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Effect of dietary wheat gluten levels on intestinal mucin flow and composition in young pigs

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ABSTRACT. The aim of the study was to investigate the effect of dietary levels of wheat gluten (WG) protein on the ileal flow of crude mucin and sugars, as well as sugar composition of crude mucin preparations in young pigs. Six cannulated young pigs were used in 3 periods × 3 diets in the Latin square design. The three isoenergetic diets containing low, medium or high WG protein level (LWG, MWG and HWG, respectively) were formulated. LWG diet was treated as a control. The three dietary WG levels were obtained by supplementing 26.7, 52.9 and 79.0 g WG/kg to the respective diets. Ileal digesta was collected during the last 3 days of each 7-day period. Xylose content in ileal digesta was higher in LWG pigs compared to two other pigs groups. Galactose content was higher in MWG pigs compared to LWG and was lower compared to HWG pigs. Xylose content decreased linearly and galactose content increased linearly with increasing dietary WG protein levels. The HWG diet increased ileal flow of crude mucin and galactose compared to the LWG diet. The flow of crude mucin and galactose increased linearly with increasing dietary WG protein levels. The content of galactose in crude mucin preparations was higher in MWG pigs than in LWG pigs and was lower than in the HWG pigs. Dietary WG protein levels had a linear effect on the content of xylose and galactose in crude mucin preparations. In conclusion, WG protein positively affected ileal flow of mucin and its sugar composition.

Introduction

Mucins are the main component of the mucus layer, covering the luminal surface of the intestinal lumen, and thus protecting the epithelium from bacterial, chemical and mechanical damage (Bansil and Turner, 2006). The mucus layer forms a dynamic balance between synthesis and secretion of mucins by goblet cells and their degradation by proteolysis and physical erosion (Miner-Williams et al., 2013). Mucins contain a peptide backbone rich in proline, threonine and serine, and oligosaccharides side chains that account for 50–90% of the mass (Lien et al., 2001). *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose, fucose and sialic acid are found in mucins, which can be divided into acidic and neutral compounds depending on the type of sugar in the chain. The content of *N*-acetylgalactosamine and *N*-acetylglucosamine in digesta and faeces and their ratio and sialic acid are considered as useful mucin markers (Lien et al., 1997; 2001). Additionally, content of sialic acid in the faeces may be treated as a biomarker of intestinal damage (Rathod and Dhok, 2024)

The level of dietary protein has an important impact on the growth and health of young pigs. A high-protein diet can favour the growth and development of piglets; however, the undigested

protein from a high-protein diet may have a negative influence on the gut, including increased permeability and proliferation of pathogenic bacteria, resulting in an increased incidence of diarrhoea (Xia et al., 2022). A high-protein diet can also increase mucin secretion in the gut, however, the observed changes depended on protein type, gut segment and analytical method applied (Święch et al., 2022). Mucin secretion may also be affected by the type of dietary protein. The stimulating effect of low-digestible protein type could be associated with a high content of fibre and antinutritional factors in plant feedstuffs and the presence of bioactive peptides in dairy products, which in turn could induce an increase in mucin secretion. A low-protein diet is generally applied to reduce diarrhoea incidence and mortality; however, it can also decrease piglet growth performance (Opapeju et al., 2008).

Wheat gluten (WG) is a high-protein plant feedstuff, poor in threonine and rich in non-essential amino acids (NEAA), especially glutamine (Blasco et al., 2005). NEAA are involved in the process of maintaining the structure and function of the gut and WG proteins are recognized as a beneficial protein source for the pig gut. It is assumed that NEAA have a sparing effect on threonine utilization for mucin synthesis and secretion (Święch et al., 2022). For this reason, NEAA are considered important for protecting the intestine with mucin.

Mucin proteins are strongly resistant to digestion in the small intestine and can be significant source of endogenous losses of protein and amino acids, such as threonine, proline, glycine and serine (Lien et al., 1997). Measuring mucin flow in the gut lumen could provide important information on protective properties of mucins depending on dietary factors.

It is known that dietary fibre, depending on its physico-chemical properties, has a different effect on mucin excretion into the ileal digesta of pigs (Piel et al., 2005). However, there are no precise data concerning the effect of the level and source of dietary protein on mucin secretion in the intestine of young pigs. It was hypothesized that dietary WG protein levels would modify mucin quality and quantity in the gut of young pigs. Therefore, the aim of the present study was to investigate the effect of dietary WG protein levels on the ileal flow of sugars and crude mucin, as well as the sugar composition of crude mucin preparations isolated from the ileal digesta of young pigs.

Material and methods

Animals and experimental procedures

All experimental procedures were approved by the 3rd Local Ethics Committee in Warsaw, Poland (approval number 4/2008). The experiment was carried out on six barrows (Polish Landrace × Duroc) weighing from 20 to 25 kg. The experiment was conducted according to the procedure described in detail by Święch (2015). Briefly, pigs were surgically fitted with a post-valvular T-caecum cannula (PVTC) in accordance with the method of van Leeuwen et al. (1991). During the first ten days after cannulation, the animals were fed increasing quantities of the LWG diet (26.7 g/kg of WG). Recovery was followed by three seven-day periods of feeding the experimental diets in 3 × 3 Latin square design with three diets and three periods. There were six replications per each diet. Two pigs were fed each diet during each period. Animals were housed individually in metabolic cages with the slatted floor and plexiglass walls in a thermally controlled room (22–23 °C) and had *ad libitum* access to the water. The feeding level was adjusted to 5% body weight (BW) corresponding to about 90% of *ad libitum* intake. Feed allowances were changed weekly according to BW. The pigs were fed twice a day at 8:00 and 20:00 with equal portions of meal diets mixed with water (1:1). Each period lasted seven days with four adaptation days and then three days of collecting ileal digesta. Ileal digesta was collected from 8:00 to 20:00 (between meals) using bags attached to the cannulas. The bags were changed approximately every hour. A mixture of EDTA (15 mM), sodium azide (1 mM) and phenylmethylsulfonyl fluoride (2 mM) was added to a representative portion (approximately 20%) of the collected ileal digesta to reduce the activity of bacterial enzymes. Samples were immediately frozen at −20 °C and freeze-dried. Samples collected from each animal in each experimental period were pooled.

Diets

The composition and nutritional value of experimental diets have been described in detail by Święch (2015). The main ingredients of diets were: wheat, maize, soybean meal, full-fat soybeans, casein, wheat gluten, rapeseed oil and maize starch. Briefly, the three WG protein levels were obtained by adding 26.7, 52.9 and 79.0 g/kg of WG to low,

medium and high WG protein diets (LWG, MWG and HWG), respectively. LWG diet was treated as a control. WG was added to the diets instead of maize starch. LWG, MWG and HWG diets contained an increasing concentration of crude protein (169, 193 and 213 g/kg, respectively) and NEAA (102.5, 119.7, 135.8 g/kg, respectively). The diets were supplemented with essential amino acids to meet the NRC (1998) requirements for pigs. The composition and nutritional value of the diets are given in Table 1.

Table 1. Composition and nutritional value of diets

Item	Wheat gluten protein levels[1]		
	LWG	MWG	HWG
Ingredients, g/kg			
constant ingredients (sum)[2]	876.50	876.50	876.50
wheat gluten[3]	26.72	52.86	72.00
maize starch	68.10	45.70	22.10
rapeseed oil	21.00	18.00	16.00
L-lysine HCl (78%)	4.30	3.90	3.40
DL-methionine (98%)	0.38	0.04	0.00
chromium oxide	3.00	3.00	3.00
Nutritional value			
metabolizable energy[4], MJ/kg	14.00	14.00	14.00
crude protein, g/kg	169	193	213
total non-essential amino acids, g/kg	102.48	119.68	135.81

[1] dietary wheat gluten protein levels: LWG – low (control), MWG – medium, HWG – high; [2] contained per kg: g: wheat 550, maize 150, soybean meal 80, full-fat soybeans 50, casein, 10, calcium phosphate 16, limestone 12, salt 3.50, vitamin-mineral mixture 5; [3] contained following total amino acids per kg: g: lysine 9.2, threonine 21.2, methionine 10.1, cysteine 15.9, tryptophan 6.5, isoleucine 44, valine 32.1, histidine 17.9, arginine 36.9, leucine 61.6, alanine 22.5, aspartic acid 31.8, glutamic acid 363.4, glycine 28.5, proline 96.8, serine 43.0, tyrosine 26.2, phenylalanine 45; [4] calculated as the sum of the metabolizable energy values of individual dietary compounds according to NRC (1998)

Chemical analysis

The content of dry matter (code No. 934.01) and nitrogen (code No. 954.01) in diets and digesta were analysed using the standard method (AOAC International, 2011). Chromic oxide content in the diets and digesta were determined according to the method of Kimura and Miller (1957).

Crude mucin content in ileal digesta was analysed according to the method described by Piel et al. (2004). Briefly, freeze-dried ileal digesta was suspended in sodium chloride solution and vortexed. The supernatants obtained by centrifugation were mixed with cold absolute ethanol, incubated overnight at −20 °C and centrifuged. The precipitates were reconstituted in sodium chloride solution, vortexed and centrifuged again using the same conditions. The precipitates were freeze-dried and weighed. Concentration of crude mucin was expressed in g/kg of dry ileal digesta. Crude mucin flow was expressed in g/kg of dry matter intake. Calculation of crude mucin flow was based on chromium oxide content in the diet and digesta using the following formula:

$$CMF = \frac{CMI \times CrD}{CrI} \times 10,$$

where: CMF – crude mucin flow expressed in g per kg of dry matter intake, CMI – concentration of crude mucin in the ileal digesta expressed in % of dry matter, CrD – concentration of chromium in the diet expressed in %, CrI – concentration of chromium in the ileal digesta expressed in %.

Contents of sugars in ileal digesta and crude mucin preparations isolated from ileal digesta were analysed as their alditol acetates according to the procedures described by Lien et al. (1997). Briefly, ileal digesta and crude mucin preparations were hydrolysed; myo-inositol and *N*-methylglucamine were applied as internal standards for neutral and acidic sugars, respectively; sugar content was analysed using a gas chromatograph as previously described (Święch et al., 2022). Concentration of individual sugars was expressed in mol/100 moles of dry ileal digesta or crude mucin preparations. Crude sugars flow was expressed in g/kg of dry matter intake. Calculation of sugars flow was based on chromium oxide content in the diet and digesta using the following formula:

$$SF = \frac{SI \times CrD}{CrI} \times 10,$$

where: SF – sugar flow expressed in g per kg of dry matter intake, SI – concentration of sugar in the ileal digesta expressed in % of dry matter, CrD – concentration of chromium in the diet expressed in %, CrI – concentration of chromium in the ileal digesta expressed in %.

Statistical analysis

The results are expressed as the mean values from six pigs. The individual pig was considered as the experimental unit. Data were statistically evaluated using the GLM procedure implemented in the Statistica 13PL software. Normal distribution of data was checked by the Shapiro-Wilk test. Homogeneity of variance was verified by Lavene's test. WG protein level was a fixed effect, and pig and period were the random effects in the model. One-way ANOVA was used to test the main effect of

dietary WG protein levels. The significance of differences between means was tested using Tukey's test. Orthogonal polynomial contrasts were used to detect the linear and quadratic effects of WG protein levels. Differences were considered significant at $P \leq 0.05$.

Results

The content of crude mucins and sugars in the ileal digesta is presented in Table 2. Xylose content in the ileal digesta was higher in the LWG group than in the other two groups fed diets with higher WG contents. Galactose content was higher in MWG group than in the LWG group and was lower than in the HWG group. Xylose content in the ileal digesta decreased linearly and galactose content increased linearly with increasing dietary WG protein levels. The flow of crude mucin and galactose was higher in the HWG pigs in comparison to the LWG pigs. The flow of crude mucin increased linearly and quadratically with increasing WG protein levels, while galactose flow increased only linearly.

The sugar composition of crude mucins preparations is listed in Table 3. The HWG diet increased galactose content in crude mucin preparations compared to the other two diets. Crude mucin preparations from the ileal digesta of MWG pigs contained also more galactose than those from LWG pigs. The content of xylose in crude mucin preparations decreased linearly and content of galactose increased linearly with increasing WG protein concentrations. There was no effect of dietary WG protein level either on the content of *N*-acetylglucosamine and

Table 2. Effect of dietary wheat gluten protein levels on the content and flow of crude mucin and sugars in the ileal digesta of young pigs

Indices	Wheat gluten protein levels[1]			SEM	*P*-value		
	LWG	MWG	HWG		main	linear	quadratic
Concentration of crude mucin in ileal digesta, g/kg							
	78.1	78.5	82.8	1.88	0.181	0.117	0.330
Concentration of sugars in ileal digesta, mol/100 moles							
arabinose	25.1	24.5	24.7	0.72	0.720	0.531	0.618
xylose	31.3^{b}	28.7^{a}	27.9^{a}	0.91	0.003	0.001	0.239
mannose	1.4	1.3	1.5	0.31	0.682	0.495	0.521
glucose	34.5	37.0	36.3	0.42	0.339	0.314	0.280
galactose	7.7^{a}	8.6^{b}	9.6^{c}	0.44	0.003	0.001	0.924
Flow of crude mucin and sugars, g/kg dry matter intake							
crude mucin	19.7^{a}	21.4^{ab}	22.7^{b}	7.30	0.041	0.013	0.007
arabinose	17.4	16.6	17.9	0.55	0.552	0.676	0.312
xylose	21.7	19.5	20.3	0.58	0.354	0.369	0.261
mannose	12.8	10.4	15.0	0.26	0.574	0.537	0.401
glucose	290.5	306.2	317.9	1.18	0.740	0.448	0.949
galactose	64.3^{a}	69.9^{ab}	83.3^{b}	0.28	0.031	0.011	0.491

[1] dietary wheat gluten protein levels: LWG – low (control), MWG – medium, HWG – high; SEM – standard error of the mean; ^{abc} means with different superscripts within a column are significantly different at $P \leq 0.05$

Table 3. Effect of dietary wheat gluten protein levels on the content of sugars in crude mucin preparations isolated from the ileal digesta of young pigs, mol/100 moles

Item	Wheat gluten protein levels[1]			SEM	*P*-value		
	LWG	MWG	HWG		main	linear	quadratic
Fucose	3.31	4.00	3.59	0.33	0.238	0.471	0.127
Arabinose	26.23	24.54	24.61	0.92	0.391	0.250	0.464
Xylose	30.75	25.63	24.89	1.92	0.093	0.047	0.359
Mannose	1.03	1.28	1.42	0.19	0.327	0.147	0.811
Glucose	7.39	8.92	7.06	0.87	0.347	0.806	0.159
Galactose	17.05^{a}	19.57^{b}	23.84^{c}	0.94	0.001	0.001	0.495
N-acetylglucosamine	6.82	8.25	7.61	0.75	0.412	0.556	0.249
N-acetylgalactosamine	7.42	9.19	8.23	0.62	0.090	0.290	0.051
Ratio[2]	0.92	0.90	0.92	0.07	0.655	0.537	0.523

[1] dietary wheat gluten protein levels: LWG – low (control), MWG – medium, HWG – high; [2] ratio of *N*-acetylglucosamine to *N*-acetylgalactosamine; SEM – standard error of the mean; ^{abc} means with different superscripts within a column are significantly different at $P \leq 0.05$

N-acetylgalactosamine or their ratio in crude mucin preparations. There was only a trend towards a higher content of *N*-acetylgalactosamine in crude mucin preparation in the MWG group.

Discussion

It is known that dietary factors including fibre, protein and anti-nutritional factors may affect the amount of mucins secreted in the gut and their sugar composition (Montagne et al., 2004). Several studies evaluated the effect of dietary fibre on mucin excretion in ileal digesta in pigs (Lien et al., 2001). It was found that the obtained results depended on both the type and level of dietary fibre. However, the influence of dietary protein on mucin secretion has been studied less frequently. It should be noted that the results of studies concerning the effect of dietary factors on mucin secretion may be difficult to compare due to differences in the sample type (mucosa scraping and digesta), animal species (pigs, calves, chickens and rats), gut segment (duodenum, jejunum, ileum and colon) and assay method (fluorometric assay, ethanol precipitation, ELISA, hexosamine assay) used in individual studies (Piel et al., 2004).

The present study found the effect of dietary WG protein level on ileal flow of crude mucin. Higher WG levels increased the ileal flow of crude mucins, but not their content in the ileal digesta. Partially contrasting results were obtained by Montagne et al. (2000a), who did not observe the effect of dietary protein level on either the content or flow of crude mucins in the ileal digesta of calves fed diets containing from 10.4 to 27.8% crude protein. These authors observed higher crude mucin content and flow only in the duodenal digesta, but not in jejunal, ileal or colonic digesta in calves fed a diet containing 20.5% crude protein in comparison to animals fed a diet containing from 10.4 and 27.8% crude protein. A negative effect of high dietary WG levels on crude mucin content in the colonic digesta was reported by Święch et al. (2022). However, in the latter study, the content of crude mucins in the ileal digesta was not determined due to insufficient amount of the collected ileal digesta. It should be noted that mucin content in ileal digesta in our study and in the above studies was determined as crude mucin by ethanol precipitation. Other proteins and polysaccharides in addition to raw mucus may also be detected as crude mucins using this method (Miner-Williams et al., 2009b). However, when mucins were determined as the number of *O*-linked oligosaccharide chains using a mucin-specific fluorometric method, the effect of WG protein level on mucin content in the ileal and colonic digesta was not found (Święch et al., 2022). The latter study reported a positive effect of WG protein level on mucin content in mucosa in the duodenum and jejunum, but not in the ileum and proximal colon.

The source of dietary protein may also modulate mucin secretion. Dietary fibre and antinutritional factors present in high-protein plant feedstuffs can induce higher mucin excretion. Replacing fish protein and part of cereals with pea, and portion of casein with wheat pea, but not black pea, increased crude mucins secretion in pigs (Piel et al., 2004; 2007). The effect of pea addition to the diet was not observed when two other methods (ELISA and hexosamine assay) were applied for mucin determination and when the results were expressed as mucin content in the ileal digesta. The inclusion of high-protein plant products such as soybean protein concentrate or potato protein concentrate into the diet of calves instead of skim milk powder increased or tended to increase the content of crude mucin in digesta and flow of crude mucin in the duodenum, but not in the jejunum or ileum (Montagne et al., 2000a). Moreover, some high-protein animal products or their enzymatic hydrolysates containing bioactive peptides were shown to stimulate mucin secretion in the rat jejunum (Trompette et al., 2003). However, storage protein isolated from common bean added to a highly digestible diet decreased crude mucin flow in the colon but not in the ileum in rats (Montoya et al., 2010).

The optimal protective functions of the mucus layer depend not only on the amount of mucins, but also on their sugar composition. The content of *N*-acetylglucosamine and *N*-acetylgalactosamine in digesta has been previously utilized as a useful mucin marker (Lien et al., 1997; 2001), because these sugars do not occur in the diet and endogenous sources other than mucins. Unfortunately, the content of *N*-acetylglucosamine and *N*-acetylgalactosamine, as well as fucose concentration were not determined in the ileal digesta samples in our study due to the too low level of these sugars in the samples, below the detection limit of the applied method. In our previous study, these sugars were also not detected in the ileal and colonic digesta of pigs, when the same method of determining mucin composition was applied as in the present study (Święch et al., 2022). The content of *N*-acetylglucosamine and *N*-acetylgalactosamine in the ileal digesta of pigs differed between studies (Miner-Wiliams et al., 2009a; Piel et al., 2004)

due to differences in diet composition and age of the animals. The ratio of *N*-acetylglucosamine to *N*-acetylgalactosamine can range from 1.2 to 1.6. White pea, but not black pea, increased *N*-acetylglucosamine content and tended to increase *N*-acetylgalactosamine content in the ileal digesta of pigs (Piel et al., 2004). In the present study, the highest WG protein level increased the content of galactose, while xylose content was reduced by both MWG and HWG. Contrasting results were obtained by Święch et al. (2022), who observed lower arabinose, xylose and mannose contents, but higher glucose content in the ileal digesta of pigs fed a diet containing the same concentration of WG protein as in the HWG diet; these authors recorded no effect of WG level on galactose content.

In the present study, with the exception of sialic acid, all mucin sugars (i.e. *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose and fucose) were detected in crude mucin preparations isolated from ileal digesta. Galactose was the dominant sugar, which in crude mucin preparations accounted for approximately 50% of the sum of four mucin sugars. These results were consistent with the studies of Lien et al. (1996) and Montagne et al. (2000b), who observed that galactose accounted for approximately 42 and 47% of the sum of mucin sugars detected in crude mucin preparation isolated from calf and human ileal digesta, respectively. The galactose content in crude mucin preparations isolated from ileal digesta of pigs and chickens was lower and accounted for approximately 30% of the sum of mucin sugars (Lien et al., 1996; Tsirtsikos et al., 2012a; b). In the current study, the galactose content in crude mucin preparations increased linearly with increasing dietary WG protein content. Increasing galactose and mannose levels were observed in mucins isolated from ileal tissue of broilers (14 but not 42-days old) fed a diet containing an increasing concentration of phytogenic feed additives (Tsirtsikos et al., 2012b). In contrast, the addition of dietary probiotic linearly decreased galactose content in mucin isolated from caecal, but not ileal tissue of 42-days old broilers (Tsirtsikos et al., 2012a). Mucins with high galactose content may be expected to better protect the pig gut against bacterial infections. This interpretation is supported by the results of Izhar et al. (1982), who observed that the addition of glucose to the incubation medium inhibited the adhesion of pathogenic bacteria to colonic cells of guinea pigs. The protective properties of galactose have not been evaluated in the latter study; however, it can be expected that galactose present in ileal mucin may have similar properties to glucose due to the chemical structure similarity of both sugars. It can be assumed that high levels of WG protein may increase the protective capacity of ileal mucins in the porcine intestine.

In this study, the proportion of *N*-acetylglucosamine and *N*-acetylgalactosamine in crude mucin preparation was nearly equal (0.90–1.00), which suggested that crude mucin isolated from ileal digesta originated primarily in the small intestine (Lien et al., 1997). The determined values were higher than those reported by Lien et al. (1997) in pigs and lower than those obtained in calves (1.0–1.2; Montagne et al, 2000a; b) and broilers (3.0–3.9; Tsirtsikos et al., 2012a; b) fed a control diet.

Here, sugars other than those found in mucin composition were also detected in crude mucin preparation. The proportion of arabinose, xylose and glucose in crude mucin preparation was high and varied from 57 to 64% of the total sugars analysed. These results were consistent with those described in the studies of Lien et al. (1996; 1997). However, the content of non-mucin sugars in the aforementioned works was significantly lower and amounted to less than 10% of the sum of all analysed sugars. These findings could indicate that crude mucin preparations obtained in our study were contaminated with dietary sugars.

Due to the high content of NEAA in WG protein, the addition of WG to the LWG diet increased NEAA levels in the MWG and HWG diets by 17 and 33%, respectively. The effect of WG protein level on mucin secretion and its composition could be partially attributed to glutamic acid, as it is the most abundant NEAA in WG protein. It is well known that glutamine and glutamate play a key role in many physiological processes in the gut, such as maintaining intestinal integrity, nutrient metabolism and immune response (Wu et al., 2011). Glutamine is a potential precursor in the synthesis of *N*-acethylglucosamine and *N*-acethylgalactosamine (Reeds and Burrin, 2001). The assumption that glutamate was responsible for the effect of WG protein on mucin secretion and its sugar composition was not supported by the results of our previous study (Święch et al., 2010), where a negative effect of monosodium glutamate, added to a low-protein threonine-deficient diet, on the number of goblet cells in the small intestine of young pigs was found. A higher number of goblet cells has the potential to increase mucin secretion capacity.

Conclusions

Dietary wheat gluten (WG) protein levels may modulate both mucin secretion and sugar composition of crude mucin in the pig intestine. High levels of WG protein increased the amount of crude mucin secreted in the ileum and its galactose content. WG protein can be considered a beneficial source of protein for maintaining the protective properties of mucins.

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Conflict of interest

The Author declares that there is no conflict of interest.

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