

# A dose-response effects of tannic acid and protein on growth performance, caecal fermentation, colon morphology, and $\beta$ -glucuronidase activity of rats

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## ABSTRACT

The aim of the present work was to study the influence of tannic acid (TA) and protein level in the diet on fermentation in the caecum of rats, activity of bacterial  $\beta$ -glucuronidase, and colon morphology. Twelve groups of six male Wistar rats were given either a control diet free of TA or diets containing 0.25, 0.5, 1, 1.5, or 2% TA. Diets contained 10 or 18% of crude protein. Body weight and feed intake were monitored during a 3-week experimental period. Tannic acid reduced protein apparent digestibility and decreased liveweight gain. Both factors affected caecal fermentation and increased volatile fatty acid production. The higher protein level increased the concentration of branched-chain fatty acids. Tannic acid reduced the activity of  $\beta$ -glucuronidase and affected colonic myenteron thickness. These results indicate that TA may have advantageous effects on the gastrointestinal tract if its amount in the diet does not exceed 1.5%.

KEY WORDS: rat, tannic acid, large intestine, morphology, VFA,  $\beta$ -glucuronidase

## INTRODUCTION

Tannins are secondary plant metabolites with different biological activities and are found in many feeds of plant origin (Mueller-Harvey, 2006). They are known to be antinutritional factors that form indigestible complexes with protein, polysaccharides, and mineral compounds, which may cause a higher flow of

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nutrients into the large intestine and thereby influence the molar ratio of volatile fatty acids produced by bacteria (Pastuszewska et al., 2000; McSweeney et al., 2001). Bacteria feed mainly on undigested carbohydrates and protein. End-products of carbohydrate fermentation are of importance to animal health. They consist of short-chain fatty acids such as acetic, propionic and butyric acids. The molar ratio of these acids can be changed depending on the type and amount of substrate for fermentation. When carbohydrates are completely fermented, bacteria start to digest protein that reaches the large intestine, which leads to formation of potentially toxic compounds such as branched-chain fatty acids, ammonia, amines and phenols. Their evolution is related to increased risk of colon cancer and it is therefore important to limit bacterial protein degradation (Le Leu et al., 2007). Colonic epithelium may also be exposed to toxins that were neutralized earlier in the liver by conjugation with glucuronic acid. Bacterial hydrolysis of glucuronides is an important enzymatic reaction, catalyzed in the colon by  $\beta$ -glucuronidase (Gadelle et al., 1985). This enzyme is related to enterohepatic circulation and activation of procarcinogens, carcinogens, mutagens and toxins. Its high activity is found to be a factor of increased risk of developing colon cancer (Jenab and Thompson, 1996). Optimal function of the large intestine is closely related to the digestive processes of intestinal bacteria. End-products from bacterial fermentation may affect intestinal morphology leading to alterations in nutrient absorption and epithelial cell turnover. Diet has the greatest influence on formation of bacterial metabolites and is one of the factors affecting colon morphology (Kuzmuk et al., 2005), but there is a lack of literature about the impact of tannins on caecal fermentation and colon morphology of monogastric animals. Tannic acid, which belongs to the group of hydrolysable tannins, is an example of such a compound. It is toxic to animals when injected into the blood stream or ingested in large doses (Khan and Hadi, 1998), but in small amounts tannic acid has a health-promoting action because of its antimutagenic, anticarcinogenic and antioxidant properties (Lopes et al., 1999; Nam et al., 2001).

The aim of this study was to examine the effect of graded levels of tannic acid and protein in the diet on fermentation processes in the caecum of rats, activity of bacterial  $\beta$ -glucuronidase, and colon morphology.

## MATERIAL AND METHODS

### *Animals and diets*

Seventy two ten-week-old male Wistar rats were divided into 12 groups so that the mean body weight in each group was similar (189 g). The animals were kept

Table 1. Formulation of diets, %

Components	Diet, group											
	10% of crude protein						18% of crude protein					
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Casein	12.9	12.9	12.9	12.9	12.9	12.9	23.3	23.3	23.3	23.3	23.3	23.3
Maize starch	53.7	53.5	53.2	52.7	52.2	51.7	43.3	43.1	42.8	42.3	41.8	41.3
Sucrose	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Pectin	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Celullose	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Soyabean oil	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
DL-methionine	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Tannic acid	0.0	0.25	0.5	1.0	1.5	2.0	0.0	0.25	0.5	1.0	1.5	2.0
Mineral mix <sup>1</sup>	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin mix <sup>2</sup>	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

<sup>1</sup> AIN-93G Mineral mix; %: calcium carbonate 35.7; monopotassium phosphate 19.6; potassium citrate monohydrate 7.078; sodium chloride 7.4; potassium sulphate 4.66; magnesium oxide 2.4; ferric citrate 0.606; zinc carbonate 0.165; manganese carbonate 0.063; copper carbonate 0.03; potassium iodate 0.001; sodium selenate, anhydrous 0.00103; ammonium molybdate 4H<sub>2</sub>O 0.000795; sodium metasilicate 9H<sub>2</sub>O 0.145; chromium potassium sulfate 12H<sub>2</sub>O 0.0275; lithium chloride 0.00174; boric acid 0.008145; sodium fluoride 0.00635; nickel carbonate 0.00318; ammonium vanadate 0.00066; powdered sugar 22.1

<sup>2</sup> AIN-93-VX Vitamin mix; g/kg: nicotinic acid 3.00; D-calcium pantothenate 1.60; pyridoxine HCl 0.70; thiamine HCl 0.60; riboflavin 0.60; folic acid 0.20; D-biotin 0.02; vit. B<sub>12</sub> (0.1% triturated in mannitol) 2.50; α-tocopherol powder (250 IU/g) 30.00; vit. A palmitate (250,000 IU/g) 1.60; vit. D<sub>3</sub> (400,000 IU/g) 0.25; phylloquinone 0.075; powdered sucrose 959.655

in individual metabolic cages for 21 days under controlled conditions of  $22\pm 1^{\circ}\text{C}$  and 12 h dark-light cycle and given free access to feed and water.

During the experimental period, body weight and feed intake were monitored. Faeces were collected every day in the 2<sup>nd</sup> and 3<sup>rd</sup> week and stored at  $-20^{\circ}\text{C}$ . After the experiment, rats were anaesthetised by  $\text{CO}_2$  and killed by cervical dislocation.

Formulation of the diets is given in Table 1. Feed containing 10% of crude protein (CP), without the addition of tannic acid (TA), was the control diet. TA powder was purchased from Sigma-Aldrich Ltd. (Poznań, Poland) and mixed with other feed components.

The animal care and experimental procedures were approved by the Local Animal Experimentation Ethics Committee.

#### *Apparent protein digestibility*

Crude protein content in diets and faeces was analysed according to standard methods (AOAC, 1990) using a Kjeltex apparatus (Tecator AB, Sweden) and coefficients of apparent protein digestibility were calculated.

#### *Measurement of volatile fatty acids (VFA)*

Volatile fatty acid concentrations in caecal digesta were determined according to the method of Ziotecki and Kwiatkowska (1973) with the following modifications: caecal digesta was mixed with 4 ml of ultra pure water and pH was measured using a WTW pH/340 pH-meter. VFAs were converted to their respective sodium salts by adjusting pH to 8.2 using 1 M NaOH. Samples were stored at  $-20^{\circ}\text{C}$ . Prior to analysis, samples were thawed at room temperature, thoroughly mixed and centrifuged for 10 min at 1000 rpm at room temperature. Supernatants were collected and formic acid was added in an amount equal to 10% of sample volume. After mixing, supernatants were centrifuged for 10 min at 10000 rpm at room temperature. 500  $\mu\text{l}$  of supernatants were transferred into chromatographic vials and mixed with isocaproic acid (internal standard; IS) at a ratio of 15  $\mu\text{l}$  of IS to 100  $\mu\text{l}$  of supernatant. Samples were analysed in duplicate, using a HP 5890 Series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) with a flame-ionization detector (FID) and Supelco Nukol fused silica capillary column (30 m x 0.25 mm i.d.; 0.25  $\mu\text{m}$ ). Helium was used as the carrier gas with a flow rate of 103 ml/min. The oven was initially kept at  $100^{\circ}\text{C}$  for 2 min, then heated at  $10^{\circ}\text{C}/\text{min}$  to  $140^{\circ}\text{C}$  and held for 20 min. The injector temperature was maintained at  $220^{\circ}\text{C}$ , while the detector was kept at  $250^{\circ}\text{C}$ . The total run time was approximately 27 min. Concentrations of individual VFA were estimated in relation to IS using a mixture of VFA standard solutions.

### *Measurement of $\beta$ -glucuronidase activity*

The  $\beta$ -glucuronidase assay was based on methods by Fishman (1974) and Jenab and Thompson (1996) with the following modifications: caecal samples (0.5 g) were homogenized for 30 s with 5 ml phosphate buffer (75 mM KH<sub>2</sub>PO<sub>4</sub> with 1% w/v bovine serum albumin, pH 6.8 at 37°C). Extracts were sonicated (2 x 30 s at room temperature) and centrifuged for 20 min at 10000 rpm at room temperature. Supernatants were collected and stored in 1 ml aliquots at -80°C. Prior to analysis the supernatants were thawed at room temperature. Reagents were added to disposable polystyrene cuvettes as follows: 200  $\mu$ l ultra pure water, 154  $\mu$ l phosphate buffer and 77  $\mu$ l phenolphthalein  $\beta$ -D-glucuronide. After 5 min of preincubation at 37°C, 31  $\mu$ l of the supernatant were added to samples and incubated for 30 min at 37°C. The reaction was terminated by the addition of 1540  $\mu$ l of glycine buffer (200 mM glycine, pH 10.4 at 37°C). Absorbance was measured at 540 nm using a UNICAM UV 300 spectrophotometer (Thermo-Spectronic, Cambridge, UK). The amount of phenolphthalein released was estimated using a standard curve for phenolphthalein.

### *Colon histometry*

Samples of colon tissue were placed in Bouin's solution (mixture of picric acid, formalin and glacial acetic acid at a ratio of 30:15:1, respectively). Subsequently, they were dehydrated, embedded in paraffin and sliced into 5  $\mu$ m sections using a microtome. Two slides were prepared from each sample; each slide contained a minimum of 4 sections that were stained with haematoxylin and eosin. Crypt depth and myenteron thickness (20 measurements per slide) were determined using a light microscope Zeiss Axio Star Plus (Carl Zeiss, Göttingen, Germany) and image analysis program Axio Vision LE Rel. 4,5 (Carl Zeiss, 2002-2005).

### *Statistical analysis*

Statistical analysis of data followed a block design, with a factorial arrangement of 2 x 6, taking into consideration the main effects of protein and tannic acid level (class variables) with an equal number of 6 replicates for each treatment. Data are presented as means and their standard error values. The effect of TA and protein, and their interactions were determined by two-way ANOVA and differences between treatments were analysed post hoc by the Least Significant Differences test using the STATGRAPHICS® Centurion XVI ver. 16.1.03 (Statistical Graphic Corp., 1982-2010) statistical package. In addition, orthogonal polynomial contrasts were used to detect linear, quadratic and cubic effects of TA supplementation on chosen parameters. The effects were considered to be significant at  $P \leq 0.05$  and  $P \leq 0.01$ . Trends between  $P \geq 0.05$  and  $P \leq 0.1$  are also presented and discussed.

RESULTS

Feed intake did not differ among rats but differences in apparent protein digestibility were noted. The addition of tannic acid caused a significant ( $P \leq 0.01$ ) reduction in apparent protein digestibility and this effect was modified by protein level in the diet (interaction  $P \leq 0.01$ ). Apparent protein digestibility for 18% CP diets was significantly higher in comparison with diets containing 10% of CP (Figure 1). Rats receiving 18% CP diets had higher liveweight gains compared with rats fed 10% CP diets and better feed efficiency (data not shown). Tannic acid decreased liveweight gains ( $P \leq 0.01$ ) and differences between control groups and groups fed on diets with 1, 1.5 and 2% TA were noticed (Figure 2).

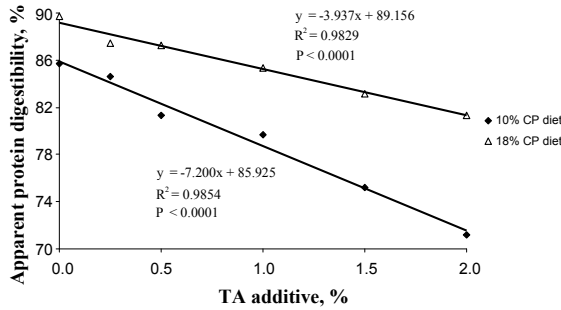


Figure 1. The relationship between the amount of tannic acid (TA) and crude protein (CP) level in the diet and the apparent protein digestibility. Effects of the dietary groups are: TA level ( $P \leq 0.0001$ ); CP level ( $P \leq 0.0001$ ); TA  $\times$  CP ( $P \leq 0.0001$ )

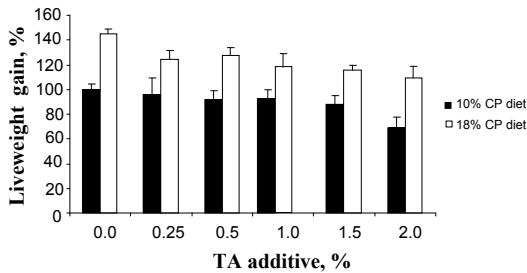


Figure 2. Liveweight gains of rats. Effects of the dietary groups are: tannic acid (TA) level ( $P = 0.006$ ); crude protein (CP) level ( $P \leq 0.001$ ); TA  $\times$  CP ( $P = 0.768$ )

Regression analysis showed a directly proportional relationship between the amount of TA in the diet and the relative weight of caecal digesta (Figure 3).

Significant differences ( $P \leq 0.01$ ) were noted between control groups and groups with 1, 1.5 and 2% TA additive. Rats fed on the diet with 2% TA differed significantly ( $P \leq 0.01$ ) from the other groups except for those diets with 1.5% added TA.

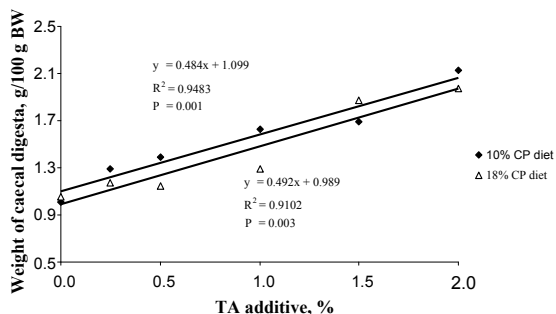


Figure 3. The relationship between the amount of tannic acid (TA) and crude protein (CP) level in the diet and a relative weight of caecal digesta of rats. Effects of the dietary groups are: TA level ( $P \leq 0.0001$ ); CP level ( $P = 0.246$ ); TA  $\times$  CP ( $P = 0.587$ )

The caecal pool of VFA (Table 2) was significantly greater in animals fed on 18% CP diets than in animals fed 10% CP diets. The addition of TA increased the caecal VFA concentration. Significant differences were found between control groups and groups with 1% ( $P \leq 0.05$ ), 1.5% ( $P \leq 0.01$ ) and 2% ( $P \leq 0.05$ ) added TA.

Table 2. Caecal concentration of volatile fatty acids (VFA) in rats

Group	Experimental factor, %		VFA concentration, $\mu$ M per caecum						
	TA	CP	C <sub>2</sub>	C <sub>3</sub>	i-C <sub>4</sub>	C <sub>4</sub>	i-C <sub>5</sub>	C <sub>5</sub>	$\Sigma$
I	0.00	10	94.3	34.6	3.1	15.8	2.1	1.9	152.0
II	0.25	10	123.0	29.9	3.6	17.8	2.9	2.7	180.1
III	0.5	10	124.6	35.3	4.1	20.2	3.6	3.4	191.2
IV	1.0	10	164.2	34.8	3.9	24.3	3.0	3.2	233.4
V	1.5	10	141.8	31.0	3.5	16.6	2.7	3.6	199.1
VI	2.0	10	136.8	35.5	3.9	15.8	3.7	2.3	198.0
VII	0.00	18	115.6	36.1	3.7	13.6	3.2	2.9	175.1
VIII	0.25	18	148.3	43.1	4.2	18.0	3.2	3.6	220.5
IX	0.5	18	126.9	39.9	4.2	15.5	4.2	3.5	194.2
X	1.0	18	158.2	42.4	4.1	15.9	3.4	3.5	227.5
XI	1.5	18	188.2	43.8	6.4	23.4	6.5	5.8	274.2
XII	2.0	18	178.2	41.3	6.8	22.5	7.0	5.5	261.3
SEM			6.0	1.5	0.2	0.9	0.3	0.2	8.0

*Significance of effects in ANOVA*

TA	0.017	0.990	0.002	0.535	0.006	0.072	0.046
CP	0.055	0.013	0.000	0.884	0.001	0.004	0.030
TA $\times$ CP	0.714	0.841	0.005	0.100	0.068	0.275	0.565

C<sub>2</sub> - acetic acid; C<sub>3</sub> - propionic acid; i-C<sub>4</sub> - isobutyric acid; C<sub>4</sub> - butyric acid; i-C<sub>5</sub> - isovaleric acid; C<sub>5</sub> - valeric acid

In all groups, acetic acid was the major metabolite and made up 62.1 to 69.5% of total VFA. Addition of 1, 1.5 and 2% of TA significantly increased the acetic acid concentration compared with control groups. Concentrations of acetic acid increased linearly ( $P \leq 0.05$ ) with increasing doses of TA in 18% CP diets and exhibited a quadratic trend ( $P \leq 0.1$ ) with TA supplementation of 10% CP diets, with the largest increase noted for the 1% TA treatment.

Propionic acid amounted to 18.1% of total VFA in caecal digesta. The concentration of propionic acid was significantly greater when 18% CP diets were fed in comparison with 10% CP diets ( $P \leq 0.05$ ). Tannic acid had no effect on propionic acid concentration and there were no functional dependencies.

Butyric acid accounted for 9.6 and 8.0% of total VFA for 10 and 18% CP diets, respectively. Based on the ANOVA results, neither TA nor CP level had a significant effect on butyric acid concentration in the caecum, although an interactive trend was noted ( $P \leq 0.1$ ). The highest butyric acid concentrations were observed at 1% TA for 10% CP diets and at 1.5% TA for 18% CP diets. Orthogonal contrasts analysis showed that addition of TA resulted in a linear increase ( $P \leq 0.05$ ) in caecal butyric acid concentration in rats fed diets with the higher level of CP.

Both factors significantly affected ( $P \leq 0.01$ ) the concentration of isobutyric acid. An interaction ( $P \leq 0.01$ ) between TA and CP level was observed. Increasing doses of TA in diets with the higher level of CP resulted in a linear response ( $P \leq 0.01$ ) in isobutyric acid concentration with the highest values noted for the 1.5 and 2% TA additions. In rats fed on 10% CP diets, a cubic trend ( $P \leq 0.1$ ) was observed with increasing TA level. Taking all groups into consideration, significant differences were noted between control groups and groups with 1.5% ( $P \leq 0.01$ ) and 2% ( $P \leq 0.05$ ) added TA.

Tannic acid and CP level had a significant ( $P \leq 0.01$ ) influence on the isovaleric acid concentration as the mean concentration of this acid was higher in caecal digesta of rats receiving 18% CP diets than in other groups. Tannic acid modified the effect of CP level on the caecal concentration of isovaleric acid and a trend towards an interaction was observed ( $P \leq 0.1$ ). Significant differences were noted between control groups and groups with 1.5% ( $P \leq 0.01$ ) and 2% ( $P \leq 0.05$ ) of TA. As in the case of isobutyric acid, a linear increase ( $P \leq 0.05$ ) of isovaleric acid concentration was observed when 18% CP diets contained TA, and a cubic response ( $P \leq 0.05$ ) was noted for increasing doses of TA in 10% CP diets.

The valeric acid concentration was the lowest. Diets with 18% of CP increased its concentration in comparison with the 10% CP diets. Tannic acid did not have a statistically significant influence on the valeric acid concentration, although functional dependencies were observed: linear ( $P \leq 0.05$ ) for 18% CP diets and quadratic ( $P \leq 0.1$ ) for 10% CP diets.

The activity of bacterial  $\beta$ -glucuronidase decreased significantly ( $P \leq 0.01$ ) when diets with TA were fed (Figure 4). The lowest  $\beta$ -glucuronidase activity was



observed in groups of rats receiving diets with 1% TA, in which was 67.9% lower than in the control group. The CP level in the diets did not affect the activity of  $\beta$ -glucuronidase.

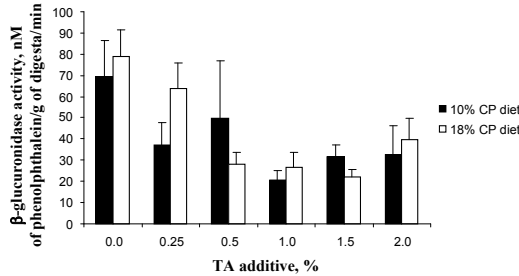


Figure 4. The activity of bacterial  $\beta$ -glucuronidase in the caecal digesta of rats. Effects of the dietary groups are: tannic acid (TA) level (P=0.002); crude protein (CP) level (P=0.673); TA  $\times$  CP (P=0.490)

Crypt depth in the colon did not significantly differ among rats (data not shown), but in relation to myenteron thickness, significant interactions between TA and CP level were noted (Figure 5). The myenteron was significantly thicker in animals fed on 18% CP diets and free of TA than in animals fed on diets with 0.5 and 2% TA. It also differed from rats receiving diets with 10% of CP with 0.25 and 1.5% TA.

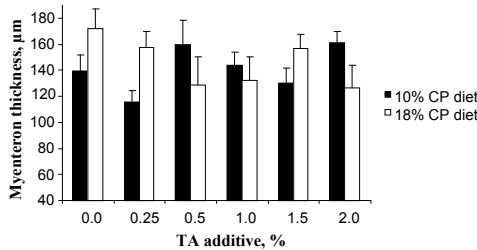


Figure 5. Myenteron thickness in the colon of rats. Effects of the dietary groups are: tannic acid (TA) level (P=0.802); crude protein (CP) level (P=0.612); TA  $\times$  CP (P=0.023)

## DISCUSSION

Protein level and the addition of tannic acid (TA) did not affect feed intake. It is generally known that the main dietary factor influencing feed intake in rats is the energy density of the diet, while the protein concentration seems to play a less

important role. TAs lack of influence may be due to its low concentration which did not reduce palatability, but the chemical structure of TA is also important. Tannic acid belongs to the group of hydrolysable tannins. A bitter taste, which negatively affects voluntary feed intake, is attributed mainly to condensed tannins (Dixon et al., 2005).

Protein and TA level influenced protein digestibility and growth of rats. Their effect may be related to amino acid availability. The negative effects of TA on growth were smaller in rats fed on the 18% CP diets in comparison with the 10% CP diets. This may be due to a higher amount of CP available for digestion in the small intestine. The higher apparent protein digestibility coefficients observed in rats fed on 18% CP diets support these results.

Nakamura et al. (2001) suggest that TA at a dose of only 0.1 g/kg of body weight causes a reduction of liveweight gain of rats. This response may result from a negative influence of TA on nutrient digestibility and absorption. The most TA-sensitive nutrient is protein (Mueller-Harvey, 2006), which can precipitate by binding to tannins. This can lead to the inhibition of enzyme activity and significant reduction of protein and dry matter digestibility (Jansman, 1993). Polyphenolic compounds may also modify brush border membrane proteins resulting in a decrease of nutrient absorption, which may be another reason for liveweight gain reduction in rats (Jansman, 1993).

The increased relative weight of caecal digesta may result from the lower digestibility of nutrients in rats receiving TA in the diets, thereby more intensive bacterial fermentation. Volatile fatty acids are an indicator of bacterial activity in the large intestine. The highest concentration of VFA was observed when 1% TA was added to 10% CP diets and 1.5% TA to 18% CP diets. The increase of the caecal VFA pool may be attributed to increased fermentation caused by higher amounts of substrates for microflora. This would be in agreement with reduced protein digestibility and increased relative weight of caecal digesta. The relationship between VFA concentration and TA level is not proportional. The caecal VFA pool at 2% TA additive is lower than at 1 and 1.5%. This may suggest that a TA supplementation of around 1 to 1.5% could be beneficial for the fermentation processes in the caecum of rats.

Acetic acid was 67% of the caecal VFA pool. This may probably result from pectin that is a major source of acetate (Pastuszewska et al., 2000) and was added to the diets to stimulate fermentation intensity. Inclusion of TA to the diet caused an increase of acetic acid concentration but did not influence the propionic acid concentration. The effect of TA was more pronounced in rats fed on diets with a higher level of CP, which does not confirm the hypothesis of Bravo et al. (1994) who suggested that TA is an inhibitor of acetic-acid-producing bacteria.

In the present study, tannic acid decreased protein digestibility, causing a higher amount of protein to reach the caecum, but it did not have a significant effect on butyric acid concentration. This acid is of particular importance as it is a major energy source for colonocytes, determines normal cell phenotype, and prevents the development of colon cancer (Topping and Clifton, 2001). The response in microbial activity in the caecum might suggest a positive effect of 1 and 1.5% TA, depending on the protein level in the diet.

The higher CP level in the diet increased branched-chain fatty acid (BCFA) concentrations in the caecum of rats. These acids are markers of proteolytic fermentation and derive from degradation of branched-chain amino acids (BCFA) of such as valine, leucine and isoleucine (Hughes et al., 2000). It is likely that the higher amount of protein reaching the caecum and stimulating the development of proteolytic microflora is the reason for the increased concentration of valeric, isovaleric, and isobutyric acids. Some of these important proteolytic species belong to the genera such as: *Bacteroides*, *Propionibacterium*, *Clostridium*, *Fusobacterium*, *Streptococcus* and *Lactobacillus* (Hughes et al., 2000). Results obtained for weaned pigs (Opapeju et al., 2009) shown that animals receiving diets with a lower CP level tended to have a smaller prevalence of the genus *Clostridium* in their colon digesta compared with pigs fed diets with higher CP levels. Our results indicate that adding 1% or 1.5% TA increases the concentration of BCFA in 18% CP diets, probably as the effect of lower protein digestibility, which was also observed by Le Leu et al. (2007) who used indigestible potato protein as only protein source in rat diets.

Bacteria in the large intestine may produce toxic metabolites, convert bile acids and digest or hydrolyse some drugs. The level of some bacterial enzymes is thought to be a factor affecting colon carcinogenesis. One of these enzymes is  $\beta$ -glucuronidase. In the present study, the TA level affected  $\beta$ -glucuronidase activity. The beneficial influence of polyphenolic compounds on  $\beta$ -glucuronidase activity was also shown by Juśkiewicz et al. (2001) and Zduńczyk et al. (2003). Inhibition is probably caused by formation of a tannin-enzyme complex (Aerts et al., 1999). Another mechanism to decrease  $\beta$ -glucuronidase activity is the inhibition of specific microflora having a high activity of this enzyme, as suggested by Jurgoński et al. (2008). The ability of TA to modify the intestinal microflora composition was shown by Chung et al. (1998), who reported that TA is an *in vitro* growth inhibitor for *Clostridium perfringens*, *Bacteroides fragilis*, *Salmonella typhimurium*, *Escherichia coli* but not for *Bifidobacterium infantis* and *Lactobacillus acidophilus*. Robertson et al. (1982) showed that bile is very important in the regulation of  $\beta$ -glucuronidase activity, and should therefore be taken into account for further studies.

Histological parameters reflect intestinal environment and are an indication of health status (Lu et al., 2008). Adding TA to the diets did not alter the colon crypt depth of rats. Therefore, it can be assumed that TA does not influence the proliferation, differentiation or functions of colonocytes. Tannic acid modifies the effect of CP level on myenteron thickness, which may point to changes in the passage rate of digesta through the large intestine. Myenteron structure may also be associated with nutrient digestibility, but the results obtained in this study are equivocal and need further research.

## CONCLUSIONS

Increasing the level of tannic acid (TA) decreased protein digestibility and caused a higher amount of undigested protein to reach the caecum, which influenced the amount and profile of volatile fatty acids. The activity of bacterial  $\beta$ -glucuronidase was influenced only by the level of TA in the diet. Considering microbial activity, it can be supposed that TA in the diet, particularly in the amounts of 1 and 1.5%, may be beneficial for fermentation processes in the caecum of rats. The negative impact on liveweight gains indicates that this compound would be more advantageous for adults than young animals. The necessary condition enabling the use of TA in the diets for rats is an appropriate level of protein.

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