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Effect of tannins on growth performance and intestinal ecosystem in weaned piglets

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Tannins are natural polyphenolic compounds that can reduce digestibility of dietary protein but also display antibacterial effects. The present study investigated, in vitro and in vivo, the effect of different levels of tannins (using a chestnut wood extract containing 75% tannins) on growth performance, intestinal microbiota and wall morphology in piglets. During a 24 h in vitro caecal fermentation, the utilisation of tannins at 0.75, 1.5, 3, and 6 g/l significantly reduced total gas production and concentrations of ammonia and volatile fatty acids and increased viable counts of enterococci and coliforms. When fed to piglets at 1.13, 2.25, and 4.5 g/kg, tannins significantly improved feed efficiency and reduced caecal concentrations of ammonia, iso-butyric, and iso-valeric acid. Viable counts of lactobacilli tended to be increased by tannins in the jejunum, while bacterial caecal counts were not affected. Depth of ileal crypts tended to decrease in piglets fed tannins at 2.25 and 4.5 g/kg. The present study showed that feeding weaned piglets with a tannin-rich wood extract can result in improved feed efficiency and reduction of intestinal bacterial proteolytic reactions. The growth-enhancing effect that tannins had on enterococci and coliforms under in vitro conditions deserves further investigation.

Keywords: caecum; microbiota; performance; pigs; tannins

1. Introduction

Tannins are natural polyphenolic compounds that are found in many vegetable feedstuffs and can be extracted from the wood of several trees (Kumar and Vaithiyathan 1990). Tannins have been known for the detrimental effect that they exert on animal growth performance when tannin-rich feedstuffs are fed to non-ruminants. In fact, tannins can reduce digestibility of dietary protein (Mariscal-Landín et al. 2004) due to their ability to form insoluble complexes with both dietary protein (Butler et al. 1984) and digestive enzymes (Jansman et al. 1994). Besides their antinutritional properties, tannins also display beneficial antibacterial (Ahn et al. 1998; Min et al. 2007) and antidiarrhoic (Palombo 2006) effects. In the first weeks after weaning, piglets frequently experience diarrhoea and low weight gain due to the relative immaturity of their gastrointestinal and immune system (Hedemann and Jensen 2004). Because bacteria-caused diarrhoea is the consequence of the
multiplication of harmful bacteria in the piglet intestine, this problem was counteracted with antibiotics and auxinic agents that may as a side-effect select antibiotic-resistant genes in the intestinal flora with the possibility of a transfer to human pathogens (Phillips et al. 2004). Because of these concerns, the use of antibiotic growth promoters has been prohibited within the European Union since January 2006, thus increasing the need for non-pharmacological alternatives to modulate the intestinal microbiota of young animals. Dietary supplementation with tannins might reduce the incidence of diarrhoea in piglets but the effect of extracted tannins on animal growth performance raises some concerns. The objective of the present study was to investigate the effect of different levels of tannins on growth response and metabolism of swine caecal microbiota \textit{in vitro} and the effect of feeding tannins on piglet growth performance, intestinal microbiota and wall morphology \textit{in vivo}.

2. Materials and methods

2.1. Animal care and use

Animal housing and care were conducted under supervision of the Veterinarian Office of the Bavarian Government. The handling protocol ensured proper care and treatment of all animals in conformity with the German law for animal protection.

2.2. In vitro study

The \textit{in vitro} study was conducted at the Department of Veterinary Morphophysiology and Animal Production (DIMORFIPA), University of Bologna, Italy, following the procedure as described in Biagi et al. (2006).

A diet (57% corn, 16% barley, and 14% soybean meal) for pigs was pre-digested \textit{in vitro} to simulate the ileal digestion as described by Vervaeke et al. (1989). This is a two-step procedure where feed is first incubated in a pepsin-HCl solution and then in a pancreatin solution. The pre-digested diet was used as substrate in the \textit{in vitro} fermentation study (Biagi et al. 2006). The caecal content of six pigs (10 months old, live weight 160 kg, fed a commercial corn-barley-soybean based diet) was collected after slaughtering, diluted with buffer (ratio 1:2; McDougall 1948) and filtered. The filtered liquid was used as inoculum. The inoculum was dispensed into five 10 ml glass syringes (5 ml of inoculum in each syringe) and five 50 ml vessels (25 ml of inoculum in each vessel) per treatment, containing 20 and 100 mg of pre-digested diet (used as control diet), respectively. Syringes and vessels were sealed and incubated at 39°C for 24 h.

There were five treatments: the control diet, and the control diet plus tannins (Farmatan®, Tanin Sevnica, Slovenia) at 0.75, 1.5, 3.0, and 6.0 g/l. In all tannin treatments, tannins were added at incubation start, prior to sealing syringes and vessels. The pH of the inoculum was adjusted to 6.7.

Farmatan is a chestnut (\textit{Castanea sativa mill}) wood extract (obtained by water extraction) containing 75% tannins, mainly gallotannins (European Food Safety Authority [EFSA] 2005). Gallotannins are hydrolysable tannins that are formed from gallic acid (and ellagic acid) which is linked to OH groups of sugars (especially glucose). Other components of Farmatan include water (6.3%), ellagic acid (13.2%), gallic acid (1.7%), simple sugars (2.6%), crude protein (0.8%), mineral substances (1.0%), and crude fibre (0.2%).
Gas production was measured as described by Menke et al. (1979), using 10 ml glass syringes and recording the cumulative volume of gas produced every 30 min. Samples of fermentation fluid were collected from each vessel at 6 and 24 h of incubation for pH and ammonia and at 24 h for volatile fatty acids (VFA) and viable counts of bacteria determinations.

### 2.3. In vivo feeding study

The in vivo feeding trial was conducted at the Division of Animal Nutrition and Production Physiology, Technical University of Munich, Germany. Forty-eight crossbred piglets (German Landrace × Pietrain) were weaned at 28 d and transported from the piggery to the barn where they were housed in individual cages in a controlled environment for a 28 d trial period. After a 4 d adaptation period during which all piglets received the same base diet, animals (8.23 ± 0.93 kg BW) were divided into four groups (12 animals per group), homogenous for weight, gender and litter. Pigs received the base diet with: (i) no addition (control diet), or with the addition of tannins (Farmatan\(^\text{TM}\)) at (ii) 1.13 g/kg, (iii) 2.25 g/kg, and (iv) 4.5 g/kg. All diets were formulated to provide the same amount of energy, protein, essential amino acids, calcium and phosphorus. No antibacterial agents were added to diets. Feed and water were provided on an ad libitum basis. Composition and chemical analyses of the experimental diets are reported in Table 1.

Animals were individually weighed and feed consumption was recorded for each pig weekly. The amount of feed wasted was recorded daily. Animal health was monitored throughout the trial.

On day 28, six animals per treatment were killed by electrical stunning followed by complete bleeding. Within 20 min after death, intestinal content (whole content of jejunum and caecum and the content of the last 100 cm of the ileum were

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Content [g/kg]</th>
<th>Chemical composition</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn meal</td>
<td>305</td>
<td>Dry matter(^{†}) [g/kg]</td>
<td>885</td>
</tr>
<tr>
<td>Wheat meal</td>
<td>273</td>
<td>Crude protein(^{†}) [g/kg]</td>
<td>190</td>
</tr>
<tr>
<td>Barley meal</td>
<td>171</td>
<td>Fat(^{†}) [g/kg]</td>
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</tr>
<tr>
<td>Soybean meal, 47% CP</td>
<td>161</td>
<td>Crude fibre(^{†}) [g/kg]</td>
<td>32.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10</td>
<td>Ca [g/kg]</td>
<td>8.5</td>
</tr>
<tr>
<td>Potato protein</td>
<td>40</td>
<td>P [g/kg]</td>
<td>6.0</td>
</tr>
<tr>
<td>CaCO(_3)</td>
<td>5.7</td>
<td>Lysine [g/kg]</td>
<td>12.0</td>
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<tr>
<td>Ca(H(_2)PO(_4))(_2)</td>
<td>5.7</td>
<td>Methionine [g/kg]</td>
<td>3.9</td>
</tr>
<tr>
<td>Premix(^#)</td>
<td>20</td>
<td>Methionine + cysteine [g/kg]</td>
<td>7.2</td>
</tr>
<tr>
<td>L-lysine HCl</td>
<td>6.6</td>
<td>Threonine [g/kg]</td>
<td>7.8</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>1.0</td>
<td>Tryptophan [g/kg]</td>
<td>2.4</td>
</tr>
<tr>
<td>L-threonine</td>
<td>1.0</td>
<td>ME [MJ/kg]</td>
<td>13.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: *As-fed; experimental diets were obtained replacing 0, 1.5, 3, and 6 g corn meal per kg diet by an equal amount of Farmatan\(^\text{TM}\) (containing 75% tannins). \(^\#\)Provided per kg of diet: Ca, 6.16 g; P, 1.68 g; Na, 1.40 g; Mg, 0.28 g; Vitamin A, 16,800 IU; Vitamin D\(_3\), 1,680 IU; Vitamin E, 56 mg; Vitamin K\(_3\), 1 mg; Thiamine, 1 mg; Riboflavin, 3.5 mg; Pyridoxine, 2.1 mg; Vitamin B\(_12\), 0.03 mg; Nicotinic acid, 16.8 mg; d-Pantothenic acid, 8.4 mg; Folic acid, 2.8 mg; Biotin, 0.22 mg; Choline, 420 mg; Fe, 140 mg; Zn, 140 mg; Cu, 28 mg; Mn, 56 mg; I, 1.68 mg; Se, 0.36 mg. \(^{†}\)Determined by analysis.
separately collected) and mucosa (samples were obtained 150 cm distal from the pylorus, 100 cm before the ileo-caecal valve, and at the apical portion of the caecum for jejunum, ileum, and caecum, respectively) were sampled for pH and ammonia determination and for intestinal mucosa morphology analysis, while VFA were determined only in caecal samples. Samples from the jejunum and the caecum were also cultured for viable counts of bacteria.

2.4. Chemical analyses of feed, faecal samples, fermentation fluid and intestinal contents

Analyses of the diets (CP, crude fibre, and ether extract) were performed according to the Association of Official Analytical Chemists standard methods (AOAC 2000; Method 954.01 for CP, Method 962.09 for crude fibre, and Method 920.39 for ether extract).

Ammonia in fermentation fluid and intestinal chyme was measured according to Searcy et al. (1967) using a commercial kit (Urea/BUN – Color, BioSystems SA, Barcelona, Spain). For the determination of VFA in the intestinal chyme, the digesta were diluted 1:2 with distilled water and centrifuged (14,000 g, 10 min) and 1 ml of the supernatant was filled in microfuge tubes and deproteinised with 50 μl perchloric acid (Merck, Darmstadt, Germany). After 3 h the samples were centrifuged again (14,000 g, 10 min). For the determination of VFA in fermentation fluid, samples were centrifuged (3,000 g, 15 min). Concentration of VFA in supernatants of fermentation fluid and digesta was determined by gas chromatography (Biagi et al. 2006).

2.5. Bacterial counts

Immediately after collection of the chyme samples, 1 g sample was diluted with 9 ml of a 1% peptone solution and homogenised. Viable counts of bacteria in chyme \( (n = 6) \) and fermentation fluid \( (n = 5) \) samples were measured by plating serial 10-fold dilutions (in 1% peptone solution) onto Lactobacillus Medium III agar plates (Medium 638, DSMZ, Germany) for lactobacilli, Difco DRCM agar plates (Beckton, Dickinson and Company, Franklin Lakes, NJ, USA) for clostridia, Azide Maltose agar plates (Biolife, Milano, Italy) for enterococci, and MacConkey agar plates (N. 1.05465, Merck, Darmstadt, Germany) for coliforms. Lactobacillus Medium III and DRCM agar plates were incubated for 48 h at 39°C under anaerobic conditions \( (\text{H}_2 + \text{approximately 4 to 10% CO}_2; \text{BBL GasPak Plus Anaerobic System Envelopes, Beckton, Dickinson and Company, Sparks, MD, USA}) \). Azide Maltose agar and MacConkey agar plates were incubated for 24 h at 39°C under aerobic conditions.

2.6. Morphological evaluations

The height of villi and depth of crypts on mucosa samples from jejunum, ileum and caecum were assessed as described in Biagi et al. (2006). Mucosa samples were fixed in 10% buffered Carson’s formalin and embedded in paraffin; histological sections of 3 mm were obtained from tissue blocks, cut perpendicular to the mucosa surface and stained with haematoxylin and eosin. Histomorphometric measurements were performed using a computer-assisted image-analysis system (Cytometrica, Byk...
Gulden, Milan, Italy) to assess height of 10 villi (except for caecum, in which villi are absent) and depth of 10 crypts of each section, on random-selected microscopic fields.

2.7. Statistical analyses

2.7.1. In vitro fermentation

A modified Gompertz bacterial growth model (Zwietering et al. 1992) was used to fit gas production data. This model assumes that substrate levels limit growth in a logarithmic relationship (Schofield et al. 1994) as follows:

\[
V = V_F \exp \left\{ -\exp \left[ 1 + \left( \frac{\mu_m V_F}{\lambda} \right) (\lambda - t) \right] \right\},
\]

where \( V \) = volume of gas produced at time \( t \), \( t \) = fermentation time, \( V_F \) = maximum volume of gas produced, \( \mu_m \) = maximum rate of gas production, which occurs at the point of inflection of the gas curve, and \( \lambda \) = the lag time, as the time-axis intercept of a tangent line at the point of inflection (Zwietering et al. 1990).

Curve fitting was performed using the program GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA). Data were analysed by ANOVA using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC, USA) in a completely randomised design. Linear and quadratic contrasts were used to determine the nature of the response exhibited to the addition of tannins. Each syringe and vessel formed the experimental unit. Differences were considered statistically significant at \( p < 0.05 \).

2.7.2. In vivo feeding trial

Data were analysed by ANOVA using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC, USA) in a completely randomised design. Linear and quadratic contrasts were used to determine the nature of the response exhibited to the feeding of tannins. Each piglet formed the experimental unit. Differences were considered statistically significant at \( p < 0.05 \).

3. Results

3.1. In vitro experiment

Gas production curves (Figure 1) were accurately described by the modified Gompertz model \( (r^2 = 0.92) \). Gas production results are shown in Table 2. Total gas production was reduced by tannins (linear, \( p < 0.05 \)), whereas maximum rate of gas production tended to be increased in tannin containing vessels \( (p = 0.06) \).

After 6 h of incubation, pH values were significantly reduced by tannins (linear, \( p < 0.001 \); Table 2), but tannin supplementation had no effect on pH at 24 h.

Ammonia concentrations were significantly reduced by tannins after 6 h (linear and quadratic, \( p < 0.001 \); Table 3) and this reduction ranged from \(-30\%\) when tannins were added at 0.75 g/l to \(-40\%\) with tannins used at 6 g/l. After 24 h of incubation, ammonia was significantly reduced by tannins (linear and quadratic, \( p < 0.001 \)) from \(-32\%\) to \(-63\%\) for tannins at 0.75 g/l and 6 g/l, respectively.

After 24 h of incubation, compared with the control, acetic acid was reduced by tannins (linear and quadratic, \( p < 0.01 \); Table 3) and the lowest acetic acid
concentration was determined at 6 g tannins per litre (−16%). Propionic acid (linear and quadratic, $p < 0.05$) and n-butyric acid (linear and quadratic, $p < 0.01$) were significantly reduced by tannins from 4–12% and from 8–36% for tannins at 0.75 and 6 g/l, respectively. Concentrations of iso-butyric and iso-valeric acid were significantly (linear and quadratic, $p < 0.001$) reduced by tannins and this reduction ranged from 21–52% and from 29–57% for tannins at 0.75 and 6 g/l, respectively. Total concentration of volatile fatty acids was significantly reduced by tannins (linear, $p < 0.001$).

Viable counts of bacteria at 6 and 24 h are represented in Figures 2 and 3, respectively. At 6 h, viable counts of coliforms were not affected by treatment; conversely, after 24 h of incubation, coliforms were increased (linear and quadratic,

---

**Table 2.** Modified Gompertz equation fitted to gas production data and pH from the 24 h *in vitro* incubation of swine caecal inoculum with different concentrations of tannins.

<table>
<thead>
<tr>
<th>Added tannins [g/kg]</th>
<th>Pooled SEM</th>
<th>$p$ of the model</th>
<th>Contrasts, $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Linear</td>
</tr>
<tr>
<td>$V_F$*</td>
<td>4.37</td>
<td>0.264</td>
<td>0.031</td>
</tr>
<tr>
<td>$\mu_m$#</td>
<td>0.29</td>
<td>0.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH at 6 h</td>
<td>7.03</td>
<td>0.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH at 24 h</td>
<td>6.80</td>
<td>0.030</td>
<td>0.472</td>
</tr>
</tbody>
</table>

Notes: *$V_F$, Maximum volume of gas produced [ml]; # $\mu_m$, Maximum rate of gas production [ml/h]. Values are least squares means of five replicates for each diet tested.
Table 3. Concentrations [mmol/l] of ammonia (after 6 and 24 h) and volatile fatty acids (after 24 h) from an *in vitro* incubation of swine caecal inoculum with different concentrations of tannins.

<table>
<thead>
<tr>
<th>Added tannins [g/kg]</th>
<th>Pooled SEM</th>
<th>p of the model</th>
<th>Contrasts, p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Linear</td>
</tr>
<tr>
<td>Ammonia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 6 h</td>
<td>12.4</td>
<td>8.66</td>
<td>8.12</td>
</tr>
<tr>
<td>at 24 h</td>
<td>20.6</td>
<td>14.1</td>
<td>9.50</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>42.8</td>
<td>44.2</td>
<td>41.8</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>16.9</td>
<td>16.3</td>
<td>15.4</td>
</tr>
<tr>
<td>iso-Butyric acid</td>
<td>1.22</td>
<td>0.96</td>
<td>0.84</td>
</tr>
<tr>
<td>n-Butyric acid</td>
<td>9.10</td>
<td>8.39</td>
<td>7.71</td>
</tr>
<tr>
<td>iso-Valeric acid</td>
<td>1.03</td>
<td>0.73</td>
<td>0.63</td>
</tr>
<tr>
<td>C2/C3*</td>
<td>2.53</td>
<td>2.71</td>
<td>2.72</td>
</tr>
<tr>
<td>n-C4/isoC4#</td>
<td>7.47</td>
<td>8.72</td>
<td>9.17</td>
</tr>
<tr>
<td>Total acids</td>
<td>72.4</td>
<td>71.7</td>
<td>67.5</td>
</tr>
</tbody>
</table>

Notes: *C2/C3, Acetic to propionic acid ratio; *n*-C4/isoC4, *n*-Butyric to iso-butyric acid ratio. Values are least squares means of five replicates for each diet tested.

Figure 2. Viable counts of coliforms (A), enterococci (B), and lactobacilli (C) at 6 h of an *in vitro* incubation of pig caecal inoculum with different concentrations of tannins (values are means ± SEM of five vessels per treatment).

$p < 0.01$ by tannins with the highest concentration of coliforms in vessels added with tannins at 3 g/l (+0.5 log CFU/ml). Lactobacilli were significantly reduced by tannin addition and the lowest counts of lactobacilli were determined at 6 g tannins.
per litre (−0.7 and −0.8 log CFU/ml after 6 and 24 h of incubation, respectively; linear, \( p < 0.01 \)). Enterococci counts were significantly increased by tannins, with the highest counts of tannins at 6 g/l (+2.2 and +2.5 log CFU/ml after 6 and 24 h of incubation, respectively; linear and quadratic, \( p < 0.05 \)). Viable counts of clostridia were not affected by treatment and averaged 7.7 and 7.8 log CFU/ml after 6 and 24 h of incubation, respectively (data not shown).

3.2. **In vivo feeding trial**

Animal live weight, average daily gain, and daily feed intake were not significantly influenced by tannins (Table 4). Feed efficiency throughout the 28 d trial was significantly improved by tannins (linear, \( p < 0.05 \)) and the lowest feed efficiency was observed in pigs fed tannins at 4.5 g/kg (−5%; Table 4).

Experimental diets had no significant effect on intestinal pH; average pH values were 5.70, 6.53, and 5.77 in jejunum, ileum, and caecum, respectively (data not shown). Caecal ammonia concentrations showed a tendency towards a reduction in pigs fed tannins (\( p = 0.07 \); Table 5), while no effect was observed on ammonia levels in chyme samples from jejunum and ileum.

Concentration of propionic acid in the caecal chyme showed a tendency towards a reduction in tannin-fed pigs (\( p = 0.06 \); Table 5). Feeding tannins to piglets reduced
linear, \( p < 0.01 \) caecal concentrations of iso-butyric (from \( -35\% \) to \( -55\% \) for tannins at 2.25 and 4.5 g/kg, respectively) and iso-valeric acid (from \( -39\% \) to \( -53\% \) for tannins at 2.25 and 4.5 g/kg, respectively). Dietary treatments also had a significant effect on \( n \)-butyric and total VFA concentrations (cubic, \( p < 0.01 \)).

Viable counts of bacteria in jejunum and caecum chyme are represented in Figure 4. Feeding tannins tended (\( p = 0.06 \)) to increase viable counts of lactobacilli in the jejunum, while caecal lactobacilli were not influenced by dietary treatment. Coliforms, enterococci and clostridia concentrations in the jejunum were under the detection limit and could not be determined. Caecal counts of coliforms showed a tendency towards an increase when pigs were fed tannins (\( p = 0.08 \)), whereas caecal clostridia and enterococci were not affected by tannin supplementation. Tannin supplementation had no effect on villi length in the jejunum and ileum (Table 6) but significantly influenced ileal crypt depth (cubic, \( p < 0.05 \)).

4. Discussion

In the present trial, the utilisation of tannins did not significantly influence animal weight gain but improved feed efficiency when tannins were fed at 4.5 g/kg of diet.
The improved utilisation of the diet in piglets fed high levels of tannins is a controversial finding because tannins have been known as antinutritional factors for their ability to reduce digestibility of dietary protein (Mariscal-Landín et al. 2004). In a study by Lizardo et al. (1995), the addition to pig diets of a tannin-rich variety of sorghum resulted in reduced animal growth performance. Conversely, other authors observed that feeding pigs with feedstuffs high in tannins such as field beans (Flis...
et al. 1999) and carob powder (Lizardo et al. 2002) did not affect animal growth. In a study with weaned pigs (Myrie et al. 2008), feeding animals with tannins at 15 g/kg of diet did not affect animal growth performance despite the fact that the ileal apparent digestibility was reduced for threonine but not for total protein. Longstaff and McNab (1991) observed that tannin-rich fieldbeans at low doses enhanced the activity of lipase in digesta from both the jejunum and ileum of young chicks. When reduced growth performance is reported in monogastric animals fed sorghum (Lizardo et al. 1995), faba beans (Rubio et al. 1990), lupins (Kim et al. 2007) as well as other tannins-rich feedstuffs, it has to be considered that, when compared to more traditional feedstuffs for pigs such as soybean meal and corn meal, these feedstuffs are generally characterised by lower digestibility of their starch (Rooney and Pflugfelder 1986; Wiseman 2006) and protein (Mariscal-Landín et al. 2002) fractions, and by the presence of significant amounts of other antinutritional factors (Huisman et al. 1990) that altogether might result in reduced animal growth.

In the present in vitro study, tannins had a strong influence on the metabolism of swine caecal microbiota. In fact, increasing concentrations of tannins linearly reduced total gas production and concentrations of acetic, propionic, and n-butyric acid, showing an inhibitory effect on the activity of caecal bacteria. Because the acetic to propionic acid ratio was not linearly influenced by tannin addition, it seems that tannins reduced total bacterial activity without favouring specific metabolic pathways. Conversely, increasing concentrations of tannins resulted in a linear increase of the n-butyric to iso-butyric acid ratio. This finding might be explained by a relatively higher carbohydrate metabolism of butyric acid producing bacteria, together with a reduction of iso-butyric acid, a metabolite deriving from protein bacterial catabolism.

Interestingly, tannins at 4.5 g/kg determined the highest rate of gas production, suggesting that, despite a general inhibitory effect, the activity of some bacteria was selectively stimulated by high levels of tannins. In fact, counts of enterococci (and, to a lower extent, of coliforms) were significantly increased by tannins, whereas lactobacilli counts were reduced. When fed to piglets, tannins did not increase enterococci caecal counts but tended to increase lactobacilli in the jejunum and coliforms in the caecum. It has been shown that several intestinal bacterial strains

<table>
<thead>
<tr>
<th>Added tannins [g/kg]</th>
<th>Pooled SEM</th>
<th>p of the model</th>
<th>Contrasts, p</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
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<td>2.25</td>
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<tr>
<td>4.5</td>
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</tbody>
</table>

Notes: Values are least squares means of six piglets for each diet tested. *Cubic (p < 0.05) when comparing 0 through 4.5 g/kg of tannins.

Table 6. Villous height and crypt depth [μm] of the intestinal mucosa in piglets that had received a diet added or not with different levels of tannins in the four weeks after weaning.
can degrade tannic, gallic, and ellagic acids and use them as a source of energy (Bhat et al. 1998; Cerda et al. 2005). Despite the relatively low content of simple sugars (2.6%) of the wood extract, it is also possible that the extract might contain some growth factors for enterococci that exerted their action in vitro but could not reach the animal hindgut when tannins were fed to piglets, as such failing to increase enterococci caecal counts. In vitro, the enhanced growth of enterococci might explain the slight reduction of lactobacilli counts due to higher competition for substrates. Nevertheless, higher enterococci counts might simply be the consequence of higher growth potential of enterococci compared to lactobacilli.

The definition of tannins refers to a group of water-soluble polyphenols that are classified as hydrolysable and non-hydrolysable (condensed) tannins (Akiyama et al. 2001). Tannin compounds differ in their antibacterial activity (Akiyama et al. 2001; Puupponen-Pimiä et al. 2005) and the composition of tannins is influenced by plant of origin and method of extraction. In vitro, antibacterial effects of tannins against pathogenic strains of Escherichia coli (Yao et al. 2006), Clostridium perfringens and Staphylococcus aureus (Ahn et al. 1998), and Helicobacter pylori (Funatogawa et al. 2004) have been reported, but in our study tannins at any concentration tested did not reduce in vitro counts of coliforms and clostridia. In the study by Ahn et al. (1998), tannins were obtained from wattle and showed antibacterial activity against different bacterial strains with MIC values ranging from 0.5–8 mg/ml that were comparable to the tannin concentrations that we used in the present trial.

The lack of in vitro antibacterial activity against coliforms and clostridia of the tannins that we used was confirmed by the in vivo study. At present, there are no data regarding the amount of dietary tannins that are absorbed in the small intestine of pigs, so that it is difficult to estimate how many tannins will reach the animal hindgut. Studies in rats fed tannins have indicated that tannic acid was mostly recovered in the faeces, but small amounts of tannin compounds were found in plasma and urine samples, showing that absorption of tannins occurred (Clifford and Brown 2005). At present, there is no evidence that tannins might exert in the animal intestine the same effective antibacterial effect that has been observed by some authors (Ahn et al. 1998; Funatogawa et al. 2004; Yao et al. 2006) under in vitro conditions.

Concentrations of ammonia, iso-butyric acid, and iso-valeric acid were significantly reduced both in vivo and in vitro, showing that tannins effectively reduced bacterial proteolytic reactions. In fact, the above isoacids are formed from the deamination of valine and leucine (Van Soest 1982) and are indicative as ammonia of bacterial protein catabolism extent. Ammonia is a toxic compound that can destroy cells and reduce villus height (Nousiainen 1991), and once absorbed must be converted to urea with the loss of energy (Eisemann and Nienaber 1990). Moreover, subacute concentrations of ammonia may reduce animal performance (Visek 1978). Several dietary supplements have been shown to reduce intestinal bacterial proteolysis: among these are non-digestible oligosaccharides and probiotics (Piva et al. 2005), organic acids (Roth and Kirchgessner 1998), and herb extracts (Ushida et al. 2002). Based on the present results, tannins seem to be a valuable alternative to the aforementioned dietary supplements in order to control intestinal bacterial proteolytic reactions in weaned pigs.

The effect of tannin supplementation on intestinal mucosa histology was also investigated. Early weaning has a dramatic negative impact on the intestinal mucosa morphology of piglets (Gu et al. 2002) and can lead to nutrient malabsorption
n-Butyric acid is the preferred energy substrate of the mucosa of the ileum (Chapman et al. 1995) and large intestine (Roediger 1980), and it has been observed that feeding piglets with sodium butyrate (Gálfí and Bokori 1990; Wang et al. 2005) can have a positive trophic effect on the intestinal mucosa, increasing the length of ileal microvilli and the depth of caecal crypts. Conversely, other authors failed to observe any trophic effect on the intestinal mucosa when sodium butyrate (Biagi et al. 2007) or butyric acid precursors (Piva et al. 2002) were fed to weaned pigs. In the present study, tannins had a cubic effect on n-butyric acid caecal concentrations and the depth of ileal crypts tended to decrease when tannins were used at 2.25 and 4.5 g/kg. At present, we do not have an explanation for this effect of tannins. Nevertheless, because crypts in the small intestine mainly have a secretory function (Wood 2006), a reduction of their surface might help reducing the severity of piglet post-weaning diarrhoea.

5. Conclusion
The present study has produced evidence that feeding weaned piglets with a tannin-rich wood extract can result in improved feed efficiency and reduction of intestinal bacterial proteolytic reactions. Nevertheless, the growth-enhancing effect that the wood extract seemed to have on coliforms deserves further investigation.

References


