The effect of raw and fermented rapeseed cake on the metabolic parameters, immune status, and intestinal morphology of turkeys

Aleksandra Drażbo,[*,1] Katarzyna Ognik,[†] Anita Zaworska,[‡] Karolina Ferenc,[§] and Jan Jankowski[*]

[*]*Department of Poultry Science, University of Warmia and Mazury in Olsztyn, 10-719, Olsztyn, Warmińsko-Mazurskie, Poland;* [†]*Department of Biochemistry and Toxicology, University of Live Science, 20-950, Lublin, Lubelskie, Poland;* [‡]*Department of Animal Nutrition and Feed Management, Faculty of Veterinary Medicine and Animal Science, Uniwersytet Przyrodniczy w Poznaniu, 60-637, Poznań, Wielkopolskie, Poland; and* [§]*Department of Physiological Sciences, Warsaw University of Life Sciences, Nowoursynowska 100, 02–797, Warsaw, Mazowieckie, Poland*

ABSTRACT This study evaluated the effects of including 15% of raw or fermented rapeseed cake (RRC and FRC, respectively) in turkey diets on the growth performance, antioxidant and immune status, and intestinal morphology of birds. Rapeseed cake was fermented using the commercial 6-phytase enzyme preparation, and dried. A total of 1,350 day-old female Hybrid Converter turkeys were randomly allocated to 3 dietary treatments with 9 replicates per treatment and 50 birds per replicate. All diets were isonitrogenous and isocaloric, and contained various protein sources. In the control group (C), soybean meal was the main source of dietary protein, and the remaining groups were fed diets containing 15% of RRC or FRC. Fermentation considerably reduced the concentrations of phytate-phosphorus and glucosinolates in rapeseed cake. In comparison with RRC, turkeys receiving FRC achieved higher average final body weight (BW), comparable with that noted in the control group. Both RRC and FRC stimulated the antioxidant system of turkeys, which was reflected in a decrease in the concentrations of lipid hydroperoxides (LOOH) and malondialdehyde (MDA), and an increase in the total antioxidant potential (FRAP) and the concentration of total glutathione (GSH + GSSG) in blood plasma, compared with the control group. Turkeys fed diets with RRC were characterized by the highest blood vitamin C concentrations, the highest activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), and lower catalase (CAT) activity. The dietary inclusion of rapeseed cake contributed to an increase in villus height and mucosal thickness in the duodenum, and a more beneficial influence was exerted by RRC. It can be concluded that the fermentation of rapeseed cake considerably reduces the concentrations of glucosinolates and phytate-phosphorus, and increases the final BW of turkeys. Diets containing 15% of RRC and FRC do not compromise metabolic parameters or immune function, and exert positive effects on antioxidant status and intestinal histomorphology in turkeys.

Key words: rapeseed cake, fermentation, redox status, intestinal morphology, turkey

2018 Poultry Science 0:1–11
http://dx.doi.org/10.3382/ps/pey250

INTRODUCTION

In modern poultry farming systems, balanced diets that meet the nutrient requirements of birds at each growth stage are essential to allow them achieve their full genetic potential. Particular attention has been paid to readily available protein sources that contribute to maximizing growth performance while maintaining good health. This is an important consideration since exposure to stressors under intensive production conditions may disturb the redox balance and induce generation of reactive oxygen species, leading to oxidative stress which has been implicated in the development of various diseases (Ognik and Wertelecki, 2012; Ognik and Krauze, 2016).

Rapeseed cake contains between 300 and 397 g of crude protein (**CP**) per kg of dry matter (**DM**) (Pastuszewska et al., 2003; Jakobsen et al., 2015), and it is rich in sulfur-containing amino acids and lysine (Mansour et al., 1993). According to Smulikowska et al. (2006), rapeseed cake is a more suitable component of broiler diets than solvent-extracted rapeseed meal (**RSM**) because the former has higher metabolizable energy value due to higher residual oil content. The production technology of rapeseed cake is also more environmentally friendly than that of RSM, which is often used in animal production (Fang et al., 2007). However, the use of rapeseed cake in poultry nutrition remains limited due to its high content of antinutritional factors

Received January 10, 2018.
Accepted May 25, 2018.
[1]Corresponding author: aleksandra.drazbo@uwm.edu.pl
This study was conducted under the Biostrateg program entitled "GUTFEED—innovative nutrition in sustainable poultry production" (Project No. 267659/7/NCBR/2015).

such as glucosinolates, phytic acid, sinapine, fiber, and tannins (Chibowska et al., 2000; Kocher et al., 2000). Rapeseed products contain approximately 200 g of total non-starch polysaccharides (**NSP**) (Knudsen, 1997) which negatively affect the bioavailability of nutrients, in particular protein, phosphorus and energy. Glucosinolates, hydrolyzed by the enzyme myrosinase, are the most toxic compounds in rapeseed. They induce generation of numerous undesirable compounds including isothiocyanates, which reduce feed intake resulting in decreased growth performance, affect liver and kidney functions, impair antioxidant capacity and immune function (Elangovan et al., 2001; Tripathi and Mishra, 2007; Hu et al., 2016; Shi et al., 2016). Various processing techniques are employed to lower the levels of antinutrional factors in RSM, including solvent extraction, chemical degradation, enzyme hydrolysis, and fermentation (Chabanon et al., 2007; Feng and Zuo, 2007; Gasiorek and Wilk, 2011). Most of the above methods have certain drawbacks such as loss of proteins, insufficient reduction of glucosinolates, high cost, and commercial infeasibility (Vig and Walia, 2001).

Fermentation improves feed quality by increasing protein availability, promoting the synthesis of vitamins and antioxidants, and reducing the content of undesirable compounds in feed ingredients (Kasprowicz-Potocka et al., 2015; Zaworska et al., 2016) including rapeseed (Bau et al., 1994; Zhang et al., 2006; Shi et al., 2016; Aljubori et al., 2017). According to Rozan et al. (1996), fermentation contributes to the degradation of 84% of carbohydrates, 30% of lignin, and 47% of total glucosinolates in RSM. Previous research (Feng et al., 2007; Xu et al., 2011) has shown that increased concentrations of biologically active compounds, including antioxidants, in fermented rapeseed products may contribute to an increase in immunoglobulin levels in poultry. Few studies have investigated the efficacy of fermented rapeseed products in broiler chicken and turkey nutrition. The experiments conducted to date revealed that fermented RSM improved the growth performance, serum biochemical parameters, and intestinal morphology of broilers (Feng et al., 2007; Chiang et al., 2010; Hu et al., 2016). Xu et al. (2012) demonstrated that diets containing 10% of fermented RSM as a substitute for soybean meal (**SBM**) in broiler diets had no adverse effects on the growth or health of birds. Xu et al. (2011) reported that SBM could be completely replaced with fermented RSM in duck diets.

In view of the above, the aim of this study was to verify the hypothesis postulating that the fermentation of rapeseed cake added at 15% to turkey diets has a beneficial influence on the growth performance, antioxidant, and immune status of birds.

MATERIALS AND METHODS

Rapeseed cake

Rapeseed cake was purchased on the domestic market, from the *Bielmar* Fat and Oil Processing Plant. It was ground and thoroughly mixed with water in a ratio of 1 : 2 in plastic containers. The fermentation of rapeseed cake was carried out using a commercial enzyme preparation of 6-phytase expressed in *Pichia pastoris*. The substrate was inoculated with enzymes (0.1% on a rapeseed cake weight basis) and mixed. Solid-state fermentation was conducted for 24 h at 30°C under anaerobic conditions. The enzymes were deactivated at 70°C within 15 min, and the fermented biomass was dried at 55°C. The amount of fermented rapeseed cake (**FRC**) was sufficient for the present experiment. The fermentation process was carried out under patent pending procedure No. 422,849.

Birds, management, and diets

The animal protocol used in this study was approved by the Local Ethics Committee (Olsztyn, Poland). The experiment was conducted at the Animal Research Laboratory in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes (OJEU, 2010). A total of 1,350 day-old female Hybrid Converted turkeys, obtained from a local commercial hatchery in Kętrzyn (Poland), were randomly allocated to 3 dietary treatments with 9 replicates per treatment and 50 birds per replicate. Turkeys were raised in pens on litter until 16 wk of age in a building with a controlled environment, and they had free access to feed and water. Indoor temperature and lighting programs were consistent with the recommendations for Hybrid Turkeys (2013).

During each of 4 feeding phases, birds were fed ad libitum isonitrogenous and isocaloric diets in pellet form, with various protein sources. In the control group (C), SBM was the main source of dietary protein, whereas the remaining groups were fed diets where SBM was partially replaced with 15% of raw rapeseed cake (**RRC**) or FRC. The diets were prepared in the "Agrocentrum" feed meal in Kałęczyn (Poland). All diets contained similar amounts of major amino acids (including lysine, methionine with cysteine, threonine), minerals (including calcium and available phosphorus), and vitamins. The nutritional value of diets was consistent with the nutrient requirements of turkeys (Hybrid Turkeys, 2013). Tables 1 and 2 present the composition of control and experimental diets prepared in successive 4-wk feeding periods.

For chemical analyses, samples of rapeseed cake were ground to pass through a 0.5-mm sieve. The samples were analyzed in duplicate for DM, CP, ether extract, crude fiber, crude ash, and phosphorus according to AOAC (2007) methods 934.01, 976.05, 920.39, 978.10, 942.05, 973.18, 984.27, and 965.17, respectively. Gross energy was determined with an adiabatic bomb calorimeter (KL 12Mn, Precyzja-Bit PPHU, Poland) standardized with benzoic acid. Phytate-phosphorus was determined as described by Haug and Lantzsch (1983).

Table 1. Composition and nutrient content of the control diet (C), in %.

	Feeding period (weeks)			
Item	1 to 4	5 to 8	9 to 12	13 to 16
Ingredients				
Wheat	51.94	52.22	62.73	72.14
Soybean meal	41.48	38.85	28.24	20.10
Soybean oil	1.66	4.09	4.95	4.74
Monocalcium phosphate	1.78	1.55	1.14	0.70
Sodium bicarbonate	0.19	0.15	0.15	0.15
Sodium chloride	0.20	0.18	0.16	0.12
Limestone	1.52	1.68	1.43	1.03
Choline chloride	0.09	0.10	0.10	0.10
L-Lysine HCL	0.43	0.46	0.46	0.37
DL-Methionine	0.33	0.30	0.23	0.19
L-Threonine	0.11	0.13	0.13	0.09
Enzymes	0.03	0.03	0.03	0.03
Vitamin-mineral premix[1]	0.25	0.25	0.25	0.25
Analyzed nutrients				
Crude protein	27.1	25.8	22.0	18.4
Crude fat	4.05	5.26	5.43	6.03
Calculated nutritional value[2]				
ME, kcal/kg	2750	2900	3050	3125
Crude fiber	2.69	2.82	2.67	2.59
Lysine	1.71	1.63	1.36	1.09
Arginine	1.74	1.58	1.28	1.05
Methionine	0.68	0.64	0.52	0.44
Methionine + Cysteine	1.11	1.05	0.88	0.77
Threonine	1.04	1.00	0.84	0.68
Tryptophan	0.34	0.31	0.26	0.224
Ca	1.30	1.10	0.90	0.65
Available P	0.70	0.50	0.40	0.30
Na	0.15	0.14	0.13	0.11
Cl	0.26	0.32	0.30	0.24

[1]Provided the following per kilogram of diet in weeks 1 to 8 and 9 to 16: vitamin A, 12,500 and 9600 IU; vitamin D_3, 5000 and 4800 IU; vitamin E, 100 and 60 mg; vitamin K_3, 4 and 3 mg; vitamin B_1, 4,5 and 2 mg; vitamin B_2, 15 and 12 mg; vitamin B_6, 5 and 5 mg; vitamin B_{12}, 16 and 0,03 mg; folic acid, 3, and 2,5 mg; pantothenic acid, 28 and 23 mg, nicotinic acid, 110 and 85 mg; biotin, 0,38 and 0,38 mg; Mn, 160 and 120 mg; Zn, 160 and 120 mg; Fe, 80 and 40 mg; Cu, 25 and 25 mg; I, 2.5 and 2 mg; Se, 0.3 and 0.3 mg, respectively.

[2]Calculated according to Polish Feedstuff Analysis Tables (Smulikowska and Rutkowski, 2005).

NSP were determined by gas-liquid chromatography (constituent neutral sugars) using an SP-2340 column and a Varian CP3380 gas chromatograph (Varian Inc., Palo Alto, CA) and by colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK), according to the procedure described by Englyst and Cummings (1984, 1988) with modifications (Slominski and Campbell, 1990). Uronic acids were determined as described by Scott (1979). Sugars (glucose—GLU, fructose, sucrose, raffinose, and stachyose) were determined as described by Slominski et al. (1993). Glucosinolates were determined by gas-liquid chromatography as described by Slominski and Campbell (1987).

Growth trial and sample collection

At the end of each 4-wk period, the body weights (**BW**) of turkeys, feed intake, and mortality rates were recorded, and each pen of 50 birds was considered an experimental unit. Daily feed intake (**DFI**), feed conversion ratio (**FCR**) and livability were calculated for each group. At the termination of the experiment, at 112 d of age, 9 birds representing the average BW per treatment were selected, tagged and fasted for 8 h. Blood samples were collected from the wing vein intravitally, and birds were sacrificed by cervical dislocation. To study intestinal histology, one square centimeter of whole thickness tissue samples from the duodenum and jejunum was taken. The samples were placed in 4% buffered formaldehyde (Sigma-Aldrich Corporation, St. Louis, Missouri, USA) for 5 d and then stored in ethanol.

Biochemical and hematological analyses

Immediately after collection, blood samples were aliquoted into test tubes containing heparin and EDTA as anticoagulants. Next, the blood samples were centrifuged at 3,000 *g* for 10 min and the plasma was collected for further analysis. The obtained plasma was stored at –20°C until analysis. The levels of hemoglobin (Hb) and hematocrit (Ht) were measured using an automated hematology analyzer (Abacuss Junior Vet, Diatron, Hungary).

The content of GLU, total protein (TP), total cholesterol (TC), triacylglycerols (TAG), urea (UREA), creatinine (CREAT), and the activities of alanine aminotransferase (ALT) and aspartate aminotrans-

Table 2. Composition and nutrient content of raw rapeseed cake (RRC) and fermented rapeseed cake (FRC) diets, in %.

	Feeding period (weeks)			
Item	1 to 4	5 to 8	9 to 12	13 to 16
Ingredients				
Wheat	42.93	43.46	53.97	63.37
Soybean meal	34.55	31.80	21.19	13.05
Rapeseed cake/Fermented rapeseed cake	15.00	15.00	15.00	15.00
Soybean oil	2.83	5.21	6.08	5.87
Monocalcium phosphate	1.70	1.50	1.09	0.65
Sodium bicarbonate	0.19	0.15	0.15	0.15
Sodium chloride	0.18	0.18	0.16	0.12
Limestone	1.40	1.53	1.28	0.88
Choline chloride	0.09	0.10	0.10	0.10
L-Lysine HCL	0.47	0.46	0.45	0.36
DL-Methionine	0.29	0.24	0.17	0.13
L-Threonine	0.10	0.09	0.09	0.05
Enzymes	0.03	0.03	0.03	0.03
Vitamin-mineral premix[1]	0.25	0.25	0.25	0.25
Analyzed nutrients				
Crude protein	27.7	25.5	22.6	18.6
Crude fat	5.90	7.56	6.99	7.51
Calculated nutritional value[2]				
ME, kcal/kg	2750	2900	3050	3125
Crude fiber	4.55	4.65	4.50	4.42
Lysine	1.74	1.63	1.36	1.09
Arginine	1.74	1.56	1.26	1.04
Methionine	0.67	0.60	0.48	0.41
Methionine + Cysteine	1.13	1.05	0.88	0.77
Threonine	1.07	1.00	0.84	0.68
Tryptophan	0.34	0.31	0.26	0.23
Ca	1.30	1.10	0.90	0.65
Available P	0.70	0.50	0.40	0.30
Na	0.15	0.14	0.13	0.11
Cl	0.26	0.31	0.30	0.24

[1]Provided the following per kilogram of diet in weeks 1 to 8 and 9 to 16: vitamin A, 12,500 and 9600 IU; vitamin D_3, 5000 and 4800 IU; vitamin E, 100 and 60 mg; vitamin K_3, 4 and 3 mg; vitamin B_1, 4,5 and 2 mg; vitamin B_2, 15 and 12 mg; vitamin B_6, 5 and 5 mg; vitamin B_{12}, 16 and 0.03 mg; folic acid, 3.0 and 2.5 mg; pantothenic acid, 28 and 23 mg, nicotinic acid, 110 and 85 mg; biotin, 0.38 and 0.38 mg; Mn, 160 and 120 mg; Zn, 160 and 120 mg; Fe, 80 and 40 mg; Cu, 25 and 25 mg; I, 2.5 and 2.0 mg; Se, 0.3 and 0.3 mg, respectively.

[2]Calculated according to Polish Feedstuff Analysis Tables (Smulikowska and Rutkowski, 2005).

ferase (AST) were measured in blood plasma using an automated biochemistry analyzer (Plasma Diagnostic Instruments, Horiba, Kyoto, Japan). The concentrations of calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) in the blood plasma of turkeys were determined by flame atomic absorption spectrometry (AAS) on a UNICAM 939 spectrometer.

The serum concentrations of free triiodothyronine (T3) and free thyroxine (T4) were determined by the radioimmunoassay using a diagnostic kit (Roche Diagnostics Gmbh, Mannheim, Germany) and the Elecsys 1010 analyzer (Instrument Center AG, Rotkreuz, Switzerland).

The activities of superoxide dismutase (**SOD**) and glutathione peroxidase (**GPx**) were determined in erythrocytes using Ransod and Ransel diagnostic kits (Randox), and catalase (CAT) activity was determined according to Aebi (1984). Total antioxidant potential (FRAP), the concentrations of total glutathione (GSH + GSSG), malondialdehyde (**MDA**), and lipid hydroperoxides (**LOOH**) in blood plasma were determined according to the methods proposed by Ognik and Wertelecki (2012).

The immune responses of turkeys were determined with the use of immunoenzymatic tests based on the plasma levels of immunoglobulin A (IgA), immunoglobulin Y (IgY), immunoglobulin M (IgM), and interleukin 6 (IL-6) using the Bigenet UMV 340 blood cell reader and kits for determining IL-6 (USCN Life Science Inc., Wuhan, China), IgA, IgM, and IgG (CusabioBiotech Co., Wuhan, China).

Histomorphometry

The intestinal segments of duodenum and jejunum were embedded in paraffin, and serial histological sections (5 μm thick) were stained with hematoxylin and eosine for histomorphometric analysis under a light microscope. In each bird, villus height, crypt depth, the thickness of tunica mucosa, and tunica muscularis were measured in 5 to 8 slides for each tissue sample (duodenum, jejunum) with an optical microscope ($\times$4 or $\times$10 objectives, OLYMPUS BX 61, Warsaw, Poland) coupled via a digital camera to a PC computer equipped with Cell P (OLYMPUS) software. In the slides, a total of 30 well-oriented villi (a population of

predominant villi) and crypts as well as the thickness of tunica mucosa and tunica muscularis (30 measurement points each) were measured for every small intestinal segment in each bird. Then, the mean value of each of the parameters was used to represent the data of a single bird. The procedure was repeated for each examined bird, and a statistical analysis was performed on three groups of birds (n = 9 in each experimental group).

Statistical analysis

For a statistical analysis of performance parameters, a single pen (n = 9) was considered as a replicate experimental unit. For analyses of biochemical, antioxidant, and histomorphometrical parameters of blood and tissues, individual birds were considered as experimental units. All analyses were performed on 27 birds representing 9 replicates from each of the 3 experimental groups.

One-way analysis of variance (**ANOVA**) was performed with the use of Statistica 10.0 software (StatSoft. Kraków, Poland). When a significant treatment effect was noted, the post-hoc Tukey test was used to determine differences between treatment groups. Data were presented as means ± SEM, and the value of $P \leq 0.05$ was considered statistically significant. In the following parameters: BW, blood T4 levels, and the villus height to crypt depth ratio in the jejunum, the post-hoc Tukey HSD test did not reveal significant differences between means in groups although P value reached 0.04. Therefore, Duncan's test was used, and the observed significant differences were considered as tendencies. The significance of differences between livability data was determined by the Kruskal–Wallis test by ranks (non-parametric ANOVA on ranks).

RESULTS

The fermentation of rapeseed cake by enzymes slightly increased the content of DM (93.5 vs. 91.2%), CP (34.9 vs. 32.5%), and CF (16.4 vs. 15.5%), and decreased the concentration of crude fat in comparison with RRC (9.30 vs. 10%). The greatest change was noted in the content of phytate-phosphorus which decreased from 0.307 to 0.016% on a DM basis. The fermentation of rapeseed cake caused a nearly 10-fold decrease in the content of glucosinolates (1.66 vs. 16.30 μmol/g) and a nearly 2-fold decrease in the content of carbohydrates (5.48 vs. 9.22%) in comparison with RRC (Table 3). The concentration of NSP was similar in groups RRC and FRC at 22.25 and 22.57%, respectively.

The growth performance of turkeys is presented in Table 4. No significant differences in DFI, FCR, or livability were observed between turkeys fed control, RRC, and FRC diets. However, a tendency towards lower average final BW of turkeys receiving RRC was observed relative to the remaining groups. Fermentation increased the final BW of turkeys, which was approximately 1.3% higher in group FRC than in group RRC, and comparable with that noted in the control group ($P = 0.043$).

Table 3. Chemical composition of rapeseed cake (RC) and fermented rapeseed cake (FRC) (%, dry matter basis).

Component	RRC	FRC
Dry matter	91.2	93.5
Crude protein	32.5	34.9
Crude fiber	15.5	16.4
Ether extract	10.0	9.30
Crude ash	6.70	6.70
Total phosphorus	1.15	1.14
Phytate-phosphorus	0.307	0.016
Gross energy (kcal/kg)	5127	5240
Non-starch polysaccharides[1]	22.2	22.6
Glucosinolates[2] (μmol/g)	16.3	1.66
Sugars[3]	9.22	5.48

[1]Including rhamnose, arabinose, xylose, mannose, galactose, glucose, and uronic acids.
[2]Including gluconapin, glucobrassicinapin, progoitrin, glucobrassicin, and hydroxyglucobrassicin.
[3]Including glucose, fructose, sucrose, raffinose, and stachyose.

Table 4. Growth performance of turkeys fed a control diet (C) and diets containing raw (RRC) or fermented rapeseed cake (FRC).

	Dietary treatment				
	C	RRC	FRC	SEM	P value
DFI, g	258	256	261	1.372	0.336
FBW, kg	10.82[a]	10.68[b]	10.83[a]	0.027	0.043
FCR, kg/kg	2.53	2.51	2.52	0.010	0.819
Livability, %	99.1	99.8	97.6	0.381	0.165

[a,b]Means within a row with different superscripts were considered as a near-significant trend

The inclusion of RRC and FRC in turkey diets did not affect the plasma concentrations of TP, TC, AST, ALT, CREAT, UREA, Ca, or Mg (Table 5). Turkeys fed diets containing RRC or FRC had higher GLU ($P = 0.001$) and Cu ($P = 0.010$) levels than control group birds. RRC increased the plasma levels of TAG in turkeys, relative to the control groups, and Zn levels relative to the FRC groups ($P = 0.029$ and $P = 0.027$, respectively). Blood T3 and T4 levels were similar in all groups, but a tendency towards decreased blood T4 levels ($P = 0.044$) was noted in turkeys fed RRC diets.

Both RRC and FRC modified selected parameters of the blood redox status in turkeys (Table 6), including a significant decrease in the concentrations of LOOH ($P = 0.001$) and MDA ($P = 0.001$), and an increase in FRAP values ($P = 0.008$) and in the concentrations of GSH + GSSG ($P = 0.001$) in blood plasma, compared with the control group. Turkeys fed diets with RRC were characterized by the highest blood vitamin C concentrations ($P = 0.001$), the highest activities of antioxidant enzymes SOD ($P = 0.002$) and GPx ($P = 0.001$), and lower CAT activity ($P = 0.027$).

A significant decrease was noted in blood Ht ($P = 0.001$) and IgM ($P = 0.001$) levels in turkeys fed diets

Table 5. Blood biochemical parameters of turkeys fed a control diet (C) and diets containing raw (RRC) or fermented rapeseed cake (FRC).[1]

	Dietary treatment				
	C	RRC	FRC	SEM	P value
GLU, mmol/L	16.1[b]	17.7[a]	17.4[a]	0.196	0.001
TP, g/L	40.4	42.0	40.9	0.578	0.545
TAG, mmol/L	1.28[b]	1.79[a]	1.32[a,b]	0.091	0.029
TC, mmol/L	3.00	2.52	2.50	0.136	0.247
AST, U/L	651	657	633	22.57	0.913
ALT, U/L	19.6	19.6	19.7	1.020	0.999
CREAT, μmol/L	7.78	8.11	8.89	0.705	0.816
UREA, μmol/L	244	233	278	12.37	0.324
T3, pg/mL	7.68	9.33	10.2	0.508	0.121
T4, ng/dl	0.847[x]	0.621[y]	0.813[x]	0.041	0.044
Ca, mmol/L	2.71	2.68	2.70	0.051	0.970
Mg, mmol/L	0.455	0.476	0.454	0.012	0.723
Fe, μmol/L	46.67	43.19	41.53	1.620	0.434
Cu, μmol/L	3.53[b]	4.71[a]	4.63[a]	0.187	0.010
Zn, μmol/L	77.7[a,b]	87.12[a]	76.74[b]	1.803	0.027

[1]Data representing mean values of 9 birds per treatment.
[a,b]Means within a row with different superscripts differ significantly ($P \leq 0.05$).
[x,y]Means within a row with different superscripts were considered as a near-significant trend; Hb- hemoglobin; Ht- hematocrit; GLU, glucose; TP, total protein; TAG, triacylglycerols; TC, total cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CREAT, creatinine; UREA, urea; T3, free triiodothyronine; T4, free thyroxine.

Table 6. Blood redox parameters of turkeys fed a control diet (C) and diets containing raw (RRC) or fermented rapeseed cake (FRC).[1]

	Dietary treatment				
	C	RRC	FRC	SEM	P value
Vit.C, μmol/L	84.8[b]	107[a]	70.5[b]	4.477	0.001
MDA, μmol/L	0.987[a]	0.506[b]	0.533[b]	0.061	0.001
GSH+GSSG, μmol/L	0.149[b]	0.279[a]	0.257[a]	0.016	0.001
FRAP, μmol/L	146[b]	212[a]	203[a]	9.988	0.008
LOOH, μmol/L	74.8[a]	54.3[b]	58.7[b]	2.524	0.001
SOD, U/gHb	2946[b]	4134[a]	3194[b]	159	0.002
GP_X, U/gHb	72.3[a]	77.7[a]	62.9[b]	1.635	0.001
CAT, U/gHb	418[a]	340[b]	372[a,b]	12.26	0.027

[1]Data representing mean values of 9 birds per treatment.
[a,b]Means within a row with different superscripts differ significantly ($P \leq 0.05$); Vit. C, vitamin C; MDA, malondialdehyde; GSH+GSS, total glutathione; FRAP, total antioxidant potential; LOOH, peroxides; SOD, superoxide dismutase; GP_X, glutathione peroxidase; CAT, catalase.

with RRC or FRC, compared with the control group (Table 7). The values of the remaining hematological and immunological parameters were similar in all groups.

The morphological parameters of the intestinal mucosa of turkeys are shown in Table 8. In groups RRC and FRC, an increase in villus height was observed in the duodenum and jejunum, but significant changes were found only in the duodenum where villus height was significantly highest in birds fed RRC ($P = 0.001$). The thickness of duodenal mucosa in group RRC was significantly higher than in the control and FRC groups ($P = 0.001$). A beneficial influence of RRC and FRC was also observed in the small intestines of turkeys, where a tendency towards a higher villus height to crypt depth ratio was noted relative to the control group ($P = 0.042$). The dietary treatments had no influence on crypt depth or wall thickness in the duodenum and jejunum of turkeys.

DISCUSSION

The use of rapeseed products in poultry nutrition is limited due to the presence of toxic and indigestible substances such as glucosinolates, phytic acid, and non-soluble carbohydrates (Rozan et al., 1996). Previous research has shown that fermentation can improve the quality of high-protein feeds by reducing their content of anti-nutritional factors (Shi et al., 2017; Goodarzi Boroojeni et al., 2018). Similar observations were made in the present study where fermentation-induced changes in the chemical composition and nutritional value of rapeseed cake. A minor increase was noted in the TP content of FRC, compared with RRC, which is consistent with the findings of other authors (Rozan et al., 1996; Vig and Walia, 2001). According to Chiang et al. (2010), this is a reflection of changes in DM content rather than an actual increase in protein content. On the other hand, an increase in CP content

Table 7. Hematological and blood immunological parameters of turkeys fed a control diet (C) and diets containing raw (RRC) or fermented rapeseed cake (FRC).[1]

	Dietary treatment				
	C	RRC	FRC	SEM	*P* value
Hb, g/L	9.22	8.95	9.34	0.123	0.421
Ht, l/L	35.8[a]	34.1[b]	33.7[b]	0.241	0.001
IgA, ng/mL	33.6	34.0	33.0	1.490	0.963
IgY, ng/mL	837.9	886.9	887.1	14.948	0.338
IgM, ng/mL	1298[a]	1007[b]	922.1[b]	36.013	0.001
Il-6, pg/mL	4.64	4.26	4.13	0.154	0.415

[1]Data representing mean values of 9 birds per treatment.
[a,b]Means within a row with different superscripts differ significantly ($P \leq 0.05$); Hb, hemoglobin; Ht, hematocrit, IgA, immunoglobulin A; IgY, immunoglobulin Y; IgM, immunoglobulin M; Il-6, interleukin 6.

Table 8. Morphology of the duodenum and jejunum of turkeys fed a control diet (C) and diets containing raw (RRC) or fermented rapeseed cake (FRC).[1]

	Dietary treatment				
	C	RRC	FRC	SEM	P value
Villus height (μm)					
Duodenum	2372[b]	2881[a]	2625[a,b]	62.5	0.001
Jejunum	1604	1767	1789	51.7	0.290
Crypt depth (μm)					
Duodenum	134	141	129	4.59	0.582
Jejunum	118	104	110	2.74	0.142
Villus height/crypt depth (μm)					
Duodenum	18.42	21.11	20.63	0.751	0.307
Jejunum	13.76[y]	16.85[x]	16.60[x]	0.570	0.042
Intestinal mucosa thickness (μm)					
Duodenum	2511[b]	3030[a]	2763[a,b]	63.6	0.001
Jejunum	1732	1885	1909	52.0	0.332
Intestinal wall thickness (μm)					
Duodenum	307	294	271	8.6	0.236
Jejunum	253	277	288	7.5	0.147

[1]Data representing mean values of 9 birds per treatment.
[a,b]Means within a row with different superscripts differ significantly ($P \leq 0.05$).
[x,y]Means within a row with different superscripts were considered as a near-significant trend.

is associated mostly with a decrease in the concentrations of non-structural carbohydrates in the biomass (Hu et al., 2016), which was also observed in our study. It should be stressed that fermentation considerably reduced the concentration of phytate-phosphorus in rapeseed cake. Our results corroborate the findings of Shi et al. (2016), who noted an 86% decrease in the content of phytate-phosphorus in fermented RSM. During the fermentation process, phytates are broken down by microbial phytase (El-Batal and Karem 2001). In the current study, fermentation considerably reduced the concentrations of glucosinolates in FRC. According to Vig and Walia (2001), the glucosinolate content of RSM decreases proportionally with fermentation time, and it can be reduced by 43.1% after 10 d. Chiang et al. (2010) and Xu et al. (2012) demonstrated that 30-d fermentation lowered isothiocyanate concentrations in RSM by 83 and 90%, respectively. The reduction of glucosinolates and their by-products during fermentation may be due to the utilization of GLU and the sulfur moieties of these compounds by microbial enzymes (Tripathi and Mishra, 2007). The fermentation of rapeseed cake had no effect on the concentrations of NSP, which is consistent with the findings of Jakobsen et al. (2015). This could be due to the fact that insoluble fiber fractions are typically more resistant to fermentation than their soluble counterparts (Bach Knudsen and Jorgensen, 2001).

The results of numerous studies indicate that high inclusion levels of rapeseed products, used as substitutes for SBM in poultry diets, can lead to decreased feed intake and BW gains, and increased mortality (McNeill et al., 2004; Gopinger et al., 2014). In the current study, diets containing 15% of RRC and FRC had no influence on feed intake or FCR, but turkeys fed RRC achieved lower final BW than birds from the control and FRC groups. Our results corroborate the findings of other authors who demonstrated that the average BW of broiler chickens and pigs fed fermented RSM were higher than in the groups receiving raw RSM and similar to the SMB group (Chiang et al., 2010; Shi et al., 2016). In our study, better growth performance in group FRC, relative to the control and RRC groups, could have resulted from the considerable reduction in the concentrations of glucosinolates and their derivatives in rapeseed cake. The glucosinolate content of poultry diets should

not exceed 2.5 μmol/g to ensure optimum performance (Mushtaq et al., 2007).

The blood biochemical and hematological parameters of turkeys were determined to evaluate the influence of experimental diets on the health status of birds. The increase in blood GLU levels, noted in turkeys fed RRC and FRC, was undesirable and could be indicative of the adverse effect of rapeseed cake on liver function. An increase in blood GLU concentrations in broiler chickens fed diets containing 10% of fermented RSM was also reported by Hu et al. (2016). The cited authors also found that fermented RSM decreased plasma TAG levels (an indicator of lipid metabolism in animals), compared with non-fermented RSM. Similar results were obtained in the present study; plasma TAG concentrations were lower in group FRC than in group RRC. This suggests that fermentation could improve the utilization of dietary fat by turkeys. Turkeys fed RRC had lower T4 concentrations, whereas T3 levels were similar in all groups. Lardy and Kerley (1994) also reported a significant linear decrease in serum T4 concentrations in response to increasing dietary levels of RSM in growing beef steers. Glucosinolates are known to decrease the levels of thyroid hormones (Hill, 1991; Taraz et al., 2006; Mikulski et al., 2012), thus slowing down metabolism. Blood T4 levels were higher in group FRC than in group RRC, which could result from a substantially lower content of isothiocyanates in FRC diets.

The increase in the blood concentrations of Cu and Zn in turkeys fed diets containing RRC and FRC points to a beneficial influence of rapeseed cake on the antioxidant status of birds. CuZn-SOD, which is localized in cytosol, contains approximately 0.30% of Cu and 0.25% of Zn (Surai, 2016). Sodium dismutase is a metalloenzyme which plays a key role in cell defense against the damaging effects of peroxides (Ognik and Krauze, 2016). In our study, SOD activity increased in both groups fed rapeseed cake, RRC and FRC. The values of other indicators, including the concentrations of GSH + GSSG and MDA, the activities of GPx and CAT, and FRAP also point to the positive effects of RRC and FRC on the redox status of turkeys; it should be noted that FRC did not exert a more beneficial influence on the redox balance than RRC. Sodium dismutase, GPx and CAT are three major antioxidant enzymes involved in scavenging oxygen free radicals (McCord, 1979). Increased SOD activity accompanied by stable or decreased activities of GPx and CAT could point to the activation of enzymatic defense mechanisms (Ognik and Krauze, 2016). MDA is the end product of lipid peroxidation; therefore, the extent of lipid peroxidation by reactive oxygen species can be monitored based on MDA levels (Sumida et al., 1989). The decrease in MDA concentrations, noted in our experiment, was associated with decreased amounts of the end products of lipid peroxidation. The beneficial effects of RRC and FRC could be related to the chemical composition of rapeseed cake which contains phenolic acids with strong antioxidant properties. According to Amarowicz et al. (2001), the antioxidant properties of phenolic acids in rapeseed oil cake contribute to inhibiting oxidation, thus maintaining its high nutritional quality. There is a general scarcity of studies investigating the effects of RRC and FRC on the redox status of turkeys. In a study by Hu et al. (2016), SOD activity and total antioxidant capacity were significantly higher in the blood of broiler chickens fed fermented RSM, compared with non-fermented RSM, but no significant differences were found in the remaining parameters of the redox status. Our findings suggest that both RRC and FRC stimulated antioxidant processes in turkeys.

Previous experiments (Feng et al., 2007; Tang et al., 2012) suggest that fermentation can increase the levels of small-size peptides in fermented RSM and improve the immune status of animals. In our study, diets containing RRC and FRC had no effect on Hb levels and decreased Ht levels in turkeys. However, the values of all hematological parameters remained within normal physiological ranges (Ognik and Sembratowicz, 2012; Ognik et al., 2016). Previous research (Matsuzaki and Chin, 2000; Xue et al., 2009; Xu et al., 2011) has revealed that fermented protein feeds, which are a rich source of polysaccharides, bioactive compounds, and lactic acid bacteria, promote immunoglobulin production and suppress the release of proinflammatory cytokines in animals. In the present study, diets with RRC and FRC significantly decreased the blood levels of IgM. IgM is produced as the first antibody isotype in the primary antibody response, and it is the major immunoglobulin class expressed on the surface of B lymphocytes in chickens (Schwarz et al., 2010). IgM can be used as an indicator of recent exposure to foreign macromolecules from infectious agents (Orlandi-Pradines et al., 2007). The results of experiments with poultry indicate that plasma IgM levels can decrease or increase in response to immunosuppression and immunostimulation. In most cases, increased IgM levels are observed during infection, accompanied by elevated levels of IgG and the proinflammatory cytokine IL-6, and oxidation reactions occur in cells (Ratcliffe, 2006; Schwarz et al., 2010; Jankowski et al., 2017). In our study, diets with RRC and FRC did not increase the levels of the remaining immunoglobulins or IL-6. The indicators of oxidative stress did not increase, either. Thus, decreased IgM levels could only be indicative of immune system stimulation.

Intestinal villi are the site where most of the nutrients are absorbed, and their height is indicative of the absorptive capacity of intestinal mucosa. Higher intestinal villi increase the area of surface contact between enterocytes and nutrients, thus improving nutrient absorption (Gopinger et al., 2014). The villus height to crypt depth ratio is a robust indicator of the absorptive capacity of the small intestine (Pluske et al., 1997; Montagne et al., 2003). An increase in the villus height to crypt depth ratio is considered beneficial for digestion and absorption, and vice versa. In the current study, both

RRC and FRC increased villus height in the duodenum and improved the villus height to crypt depth ratio in the jejunum of turkeys. Surprisingly, the thickness of duodenal mucosa was highest in turkeys fed RRC, not FRC. In other studies (Chiang et al., 2010; Hu et al., 2016), the dietary inclusion of 10% of fermented RSM enhanced small intestinal structure and function in broilers, compared with non-fermented RSM. Previous research suggests that fermented feed significantly increases the total counts of beneficial bacteria in the gastrointestinal tract of broiler chickens and turkeys (Firman et al., 2013; Naji et al., 2015). According to Xu et al. (2003), increased villus height and villus height to crypt depth ratio might be associated with increased counts of beneficial gut bacteria such as lactobacilli. The increased villus height to crypt depth ratio can produce an intestinal structure with improved absorptive and hydrolysis potential, which requires fewer nutrients for intestinal maintenance. Thus, fermented feed can promote the growth of intestinal epithelial cells, increase the surface area for nutrient absorption, and improve the efficiency of nutrient utilization. The results of our study are interesting because FRC exerted a beneficial influence on intestinal morphometry, as expected, but RRC also had a positive effect on the analyzed parameters. Previous studies have only shown that the inclusion of 15 to 20% of RSM in broiler chicken diets had no adverse effect on the morphometric parameters of the jejunum (Perić et al., 2015) and did not alter the integrity of the intestinal mucosa (Gopinger et al., 2014).

CONCLUSIONS

It can be concluded that fermentation had a beneficial influence on the chemical composition of rapeseed cake because it considerably reduced the concentrations of glucosinolates and phytate-phosphorus. In comparison with RRC, FRC increased the final BW of turkeys. Both RRC and FRC can be added to turkey diets at 15% without compromising metabolic parameters or immune function, while exerting a positive effect on antioxidant status and intestinal histomorphology in turkeys.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Bogdan A. Slonimski and Dr. Anna Rogiewicz from the Department of Animal Science at the University of Manitoba, Winnipeg, Canada, for technical support.

REFERENCES

Aebi, H. 1984. Catalase in vitro. Methods Enzymol 105:121–126.

Aljubori, A., Z. Idrus, A. F. Soleimani, N. Abdullah, and L. J. Boo. 2017. Response of broiler chickens to dietary inclusion of fermented canola meal under heat stress condition. Italian J. Anim. Sci. 16:546–551.

Amarowicz, R., J. Fornal, M. Karamać, and F. Shahidi. 2001. Antioxidant activity of extracts of phenolic compounds from rapeseed oil cakes. J. Food Biochem. 8:65–74.

AOAC. 2007. Official Methods of Analysis of AOAC International. 18th ed. Association of Official Analytical Chemists, Washington, DC.

Bau, H. M., C. Villaume, C. F. Lin, J. Evrard, B. Quemener, J. P. Nicolas, and L. Mejean. 1994. Effect of a solid-state fermentation using Rhizopus oligosporus sp.T-3 on elimination of antinutritional substances and modification of biochemical constituents of defatted rapeseed meal. J. Sci. Food Agric. 65:315–322.

Chabanon, G., I. Chevalota, X. Framboisiera, S. Chenub, and I. Marc. 2007. Hydrolysis of rapeseed protein isolates: kinetics, characterization and functional properties of hydrolysates. Process Biochem. 42:1419–1428.

Chiang, L., W. Q. Lu, X. S. Piao, J. K. Hu, L. M. Gong, and P. A. Thacker. 2010. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian-Aust. J. Anim. Sci. 23:263–271.

Chibowska, M., S. Smulikowska, and B. Pastuszewska. 2000. Metabolisable energy value of rapeseed meal and its fractions for chickens as affected by oil and fibre content. J. Anim. Feed Sci. 9:371–378.

Elangovan, A. V. S., V. S. Verma, V. R. B. Sastry, and S. D. Singh. 2001. Effect of feeding high glucosinolate rapeseed meal to laying Japanese quail. Asian-australas. J. Anim. Sci. 14: 1305–1307.

El-Batal, A. I., and H. A. Karem. 2001. Phytase production and phytic acid reduction in rapeseed meal by *Aspergillus niger* during solid state fermentation. Food Res. Int. 34:715–720.

Englyst, H. N., and J. H. Cummings. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 109:937–942.

Englyst, H. N., and J. H. Cummings. 1988. Improved method for the determination of dietary fiber as non-starch polysaccharidesin plant foods. J. AOAC Int. 71:808–814.

Fang, Z. F., J. Peng, T. J. Tang, Z. L. Liu, J. J. Dai, and L. Z. Jin. 2007. Xylanase supplementation improved digestibility and performance of growing pigs fed Chinese double-low rapeseed meal inclusion diets: in vitro and in vivo studies. Asian-Australas. J. Anim. Sci. 11:1721–1728.

Feng, J., X. Liu, Y. Y. Liu, Z. R. Xu, and Y. P. Lu. 2007. Effects of *Aspergillus oryzae* 3.042 fermented soybean meal on growth performance and plasma biochemical parameters in broilers. Anim. Feed Sci. Technol. 134:235–242.

Feng, D., and J. Zuo. 2007. Nutritional and anti-nutritional composition of rapeseed meal and its utilization as a feed ingredient for animal. Pages 265–270 in Proc. 12th IRC Rapeseed Conf. Wuhan, China.

Firman, J. D., D. More, and D. Mclntyre. 2013. Effects of dietary inclusion of a *Saccharomyces cerevisiae* fermentation product on performance and gut characteristics of male turkeys to market weight. Int. J. Poult. Sci. 12:141–143.

Gasiorek, E., and M. Wilk. 2011. Possibilities of utilizing the solid by-products of biodiesel production - a review. Pol. J. Chem. Technol. 13:58–62.

Goodari Boroojeni, F., K. Kozłowski, J. Jankowski, M. Senz, M. Wiśniewska, D. Boros, A. Drażbo, and J. Zentek. 2018. Fermentation and enzymatic treatment of pea for turkey nutrition. Anim. Feed Sci. Technol. 237:78–88.

Gopinger, E., E. G. Xavier, M. C. Elias, A. A. Catalan, M. L. Castro, A. P. Nunes, and V. F. Roll. 2014. The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens. Poult. Sci. 93:1130–1136.

Haug, W., and H. J. Lantzsch. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34:1423–1426.

Hill, R. 1991. Rapeseed meal in the diets of ruminants. Nutr. Abst. Rev. 61:139.

Hu, Y., Y. Wang, A. Li, Z. Wang, X. Zhang, T. Yun, L. Qiu, and Y. Yin. 2016. Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. Food Agric. Immunol. 27:182–193.

Hybrid Turkeys. 2013. A Hendrix Genetix. Available at: www.hybridturkeys.com.

Jakobsen, G. V., B. B. Jensen, K. E. B. Knudsen, and N. Canibe. 2015. Improving the nutritional value of rapeseed cake and wheat dried distillers grains with solubles by addition of enzymes during liquid fermentation. Anim. Feed Sci. Technol. 208:198–213.

Jankowski, J., K. Ognik, M. Kubińska, A. Czech, J. Juśkiewicz, and Z. Zduńczyk. 2017. The effect of DL-, L-isomers and DL-hydroxy analog administered at 2 levels as dietary sources of methionine on the metabolic and antioxidant parameters and growth performance of turkeys. Poult. Sci. 96:3229–3238.

Kasprowicz-Potocka, M., A. Zaworska, A. Frankiewicz, W. Nowak, P. Gulewicz, Z. Zduńczyk, and J. Juśkiewicz. 2015. The nutritional value and physiological properties of diets with raw and *Candida utilis* fermented lupine seeds in rats. Food Technol. Biotechnol. 53. doi: 10.17113/ftb.53.03.15.3979.

Kocher, A., M. Choct, M. D. Porter, and J. Broz. 2000. The effects of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. Poult. Sci. 79:1767–1774.

Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319–338.

Knudsen, K. E., and H. Jørgensen. 2001. Intestinal degradation of dietary carbohydrates from birth to maturity. Pages 109–120 in Digestive Physiology of Pigs, Vol. 26. J. E. Lindberg, and B. Ogle, eds. CABI Publishing, Wallingford.

Lardy, G. P., and M. S. Kerley. 1994. Effect of increasing the dietary level of rapeseed meal on intake by growing beef steers1. J. Anim. Sci. 72:1936–1942.

Mansour, E. H., E. Dworschák, A. Lugasi, Ö. Gaál, É. Barna, and A. Gergely. 1993. Effect of processing on the antinutritive factors and nutritive value of rapeseed products. Food Chem. 47:247–252.

Matsuzaki, K., and J. Chin. 2000. Modulating immune responses with probiotic bacteria. Immunol Cell Biol 78:67–73.

McCord, J. M. 1979. Superoxide, superoxide dismutase and oxygen toxicity. Rev. Biochem. Toxicol. 1:109–124.

McNeill, L., K. Bernard, and M. G. MacLeod. 2004. Food intake, growth rate, food conversion and food choice in broilers fed on diets high in rapeseed meal and pea meal, with observations on sensory evaluation of the resulting poultry meat. Br. Poult. Sci. 45:519–523.

Mikulski, D., J. Jankowski, Z. Zdunczyk, J. Juskiewicz, and B. A. Slominski. 2012. The effect of different dietary levels of rapeseed meal on growth performance, carcass traits, and meat quality in turkeys. Poult. Sci. 91:215–223.

Montagne, L., J. R. Pluske, and D. J. Hampsom. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108:95–117.

Mushtaq, T., M. Sarwar, G. Ahmad, M. Mirza, H. Nawaz, M. H. Mushtaq, and U. Noreen. 2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance digestibility carcass and immunity responses of broiler chickens. Poult Sci. 86:2144–2151.

Naji, S. A. H., J. K. M. Al-Gharawi, and I. F. B. Al-Zamili. 2015. The effect of starting age of feeding wetting fermented feed on the intestinal flora, humoral and cellular immunity of broiler chicks. Int. J. Adv. Res. 3:41–49.

Ognik, K., E. Chlewińska, and A. Czech. 2016. The effect of adding hesperidin, diosmin, quercetin and resveratrol extracts to feed for turkey hens on selected immunological and biochemical blood indices. Ann. Anim. Sci. 16:1–14.

Ognik, K., and M. Krauze. 2016. The potential for using enzymatic assays to assess the health of turkeys. Worlds Poult. Sci. J. 72:535–550.

Ognik, K., and I. Sembratowicz. 2012. Effect of Aloe-plus preparation supplement on hematological and immunological blood parameters and performance of turkey hens. Turk. J. Vet. Anim. Sci. 36:491–498.

Ognik, K., and T. Wertelecki. 2012. Effect of different vitamin E sources and levels on selected oxidative status indices in blood and tissues as well as on rearing performance of slaughter turkey hens. J Appl. Poult. Res. 21:259–271.

OJEU. 2010. Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. OJEU 20.10.2010, Series L. Off. J. Eur. Union 276:33–79.

Orlandi-Pradines, E., L. Almeras, L. Denis de Senneville, S. Barbe, F. Remoue, C. Villard, S. Cornelie, K. Penhoat, A. Pascual, C. Bourgouin, D. Fontenille, J. Bonnet, N. Corre-Catelin, P. Reiter, F. Pages, D. Laffite, D. Boulanger, F. Simondon, B. Pradines, T. Fusai, and C. Rogier. 2007. Antibody response against saliva antigens of Anopheles gambiae and Aedes aegypti in travellers in tropical Africa. Microbes Infect. 9:1454–1462.

Pastuszewska, B., G. Jabłecki, L. Buraczewska, P. Dakowski, M. Taciak, R. Matyjek, and A. Ochtabińska. 2003. The protein value of differently processed rapeseed solvent meal and cake assessed by in vitro methods and in tests with rats. Anim. Feed Sci. Technol. 106:175–188.

Perić, L., M. Ivković, D. Žikić, and I. Jajić. 2015. Effect of rapeseed meal on nutrient digestibility and gut morphology in broiler chickens. Sci. Papers Anim. Sci. Biotechnol. 48:36–39.

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock Prod. Sci. 51:215–236.

Ratcliffe, M. J. 2006. Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development. Dev. Comp. Immunol. 30:101–118.

Rozan, P., C. Villaume, and H. M. Bau. 1996. Detoxication of rapeseed meal by *Rhizopus Oligosporus sp-T3*: A first step towards rapeseed protein concentrate. Int. J. Food Sci. Tech. 31:85–90.

Schwarz, A., N. Medrano-Mercado, P. F. Billingsley, G. A. Schaub, and J. M. Sternberg. 2010. IgM-antibody responses of chickens to salivary antigens of *Triatoma infestans* as early biomarkers for low-level infestation of triatomines. Int. J. Parasitol. 40:1295–1302.

Scott, R. W. 1979. Colorimetric determination of hexuronic acids in plant materials. Anal. Chem. 51:936–941.

Shi, C., J. He., J. Wang, J. Yu, B. Yu, X. Mao, P. Zheng, Z. Huang, and D. Chen. 2016. Effects of *Aspergillus niger* fermented rapeseed meal on nutrient digestibility, growth performance and serum parameters in growing pigs. Anim. Sci. J. 87:557–563.

Shi, C., Y. Zhang, Z. Lu, and Y. Wang. 2017. Solid-state fermentation of corn-soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* for degrading antinutritional factors and enhancing nutritional value. J. Anim. Sci. Biotechnol. 8:50.

Slominski, B. A., and L. D. Campbell. 1987. Gas chromatographic determination of indole glucosinolates–a re-examination. J. Sci. Food Agric. 40:131–143.

Slominski, B. A., and L. D. Campbell. 1990. Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 53:175–184.

Slominski, B. A., W. Guenter, and L. D. Campbell. 1993. New approach to water-soluble carbohydrate determination as a tool for evaluation of plant cell wall-degrading enzymes. J. Agric. Food Chem. 41:2304–2308.

Smulikowska, S., J. Czerwiński, and A. Mieczkowska. 2006. Nutritional value of rapeseed expeller cake for broilers: effect of dry extrusion. J. Anim. Feed Sci. 15:445–453.

Smulikowska, S., and A. Rutkowski, eds. 2005. Recommended Allowances and Nutritive Value of Feedstuffs. Poultry Feeding Standards (in Polish). 4th edition. The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna (Poland) and Polish Branch of WPSA.

Sumida, S., K. Tanaka, H. Kitao, and F. Nakadomo. 1989. Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. Int. J. Biochem. 21:835–838.

Surai, P. F. 2016. Antioxidant systems in poultry biology: superoxide dismutase. J. Anim. Nutr. 1:1–16.

Tang, J. W., H. Sun, X. H. Yao, Y. F. Wu, X. Wang, and J. Feng. 2012. Effects of replacement of soybean meal by fermented cottonseed meal on growth performance, serum biochemical parameters and immune function of yellow-feathered broilers. Asian-australas. J. Anim. Sci. 25:393–400.

Taraz, Z., S. M. A. Jalali, and F. Rafeie. 2006. Effects of replacement of soybean meal with rapeseed meal on organs weight, some blood biochemical parameters and performance of broiler chicks. Int. J. Poult. Sci. 5:1110–1115.

Tripathi, M. K., and A. S. Mishra. 2007. Glucosinolates in animal nutrition: a review. Anim. Feed Sci. Technol. 132:1–27.

Vig, A. P., and A. Walia. 2001. Beneficial effects of *Rhizopus oligosporus* fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (*Brassica napus*) meal. Bioresour. Technol. 78:309–312.

Xu, Z. R., C. H. Hu, M. S. Xia, X. A. Zhan, and M. Q. Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82:1030–1036.

Xu, F. Z., L. M. Li, J. P. Xu, K. Qian, Z. D. Zhang, and Z. Y. Liang. 2011. Effects of fermented rapeseed meal on growth performance and serum parameters in ducks. Asian-Aust. J. Anim. Sci. 24:678–684.

Xu, F. Z., X. G. Zeng, and X. L. Ding. 2012. Effects of replacing soybean meal with fermented rapeseed meal on performance, serum biochemical variables and intestinal morphology of broilers. Asian-Aust. J. Anim. Sci. 25:1734–1741.

Xue, Z. H., W. C. Yu, M. C. Wu, and J. H. Wang. 2009. *In Vivo* Antitumor and antioxidative effects of a rapeseed meal protein hydrolysate on an S180 Tumor-bearing murine model. Biosci. Biotechnol. Biochem. 73:2412–2415.

Zaworska, A., M. Kasprowicz-Potocka, A. Frankiewicz, Z. Zduńczyk, and J. Juśkiewicz. 2016. Effects of fermentation of narrow-leafed lupine (*L. angustifolius*)seeds on their chemical composition and physiological parameters in rats. J. Anim. Feed Sci. 25:326–334.

Zhang, W., Z. Xu, J. Sun, and X. Yang. 2006. A study on the reduction of gossypol levels by mixed culture solid substrate fermentation of cottonseed meal. Asian-Aust. J. Anim. Sci. 19:1314–1321.