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The effect of fibre level in the mixture on the state of intestinal epithelium of fatteners

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Abstract: *The effect of fibre level in the mixture on the state of intestinal epithelium of fatteners.* The purpose of the work was to determine the effect of the fibre content in the mixture on morphometric traits of intestinal epithelium of fatteners. In one-phase fattening, 14 crossbred pigs were classified into two groups and *ad libitum* fed the mixtures, differing in the composition and nutritional value, including the level of crude fibre (control group – C – 3.4%; experimental group – E – 12%) but with the maintained energy-protein ratio (1 : 13). At the age of 180 days, the fattening was completed; the weight of the pigs from C group was equal to ca. 100 kg and that one of the pigs from group E was by ca. 26 kg lower. The rate of growth of pigs from group E as compared to the animals from group C was slower ($P \leq 0.01$). After slaughter, morphometric evaluation of the scrapes from the following three segments of the small intestine was carried out: duodenum, jejunum and ileum. There was found a lower mitosis index (the number of divisions per one crypt) of the intestinal epithelium cells of the pigs from group E as compared to group C (duodenum, $P \leq 0.01$). The height of the epithelial cells in crypts and depth of crypts in three examined segments of small intestine (D, J, I) was higher in group E vs. group C ($P \leq 0.05$; $P \leq 0.01$), whereas the villus height was lower in group E vs. group C. The fibre level in the mixture affected the direction and level of changes in the examined indicators, characterizing the morphometric traits of intestinal epithelium.

Key words: fatteners, nutrition, fibre level, intestinal epithelium

INTRODUCTION

The application of the mixtures with the increased fibre content, containing e.g. dried substances and/or pectins in the nutrition of monogastric animals constitutes the basis for the studies on the physiology of nutrition and digestion and utilization of nutrients and also, on the morphology and morphometry of intestines in the pigs and model animals (Jin et al. 1994, Pluske et al. 1997, Wenk 2001, Fusch et al. 2003, Hedemann et al. 2006, Drzikova et al. 2007, Rekiel et al. 2007, Serena et al. 2008, Rekiel et al. 2010, Hanczakowska and Świątkiewicz 2012, Święch et al. 2012).

Feed materials, containing considerable quantities of fibre, dried grass and cotyledonous plants, wheat brans, oats grain are not employed in intensive production of slaughter pigs, or they are administrated in small quantities. It results from limitation of digestibility and assimilability of nutrients (Fusch et al.

2003). For growing pigs, piglets, weaners and fatteners under intensive production, it is recommended to employ full-ration mixtures with a low content of fibre and high digestibility. In extensive management, *inter alias*, ecological one, feed materials with a higher fibre content are used. Nutritional preferences of the pigs differing in age and physiological condition, connected with the production systems (intensive, organic) are the basis for the studies on feed materials (Fabijańska et al. 2003). They concern also the effect of diet, containing nutritional fibre on development of gastrointestinal tract. Depending on the source and level of fibre in the mixtures, the growth of small intestine and internal organs, liver and pancreas was examined in the rats (Faraldo Corrêa et al. 2009); in the pigs, the length and volume of gastrointestinal tract was studied (Jin et al. 1994). As affected by the feed components and the present fibre, intestinal mucus is changed. Its optimal level stimulates growth of intestinal villi and increases the number of crypts. In the crypts, the epithelial cells are proliferating and the factors of antibacterial resistance are produced (Paneth cells produce peptides – defensins) as well as endocrine factors, e.g. chromogranine A. The changes within the intestinal crypts increase or lower proliferating activity of enterocytes, contributing to the change in secreting activity of bactericidal factors and secretion of mucus (Mc Cullough et al. 1998, Ayabe et al. 2000).

The aim of the work was to determined the effect of fibre content in the mixture on the morphometric traits of intestinal epithelium of fatteners.

MATERIAL AND METHODS

One-phase fattening of 14 crossbred pigs of 4 breeds (PL × PLW) × (DUR × PIETR) was carried out; the animals were *ad libitum* fed from the initial body weight of ca. 25 kg in the intensive system (control group – C), or in the extensive system (experimental group – E). The groups consisted of 7 animals in each group, including 4 gilts and 3 barrows, from 7 litters. The mixtures for animals from groups C and E differed in composition and nutritional value (AOAC 1990), including crude fibre content (C – 3.4%; E – 12%) but they had the same ratio of energy to protein (Table 1).

TABLE 1. Composition of raw materials of the mixtures and their nutrive values

Specification		Group	
		C	E
Content			
Barley meal	%	64.0	64.0
Wheat meal		15.0	–
Extraction soy meal		13.5	–
Lard		7.0	–
Premix		0.5	0.5
Dried papilionaceous plants		–	35.5
Nutrive value			
Metabolizable energy (MJ/kg)		13.53	9.81
Crude protein (%)		17.75	12.80
Crude fiber (%)		3.4	12.0
Ratio of energy to protein (–)		1 : 13.1	1 : 13.0

After completion of fattening (age of 180 days), the animals were subject to standard slaughter procedures. From all pigs, the following samples from three segments of small intestines were *post mortem* collected: duodenum (D), the first loop of jejunum (J) and ileum (I), with dimensions of 5 × 20 mm each. The collected material was washed out with 0.9% solution of physiological salt and then, preserved in 10% buffered formalin solution (for 24 h). The samples were immersed in paraffine (paraplast--Sigma). To protect material, Vacuum Infiltration Processor (V.I.P.) by SAKURA company was used.

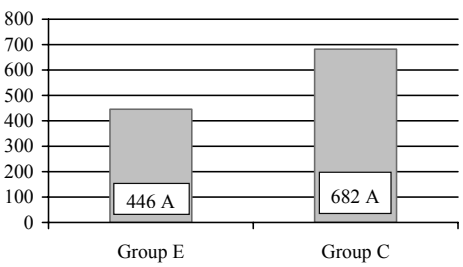
Paraffine blocs, containing specimens of intestines were cut into series of scrapes of 4 μm thickness in microtome and placed on basic slides, covered with protein (for histological staining). Staining of the scrapes was carried out using a review method: hematoxyline – eosine (H-E), using colouring machine DIVERSIFIED STAINER DRS--601 by SAKURA company. Then, the preparations were closed with a band, using closing device Coverslipper by SAKURA company.

The evaluation of the preparations with magnitude of 400× was performed with the use of light microscope BX 50 Olimpus. The number of partitions of enterocytes in crypt (mitotic index per 1 crypt), counting each time 100 cells in three repetitions was calculated. The height of villi and depth of crypts was also determined. The height of epithelial cells in crypts was evaluated, using analysis of the image AnalySIS 3.0. The result was expressed as the mean for 10 measured cells.

The number of cells' division per 1 crypt – mitotic index, height of epithelium cells in crypts, villus height, depth of crypts in each intestine segment (duodenum, jejunum, ileum) among the groups were compared with the Wilcoxon test with the Bonferroni correction using R software.

RESULTS AND DISCUSSION

The rate of growth of pigs from group E as compared to the animals from group C was slower (446 vs. 682; $P \leq 0.01$) – Figure 1. The results of the own studies are confirmed by the results of another



A, A – $P \leq 0.01$.

FIGURE 1. Growth rate (g)

experiment (Fusch et al. 2003) in which various levels of fibre were employed in the mixtures for weaners. The increased participation of the fibre in feed for growing pigs caused abbreviation and decrease of the absorptive area of small intestine and prolongation of colon what slowed down the growth rate of the animals. Lower by 25.9 kg body weight of

the pigs from group E vs. group C (extensively fed in comparison to those ones fed intensively) as being found in own studies, confirmed a negative effect of the increased fibre level in the mixture on physiology of digestion and, in effect, on the growth rate (Wenk 2001).

The correct histological structure of intestinal wall was recorded. The detailed evaluation included image of intestinal crypts and border of mucus and intestinal villi. Any univocal directional changes in the research groups E and C, as being expressed by value of the examined indicators (number of partitions into 1 crypt, height of epithelial cells in crypts and height of villi and depth of crypts) in small intestine (Table 2) were recorded.

The number of partitions per 1 crypt was higher in group C vs. group E in one from three examined segments of small intestine – in duodenum ($P \leq 0.01$). Values of the two examined indicators: height of enterocytes in crypts and depth of crypts revealed domination in group E vs. group C in each from three studied segments of small intestine. In D, J, I segments of intestine, the height of epithelial cells in crypts was higher in group E vs. group C by 0.20 (0.94%), 2.84 (13.58%) at $P \leq 0.05$, and by 4.12 (22.34%) at $P \leq 0.01$, respectively; the depth of crypts was higher by 80.92 (22.01%), 51 (13.74%) and 48 (14.81%) at $P \leq 0.05$, respectively. In D, J, I segments of intestine, villi were shorter in group E vs. group C by 6.67 (1.48%), 6.04 (1.46%), 5.7 (1.31%).

Nutritional factors and bacteria, present in intestines, may modify their microstructure and change functioning of mucus (Babińska et al. 2005). It was also demonstrated that the level of fibre and feed components changed microflora of intestinal content of growing pigs (Pluske et al. 1997). The results, obtained in own studies, indicate that the increased level of fibre in the mixture (group E) changed

TABLE 2. Morphometry of intestines of fatteners mixtures with different fibre level (mediana and IQR)

Item	Intestine segment	Group	
		C	E
Number of cells' division per 1 crypt – mitotic index	Duodenum (D)	2 (2.00) A	1 (1.00) A
	Jejunum (J)	2 (1.00)	2 (0.50)
	Ileum (I)	2 (2.00)	2 (1.00)
Height of epithelium cells in crypts (μm) – magnif. 20×	Duodenum (D)	21.35 (4.73)	21.55 (6.01)
	Jejunum (J)	20.91 (5.39) a	23.75 (8.55) a
	Ileum (I)	18.44 (4.33) A	22.56 (5.09) A
Villus height (μm) – magnif. 4×	Duodenum (D)	450.49 (220.78)	443.82 (154.04)
	Jejunum (J)	413.12 (118.42)	407.08 (99.97)
	Ileum (I)	433.89 (101.53)	428.19 (82.40)
Depth of crypts (μm) – magnif. 4×	Duodenum (D)	367.58 (129.76)	448.50 (119.50)
	Jejunum (J)	374.62 (97.75) a	426.10 (87.42) a
	Ileum (I)	326.60 (146.27)	374.96 (107.02)

A, A – $P \leq 0.01$; a, a – $P \leq 0.05$.

the structure of intestinal epithelium of fatteners and its morphometric parameters. It was a "response" to different mixture which was administrated to experimental pigs. Slower rate of growth of pigs from group E vs. group C resulted from worse utilization (conversion) of nutrients in feed with the increased fibre level. The obtained results indicate and confirm the limited capacities of utilizing hardly digestible feed components by monogastric animals where enzymatic digestion is dominating. In case of pigs, the participation and activity of favourable intestinal microflora in digestion process is small (Pluske et al. 1997, Babińska et al. 2005).

CONCLUSIONS

The pigs from the experimental group as compared to the animals from the control group were characterized by slower growth rate ($P \leq 0.01$). It could be associated, *inter alias,* with morphological changes of intestinal epithelium. The number of partitions per 1 crypt was higher in group C vs. group E in case of duodenum ($P \leq 0.01$). The height of the epithelial cells in crypts and depth of crypts in three examined segments of small intestine (D, J, I) was higher in group E vs. group C ($P \leq 0.05$; $P \leq 0.01$), whereas the villus height was lower in group E vs. group C. The results indicate the effect of differentiated content of fibre (derivate of diet components) on morphometric traits of intestinal epithelium. Preliminary results require confirmation on a larger sample.

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Streszczenie: *Wpływ poziomu włókna w mieszance na stan nabłonka jelit tuczników.* Celem pracy było określenie wpływu zawartości włókna w mieszance na cechy morfometryczne nabłonka jelit tuczników. W tuczu jednofazowym, 14 świń mieszańców podzielono na dwie grupy i żywiono do woli mieszankami różniącymi się składem i wartością pokarmową, w tym zawartością włókna surowego (grupa kontrolna – C – 3,4%, grupa doświadczalna – E – 12%), ale o zachowanym stosunku energetyczno-białkowym (1 : 13). W wieku 180 dni zakończono tucz; świnie z grupy C ważyły ok. 100 kg, a z grupy E o ok. 26 kg mniej. Tempo wzrostu świń z grupy E w porównaniu z grupą C było wolniejsze ($P \leq 0,01$). Poubojowo dokonano oceny morfometrycznej skrawków trzech odcinków jelita cienkiego: dwunastnicy, jelita czczego i jelita biodrowego. Stwierdzono niższy indeks mitotyczny (liczba podziałów na 1 kryptę) komórek nabłonka jelit świń (dwunastnica, $P \leq 0,01$) otrzymujących mieszankę o dużej zawartości włókna (grupa E) w porównaniu z grupą otrzymującą jego standardową zawartość w paszy (grupa C). Wysokość komórek nabłonka w kryptach i głębokość krypt w trzech badanych odcinkach jelita cienkiego (dwunastnica, jelito czcze i jelito biodrowe) miały większe wartości w grupie E w porównaniu z grupą C ($P \leq 0,05$; $P \leq 0,01$), a wysokość kosmków mniejszą. Poziom włókna w mieszance wpłynął na kierunek i poziom zmian badanych wskaźników charakteryzujących cechy morfometryczne nabłonka jelit.

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