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Effect of diets with different contents of sunflower meal without or with exogenous enzymes supplementation on gastrointestinal tract response of growing turkeys

J. Juśkiewicz[1,4], J. Jankowski[2], A. Lecewicz[2], B. Slominski[3] and Z. Zduńczyk[1]

[1]Institute of Animal Reproduction and Food Research, Polish Academy of Sciences
Tuwima 10, 10-747 Olsztyn, Poland
[2]University of Warmia and Mazury, Department of Poultry Science
10-718 Olsztyn, Poland
[3]University of Manitoba, Department of Animal Science
Fort Garry Campus 12, Dafoe Road 201, Winnipeg, Manitoba, Canada

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ABSTRACT

The present experiment was conducted to investigate the effect of a different dietary content of sunflower meal (SFM) and the efficiency of an non-starch polysaccharide (NSP)-degrading enzyme preparation on growth and gut function of young turkeys. A total of 1512 one-day-old male turkey poults were randomly assigned to 8 dietary treatments, with 7 pens per treatment and 27 birds per pen. Experimental diets with a different content of SFM (0, 7, 14 and 21%; SFM_0, SFM_7, SFM_{14} and SFM_{21} groups, respectively) were administered in two variants, with and without NSP-degrading enzymes (E+ and E_0 treatments, respectively). Diets fed to the turkeys for 8 weeks were isonitrogenous and isocaloric, but they differed substantially with regard to crude fibre content (in average 2.98, 3.97, 4.64 and 5.64% in the SFM_0, SFM_7, SFM_{14} and SFM_{21} groups, respectively). The enzyme preparation applied to a diet caused a tendency towards lower ileal viscosity (P=0.099) and a significant decrease in caecal total volatile fatty acids concentration,

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[4] Corresponding author: e-mail: j.juskiewicz@pan.olsztyn.pl

despite of the observed increase in activities of bacterial α-glucosidase, α-galactosidase and β-galactosidase in the E+treatment. At the same time, two-way ANOVA revealed that following the dietary inclusion of SFM at the amount of 14 and 21%, a significant decrease was observed in final body weight, small intestine and caecal tissue mass, caecal digesta mass, as well as the rate of bacterial production of volatile fatty acids in the caeca. Such an effect was not recorded when SFM was applied at a dose of 7%. In conclusion, sunflower meal rich in crude fibre added at the level of 14-21% to a diet for growing turkeys may induce undesirable processes manifested in the decrease in relative mass of small intestine and caecal tissues, as well as potent inhibition of the fermentation processes in the caeca. Our study showed additionally that high fibre sunflower meal could be used at a dose of up to 7% without any adverse effects on the gastrointestinal physiology of the growing turkeys.

KEY WORDS: sunflower meal, gastrointestinal tract, caecal fermentation, turkey

INTRODUCTION

Soyabean meal (SBM) has been reported to presumably be the only common protein supplement that is typically included in poultry rations with no limitation as to the quantity used (Bach Knudsen, 2001). In contrast, other studies have shown that dietary SBM added at a high level may lead to a decrease in intestinal absorptive surface area and an undesirable increase in litter moisture concentration in young turkeys due to relatively high amounts of raffinose family oligosaccharides (Juśkiewicz et al., 2009). Having in mind that effect as well as the fact that protein is often one of the most expensive components of poultry diets, nutritionists have started a search for alternative ingredients which have potential as cost-effective protein sources, sometimes underutilized in poultry production (Mushtaq et al., 2006). It is well known that sunflower oil is considered one of the most healthful vegetable oils for humans, thus the availability of sunflower meal (SFM), as a by-product, is relatively high (Vieira et al., 1992; Stodolak et al., 2009). SFM obtained from processed intact sunflower seeds is rich in protein but also has a high content of fibre. Moreover, diets formulated with SFM can be deficient in lysine. The latter could be overcome by an appropriate supplementation. But high level of fibre, causing low dietary energy values, may excessively reduce the time of feed passage throughout the digestive system and diminish nutrients utilization (Wenk, 2001). Among poultry, turkeys seem to be most sensitive to a high content of crude fibre in a diet. Experiments concerning SFM in turkeys' feeding are sparse, thus the recommended level of dietary SFM for growing turkeys have not been explicitly established. Moreover, studies focused not only on the growth and performance response but with a deeper look into physiology have increasingly been in great demand. In the light of the above, the aim of this study was to investigate the growth and the physiological response of the gastrointestinal tract

of young turkeys (to 8 wk of age) to diets with different contents of sunflower meal, applied without or with NSP-degrading enzymes. As the experimental diets differed substantially in non-digestible carbohydrate fractions, great attention was paid to the development and bacterial fermentation processes in the caeca of the birds.

MATERIAL AND METHODS

Birds and housing

The experimental procedure was approved by the Local Ethics Committee. The study was conducted at the research laboratory of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn. The experimental materials comprised a total of 1512 heavy-type Big 6 turkeys purchased as one-day-old male poults at the "Grelavi" Hatchery in Kętrzyn. The birds were randomly assigned to 8 dietary treatments, with 7 pens per treatment and 27 birds per pen, and they were raised on deep litter. At the end of 8-wk period, birds were weighed, and feed consumption was recorded. Weight gain and feed efficiency were calculated. For performance indices, each pen was considered an experimental unit. Light was provided for 16 h per day. Indoor temperature was 32°C at the beginning of the experiment and 22°C at the end of week 8. At the beginning of the study, poults were administered Aviffa-RTI - live vaccine against infectious rhinotracheitis. During two rearing periods (weeks 1-4 and 5-8), birds were fed isoenergetic diets containing 28 and 26% crude protein, respectively, covering turkeys nutrient requirement of (NRC, 1994). They had free access to feed and water.

Diets

Commercially available soyabean (from a local feed company) and sunflower (from WIOJL-AGRO Co., Winnica, Ukraine) meals were used in this experiment. The composition of experimental diets with a different content of SFM (0, 7, 14 and 21%) is given in Table 1. The levels of other components, including SBM, were determined so as to meet the nutrient requirement of turkeys (NRC, 1994) aged 1-4 and 5-8 weeks. Each diet was prepared in two variants, with and without non-starch polysaccharide (NSP)-degrading enzymes. The enzymatic preparation supplied, U: pectinase 500, cellulose 40U, xylanase 1600, glucanase 800, mannanase 200, galactanase 20, and other minor enzymes per kg diet (Superzyme CS, Canadian Bio-Systems Inc., Calgary, AB, Canada). The lysine, arginine, methionine, threonine, tryptophan, mineral and vitamin contents were

similar in all dietary treatments. The average content of crude fibre in the diets was approximately 2.98, 3.97, 4.64 and 5.64 in the SFM_0, SFM_7, SFM_{14} and SFM_{21} groups, respectively. In terms of total NSP the aforementioned diets contained approximately 11.17, 11.93, 12.68 and 13.43 of total NSP, respectively.

Table 1. Composition of experimental diets

	Period of feeding 1-4 weeks				Period of feeding 5-8 weeks			
	SFM_0	SFM_7	SFM_{14}	SFM_{21}	SFM_0	SFM_7	SFM_{14}	SFM_{21}
Ingredient, g/kg								
wheat	245.8	211.9	178.5	144.3	244.9	207.9	171.0	134.0
maize	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
soyabean meal	433.0	384.0	335.0	286.0	471.5	424.5	377.4	330.2
sunflower meal (SFM)	-	70.0	140.0	210.0	-	70.0	140.0	210.0
potato protein	50.0	50.0	50.0	50.0	-	-	-	-
soyabean oil+animal fat (1:1)	24.5	37.5	50.0	63.0	25.5	59.1	72.7	86.3
sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
sodium chloride	2.6	2.6	2.6	2.6	2.1	2.1	2.2	2.2
limestone	18.0	17.5	17.0	16.7	14.4	14.1	13.7	13.4
monocalcium phosphate	20.0	20.0	20.0	20.0	14.8	14.9	15.1	15.2
DL-methionine	1.7	1.5	1.3	1.1	1.5	1.4	1.2	1.1
L-lysine-HCl	0.4	1.0	1.6	2.3	1.3	2.0	2.7	3.4
L-threonine	-	-	-	-	-	-	0.1	0.2
mineral and vitamin premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Calculated composition, g/kg								
crude protein	279.9	280.0	280.1	279.8	260.0	260.0	260.0	260.0
calcium	12.1	12.1	12.0	12.0	10.0	10.0	10.0	10.0
available phosphorus	8.8	8.6	8.4	8.2	5.0	5.0	5.0	5.0
lysine	16.0	16.0	16.0	16.0	15.0	15.0	15.0	15.0
methionine	6.0	6.0	6.0	6.0	5.2	5.2	5.2	5.2
methionine + cystine								
threonine	11.0	10.9	10.9	10.9	9.6	9.5	9.5	9.5
arginine	17.8	18.2	18.6	18.9	17.2	17.4	17.6	17.8
tryptophan	3.6	3.6	3.6	3.6	3.3	3.3	3.2	3.2
sodium	1.7	1.7	1.7	1.7	1.5	1.5	1.5	1.5
crude fibre	32.7	40.6	48.5	56.4	34.3	42.5	50.8	59.2
ME, MJ/kg	11.72	11.72	11.71	11.71	12.14	12.14	12.14	12.14
Analysed composition, g/kg								
crude protein	275.0	282.2	280.4	281.3	262.4	265.8	267.6	272.5
crude fibre	28.8	42.5	46.1	55.5	30.7	36.9	46.7	57.2
non-starch polysaccharides	108.3	115.8	123.3	130.7	115.1	122.7	130.3	137.8
total fibre	141.0	151.2	164.4	171.5	148.5	158.5	168.8	178.9
ether extract	48.0	56.3	70.1	80.5	69.7	77.9	90.8	106.7

[1] for 1 to 4 and 5 to 8 week of feeding the vitamin and mineral premix supplied per kg of diet, IU: vit. A 15.000, and 13.000; mg: vit. E 40 and 35, respectively. For 1 to 8 week of feeding the vitamins and mineral premix supplied per kg of diet, mg: Se 0.3, Mn 150, Zn 90, Fe 60, Cu 15, I 1, diclazuril 1, IU: vit. D_3 4,500; mg: vit K_3 2.5, thiamin 3.5, riboflavin 10, vit. B_6 6, vit B_{12} 0.03, folic acid 2, biotin 0.36, niacin 75, pantothenic acid 21, choline 600

For chemical analysis, the samples were ground to pass through a 0.5 mm sieve. Feed ingredient, diet or digesta samples were analysed in duplicate for dry matter, crude protein (CP), fat, and crude fibre (CF) using AOAC (2005) methods 934.01, 976.05, 920.39 and 978.10, respectively. NSP were determined by gas-liquid chromatography using the procedure described by Slominski and Campbell (1990). Briefly, 100 mg samples were boiled with 2 ml dimethylsulphoxide for 1 h and then incubated at 45°C overnight with a sodium acetate buffer solution (pH 5.2) of starch-degrading enzymes: amylase, pullulanase and amyloglucosidase (Sigma Chemical Co., St Louis, MO). Ethanol was then added and the mixture was left for 1 h at room temperature before being centrifuged at 1990 *g*, for 10 min at 21°C. The supernatant was discarded and the dried residue was dissolved in 1 ml of 12 M sulphuric acid and incubated for 1 h at 35°C. Six ml of water and 5 ml of myoinositol (internal standard) solution were then added and the mixture was boiled for 2 h. One ml of the hydrolysate was taken and neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetate anhydride in the presence of 1-methylimidazole. Component sugars were separated using a SP-2340 column and a Varian CP 3380 Gas Chromatograph (Varian Canada Inc., Mississauga, Ontario, Canada).

Sample collection and chemical analyses

After 8 weeks of feeding, 10 turkeys that represented an average body weight for each group were killed by cervical dislocation. After laparatomy, segments of the digestive tract (crop, ventriculus, small intestine, caeca and colon) were removed and weighed. As soon as possible after euthanasia (about 20 min) pH was measured in each segment using a microelectrode and pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal). Samples of ileal (middle, 1/3 section of ileum) and caecal contents were used for immediate analysis (ammonia, dry matter, viscosity, volatile fatty acids - VFA), while the rest of the caecal digesta was transferred to tubes and stored at -70°C. The ventriculus (gizzard), small intestine, caeca and colon were flushed with water, blotted on filter paper and weighed as the tissue mass.

Dry matter of digesta was determined at 105°C. The total contents of the small intestine were collected, mixed on a vortex, and centrifuged at 7.211 *g* for 10 min. The supernatant fraction (0.5 ml) was placed in a Brookfield LVDV-II+cone-plate rotational viscometer (CP40; Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) and the viscosity of all samples was measured at a fixed temperature of 39°C and at a shear rate of 60 per min. The viscosity value was recorded as an apparent viscosity. In fresh caecal digesta, ammonia was extracted, trapped in a solution of boric acid in the Conway's dishes, and determined by

direct titration with sulphuric acid (Hofirek and Haas, 2001).

Caecal digesta samples were subjected to VFA analysis using gas chromatography (Shimadzu GC-2010, Shimadzu, Kyoto, Japan). The samples (0.2 g) were mixed with 0.2 ml formic acid, diluted with deionized water and centrifuged at 7.211 *g* for 10 min. The supernatant was loaded onto a capillary column (SGE BP 21.30 m × 0.53 mm) using an on-column injector. The initial oven temperature was 85°C and was raised to 180°C by 8°C/min and held there for 3 min. The temperatures of flame ionization detector and the injection port were 180 and 85°C, respectively. The sample volume for GC analysis was 1 µl. Caecal VFA pool size was calculated as the sum of VFA concentration in digesta and caecal digesta mass.

The bacterial glycolytic activity in the caecal digesta was measured by the rate of *ρ*- or *o*-nitrophenol release from their nitrophenylglucosides according to the modified method of Djouzi and Andrieux described by Juśkiewicz et al. (2006). The following substrates were used: *ρ*-nitro-phenyl-*α*-D-glucopyranoside (for *α*-glucosidase), and *ρ*-nitrophenyl-*β*-D-glucopyranoside (for *β*-glucosidase),*ρ*-nitrophenyl-*α*-D-galactopyranoside(*α*-galactosidase),*o*-nitrophenyl-*β*-D-galactopyranoside (*β*-galactosidase), and *ρ*-nitrophenyl-*β*-D-glucuronide (for *β*-glucuronidase). The reaction mixture contained 0.3 ml of a substrate solution (5 mM) and 0.2 ml of a 1:10 (v/v) dilution of the caecal sample in 100 mM phosphate buffer (pH 7.0) after centrifugation at 7.211 *g* for 15 min. Incubation was carried out at 37°C and *ρ*-nitrophenol was quantified at 400 nm and at 420 nm (*o*-nitrophenol concentration) after the addition of 2.5 ml of 0.25 M cold sodium carbonate. The enzymatic activity (*α*- and *β*-glucosidase, *α*- and *β*-galactosidase, and *β*-glucuronidase) was expressed as µmol product formed per min (IU) per g of digesta.

Statistical analysis

The STATISTICA software package version 8.0 (StatSoft Corp., Krakow, Poland) was used to determine if variables differed among treatment groups. Two-way ANOVA was performed to assess the effects of the dietary level of sunflower meal (0, 7, 14 and 21%), of dietary addition of enzyme mixture preparation (without and with supplementation) and of the interaction between SFM level and NSP-degrading enzyme addition (D × E) (Snedecor and Cochran, 1989). When the ANOVA indicated significant treatment effects, means were separated using Duncan's multiple range test. In the Tables, results are presented as mean values with pooled standard errors. Data were checked for normality before statistical analysis was performed. Differences were considered to be significant at $P \leq 0.05$.

RESULTS

After 8 weeks, the body weights of turkeys fed diets containing 14 and 21% SFM were significantly lower, compared with the control group (Table 2). Moreover, the birds fed the diet containing 21% of SFM were also significantly lighter than those from the SFM_7 treatment. A tendency (P=0.068) towards lower diet intake in SFM groups was observed during eight weeks of feeding. Neither SFM levels nor enzymatic supplementation affected the feed conversion ratio during the experimental period covering 8 weeks.

Table 2. The effect of different content of sunflower meal[1] (SEM) in diets on the growth performance of turkeys aged 8 weeks of age

Dietary treatment	Diet intake, kg	Body weight, kg	Feed efficiency ratio
Subgroup			
SFM_0E_0	8.01	4.13	1.94
SFM_0E+	7.58	4.21	1.80
SFM_7E_0	7.64	4.11	1.86
SFM_7E+	7.58	4.14	1.83
$SFM_{14}E_0$	7.22	4.01	1.80
$SFM_{14}E+$	7.60	4.02	1.89
$SFM_{21}E_0$	7.29	3.90	1.87
$SFM_{21}E+$	7.40	3.96	1.95
SEM[2]	0.055	0.025	0.018
Diet (D)			
SFM_0	7.80	4.17[a]	1.87
SFM_7	7.60	4.13[ab]	1.84
SFM_{14}	7.42	4.01[bc]	1.85
SFM_{21}	7.51	3.93[c]	1.91
P value	0.068	0.003	0.549
Enzymes mixtures (E)			
E_0	7.54	4.03	1.87
E+	7.65	4.09	1.87
P value	0.276	0.304	0.938
Interaction D×E	0.059	0.960	0.087

[1] SFM was applied at following dietary levels: 0, 7, 14 and 21% (SFM_0, SFM_7, SFM_{14} and SFM_{21} respectively); [2] SEM - standard error of the mean (SD for all birds divided by the square root of turkey number); values within each column with the same superscript letter are not different at P≤0.05

At 8 weeks of age, regardless of the addition of NSP-degrading enzymes, the inclusion of SFM to a diet caused a tendency towards lower ventriculus pH (P=0.069) and relative tissue mass (P=0.089), when compared to the control

treatment without SFM addition (Table 3). The turkeys fed diets containing 14 and 21% SFM were characterized by a significantly lower small intestinal tissue weight than those fed the 0 and 7% SFM diets ($P<0.05$). Dietary SFM had no effect on ileal digesta pH, dry matter concentration nor viscosity value ($P<0.05$). When diets supplementation with the enzyme preparation was taken into consideration, the effect of this additive on indices of upper gastrointestinal tract functioning was negligible, except numerically lower ileal viscosity (1.26 vs 1.36 mPa·s; $P=0.099$).

Table 3. Indices of upper GIT functioning in turkeys fed diets with increasing levels of sunflower meal[1] (SFM) without or with inclusion of feed enzymes (E)

	Crop	Ventriculus		Small intestine			
	pH of digesta	pH of digesta	tissue, g/kg BW	pH of ileal digesta	tissue g/kg BW	dry matter (ileal), %	viscosity (ileal), mPa·s
Subgroup							
SFM_0E_0	4.88	3.78	13.39	5.63	24.56	15.69	1.37
SFM_0E+	4.96	3.83	12.76	5.32	23.71	16.55	1.30
SFM_7E_0	4.91	3.71	12.77	5.53	24.57	17.19	1.44
SFM_7E+	4.92	3.62	12.58	5.23	23.72	15.68	1.27
$SFM_{14}E_0$	4.76	3.45	10.51	5.43	22.74	15.02	1.29
$SFM_{14}E+$	4.98	3.52	11.77	5.23	21.36	15.44	1.22
$SFM_{21}E_0$	4.93	3.58	10.79	5.54	22.74	16.77	1.33
$SFM_{21}E+$	4.87	3.61	10.45	5.26	21.88	16.32	1.23
SEM[2]	0.056	0.052	0.717	0.077	0.393	0.277	0.020
Diet (D)							
SFM_0	4.92	3.81	13.08	5.48	24.13[a]	16.12	1.34
SFM_7	4.91	3.67	12.68	5.38	24.15[a]	16.44	1.36
SFM_{14}	4.87	3.49	11.14	5.33	22.05[b]	15.23	1.26
SFM_{21}	4.90	3.60	10.62	5.40	22.31[b]	16.55	1.28
P value	0.505	0.064	0.089	0.509	<0.05	0.324	0.143
Enzymes mixtures (E)							
E_0	4.87	3.63	11.87	5.53	23.66	16.17	1.36
E+	4.93	3.65	11.89	5.26	22.67	16.00	1.26
P value	0.145	0.386	0.768	0.345	0.193	0.705	0.099
Interaction D×E	0.295	0.677	0.479	0.799	0.966	0.126	0.525

[1] SFM was applied at following dietary levels: 0, 7, 14 and 21% (SFM_0, SFM_7, SFM_{14} and SFM_{21} respectively); [2] SEM - standard error of the mean (SD for all birds divided by the square root of turkey number); values within each column with the same superscript letter are not different at $P\leq0.05$

After 8 weeks of experimental feeding, the lowest caecal tissue mass was found in turkeys fed the diet with highest SFM content (SFM_{21} < SFM_0 and SFM_7; P<0.05; Table 4). The mass of the caecal digesta was noted to be the highest in the control treatment and was decreasing as follows: SFM_0^a> SFM_7^b> SFM_{14}^c> SFM_{21}^c. The low dietary level of SFM (7%) elicited a significant increase in caecal dry matter concentration as compared to the other groups (P<0.05). The caecal ammonia concentration as well as colonic pH and tissue mass were not affected by the dietary treatments, either by SFM or enzyme preparation addition. The dietary application of the NSP-degrading enzymes mixture resulted in a significant increase in the pH of caecal digesta (E+ > E_0; P<0.05). The activity of bacterial β-glucosidase and β-glucuronidase was not affected by the level of dietary SFM or the application of exogenous enzymes (Table 5). On the other hand, both main

Table 4. Indices of lower GIT functioning in turkeys fed diets with increasing levels of sunflower meal[1] (SFM) without or with inclusion of feed enzymes (E)

	Caeca					Colon	
	pH of digesta	tissue, g/kg BW	digesta, g/kg BW	dry matter, %	ammonia, mg/g	pH of digesta	tissue, g/kg BW
Subgroup							
SFM_0E_0	5.93	4.59	4.71	15.68	0.73	4.92	3.56
SFM_0E+	5.91	4.38	4.79	15.82	0.70	4.90	3.48
SFM_7E_0	5.94	4.23	3.79	16.42	0.72	4.86	3.45
SFM_7E+	6.06	3.98	3.68	17.63	0.69	4.86	3.43
$SFM_{14}E_0$	5.78	3.94	2.23	15.44	0.71	4.84	3.59
$SFM_{14}E+$	6.16	3.83	2.06	15.59	0.68	4.79	3.53
$SFM_{21}E_0$	5.99	3.62	1.97	15.13	0.68	5.12	3.57
$SFM_{21}E+$	6.23	3.57	1.78	14.87	0.60	5.05	3.64
SEM[2]	0.062	0.084	0.249	0.426	0.039	0.073	0.072
Diet (D)							
SFM_0	5.92	4.49^a	4.75^a	15.75^b	0.71	4.91	3.52
SFM_7	6.00	4.11^a	3.74^b	17.03^a	0.71	4.86	3.44
SFM_{14}	5.97	3.89^{ab}	2.15^c	15.52^b	0.70	4.82	3.56
SFM_{21}	6.11	3.60^b	1.88^c	15.00^b	0.64	5.08	3.61
P value	0.756	<0.05	<0.05	<0.05	0.310	0.232	0.666
Enzymes mixtures (E)							
E_0	5.91^b	4.09	3.18	15.67	0.71	4.94	3.54
E+	6.09^a	3.94	3.08	15.98	0.67	4.90	3.52
P value	<0.05	0.314	0.789	0.291	0.407	0.945	0.742
Interaction D×E	0.482	0.527	0.550	0.385	0.460	0.981	0.628

[1]SFM was applied at following dietary levels: 0, 7, 14 and 21% (SFM_0, SFM_7, SFM_{14}, and SFM_{21}, respectively); [2]SEM, standard error of the mean (SD for all birds divided by the square root of turkey number); values within each column with the same superscript letter are not different at P≤0.05

Table 5. Activity of bacterial enzymes in caecal digesta (U/g) of turkeys fed diets with increasing levels of sunflower meal[1] (SFM) without or with inclusion of feed enzymes (E)

Dietary treatment	Glucosidase		Galactosidase		β-glucuronidase
	α-	β-	α-	β-	
Subgroup					
SFM_0E_0	0.17	0.06	0.46	0.74	0.27
SFM_0E+	0.22	0.06	0.66	1.46	0.21
SFM_7E_0	0.26	0.09	0.63	0.99	0.30
SFM_7E+	0.31	0.08	0.90	1.61	0.26
$SFM_{14}E_0$	0.24	0.06	0.54	0.79	0.32
$SFM_{14}E+$	0.31	0.06	0.71	0.95	0.27
$SFM_{21}E_0$	0.21	0.05	0.31	0.68	0.32
$SFM_{21}E+$	0.21	0.05	0.32	0.74	0.26
SEM[2]	0.009	0.007	0.030	0.062	0.023
Diet (D)					
SFM_0	0.19^b	0.06	0.56^b	1.10^a	0.24
SFM_7	0.29^a	0.08	0.77^a	1.30^a	0.28
SFM_{14}	0.27^a	0.06	0.63^b	0.87^{ab}	0.30
SFM_{21}	0.21^b	0.05	0.32^c	0.71^b	0.29
P value	<0.05	0.417	<0.05	<0.05	0.843
Enzymes mixtures (E)					
E_0	0.22^b	0.07	0.49^b	0.80^b	0.30
E+	0.26^a	0.06	0.65^a	1.19^a	0.25
P value	<0.05	0.801	<0.05	<0.05	0.307
Interaction D×E	0.341	0.995	0.129	0.111	0.998

[1]SFM was applied at following dietary levels: 0, 7, 14 and 21% (SFM_0, SFM_7, SFM_{14} and SFM_{21}, respectively); [2] SEM - standard error of the mean (SD for all birds divided by the square root of turkey number); values within each column with the same superscript letter are not different at P≤0.05

enzymes (Table 5). On the other hand, both main experimental factors (SFM and enzyme preparation) significantly influenced the activities of bacterial α-glucosidase, α-galactosidase and β-galactosidase in the caecal digesta. When the level of SFM in a diet was taken into account, the highest activity of α-glucosidase was observed in turkeys fed diets with low and moderate SFM content (SFM_7, SFM_{14} > SFM_0, SFM_{21}; P<0.05). The birds from the SFM_7 and SFM_{21} groups displayed the highest and the lowest α-galactosidase activity, respectively (in both cases P<0.05 vs other treatments). The SFM_{21} group was also characterized by the lowest β-galactosidase activity (P<0.05 vs SFM_0 and SFM_7 groups). The supplementation of diets with the NSP-degrading

enzyme preparation resulted in enhanced activities of bacterial α-glucosidase, α-galactosidase and β-galactosidase in the caecal digesta of the turkeys ($P<0.05$). Regardless the addition of the NSP-degrading enzyme preparation, the increased SFM content in a diet up to 14-21% caused a significant decrease in the total concentration as well as content (pool) of VFA in the caeca in comparison to the control and SFM_7 treatments (Table 6). It was mainly due to the enhanced concentration of acetic acid and - to a lower extent - of valeric acids (SFM_0 and SFM_7 vs SFM_{14} and SFM_{21} groups; $P<0.05$). Moreover, the SFM_7 treatment was accompanied by the highest caecal propionate concentration as compared with the other groups ($P<0.05$). When the enzyme preparation applied

Table 6. Concentration (μmol/g fresh digesta), total content (μmol/kg body weight) of volatile fatty acids (VFA) in the caeca of turkeys fed experimental diets

Subgroup, Treatments	Total VFA	C2	C3	C4i	C4	C5i	C5	VFA pool
Subgroup								
SFM_0E_0	133	84.0^a	10.6^c	1.71	31.3	1.45	4.07	627
SFM_0E+	120	69.8^b	13.9^c	1.87	27.2	2.37	4.93	574
SFM_7E_0	140	80.6^a	22.8^a	1.76	27.4	2.10	5.68	531
SFM_7E+	131	78.7^{ab}	17.8^b	1.85	25.9	2.28	4.73	182
$SFM_{14}E_0$	121	71.5^{ab}	14.5^{bc}	1.63	27.7	1.71	4.36	270
$SFM_{14}E+$	104	64.5^b	12.4^c	1.35	21.6	1.73	2.87	214
$SFM_{21}E_0$	111	66.9^b	13.1^c	1.64	24.5	1.65	3.11	219
$SFM_{21}E+$	115	71.3^{ab}	13.0^c	1.63	23.8	2.06	2.85	205
SEM[2]	2.342	1.510	0.630	0.073	0.853	0.098	0.211	29.25
Diet (D)								
SFM_0	127^a	76.9^a	12.3^b	1.79	29.3	1.91	4.50^a	601^a
SFM_7	136^a	79.7^a	20.3^a	1.81	26.7	2.17	5.21^a	507^a
SFM_{14}	113^b	68.0^b	13.5^b	1.49	24.7	1.72	3.62^b	242^b
SFM_{21}	113^b	69.1^b	13.1^b	1.64	24.2	1.86	2.98^b	212^b
P value	<0.05	<0.05	<0.05	0.386	0.330	0.079	<0.05	<0.05
Enzymes mixtures (E)								
E_0	126^a	75.8	15.3	1.69	27.7^a	1.73^b	4.31	412
E+	118^b	71.1	14.3	1.68	24.6^b	2.11^a	3.85	369
P value	<0.05	0.663	0.663	0.982	<0.05	<0.05	0.284	0.242
Interaction D×E	0.173	<0.05	<0.05	0.699	0.474	0.076	0.061	0.944

[1] SFM was applied at following dietary levels: 0, 7, 14 and 21% (SFM_0, SFM_7, SFM_{14} and SFM_{21}, respectively); [2] SEM - standard error of the mean (SD for all birds divided by the square root of turkey number); values within each column with the same superscript letter are not different at $P\leq0.05$

to a diet was taken into consideration, the E+ treatment caused a significant decrease in the total VFA and butyric acid as well as an increase in isovaleric acid concentrations ($P<0.05$ vs E_0 treatment). An interaction between SFM content and enzymatic supplementation affected the caecal concentrations of acetic and propionic acids. In contrast to the control treatment, the addition of enzymes to diets with a low (7%), moderate (14%) and high (21%) sunflower meal content did not affect C_2 caecal concentration significantly ($P<0.05$). The concentration of this acid in the SFM_0E_0 subgroup was significantly higher than in the birds fed the SFM_0E+ diet. Alike differences were noted between subgroups SFM_7E_0 and SFM_7E+ with regard to the concentration of caecal propionic acid.

DISCUSSION

At the termination of the performance part of this study we observed that the body weight (BW) of 8-wk turkeys fed diets with a relatively high sunflower meal (SFM) content may be depressed in comparison to the BW of control birds receiving the soyabean meal-based diet. Therefore, a special attention was paid to the gastrointestinal tract response of growing turkeys fed diets with different contents of SFM added at the expense of soyabean meal and partly of wheat. The efficacy of NSP-degrading enzymes application was assessed as well.

As for the applied enzyme supplementation, the data obtained revealed that the enzymatic mixture added to a diet caused a tendency towards lower ileal viscosity ($P=0.099$) and a significant decrease in caecal total volatile fatty acids (VFA) concentration, despite an increase in the activities of bacterial α-glucosidase, α-galactosidase and β-galactosidase observed in the E+ treatment. The decrease in VFA concentration was probably the main reason for an increase in caecal pH value observed in E-supplemented treatment. The minor effect of the enzyme preparation on ileal viscosity was in accordance with a relatively small content of soluble NSP in experimental diets. It should be emphasized, however, that the viscosity values observed in the current study were relatively low (1.22-1.44 mPa·s) and lower than the values of 3.0 to 5.0 mPa·s observed in broiler chickens and growing turkeys fed wheat-based diets (Juśkiewicz et al., 2005; Meng et al., 2005). Furthermore, such low intestinal viscosity values have been reported to have a minimal, if any, effect on growth performance of broilers (Liang and Liu, 1988).

Following the increasing dietary level of SFM in a diet, a considerable decrease in relative tissue mass of ventriculus, small intestine, and caeca, expressed as a percentage of BW, was observed after 8 weeks of feeding in turkeys exposed to the higher doses of SFM. It is common knowledge that it is not only the level

of dietary fibre that is of paramount importance for gastrointestinal tract (GIT) segments development, but the type or the source of fibre also plays a significant role in digestion and absorption (Wenk, 2001). In comparison to soyabean meal, sunflower meal used in this study contained more total NSP (27.0 vs 17.9%), more lignin (8.6 vs 0.5%), and more crude fibre (15.3 vs 4.8%). SBM exceeded SFM only in respect of the content of water-soluble NSP (1.05 vs 0.78%). Therefore, diets with SFM were characterized by an increasing level of crude fibre, lignin and total NSP, but a lower content of water-soluble NSP (Table 1). Our findings are in contradiction with the suggestion made by Dusterhoft et al. (1992) that the presence in the sunflower seeds of a substantial amount of cell-wall material, mainly cellulose, pectic polysaccharides and 4-0-methyl-glucuroxylans, with small amounts of glucomannans and fucoxyloglucans, could exert a trophic effect on the intestinal mucosa, increasing the intestinal size. Other authors reported a different response of intestinal organs to sunflower products. Arija et al. (2000) showed the shortening and thickening of the jejunal villi caused by the inclusion of full-fat sunflower kernels in chicken diets. In turn, Brenes et al. (2008) observed a decrease in the size of the small intestine (particularly the duodenum) and caeca in chickens fed 15% high oleic acid sunflower seeds. One explanation to the lower weight of GIT segments, observed in our study, could be faster passage of digesta through the gastrointestinal tract in the SFM-fed birds, as supported by the significantly decreased digesta mass in the caeca. Another reason for the foregoing effect might be the lower content of soluble polysaccharides in diets containing sunflower meal in comparison to the control SBM-containing diet. It is well established that water-soluble polysaccharides may evoke a considerable increase in the weight of caecal tissue and digesta in monogastric animals, in a big part due to an increased bacterial number and greater fermentation in the lower parts of GIT (Juśkiewicz and Zduńczyk, 2004). Some researchers reported that a diet supplementation with non-digestible carbohydrate fractions may result in an enhanced glycolytic activity of microorganisms living in the large intestines of monogastric animals (Monsan and Paul, 1995). In our experiment, the highest activities of bacterial α-glucosidase, α-galactosidase and β-galactosidase were observed when SFM_7 treatment was applied. β-galactosidase, α-galactosidase and α-glucosidase activities can improve the fermentation of lactose, raffinose family oligosaccharides and resistant starch, leading to VFA and lactic acids which are the source of energy for the tissues. However, the activities of potentially-harmful β-glucuronidase and β-glucosidase were similar in all groups, which should be considered as a positive effect of sunflower meal addition to a diet for turkeys. Both β-glucuronidase and β-glucosidase have been implicated in the generation

of mutagens or carcinogens in the hindgut (Campbell et al., 1997). It could be assumed that well-balanced content of dietary insoluble and soluble non-digestible carbohydrates may positively affect the activity of enzymatic microbiota.

When large amounts of fermentable carbohydrates were fed, the increased caeca weights (especially tissue) were considered as a positive physiological response associated with some beneficial changes in the large intestine, e.g., acidification of digesta, increased VFA contents (Jankowski et al., 2009). In the present study, the application of diets containing increasing levels of SFM was indeed in reverse relation to VFA concentration and pool. In the presence of different fermentable carbohydrates, the VFA pool size rather than the individual fatty acid concentration has been postulated to be the best indicator of the intensity of colonic fermentation (Campbell et al., 1997). In the 8-wk-old turkeys, the VFA pool in the birds fed the SFM_{14} and SFM_{21} treatments was more than 2+fold lower than that of the birds fed the SFM_7 and SFM_0 diets. Our recent study on growing turkeys demonstrated that, in contrast to dietary crude fibre applied at the doses of 3.5 or 5.3%, the different dietary level of soyabean α-gactosides (0.06, 0.93, 1.69 and 2.33%) caused a distinct response of turkeys caeca (Zduńczyk et al., in press). In that study we concluded that soyabean raffinose-family oligosaccharides seemed to greatly affect the development and physiology of the GIT, and that their presence in the amount of approximately 1% in a diet for growing turkeys ought to be considered as nutritionally advisable. The present experiment proved that sunflower meal added at the level of 14 or 21% to a diet delivered excessive amounts of crude fibre, lignin, and water-insoluble non-starch polysaccharides, which in turn inhibited the fermentation processes in the caeca and, to some extent, impaired the development of the upper GIT segments in growing turkeys.

CONCLUSIONS

In conclusion, sunflower meal (SFM) rich in crude fibre and lignin added at the level of 14-21% to a diet for growing turkeys may induce undesirable processes in the gastrointestinal tract development manifested in decreased relative mass of small intestine and caecal tissues, as well as in potent inhibition of the fermentation processes in the caeca. Having in mind a worsening in birds' growth observed during the performance stage of the study, it should be concluded that the dietary SFM level at 14% and more seems to be an overdose for young growing turkeys. On the other hand, our study showed also that high fibre SFM could be an effective alternative substitute of soyabean meal for growing turkeys when applied at the dietary level of up to 7%.

REFERENCES

AOAC, 2005. Association of the Official Analytical Chemists, Official Methods of Analysis. 18th Edition. Arlington, VA

Arija I., Viveros A., Brenes A., Canales R., Pizarro M., Castano M., 2000. Histological alterations in the intestinal epithelium caused by the inclusion of full-fat sunflower kernels in broiler chicken diets. Poultry Sci. 79, 1332-1334

Bach Knudsen K.E., 2001. The nutritional significance of "dietary fibre" analysis. Anim. Feed Sci. Tech. 90, 3-20

Brenes A., Centeno C., Viveros A., Arija I., 2008. Effect of enzyme addition on the nutritive value of high oleic acid sunflower seeds in chicken diets. Poultry Sci. 87, 2300-2310

Campbell J.M., Fahey Jr. G.C., Wolf B.W., 1997. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J. Nutr. 127, 130-136

Dusterhoft E.M., Posthumus M.A., Voragen A.G.J., 1992. Non-starch polysaccharide from sunflower (*Helianthus Annuus*) meal and palm-kernel (*Elaeis guineensis*) meal. II. Investigation of the structure of major polysaccharides. J. Sci. Food Agr. 59, 151-160

Hofirek B., Haas D., 2001. Comparative studies of ruminal fluid collected by oral tube or by puncture of the caudorental ruminal sac. Acta Vet. Brno 70, 27-33

Jankowski J., Juśkiewicz J., Gulewicz K., Lecewicz A., Slominski B.A., Zduńczyk Z., 2009. The effect of diets containing soybean meal, soybean protein concentrate, and soybean protein isolate of different oligosaccharide content on growth performance and gut function of young turkeys. Poultry Sci. 88, 2132-2140

Juśkiewicz J., Jankowski J., Zduńczyk Z., Lecewicz A., Przybylska-Gornowicz B., Zięba M., 2009. Effect of diets with different contents of soybean α-galactosides and crude fibre on modification of duodenal microstructure and selected parametrs of nutrient utilization in young turkeys. Pol. J. Vet. Sci. 12, 455-463

Juśkiewicz J., Jankowski J., Zduńczyk Z., Mikulski D., 2006. Performance and gastrointestinal tract metabolism of turkeys fed diets with different content of fructooligosaccharide. Poultry Sci. 85, 886-891

Juśkiewicz J., Mikulski D., Jankowski J., Zduńczyk Z., 2005. Gastrointestinal responses of turkeys to the addition of exogenous xylanase and glucanase to diets. J. Anim. Feed Sci. 14, Suppl. 1, 451-454

Juśkiewicz J., Zduńczyk Z., 2004. Effects of cellulose, carboxymethylcellulose and inulin fed to rats as single supplements or in combinations on their caecal parameters. Comp. Biochem. Physiol. Pt. A 139, 513-519

Liang C., Liu Y., 1988. AME examined in relation to wheat-fed broilers. Feedstuffs 60 (5), 12-15

Meng X., Slominski B.A., Nyachoti M., Campbell L.D., Guenter W., 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poultry Sci. 84, 37-47

Monsan P.F., Paul F., 1995. Oligosaccharide feed additives. In: R.J. Wallace, A. Chesson (Editors). Biotechnology in Animal Feeds and Feeding. Weinheim and New York: VCH Verlagsgesellschaft mBH, pp. 233-245

Musthaq T., Sarwar M., Ahmad G., Nisa M.U., Jamil A., 2006. The influence of exogenous multienzyme preparation and graded levels of digestible lysine in sunflower meal-based diets on the performance of young broiler chicks two weeks posthatching. Poultry Sci. 85, 2180-2185

NRC, 1994. Nutrient Requirements of Poultry. National Research Council. 9th Edition. National Academy Press. Washington, DC

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

Snedecor G.W., Cochran W.G., 1989. Statistical Methods. 8th Edition. Ames, Iowa (USA)

Stodolak B., Starzyńska-Janiszewska A., Pustkowiak H., Mickowska B., 2009. Effect of sunflower seeds addition on the nutritional value of grass pea tempeh. Pol. J. Food Nutr. Sci. 59, 145-150

Vieira S.L., Penz Jr. A.M., Leboute E.M., Corteline J., 1992. A nutritional evaluation of a high fiber sunflower meal. J. Appl. Poultry Res. 1, 382-388

Wenk C., 2001. The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Tech. 90, 21-33

Zduńczyk Z., Jankowski J., Juśkiewicz J., Lecewicz A., Slominski B., (in press). Application of soybean meal, soy protein concentrate and isolate differing in α-galactosides content to low- and high-fibre diets in growing turkeys. J. Anim. Physiol. Anim. Nutr. (DOI: 10.1111/j.1439-0396.2009.00939)