samples were extracted in order to analyze fatty acids; this fat was hydrolyzed by means of 0.5 M KOH in methanol and esterified with 5% HCL in methanol [18]. Next, 0.2 mL of hexane was added, and fatty acid methyl esters were separated and quantified using the gas chromatography apparatus (CHROM 5, Laboratorni Pristroje, Prague. Czech Republik) equipped with a 2.5- m column, a flame ionization detector, and an integrator. The identification of fatty acid peaks was carried out by comparison with retention times of authentic fatty acid standards purchased from Fluka.

The apparent digestibility of nutrients was calculated following the total collection method using the following equation [19]:

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

where $AD_{nutrient}$ is the apparent digestibility coefficient of the nutrient (%) , Q_{feed} is the quantity of ingested feed (g), N_{feed} is the dietary concentration of the nutrient (%), Q_{feces} is the quantity of feces produced (g), and N_{feces} is the concentration of the nutrient (%).

The data obtained were statistically tested using analysis of variance. When the differences between means were significant at $P< 0.05$, Duncan's multiple range test was used. Statistica 8 was used for statistical analyses.

RESULTS AND DISCUSSION

The rapeseed cakes obtained by pressing the Kana cultivar contained 25.61% crude protein (CP), 28.67% ether extract (EE) and 10.65% crude fiber (CF). The saturated fatty acids were composed of 3.65% of total lipid and unsaturated fatty acids amounted to 96.31% (Table **2**). An erucic acid level of 0.01% was identified in the fatty acid composition. The total glucosinolate in the rapeseed was 13.0 $\mu mol \cdot g^{-1}$ and the alkenyl glucosinolates was 8.8 $\mu mol \cdot g^{-1}$. A high amount of progoitrin (5.7 $\mu mol \cdot g^{-1}$) was found. Except for EE, the contents of basic nutrients in Kana cultivar rapeseed cakes were similar to the content specified by the NRC [20] and their chemical composition was similar to other double-zero cultivars [21]. The Kana cultivar rapeseed cakes contained 286.7 g kg^{-1} of ether extract which was higher than that of the rapeseed cakes evaluated by

Table 2: Chemical Composition of Rape Cake of Kana Cultivar

Specification	Content
Dry matter, $g \cdot kg^{-1}$	953.7
Crude ash, $g \cdot kg^{-1}$	52.5
Crude protein, $g \cdot kg^{-1}$	256.1
Crude fat, $g \cdot kg^{-1}$	286.7
Crude fiber, $g \cdot kg^{-1}$	106.5
N-free-extracts, $g \cdot kg^{-1}$	251.9
Crude phosphorus, $g \cdot kg^{-1}$	85.2
Gross energy, $MJ \cdot kg^{-1}$	24.26
Fatty acids (% of total acids)	
$C_{16:0}$	2.74
$C_{18:1\ n-9}$	75.53
$C_{18:2\ n-6}$	14.62
$C_{18:3\ n-3}$	5.55
$C_{22:1\ n-9}$	0.01
-saturated	3.65
- unsaturated	96.31
-monounsaturated	76.14
Glucosinolates, $\mu mol \cdot g^{-1}$	
progoitryn	5.7
gluconapin	2.6
glucobrassicanapin	0.4
napoleiferyn	0.1
4-OH-glucobrassicin	4.0
total glucosinolates	13.0
alkenyl glucosinolates	8.8

Table 3: Chemical Composition and Gross Energy Content in Body of Broiler Chickens

Age	Group	Dry matter, %	Crude ash, %	Crude protein, %	Crude fat, %	Crude phosphorus, g·kg^{-1}	Gross energy, MJ·kg^{-1}
1-day	"0"	24.66^{a}	1.79^{a}	15.57^{b}	6.79^{a}	2.70^{b}	5.61^{b}
SEM		0.44	0.03	0.26	0.31	0.04	0.14
21-days	C	31.14^{b}	2.70^{a}	19.03^{a}	8.41^{a}	4.83^{a}	8.11^{a}
	RC	29.95^{b}	2.73^{a}	19.15^{a}	7.05^{a}	4.76^{a}	7.50^{a}
	RC+A	29.29^{b}	2.53^{a}	18.28^{ab}	7.42^{a}	4.49^{a}	7.87^{a}
SEM		1.49	0.14	0.99	0.67	0.27	0.46

"0"- one-day-old broilers chickens on beginning experiment.
C-control group fed wheat-soybean diet without rape cake.
RC-group fed diet with 15% of rape cake.
RC+A-group fed diet with 15% of rape cake supplemented of xylanase.
^{a,b}means in columns followed by different letters are significantly different (p≤ 0.05).
SEM- pooled standard error of mean.

Smulikowska *et al*. due to the kind of press used to obtain the oil [22]. Podkówka *et al*. reported that the variability of nutrients in rapeseed cakes [23], and of EE in particular, depended on the technology used for expression. The content of saturated and unsaturated fatty acids in a lipid fraction of the Kana cultivar was higher than in other double-zero cultivars [24]. Korol *et al*. presented some differences [24], especially in relation to palmitic, oleic, eicosenoic and erucic acid contents, in rapeseeds. Total glucosinolates (13 μmol·g^{-1}) did not exceed 25 μmol·g^{-1}, which confirmed that Kana was a double-zero cultivar. In the domestic and foreign rapeseeds analysed by Łukaszewski and Petkov [25] the content of glucosinolates ranged from 7.96 to 32.05 μmol·g^{-1}.

The results of the chemical composition analyses of the broiler' bodies on days 1 and 21 of life are presented in Table **3**. The dry matter, crude protein, crude phosphorus and energy content in the 1-day-old chicken bodies were significantly lower (P< 0.05), than those of the 21-day-old broilers. The ether extract and crude ash contents were also lower, but these differences were not significant. On the other hand, the nutrient and energy contents of the 21-day-old chickens' bodies did not differ significantly between treatments (Table **3**).

The BWs of the broiler chickens at 21 days ranged from 481 to 529 g and feed intake from 1to 21day ranged from 790 to 831 g per bird (Table **4**); these were similar across the groups. Zeb *et al*. ascertained that weight gain was not significantly lower in chickens fed diets containing up to 15% rapeseed meal [26]. Kocher *et al.* showed that the canola meal could be introduced into broiler diets instead of SBM without loss

Table 4: Body Weight, Feed Intake and Degree of Deposition of Dry Matter, Crude Protein, Crude Fat, Crude Phosphorus and Energy in Broiler Chickens

Specification	Experimental groups			
	C	RC	RC+A	SEM
Body weight of chickens on first day of life, g	39^{a}	39^{a}	39^{a}	0.63
Body weight of chickens on 21day of life, g	505^{a}	481^{a}	529^{a}	16.07
Feed intake, g	790^{a}	794^{a}	831^{a}	33.1
Deposition in chickens body, % of intake:				
- dry matter	17.94^{a}	14.35^{a}	14.33^{a}	1.44
- crude protein	48.31^{a}	39.85^{a}	40.62^{a}	3.82
- crude fat	126.77^{a}	58.04^{b}	48.75^{b}	18.54
- crude phosphorus	33.10^{a}	28.88^{a}	27.68^{a}	2.57
- energy	25.35^{a}	18.11^{a}	18.25^{a}	2.25

C -control group fed wheat-soybean diet without rape cake.
RC-group fed diet with 15% of rape cake.
RC+A-group fed diet with 15% of rape cake supplemented of xylanase.
^{a,b}means in the rows marked with different letters are significantly different at p≤ 0.05.
SEM- pooled standard error of mean.

of bird performance [10]. Kocher *et al*. stated that the application of the commercial feed Roxazyme G at a quantity of 0.15 kg^{-1} or Ronozyme VP at 0.4 kg^{-1} to sorghum-canola meal diets had no significant effects on the growth [10], feed intake or feed conversion ratio, whereas Rassmussen and Petterson and Guenter *et al*. showed that the addition carbohydrase preparations to a canola-soybean diet significantly improved weight gain, but the control diets used in those studies were deficient in energy and protein [19, 27]. According to Śliwiński *et al*., the total application of xylanase at a level of 0.01% and glucanase 0.001% to triticale mixtures was effective and improved chicken BW gain [28], but results depended on the type and amount of enzyme and substratum.

The degree of deposition of nutrients and energy in the chickens is shown in Table **4**. The highest depositions of nutrients and energy compared to quantity consumed were found in the control group. The obtained results for DM, CP, crude phosphorus and energy from the control group were not significantly different compared to the other groups. Deposition of the nutrients and energy in chickens from the groups fed diets containing rapeseed cakes was only a little lower than in the control group. Dry matter, crude protein, crude phosphorus and energy in the broilers bodies from these groups were deposited similarly to in the control group. There were no significant differences in nutrient and energy deposition between the groups fed a diet containing rapeseed cake with and without the xylanase enzyme preparation. The significant differences between the groups were related to the amount of EE ($P < 0.05$). The highest deposition of crude fat compared to the quantity consumed was found in the control group. The degree of deposition of crude protein in the chickens' bodies in this experiment was on the level from 39.85 to 48.31%. The degree of deposition of crude protein from a wheat- rapeseed diet supplemented with amino acids or phytase preparation was on the level of about 40% [29, 30]. The phytase application to rape cake diet with contained less P and Ca significantly improved the deposition of total phosphorus in chickens bodies at 1to 21 days of age [30]. The main factor that may decrease protein utilization from rapeseed cake was the glucosinolate content [22]. The present results show that the degree of deposition of fat differed significantly between groups, and the highest deposition of fat in the chickens' bodies was found in the control group. The digestibility of fiber components of rapeseed products maybe factor responsible for depressing availability nutrients and ME value. Słomiński and Campbell proved that the digestibility of NSP fraction of canola meal is very low [31].

The digestibility coefficients are shown in Table **5**. The differences in the digestibility of DM, EE and crude phosphorus of the diets were statistically significant (Table **5**). The highest digestibility of DM, EE and crude phosphorus ($P < 0.05$) was found in the control group. There was not a significant increase in the digestibility of the supplemented diet containing the rapeseed cakes with the xylanase enzyme preparation. This shows that the digestibility of the nutrients in the diet containing rapeseed cake supplemented with enzyme preparation was similar to that of the diet without xylanase (Table **5**), but a tendency for improved nutrient digestibility as a result of xylanase addition was observed. The digestibility of DM (from 71.82% to 76.69%), EE (from 72.35% to 89.12%) and crude phosphorus (from 60.05% to 70.98%) in this study was significantly different between groups. The highest digestibility of nutrients was found in the control (wheat-soy diet) group. Supplementing the wheat-soy-rapeseed cake diet with xylanase did not significantly improve the digestibility of nutrients in comparison to the diet without xylanase, but it was a little higher.

Table 5: Apparent Digestibility of Nutrients in Experimental Diets

Groups	Apparent digestibility coefficients, %				
	Dry matter	**Crude protein**	**Crude fat**	**N-free-extracts**	**Crude phosphorus**
C	76.69^{a}	88.40^{a}	89.12^{A}	76.61^{a}	70.98^{a}
RC	71.82^{b}	83.72^{a}	72.35^{B}	66.19^{a}	60.05^{b}
RC+A	73.26^{b}	86.80^{a}	73.12^{B}	73.86^{a}	61.76^{b}
SEM	3.73	1.64	5.36	3.67	5.75

C -control group fed wheat-soybean diet without rape cake.
RC-group fed diet with 15% of rape cake.
RC+A-group fed diet with 15% of rape cake supplemented of xylanase.
^{a,b}.. – means in columns followed by different letters are significantly different (p≤ 0.05).
^{A,B}.. - means in columns followed by different letters are significantly different (p≤ 0.01).
SEM- pooled standard error of mean.

Juanpere *et al*. observed the improvement of DM and starch digestibility in wheat-based diets when xylanase was added to the diets. Simultaneous application of phytase and xylanase had no significant influence on apparent digestibility of nutrients at broilers' wheat – rapeseed cake diet [32, 33].

Results of a study conducted by Kocher *et al*. indicated that commercial enzyme preparations had some effects when they were applied in diets containing a high concentration of canola meal [5]. These effects could only be seen after detailed analyses and did not result in significant improvement of broiler growth performance.

CONCLUSIONS

15% of Kana cultivar rapeseed cakes supplemented with xylanase enzyme preparation could replace part of the SBM in wheat diets without any detrimental effects on BW gain in the first 3 weeks of life, however, deposition of fat was severely degraded by this treatment and digestibility of diet was also lowered.

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