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Original Research Article

The effect of the dietary inclusion of pea seeds of colored-flowered and white-flowered varieties on gastrointestinal function in turkeys

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ABSTRACT

This study investigated the effects of dietary replacement of soybean meal (SBM) with graded levels of pea seeds (PS) on the gastrointestinal function of turkeys. Seeds of 2 pea varieties, a colored-flowered variety and a white-flowered variety (CFP and WFP, respectively) were fed to 56-d-old birds for 8 wk. A total of 539 female Hybrid turkeys were allocated to 7 groups, each group consisted of 7 pens with 11 birds per pen. The experiment had a 2-factorial design, with 3 dietary inclusion levels of PS (100, 200 and 300 g/kg) and 2 pea varieties (CFP and WFP). The control group (diets without PS) was compared with CFP and WFP treatments by simple contrast analysis. In comparison with CFP seeds, WFP seeds contained 7-fold less tannins (0.67 vs. 4.66 g/kg) and less non-starch polysaccharides (NSP, 117.8 vs. 132.7 g/kg), but more trypsin inhibitors (1.34 vs. 0.98 g/kg) and starch (489 vs. 455 g/kg). A rise in the PS content of diets from 100 to 200 and 300 g/kg increased the weight of the small intestine ($P = 0.031$) and the dry matter (DM) content of intestinal digesta ($P = 0.001$), but it had no effect on the pH of digesta. Only the highest PS content differentiated the concentrations of short-chain fatty acids (SCFAs) in the small intestinal digesta (WFP > CFP, $P = 0.008$), whereas PS did not cause any changes in the morphological parameters of the small intestinal mucosa. The dietary inclusion of PS had no influence on the levels of acetate, butyrate, putrefactive SCFAs or total SCFAs in the cecal contents. Apart from increasing the activities of β-glucosidase ($P = 0.017$) and β-galactosidase ($P = 0.025$), pea varieties did not affect the activities of the analyzed cecal microbial enzymes. However, CFP seeds decreased the DM content ($P = 0.041$) and increased the pH of cecal digesta, compared with WFP seeds ($P = 0.013$). The results of this study, pointing to a few differences in the functional parameters of the small intestine and cecum, indicate that tannins are not a factor differentiating the suitability of CFP and WFP seeds in the nutrition of finisher turkeys. The inclusion of PS at 200 and 300 g/kg of the diet reduces the content of SBM and wheat in turkey diets, which has a positive effect on gastrointestinal function.

1. Introduction

Contemporary fast-growing turkeys have high protein requirements, approximating 30% of the diet in the first month of rearing (Hybrid Turkeys, 2020), which results in a high content of soybean meal (SBM) or its substitutes in the diet. It is estimated that SBM accounts for 84% of the high-protein oilseed meal used in compound livestock rations worldwide (FAO Faostat, 2020). Typical SBM-cereal-based diets for young turkeys contain up to 50% SBM (Nalle et al., 2010; Zduńczyk et al., 2018) and, consequently, more

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than 5% of ingestible α-galactosides (Baker, 2000). Such diets contribute to an undesirable increase in the rate of cecal fermentation and in excreta moisture content (Jankowski et al., 2009). Therefore, attempts have been made to replace SBM with alternative protein sources in poultry diets, including protein crops such as legumes (Laudadio and Tufarelli, 2010), in particular those grown locally in organic farming systems where SBM (mostly derived from genetically modified plants) is not used (Vicenti et al., 2009; Jezierny et al., 2010).

One of the local protein sources in poultry diets could be peas (*Pisum sativum* L.) grown in many regions of the world, including Europe (Watson et al., 2017), South America (Bingol et al., 2016) and North America (Johnson et al., 2014). Research has shown (Palander et al., 2006) that peas are a better source of protein than lupines or beans in terms of amino acid digestibility. Legumes have a lower content of sulfur-containing amino acids and tryptophan (Gatel, 1994) and a higher content of arginine than SBM (Spielmann et al., 2008). Due to the addition of crystalline amino acids to the diet, the biological value of protein can be adjusted to the needs of monogastric animals (Stein et al., 2004). However, the presence of antinutritional factors (Castell et al., 1996), in particular tannins (Gdala et al., 1992), can limit wider use of field pea (*P. sativum arvense*) seeds in animal nutrition. In general, the color of pea flowers is related to the tannin content of seeds, which ranges from 7 to 12 g/kg (Smulikowska et al., 2001). Only a few colored-flowered varieties of field pea contain approximately 1 g/kg of tannins, the amount typical of white-flowered varieties of garden pea (Konieczka et al., 2014). Another factor compromising the nutritional value of pea seeds (PS) fed to animals can be the low digestibility of pea starch (which accounts for over 50% of seed DM), which increases after hydrothermal treatment, e.g. extrusion (Nalle et al., 2011). The apparent metabolizable energy of SBM is approximately 28% lower in poultry than in swine (NRC 1994) due to the lower utilization of low-digestible oligosaccharides and polysaccharides in the gastrointestinal tract (GIT) of poultry (Nalle et al., 2010). Therefore, it appears that in genetically improved pea varieties, the suitability of seeds for feeding monogastric animals is determined by the content of components that are not digested in the upper GIT but undergo hindgut fermentation (mainly polysaccharides and resistant starch) rather than by antinutritional factors (Konieczka et al., 2014). Thus, the rate and conditions of fermentation processes in the GIT of poultry fed diets containing PS are important considerations.

In a study of pigs, long-term intake of pea fiber improved colonic function via altering colonic barriers, colonic immunity, and metabolism-related protein gene expressions (Che et al., 2014). In another experiment, however, microbial activity and intestinal morphology did not change considerably in response to the partial replacement of SBM with PS (Tuśnio et al., 2017). In a similar study of chickens (Czerwiński et al., 2010), the inclusion of 150 g/kg raw white-flowered pea into broiler diets did not affect the concentrations of short-chain fatty acids (SCFAs) in the ileal digesta, but increased their levels in the cecal digesta. However, similar studies have not been conducted on turkeys, birds that have a longer rearing period and are fed diets with higher protein content. Zduńczyk et al. (2020) have recently shown that white-flowered peas at 300 g/kg at the expense of wheat and SBM could be effectively used in the diets of young turkeys (up to 8 wk of age) without any negative effects on the gastrointestinal function or final body weight (BW).

The aim of this experiment was to investigate the response of the GIT of turkeys to graded dietary inclusion levels of peas (100, 200 and 300 g/kg) of 2 varieties differing in flower color and tannin content.

2. Materials and methods

2.1. Ethics statement

The experiment was conducted at the Animal Research Laboratory (Department of Poultry Science, University of Warmia and Mazury, Olsztyn, Poland) in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol for this study was approved by the Local Ethics Committee.

2.2. Birds, management, and experimental diets

The experiment had a completely randomized design. Female Hybrid Converter turkeys aged 56 d old (a total of 539 birds) were placed in pens on litter (wood shavings) and were assigned to 7 dietary treatments. Each experimental group comprised 77 turkeys, with 7 replicate pens and 11 birds per pen. The birds were distributed among the treatments so as the average values of group BW did not differ significantly between the treatments at the beginning of the experiment. The turkeys from each treatment were weighed on an electronic weighbridge (RADWAG WPT/4 F300C8) with a readability of 0.1 kg. The average initial BW of birds in all groups was similar (3.90 kg; SD = 0.14; $P = 0.925$). The room conditions were consistent with the management recommendations for Hybrid Turkeys (2020).

The birds received 7 diets (Table 1) throughout 2 feeding phases, from 9 to 12 wk and from 13 to 16 wk of age. The control diet without PS (PS_0) contained SBM as the main high-protein component. In the remaining 6 experimental diets, SBM was partially replaced with 100, 200 or 300 g/kg of PS of the colored-flowered variety (subgroups CFP_{100}, CFP_{200} and CFP_{300}, respectively) and the white-flowered variety (subgroups WFP_{100}, WFP_{200} and WFP_{300}, respectively). Certified PS of colored-flowered variety Turnia (*P. sativum arvense*) and white-flowered variety Tarchalska (*P. sativum hortense*) were obtained from the Plant Breeding Station in Strzelce (Poland). The chemical composition of PS, and the analytical methods are presented in Table 2. Before inclusion in the diets, raw PS with hulls were ground to pass through a 3-mm sieve in a hammer mill (Jesma Co., Sprout Matador, Denmark). The SBM used in the diets came from the same batch as in the parallel studies whose results have already been published (Zdunczyk et al., 2020), which contained 542 g proteins, 0.9 g of starch, 163.1 g of total fiber and 87.7 g of oligosaccharides on a dry matter (DM) basis. The diets were formulated to be iso-caloric for energy and iso-nitrogenous for protein, and to meet the nutrient requirements of commercial turkeys at the appropriate stage of rearing (Smulikowska and Rutkowski, 2018; in Polish). The same amount of rapeseeds (80 and 100 g/kg in the first and second stage of the experiment, respectively) and different amounts of tallow (from 18.4 to 28.0 g/kg in the first stage and from 13.4 to 23.1 g/kg in the second stage of the experiment) were used to balance the energy value of the diets. All experimental diets were prepared as 3.5 mm pellets at 65 °C by the local feed mill. The trial lasted 8 wk, from 9 to 16 wk of age. Throughout the experiment, the turkeys had unrestricted access to feed and water which was available ad libitum.

2.3. Sampling collection and investigations

During the trial, the BW of turkeys and feed consumption were recorded on a pen basis at 12 and 16 wk of age. Daily feed intake (DFI) per bird was calculated on a pen total feed consumption basis for the entire experimental period and for the number of days in the period. Feed conversion ratio (FCR; kilogram of feed/kilogram

Table 1
Composition and nutrient levels of the experimental diets for female turkeys from 9 to 16 wk of age (g/kg, as-fed basis).

Item	9 to 12 wk of age							13 to 16 wk of age						
	PS_0	CFP_{100}	CFP_{200}	CFP_{300}	WFP_{100}	WFP_{200}	WFP_{300}	PS_0	CFP_{100}	CFP_{200}	CFP_{300}	WFP_{100}	WFP_{200}	WFP_{300}
Ingredients														
Wheat	674.9	600.4	525.6	451.1	605.4	535.9	466.4	714.3	639.6	565.2	490.6	644.7	575.3	505.9
Soybean meal (48.3% of CP)	184.8	160.7	136.7	112.6	157.8	130.9	103.9	136.8	112.7	88.5	64.3	109.8	82.8	55.6
Pea seeds		100.0	200.0	300.0	100.0	200.0	300.0		100.0	200.0	300.0	100.0	200.0	300.0
Full-fat rapeseed (20.7% of CP)	80.0	80.0	80.0	80.0	80.0	80.0	80.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Tallow	28.0	27.1	26.1	25.2	24.8	21.6	18.4	23.1	22.1	21.1	20.2	19.9	16.7	13.4
Sodium bicarbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sodium chloride	2.2	2.2	2.3	2.3	2.2	2.3	2.3	2.2	2.3	2.3	2.3	2.3	2.3	2.3
Limestone	13.2	13.1	13.1	13.0	13.2	13.1	13.1	10.9	10.9	10.8	10.8	10.9	10.9	10.9
Monocalcium phosphate	7.4	7.8	8.1	8.4	7.8	8.1	8.4	4.2	4.5	4.9	5.2	4.5	4.8	5.2
Choline chloride	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
DL-Methionine[1]	1.4	1.5	1.7	1.9	1.5	1.6	1.8	0.8	1.0	1.2	1.4	1.0	1.1	1.3
L-Lysine[2]	3.3	2.5	1.8	1.0	2.6	1.9	1.2	3.3	2.6	1.8	1.0	2.6	1.9	1.2
L-Threonine[2]	0.6	0.5	0.4	0.3	0.5	0.4	0.3	0.2	0.1			0.1		
Vitamin-mineral premix[3]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Nutrient levels[4]														
Crude protein	191.1	189.4	188.6	190.7	192.0	191.4	188.0	177.0	175.5	176.8	175.1	178.2	175.4	176.6
AME, MJ/kg	12.97	12.97	12.97	12.97	12.97	12.97	12.97	13.18	13.18	13.18	13.18	13.18	13.18	13.18
Lysine	11.0	11.0	11.0	11.0	11.0	11.0	11.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methionine and cysteine	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Threonine	7.0	7.0	7.0	7.0	7.0	7.0	7.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Arginine	5.7	6.9	8.2	9.5	7.2	8.7	10.2	6.0	7.3	8.6	9.8	7.5	9.0	10.6
Calcium	8.0	8.0	8.0	8.0	8.0	8.0	8.0	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Available phosphorus	3.5	3.5	3.5	3.5	3.5	3.5	3.5	2.8	2.8	2.8	2.8	2.8	2.8	2.8

AME = apparent metabolisable energy.
[1] It contains 990 g methionine/kg product (Evonik Degussa GmbH, Essen, Germany).
[2] It contains 780 g lysine hydrochloride/kg product and 985 g threonine/kg product (Ajinomoto Eurolysine S.A.S, Amiens, France).
[3] Vitamin-mineral premix provided following per kilogram of the diets from 9 to 12 wk of age and 13 to 16 wk of age: retinol 2.88 and 2.52 mg, cholecalciferol 0.10 and 0.09 mg, α-tocopheryl acetate 80 and 70 mg, vitamin K_3 4.8 and 4.2 mg, thiamine 4.0 and 3.5 mg, riboflavin 6.4 and 5.6 mg, pyridoxine 4.8 and 4.2 mg, cobalamin 0.024 and 0.021 mg, biotin 0.24 and 0.21 mg, pantothenic acid 20 and 18 mg, nicotinic acid 64 and 56 mg, folic acid 2.4 and 2.1 mg, Fe 48 and 42 mg, Mn 96 and 84 mg, Zn 88 and 77 mg, Cu 16 and 14 mg, I 2.4 and 2.1 mg, Se 0.24 and 0.21 mg, respectively.
[4] Crude protein was determined analytically, and the content of the remaining nutrients was calculated based on the analyzed chemical composition of soybean meal and pea seeds and according to the Polish Feedstuff Analysis Tables (Smulikowska and Rutkowski, 2018).

of BWG) was calculated from BW gain and feed consumption. The weights of dead birds were used to adjust average BW gain, DFI and FCR. The performance parameters were determined for the entire 8 wk experiment.

At the termination of the experiment at 16 wk of age, 7 turkeys representing average group BW were selected from each dietary treatment and were sacrificed after electrical stunning. The segments of the GIT (small intestine and cecum), including the contents, were collected and weighed. The pH values of the ileum and cecal digesta were measured immediately after digesta collection (electrode pH/ION meter, model 301, Hanna Instruments, Woonsocket, RI, USA). Fresh samples of ileal (middle section of the ileum) and cecal contents were used for immediate analysis (ileal and cecal DM, ileal viscosity, cecal ammonia). The DM content of digesta was determined at 105 °C and digesta viscosity was measured in the supernatant fraction using the cone/plate viscometer (model LVDV II+, Brookfield Engineering Laboratories, Stoughton, MA, USA). Ammonia was extracted from fresh cecal digesta, trapped in a solution of boric acid in Conway dishes and determined by direct titration with sulfuric acid. The remaining portions of the ileal and cecal contents were used immediately for the determination of enzymatic activity in the gut microbiota (approximately 0.5 g) and SCFA concentrations (0.5 g).

2.4. Activity of intestinal microbiota

The activity of gut microbiota was measured based on the activity of bacterial enzymes and the concentrations of SCFAs. Extracellular bacterial enzymatic activity in the ileal and cecal digesta was determined spectrophotometrically by the rate of p- or o-nitrophenol release from their respective nitrophenylglucosides according to the protocol described by Juskiewicz et al. (2006). The activity of the following microbial enzymes was assessed: α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase, β-xylosidase

Table 2
Chemical composition of pea seeds of colored-flowered (CFP) and white-flowered (WFP) varieties (g/kg).

Item	As-is		In DM	
	CFP	WFP	CFP	WFP
Dry matter (AOAC, 2005; procedure 934.01)	889.4	878.6		
Ash (AOAC, 2005; procedure 942.05)	20.5	20.0	23.0	22.8
Crude protein (AOAC, 2005; procedure 976.05)	181.2	189.3	203.7	215.5
Ether extract (AOAC, 2005; procedure 920.39)	14.0	9.9	15.7	11.3
Starch (AOAC, 2005; procedure 996.11)	455.0	489.0	511.6	556.6
Fiber fractions[1]				
Neutral detergent fiber	108.5	89.9	122.0	102.3
Acid detergent fiber	77.6	62.9	87.2	71.6
Total fiber	166.3	149.3	187.0	169.9
Lignin and polyphenols	27.8	27.4	31.3	31.2
Non-starch polysaccharides (NSP)	132.7	117.8	149.2	1341
NSP component sugars				
Arabinose	25.4	27.5	28.6	31.3
Xylose	12.9	10.7	14.5	12.2
Mannose	5.2	0.9	5.8	1.0
Galactose	5.2	5.1	5.8	5.8
Glucose	60.1	52.1	67.6	59.3
Uronic acids	28.2	21.5	31.7	24.5
Antinutritional factors				
Rafinose family oligosaccharides[1]	33.4	29.3	37.5	33.3
Activity of trypsin inhibitors (TIA)[2]	0.98	1.34	1.10	1.52
Tannins[3]	4.66	0.67	5.24	0.76

[1] As described by Slominski et al. (1993).
[2] According to Kakade et al. (1974).
[3] According to the method of Jeruminas (1972) modified by Adams and Novellie (1975).

and α-arabinopyranosidase. The remaining samples were stored in test tubes at −70 °C until analysis. Ileal and cecal SCFA concentrations were analyzed by gas chromatography (Shimadzu GC-2010, Kyoto, Japan) on a capillary column (SGE BP21, 30 m × 0.53 mm, SGE Europe Ltd., Kiln Farm Milton Keynes, UK) as described previously (Juskiewicz et al., 2006). All analyses were performed in duplicate.

2.5. Architecture of the intestinal wall

The functional status of the gut was additionally assessed based on the morphometric analyses of the duodenal and ileal walls, including mucosa thickness, the height of intestinal villi, and the depth of the crypts of Lieberkuhn. The protocol of tissue preparation for the morphometric examination has been described in detail by Przybylska-Gornowicz et al. (2015). In brief, intestinal wall samples of 1 cm (2 per each bird) were collected from the middle part of the duodenal loop and the jejunum. The specimens were fixed in 4% paraformaldehyde in phosphate buffer for 48 h and embedded in paraffin. The 4-μm-thick sections were stained using the hematoxylin and eosin method (HE), the periodic acid Schiff method (PAS), and the methyl green-pyronine method (MGP). The specimens were analyzed using Panoramic Viewer 1.12 (3D-Histech, Hungary) and AxioVision 4.8 software (Carl Zeiss, Jena, Germany).

2.6. Statistical analysis

The results were analyzed statistically using the same procedures that were used in previous research (Zduńczyk et al., 2018). The data were subjected to 2-way ANOVA to examine the following effects: a) interaction between pea variety and inclusion dose (V × D); b) main effect of pea variety (CFP vs. WFP, V effect); and c) main effect of PS inclusion dose (100, 200 and 300 g/kg; D effect). When a significant interaction effect was noted, the post-hoc Tukey's test was applied to determine the differences between groups CFP_{100}, WFP_{100}, CFP_{200}, WFP_{200}, CFP_{300}, and WFP_{300}. In addition, a simple contrast analysis was used to compare the control diet PS_0 vs. all CFP diets or all WFP diets. Statistical analysis was performed using the STATISTICA Software, ver. 12.0 (StatSoft Inc, 2014) at a significance level of $P < 0.05$. The results were presented as mean and the pooled standard error of the mean (SEM).

3. Results

3.1. Chemical composition of feedstuffs and experimental diets

In comparison with CFP seeds, WFP seeds contained slightly more crude protein (CP; 189.3 vs. 181.2 g/kg), more starch (489 vs. 455 g/kg), and less neutral detergent fiber and total fiber (89.9 vs. 108.5 and 149.3 vs. 166.3 g/kg, respectively) (Table 2). In the group of antinutritional factors, WFP seeds had a higher content of trypsin inhibitors (1.34 vs. 0.98 g/kg), but a nearly 7-fold lower content of tannins than CFP seeds (0.67 vs. 4.66 g/kg) (Table 2). In addition, WFP seeds contained less non-starch polysaccharides (NSP; 117.8 vs. 132.7 g/kg) than CFP seeds. As regards NSP component sugars, the only difference was the higher mannose content of CFP seeds, compared with WFP seeds.

The incorporation of 100, 200 and 300 g/kg of CFP seeds reduced the SBM content of diets by approximately 24, 48 and 72 g/kg, respectively, and it also reduced the amount of wheat in the diets by around 75, 149 and 224 g/kg, respectively (Table 1). Due to the higher CP content of WFP seeds, the diets containing seeds of this variety contained slightly less SBM, and slightly more wheat than the diets containing CFP seeds.

3.2. Parameters of small intestinal function

Two-way ANOVA revealed that the weight of the small intestine including the contents, the DM content and pH of digesta were not affected ($P > 0.05$) by pea variety, and some differences ($P < 0.05$) were noted between turkeys fed diets with different PS content (Table 3). A rise in the PS content of diets from 100 to 200 and 300 g/kg increased the weight of the small intestine ($P = 0.031$) and the DM content of intestinal digesta ($P = 0.001$), but it had no effect ($P > 0.05$) on the pH of digesta.

Simple contrasts were used to evaluate the effects of diets without and with different inclusion levels of PS of different varieties on the parameters of small intestinal function in turkeys. A significant decrease ($P = 0.033$ and $P = 0.025$, respectively) in the weight of the small intestine including the contents was noted in turkeys fed diets containing CFP and WFP seeds compared with those fed the PS_0 diet. In turn, a significant increase ($P = 0.015$ and $P = 0.016$, respectively) in the DM content of the small intestinal digesta in turkeys fed CFP and WFP diets vs the PS_0 diet resulted from the effects exerted by the medium and high dietary inclusion levels of PS. The contrast analysis revealed a significant increase ($P = 0.006$) in the viscosity of small intestinal digesta in WFP treatments vs. the control group (PS_0) but such an effect was not observed ($P = 0.396$) when CFP seeds were added to the diet. In comparison with the control group (PS_0), the inclusion of CFP and WFP seeds in experimental diets significantly increased ($P = 0.002$ and $P = 0.001$, respectively) the pH of small intestinal digesta. In all dietary treatments, total SCFA concentrations in the ileal digesta were very low, at 7.10 μmol/g in group PS_0 and from 4.34 to 8.70 μmol/g in PS diets. The concentrations of acetate and total SCFAs were affected by the interaction between the experimental factors: WFP seeds significantly increased ($P = 0.004$ and $P = 0.008$, respectively) the concentrations of acetate and total SCFAs but only at the highest inclusion level. Unlike the highest CFP content, the highest WFP content contributed to the highest proportion of acetate ($P = 0.009$ for V × D interaction) in total SCFAs. The V × D interaction was also noted for the calculated percentage of propionic acid and butyric acid in the SCFA profile ($P = 0.020$ and $P = 0.019$, respectively). The percentage of propionic acid was highest in group CFP_{200} and lowest when PS of the white-colored variety were included in turkey diets at 300 g/kg. In the case of butyric acid, a significant difference was noted between 100 g/kg subgroups ($WFP_{100} > CFP_{100}$; $P < 0.05$). Two-way ANOVA revealed that the small intestinal concentrations of putrefactive SCFAs were elevated ($P = 0.006$) in turkeys fed diets with the lowest PS content as compared with both higher inclusion rates of PS. Neither pea variety nor the dietary inclusion level of PS affected ($P > 0.05$) propionate or butyrate concentrations in the small intestinal digesta. The contrast analysis demonstrated that in comparison with group PS_0, the dietary application of CFP seeds decreased the concentrations of acetic acid ($P = 0.001$) and total SCFAs ($P = 0.010$), and increased ($P = 0.001$) propionic acid concentration in the small intestinal digesta. As regards WFP seeds, the contrast analysis revealed elevated propionic acid concentration ($P = 0.001$) as compared with group PS_0.

The thickness of the mucosa, the depth of the crypts of Lieberkuhn, and the height of small intestinal villi in turkeys fed diets containing different SBM and PS levels were similar in all experimental treatments (Table 4). No interactions between the experimental factors, i.e. pea variety and pea inclusion level, were found in any of the histological parameters.

3.3. Cecal function parameters

Tissue mass, the weight of cecal contents and ammonia concentration in the cecal digesta were similar for both pea varieties

Table 3
Selected parameters of small intestinal function in turkeys fed diets without and with different levels of pea seeds of different varieties ($n = 7$).

Item[1]	Total mass, g/kg BW	Dry matter, %	Viscosity, mPa·s	pH	SCFAs, μmol/g					SCFA profile, % of ΣSCFA		
					Acetic	Propionic	Butyric	Total	PSCFAs	Acetic	Propionic	Butyric
PS_0	17.6	20.0	2.53	6.26	6.50	0.48	0.035	7.10	0.078	91.6	6.84	0.504
CFP_{100}	15.1	19.9	2.47^{b}	6.69	5.33^{b}	1.01	0.028	6.47^{b}	0.103	81.9^{ab}	16.0^{ab}	0.455^{b}
WFP_{100}	14.9	19.0	3.55^{a}	6.92	4.21^{b}	0.97	0.049	5.31^{bc}	0.087	79.1^{b}	18.3^{ab}	0.954^{a}
CFP_{200}	16.8	23.1	2.97^{b}	6.70	4.46^{b}	1.19	0.046	5.75^{bc}	0.049	77.6^{b}	20.6^{a}	0.822^{ab}
WFP_{200}	16.4	23.0	2.95^{b}	6.90	5.06^{b}	0.87	0.032	6.04^{bc}	0.072	83.5^{ab}	14.7^{ab}	0.530^{ab}
CFP_{300}	16.3	23.2	2.66^{b}	6.94	3.34^{b}	0.81	0.035	4.34^{c}	0.052	78.6^{b}	19.2^{ab}	0.851^{ab}
WFP_{300}	16.6	24.1	2.82^{b}	7.13	7.52^{a}	1.08	0.053	8.70^{a}	0.049	86.1^{a}	12.7^{b}	0.610^{ab}
SEM	0.252	0.366	0.078	0.060	0.251	0.046	0.003	0.258	0.005	0.863	0.837	0.056
Variety												
CFP	16.1	22.0	2.70	6.78	4.41	1.00	0.037	5.52	0.068	79.4	18.6	0.709
WFP	16.0	22.0	3.11	6.98	5.59	0.97	0.044	6.68	0.069	82.9	15.3	0.698
Dose												
100 g/kg	15.0^{b}	19.5^{b}	3.01	6.80	4.77	0.99	0.039	5.89	0.094^{a}	80.5	17.1	0.704
200 g/kg	16.6^{a}	23.0^{a}	2.96	6.80	4.76	1.03	0.039	5.90	0.060^{b}	80.6	17.7	0.676
300 g/kg	16.4^{a}	23.6^{a}	2.74	7.04	5.48	0.95	0.044	6.52	0.050^{b}	82.4	16.0	0.731
ANOVA *P*-value												
Variety (V)	0.853	0.951	0.006	0.061	0.004	0.699	0.195	0.008	0.910	0.015	0.019	0.928
Dose (D)	0.031	0.001	0.247	0.136	0.237	0.683	0.729	0.374	0.006	0.461	0.590	0.934
V × D interaction	0.816	0.367	0.006	0.987	0.001	0.062	0.055	0.001	0.343	0.009	0.020	0.019
Contrast *P*-value[2]												
PS_0 vs. CFP	0.033	0.015	0.396	0.002	0.001	0.001	0.818	0.010	0.492	0.001	0.001	0.215
PS_0 vs. WFP	0.025	0.016	0.006	0.001	0.104	0.001	0.233	0.481	0.545	0.001	0.001	0.241

SCFAs = short-chain fatty acids; PSCFAs = putrefactive SCFAs (C4i + C5i + C5); Σ = sum; SEM = standard error of the mean (SD divided by the square root of replication number, $n = 42$).
[a, b, c] Values in a column with different letter superscripts differ significantly ($P < 0.05$).
[1] Diets PS_0, CFP_{100}, CFP_{200}, CFP_{300}, WFP_{100}, WFP_{200}, WFP_{300} contained 0, 100, 200, 300 g/kg of pea seeds of colored-flowered (CFP) or white-flowered (WFP) varieties, respectively.
[2] The control PS_0 group was compared with CFP and WFP treatments by simple contrast analysis.

Table 4
Mucosa thickness, the depth of the crypts of Lieberkuhn, and the height of small intestinal villi in turkeys fed diets containing different levels of pea seeds (μm, $n = 7$).

Item[1]	Duodenum				Jejunum			
	Mucosa thickness	Villus height	Crypt depth	Vh/Cd	Mucosa thickness	Villus height	Crypt depth	Vh/Cd
PS_0	2,815	2,784	258	10.75	1,710	1,551	199	7.78
CFP_{100}	2,867	2,719	248	11.23	1,748	1,443	197	7.35
WFP_{100}	2,810	2,510	234	10.68	1,839	1,555	198	7.90
CFP_{200}	2,729	2,532	247	10.21	1,658	1,413	197	7.16
WFP_{200}	2,878	2,738	242	11.34	1,668	1,507	199	7.59
CFP_{300}	2,973	2,718	242	11.43	1,713	1,640	200	8.23
WFP_{300}	2,781	2,653	240	11.11	1,705	1,508	201	7.51
SEM	58.38	46.82	4.52	0.21	31.38	28.83	2.27	0.14
Variety								
CFP	2,856	2,657	246	10.96	1,707	1499	198	7.58
WFP	2,823	2,634	239	11.05	1,737	1523	199	7.66
Dose								
100 g/kg	2,838	2,614	241	10.96	1,793	1499	197	7.62
200 g/kg	2,803	2,635	245	10.78	1,663	1460	198	7.37
300 g/kg	2,877	2,686	241	11.28	1,709	1574	201	7.87
ANOVA *P*-value								
Variety (V)	0.787	0.828	0.512	0.862	0.667	0.685	0.868	0.782
Dose (D)	0.890	0.847	0.948	0.699	0.330	0.305	0.896	0.420
D × V interaction	0.528	0.268	0.903	0.325	0.833	0.202	0.995	0.183
Contrast *P*-value[2]								
PS_0 vs. CFP	0.833	0.395	0.397	0.755	0.975	0.561	0.941	0.637
PS_0 vs. WFP	0.969	0.317	0.183	0.660	0.786	0.759	0.961	0.786

Vh/Cd = villus height/crypt depth ratio; SEM = standard error of the mean (SD divided by the square root of replication number, $n = 42$).
[1] Diets PS_0, CFP_{100}, CFP_{200}, CFP_{300}, WFP_{100}, WFP_{200}, WFP_{300} contained 0, 100, 200, 300 g/kg of pea seeds of colored-flowered (CFP) or white-flowered (WFP) varieties, respectively.
[2] The control PS_0 group was compared with CFP and WFP treatments by simple contrast analysis.

(Table 5). Two way-ANOVA revealed that turkeys fed diets with WFP seeds were characterized by higher ($P = 0.041$) DM content of cecal digesta than birds fed diets with CFP seeds. In comparison with CFP seeds, WFP seeds decreased the pH of cecal digesta ($P = 0.013$). Cecal ammonia concentration was lower ($P = 0.018$) when the dietary inclusion of PS was increased from 100 to 200 and 300 g/kg. In comparison with the control group (PS_0), the dietary addition of CFP and WFP seeds significantly increased the DM content of cecal digesta ($P = 0.047$ and $P = 0.008$, respectively).

Apart from increasing the activity of β-glucosidase and β-galactosidase, pea varieties did not affect the activity of the analyzed cecal microbial enzymes (Table 6). In comparison with CFP seeds, dietary WFP seeds increased the activity of β-glucosidase

Table 5
Cecal function parameters in turkeys fed diets containing different levels of pea seeds ($n = 7$).

Item[1]	Tissue mass, g/kg of BW	Cecal contents, g/kg of BW	Ammonia, mg/g	Dry matter, %	pH
PS_0	3.01	2.85	0.219	11.1	6.15
CFP_{100}	2.62	2.28	0.245	11.5	6.27
WFP_{100}	2.63	1.81	0.248	15.1	5.84
CFP_{200}	2.61	2.54	0.156	14.4	6.10
WFP_{200}	2.84	1.88	0.188	14.1	5.82
CFP_{300}	2.85	1.85	0.180	13.4	6.19
WFP_{300}	2.77	2.62	0.200	14.7	5.89
SEM	0.067	0.129	0.010	0.367	0.061
Variety					
CFP	2.69	2.22	0.193	13.1[b]	6.19[a]
WFP	2.74	2.11	0.212	14.6[a]	5.85[b]
Dose					
100 g/kg	2.62	2.04	0.246[a]	13.3	6.06
200 g/kg	2.72	2.21	0.172[b]	14.3	5.96
300 g/kg	2.81	2.23	0.190[b]	14.1	6.03
ANOVA *P*-value					
Variety (V)	0.623	0.612	0.392	0.041	0.013
Dose (D)	0.375	0.754	0.018	0.510	0.810
V × D interaction	0.456	0.060	0.857	0.100	0.873
Contrast *P*-value[2]					
PS_0 vs. CFP	0.143	0.102	0.361	0.047	0.829
PS_0 vs. WFP	0.221	0.054	0.786	0.008	0.106

SEM = standard error of the mean (SD divided by the square root of replication number, $n = 42$).
[a, b] Values in a column with different letter superscripts differ significantly ($P < 0.05$).
[1] Diets PS_0, CFP_{100}, CFP_{200}, CFP_{300}, WFP_{100}, WFP_{200}, WFP_{300} contained 0, 100, 200, 300 g/kg of pea seeds of colored-flowered (CFP) or white-flowered (WFP) varieties, respectively.
[2] The control PS_0 group was compared with CFP and WFP treatments by simple contrast analysis.

($P = 0.017$) and the activity of β-galactosidase, in the latter case only at the lowest PS content (see V × D interaction, $P = 0.047$). A nearly significant ($P = 0.060$) difference in the activity of β-glucuronidase was also noted (10.1 in groups CFP vs. 14.2 μmol/h/g in groups WFP). Enzyme activities were also affected by the PS content of diets: the activity of α-glucosidase, α-galactosidase and β-glucuronidase in the cecal digesta increased (*P* value from 0.011 to 0.049) with increasing inclusion levels of PS. The contrast analysis demonstrated that dietary CFP increased ($P = 0.034$) the extracellular activity of cecal bacterial β-glucuronidase in comparison with control PS_0 birds. At the same time, as compared with group PS_0, the dietary inclusion of WFP seeds significantly increased the activity of the following cecal bacterial enzymes: β-glucosidase ($P = 0.001$), α-galactosidase ($P = 0.008$), β-galactosidase ($P = 0.006$), β-glucuronidase ($P = 0.001$), β-xylosidase ($P = 0.029$) and α-arabinopyranosidase ($P = 0.009$).

Table 6
Activity of selected glycolytic bacterial enzymes in the cecal digesta of turkeys (μmol/h per g, $n = 7$).

Item[1]	Microbial enzymes						
	α-gluco	β-gluco	α-gal	β-gal	β-glucu	β-xylo	α-arabino
PS_0	26.0	0.71	3.92	7.51	4.03	3.84	11.6
CFP_{100}	23.7	1.27	6.15	7.10[b]	7.97	3.72	13.8
WFP_{100}	30.1	3.56	8.54	19.7[a]	11.4	8.33	23.7
CFP_{200}	28.2	1.48	5.96	12.2[a,b]	10.1	4.46	18.7
WFP_{200}	32.8	2.87	9.71	12.1[a,b]	11.7	6.82	21.8
CFP_{300}	41.1	2.60	11.9	14.1[a,b]	12.3	8.56	25.2
WFP_{300}	36.7	2.93	14.5	16.5[a,b]	19.4	6.66	25.6
SEM	1.576	0.258	0.908	1.099	1.057	0.540	1.531
Variety							
CFP	31.0	1.79[b]	8.00	11.1	10.1	5.58	19.2
WFP	33.2	3.11[a]	10.9	16.1	14.2	7.27	23.7
Dose							
100 g/kg	26.9[b]	2.41	7.35[b]	13.4	9.60[b]	6.03	18.7
200 g/kg	30.5[b]	2.17	7.84[b]	12.1	10.9[a,b]	5.64	20.3
300 g/kg	38.9[a]	2.76	13.8[a]	15.3	15.8[a]	7.61	25.4
ANOVA *P*-value							
Variety (V)	0.488	0.017	0.132	0.025	0.060	0.135	0.177
Dose (D)	0.011	0.662	0.029	0.480	0.049	0.318	0.225
V × D interaction	0.336	0.331	0.950	0.047	0.555	0.064	0.465
Contrast *P*-value[2]							
PS_0 vs. CFP	0.258	0.135	0.110	0.231	0.034	0.259	0.089
PS_0 vs. WFP	0.106	0.001	0.008	0.006	0.001	0.029	0.009

α-gluco = α-glucosidase; β-gluco = β-glucosidase; α-gal = α-galactosidase; β-gal = β-galactosidase; β-glucu = β-glucuronidase; β-xylo = β-xylosidase; α-arabino = α-arabinofuranosidase; SEM = standard error of the mean (SD divided by the square root of replication number, $n = 42$).
[a, b] Values in a column with different letter superscripts differ significantly ($P < 0.05$).
[1] Diets PS_0, CFP_{100}, CFP_{200}, CFP_{300}, WFP_{100}, WFP_{200}, WFP_{300} contained 0, 100, 200, 300 g/kg of pea seeds of colored-flowered (CFP) or white-flowered (WFP) varieties, respectively.
[2] The control PS_0 group was compared with CFP and WFP treatments by simple contrast analysis.

Two-way ANOVA showed that the inclusion of PS in turkey diets did not cause any changes in the concentrations of acetate, butyrate, putrefactive SCFAs, total SCFAs or the SCFA pool in the cecal digesta (Table 7). The cecal concentration of propionic acid and the share of propionate in the total SCFA tool were higher (both $P = 0.001$) at the highest dietary PS content, compared with the lowest and medium levels of SBM replacement with PS. The contrast analysis revealed that WFP seeds decreased the cecal concentration of propionic acid ($P = 0.047$) and C3 percentage ($P = 0.027$), compared with PS0 birds.

3.4. Growth performance of turkeys

There were no interactions between pea variety and inclusion level in any of the measured variables of turkey performance (Table 8). Similarly, the contrast analysis revealed no differences ($P > 0.05$) between the control group (PS_0) vs. CFP and WFP treatments. Mortality was low and not related to the dietary treatments. Over the experimental period, one turkey died in group PS_0, and one turkey died in groups CFP and WFP each. An analysis of the growth performance of turkeys indicated that the dietary inclusion of PS at up to 300 g/kg as a substitute for SBM had no effect on feed intake or BW gain. Two-way ANOVA revealed that the FCR deteriorated significantly ($P = 0.012$) when the PS content of diets increased from 100 to 200 and 300 g/kg. Additionally, the values of the FCR were higher ($P = 0.042$) in CFP treatments than in WFP treatments.

4. Discussion

4.1. Chemical composition of feedstuffs and experimental diets

In general, the levels of condensed tannins in the seeds of older pea varieties have been associated with colored flowers (Grosjean et al., 1999). In Polish varieties, the content of tannins was determined at 7 to 8 g/kg in colored-flowered peas and at only 1.6 to 2.6 g/kg in white-flowered peas (Gdala et al., 1992). In the present study, the tannin content of seeds of colored-flowered and white-flowered pea varieties was lower, at 4.66 and 0.67 g/kg, respectively. This is in line with the modern trend of reducing the content of these compounds in the process of genetic improvement of new pea varieties (Jezierny et al., 2010). A pink-flowered pea variety with a tannin content of 0.96 g/kg has recently been introduced into cultivation (Konieczka et al., 2014). It is believed that modern pea cultivars containing around 1 g of tannins/kg of seeds could be accepted as feed component for chickens (Smulikowska et al., 2001; Konieczka et al., 2014).

In an earlier study, the TIA value of white-flowered Polish pea varieties averaged 2.10 g/kg (Zduńczyk et al., 1997). In other studies (Smulikowska et al., 2001), the average TIA value of PS reached 1.01 g/kg, and it was not determined by flower color. In the current study, the TIA value of seeds of colored-flowered and white-flowered pea varieties was 0.98 and 1.34 g/kg, respectively. A higher TIA value of white-flowered peas, relative to colored-flowered peas (2.68 vs. 1.31 g/kg), was also reported by Grosjean et al. (1999). The TIA values of PS noted in our study were low, ranging from 2 to 5 g/kg, which is an acceptable level for SBM used in chicken nutrition (Huisman and Jansman, 1991).

Previous research (Nalle et al., 2010; Mikulski et al., 2017; Zduńczyk et al., 2018) has shown that the replacement of SBM with feed components with lower protein content such as faba beans or peas increases the share of protein feedstuffs in the diet even to 50%. In the present experiment, the inclusion of 100, 200 and 300 g/kg of CFP seeds in turkey diets decreased SBM content by 24, 48 and 72 g/kg, respectively, but the total content of protein feed components increased from 265 g/kg to 340 and 495 g/kg, respectively. At the same time, the wheat content of PS diets decreased from around 70 g to around 220 g/kg.

Table 7
Effects of dietary treatments on the concentrations of short-chain fatty acids (SCFAs) in the cecal digesta of turkeys at 112 days of age ($n = 7$).

Item[1]	SCFAs, μmol/g				SCFA pool, μmol/kg BW	PSCFAs, μmol/g	SCFA profile, % of ΣSCFA		
	Acetic	Propionic	Butyric	Total			Acetic	Propionic	Butyric
PS_0	131	11.8	38.1	184	527	2.93	71.5	6.23	20.7
CFP_{100}	115	7.74	37.2	163	374	2.95	70.8	4.85	22.6
WFP_{100}	134	7.77	39.8	184	340	2.31	72.9	4.27	21.5
CFP_{200}	139	6.98	45.0	193	498	2.60	71.7	3.67	23.2
WFP_{200}	135	7.15	41.0	185	351	2.48	72.5	3.86	22.2
CFP_{300}	142	13.2	37.7	196	366	3.66	72.6	6.80	18.6
WFP_{300}	143	10.9	41.1	198	515	3.14	72.0	5.67	20.7
SEM	3.01	0.582	1.533	4.13	27.3	0.159	0.480	0.272	0.577
Variety									
CFP	132	9.30	40.0	184	413	3.07	71.7	5.10	21.5
WFP	137	8.60	40.6	189	402	2.64	72.5	4.60	21.5
Dose									
100 g/kg	124	7.75[b]	38.5	173	357	2.63	71.9	4.56[b]	22.0
200 g/kg	137	7.07[b]	43.0	189	425	2.54	72.1	3.76[b]	22.7
300 g/kg	142	12.1[a]	39.4	197	441	3.40	72.3	6.23[a]	19.7
ANOVA *P*-value									
Variety (V)	0.415	0.426	0.833	0.588	0.832	0.190	0.481	0.243	0.995
Dose (D)	0.085	0.001	0.476	0.098	0.362	0.070	0.933	0.001	0.102
D × V interaction	0.330	0.440	0.588	0.420	0.065	0.785	0.594	0.460	0.487
Contrast *P*-value[2]									
PS_0 vs. CFP	0.943	0.111	0.678	0.990	0.166	0.780	0.868	0.122	0.683
PS_0 vs. WFP	0.506	0.047	0.576	0.687	0.131	0.545	0.506	0.027	0.680

PSCFAs = putrefactive SCFAs (C4i + C5i + C5); Σ = sum; SEM = standard error of the mean (SD divided by the square root of replication number, $n = 42$).
[a, b] Values in a column with different letter superscripts differ significantly ($P < 0.05$).
[1] Diets PS_0, CFP_{100}, CFP_{200}, CFP_{300}, WFP_{100}, WFP_{200}, WFP_{300} contained 0, 100, 200, 300 g/kg of pea seeds of colored-flowered (CFP) or white-flowered (WFP) varieties, respectively.
[2] The control PS_0 group was compared with CFP and WFP treatments by simple contrast analysis.

Table 8
Effects of dietary treatments on the growth performance of turkeys at 9 to 16 wk of age ($n = 7$).

Item[1]	BW 16 wk, kg/bird	DBWG, g/bird	DFI, g/bird	FCR, g feed/g BWG
PS_0	10.89	123.7	372.3	3.01
CFP_{100}	10.84	124.1	369.7	2.98
WFP_{100}	10.91	125.8	374.9	2.98
CFP_{200}	10.73	123.3	380.7	3.09
WFP_{200}	10.80	124.8	377.3	3.03
CFP_{300}	10.59	119.0	374.4	3.15
WFP_{300}	10.78	123.5	374.1	3.03
SEM	0.041	0.769	2.025	0.015
Variety				
CFP	10.72	122.1	374.9	3.07[a]
WFP	10.83	124.7	375.4	3.01[b]
Dose				
100 g/kg	10.88	124.9	372.3	2.98[b]
200 g/kg	10.76	124.1	379.0	3.06[a]
300 g/kg	10.68	121.3	374.2	3.09[a]
ANOVA *P*-value				
Variety (V)	0.227	0.108	0.912	0.042
Dose (D)	0.216	0.154	0.439	0.012
D × V interaction	0.817	0.688	0.716	0.238
Contrast *P*-value[2]				
PS_0 vs. CFP	0.183	0.507	0.687	0.155
PS_0 vs. WFP	0.623	0.662	0.633	0.993

BW = body weight; DBWG = daily body weight gain; DFI = daily feed intake; FCR = feed conversion ratio; SEM = standard error of the mean (SD divided by the square root of replication number, $n = 42$).
[a, b] Values in a column with different letter superscripts differ significantly ($P < 0.05$).
[1] Diets PS_0, CFP_{100}, CFP_{200}, CFP_{300}, WFP_{100}, WFP_{200}, WFP_{300} contained 0, 100, 200, 300 g/kg of pea seeds of colored-flowered (CFP) or white-flowered (WFP) varieties, respectively.
[2] The control PS_0 group was compared with CFP and WFP treatments by simple contrast analysis.

4.2. Parameters of small intestinal function

The replacement of SBM with PS, accompanied by a decrease in the wheat content of turkey diets, affected selected parameters of small intestinal function. The weight of the small intestine including the contents increased with increasing dietary inclusion levels of PS (from 100 to 200 and 300 g/kg), but this increase was below the value noted in group PS0. A numerical increase was also observed in the DM content of small intestinal digesta, but no significant differences were found relative to the control group. A significant increase in the viscosity of small intestinal digesta was noted only in group WFP100, compared with the remaining groups (see V × D interaction). This difference is difficult to explain because the DM content of intestinal digesta was similar in both 100 g/kg subgroups. There is no evidence in the available literature that differences in the polyphenol content of poultry diets, analogous to differences between PS of white- and colored-flowered varieties, may affect the physicochemical properties of intestinal digesta, including viscosity. Amerah et al. (2015) reported that a different content of rapeseed meal in chicken diets did not affect the viscosity of the intestinal contents. This is an important consideration in the interpretation of the results of the present experiment where rapeseed was used as an energy component in the same amount in all diets. In the remaining treatments, digesta viscosity did not exceed 3 mPa•s, which is comparable with the values noted in experiments where SBM was partially replaced with faba beans (Przywitowski et al., 2017; Mikulski et al., 2017) or peas (Zduńczyk et al., 2020). The results of similar studies indicate that the viscosity of small intestinal digesta in the range of 2 to 3 mPa•s can be treated as a normal physiological state of the intestines in turkeys, at which the antinutritional effect is not observed (Jankowski et al., 2013). Taking into account the fact that WFP treatments excelled CFP treatments in terms of FCR values, the above difference in small intestinal viscosity could be considered below the antinutritional threshold value.

The changes in the content of SBM, PS and wheat in turkey diets, discussed above, could increase the concentrations of polysaccharides fermented by gut microbiota, in particular amylase-resistant starch (RS). In comparison with cereal starch, starch from legume seeds is characterized by higher amylase content and greater polymer weight, which decreases the intestinal digestibility of this polysaccharide (Svihus et al., 2005). According to Goodarzi Boroojenji et al. (2018), raw PS contain 3.25% RS, but other studies (Hejdysz et al., 2016) have demonstrated that the RS content of grain legumes can be much higher. Therefore, higher dietary inclusion levels of starch-rich legume seeds may enhance fermentation processes in the GIT of turkeys, as demonstrated by an experiment with faba beans (Mikulski et al., 2017). Another factor that can stimulate fermentation in the GIT of poultry is increased NSP content of diets. In the present study, the levels of acetate, propionate and total SCFAs in the small intestinal digesta increased only in response to the highest content of WFP seeds, compared with CFP seeds, which could result from the opposing effects of tannins and non-digestible oligosaccharides and polysaccharides. Díaz Carrasco et al. (2018) demonstrated that tannin-fed chickens were characterized by a drastic decrease in the counts of *Bacteroides* spp., accompanied by an increase in the counts of certain members of the order *Clostridiales*, predominantly belonging to the families Ruminococcaceae and Lachnospiraceae. Other polyphenolic compounds can also suppress the activity of selected groups of gut microbiota and enzymes (Negi and Jayaprakasha, 2003; Mateos et al., 2012; Klinder et al., 2016), whereas readily fermentable polysaccharides such as RS stimulate gut fermentation (Montagne et al., 2003). In the current study, the increase in SCFA concentrations noted in the WFP_{300} treatment, relative to CFP_{300}, suggests that the high starch content of peas stimulated fermentation processes at low dietary tannin levels. In previous experiments where turkeys were fed diets containing grain legumes (Mikulski et al., 2017; Zduńczyk et al., 2018), SCFA levels in the small intestinal contents below 10 μmol/g had no adverse effects on

the physicochemical properties of digesta, and no undesirable bacterial overgrowth was observed in the small intestine.

Many biological components of the diet, including polyphenols, can affect the condition of the mucosa and the microscopic structure of intestines in poultry (Viveros et al., 2011). The intestinal mucosa plays a key role in the digestion and absorption of nutrients, and it protects the host against harmful substances and pathogens (Celi et al., 2017). An important parameter for the estimation of the absorptive capacity of the small intestine in chickens is the ratio of villus height to crypt depth because nutrient absorption increases with an increase in this ratio (Montagne et al., 2003). In experiments conducted on chickens, diets supplemented with tannin extract from faba bean seeds contributed to histological lesions (Ortiz et al., 1994). A sorghum-based diet containing high tannin levels also decreased villus height and crypt depth in the first period of chicken feeding; however, dietary tannin levels are generally not a limiting factor in GIT development (Nyamambi et al., 2007). Another study revealed undesirable changes in intestinal mucosa architecture in chickens fed diets with high-tannin faba beans (Tomaszewska et al., 2018). In the present experiment, mucosa thickness, crypt depth and the height of small intestinal villi were similar in turkeys fed diets containing different SBM and PS levels. Other studies (Smits and Annison, 1996; Teirlynck et al., 2009) have shown that increased intestinal viscosity might change the morphology of ileal villi. In this experiment, the difference in digesta viscosity between the WFP_{100} treatment and the remaining treatments was not confirmed by differences in intestinal histology.

4.3. Cecal function parameters

In the present experiment, the only parameter of ceca that differentiated the subgroups of turkeys receiving diets with PS of different varieties was the higher pH of digesta noted for CFP seeds. The concentrations of SCFAs in the cecal digesta were similar in groups CFP and WFP. A significant ($P = 0.041$) difference was found in the DM content of digesta (13.1% in CFP treatments vs. 14.6% in WFP treatments). The higher pH of digesta in subgroups CFP could result from increased hydration of cecal digesta, which decreased the acidifying effect of SCFAs on the digesta. An increase in the PS content of turkey diets (from 100 to 200 and 300 g/kg) led to a desirable decrease in ammonia concentration in the cecal digesta. Similar observations were made by Shim et al. (2007) who found that insoluble fiber present in legumes was slowly fermented in the large intestine and could reduce proteolytic fermentation. This fact could also explain the reduced concentrations of ammonia and putrefactive SCFAs in the lower gut of birds fed diets with an increased content of PS in the present study. An experiment with growing pigs (Jha and Leterme, 2012) revealed that pea fiber increased SCFA levels and decreased ammonia concentration in the intestines, and reduced fecal N excretion.

It is well known that the enzymatic activity of gut microbiota is enhanced by a higher dietary content of readily fermentable oligo- and polysaccharides (Flint et al., 2012; Apajalahti and Vienola, 2016), and reduced by a higher content of polyphenolic compounds (Negi and Jayaprakasha, 2001; Hanhineva et al., 2010) and their complexes with polysaccharides (Sellimi et al., 2017). In the present experiment, the inhibitory effect of polyphenols on the enzymatic activity of gut microbiota could explain the lower ($P < 0.017$) activity of β-glucosidase in CFP treatments as compared with WFP treatments. The dietary inclusion of PS exerted a similar effect on β-galactosidase activity, which was lower ($P = 0.047$) in the CFP_{100} treatment than in the WFP_{100} treatment. It can be assumed that the inhibitory effect of CFP_{100} was caused by the quantitative relationship between PS as a source of additional dietary polyphenols and the amounts of readily fermentable starch, oligosaccharides and NSP available to intestinal bacteria. Interestingly, such an effect was not noted in the treatments with medium and high levels of PS when the content of substrates available for microbiota was higher. A comparison of CFP and WFP treatments vs. the control group without PS revealed that the inclusion of PS in turkey diets stimulated the activity of all analyzed enzymes produced by cecal microbiota, including those involved in fermentation (α-glucosidases, α- and β-galacosidases, β-xylosidase and α-arabinofuranosidase) as well as β-glucuronidase and β-glucosidase, which are capable of deconjugating toxins and their lower activity may lead to reduced exposure to carcinogens (Desrouillères et al., 2015). The increased activity of gut microbial enzymes, noted in this study, points to the presence of readily fermentable polysaccharides in PS diets. At the same time, similar SCFA levels in the intestinal digesta suggest that the amount of available substrate was not significantly different. Therefore, the inclusion of PS in diets changed the composition of polysaccharides rather than their concentrations.

In the present study, the cecal concentration of propionic acid and the share of propionate in total SCFAs were higher at the highest dietary inclusion of PS, compared with the lowest and medium levels of SBM replacement with PS, whereas the experimental factors did not affect butyric acid concentration. According to Topping and Clifton (2001), the fermentation of some RS types favors butyrate production but RS is less effective than NSP in stool bulking. In the current experiment, the amount of cecal digesta and butyrate concentration in the digesta were comparable in all dietary treatments. Kubena et al. (2001) demonstrated that the concentration of propionic acid produced in the ceca of young chicks may be an important part of the mechanism (s) that inhibit GIT colonization by anaerobic bacteria. Although carbohydrates are the main precursors of propionic acid, it can be synthesized from a wide range of substrates, including proteins (Al-Lahham et al., 2010). This could also explain the lower ammonia concentration in the cecal digesta of turkeys fed diets with increased PS content, observed in the present study.

4.4. Growth performance of turkeys

Our previous experiment (Zduńczyk et al., 2020) has shown that the seeds of white-flowered peas at 300 g/kg at the expense of wheat and SBM can be effectively used in diets for young turkeys (up to 8 wk of age) without any negative effects on the digestive function or final BW. In the present study, the dietary inclusion of PS at up to 300 g/kg as a substitute for SBM had no effect on feed intake or BW gain. FCR deteriorated significantly ($P = 0.012$) when the PS content of diets increased, particularly in turkeys fed diets with the highest and medium levels of CFP seeds ($P = 0.042$) relative to the lowest level. The difference in FCR between CFP and WFP treatments was statistically significant, but relatively small (3.07 vs. 3.01 kg/kg), which implies that the use of both types of seeds in turkey nutrition may be determined by economic factors, primarily their price.

5. Conclusions

It can be concluded that PS of colored-flowered and white-flowered varieties differing in the content of tannins and, to a lesser extent, trypsin inhibitors and fiber fractions, do not induce undesirable changes in gastrointestinal function in finisher turkeys. Selected physiological parameters of turkeys indicate that diets with increased PS content (200 and 300 g/kg vs. 100 g/kg) have a more beneficial influence on small intestinal and cecal functions. Despite slight differences in the physiological parameters of the

GIT, the use of PS of the white-flowered variety resulted in better feed conversion (FCR 3.01 vs. 3.07, $P = 0.042$).

Author contributions

Zenon Zduńczyk: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization. **Dariusz Mikulski**: Methodology, Investigation, Data Curation, Software, Writing - Review & Editing, Supervision. **Jan Jankowski**: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **Bogdan A. Slominski**: Methodology, Resources, Data Curation, Writing - Review & Editing. **Jerzy Juśkiewicz**: Methodology, Resources, Writing - Review & Editing, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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