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RESPONSE OF THE CAECAL MICROFLORA OF TURKEYS FED DIETS WITH A DIFFERENT CONTENT OF HIGH-FIBRE SUNFLOWER MEAL*

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Abstract

The effect of different dietary content of sunflower meal (SFM; 0, 7, 14 and 21%) on fermentation processes and caecal microflora in 8-week-old male turkeys was investigated. All diets were isonitrogenous and isocaloric, but they differed substantially with regard to crude fibre content (2.92%, 4.18%, 4.72% and 5.56% on average in groups SFM_0, SFM_7, SFM_{14} and SFM_{21}, respectively). After 8 weeks of feeding, with increasing levels of SFM a linear decrease ($P<0.05$) was observed in feed intake and final body weight of turkeys. The dietary inclusion of SFM_{14} and SFM_{21} caused a significant decrease in caecal relative tissue mass, caecal digesta weight and the rate of bacterial production of SCFA in the caeca, as compared with the control and SFM_7 groups. The highest activity of bacterial α-glucosidase, α-galactosidase and β-galactosidase was noted in the SFM_7 group. In comparison with the soybean meal-based diets, the counts of *Escherichia coli* and *Bacteroides-Prevotella* significantly increased in the SFM_{21} treatment. The results of this study demonstrate that turkey diets can be effectively supplemented with high-quality sunflower meal at a concentration of approximately 70 g/kg. High-fibre sunflower meal added at a level of 21% to a diet for growing turkeys may cause undesirable changes in the caecal microflora activity and profile as manifested in the potent inhibition of SCFA production and enhanced growth of *Bacteroides* and *Escherichia coli* populations. It should be noted, however, that at 8 weeks of age the body weight of turkeys fed diets containing 210 g/kg of SFM could be lower by 6% from those receiving the soybean meal-based diets.

Key words: turkey, sunflower meal, caeca fermentation, microflora, performance

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Changes in diet composition might affect the rate of food passage, microbial growth and the health of the gastrointestinal tract (Santos et al., 2006). In broiler chickens, when the diet contained indigestible carbohydrates which increase the viscosity of the GIT digesta, active microflora was already present in the ileum, as measured by the level of short-chain fatty acid production (Choct et al., 1995). It has long been a well known fact that high levels of non-starch polysaccharides (NSP), especially the water-soluble fraction of NSP correlated closely with the viscosity of digesta in the small intestine, result in decreased nutrient digestion and absorption and cause lower weight gain and poorer feed conversion in poultry (Bedford and Classen, 1992).

The main source of indigestible carbohydrates are cereals; however, high-protein components can significantly increase the pool of these carbohydrates. Soybean meal (SBM), commonly used in animal nutrition, contains over 20% of NSP and up to 6% of oligosaccharides (Bach Knudsen, 1997). Potential substitutes of SBM, including sunflower meal (SFM), usually contain more indigestible carbohydrates, determined as NSP or crude fibre (CF). The CF content of SFM, depending on the chemical composition of seeds and processing technology, ranges from 19% to over 30% (Villamide and San Juan, 1998), i.e. it is several times higher compared with SBM (Grieshop et al., 2003). For this reason, SFM inclusion in the diet results in a significant increase in the content of indigestible carbohydrates, mainly NSP, whose physiological properties are little known.

Most of the carbohydrates that may be fermented in the avian GIT are classified as dietary fibre (DF). The DF consists of NSPs together with non-carbohydrate compounds including lignin, protein, fatty acids, and waxes to which DF is intricately bound (Bach Knudsen, 2001). The results of experiments indicate that excessive amounts of dietary fibre adversely affect nutrient digestibility and reduce the growth performance of broiler chickens and turkeys (Santos et al., 2004). It was also observed that dietary NSP significantly increased the intestinal populations of pathogenic bacteria at the expense of beneficial ones (Langhout et al., 2000). The inclusion of fibre in diets may also have positive non-nutritional effects in animals. Viveros et al. (1994) reported that high concentrations of NSP might stimulate the development of goblet cells on the epithelial gut surface. Persia et al. (2002) also demonstrated that dietary fibre is beneficial to the intestinal microbial ecology and may suppress the colonization of enteric pathogens that adversely affect the health and welfare of turkeys. These beneficial properties of dietary fibre may be associated with the end-products of fermentation. It is well accepted that the caeca are the principal site of fermentation processes in the avian (broiler chicken and turkey) gastrointestinal tract. In the caeca, undigested carbohydrates (DF fractions) can be fermented by the commensal microflora and transformed into short-chain fatty acids (SCFA) and gases (Montagne et al., 2003; Józefiak et al., 2004; Zduńczyk et al., 2010).

The above controversy indicates the need for further investigations into this area, especially since the physiological consequences of an increased crude fibre content of diets for young turkeys, supplemented with sunflower meal, remain poorly known.

Therefore, the objective of the present study was to investigate the response of the microbial ecosystem, indices of fermentation processes in the caeca and performance in young turkeys (to 8 weeks of age) to diets with a different content of sunflower meal used as a substitute for soybean meal.

Material and methods

The experimental procedure was approved by the Local Ethics Committee in the University of Warmia and Mazury in Olsztyn. A total of 756 one-day-old heavy-type Big-6 turkey males were randomly assigned to four dietary treatments, each of 7 pens per treatment and 27 birds per pen, and they were raised on deep litter. Briefly, turkeys were housed in an environmentally-controlled room with a 16-h light and 8-h dark cycle and a temperature from 32°C at the beginning to 22°C at the end of week 8. At the beginning of the experiment, the poults were vaccinated against infectious rhinotracheitis using the Aviffa-RTI vaccine.

In two rearing periods (weeks 1–4 and 5–8), turkeys were fed isoenergetic diets containing 28 and 26% crude protein, respectively, covering the nutrient requirements for turkeys (NRC, 1994). They had free access to feed and water. Commercially available soybean (from the local feed company) and sunflower (from WIOJL-AGRO Co., Vinnytsia, Ukraine) meals were used in this experiment. Diets varied with respect to the content of structural carbohydrates determined as non-starch polysaccharides, crude fibre, total dietary fibre and lignin. In addition, diets differed in crude fat content, because the inclusion of SFM which contributes less energy than SBM required larger quantities of high-fat feed in the ration.

Table 1. Chemical composition of sunflower meal and soybean meal used in the study (g/kg, as fed basis)

Component	Soybean meal	Sunflower meal
Crude protein	467.5	377.8
Ether extract	25.9	19.1
Ash	58.9	64.8
Oligosaccharides	56.2	15.4
Dietary fibre fractions		
Crude fibre	47.7	152.7
Nonstarch polysaccharides (NSP)	178.5	270.2
Water-soluble NSP	10.5	7.8
Water-insoluble NSP	168.0	262.4
Lignin	5.0	85.7
Total dietary fibre	197.4	350.5

Table 2. Composition of experimental diets

	Starter (0 to 4 wk)				Grower (5 to 8 wk)			
	SFM_0	SFM_7	SFM_{14}	SFM_{21}	SFM_0	SFM_7	SFM_{14}	SFM_{21}
Ingredient (g/kg):								
Wheat	254.8	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
Soybean meal (SBM)	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	-	-	-	-
Soybean 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
Vitamin and mineral premix1	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	280.2	260.0	260.0	260.0	260.0
Lysine	16.0	16.0	16.0	16.0	15.0	15.0	15.0	15.0
Methionine + Cystine	10.5	10.5	10.5	10.5	9.5	9.5	9.5	9.5
Threonine	11.0	10.9	10.9	10.9	9.6	9.5	9.5	9.5
Tryptophan	3.6	3.6	3.6	3.6	3.3	3.3	3.2	3.2
Calcium	12.1	12.1	12.0	12.0	10.0	10.0	10.0	10.0
Available phosphorus	6.1	6.0	6.0	6.0	5.0	5.0	5.0	5.0
Sodium	1.7	1.7	1.7	1.7	1.5	1.5	1.5	1.5
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	279.0	282.2	280.4	281.3	268.1	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 dietary 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 weeks of feeding the vitamin and mineral premix supplied per kg of diet, IU: vit. A (all-*trans* retinol acetate) 15,000 and 13,000; mg: vit. E (all-*rac*-*α*-tocopherol acetate) 40 and 35, respectively. For 1 to 8 weeks 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 (menadione nicotinamide bisulfate) 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 chloride 600.

For chemical analysis, the samples were ground to pass through a 0.5 mm sieve. Feed ingredient (SFM and SBM) or diet samples were analysed in duplicate for dry matter, crude protein, fat, and crude fibre using AOAC (2005) methods. NSP, were determined by gas-liquid chromatography using the procedure described by Słomiński and Campbell (1990). The composition of feed ingredients (SFM and SBM) and experimental diets with a different content of SFM (0, 7, 14 and 21%) is

given in Tables 1 and 2, respectively. After 8 weeks of feeding, 10 birds representing an average body weight of each group were sacrificed by cervical dislocation. After laparatomy, the caeca were removed and weighed. As soon as possible after euthanasia (ca. 20 min), caecal pH was measured using a microelectrode and a pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal). Samples of the caecal contents were used for immediate analysis of dry matter, ammonia, bacterial enzymes and volatile fatty acids (VFA), while the rest of the caecal digesta were transferred to tubes and stored at –70°C. The caeca were flushed with water, blotted on filter paper and weighed as the tissue mass.

Dry matter of caecal digesta was determined at 105ºC. In fresh samples, ammonia was extracted, trapped in a solution of boric acid in Conway dishes, and determined by direct titration with sulphuric acid (Hofirek and Haas, 2001). Bacterial glycolytic activity in the caecal digesta was measured by the rate of *ρ*- or *o*- nitrophenol release from their nitrophenylglucosides according to the method described by Zduńczyk et al. (2006). 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.

Caecal digesta samples were assayed for the concentrations of volatile fatty acids (VFA) by 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 it was raised to 180ºC by 8ºC/min and held there for 3 min. The temperatures of the flame ionization detector and the injection port were 180 and 85ºC, respectively. The sample volume for GC analysis was 1 µL.

The caecal microflora was investigated by fluorescent in situ hybridization (FISH) with specific 16S rRNA-targeted oligonucleotide probes (Table 3). Samples of digesta were weighed and they were immediately placed in sterile bags with phosphate-buffered saline (PBS) to give a 20-fold dilution. After decantation, the supernatant was collected and fixed overnight at 4°C with a 4% (wt/vol) fresh paraformaldehyde solution. The samples were filtered through 0.2 µm pore-size polycarbonate membrane filters (Millipore), by applying gentle vacuum (less than 80 mmHg) to avoid the disruption of fragile cell. Oligonucleotide probes were synthesized with Cy3 fluorescent dye at 5' end (Thermo ElectronGmbH, Germany). Permeabilization of membrane filters was conducted with 1 ml of TE-HIS buffer (100 mM Tris-HCL [pH 8.0], 50 mM EDTA) and lysozyme solution (1 mg ml^{-1}) in fresh TE-HIS buffer for 10 min. at room temperature to identify Gram-positive bacteria. Formamide concentration and hybridization temperature were applied according to references, to achieve optimal stringency (Olsen et al., 2008). Bacterial cells on the filter sections were observed with an epifluorescence microscope (BX 51, Olympus, Japan) equipped with filter sets for DAPI (Ex 330 – 380 nm, DM – 400 nm, BA – 420 nm) and for CY3 (Ex 450 – 490 nm, DM – 505 nm, BA – 520 nm). The total number of bacteria present in caecal digesta was determined by the method of Porter and Feig (1980). In our study, automated image analysis software was developed for bacteria after staining with DNA-intercalating dye 4'–6-diamidino 2-phe-

nylindole (DAPI), and after *in situ* hybridization with Cy3-labelled rRNA-targeted oligonucleotide probes.

Table 3. FISH oligonucleotide probes[1]

Probe	Sequence (5`-3`)	Groups targeted
Eub338	GCTGCCTCCCGTAGGAGT	*Bacteria*
Non338	GCACGGAGGGCATCCTCA	Negative control
Bif164	CATCCGGCATTACCACCC	*Bifidobacterium*
Bac1080	GCACTTAAGCCGACACCT	*Bacteroides, Porphyromonas, Prevotella*
Lab158	GGTATTAGCA(C/T)CTGTTTCCA	*Lactobacillus-Enterococcus*
Erec482	GCTTCTTAGTCAGGTACCG	*Eubacterium rectale-Clostridium coccoides*
Chis150	TTATGCGGTATTAATCTYCCTTT	*Clostridiaceae*
E367	GACCTCGGTTTAGTTCACAGA	*Escherichia coli*

[1]FISH = fluorescent in situ hybridization.

Data were checked for normality before statistical analysis using Shapiro-Wilk test. In the case of bacterial count, a trait that does not show normal distribution, the statistical analysis was preceded by data transformation (x = log 10y, where y = trait value), and was returned to the original scale. Statistical analyses of the effects of dietary SFM were achieved as a completely randomized design using the General Linear Model procedure of Statistica 8.0 software. The linear and quadratic contrasts between diet groups were estimated for testing the trends. In case of a significant treatment effect, the post-hoc Newman-Keuls test was used to determine differences between SFM treatment groups. Test results were considered significant if $P<0.05$. All data were expressed as mean values with pooled standard errors.

Results

In comparison to soybean meal, sunflower meal used in this study (Table 1) 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 2).

After 8 weeks of experimental feeding, with increasing levels of SFM a linear decrease ($P<0.05$) was observed in feed intake and final body weight of turkeys (Table 4). The body weight and feed intake of turkeys fed diets containing 21% SFM were significantly lower, compared with the control treatment.

With increasing levels of SFM in a diet, there was a linear decrease ($P<0.05$) in the caecal tissue and caecal digesta weight (Table 5). The lowest caecal tissue mass was found in turkeys fed a diet with the highest SFM content ($SFM_{21} < SFM_0$ and SFM_7; $P<0.05$). The highest mass of the caecal digesta was noted in the control treatment and it decreased as follows: $SFM_0^a > SFM_7^a > SFM_{14}^b > SFM_{21}^b$. The caecal pH values, dry matter and ammonia concentrations as well as the activity of bacte-

rial *β*-glucosidase and *β*-glucuronidase in the caecal digesta were not affected by the dietary treatments. The highest activity of α-glucosidase was observed in turkeys fed diets with a low and moderate SFM content (SFM_7, SFM_{14} > SFM_0; P<0.05). SFM_7 group birds displayed the highest α-galactosidase (P<0.05 vs. SFM_0 and SFM_{21}) and *β*-galactosidase (P<0.05 vs. all treatments) activities. The SFM_{21} group was characterized by the lowest α-galactosidase activity (P<0.05 vs. all groups).

Table 4. The effect of different content of sunflower meal in diets on the growth performance of turkeys at 8 weeks of age

	Dietary treatment1				SEM[2]	P-value	
	SFM_0	SFM_7	SFM_{14}	SFM_{21}		linear	quadratic
Diet intake (kg/bird)	7.77 a	7.41 ab	7.04 b	7.14 b	0.098	0.008	0.197
Body weight (kg)	4.13 ab	4.11 ab	4.01 bc	3.90 c	0.036	0.016	0.511
Feed conversion ratio (kg/kg)	1.94	1.86	1.81	1.87	0.029	0.337	0.226

[1] SFM was applied at the 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).

Means within a row with different superscripts differ at P<0.05.

Table 5. Indices of caeca function in turkeys fed experimental diets

	Treatment[1]				SEM[2]	P-value linear	P-value quadrat
	SFM_0	SFM_7	SFM_{14}	SFM_{21}			
Tissue weight[3]	4.59 a	4.23 ab	3.94 bc	3.62 c	0.111	0.001	0.930
Digesta weight[3]	4.71 a	3.79 a	2.23 b	1.97 b	0.268	0.001	0.380
Dry matter (%)	15.7	16.4	15.4	15.1	0.432	0.516	0.560
Ammonia (mg/100g)	73.0	71.7	71.3	68.5	1.988	0.450	0.863
pH of digesta	5.91	6.06	6.16	6.23	0.078	0.148	0.781
Bacterial enzyme activity (U/g of the digesta)							
α-glucosidase	0.17 b	0.26 a	0.24 a	0.21 ab	0.012	0.318	0.016
β-glucosidase	0.06	0.09	0.07	0.05	0.011	0.650	0.422
α-galactosidase	0.46 b	0.63 a	0.55 ab	0.31 c	0.031	0.020	0.002
β-galactosidase	0.74 b	0.99 a	0.79 b	0.68 b	0.037	0.214	0.009
β-glucuronidase	0.27	0.30	0.32	0.32	0.035	0.598	0.808
Concentration of VFA (μmol/g of fresh digesta)							
Acetate	84.0 a	80.6 ab	71.5 bc	66.9 c	2.067	0.001	0.850
Propionate	10.6 b	22.8 a	14.5 b	13.1 b	1.030	0.882	0.001
Iso-butyrate	1.71	1.76	1.63	1.64	0.097	0.718	0.911
Butyrate	31.3	27.4	27.7	24.5	1.329	0.102	0.890
Iso-valerate	1.45	2.10	1.71	1.65	0.117	0.823	0.134
Valerate	4.07 b	5.68 a	4.36 ab	3.10 b	0.290	0.071	0.008
Total VFA	133 ab	140 a	121 bc	111 c	3.246	0.001	0.108
VFA pool (μmol/kg of BW)	627 a	534 a	282 b	218 b	41.21	0.001	0.811

[1]SFM was applied at the 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). 3g/kg of BW.

Means within a row with different superscripts differ at P<0.05.

An increase in the SFM content of the diet caused a linear decrease (P<0.05) in the total concentration as well as content (pool) of VFAs in the caecal digesta of turkeys. VFA concentrations in the SFM_{21} group were also significantly lower than in the control birds. In comparison with the control and SFM_7 groups, the total concentrations of VFAs in the caeca and total caecal VFA pool, expressed as μmol per kg of body weight, was significantly diminished by the dietary treatments with a moderate (14%) and high (21%) content of sunflower meal. This was mainly due to the enhanced production of acetic acid and, to a lower extent, of other acids.

Table 6. Median bacterial number, cells/g-1of wet weight of turkey caeca (x 109)

Bacterial group or parameter	Treatment[1]				SEM[2]	P-value	
	SFM_0	SFM_7	SFM_{14}	SFM_{21}		linear	quadratic
Total cells	12.65	14.19	11.66	14.31	0.656	0.677	0.678
Bacteria	8.90	9.68	8.09	9.33	0.439	0.941	0.801
Bacteroides/Prevotella	0.67 b	0.55 b	0.69 b	2.34 a	0.256	0.014	0.055
Eubacterium rectale/ Clostridium coccoides	2.12	2.87	1.85	2.39	0.204	0.913	0.803
Bifidobacterium	1.61	1.77	0.98	0.72	0.202	0.056	0.602
Lactobacillus/Enterococcus	2.18	2.27	1.72	1.13	0.254	0.112	0.514
Escherichia coli	0.03 b	0.04 b	0.01 b	0.17 a	0.022	0.030	0.080
Clostridiaceae	0.01	0.02	0.03	0.04	0.006	0.089	0.869

[1] SFM was applied at the 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).

Means within a row with different superscripts differ at P<0.05.

An increase in the SFM content of the diet caused a linear increase (P<0.05) in the numbers of *Bacteroides-Prevotella* and coliforms in the caeca of turkeys (Table 6). It corresponded with a tendency towards lower counts of *Bifidobacterium* (P = 0.056) and *Lactobacillus/Enterococcus* groups (P = 0.112). The highest count of *Bacteroides-Prevotella* and coliforms was noted in the caeca of turkeys fed SFM_{21} diet, compared with the groups fed diets with SFM_0, SFM_7, SFM_{14}.

Discussion

At the termination of the performance part of this study we observed that the body weight (BW) of 8-week-old turkeys fed diets with a relatively high SFM content may be depressed in comparison to the BW of control birds receiving the SBM-based diet. Therefore, a special attention was paid to the GIT response of turkeys fed diets with different contents of SFM added at the expense of soybean meal and partly of wheat.

Following the increasing dietary levels of sunflower meal in the diet, a considerable decrease in the relative tissue mass of the caeca, expressed as a percentage of BW, was observed after 8 weeks of feeding in turkeys exposed to higher doses of

SFM. In another experiment with 8-week-old turkeys, high-fibre diets (5.3%) containing soybean hulls caused a decrease in the duodenal villus height and number of goblet cells in comparison to the dietary 3.5%-fibre treatment (Juśkiewicz et al., 2009), but did not greatly affect the caecal physiological parameters (Zduńczyk et al., 2010). Other authors reported a different response of intestinal organs to sunflower products. 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. In turn, Arija et al. (2000) showed the shortening of the jejunal villi, caused by the inclusion of full-fat sunflower kernels in chicken diets. The results of a well documented study conducted by Sklan et al. (2003) revealed significant changes in the duodenum morphology in turkeys fed diets with 3, 6 and 9% crude fibre content. An increased level of dietary fibre from 3 to 6% caused villus elongation, but further increase in dietary fibre resulted in an opposite effect. Tabook et al. (2006) reported that the high-fibre diet caused a significant increase in the weight of ileal tissue when compared with the low-fibre diet. It is common knowledge that it is not only the level of DF that is of paramount importance for GIT segments development, but the source of DF also plays a significant role in digestion and absorption (Wenk, 2001). In comparison with soybean meal, sunflower meal used in this study contained more total non-starch polysaccharides, water-insoluble NSP, lignin and crude fibre. SBM had only a higher content of water-soluble NSP than SFM (Table 1). One explanation to the lower weight of caecal tissue, observed in our study, could be a faster passage of digesta through the gastrointestinal tract in SFM-fed birds, as supported by the significantly decreased digesta mass in the caeca. Another reason for the foregoing effect might be a lower concentration of soluble polysaccharides in diets containing sunflower meal in comparison with the control SBM-supplemented 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, largely due to increased bacterial counts and a higher rate of fermentation in the lower parts of the GIT (Juśkiewicz and Zduńczyk, 2004). The latter explanation is supported by the lowest short-chain fatty acid concentrations and production (pool) in the caeca of turkeys from the SFM_{21} treatment. In addition to energy-yielding activity, SCFA in the caeca can provide other benefits, e.g., a lower digesta pH and this may inhibit some pathogenic bacteria by dissipating the proton motive force across the bacterial cell membrane (Józefiak et al., 2004).

In our experiment, the highest activities of bacterial α-glucosidase, α-galactosidase and β-galactosidase were observed in the SFM_7 treatment. b-galactosidase, α-galactosidase and α-glucosidase activities can improve the fermentation of lactose, raffinose family oligosaccharides and resistant starch, leading to the production of SCFAs and lactic acid which are a source of energy for the tissues. However, the activities of potentially harmful ß-glucuronidase and ß-glucosidase were similar in all groups, which should be considered as a positive effect of sunflower meal added to turkey diets. Both ß-glucuronidase and ß-glucosidase have been implicated in the generation of mutagens or carcinogens in the hindgut (Salminen et al., 1998).

There is no doubt that the caeca are the principal site of fermentation processes in the avian gastrointestinal tract, but the relative fermentability of different fibres is

dependent on a number of physiochemical properties (Montagne et al., 2003; Gibson, 2004). In the present study, the application of diets containing increasing levels of SFM, rich in insoluble fibre, was in a reverse relationship with SCFA concentrations and pool. In the presence of different fermentable carbohydrates, the SCFA 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 8-week-old turkeys, the SCFA pool was more than 2-fold lower in birds fed SFM_{14} and SFM_{21} diets than in those fed SFM_7 and SFM_0 diets. The present experiment showed 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 of growing turkeys and, to some extent, impaired the GIT development. Considering that the lowest productivity was observed in turkeys exposed to highest dose of SFM, a conclusion also could be drawn that slow rate of caecal fermentation did not promote turkeys' growth in this study.

Although other parts of the digestive tract of poultry might also be important sites for pathogen-host microbiota interactions, the caeca have received most of the attention because the microbiota of the caeca is very diverse and caecal digesta contain the largest number of bacteria (Mead, 1997). The majority of research on poultry GIT microbiology has been conducted with chickens. In one of the few reports regarding turkeys, Bedburry and Duke (1983) demonstrated that a high-fibre diet significantly increased the concentration of microorganisms in the caecum, compared with a low-fibre diet. The percentages of *Peptostreptococcus* were significantly greater ($P<0.05$) in HF-fed turkeys, and of *E. coli* – in LF-fed turkeys ($P<0.05$). Another experiment performed on turkeys indicated an increase in cellulolytic bacteria in the caeca of birds fed a high-fibre diet (Duke et al., 1984).

Broiler chicken diets containing maize, sorghum, barley, oats, or rye had various effects, i.e. maize- and sorghum-based diets increased the numbers of *Enterococcus*, a barley-based diet increased the counts of *Lactobacillus*, an oats-based diet enhanced the growth of *Escherichia* and *Lactococcus*, and a rye-based diet increased the numbers of *Streptococcus* in broilers (Apajalahti, 2004). Hűbener et al. (2002) found a higher number of lactic acid bacteria in the caecal contents of 42-day-old chickens fed diets containing wheat and rye as the main NSP source, in comparison with maize. While comparing a maize-based diet with a diet containing wheat and barley, Mathlouthi et al. (2002) observed lower counts ($P\leq0.01$) of *Escherichia coli* bacteria in the caecal contents of chickens. Apajalahti et al. (2004) found that the inclusion of rye instead of maize in the ration significantly increased the population size of *Escherichia coli* in the small intestines of chickens. In our experiment the lowest counts of *Bifidobacterium* and *Lactobacillus/Enterococcus* groups as well as the highest numbers of the potential pathogenic bacteria *Bacteroides/Prevotella* and *Escherichia coli* were found in turkeys fed the SFM_{21} diet. It could be assumed that in the critical period, namely 8th week of age, a high level of sunflower meal in the diet for growing turkeys did not support the health-promoting effects of gastrointestinal microflora.

In conclusion, sunflower meal rich in crude fibre and lignin, added at a level of 21% to the diet for growing turkeys, may cause undesirable changes in caecal microflora activity and composition, manifested in a decrease in the production of short-chain fatty acids and an increase in the population size of *Bacteroides-Prevotella* and *Escherichia coli*. As a consequence, at 8 weeks of age the body weight of turkeys fed diets containing 210 g/kg of SFM were lower by 6% from those receiving the soybean meal-based diets. On the other hand, our study showed that high-fibre sunflower meal could be an effective alternative to soybean meal in diets for growing turkeys when applied at a level of up to 7%.

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Reakcja ekosystemu jelit ślepych indyków na zróżnicowaną zawartość w mieszance poekstrakcyjnej śruty słonecznikowej

STRESZCZENIE

Badano wpływ różnej zawartości (0, 7, 14 i 21%) poekstrakcyjnej śruty słonecznikowej (SFM) na procesy fermentacyjne i populację mikroflory jelit ślepych u 8 tygodniowych indorów. Wszystkie diety były izobiałkowe i izokaloryczne, ale różniły się głównie w odniesieniu do zawartości włókna surowego – 2,92%, 4,18%, 4,72% i 5,56%, odpowiednio w grupach SFM_0, SFM_7, SFM_{14} i SFM_{21}. Po 8 tygodniach odchowu stwierdzono, że zwiększająca się zawartość SFM w dietach spowodowała istotne statystycznie zmniejszenie spożycia paszy i masy ciała indyków. W porównaniu z grupą SFM_0 i SFM_7, u indyków żywionych dietami SFM_{14} i SFM_{21} stwierdzono istotne zmniejszenie masy tkanki i treści jelit ślepych oraz produkcji lotnych kwasów tłuszczowych (LKT). Najwyższą aktywność bakteryjnej α-glukozydazy, α-galaktozydazy i β-galaktozydazy stwierdzono w grupie żywionej niską ilością (7%) śruty słonecznikowej. W grupie SFM_{21} zwiększyła się znacząco liczebność bakterii *Escherichia coli* i *Bacteroides-Prevotella*. Uzyskane wyniki wskazują, że diety dla indyków mogą być efektywnie uzupełnione wysokogatunkową śrutą słonecznikową w ilości około 70 g/kg. Żywienie indyków dietami zawierającymi 210 g/kg SFM może powodować niekorzystne zmiany w aktywności i profilu mikroflory jelit ślepych, objawiające się zmniejszoną produkcją LKT oraz zwiększeniem populacji *Bacteroides* i *Escherichia coli*. Skutkiem tego może być mniejsza o około 6% masa ciała 8 tygodniowych indyków, w porównaniu z żywionymi dietami z udziałem śruty sojowej.