



Effect of including carob pulp in the diet of fattening pigs on the fatty acid composition and oxidative stability of pork

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ARTICLE INFO

Article history:

Received 13 February 2014

Received in revised form 7 July 2014

Accepted 25 September 2014

Available online 16 October 2014

Keywords:

Carob pulp

Pork

Fatty acids

Lipid oxidation

Colour stability

ABSTRACT

The effect of feeding pigs with carob pulp on meat quality was investigated. Nine pigs were finished on a conventional concentrate-based diet (control), while two groups received a diet comprising of the same ingredients with the inclusion of 8% or 15% carob pulp (Carob 8% and Carob 15%, respectively). Feeding carob-containing diets reduced the concentration of saturated fatty acids in the muscle, increased the concentration of monounsaturated fatty acids in meat ($P < 0.01$) and of n-3 polyunsaturated fatty acids (PUFAs) and reduced the n-6/n-3 PUFA ratio ($P < 0.001$). The meat underwent slow oxidative deterioration over 9 days of storage. However, the Carob 15% treatment increased meat susceptibility to lipid oxidation across storage ($P = 0.03$), while the dietary treatment did not affect meat colour stability. In conclusion, feeding pigs with carob pulp could represent a strategy, in the Mediterranean areas, to naturally improve meat nutritional value and to promote the exploitation of this local feed resource.

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1. Introduction

Carob tree (*Ceratonia siliqua* L.) is native to the Mediterranean areas and mainly grown in Italy, Spain, Portugal, Greece and Morocco (FAO, 2011). Traditionally, carob fruits have been used in human and animal nutrition. Nowadays, carob fruit constituents found applications in food, pharmaceutical and cosmetic industries, with special interest being devoted to carob gum which is produced from the seeds (Dakia, Blecker, Robert, Wathelet, & Paquot, 2008). Therefore, as the seeds are considered the most valuable part of the fruits, carob pulp can be considered, in some instances, as a by-product resulting from the processing procedure of the pods (Vekiri, Ouzounidou, Ozturk, & Görkc, 2011) and could find valuable application as local and cheap feed resource for livestock nutrition in the areas of production.

Carob pulp contains high levels of sugars, particularly low molecular weight carbohydrates, such as sucrose, thus representing a potentially good source of energy in animal diets (Marakis, 1996). On the other hand, carob pulp is characterized by a rather low protein and fat content (Avallone, Plessi, Baraldi, & Monzani, 1997). Nevertheless, carob pulp has a favourable fatty acid composition due to the presence of essential fatty acids, such as linoleic and alpha-linolenic acids (Ayaz et al., 2009) and might represent a natural source of desirable fatty acids in the diets of concentrate-fed animals. Studies so far conducted to evaluate the possibility of feeding carob pulp to livestock have mainly focused on ruminants (Priolo, Waghorn, Lanza, Biondi, & Pennisi, 2000;

Silanikove et al., 2006) and highlighted that the main limitation to the inclusion of high levels of carob pulp in the diet is its high content of condensed tannins. Highly polymerized condensed tannins are plant secondary compounds, belonging to the heterogeneous group of phenolic compounds, which are receiving considerable attention in animal nutrition. On one hand, tannins can form complexes with proteins and carbohydrates and, when present at high levels in the diet, can act as antinutritional factors for ruminants and monogastric animals (Jezierny, Mosenthin, & Bauer, 2010; Makkar, 2003). On the other hand, the dietary administration of tannins to pigs has been shown to exert positive effects on the functionality of the gastrointestinal tract (Biagi, Cipollini, Paulicks, & Roth, 2010). Furthermore, tannins are known as potent antioxidants (Hagerman et al., 1998) and, although their efficacy *in vivo* is still under debate, the dietary administration of tannin-rich feeds to animals is being explored as a strategy to provide natural antioxidants in the diet to improve meat quality traits such as oxidative stability (Vasta & Luciano, 2011). Some studies have elucidated the chemical composition of the phenolic compounds occurring in carob pods and have demonstrated that most of these compounds possess strong antioxidant capacity (Kumazawa et al., 2002; Owen et al., 2003; Papagiannopoulos, Wollseifen, Mellenthin, Haber, & Galensa, 2004). Bastida et al. (2009) were able to extend the storage stability of pork meat by using carob extract as a functional ingredient in meat preparation.

Some studies assessed the effect of feeding phenolic-rich plant extracts or tannin-rich feeds, such as chestnut, to pigs on the performance, antioxidant status and meat and fat quality traits (Bermúdez, Franco, Franco, Carballo, & Lorenzo, 2012; Frankič & Salobir, 2011; Pugliese

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et al., 2013; Rossi et al., 2013). However, due to the high variability of the phenolic compound profiles between different natural sources, it is not possible to directly extrapolate hypotheses from the above studies on the possible effects that feeding pigs with carob could exert on meat quality. Very limited information is available on the effect of feeding carob pulp to monogastric animals on meat quality, despite the fact that there is evidence of the use of carob pulp in pig feeding since the New Testament (Luke, 15, 16). To the best of our knowledge, only one study investigated the effects of the dietary administration of carob pulp to growing pigs on some meat quality traits including intramuscular fatty acid composition (Kotrotsios, Christaki, Bonos, & Floru-Paneri, 2012), while information on the effect of dietary carob pulp on meat oxidative stability has not yet been provided.

Therefore, the objective of the present investigation was to evaluate the effect of the inclusion of two different levels of carob pulp in the finishing diet of pigs on the intramuscular fatty acid composition and oxidative stability of meat.

2. Materials and methods

2.1. Animals and diets

Twenty-seven Pietrain \times Large White barrows, born at the end of January 2012, and weaned at 5 weeks of age (7 kg live weight, approximately) were used. Pigs were fed with commercial starter concentrates until 60 days of age and, subsequently, animals were grown on commercial grower concentrates until 180 days of age. Then, pigs were randomly assigned to one of three experimental treatments (with 9 animals in each group). One group (control) was fed with a commercial finishing concentrate-based diet. The other two groups received a diet comprising of the same ingredients as the control diet with 8% or 15% carob pulp was also included (groups Carob 8% and Carob 15%, respectively). The composition of the experimental diets is described in

Table 1
Ingredient and chemical composition of the experimental concentrates.

	Control	Carob 8%	Carob 15%
<i>Ingredients (%)</i>			
Corn	23.5	30	36
Barley	34.7	22.5	12.5
Soya bean meal	10	13	16
Faba bean	11	9	6.6
Wheat middlings	15	11.7	8
Carob pulp	0	8	15
Soybean oil	3	3	3
Premix ^a	2.8	2.8	2.9
<i>Chemical composition</i>			
Calculated digestible energy (MJ/kg)	13.43	13.18	13.10
Dry matter (DM) ^b	90.3	89.1	90.1
Ash ^c	9.9	7.2	5.5
Crude protein (CP) ^c	16.3	16.9	18.4
Neutral detergent fibre (NDF) ^c	26	20.9	19.4
Ether extract (EE) ^c	6.49	5.49	5.24
Total phenolic compounds ^d	2.76	2.90	3.16
<i>Fatty acid composition (% of total fatty acids)</i>			
C12:0	0.33	0.81	0.72
C14:0	0.75	0.52	0.42
C16:0	11.17	13.64	12.50
C16:1	0.38	0.75	0.53
C18:0	2.66	3.47	3.06
cis-9 C18:1	16.26	13.27	14.75
cis-9, cis-12 C18:2	41.58	35.21	34.18
cis-6, cis-9, cis-12 C18:3 n-6	1.44	0.55	0.50
cis-9, cis-12, cis-15 C18:3 n-3	25.45	31.77	33.33

^a Included: calcium carbonate, sodium chloride, total phosphorus (dicalcium phosphate, calcium phosphate), vitamin premix, lysine, methionine, threonine.

^b Expressed as g/100 g of fresh weight.

^c Expressed as g/100 g of DM.

^d Expressed as mg of tannic acid equivalents/g of DM.

Table 1. During the experimental feeding phase (120 days), animals received 2 kg of concentrate (as fed)/day/head and had *ad libitum* access to water. All animals were slaughtered in a commercial abattoir at 300 days of age. Animals were electrically stunned and exsanguinated, the carcass weight was recorded and the muscle *longissimus thoracis et lumborum* (LTL; approximately 400 g) was removed from each carcass and immediately transported and refrigerated to the laboratory.

The experimental protocol was approved by the Animal Welfare Committee of the University of Catania and animals were handled by specialized personnel following the guidelines of the European Parliament and Council (2010/63/EU Directive).

2.2. Analyses of feedstuffs

Samples of the experimental diets and of carob pulp, collected during the trial, were analysed for neutral detergent fibre (NDF) according to Van Soest, Robertson, and Lewis (1991). Furthermore, according to AOAC (1995), feedstuffs were also analysed for ash, crude protein and crude fat (ether extract). Following the procedure described by Makkar, Blümmel, Borowy, and Becker (1993), total phenolic compounds were extracted from the feed samples using aqueous acetone (70% v/v), analysed by means of the Folin–Ciocalteu reagent and expressed as tannic acid equivalents. The fatty acid composition of the feedstuffs was analysed by gas-chromatography using the method described by Sukhija and Palmquist (1988) and was expressed as g/100 g of total fatty acids.

2.3. Analyses of meat samples

Fresh LTL samples were divided into two 200-g portions. One portion was immediately vacuum-packaged and stored at -30°C pending analysis of intramuscular fatty acid composition. The remaining portion was vacuum-packaged and stored at 4°C . After 24 h of refrigerated storage, bags were opened and the ultimate pH of LTL was measured using an Orion 9106 pH-meter equipped with a penetrating electrode (Orion Research Incorporated, Boston, MA). Then, each muscle was divided into 3 sub-samples (2 cm thickness) using a knife. The sub-samples were placed in polystyrene trays, covered with PVC film and stored in the dark at 4°C . Colour and lipid oxidation measurements were performed after 2 h of blooming (day 0) and after 5 and 9 days of storage, using one sub-sample for each day of storage. All the analyses were performed as described below.

2.3.1. Intramuscular fatty acid composition

Intramuscular lipids were extracted according to the method used by Folch, Lees, and Stanley (1957). Briefly, 5 g of LTL was blended with extraction solvent chloroform/methanol (2:1, v/v) twice, filtered, placed in separator funnels and mixed with saline solution (0.88% KCl). After separation into two phases, the chloroform lipid fraction was collected and washed with distilled water/methanol (1:1, v/v). After a further filtration and evaporation by means of a rotary evaporator, lipid extracts were transferred to test tubes for subsequent gas chromatographic analysis. Duplicates of 100 mg of lipid extract were methylated adding 1 ml of hexane and 0.05 ml of 2 N methanolic KOH. Nonanoic acid (C9:0) was used as an internal standard. Gas chromatographic analysis was performed using a Varian model Star 3400 CX instrument equipped with a CP 88 capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.25 μm). Operating conditions were: a helium flow rate of 0.7 ml/min, a FID detector set at 260°C , a split-splitless injector at 220°C with an injection rate of 120 ml/min, and an injection volume of 1 μl . The temperature programme of the column was: 4 min at 140°C and a subsequent increase to 220°C at $4^{\circ}\text{C}/\text{min}$. Retention time and area of each peak were computed using the Varian Star 3.4.1. software. The individual fatty acid peaks were identified by comparison of retention times with those of known

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