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Digestibility and metabolic utilisation of diets containing chestnut tannins and their effects on growth and slaughter traits of heavy pigs

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ABSTRACT

This research aims at evaluating the effects of the dietary addition of a supplement containing 75% of chestnut tannins (CT) on growth performance, slaughter traits (Experiment 1) and on nutrient utilisation (Experiment 2) of Italian heavy pigs. Exp. 1 compared a control (C) diet with diets containing 0.15 or 0.30% of CT supplement. Forty-two barrows (91 ± 6 kg of BW) were divided into pairs and kept in 21 partially-slatted pens equipped with individual feeding (7 pens/diet). Animals were slaughtered at a BW of 174 ± 6 kg. The CT inclusion did not modify the performance and the slaughter traits but lowered the intensity of red colour ($p < .05$) and brightness ($p < .05$) of the gastric mucosa. Exp. 2 measured digestibility of nutrients, nitrogen (N) balance and energy utilisation of a C diet compared with low protein diets containing 0 or 0.53% of CT supplement (low protein, LP and low protein plus tannins, LPT, respectively). Eighteen barrows (BW 153 ± 4 kg) were housed in 6 metabolic cages in 3 periods of 14 days, with 6 animals per diet. LPT pigs produced less urine than LP (2132 vs. 2561 g/d, $p < .05$) and both were lower than C (2978 g/d, $p < .05$). LP and LPT diets had similar digestibility (87.6 vs. 87.8% for DM, and 85.1 vs. 83.9% for CP). N and energy balance were similar among all diets (34.8, 39.0, 39.2% retained N, and 37.6, 35.7, 37.3% retained energy, for C, LP and LPT, respectively). In conclusion, tannins do not exert anti-nutritional effect at the concentrations applied.

HIGHLIGHTS

- Dietary chestnut tannin (CT) addition (at 1.5–5.3 g/kg) does not modify digestibility, metabolic use of nutrients and performance of heavy pigs.
- Dietary CT addition reduces urine volumes of animals with potential impact on farm slurry management.
- Changes of colour and hypertrophy of gastric mucosa of pigs fed CT are proxy indicators of metabolic reactions to be further investigated.

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Introduction

Tannins are a complex category of secondary plant compounds, classified as hydrolysable or condensed, bioactive in the digestive tract, having antimicrobial effects (Scalbert 1991) and binding properties with protein substances. Recently, tannins fed to pigs were also studied as compounds able to reduce boar taint (Čandek-Potokar et al. 2015; Bee et al. 2017). Pigs from extensive open pasture systems in the Mediterranean area consume high amounts of tannins contained in the acorns from *Quercus* species. Acorns are rich in hydrolysable tannins that stimulate the pigs to secrete,

through the saliva and the gastric wall, rich proline proteins (RPP); these proteins have a high affinity for tannins and act as protection against the negative effects of these plant compounds (Cappai et al. 2010, 2013; Cappai, Wolf, Pinna, et al. 2014; Cappai, Wolf, Dimauro, et al. 2014).

Hydrolysable tannins are also extracted from chestnut wood (*Castanea sativa* L). Chestnut tannins (CT) fed to pigs reduced the apparent digestibility, but did not determine negative effects in terms of N retention (Antongiovanni et al. 2007) or growing performance (Prevolnik et al. 2012; Bee et al. 2017). Our hypothesis

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is that CT could induce a physiological reaction in pigs like that observed for acorns, with a recycling of proteins of low biological value (e.g. RPP) in the gut and a reduction of the urinary N losses. A shift of N excretion from urine to faeces reduces the energetic cost of excretion and is important for the environmental sustainability, given the low ammonia volatilisation intensity from faecal N in comparison with urine N (Galassi et al. 2010).

Therefore, in this paper we present two trials aimed at evaluating the effects of CT addition to diets for Italian heavy pigs. A first feeding trial (Exp.1) tested the performance and slaughter traits, and some biological indicators (e.g. stomach and parotid measures) of the reaction of animals to tannin feeding, while a second metabolic trial (Exp.2) aimed at evaluating the potential benefit of the CT addition in low protein diets added with essential amino acids. These diets have been chosen because of the increasing attention for rations environmentally friendly, and to study the effect of tannins on diets added with free essential amino acids.

Material and methods

Two *in vivo* experiments were performed at the University of Udine (Italy), Department of Agricultural, Food, Environmental and Animal Sciences (Exp. 1) and at the University of Milan (Italy), Department of Agricultural and Environmental Sciences (Exp. 2). In both experiments, the Gruppo Mauro Saviola Srl (Radicefani, Siena, Italy) provided the SaviotanFeed supplement, extracted from chestnut wood, which was used in compound feed formulations as a source of CT (750 g of tannic acid equivalent/kg DM). The chemical composition and gas chromatographic profile of the CT is available by Campo et al. (2012).

Feeding trial and slaughter traits (Exp. 1)

Forty-two Italian Large White × Italian Duroc barrows (91 ± 6 kg of BW and ~5 months of age) were transferred into the experimental farm, where they were divided into pairs and kept in 21 partially-slatted pens (1.2 × 3 m) equipped with individual feeding and free access to water. After arrival, all animals were fed a commercial compound feed for 5 d, containing cereal meals, wheat bran and soybean meal (80:8:9) and supplemented with antibiotics to prevent intestinal disease (575 and 200 mg of amoxicillin and colistin/kg of compound feed, respectively). In the subsequent 9 d the pigs were progressively adapted to the three

experimental rations: one group of 14 animals (7 pens) received the control compound feed (C, group) and the other two groups (7 pens for each group) received the experimental pelleted compound feed containing the commercial product SaviotanFeed (having 75% of tannins) at the proportion of 0.15 or 0.30% (T15 and T30 groups, respectively, Table 1). The pigs were weighed at the end of the second week (beginning of the trial), and consecutively every two weeks and the day before the slaughter. The individual daily amount of each experimental compound feed was prepared every day in 2 equal meals (at 09:00 am and 05:00 pm) and the pigs were fed at restricted level (daily DMI from about 8.0–6.2% BW^{0.75} during the whole growth from 90 to 170 kg BW). No feed refusals were recorded, and samples of the experimental compound feeds were collected and analysed every 4 weeks during the growing period.

The animals were slaughtered at an average BW of 174 ± 6 kg by electrical stunning and were exsanguinated, scalded at 65 °C, skinned, eviscerated, and split at the centre of the vertebral column according to the standard slaughtering procedures. The whole carcasses and some main cuts (hams, loins and backfat) were weighted before cooling. Backfat thickness was measured before cooling at 8 cm aside from the central line of the carcass between the third and the fourth last rib by a calliper. At slaughtering, the whole stomach

Table 1. Composition of the experimental diets.

| Diets ^a | Exp. 1 | | | Exp. 2 | | |
|--------------------------|--------|-------|-------|--------|-------|-------|
| | C | T15 | T30 | C | LP | LPT |
| Ingredient (g/kg) | | | | | | |
| Corn meal | 550.0 | 550.0 | 550.0 | 496.7 | 544.2 | 538.9 |
| Barley meal | 230.0 | 230.0 | 230.0 | 123.0 | 123.0 | 123.0 |
| Wheat meal | – | – | – | 101.0 | 101.0 | 101.0 |
| Wheat bran | 80.0 | 78.5 | 77.0 | 79.2 | 79.2 | 79.2 |
| Soya bean meal extracted | 90.0 | 90.0 | 90.0 | 79.2 | 29.7 | 29.7 |
| Wheat middlings | – | – | – | 59.4 | 59.4 | 59.4 |
| Cane molasses | – | – | – | 36.1 | 36.1 | 36.1 |
| SaviotanFeed | – | 1.5 | 3.0 | – | – | 5.3 |
| CaCO ₃ | 12.0 | 12.0 | 12.0 | 9.9 | 9.9 | 9.9 |
| NaHCO ₃ | – | – | – | 2.5 | 2.5 | 2.5 |
| NaCl | 4.0 | 4.0 | 4.0 | 2.0 | 2.0 | 2.0 |
| CaHPO ₄ | 8.0 | 8.0 | 8.0 | 2.0 | 2.0 | 2.0 |
| Supplement ^b | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Lignosulphate | – | – | – | 3.0 | 3.0 | 3.0 |
| Emulsifier | – | – | – | 0.5 | 0.5 | 0.5 |
| L-Lysine HCL | 1.0 | 1.0 | 1.0 | 0.5 | 2.0 | 2.0 |
| L-Threonine | – | – | – | 0.0 | 0.5 | 0.5 |

^aDiets: C: control, T15 = SaviotanFeed 1.5 g/kg, T30 = SaviotanFeed 3.0 g/kg, LP: low protein, LPT: low protein plus tannins (5.3 g/kg SaviotanFeed).

^bSupplied per kilogram DM of complete diet: vitamin A 10,000 U; vitamin D₃ 1000 U; vitamin E 7.5 mg; vitamin K₃ 2.5 mg; vitamin B₁ 1 mg; vitamin B₂ 2 mg; vitamin B₃ 15 mg; vitamin B₆ 1.5 mg; B₁₂ 0.01 mg; pantothenic acid 7.5 mg; folic acid 0.25 mg; biotin 0.1 mg; choline chloride 375 mg; Fe 150 mg from FeCO₃; I 0.5 mg from Ca(IO₃)₂; Mn 50 mg from MnO₂; Cu 10 mg from CuSO₄·5H₂O; Zn 110 mg from ZnSO₄·H₂O; Se 0.1 mg from Na₂SeO₃; Mo 0.5 mg from Na₂MoO₄.

and a sample of the parotid gland were collected from each pig. The stomachs were stored at -20°C and after thawing organs were opened (Mason et al. 2013) along the greater curvature (curvature ventricles major), emptied, gently rinsed, weighed and orthogonal photos of the outstretched stomach were taken by stand. The internal mucosa of the stomachs was examined for the presence of lesions referred to gastritis, which were classified giving them a score of intensity from 1 (mild lesion) to 5 (severe gastritis) and gathered into four categories (Marcato 2002): (1) hyperplastic: mucosa thickened and covered with mucus; (2) follicular: mucosa with lymphatic follicles swollen and grey-whitish colour; (3) atrophic: mucosa having a flattened epithelium, with the reduction or disappearance of the folds and with increased connective tissue; (4) simple: superficial inflammation of the lamina propria, which can be replaced by connective tissue with no presence of lymphoid follicles.

Later, the pictures were used to measure the internal surface area of the stomachs by using a dedicated open source software (ImageJ 1.48, source freely available. Developed by Rasband W., National Institute of Mental Health, Bethesda, MD, USA), and the internal surface colour of the stomachs, according to the additive model RGB, which measures the intensity of red, green and blue colours of each pixel by giving an intensity value ranging from 0 to a maximum of 255 (highest intense colour).

The left parotid gland of each pig was surgically removed from the carcass and cleaned from the fat, lymph nodes and associated vessels.

Metabolic trial (Exp. 2)

Three experimental pelleted diets (Table 1) were fed to pigs: a control diet (C), containing cereal meals (corn, barley and wheat), soy bean meal, wheat bran, wheat middlings, cane molasses, minerals and supplements, and two low protein diets: without (low protein, LP) and with (low protein plus tannins, LPT) tannins, containing five percentage points less of soy bean meal in comparison to the C diet. The LPT diet contained 5.3 g/kg of SaviotanFeed to have 4 g/kg of tannic acid equivalent. The C diet was formulated to represent a typical diet given to finishing heavy pigs in the commercial Italian pig farms.

The low protein diets were formulated to have the same standardised ileal digestible (SID) Lys content of the C diet and all diets were formulated to have a sufficient content of the essential amino acids in accordance with what is recommended by NRC (2012) for

Table 2. Nutrient content (g/kg DM, unless otherwise indicated) of the experimental diets.

| Diet ^a | Exp. 2 | | | |
|--|--------|--------|--------|--------|
| | Exp. 1 | C | LP | LPT |
| Analysed nutrient composition | | | | |
| DM, g/kg as fed | 880.00 | 866.00 | 865.00 | 866.00 |
| CP (N \times 6.25) | 137.00 | 142.00 | 122.00 | 123.00 |
| aNDFom ^b | 138.00 | 112.00 | 112.00 | 113.00 |
| ADFom ^c | – | 30.60 | 30.20 | 30.70 |
| EE ^d | 27.00 | 33.20 | 38.30 | 37.30 |
| Ash | 50.00 | 53.40 | 51.00 | 50.90 |
| Starch | – | 533.00 | 563.00 | 559.00 |
| GE, MJ/kg DM | 17.89 | 18.31 | 18.15 | 18.10 |
| ME ^e , MJ/kg DM | – | 15.59 | 15.49 | 15.48 |
| NE ^f , MJ/kg DM | – | 10.83 | 10.32 | 10.57 |
| Calculated SID amino acid content ^g | | | | |
| Lysine | 5.50 | 5.30 | 5.30 | 5.30 |
| Methionine | 1.90 | 2.10 | 1.80 | 1.80 |
| Threonine | 3.70 | 3.80 | 3.60 | 3.60 |
| Tryptophan | 1.10 | 1.20 | 0.90 | 0.90 |

^aDM: dry matter; CP: crude protein; GE: gross energy; Diets: C: control; LP: low protein; LPT: low protein plus tannins.

^baNDFom: neutral detergent fibre assayed with amylase and expressed exclusive of residual ash.

^cADFom: acid detergent fibre expressed exclusive of residual ash.

^dEE: ether extract.

^eME: Metabolisable energy, determined by respiratory chambers.

^fNE: Net energy, determined by respiratory chambers, assuming a requirement of 261 kJ/kg BW^{0.75} for maintenance (Noblet et al. 1993).

^gSID: Standardised ileal digestible according to NRC (2012).

animals weighing 135 kg, the heaviest fattening animals considered by the NRC system.

Eighteen barrows (Italian Landrace \times Italian Large White), chosen for size homogeneity and having the same father, were used. The pigs were randomly housed in 6 pens (3 \times 3 m), with 3 animals per pen, and 2 pens per feeding treatment. After 18 d from arrival, to determine total tract apparent digestibility, N and energy balance, one pig per each pen was moved to the individual metabolic cage, in 3 consecutive periods. Each period lasted 14 d: 7 d of adaptation and 7 d of separate collection of excreta (test period). Consequently, all animals were tested. Between the beginning of the first test period and the end of the third, the average weight of the 18 pigs was 153 kg (\pm 4).

During each 7 d testing period the animals in the metabolic cages were placed individually in an open-circuit respiration chamber (described by Crovetto 1984) to measure respiratory exchanges over three 24 h cycles.

Heat production (HP) for each animal was calculated from Brouwer's equation (Brouwer 1965):

$$\text{HP(kJ/d)} = (16.175 \text{ O}_2) + (5.021 \text{ CO}_2) - (2.167 \text{ CH}_4) - (5.987 \text{ N})$$

where O₂, CO₂ and CH₄ are the volumes (l/d) of the gases at standard temperature (0°C) and pressure

(760 mm Hg) conditions, consumed or produced during respiration, and N is the urinary nitrogen (g/d).

Digestibility trials were conducted according to the indications provided by the Italian Animal Science and Production Association (ASPA 1982). Feeding was always restricted, as usually done in practice for the production of the Italian heavy pig. The average fed dry matter was 6.7% of the $BW^{0.75}$ of the animals, with feed intakes like those of the animals in the piggery. Animals were fed at 08:00 am and 05:00 pm each day, and had free access to water.

During each collection period, a sample of each diet was taken every day in order to obtain a pooled sample for chemical analysis. No feed residue was found in the troughs. Every day, during the collection periods, faeces and urine from each animal were collected, separately weighed, sampled on weight basis (20% for faeces, 10% for the urine), pooled for each animal, and used for analysis. To keep the urine pH below 2.5 and avoid ammonia loss, 150 ml of a 20% v/v H_2SO_4 solution were added daily to each animal's urine collection vessel.

Chemical and statistical analysis (Exp. 1 and 2)

Urine, faeces and parotid gland tissues were frozen ($-20^{\circ}C$) for the subsequent chemical analyses. After thawing, a fresh sample of faeces and parotid gland tissue was directly used for the N analysis, while the remaining samples were dried in a forced air oven (72 h at $60^{\circ}C$). All dry samples were milled through 1 mm screen (Pulverisette, Fritsch, Idar-Oberstein Germany).

Samples of experimental diets (Exp.1 and 2) and faecal samples (Exp. 2) were assayed in two replications for residual DM, CP, ether extract (EE) and total ash content (methods 930.15, 976.05, 954.02 and 942.05, AOAC 2000, respectively). Samples of parotid glands (Exp. 1) were assayed in two replications for residual DM. The aNDFom content of experimental diets (Exp. 1 and 2) was measured by a fibre analyser (Ankom II Fiber Analyzer, Ankom Technology Corporation, Fairport, NY) following the procedure of Mertens (2002).

Samples of diets of Exp. 2 were also analysed for the starch content using the Megazyme kit K-TSTA (Megazyme International Ireland Ltd., Wicklow, Ireland) for total starch assay procedure, according to the method 996.11 (AOAC 2000). Gross energy of feeds, faeces and urine was measured using an adiabatic bomb calorimeter (IKA 4000, Staufen, Germany).

All the data were statistically analysed by GLM procedure of SAS statistical package (SAS, 2012). The data from Exp. 1 were analysed as one-factor design (e.g. dietary treatment) by using the pen as the experimental unit. Data from Exp. 2 were initially analysed by a model which included dietary treatment and period, and their interaction. Later on, the effect of the period and the interaction were excluded from the model since resulted not significant. For all analysis, a comparison of the treatment means was conducted using the Student-Newman-Keuls multiple range test (SNK option in the MEANS statement).

Significance was declared at $p < .05$ and trend at $p < .10$.

Results and discussion

Diets composition (Exp. 1 and 2)

The diet compositions (Table 1) were similar in both experiments, because they were based on common ingredients and on a large presence of cereal kernel meals (750–800 g/kg). Differences concerned the use in Exp. 2 of wheat meal, by-products in partial substitution of barley (wheat middling and cane molasses), sodium bicarbonate and lower levels of some salts (sodium chloride and dicalcium phosphate). Overall, the chemical composition of diets was very similar in the two experiments (Table 2) in terms of NDF, ash and EE (137 vs. 112, 60 vs. 51–53 and 29 vs. 33–38 g/kg DM, respectively) and the CP content of diets of Exp.1 was similar to that of the control diet of Exp. 2 (142 vs. 139 g/kg DM).

In Exp. 1 the only difference between diets concerned the inclusion of the additive (0.15 or 0.30%) and this did not modify the gross chemical composition. In Exp. 2, due to the replacement of part of the soy bean meal with maize meal, in comparison with the C diet the two LP diets resulted to have 14% less protein content (142 vs. 122–123 g/kg DM) and higher starch and lipid contents. The energy content (in terms of GE, ME and NE) determined for the three diets was similar.

In vivo performance, slaughter traits and measures on stomach and parotid glands (Exp. 1)

The feeding trial covered the last part of the fattening phase of the Italian heavy pigs. The reason to focus on this last part of the growth of animals was because the previous papers already considered the effects of CT in a first part of growing of animals (Štukelj et al. 2010; Prevolnik et al. 2012; Bee et al. 2017).

Table 3. Effect of dietary addition of SaviotanFeed on growth performance, slaughter and stomach traits and parotid gland composition of finishing heavy pigs (Exp. 1).

| Item | Diet ^a | | | SEM | <i>p</i> |
|---|----------------------|----------------------|----------------------|---------|----------|
| | C | T15 | T30 | | |
| Initial live weight, kg | 90.100 | 89.300 | 93.000 | 4.500 | .293 |
| Final live weight, kg | 175.100 | 172.800 | 174.500 | 7.110 | .825 |
| Daily DM intake, g/(kg PV ^{0.75} .d) | 70.300 | 70.900 | 69.800 | 2.470 | .675 |
| Daily gain, g | 765.000 | 752.000 | 734.000 | 39.100 | .345 |
| Slaughter traits | | | | | |
| carcass weight, kg | 140.800 | 139.900 | 141.800 | 5.800 | .826 |
| carcass dressing, % | 80.400 | 80.900 | 81.300 | 0.780 | .123 |
| back fat thickness, mm | 29.200 | 28.500 | 28.700 | 2.570 | .864 |
| hams weight, kg | 35.400 | 35.000 | 35.300 | 1.460 | .898 |
| loins weight, kg | 14.100 | 14.000 | 14.100 | 0.820 | .970 |
| backfat weight, kg | 10.800 | 11.100 | 10.400 | 1.340 | .692 |
| Stomach dimension | | | | | |
| empty weight, g | 692.000 | 659.000 | 726.000 | 63.000 | .162 |
| internal area, cm ² | 1168.000 | 1077.000 | 1135.000 | 106.000 | .295 |
| Stomach internal colour ^b | | | | | |
| red | 194.000 ^d | 188.000 ^e | 188.000 ^e | 3.590 | .004 |
| green | 119.000 | 111.000 | 110.000 | 7.420 | .058 |
| blue | 79.000 | 74.000 | 74.000 | 6.360 | .198 |
| brightness | 137.000 ^d | 130.000 ^e | 129.000 ^e | 5.330 | .019 |
| Stomach gastritis ^c | | | | | |
| hyperplastic | 2.570 | 4.140 | 4.140 | 1.310 | .058 |
| follicular | 1.140 | 0.860 | 1.210 | 1.020 | .788 |
| atrophic | 0.070 | 0.000 | 0.000 | 0.110 | .387 |
| simple | 0.210 | 0.000 | 0.000 | 0.230 | .154 |
| Parotid gland composition | | | | | |
| dry matter | 36.000 | 35.800 | 35.300 | 2.770 | .890 |
| crude protein, % DM | 52.500 | 52.500 | 53.300 | 5.990 | .960 |

^aDiets: C: control, T15 = SaviotanFeed 1.5 g/kg and T30 = SaviotanFeed 3.0 g/kg.

^bIntensity value ranging from 0 to a maximum of 255.

^cScore of intensity from 1 (mild lesion) to 5 (severe gastritis).

^{d,e}Within rows, means without a common superscript differ ($p < .05$).

The animals showed a satisfactory daily gain (Table 3), like that reported in our previous trials with heavy pigs in the same stage of fattening (Zanfi et al. 2014) and the inclusion of the additive did not modify the *in vivo* animal performance. Also, at slaughter animals of different experimental groups showed similar carcass dressing, backfat thickness and weights of the main cuts (hams, loins and back fat).

The additive had no effects on stomach size (e.g. empty weight and internal surface area), while there were significant differences in terms of measurable intensity of colour in the internal stomach mucosa (ranging from 0 to 255, minimum and maximum intensity colour, respectively).

At both inclusion levels, CT significantly reduced the intensity of red (from 194 to 188, $p < .05$) and tended to decrease the intensity of green (from 119 to 111–110, $p = .058$). Overall, also the brightness was reduced (from 137 to 129–130, $p < .05$).

Cappai et al. (2013) investigated the morphology and histology of gastric mucosa in pigs fed acorns and described a discolouration of the internal gastric mucosa in animals fed diets containing tannins, as in

Table 4. Live weight, DM intake, excretions and apparent digestibility of experimental diets administered to 18 finishing heavy pigs (6 animals per diet) in Exp. 2.

| Item | Diet ^a | | | SEM | <i>p</i> |
|-------------------------|-----------------------|-----------------------|-----------------------|---------|----------|
| | C | LP | LPT | | |
| Average weight, kg (kg) | 152.900 | 152.600 | 153.700 | 1.050 | .761 |
| DM intake, g/d | 2950.000 | 2951.000 | 2966.000 | 10.300 | .477 |
| Faecal DM, g/d | 361.000 | 365.000 | 361.000 | 17.400 | .983 |
| Urine, g/d | 2978.000 ^e | 2561.000 ^f | 2132.000 ^g | 126.000 | .004 |
| Digestibility, % | | | | | |
| DM | 87.700 | 87.600 | 87.800 | 0.630 | .970 |
| OM | 89.900 | 89.800 | 89.900 | 0.530 | .979 |
| CP | 86.500 | 85.100 | 83.900 | 0.820 | .116 |
| EE ^b | 72.600 | 76.300 | 76.900 | 1.320 | .076 |
| aNDFom ^c | 56.000 | 53.900 | 57.500 | 2.510 | .610 |
| ADFom ^d | 36.500 | 33.000 | 32.500 | 4.220 | .774 |
| Ash | 47.600 | 47.100 | 48.200 | 2.650 | .964 |
| Gross energy | 88.400 | 88.100 | 88.300 | 0.580 | .942 |

^aDM: dry matter; OM: organic matter; CP: crude protein; Diets: C: control; LP: low protein; LPT: low protein plus tannins.

^bEE: ether extract.

^caNDFom: neutral detergent fibre assayed with amylase and expressed exclusive of residual ash.

^dADFom: acid detergent fibre expressed exclusive of residual ash.

^{e,f,g}Within rows, means without a common superscript differ ($p < .05$).

present work. Those authors associated the variation of the colour to secretion of mucin, rich in proline from the cardiac mucosa glands. In accordance with such finding, in pigs fed tannins we detected a tendency ($p = .058$) for an increase of hyperplastic gastritis, which is described by Marcato (2002) as mucosa thickened and covered with mucus.

Cappai et al. (2010) found a significant increase of the CP content of parotid glands in young growing pigs fed the acorns, which was associated to a greater dimension of glands and to a higher proline content. In contrast, in our trial, we were not able to find a difference in CP contents of parotid glands due to dietary tannin addition and this could be due to the lower tannin content of our diets. Moreover, our measures were done on mature animals and Cappai, Wolf, Dimauro, et al. (2014) demonstrated that the hypertrophy of the parotid glands due to tannin feeding is age-dependent, with a decreasing effect according to the increase of pig age.

Diets, ingesta and excreta, and digestibility (Exp. 2)

Feed intake (Table 4) was similar for the three diets (on average 2956 g DM/head daily) and the same holds true for the excretion of faeces (on average 362 g DM/head daily).

Throughout the whole experiment, we clearly observed a high production of saliva by the pigs fed tannins, although, unfortunately, we could not measure the saliva production. According to previous

Table 5. Effects of the experimental diets on N and energy balance of finishing heavy pigs (6 animals per diet) in Exp. 2.

| Item | Diet ^a | | | SEM | <i>p</i> |
|---------------------------|---------------------|----------------------|---------------------|-------|----------|
| | C | LP | LPT | | |
| N Intake (NI), g/d | 67.100 ^b | 57.600 ^c | 58.500 ^c | 0.530 | <.001 |
| Faecal N | | | | | |
| g/d | 9.100 | 8.600 | 9.400 | 0.490 | .463 |
| % NI | 13.500 | 14.900 | 16.100 | 0.820 | .116 |
| Urinary N | | | | | |
| g/d | 34.600 ^b | 26.500 ^c | 26.100 ^c | 1.180 | <.001 |
| % NI | 51.600 ^b | 46.100 ^{bc} | 44.700 ^c | 1.840 | .042 |
| Excreted N | | | | | |
| g/d | 43.700 ^b | 35.100 ^c | 35.500 ^c | 1.180 | <.001 |
| % NI | 65.100 | 61.000 | 60.800 | 1.930 | .227 |
| Retained N | | | | | |
| g/d | 23.400 | 22.500 | 23.000 | 1.270 | .899 |
| % NI | 34.800 | 39.000 | 39.200 | 1.930 | .227 |
| Energy intake (EI), MJ/d | 54.000 | 53.600 | 53.700 | 0.463 | .777 |
| Energy in faeces | | | | | |
| MJ/d | 6.490 | 6.360 | 6.270 | 0.292 | .966 |
| % EI | 11.600 | 11.900 | 11.700 | 0.583 | .942 |
| Energy in urine | | | | | |
| MJ/d | 1.480 ^b | 1.260 ^c | 1.290 ^c | 0.045 | .009 |
| % EI | 2.730 ^b | 2.360 ^c | 2.400 ^c | 0.079 | .008 |
| Energy in CH ₄ | | | | | |
| MJ/d | 0.270 | 0.260 | 0.270 | 0.038 | .988 |
| % EI | 0.500 | 0.490 | 0.500 | 0.069 | .989 |
| Energy metabolised | | | | | |
| MJ/d | 46.000 | 45.700 | 45.900 | 0.609 | .925 |
| % EI | 85.100 | 85.300 | 85.400 | 0.614 | .944 |
| Heat production | | | | | |
| MJ/d | 25.700 | 26.500 | 25.800 | 0.558 | .529 |
| % EI | 47.500 | 49.600 | 48.100 | 0.975 | .341 |
| Energy retained | | | | | |
| MJ/d | 20.300 | 19.100 | 20.000 | 0.734 | .502 |
| % EI | 37.600 | 35.700 | 37.300 | 1.251 | .518 |

^aDiets: C: control; LP: low protein; LPT: low protein plus tannins.

^{b,c}Within rows, means without a common superscript differ ($p < .05$).

research (Albar and Granier 1996; Carpenter et al. 2004) urine production was lower ($p < .05$) for LP and LPT diets in comparison with C, with the lowest value for the LPT diet, significantly lower also in comparison with LP. The lower urine production of the LPT fed pigs in comparison with the LP fed ones, is probably correlated to the high production of saliva by the pigs fed tannins. A high rate of salivation in presence of tannins was found also by Cappai et al. (2010, 2013) and Cappai, Wolf, Pinna, et al. (2014) in diets rich in tannins provided by acorns.

Tannins stimulate the secretion, through the saliva and the gastric wall, of rich proline proteins (RPP) which have a small size, an open loose structure and a high affinity for tannins. As a result, the RPP secreted in the gut neutralise the tannins and increase the endogenous N losses in the faeces. Diets rich in tannins, therefore, should increase proline excretion also and consequently decrease proline digestibility. This is confirmed by Mariscal-Landin et al. (2004), where in a work with sorghum-based found that 'proline was the only amino acid in which coefficient of apparent ileal digestibility decreased as tannin levels increased'.

From Table 4 it appears that digestibility is similar among the three diets. For protein digestibility, which is normally reported in the literature to decrease with tannins (Antongiovanni et al. 2007), we registered only no significant decrease with LPT diet ($p = .116$). However, it must be underlined that Mariscal-Landin et al. (2004) showed a clear reduction in apparent ileal digestibility only with tannins used in concentration much higher (47–57 g/kg DM) than those adopted in this study (4.6 g/kg DM).

Nitrogen and energy balances (Exp. 2)

As expected from literature (Zervas and Zijlstra 2002a,b; Galassi et al. 2015), low protein diets determined a significant reduction in N intake and N excretion, and a partial shift in the site of N excretion from urine to faeces (Table 5). The daily faecal N excretion was similar for the 3 diets while urine N production was lower ($p < .05$) for LP and LPT diets in comparison with C. This fact is important for manure pollution, given the lower ammonia volatilisation intensity from faecal N (Galassi et al. 2010). Considering the LPT diet, tannins addition did not change the pattern of N utilisation by pigs.

Altogether, LP and LPT diets achieved a N retention similar to that of the C diet. This confirms the convenience of reducing the protein content of the diet, if the required amounts of the essential amino acids are supplied.

The energy balance data (Table 5) were not statistically different between the diets, except for a lower urinary loss for the two low protein diets in comparison with the C diet. The data obtained reveal a high energy digestibility for all the diets, including that added with tannins. Energy losses as urine, methane and heat are also similar among the dietary treatments and consistent with those of previous experiments (Galassi et al. 2005; Galassi et al. 2015).

The results of the N and energy balances indicate that tannins, at the level used in this experiment, did not reduce N and energy retention: 39.0 vs. 39.2% of N intake, and 35.7 vs. 37.3% of energy intake for LP and LPT diets, respectively.

Conclusions

Chestnut tannins did not modify pig performance (growth and slaughter traits) and metabolic N and energy utilisation. Hence, CT cannot be considered anti-nutritional factors when included at the levels used in the two experiments.

A clear reduction of the urine yield was registered for the animals fed tannins, whilst some reliefs on gastric mucosa (colour and hypertrophy) indicate that tannins increase the stomach mucus secretion. The above-mentioned physiological effects are indicators of a metabolic reaction of pigs to CT feeding, although higher CT dosages would be required to have an impact on pig performances.

Animal ethics

Animals of both trials were cared for in accordance with the Italian laws on the protection of animals used for scientific purposes (Exp. 1 legislative decree 116/1992, prot. 777 20/12/2013; Exp. 2 legislative decree 26/2014, n. 749 22/07/2015).

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