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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A B S T R A C T

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 \(\beta\)-glucosidase \((P = 0.017)\) and \(\beta\)-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.

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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
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 (Vincenti 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 of chickens (Czerwiński et al., 2010), the inclusion of 150 g/kg raw

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 (PS0) 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 CFP100, CFP200 and CFP300, respectively) and the white-flowered variety (subgroups WFP100, WFP200 and WFP300, 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 (Jesa 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 Rutzkowiak, 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
Cu 16 and 14 mg, I 2.4 and 2.1 mg, Se 0.24 and 0.21 mg, respectively.

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.

LVDV II the supernatant fraction using the cone/plate viscometer (model

¼ treatment and were sacri
ging average group BW were selected from each dietary
weights of dead birds were used to adjust average BW gain, DFI and

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 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-xylanase.

of the REM – apparent metabolizable energy.

1 It contains 990 g methionine/kg product (Evonik Degussa GmbH, Essen, Germany).

2 According to Kakade et al. (1974).

3 According to the method of Jeruminas (1972) modified by Adams and Novellie (1975).

4 Crude protein was determined analytically, and the content of the remaining nutrients was calculated based on 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-xylanase.

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-xylanase.

### 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 |
|------|------------------|------------------|
|      | PS0  CFP100  CFP200  CFP300  WFP100  WFP200 | PS0  CFP100  CFP200  CFP300  WFP100  WFP200 |
| Ingredients | | | |
| Wheat | 674.9 | 600.4 | 525.6 | 451.1 | 505.4 | 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 | 0.000 | 200.0 | 300.0 | 400.0 | 200.0 | 300.0 | 400.0 | 200.0 | 300.0 | 400.0 | 200.0 | 300.0 | 400.0 |
| Full-fat rapeseed (20.7% of CP) | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 |
| Tallow | 28.0 | 27.1 | 26.1 | 25.2 | 24.8 | 24.6 | 24.4 | 24.2 | 24.0 | 23.8 | 23.6 | 23.4 | 23.2 |
| 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 |
| Sodium chloride | 2.2 | 2.2 | 2.3 | 2.3 | 2.2 | 2.2 | 2.3 | 2.3 | 2.2 | 2.3 | 2.2 | 2.3 | 2.3 |
| Limestone | 13.2 | 13.1 | 13.1 | 13.0 | 13.2 | 13.1 | 13.2 | 13.1 | 13.1 | 13.2 | 13.1 | 13.2 | 13.1 |
| 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 |
| DL-Methionine | 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 | 3.3 | 2.5 | 1.8 | 1.0 | 2.9 | 1.2 | 3.3 | 2.6 | 1.9 | 1.2 | 3.3 | 2.6 | 1.9 | 1.2 |
| L-Threonine | 0.6 | 0.5 | 0.4 | 0.3 | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Vitamin-mineral premix | 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 |

### 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 | 876.6 | 23.0 | 22.8 |
| Ash (AOAC, 2005; procedure 920.45) | 20.5 | 20.3 | 20.3 | 20.5 |
| Ether extract (AOAC, 2005; procedure 920.39) | 181.2 | 189.3 | 203.7 | 215.5 |
| Starch (AOAC, 2005; procedure 996.11) | 450.0 | 489.0 | 511.6 | 556.6 |
| Fiber fractions | | | | |
| Neutral detergent fiber | 108.5 | 99.9 | 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 | 134.1 |
| NSP component sugars | | | | |
| Arabinoxylanes | 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 | | | | |
| Rafinesque family oligosaccharides | 33.4 | 29.3 | 37.5 | 33.3 |
| Activity of trypsin inhibitors (TIA) | 0.98 | 1.34 | 1.10 | 1.52 |
| Tannins | 4.66 | 0.67 | 5.24 | 0.76 |
and a-arabinoplyranosidase. 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 (Juszkiewicz 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-His-tech, 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 CFP100, WFP100, CFP200, WFP200, CFP300, and WFP300.

In addition, a simple contrast analysis was used to compare the control diet PS0 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 R < 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 (6.67 vs. 46.66 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, the variety contained 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 PS0 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 PS0 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 (PS0) but such an effect was not observed (P = 0.396) when CFP seeds were added to the diet. In comparison with the control group (PS0), 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 710 μmol/g in group PS0, 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 pH × 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 CFP100, 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 (WFP100 > CFP100; 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 PS0, 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 PS0.

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
with CFP seeds, WFP seeds decreased the pH of cecal digesta 

WFP seeds were characterized by higher (\( n = 42 \)) 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 (\( n = 7 \)).

| Item \(^1\) | Total mass, g/kg BW | Dry matter, % | Viscosity, mPa.s | pH | SCFAs, \( \mu \)mol/g | SCFA profile, % of SCFA |
|-------------|---------------------|---------------|------------------|----|----------------------|-------------------------|
| 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 | 81.4\(^b\) | 16.0\(^b\) | 0.454\(^b\) |
| WFP \(_{100}\) | 14.9                | 19.0          | 3.54\(^b\) | 6.92 | 4.21\(^b\) | 0.97 | 0.049 | 5.31\(^b\) | 0.087 | 79.1\(^b\) | 18.3\(^b\) | 0.954\(^b\) |
| CFP \(_{200}\) | 16.8                | 23.1          | 2.97\(^b\) | 6.70 | 4.46\(^b\) | 1.19 | 0.046 | 5.75\(^b\) | 0.049 | 77.6\(^b\) | 20.6\(^b\) | 0.822\(^b\) |
| WFP \(_{200}\) | 16.4                | 23.0          | 2.95\(^b\) | 6.90 | 5.06\(^b\) | 0.87 | 0.032 | 6.04\(^b\) | 0.072 | 83.5\(^b\) | 14.7\(^b\) | 0.530\(^b\) |
| CFP \(_{300}\) | 16.3                | 23.2          | 2.66\(^b\) | 6.94 | 3.34\(^b\) | 0.81 | 0.035 | 4.34\(^b\) | 0.052 | 78.6\(^b\) | 19.2\(^b\) | 0.851\(^b\) |
| WFP \(_{300}\) | 16.6                | 24.1          | 2.82\(^b\) | 7.13 | 7.52\(^b\) | 1.08 | 0.053 | 8.70\(^b\) | 0.049 | 86.1\(^b\) | 12.7\(^b\) | 0.610\(^b\) |
| SEM         | 0.252               | 0.366         | 0.078            | 0.716 | 0.251 | 0.046 | 0.003 | 0.258 | 0.005 | 0.863 | 0.837 | 0.056 |

\( \text{SEM} = \text{standard error of the mean (SD divided by the square root of replication number, } n = 42) \)

\( ^{1}\) 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 3

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

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 (\( \mu \)m, \( n = 7 \)).

| 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 |

\( \text{Vh/Cd} = \text{villus height/crypt depth ratio; SEM} = \text{standard error of the mean (SD divided by the square root of replication number, } n = 42) \)

\( ^{3}\) 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.

\( ^{4}\) 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 

**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 \( \beta \)-glucosidase and \( \beta \)-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 \( \beta \)-glucosidase...
significantly (10.1 in groups CFP vs. 14.2 at the lowest PS content (see Table 5)). Enzyme activities were also affected by the PS content of the dietary inclusion of WFP seeds significantly (P < 0.009). A nearly significant (P = 0.060) difference in the activity of β-glucuronidase was 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 PS0 birds. At the same time, as compared with group PS0, 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-xyllosidase (P = 0.029) and α-arabinopyranosidase (P = 0.009).

Table 5
Cecal parameter functions in turkeys fed diets containing different levels of pea seeds (n = 7).

| Item1 | Tissue mass, g/kg of BW | Cecal contents, g/kg of BW | Ammonia, mg/g | Dry matter, % | pH |
|-------|------------------------|---------------------------|--------------|--------------|----|
| PS0   | 3.01                   | 2.85                      | 0.219        | 11.1         | 6.15|
| CFP100| 2.62                   | 2.28                      | 0.245        | 11.5         | 6.27|
| WFP100| 2.63                   | 1.81                      | 0.248        | 15.1         | 5.84|
| CFP300| 2.61                   | 2.54                      | 0.156        | 14.4         | 6.10|
| WFP200| 2.84                   | 1.88                      | 0.188        | 14.1         | 5.82|
| CFP100| 2.85                   | 1.85                      | 0.180        | 13.4         | 6.19|
| WFP300| 2.77                   | 2.62                      | 0.200        | 14.7         | 5.89|
| SEM   | 0.067                  | 0.129                     | 0.010        | 0.367        | 0.061|

| Variety | CFP | WFP | SEM |
|---------|-----|-----|-----|
| Dose    |     |     |     |
| 100 g/kg| 2.62| 2.04| 0.246a|
| 200 g/kg| 2.72| 2.21| 0.172b|
| 300 g/kg| 2.81| 2.23| 0.190b|

ANOVA P-value

| Variety (V) | Dose (D) | V × D interaction |
|-------------|----------|-------------------|
| PS0 vs. CFP | 0.143    | 0.102             |
| PS0 vs. WFP | 0.221    | 0.054             |

SEM = standard error of the mean (SD divided by the square root of replication number, n = 42).

P<0.017) and the activity of β-galactosidase, in the latter case only

1 Diets PS0, CFP100, CFP200, CFP300, WFP100, WFP200, WFP300 contained 0, 100, 200, 300 g/kg of pea seeds of colored-flowered (CFP) or white-flowered (WFP) varieties, respectively.

2 The control PS0 group was compared with CFP and WFP treatments by simple contrast analysis.

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

| Item1 | Microbial enzymes...
|-------|------------------|
| n-gluc | β-gluc | α-gal | β-gal | β-gluc | β-xyl | α-arab |
| PS0    | 26.0   | 0.71  | 3.92  | 7.51   | 4.03  | 3.84  | 11.6   |
| CFP100 | 23.7   | 1.27  | 6.15  | 7.10a  | 7.97  | 3.72  | 13.8   |
| WFP100 | 30.1   | 3.56  | 8.54  | 19.7a  | 11.4  | 8.33  | 23.7   |
| CFP200 | 28.2   | 1.48  | 5.96  | 12.2a  | 10.1  | 4.46  | 18.7   |
| WFP200 | 32.8   | 2.87  | 9.71  | 12.1a  | 11.7  | 6.82  | 21.8   |
| CFP300 | 41.1   | 2.60  | 11.9  | 14.1a  | 12.3  | 8.56  | 25.2   |
| WFP300 | 36.7   | 2.93  | 14.5  | 16.5a  | 19.4  | 6.66  | 25.6   |
| SEM    | 1.576  | 0.258 | 0.908 | 1.099  | 1.057 | 0.540 | 1.531  |

| Variety | CFP | WFP |
|---------|-----|-----|
| Dose    |     |     |
| 100 g/kg| 26.9a| 2.41 |
| 200 g/kg| 30.5a| 2.17 |
| 300 g/kg| 38.9a| 2.76 |

ANOVA P-value

| Variety (V) | Dose (D) | V × D interaction |
|-------------|----------|-------------------|
| PS0 vs. CFP | 0.258   | 0.135             |
| PS0 vs. WFP | 0.106   | 0.001             |

SEM = standard error of the mean (SD divided by the square root of replication number, n = 42).

1 Values in a column with different letter superscripts differ significantly (P < 0.05).

2 The control PS0 group was compared with CFP and WFP treatments by simple contrast analysis.

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Two-way ANOVAs 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 $P = 0.047$) 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 ($P = 0.001$) 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.

### 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 (Jezierski 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 CFPS 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

| Item | SCFAs, µmol/g | SCFA pool, µmol/kg BW | PSCFAs, µmol/g | SCFA profile, % of SCFAs |
|------|---------------|-----------------------|----------------|-------------------------|
|      | Acetic | Propionic | Butyric | Total |                   | Acetic | Propionic | Butyric |
| PS0  | 131    | 11.8     | 38.1      | 184   | 527           | 2.93   | 71.5       | 6.23      | 20.7     |
| CFP  | 115    | 7.74     | 37.2      | 163   | 374           | 2.95   | 70.8       | 4.85      | 22.6     |
| WFP100 | 134    | 7.77     | 39.8      | 184   | 340           | 2.31   | 72.9       | 4.27      | 21.5     |
| CFP200 | 139    | 6.98     | 45.0      | 193   | 498           | 2.60   | 71.7       | 3.67      | 23.2     |
| WFP200 | 135    | 7.15     | 41.0      | 185   | 351           | 2.48   | 72.5       | 3.86      | 22.2     |
| CFP300 | 142    | 13.2     | 37.7      | 196   | 366           | 3.66   | 72.6       | 6.80      | 18.6     |
| WFP300 | 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     | 38.5      | 173   | 357           | 2.63   | 71.9       | 4.56      | 22.0     |
| 200 g/kg | 137    | 7.07     | 43.0      | 189   | 425           | 2.54   | 72.1       | 3.76      | 22.7     |
| 300 g/kg | 142    | 12.1     | 39.4      | 197   | 441           | 3.40   | 72.3       | 6.23      | 19.7     |
| ANOVA P-value |        |          |           |       |               |        |            |           |           |
| Variety (V) | 0.415   | 0.426    | 0.833     | 0.588 | 0.832         | 0.190  | 0.481      | 0.243     | 0.099     |
| Dose (D) | 0.085   | 0.001    | 0.476     | 0.098 | 0.362         | 0.070  | 0.933      | 0.001     | 0.102     |
| Contrast P-value | 0.330   | 0.440    | 0.588     | 0.420 | 0.065         | 0.785  | 0.594      | 0.460     | 0.487     |
| PS0 vs. CFP | 0.943   | 0.111    | 0.678     | 0.990 | 0.166         | 0.780  | 0.868      | 0.122     | 0.683     |
| PS0 vs. WFP | 0.506   | 0.047    | 0.576     | 0.687 | 0.131         | 0.545  | 0.506      | 0.027     | 0.680     |

PSCFAs – putrefactive SCFAs (C4i + CSI + C5i); Σ – sum; SEM – standard error of the mean (SD divided by the square root of replication number, $n = 42$).

1. Values in a column with different letter superscripts differ significantly ($P < 0.05$).
2. The control PS0 group was compared with CFP and WFP treatments by simple contrast analysis.
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 $V \times D$ 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 (CFP) or white-flowered (WFP) 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.

### 4.2. Parameters of small intestinal function

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 WFP300 treatment, relative to CFP300, 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; Teislyneck 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 be accompanied by a decrease in the DM content of digesta and a more pronounced effect on the activity of gut microbial enzymes. In the present study, the only parameter of ceca that differentiated turkeys fed diets with PS of different varieties was the higher pH of digesta noted for CFP seeds. 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 growth 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 the WFP and CFP 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

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-galactosidases, 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 and butyric acids 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.
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 Zdunczyk: 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, Supervision. Jerzy Juskiewicz: 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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