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Effects of dietary fat saturation on fatty acid composition and gene transcription in different tissues of Iberian pigs

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ABSTRACT

The effect of two diets, respectively enriched with SFA (*S*) and PUFA (*P*), on FA tissue composition and gene expression was studied in fattened Iberian pigs. The FA composition of adipose, muscular and liver tissues was affected by dietary treatment. *S* group showed higher MUFA and MUFA/SFA ratio and lower PUFA and $n-6/n-3$ ratio than *P* group in all analyzed tissues. In muscle and liver the extracted lipids were separated into neutral lipids and polar lipid fractions which showed significantly different responses to the dietary treatment, especially in liver where no significant effect of diet was observed in NL fraction. The expression of six candidate genes related to lipogenesis and FA oxidation was analyzed by qPCR. In liver, *stearoyl CoA desaturase (SCD)*, *acetyl CoA carboxylase alpha (ACACA)* and *malic enzyme 1* (*ME1)* genes showed higher expression in *S* group. *SCD*, *ACACA*, *ME1*, and *fatty acid synthase (FASN)* gene expression levels showed a wide variation across the tested tissues, with much higher expression levels observed in adipose tissue than other tissues. Tissue FA profile and gene expression results support the deposition of dietary FA, the lipogenic effect of dietary saturated fat in liver and the employment of saturated dietary fat for endogenous synthesis of MUFA in all the analyzed tissues.

1. Introduction

Besides fat quantity, fatty acid (FA) composition of muscle and adipose tissues determines sensorial, technological and nutritional aspects of meat influencing its perception by the consumers (Webb & O'Neill, 2008; Wood et al., 2008). From a nutritional point of view, medical recommendations are now shifting from the reduction of fat intake towards increasing fat quality in order to maintain cardiovascular health. Meat is a primary source of dietary fat and especially of saturated fatty acids (SFA). High consumption of SFA has been associated with obesity, high plasma cholesterol and cardiovascular diseases (Chizzolini, Zanardi, Dorigoni, & Ghidini, 1999; Katan, Zock, & Mensink, 1994), while replacing SFA with MUFA or PUFA reduces the risk of coronary heart disease (de Lorgeril & Salen, 2012). At the same time, long-chain polyunsaturated fatty acids (PUFA) have been implicated in the prevention of different diseases (Nguyen, Nuijens, Everts, Salden, & Beynen, 2003; Wood et al., 2003), although nutritionists tend to focus more on the PUFA/SFA ratio and the ratio $n-6/n-3$ rather than the content of particular FAs (Jimenez-Colmenero, Ventanas, & Toldra, 2010). Although producers and consumers differ about the importance of animal FA profile in meat quality (Webb & O'Neill, 2008), an increasing number of consumers prefer meat products with higher ratios of PUFA and MUFA relative to SFA and with favorable balance between $n-6$ and $n-3$ PUFA, because of their beneficial effects on disease prevention (Kallas, Realini, & Gil, 2014; Wood et al., 2003). Hence, there has been much interest in finding ways to manipulate the FA composition of meat in order to produce functional foods (Coates, Sioutis, Buckley, & Howe, 2009). Different aspects such as feeding system, age, sex or the genetic type influence this composition, dietary manipulation of the FA profile being the most effective procedure of altering the fat composition of pig meat products (Kouba, Enser, Whittington, Nute, & Wood, 2003; Morel, McIntosh, & Janz, 2006).

In monogastric meat animal species most dietary fatty acids are absorbed directly, unchanged from the intestine (Enser, Richardson, Wood, Gill, & Sheard, 2000), and deposited in muscle and adipose tissues. Moreover, tissue fatty acid composition is also dependent on endogenous synthesis which may be also influenced by dietary composition. Changes in dietary fat have different impacts on the expression of genes related to lipid metabolism (Jump et al., 2005). Most studies have been performed in rodents, in which PUFA and SFA enriched diets have been shown to alter the transcription of genes related to

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lipogenesis, FA desaturation and β-oxidation, with dietary SFA inducing lipogenic genes (Sampath, Miyazaki, Dobrzyn, & Ntambi, 2007), while PUFA upregulate FA oxidation genes and downregulate those of lipid synthesis (Proud, Hundal, & Taylor, 2004; Sessler & Ntambi, 1998). These effects are highly dependent on the species, age of the animal and studied tissue (Ding, Lapillonne, Heird, & Mersmann, 2003; Duran-Montgé, Theil, Lauridsen, & Esteve-Garcia, 2009a, 2009b). In mouse and humans, the main effects of dietary FAs on transcription of lipogenic genes have been observed at the hepatic level (Azain, 2004) since the liver is the most important organ regulating fatty acid metabolism in these species. However, in pigs, adipose tissue is the most important organ in fat synthesis (O'Hea & Leveille, 1969).

Some previous studies have also shown the effects of SFA and PUFA enriched diets on composition and gene transcription in different pig breeds, with no consistent results (Duran-Montgé et al., 2009a, 2009b; Iyer, Sarmah, Tamuli, Das, & Kalita, 2012; Mitchaothai et al., 2007). Thus, there is no clear understanding on how the dietary FA composition has an impact on different tissues, their relative influence on the direct deposition of FA or on its endogenous synthesis. Moreover, knowledge of their effects on the Iberian pig breed is scarce. The Iberian breed is characterized by its high lipogenic potential and specific tissue FA profile which is conditioned by its feeding system and by its own metabolism (López-Bote, 1998). In this sense, the breed shows a high desaturation capacity (Daza, Rey, Ruiz, & López-Bote, 2005; Pérez-Palacios, Ruiz, Tejeda, & Antequera, 2009; Rey, Daza, López-Carrasco, & López-Bote, 2006; Ventanas, Tejeda, & Estévez, 2008) and presents high levels of monounsaturated fatty acids (MUFA) in fat tissues. In these fatty pigs, the energy and fat metabolism could be differentially affected by diet composition with respect to lean-type breeds (Barea, Isabel, Nieto, López-Bote, & Aguilera, 2013). Our previous work has shown that enrichment of diet with MUFA influences tissue composition but has minor influence on gene transcription in Iberian pigs, at least in the long term (Óvilo, Benítez, Fernández, Isabel, et al., 2014). According to our previous results, the effects of oleic acid supplementation on candidate gene transcription seem to be more important in adipose than in other tissues.

Therefore, the objective of this study was to evaluate the effect of two diets, respectively enriched with SFA and PUFA, on FA tissue composition and gene expression of six candidate genes involved in lipogenesis and FA metabolism in adipose, muscular, hepatic and cardiac tissues of fattening Iberian pigs. Gene expression differences between tissues were also studied.

2. Material and methods

2.1. Animals

The current study was carried out at the facilities of the CIA Dehesón del Encinar (Toledo, Spain), under a Project License from the INIA Scientific Ethic Committee. Animal manipulations were performed according to the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 about the protection of animals used in experimentation.

The study comprised 27 barrows born in 15 contemporary litters of the Iberian Torbiscal strain, which were fed a barley-wheat bran-soybean meal-based diet during a pre-experimental period of 14 weeks. When pigs reached 60 kg (SD = 4 kg) of body weight (BW) they were randomly allotted to two groups, with full-sibs being split into the two groups, and were fattened with two different isocaloric and isoproteic diets. The crude energy content was 3100 Kcal per kg of feed for both diets. Each experimental diet was respectively enriched in saturated FA (group *S*, n = 13), with the inclusion of 5% hydrogenated lard, and polyunsaturated FA (group *P*, n = 14), with the inclusion of 5% sunflower oil. Feed composition is shown in (Table 1). During this fattening period, the pigs were raised outdoors in two separated fenced areas. Animals were manually fed twice a day and water was provided *ad libitum*. One long feeder was available for each experimental group (80 cm/animal), which allowed all animals to eat at the same time. The average daily ration was increased from 1.5 to 3.53 kg along 25 weeks until the pigs reached 150 kg (SD = 12 kg) of BW. Animals were then stunned and slaughtered at a local slaughterhouse (Alcaudete de la Jara, Toledo, Spain). After slaughter, carcasses were scalded and eviscerated. Carcass weight was recorded and backfat thickness was measured on the left side of each carcass at the level of the last rib, using a vernier caliper. Tissue samples were collected from backfat, *longissimus thoracis* muscle, heart (left ventricle) and liver (right lateral lobe) and stored at −80 °C. The time elapsed since the stunning of the animals to the samples storing did not exceed 3 h. Backfat samples and *longissimus* muscle samples were taken at the level of the last rib. Backfat samples were separated into outer and inner layers, which were separately analyzed for fatty acid composition.

Table 1
Ingredients, calculated chemical composition and analyzed fatty acid composition of the experimental diets (g/kg; as-fed basis).

Diet	Saturated (S)	Polyunsaturated (P)
Ingredients		
Barley	688.7	688.7
Sunflower meal, 30% CP[a]	121.7	121.7
Soyabean meal, 44% CP	86.8	86.8
Wheat bran	25.5	25.5
Hydrogenated fat	50.0	–
Sunflower oil	–	50.0
Sodium choride	3.0	3.0
Calcium carbonate	7.0	7.0
Bicalcium phosphate	13.0	13.0
Lysine (50%)	1.3	1.3
Vitamin and mineral premix	3.0	3.0
Chemical composition, g/kg of feed		
Moisture	94.4	94.4
Lipids	67.1	68.6
Crude protein	157.0	157.0
Crude fiber	66.5	66.5
Nitrogen-free extractives	360.4	360.4
Ash	50.9	50.9
Main fatty acids, g/kg of feed		
C14:0	0.9	0.2
C16:0	40.4	11.5
C18:0	4.1	3.1
C18:1*n*−9	29.2	24.4
C18:2*n*−6	13.7	50.8
C18:3*n*−3	0.7	1.3

[a] CP: crude protein.

2.2. Tissue and feed FA composition analyses

Lipid extracts from subcutaneous fat (inner and outer layers), were extracted by the procedure proposed by Bligh and Dyer (1959) whereas the extracted lipids from *longissimus* muscle and liver were separated into neutral lipids (NL) and polar lipids (PL) using aminopropyl minicolumns, following the method used by Segura and Lopez-Bote (2014). Fat extracts were methylated in the presence of sulfuric acid and analyzed by gas chromatography as described elsewhere (López-Bote, Rey, Sanz, Gray, & Buckley, 1997) using a Hewlett Packard HP-6890 (Avondale, PA, USA) gas chromatograph equipped with a flame ionization detector and capillary column (HP-Innowax, 30 m × 0.32 mm i.d. and 0.25 μm polyethylene glycol-film thickness). A temperature program of 170 to 245 °C was used. The injector and detector were maintained at 250 °C. The carrier gas (helium) flow rate was 2 ml/min. Results were expressed as grams per 100 g of detected FAMEs.

Dietary FAs were extracted and quantified by the one-step procedure as described by Sukhija and Palmquist (1988) in lyophilized samples. Pentadecanoic acid (C15:0) (Sigma, Alcobendas, Madrid,