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Supplementing entire male pig diet with hydrolysable tannins: Effect on carcass traits, meat quality and oxidative stability

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ABSTRACT

The purpose of the present study was to investigate the potential impact on carcass and meat quality of a sweet chestnut wood extract (SCWE)diet supplement for pigs, in particular on oxidative stability and fatty acid composition. Entire (non-castrated) male pigs (n = 24) were assigned to treatment groups within litter and offered one of 4 finisher diets on an ad libitum basis: T0 (control), T1, T2 or T3, supplemented with 0, 1, 2 or 3% of commercially available SCWE, respectively. The highest SCWE supplementation reduced carcass fat deposition and water holding capacity of meat (higher thawing loss). In fresh meat, SCWE supplementation increased lipid (malondialdehyde) and protein oxidation (carbonyl groups in myofibril isolates). With regard to fat tissue, SCWE supplementation increased the proportion of polyunsaturated fatty acids.

1. Introduction

Tannins are secondary plant metabolites with great structural diversity (classified as hydrolysable, condensed or complex tannins) exerting different physiological effects according to their form, the amount ingested and the animal species involved (for detailed review see Mueller-Harvey, 2006). In certain amounts tannins are considered anti-nutritive substances because they precipitate proteins, inhibit digestive enzymes and affect the utilisation of vitamins and minerals (Chung, Wei, & Johnson, 1998). In addition they reduce feed palatability and consequently feed intake and animal performance (Jansman, 1993; Mueller-Harvey, 2006). On the other hand, certain tannins are, due to their antimicrobial properties, commonly fed to monogastric animals as antihelmintic, antimicrobial and antiviral agents, and as supportive treatment for diarrhoea (Mueller-Harvey, 2006; Redondo, Chacana, Dominguez, & Fernandez Miyakawa, 2014). Tannins may work as antioxidants to scavenge free radicals (Riedl, Carando, Alessio, McCarthy, & Hagerman, 2002). Sweet chestnut (*Castanea sativa* Mill.) wood extract (SCWE), consisting mainly of hydrolysable tannins, was shown to possess reducing and antioxidant capacity in in vitro trials (Lampire et al., 1998), was able to reduce in vivo oxidative stress in pigs and poultry (Frankič & Salobir, 2011;Voljč, Levart, Žgur, & Salobir, 2013) and increased oxidative stability of meat through preserving vitamin E (Voljč et al., 2013). In pigs, it was demonstrated that hydrolysable tannins, besides reducing total protein digestibility (Antongiovanni, Minieri, & Petacchi, 2007; Salobir, Kostanjevec, Štruklec, & Salobir, 2005), inhibit protein fermentation in the colon (Biagi, Cipollini, Paulicks, & Roth, 2010) and reduce intestinal production of skatole, a boar taint compound (Čandek-Potokar et al., 2015). Because of the possible effects on the digestion of protein and other nutrients, and due to its antioxidant properties, tannin supplementation is likely to affect carcass and meat quality. However, scientific literature on this topic is still lacking, especially in relation to pigs. Apart from the research dealing with pigs fed with acorns and chestnuts (García-Valverde, Nieto, Lachica, & Aguilera, 2007; Pugliese et al., 2009; Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2011), other literature data refers either to ruminants (Luciano et al., 2009, 2011; Vasta, Nudda, Cannas, Lanza, & Priolo, 2008), rabbits (Gai et al., 2009; Liu, Dong, Tong, & Zhang, 2011; Liu, Zhou, Tong, & Vaddella, 2012; Liu et al., 2009) or poultry (Schiavone et al., 2008). Moreover, so far the reported dosages have been low, and despite the fact that pigs (e.g. Iberian) can ingest relatively high levels of tannins, information about the effect of tannins (type and concentration) is lacking. Therefore, the present study was conducted to investigate the potential impact on carcass and meat quality of supplementing the diet of pigs with SCWE rich in hydrolysable tannins, considering also fatty acid composition and oxidative stability.

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Table 1
Chemical and fatty acid composition (% of total fatty acids) of feed mixtures.

	Treatment group[a]			
	T0	T1	T2	T3
Dry matter (g/kg)	892	885	881	885
Crude ash (g/kg)	48	45	46	45
Crude protein (g/kg)	175	169	166	164
Crude fat (g/kg)	26	29	28	27
Crude fibre (g/kg)	52	47	49	50
Nitrogen free extract (g/kg)	593	595	591	599
α-tocopherol (mg/kg)	67.3	70.4	71.4	69.6
γ-tocopherol (mg/kg)	20.9	21.3	21.7	21.9
Main fatty acid composition (% of the total fatty acids)[b]				
C 12:0	< 0.01	< 0.01	< 0.01	< 0.01
C 14:0	0.09	0.08	0.08	0.08
C 16:0	14.0	13.7	13.6	13.7
C 18:0	2.2	2.4	2.3	2.3
Σ C18:1	27.9	27.7	28.0	27.9
C 18:2 n − 6	51.2	50.9	51.1	51.0
C 18:3 n − 3	2.7	3.3	3.1	3.1
Σ SFA	17.4	17.2	17.1	17.2
Σ MUFA	28.7	28.5	28.7	28.6
Σ PUFA	53.9	54.2	54.2	54.1
Σ n − 3 PUFA	2.7	3.3	3.1	3.1
Σ n − 6 PUFA	51.3	50.9	51.1	51.0
Σ n − 6/Σ n − 3 PUFA	19.2	15.5	16.4	16.6

[a] The control group (T0) received feed without supplementation, while the experimental groups T1, T2 and T3 were offered the same diet supplemented with 1%, 2% and 3% of commercially available sweet chestnut wood extract, respectively. Diet T0 was composed of maize (62%), soya meat (13%), wheat meal (8%), rapeseed meal (7%), sunflower meal (5%), molasses (2%), $CaCO_3$ (1.1%), NaCl (0.6%), lysine (1%), methionine (0.3%), $Ca(H_2PO_4)_2$ (0.17%).

[b] Only predominant fatty acids are listed, but the sum of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are computed using all analysed fatty acids.

2. Materials and methods

2.1. Animals and diets

Experimental design, animals and feed composition are described in detail elsewhere (Čandek-Potokar et al., 2015). As explained therein, the work was undertaken with full owner compliance and within the normal running of the farm. The study was performed following the Slovenian law on animal protection (Zakon o zaščiti živali, 2007) and was not subject to ethical protocols according to Directive 2010/63/EU (2010), i.e. approved feed additives were used (European Union Register of Feed Additives, 2013) and tissue samples were taken after the slaughter. Briefly, 24 crossbred (Large White × Landrace) entire male pigs were allocated within litters to four treatment groups, housed individually with ad libitum access to feed and water. All the animals were fed a commercial feed mixture (Table 1) that was calculated to contain 13.2 MJ ME/kg and to meet requirements according to NRC (2012). The control group (T0) received feed without supplementation, while the experimental groups T1, T2 and T3 were offered the same feed supplemented with 1%, 2% and 3% of commercially available SCWE (Farmatan®, Tanin Sevnica d.d., Sevnica, Slovenia), respectively. Sweet chestnut wood extract is rich in hydrolysable tannins, mainly gallotannins (Bee et al., 2016; Biagi et al., 2010). The analysis of SCWE for total phenols – determined by the Folin-Ciocalteau colourimetric method (Rigo et al., 2000) and expressed as gallic acid equivalents – showed the content of 43.6%. Feed samples were taken at the beginning and at the end of the experiment, pooled and used for proximate analysis and determination of fatty acid composition. Proximate analysis (moisture, crude ash, crude protein, crude fat, crude fibre) of feed was performed according to standard procedures (AOAC, 2000): dry matter (in oven drying at 95–100 °C AOAC method 934.01), crude protein (the copper catalyst Kjeldahl method AOAC method 984.13), crude fat (AOAC method 920.39), crude fibre (fritted glass crucible method, AOAC method 978.10) and crude ash (AOAC method 942.05). Animals were fed experimental diets for 70 days, including a 5-day transitional period. At the age of 193 days and weight of 122.5 kg, the experimental pigs were slaughtered in one batch using routine abattoir procedure (CO_2 stunning, dehairing). Feed was withdrawn one day prior to slaughter.

2.2. Carcass and meat quality measurements

After the slaughter, pigs were eviscerated, leaf (i.e. subperitoneal) fat was removed, carcasses split apart, weighed and classified by the official classification body, using a method approved for Slovenia (OJ EU L56/28, 2008). The method consists of measuring minimal fat thickness over *gluteus medius* muscle (backfat thickness) and the shortest distance between cranial end of *gluteus medius* and dorsal edge of vertebral canal at the carcass split line with a digital caliper and enables calculation of lean meat content. Measurement of pH (pH 3) was taken in *longissimus lumborum* muscle (LL) at the level of last rib 3 h post mortem using a MP120 Mettler-Toledo pH meter (Mettler-Toledo GmbH, Schwarzenbach, Switzerland). The carcasses were cooled overnight at 0–2 °C until the internal temperature dropped below 7 °C. A day after the slaughter the carcasses were cut at the level of last rib perpendicularly to the spine. The measurements of CIE L*, a*, b* colour parameters (using Minolta Chroma Meter CR-300, Minolta Co. Ltd., Osaka, Japan) and ultimate pH were performed on a freshly cut surface of LL. A digital photo of the cross-section was taken and the measurements of loin eye area and area of the corresponding fat performed using LUCIA.NET 1.16.5 software (Laboratory Images s.r.o., Prague, Czech Republic) performed as described in Batorek et al. (2012). Caudally from last rib, two 2.5 cm thick chops of LL were taken, trimmed of epimysium and external fat and used for the determination of drip loss, chemical composition, thawing loss, cooking loss and shear force. Drip loss was determined according to the EZ method (Christensen, 2003). In short, two cylindrical samples of LL were excised, weighed and stored in plastic containers at 4 °C. Drip loss was expressed as the difference (%) from the initial sample weight after 24 and 48 h. For determination of chemical composition, LL samples were minced and the moisture, intramuscular fat (IMF) and protein content were determined using near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA) using internal calibration (Prevolnik et al., 2005). The second LL chop was weighed, vacuum packed and frozen at − 20 °C until analysis. Samples were thawed (overnight at 4 °C), gently drained with a paper towel, weighed and the difference in weights used for thawing loss calculation. The same samples were then cooked in a thermostatic bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72 °C, drained and reweighed for cooking loss evaluation, and cooled overnight at 4 °C. The next day, shear force was measured on two 2.5-cm-wide cylindrical cores excised from the sample using TA Plus texture analyzer (Ametek Lloyd Instruments Ltd., Bognor Regis, UK) equipped with a 60° V-shaped blade at a crossheaf speed of 3.3 mm/s.

2.3. Preparation of meat and subcutaneous fat samples for chemical analysis

Muscle and fat samples were trimmed of other tissues. Each muscle sample was divided into two parts: one was homogenised in liquid nitrogen, the other was transferred to 50-mL polypropylene containers with covers, cooked in a water bath (LCS 0081 Lauda, Bartelt GmbH, Graz, Austria) for 60 min at 90 °C, and then cooled to room temperature in a cold water bath. All fat and muscle samples were frozen in liquid nitrogen and homogenised using a laboratory knife mill (Grindomix GM200, Retsch GmbH and Co., Haan, Germany). Concentration of carbonyls was determined in myofibril isolates from fresh muscle

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