The Veterinary Journal 204 (2015) 162–167

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Hydrolysable tannin fed to entire male pigs affects intestinal production, tissue deposition and hepatic clearance of skatole

M. Čandek-Potokar [a,b], M. Škrlep [a,*], N. Batorek Lukač [a], G. Zamaratskaia [c], M. Prevolnik Povše [b], Š. Velikonja Bolta [a], V. Kubale [d], G. Bee [e]

[a] Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia
[b] Faculty of Agriculture and Life Sciences, University of Maribor, Pivola 10, 2311 Hoče, Slovenia
[c] Uppsala BioCenter, Department of Food Science, Swedish University of Agricultural Sciences, P.O. Box 7051, SE-750 07 Uppsala, Sweden
[d] Veterinary Faculty, University of Ljubljana, Gerbičeva ulica 60, 1000 Ljubljana, Slovenia
[e] Agroscope Institute for Livestock Sciences, Route de la Tioleyre 4, 1725 Posieux, Switzerland

ARTICLE INFO

Article history:
Accepted 13 February 2015

Keywords:
Pig
Androstenone
Cytochrome P450
Skatole
Tannins

ABSTRACT

The effect of adding hydrolysable tannins to the diet of fattening boars was studied. Performance, reproductive organ weights, salivary gland morphology, boar taint compounds and skatole metabolism were evaluated. At 123 days of age and 52 ± 6 kg liveweight, 24 Landrace × Large White boars were assigned within a litter to four treatment groups: control (T0 fed mixture with 13.2 MJ/kg, 17.5% crude proteins) and three experimental diets for which the T0 diet was supplemented with 1%, 2% and 3% of hydrolysable tannin-rich extract (T1, T2 and T3, respectively). Pigs were kept individually with ad libitum access to feed and water and slaughtered at 193 days of age and 122 ± 10 kg liveweight.

Adding hydrolysable tannins to the diet had no negative effect on growth performance at 1% and 2%, whereas the 3% inclusion reduced feed intake and resulted in an adaptive response of the salivary glands (particularly parotid gland hypertrophy). Relative to T0, fat tissue skatole concentration was increased in the T1 group, but was similar in T2 and T3. Across treatments tissue skatole concentrations were proportional to the activity of hepatic CYP450. The results indicate the potential of tannin supplementation to reduce boar taint although further investigations are needed in order to establishing optimal dosage.

Introduction

Reduction of boar taint in intact boars could be achieved by decreasing the accumulation in fat tissue of its two main components, namely, androstenone and skatole. Androstenone is a steroid produced by the Leydig cells of the testis (Gower, 1972) and is largely under genetic influence (Robic et al., 2008). There is no clear relationship between nutrition and androstenone, although altering growth intensity could indirectly influence it. A decreased concentration of androstenone in fat was observed in boars fed chicory root and was found to be related to higher activity of the androstenone-degrading enzyme 3β-hydroxysteroid dehydrogenase/Δ(5→4)isomerase (3βHSD) (Rasmussen et al., 2012).

The accumulation of the crystalline amine skatole in adipose tissues depends on the rate of microbial metabolism of tryptophan in the large intestine, its intestinal absorption and hepatic metabolism (Zamaratskaia and Squires, 2009). Skatole concentrations in fat can be altered by influencing microbial nitrogen metabolism or by changing intestinal contents, or the rate of passage and absorption (Jensen, 2006). Pigs fed chicory root or chicory inulin (Rideout et al., 2004; Jensen and Hansen, 2006; Aluwe et al., 2013), sugar beet pulp (Jensen et al., 1995; Knarreborg et al., 2002) or raw potato starch (Claus et al., 2003; Zamaratskaia et al., 2005) all exhibited decreased concentrations of skatole in intestinal contents, faeces, blood plasma and fat tissue.

Diets containing proteins with low pre-caecal digestibility stimulate skatole production (Jensen et al., 1995). Once it is absorbed from the intestine into the bloodstream, skatole is metabolised by the liver, mainly through two cytochrome P450 isoenzymes CYP2E1 and CYP2A, and aldehyde oxidase (Babol et al., 1998; Diaz and Squires, 2000a, 2000b). Hepatic metabolism of skatole is reduced by androstenone via its inhibiting effect on the CYP450 enzymes (Doran et al., 2002; Zamaratskaia et al., 2007; Chen et al., 2008). However, the effect of diet on hepatic skatole metabolism requires more clarification.

Tannins are secondary metabolites found in many plants (Serrano et al., 2009) and are dietary components that could potentially influence skatole production. They have high structural diversity with two key structural groups identified (Haslam, 2007): namely, oligomeric flavan-3-ols or protoanthocyanidins (condensed tannins) and poly-3,4,5-trihydroxyaroyl esters or gallotannins and ellagitannins

* Corresponding author. Tel.: +386 1 2805 234.
E-mail address: martin.skrlep@kis.si (M. Škrlep).

http://dx.doi.org/10.1016/j.tvjl.2015.02.012
1090-0233/

(hydrolysable tannins). Although tannins were initially considered purely anti-nutritive agents (Chung et al., 1998), it is now known that, due to their structural diversity, the effect of tannins can vary according to the type of tannin, amount fed and the animal species involved.

Some tannins, at appropriate concentrations in the intestines, are known to exert anthelmintic, antibacterial and antiviral effects and are used as a supportive treatment for diarrhoea (Goel et al., 2005; Mueller-Harvey, 2006). Tannins also exert an antioxidant (Frankič and Salobir, 2011) and hepatoprotective potential (Pithayanukul et al., 2009). Pigs fattened on acorns or chestnuts in traditional silvo-pastoral systems are known to consume tannin-rich feedstuffs, as they can quickly adapt to high-tannin diets by increasing salivary secretion (Cappai et al., 2010).

Tannins have been shown to affect gut microflora and protein digestibility (Chung et al., 1998; Goel et al., 2005), but data relating tannins to skatole production are scarce and absent for pigs. In ruminants, reduced concentrations of skatole in the plasma and milk of ewes (Roy et al., 2002) and reduced meat flavour in lambs (Priolo et al., 2009) have been reported after tannin supplementation. Recently, Fischer and Wüst (2012) demonstrated very low skatole concentrations in the subcutaneous fat of wild boars despite high androstenone concentrations, which the authors ascribed to the woodland diet and/or intestinal microbiota. It is therefore reasonable to assume that dietary tannins could affect the intestinal synthesis and hepatic clearance of skatole as well as its accumulation in the adipose tissue.

The objective of the present study was to evaluate the effect of adding hydrolysable tannins to the diet of entire male pigs on intestinal skatole formation, skatole degradation in the liver and deposition of skatole and androstenone in adipose tissue. Additionally, production traits, reproductive organ weights, and salivary gland morphology were monitored in order to test for potentially negative aspects of tannin ingestion.

Materials and methods

Animals and fattening trial

The experiment was carried out following the Slovenian law on animal protection (Zakon o zaščiti živali, 2007). The work was undertaken with full owner compliance and within the normal running of the farm. The study was not subject to ethical protocols according to Directive 2010/63/EU (2010)[1] i.e. approved food additives were used (European Union Register of Feed Additives, 2013)[2], and the intestinal content and tissues were sampled after slaughter.

At a mean age of 123 days, 24 crossbred (Large White × Landrace) entire male pigs (EM) were allocated within litters to four treatment groups, housed individually (1.3 × 2.1 m^2 pens with slatted walls) with ad libitum access to feed and water. The control group (T0) received a commercial feed mixture, while experimental groups T1, T2 and T3 were offered the same feed supplemented with 1%, 2% and 3% of chestnut wood extract Farmatan (Tanin Sevnica), respectively (Table 1). Farmatan is rich in hydrolysable tannins, mainly gallotannins (Biagi et al., 2010). The pigs were slaughtered at 193 days of age. In the experimental period individual feed intake was determined daily and bodyweight (BW) recorded weekly.

Slaughter and sampling

Feed was withdrawn 1 day before slaughter. Pigs were slaughtered in one batch in a commercial abattoir using CO_2 stunning. On the slaughter line, samples of liver, and the contents of the caecum, ascending and descending colon were collected and immediately frozen in liquid nitrogen. Testes, bulbourethral and salivary (parotid and mandibular) glands were dissected and weighed. Samples of subcutaneous fat were taken at the site of the last rib and frozen at –20 °C until analysis.

[1] See: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&from=EN (accessed 10 February 2015).

[2] See: http://ec.europa.eu/comm/food/food/animalnutrition/feedadditives/registeradditives_en.htm (accessed 10 February 2015).

Table 1
Ingredients (%) and chemical composition of experimental feed mixtures.

Ingredients[a]	T0	Diet composition	T0	T1	T2	T3
Maize	62.0	Farmatan, %	0.0	1.0	2.0	3.0
Soya meal	13.0	DM, g/kg	892.5	884.8	881.4	884.9
Wheat meal	8.0	Crude protein, g/kg DM	174.5	168.7	166.4	164.4
Rapeseed meal	7.0	Crude fat, g/kg DM	25.9	28.7	28.2	26.7
Sunflower meal	5.0	Crude fibre, g/kg DM	51.5	46.6	49.4	50.0
Molasses	2.0	Crude ash, g/kg DM	47.6	45.4	46.5	45.2
Calcium carbonate	1.1	ME, MJ/kg	13.2	13.3	13.1	13.2
Sodium chloride	0.6					
Lysine	1.0					
Methionine	0.3					
Monocalcium phosphate	0.17					

Farmatan, hydrolysable tannin-rich chestnut wood extract; DM, dry matter; ME, metabolisable energy.

[a] L-Lysine HCl and DL-methionine were added to the diet to reach a level in the composed diet of 1.0% and 0.3% of digestible lysine and methionine, respectively.

Chemical analyses of intestinal content

The pH of the intestinal content was determined after diluting 1 g of sample in 4 mL of water and vortexing for 20 s. Samples were centrifuged (3000 *g* for 15 min at 20 °C) and the pH was measured in supernatant using an MP120 pH meter (Mettler-Toledo).

Intestinal dry matter (DM) content was determined by drying 5 g of sample at 130 °C in a vacuum oven (SP-105C, Kambič) until a constant weight was reached; this was expressed as percentage of primary weight.

For the analysis of ammonia nitrogen, approximately 1 g of sample was weighed into a Kjeldahl digestion tube and alkalised with 2 g of magnesium sulphate. The tube was connected to a distillation unit (Kjeltec 2300 Nitrogen Analyser, Foss Tecator), to which 50 mL of deionised water was added prior to the start of the steam distillation. Boric acid (1%, m/v) containing a mixture of indicators (i.e., bromocresol and methyl red) was used as the collecting liquid for the liberated ammonia. The titration of ammonia was performed automatically using 0.15 M hydrochloric acid. Total nitrogen was determined according to ISO 5983-1 (2005) using Tecator 2020 Digestor apparatus (Foss Tecator) for the digestion phase, with the distillation and titration procedures being the same as described for ammonia nitrogen.

Skatole and indole concentrations of intestinal content were measured according to the modified method of Denhard et al. (1991). In brief, 5 g of sample was suspended in 20 mL of methanol and vortexed for 30 s. After centrifugation for 15 min at 2000 *g*, 4 mL of the supernatant was collected and 400 µL of 2-methylindole standard (0.15 mg/mL) added. Afterwards, 2 mL of the extract was combined with 4 mL of Tris buffer (0.5 M Tris, 0.05 M NaCl, pH 8.3) and the mixture cleaned on a Supelclean LC-18 SPE column (Supelco). The separation and quantification of skatole was performed using high performance liquid chromatography (HPLC; HP 1200, Agilent Technologies) equipped with a fluorescence detector (λ_{ex} = 285 nm, λ_{em} = 340 nm). The mobile phase was 10 mM phosphate buffer (pH 6.0)–methanol (55:45, v:v). The flow rate was 1.2 mL/min, injection volume 1 µL and the column temperature 35 °C. The column type used was Sunfire C18, 3.5 µm, 75 × 4.6 mm (Waters).

Analyses of androstenone and skatole in subcutaneous fat

The concentrations of androstenone and skatole in subcutaneous fat were measured by HPLC according to the procedure described by Batorek et al. (2012). Concentrations were expressed as µg/g of liquid fat; the detection limits were 0.03 µg/g for skatole and 0.24 µg/g for androstenone.

Catalytic activities of CYP1A1, CYP1A2, CYP2A19, CYP3A and CYP2E1

Hepatic microsomes were prepared using a calcium aggregation method (Rasmussen et al., 2011). Microsomal protein was determined using a commercially available kit (Bio-Rad Laboratories) according to the manufacturer's instructions. Catalytic activities of CYP1A1, CYP1A2, CYP2A19, CYP3A and CYP2E1 in hepatic microsomes were estimated using specific probe substrates. The activities of CYP1A1 and CYP1A2 were determined as the rates of 7-ethoxyresorufin O-deethylation and 7-methoxyresorufin O-demethylation, respectively, and the activities of CYP2A19 and CYP2E1 as the rates of coumarin and p-nitrophenol hydroxylation, respectively (Zamaratskaia et al., 2009). The activity of CYP3A was determined as the rate of 7-benzyloxyresorufin O-debenzylation (Zlabek and Zamaratskaia, 2012).

Incubation parameters for each isoform including linearity with incubation time and protein content, and optimal substrate concentrations were initially determined using pooled hepatic microsomes. The reactions were conducted at 37 °C and were started by the addition of 1 mM NADPH. All assays were performed in duplicate. The amount of formed metabolites was measured in duplicate on an HPLC system consisting of a pump (L-7100), autosampler (L-7200), fluorescence (L-7485) and