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IMPACT OF SUPPLEMENTING PIG DIET WITH TANNINS ON HISTOLOGICAL CHARACTERISTICS OF SMALL INTESTINE AND GROWTH PERFORMANCE OF FATTENING PIGS

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ABSTRACT

The aim of the present preliminary study was to examine the effect of tannins (0.2% of chestnuts or oak tannins) as a supplement in pigs' diet on histological characteristics of small intestine and growth performance of fattening pigs. A total of 75 pigs were involved in study during fattening period from 30 to 120 kg divided into three groups: CONTROL (n = 25), CONTAN (n = 25) and FARMATAN (n = 25). At slaughter, small intestines of two animals per group were dissected into three parts (duodenum, jejunum and ileum). Different histometric characteristics such as height of mucosa, height of villi, depth of crypts, height of necrosis and height of epithelium were estimated on tissue cross section. Daily gain was calculated as growth characteristics of pigs. In the present study necroses of small intestine villi were generally present by experimental animals. Results showed that 0.2% of FARMATAN – chestnut tannin (F) in the pig diet reflected in higher growth performance and lower proportion of necrosis (16:17%) in comparison to CONTROL pigs with diet without supplemented tannins. Although pigs of CONTROL group showed negligible differences in small intestine wall histological characteristics comparing to FARMATAN, their growth performances were significantly different. It could be speculated, that higher villi, lower proportion of necrosis and narrower crypt/villi ratio may led to better growth performance but small numbers of investigated pigs in present study make difficulties to give solid conclusions.

Key words: pigs / tannins / small intestine / histology / growth

1 INTRODUCTION

Tannins are water-soluble plant polyphenols compounds and can be extracted from the wood of oak and chestnut. According to their chemical structure, they are divided into four major classes: proanthocyanidins or condensed tannins, hydrolysable tannins, phlorotannins found in marine brown algae and complex tannins. Tannins have a range of detrimental effects in animals, among others reduce feed intake, digestibility of crude proteins and decrease growth performance in monogastric species (Treviño *et al.*, 1992; Smulikowska *et al.*, 2001). Tannins also demonstrated positive antibacterial properties reduce gastrointestinal parasites in mammals (Min *et al.*, 2005; Choi *et al.*, 2009) and diarrhea in piglets by preventing the dissemination of intestinal parasites and viruses (Palombo, 2006).

After weaning of piglets marked changes occur in the structure and function of small intestine that are associated with poor growth performance due to temporary decrease in digestive and absorptive capacity (Pluske *et al.*, 1997). Only a few detailed nutritional studies have been published on the use of tannins in finishing stage of pig fattening and their possible effect on growth performance and histomorphometric characteristics of small intestine. Therefore, the aim of the present study was to find out whether the supplementation of 0.2% of tannins in pig feed has any effects on growth performance and height of villi's, depth of crypts and incidence of necrosis.

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In addition, relationships between growth rate and small intestinal wall characteristics were investigated.

2 MATERIAL AND METHODS

The study was conducted on fattening pigs from weaning to the final body weight (approx. 120 kg). Altogether, 75 animals were included in the analyses. In the first stage of the experiment (up to ≈30 kg BW) piglets were fed with starter and following grower complete feed mixture without tannins supplements. Later, tannins were added as a feed supplement in complete fattening feed mixtures until final BW. In present trial a commercial preparation of FARMATAN and CONTAN (Tanin Sevnica d.d., Sevnica, Slovenia) were used as 0.2% supplement in pigs diet. Three experimental groups were formed: first without tannins as CONTROL, second with CONTAN (i.e. oak tannin: 2 kg/t_{CF}) (C) and third with FARMATAN (F) added to CF (chestnut tannin: 2 kg/t_{CF}). After each fattening stage two animals from each group were sacrificed. Twenty animals were included in the histometrical analyses of the small intestine. From each sacrificed animal samples of jejunum and ileum were taken and stained with haematoxylin and eosin. Histometry was carried out with standardised methodology (Biagi *et al.*, 2006), using optical (light) microscope Olympus BX50 with Olympus DP10 digital camera incorporated. On each histological slide 5 parameters were measured on approx. 15–20 (min. 10) different position per one part of tissue cross-section sample (approx. 30–40 per animal): height of mucosa (magnification 40×), height of villi (40×), depth of crypts (40×), height of necrosis (40×) and height of epithelium (400×). Proportion of necrosis, relatively to the height of villi was calculated and included in analyses. Due to their similarity in measured histometrical parameters jejunum and ileum were treated as one unit. For all animals daily gain (DG) from weaning to slaughter was calculated. Additionally, average DG was calculated, both for animals with necrotic villi and without necrosis, DG was analysed for differences between groups. ANOVA post-hoc Tukey HSD test and correlation analysis (Pearson correlation coefficient) were performed in IBM SPSS Statistics 20 software.

3 RESULTS AND DISCUSSION

Main focus of the present study was put on necrosis of small intestine villi, particularly jejunum and ileum, which are apparently uncommon in natural cases (Songer and Uzal, 2005). In the present study, necrosis was generally present and its degree was in significant negative correlation with DG, presuming due to reduction of absorptive surface of small intestine mucosa (Table 1). Indeed, animals without necrosis had significantly higher DG than animals with necrotic mucosa (904 ± 135 g vs. 794 ± 170 g; $P < 0.01$, respectively). Necrosis often occurs after specific bacterial infection and becomes deeper with progression of infection, i.e. *Clostridium spp*. (Songer and Uzal, 2005) or *Clamydia spp*. (Rogers and Andersen, 1996). Consequently, necrosis can affect growth rate, feed conversion and daily health status of pigs (Holyoake *et al.* 1996). Moreover, villi's atrophy could occur also due to higher level of feed intake after weaning, rather than to the composition of the diet (van Beers-Schreurs *et al.*, 1998). Changes in villi's size due to shortening that occurs because of increased rate of cell loss in absorptive apical part is associated with increased crypt-cells proliferation rate, and therefore increased crypt depth, i.e. crypt hyperplasia (Pluske *et al.*, 1997). Relative crypt depth in relation to villi height showed negative correlation with DG. Crypts were deeper when necroses were present in the higher extent and were in significant negative correlation with DG. This is not surprising, seeing that crypts generated young epithelium cells and they

Table 1: *Correlation between histological characteristics of small intestine and daily gain*

	HEIGHT OF VILLI [um]	HEIGHT OF EPITHELIUM [um]	PROPORTION OF NECROSIS	CRYPT/ VILLI RATIO	DAILY GAIN [kg/day]
HEIGHT OF MUCOSSA [um]	0.495**	0.394**	0.052	0.020	−0.215*
HEIGHT OF VILLI [um]		0.054	−0.198*	−0.673**	0.288**
HEIGHT OF EPITHELIUM [um]			0.008	0.013	−0.327**
PROPORTION OF NECROSIS				0.130	−0.304**
CRYPT DEPTH/HEIGHT OF VILLI RATIO					−0.370**

** $P < 0.01$; * $P < 0.05$

Table 2: *Daily gain (DG) and histological characteristics of experimental groups of fattening pigs from approx. 90 kg to120 kg*

Items	CONTROL	CONTAN	FARMATAN
[1]DG (g/day)	660 ± 301[a]	770 ±159[ab]	897 ± 88[b]
[2]DG (g/day)	727 ± 143[a]	751 ± 112[ab]	909 ± 46[b]
HEIGHT OF MUCOSA [um]	906 ± 100[a]	938 ± 78[a]	902 ± 110[a]
HEIGHT OF VILLI [um]	465 ± 66[a]	587 ± 80[b]	491 ± 92[a]
HEIGHT OF EPITHELIUM [um]	37 ± 7[a]	32 ± 7[b]	37 ± 7[a]
PROPORTION OF NECROSIS [%]	17 ± 23[a]	21 ± 23[a]	16 ± 27[a]
CRYPT/VILLI RATIO	0.8 ± 0.3[a]	0.6 ± 0.2[b]	0.6 ± 0.2[b]

[a, b] different values when $P < 0.05$; [1] DG for final fattening stage 90–120 kg; [2] DG throughout experiment 30–120 kg

consequently respond with intensive renovation of epithelium layer when necroses are present. Moreover, hypertrophy of epithelium cells or epithelium cells proliferation is also one of the responding mechanisms on spreading necroses. Namely, unabsorbed nutrients could operate a feedback control of epithelial cell proliferation (Sagor *et al.*, 1982; Pluske *et al.*, 1997). Results showed that height of epithelium was in significant negative correlation with DG. According to our results only height of villi was in significant positive correlation with DG, in contrast to the others already mentioned histological characteristics (Table 1).

CONTROL had the lowest DG in comparison to FARMATAN and CONTAN during whole fattening period (Table 2). Pigs supplemented with FARMATAN had the highest DG ($P < 0.05$), and similar histological characteristics of small intestinal wall as CONTROL pigs ($P \geq 0.05$). Interestingly, the CONTAN pigs showed significantly different small intestine wall characteristics, except proportion of necrosis, which was the highest (20.5%) and the lowest in FARMATAN (16%).

4 CONCLUSIONS

Height of epithelium of villi in our experiment was due to proliferation of cells in the crypts (intestinal glands) and following the hypertrophy of epithelial cells, probably with incomplete differentiation into the active form, which is required for effective nutrients digestion. Villi height was positively correlated with daily gain, which is understandable. Negative correlation of mucosal height with the daily gain can be attributed to the increased proportion of intestinal crypts in the mucosa, because the proliferation of cells in the crypts is primarily intended for reconstruction of damaged epithelial villi. It could be speculated, that higher villi, lower proportion of necrosis and narrower crypte/villi ratio may led to better growth performance. We are aware that the small numbers of investigated pigs in present study makes it difficult to give solid conclusions.

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