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EFFECT OF CHESTNUT (*CASTANEA SATIVA* MILL.) WOOD TANNINS AND ORGANIC ACIDS ON GROWTH PERFORMANCE AND FAECAL MICROBIOTA OF PIGS FROM 23 TO 127 DAYS OF AGE

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Abstract

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The aim of the study was to investigate the effects of the supplementation of chestnut (*Castanea sativa* Mill.) tannin and/or organic acids in diets for pigs on their growth performance and the faecal microbial composition from 23 to 127 days of age. The dietary treatments consisted of a basal diet (control (C)), the basal diet added organic acids (OA), and the basal diet added organic acids plus tannin (OA+T). Animals were weighed on days 23, 82 and 127. Pigs fed the OA and OA+T diets showed during the grower period significantly higher daily gain (ADG)(P<0.05), improved feed conversion ratio(FCR) (P<0.05) and lower levels of *E. coli* (P<0.05) compared with C pigs. From 82-127 days only the pigs fed the OA+T diet significantly diminished (P<0.05) the total count of *E. coli*. (5.21 ± 0.24 vs. 4.86 ± 0.28 vs. 4.24 ± 0.28 $\log_{10}$cfu/g; respectively for C, OA and OA+T; P<0.05), elevated the total count of Lactic acid bacteria (6.85 ± 0.10 vs. 7.15 ± 0.49 vs. 7.95 ± 0.50 $\log_{10}$cfu/g; respectively for C, OA and OA+T; P<0.05)and significantly increased ADG (P<0.05) (687 ± 23 vs. 712 ± 11 vs. 766 ± 12 g/day; respectively for C, OA and OA+T; P<0.05). The results from the present study indicate that the supplementation of chestnut wood tannins and organic acids can improve the growth performance in period from 82-127 days mainly by reducing harmful *E. coli* counts and by increasing counts of beneficial Lactic acid bacteria.

Key words: pig, tannins (*Castanea sativa* Mill.), growth, microbiota

Introduction

Weaning is a critical period for pig production as the piglets are submitted to social, dietary and environmental challenges. These factors can lead to reduced post-weaning performance, usually expressed by the proliferation of pathogenic microorganisms in the digestive tract accompanied by digestive disorders (Metzler et al., 2005). To avoid such post-weaning problems, the uses of in feed antibiotics have been adopted to treat gastrointestinal diseases and as growth promoters in weaned piglets (McEwen and Fedorka-Cray, 2002). On the other hand, there is growing public concern about the use of antibiotics in livestock production. In the view of increasing the number of antibiotic-resistant pathogens and antibiotic residues in animal products, the European Union banned the total use of antibiotics in livestock production on January, 2006. Therefore, producers have increased the use of alternative feed additives in order to improve health and enhance the growth performance of pigs (Verstegen and Williams, 2002). These include direct-fed microbes (van Heugten et al., 2003; Li et al., 2006), organic acids (Kim et al., 2005), herbal extracts and dietary spices (Manzanilla et al., 2004; Shan et al., 2007), herbal extracts and organic acids (Namkung et al., 2004), nucleotide supplementation (Martinez-Puig et al., 2007), mannan oligosaccharides (Kogan and Kocher, 2007), fructo-oligosaccharide (Mikkelsen et al., 2003), oligofructose (Pellikaan et al., 2007) and tannins (Mitaru et al., 1984). Therefore, the use of tannins could be an alternative as an antimicrobial additive in pig feed. These are water-soluble plant polyphenols, which precipitate proteins. According to their chemical structure, they are divided into two major classes: condensed tannins and hydrolysable tan-

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nins. Tannins have a range of effects in animals, among others reduce feed intake, digestibility of crude proteins and decrease growth performance in monogastric species (Treviòo et al., 1992; Smulikowska et al., 2001). Moreover, previously has been demonstrated that tannins reduce gastrointestinal parasites in mammals (Min et al., 2005; Choi et al., 2009). Contrary to a large body of literature for ruminants, there have been a fewer detailed nutritional studies published on the use of tannin in pig feeding.

Therefore, the aim of the present study was to determine whether organic acids and/or organic acids plus tannins have potential antimicrobial effects on most common faecal pathogens and hence a positive effect on growth performance and feed conversion ratio of pigs from weaning until 127 days of age.

Materials and Methods

The experiment was performed according to the Council directive for minimum standards for the protection of pigs (91/630/EEC) and Slovenian Law Regulating the Protection of Animals (1999).

Animals, housing and diets

504 crossbred pigs (Swedish Landrace x Large White) were included in present study. Within the first 48 h after birth litter size was, standardize to 10 piglets using cross fostering. During the first week of age, piglets were handled for teeth clipping and tail docking. They received an intramuscular injection of 200mg of iron dextran and males' piglets were castrated. During the whole lactation period, piglets had free access to water.

All animals were ear tagged and weighed on days 23, 82 and 127. After weaning, the piglets were moved into a prefattening house. Pens had a slatted plastic floor and sidewalls equipped with a nipple drinker. The piglets had *ad libitum* access to water and feed. Feeding was carried out using automatic dosing machines. The boxes had natural daylight and additional artificial light (>60 lux) during daytime. A thermostat controlled ambient temperature in the stall. Pigs remained in the pre-fattening stall until they reached the age of 82 days (~30 kg body weight).

When the piglets reached 30 kg body weight, they were transferred to the finisher facilities. Pigs were housed in pens, which had one third of solid concrete floors for lying and two thirds of area had concrete slatted floor. Pigs had *ad libitum* access to water and feed. Feeding was carried out using automatic dosing machines. The boxes had natural daylight and additional artificial light (>60 lux) during daytime.

During the experiment, piglets received different feed basal diets (Table 1). The pre-starter meal was a supplemental feed for suckling and weaning piglets aged 23-42 days. During the growing period from 42-63 days (i.e., body weight 12-20 kg), the starter feed was fed. At 9 weeks, the pigs started to obtain a grower mixture up to 25 kg body weight and thereafter pigs received Pork-25 diets.

The pigs (both sex) were randomly divided at weaning time into three groups according to fed diet. Each dietary treatment was applied to twelve replicate pens with fourteen pigs (7 gilts and 7 barrows) per pen. First group named control (C, n=168) were fed with a basal diet (Panvita, Proizvodnja krme d.o.o., Slovenia). Second group (OA, n=168) were fed with the same basal diet with added 0.35% commercial acidificant FraAcidDry (Perstop Franklin; Waspik, Netherlands), which contains lactic acids, citric acid, formic acid, fumaric acid and ammonium formate. Third group (OA+T,

Table 1
Percentage composition and chemical content (g, mg and IU per kg of fed) of the experimental diets (as-fed-basis)

	Prestarter	Starter	Grower	Pork-25
Crude protein, %	17.5	18.0	18.0	17.0
Crude fat, %	4	3.0	4.0	3.5
Crude fibre, %	5	4.5	5.0	5.0
Crude Ash, %	6.5	6.0	5.0	5.0
Lysine, %	1.28	1.28	1.15	1.12
ME, MJ/kg	13.6	13.6	13.6	13.8
Vitamin A, IU	20000	20000	20000	10000
Vitamin D3, IU	2000	2000	2000	2000
Vitamin E, mg	200	200	200	150
Vitamin K3, mg	4	4	4	2.5
Vitamin B1, mg	4	4	4	4
Vitamin B2, mg	8	8	8	5
Vitamin B3, mg	40	40	40	25
Vitamin B5, mg	25	25	25	15
Vitamin B6, mg	5	6	6	4
Vitamin B12, ìg	50	50	50	30
Folic acid, mg	1.5	1.5	1.5	1.0
Vitamin H, ìg	300	300	300	50
Holin chloride, mg	400	400	400	300
Vitamin C, mg	100	100	100	100
J, mg	2.5	2.5	2.5	1.0
Mn, mg	100	100	100	80
Zn, mg	150	110	110	110
Co, mg	1	1	1	0.8
Fe, mg	150	150	150	140
Cu, mg	160	140	160	15
Se, mg	0.4	0.4	0.4	0.4
Mg, mg	200	200	200	150

n=168) were fed with the same basal diet with added 0.35% additive containing 0.19% tannin extracted from chestnut tree (*Castanea sativa* Mill.) (Tanin Sevnica d.d., Slovenia) and overall 0.16% of five acids (formic acid, lactic acid, DL-citric acid and DL-malic acid, phosphorus acid) (Table 1).

The pigs were observed throughout the experiment trial and health status was recorded daily. Mortality was recorded in all three groups during the trial.

Faecal samples and bacterial counts

Each faecal sample was taken *per rectum* using rubber gloves and placed in a sterile plastic container. Faecal samples were taken from six randomly chosen pigs from each pen at 7, 23, 82 and 127 days of age and kept at 4°C for no longer than 4 h. Samples were diluted in 1x PBS (pH 7.0) to 0.1g of faeces/mL. 100 mL aliquots of serial dilutions (10^{-1} to 10^{-8}) were placed on selective agar plates and incubated according to the manufacturer's instructions. For counting *Escherichia coli* HiCrome (TM) ECC selective agar was used; for detection of *Campylobacter* spp. HPA standard identification protocol BSOPID23 was applied (http://www.hpa-standard-methods.org.uk/pdf_sops.asp) and for counting of lactic acid bacteria, MRS broth (Oxoid, Basingstoke, England) was used and bacteria were grown under anaerobic conditions using the Anaerogen (Oxoid,Basingstoke, England) system for 24h at 37°C as previously described (Pipenbaher et al., 2009). All agars were purchased from Fluka (Sigma-Aldrich, St Louis, MO, USA) and prepared according to the manufacturer's instructions. The bacterial population was transformed (log_{10}) before statistical analysis and expressed as log_{10} cfu/g.

Statistical analysis

Data were analyzed using the General Linear Model (GLM) in SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). For average daily gain (ADG), body weight at specific age (BW), feed conversion ratio (FCR) and counts of specific microorganisms per faecal sample, the statistical model included the main effects of diet and age. All data are presented as mean ± SEM. Multiple comparisons of the observed means were based on Duncan's Post Hoc test. Statistically significant differences are shown at $P<0.05$.Pearson's correlation coefficients were estimated between ADG and transformed (log_{10}) sizes of specific microbial populations at different fattening periods.

Results and Discussion

Supplementation of chestnut tannin (*Castanea sativa* Mill.) in the diet in the present study was in order to induce pig better growth performance and to increase the total concentration of beneficial bacteria (i.e. lactic acid bacteria) and decrease of the pathogenic bacteria (i.e. *E. coli*, *Campylobacter* spp.) during the post-weaning period and in the early fattening stage. However, it has been reported that tannins present in diets could impact negatively on animal production due to reducing feed intake, growth rate and feed efficiency (Mueller-Harvey, 2006). Preliminary study of Antongiovanni et al. (2007) showed that tannin concentration of 2.5 g per kg of feed in 40 days of feeding trial, from the average body weight 76 kg to about 110 kg had no significant negative influence on ADG, but significantly decreased the apparent digestibility of dry matter and nitrogen digestibility. Indeed, data presented in literature also indicated that 4% or more of tannin from sorghum in diets for growing pigs had a negative effect on the coefficient of apparent ileal digestibility and coefficient of standardized ileal digestibility of proteins (Mariscal-Landín et al., 2004). Inclusion of faba beans hulls (*Vicia faba* L.) in the feed of young pigs with either low or high tannin contents reduced the true digestibility of dietary protein and increased the endogenous excretion of secreted proteins (Jansman et al., 1995). Although, an addition of 1.1%, 2.2% and 4.5% of sweet chestnut (*Castanea sativa* Mill.) tannins in the feed significantly reduced apparent protein digestibility in pigs, but did not reduce the protein-related efficiency of phytase action in growing pigs (Salobir et al., 2005).

In the pre-fattening period, the OA or OA+T treatments improved pig growth performance significantly in comparison to control (Table 2). The pigs fed OA and OA+T diet had 51% and 46% higher ADG, respectively, than C pigs. At the same time, their FCR was approx. 21% improved in comparison with C pigs. Improvements in the performance of pigs fed with organic acids have been well documented. The organic acid added to pigs grower diets influence on improved daily gain (Jongbloed et al., 2000; Øverland et al., 2007; Partanen et al., 2007; Walsh et al., 2007). This amelioration in growth performance of pigs has been hypothesized to be related to the lowering gastric pH (Risley et al., 1992), stimulating the secretion of digestive enzyme and increasing gastric retention time (Partanen and Mroz, 1999), stimulating intermediary metabolism, acting as energy source for the gastrointestinal tract (Kim et al., 2005), and reducing the number of pathogens (Tsiloyiannis et al., 2001; Li et al., 2008). These higher daily gains may be assigned to higher feed intake as well as higher apparent nutrient digestibility in pigs feed with OA and OA+T. In contrast to our results, Štukelj et al. (2010) observed very high ADG almost 1000g/day in trial period from 7 wk to 10 wk of age, with no effect of added 0.3% commercial acidificant FraAcid as well as 0.3% additive containing tannin and organic acids on ADG and BW of pigs. Conversely, Biagi et al. (2010) found that

Table 2
Comparison of effect of diets on pigs' growth performance

Items	C	OA	OA+T
BW, kg			
Day 23	7.43 ±0.32[a]	7.16 ± 0.10[a]	7.30 ± 0.09[a]
Day 82	23.53±0.88[b]	30.33 ± 0.43[a]	31.24 ± 0.40[a]
Day 127	56.84± 1.71[a]	62.73 ± 0.73[b]	66.32 ± 0.76[c]
ADG, g/day			
Days 23 - 82	269 ±13[b]	392 ± 6[a]	407 ± 6[a]
Days 82 - 127	687 ± 23[a]	712 ± 11[b]	766 ± 12[c]
FCR, kg/kg			
Days 23 - 82	2.47 ± 0.04[a]	1.90 ± 0.12[b]	2.01 ± 0.02[b]
Days 82 - 127	2.72 ± 0.06[a]	3.03 ± 0.02[a]	2.99 ± 0.06[a]

BW=body weight; ADG=average daily gain; FCR=feed conversion ratio; [a,b,c]Different letters indicate statistical differences between columns at $P<0.05$.

when fed weaned piglets at 1.13, 2.25 and 4.5 g/kg, tannins (Farmatan®) significantly improved feed efficiency and ADG in 28 days trial period. Similarly improvements of ADG and FCR have been reported in broilers (Schiavone et al., 2007, 2008) when natural extract of chestnut (Silvafeed ENC®) was added at 0.15 and 0.20% to commercial feed. Contradictory results are presented in literature about the effect of organic acids on pig growth performance. Not only Štukelj et al. (2010), but also Ettle et al. (2004) reported that acidifying the diets had no effect on growth development and feed conversion ratio, as well as feed intake in six weeks long post-weaning period. Furthermore, no effects of the addition of organic acids and their salts to diets on daily gain or feed intake during the later growing period (28 and 113 kg initial and final body weight, respectively) were observed (Canibe

Table 3
***Escherichia coli, Campylobacter* spp. and lactic acid bacteria in faeces of pigs in lactation and fed with different diets in pre-fattening and fattening period**

Period	Group	Age, days	*Escherichia coli*	*Campylobacter* spp.	Lactic acid bacteria
Lactation					
	C	7	5.39 ± 0.23[a]	4.37 ± 0.31[a]	5.28 ± 0.16[a]
	C	23	5.73 ± 0.08[a]	4.34 ± 0.23[a]	5.67 ± 0.22[b]
Pre-fattening					
	C	82	6.28 ± 0.25[c]	5.81 ± 0.26[c]	6.67 ± 0.15[a]
	OA	82	5.28 ± 0.24[a]	6.34 ± 0.39[c]	6.79 ± 0.55[a]
	OA + T	82	3.94 ± 0.25[b]	4.84 ± 0.08[b]	7.32 ± 0.06[a]
Fattening					
	C	127	5.21 ± 0.24[a]	4.85 ± 0.26[a]	6.85 ± 0.10[a]
	OA	127	4.86 ± 0.28[ba]	5,16 ± 0.29[a]	7.15 ± 0.49[a]
	OA + T	127	4.24 ± 0.28[b]	4.49 ± 0.25[a]	7.95 ± 0.50[b]

C=control basal diet; OA=basal diet added organic acid (OA); OA+T=basal diet added organic acid and tannin; Values are compared within columns and only within specific period between different diets; [a,b,c]Different letters indicate statistical differences at $P<0.05$

Table 4
Pearson´s correlation coefficients between diets, contents of microorganisms in faeces of pigs and growth rate

Impact	Diet	LB day 82	LB day 127	EC day 82	EC day 127	CAM day 82	CAM day 127	ADG days 82-127
Diet	1	0.290	0.033	-0.777**	-0.151	-0.854**	-0.230	0.174**
LB day 82		1	0.464	0.115	0.452	0.136	0.448	-0.346
LB day 127			1	-0.020	0.703**	-0.104	0.660**	-0.019
EC day 82				1	0.332	0.793**	0.332	-0.724*
EC day 127					1	0.282	0.927**	-0.413
CAM day 82						1	0.310	-0.892**
CAM day 127							1	-0.416
ADG days 82-127								1

LB 82 days - Lactic acid bacteria on day 82; LB 127 days - Lactic acid bacteria on day 127; EC 82 days – *Escherichia coli* on day 82; EC 127 days - *Escherichia coli* on day 127; CAM 82 days - *Campylobacter* spp. on day 82; CAM 127 days - *Campylobacter* spp. on day 127; ADG 82-127 days - average daily gain at days 82-127; *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed) et al., 2001). The possible explanation for diverse influences of organic acids on pig growth performance could also be related to the combination and doses of organic acids, composition of the basal diet used different salts used in trials and duration of feeding trial (Štukelj et al., 2010).

In addition, weaning is generally followed by short post-weaning disruption of growth; which it is not only a consequence of the piglet's low intake of solids, but also of an immature gastrointestinal tract (GIT) (Richards et al., 2005). The lack of no significant differences in BW and ADG observed between OA and OA+T-group of pigs in this period could be attributed to similar effects of added organic acids in both treatments on early growth performance. Nevertheless, by day 82, however, the feed intake would be sufficient to support a larger bacterial population. Interestingly, organic acids reduced the number of *E. coli*, but not the number of *Campylobacter* spp. in-group fed with basal feed supplemented with OA. Only the presence of tannin and OA in fed together had significant effect on reduction on both *E. coli* and *Campylobacter* spp. in pigs' faeces. The same effect of OA + tannin treatment on significant diminishing of *E. coli* and *Campylobacter* spp. in pigs' faeces was noticed likewise later in fattening period up to 127 days. Reduction of *E. coli* and *Campylobacter* spp. number in faeces of pigs with this treatment was followed by significant increase of counts of Lactic acid bacteria. These bacteria are normally considered antagonistic. It has been reported that greater lactobacilli number should inhibit the proliferation of *E. coli* (Cranwell et al., 1976). Consequently, the greater number of lactic acid bacteria of pigs fed with sweet chestnut tannins might inhibit the colonization and proliferation of *E. coli* and *Campylobacter* spp. by blocking possible intestinal receptors of *E. coli* and by secreting toxic metabolites (Danielson et al., 1989).

Although the level of feed intake is low shortly after weaning, it would result in little intestinal digesta to support bacterial growth. In case, when the amount of undigested substrate increases, the colonization resistance is disturbed and microbial such as *E. coli* can proliferate and induce the diarrhoea (Kim et al., 2005). In present study, no diarrhoea was noticed. It is worth to emphasize that it has been published decades ago that *Castaneva sativa* was used as medical treatment (max 1g mixed with rise and skim milk) against pig`s diarrhoea (Steuert, 1909) (Tables 2 and 3).

Relationships between ADG and count of *Escherichia coli*, *Campylobacter* spp. and lactic acid bacteria at different days of observations were estimated using Pearson's correlation analysis (Table 4).

Significantly strong negative correlations were found between the effect of organic acids and tannins and the *E. coli* and *Campylobacter* spp. concentrations on day 82 ($r = -0.777$, $r = -0.854$ respectively; $P<0.01$). A weaker, but significant positive relationship was estimated between the effect of diet and ADG at days 82-127 ($r = 0.174$, $P<0.01$). The ADG at days 82-127 showed a strongly negative correlation with the numbers of *Campylobacter* spp. and *E. coli* in faeces ($r = -0.892$, $P<0.01$; $r = -0.724$, $P<0.05$, respectively). The present data shows an improvement in daily gain due to reduction in count of *E. coli* and *Campylobacter* spp.

Conclusions

The present study demonstrated that supplementation with chestnut (*Castanea sativa* Mill.) tannin and organic acids (OA+T) in the feed of young pigs is effective in reducing pathogens in faeces and in improving the growth performance after weaning. Additionally, OA+T pigs in period from 23–82 days of fattening increased growth performance significantly mainly via reducing pathogenic bacteria. Nevertheless, OA+T diet of pigs had no effect on reducing FCR in pre-fattening period in comparison to OA diet, but significantly improved FCR in comparison to C diet.

In the present study, pigs fed the control diets had significantly lower body weight and daily gain all over the study in comparison to the other two treatments.

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