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Hepatic lipogenic enzyme expression in pigs is affected by selection for decreased backfat thickness at constant intramuscular fat content

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ABSTRACT

This study investigated (i) whether genetic selection for decreased backfat thickness at constant intramuscular fat (IMF) affects the expression of lipogenic enzymes in pig liver and (ii) whether expression of the hepatic lipogenic enzymes is related to subcutaneous fat and IMF fatty acid composition. The enzymes investigated were fatty acid synthase (FAS), stearoyl-CoA desaturase and Δ6-desaturase (Δ6d). Experiments were conducted on 30 barrows (15 controls and 15 selected). Selected pigs had lower backfat thickness, which was accompanied by a reduced expression of the hepatic FAS and Δ6d when compared to control pigs. There was a trend towards a positive relationship between FAS and Δ6d protein expression and saturated and polyunsaturated fatty acids content respectively, in subcutaneous fat but not in muscle. It was concluded that selection against backfat thickness is associated with changes in expression of the hepatic FAS and Δ6d proteins. The changes in protein expression did not influence significantly the tissue fatty acid composition under these conditions.

1. Introduction

Intramuscular fat (IMF) content is one of the important characteristics of meat quality (Fernandez, Monin, Talmant, Mourot, & Lebret, 1999). The amount and type of IMF have direct impact on human health (Valsta, Tapanainen, & Männistö, 2005; Webb & O´Neill, 2008). During the last decades IMF content in pigs has been continuously decreasing as a result of selection towards leaner genotypes which compromised eating quality of pork (Hermesch, 2004; Merks, 2000). Although it is known that there is a positive relationship between IMF and subcutaneous backfat (Solanes, Reixach, Tor, Tibau, & Estany, 2009), there is evidence that genetic correlation between these two fat depots is not always strong and that it is possible to manipulate IMF and subcutaneous fat independently by dietary or genetic means (Doran et al., 2006; Solanes et al., 2009). Manipulation of IMF and subcutaneous fat deposition is associated with regulation of expression of lipogenic enzymes in these tissues (Cánovas, Estany, Tor, Pena, & Doran, 2009; Doran et al., 2006; Ntawubizi, Raes, Buys, & De Smet, 2009). Lipogenesis involves the biosynthesis of palmitic fatty acid (C16:0), which in turn, may be converted into a range of longer chain of fatty acids via elongation and/or desaturation reactions. Long chain polyunsaturated fatty acid (PUFA) can also be produced via desaturation and elongation of the essential precursors, linoleic and α-linolenic acids. Therefore, lipogenesis and biosynthesis of PUFA from precursors are important processes that influence tissue fatty acid composition (Ntawubizi et al., 2010).

In spite of increasing body of evidence that IMF and subcutaneous fat depositions can be regulated independently, the molecular mechanisms underlying fat partitioning in pigs remain unclear. Furthermore it is unknown whether depositions of IMF and subcutaneous fat can be influenced by lipogenic processes taking place in other organs, and particularly in the liver. Liver, alongside with subcutaneous fat, plays an important role in mediating fatty acid metabolism in pigs, in particular, in the synthesis of triglycerides, which are packed into lipoproteins to be distributed to peripheral tissues (Bergen & Mersmann, 2005; Dodson et al., 2010; Reiter et al., 2007). The main objectives of this study were (i) to investigate whether genetic selection for decreased subcutaneous fat thickness at constant IMF affects the expression of hepatic lipogenic enzymes in pigs; and (ii) to determine whether expression of the hepatic lipogenic enzymes is related to fatty acid compositions of subcutaneous fat and IMF. The study focused on the expression of the key lipogenic enzymes fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), and Δ6-desaturase (Δ6d) catalyzing the biosynthesis of saturated (SFA), monounsaturated (MUFA), and PUFA respectively.

2. Materials and methods

All the experimental procedures related to animal trials were approved by the Ethics Committee for Animal Experimentation of the University of Lleida, Spain. The pigs were reared and slaughtered at the facilities of the commercial company Grupo Batallé, Spain.

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2.1. Animals, diet and sample collection

The experiment was conducted on 30 purebred Duroc barrows (the selected and the control groups, 15 animals per group) randomly chosen from 2 larger groups as described below. The groups differed in backfat thickness, but not in IMF content (Reixach, Tor, Díaz, & Estany, 2008; Reixach, Tor, & Estany, 2009). The genetically selected and the control groups were constituted according to the mid-parent (litter) breeding values for backfat thickness at 180 days of age and for IMF content in the *gluteus medius* muscle adjusted for carcass weight, which were predicted using the model described in Solanes et al. (2009). Litters in the selected group were selected against backfat thickness while maintaining IMF content to the values most similar to those in the control group. At the age of 11 wk, two barrows per litter were randomly taken and moved to a finishing facility, where they were kept in pens with 12 animals per pen until the age of 30 wk. This selection process was repeated in 4 consecutive batches, in which pigs in both groups were raised under same conditions and managed in the same way. Pigs used in the present study were randomly taken from the last batch.

During the fattening period pigs were given ad libitum access to the feed. A commercial pelleted finishing diet (Esporc, Ruidarenes, Girona, Spain) was given from the day 160 onwards. Composition of this diet is given in Table 1. Feed analyses were performed in triplicate. Dry matter (DM) was determined by oven-drying at 100 to 102 °C for 24 h. Ash content was determined by muffle-heating at 550 °C until constant weight. Crude protein was analyzed by the Kjeldahl method (AOAC International, 2000), crude lipid content was determined by Soxhlet fat analysis (AOAC International, 2000), and crude fiber was analyzed by acid and alkaline digestions with a Dosi-Fiber extractor (Selecta, Barcelona, Spain; AOAC International, 2000). Analyses of FA composition of diet were performed as described in Section 2.2. after extracting the total lipids by the method of Hanson and Olley (1963). Weight of the pigs was recorded at 26 and 29 wk of age, and their SF thickness was ultrasonically measured at 5 cm off the midline at the position of the last rib (Piglog 105, SFK-Technology, Herlev, Denmark). All the pigs were slaughtered at 30 wk of age, and carcass backfat was measured at 6 cm off the midline between the third and fourth last ribs using an on-line ultrasound automatic scanner (AutoFOM, SFK-Technology, Herlev, Denmark).

Samples of pig liver and subcutaneous fat were collected immediately after slaughter, snap-frozen and stored at −80 °C until analyzed. The samples of subcutaneous fat were taken at the level of the third and fourth ribs. Samples of muscle were collected after chilling the carcasses for about 24 h at 2 °C, vacuum packed, and stored in deep freeze until required. It has been previously demonstrated that these storage conditions do not affect protein expression (Doran et al., 2006).

Table 1
Composition of the diet during the finishing period.

Item	Amount
Dry matter (DM), g/kg	895.4
Crude protein, g/kg of DM	159.9
Crude lipid, g/kg of DM	68.4
Crude fiber, g/kg of DM	58.8
Ash, g/kg of DM	66.4
Fatty acid composition, mg/g of fatty acids	
C14:0, myristic	11.1
C16:0, palmitic	201.2
C18:0, stearic	69.9
C20:0, arachidic	2.2
Total SFA	284.4
C16:1, n−7 palmitoleic	23.8
C18:1, n−9 oleic	380.7
C20:1, n−9 eicosenoic	9.6
Total MUFA	414.1
C18:2, n−6 linoleic	258.8
C18:3, n−3 linolenic	23.9
C20:2, n−6 eicosadienoic	6.6
C20:4, n−6 arachidonic	2.3
Total PUFA	291.6

2.2. Analysis of fatty acid composition by gas chromatography

Once defrosted, the samples of liver, muscle, and subcutaneous fat were freeze-dried and homogenized by mixing with sand and using a glass stirring rod. Dry matter was calculated as the weight difference before and after freeze-drying. A representative aliquot from the homogenized samples was used for determining FA composition. Fatty acid methyl esters (FAME) were directly obtained by transesterification using a solution of 20% boron trifluoride in methanol (Rule, 1997). Methyl esters were determined by gas chromatography using a capillary column SP2330 (30 m × 0.25 mm fused silica capillary coated with a 0.20 μm film of poly 80% bicyanopropyl-20% cyanopropylphenyl siloxane, Supelco, Bellefonte, PA) and a flame ionization detector with helium as carrier gas at 1 mL/min. The oven temperature program increased from 150 to 225 °C at 7 °C per min and injector and detector temperatures were both 250 °C, (Bosch, Tor, Reixach, & Estany, 2009). Response factors of methyl esters were calculated under the same chromatographic conditions using a commercially available standard mixture of FAME. Fatty acid quantification was carried out through area normalization by using as internal standards 1,2,3-tripentadecanoylglycerol, for muscle and subcutaneous fat, and the 1,2,3-triundecanoylglycerol, for the liver. Intramuscular fat was calculated as the sum of each individual FA expressed as triglyceride equivalents (AOAC International, 2000). In the case of liver, qualitative analysis was also performed using a GC–MS system (Agilent 6890N GC coupled to a 5973 Mass Selective Detector; Agilent Technologies España, S.L. Las Rozas, Spain). The analytical column and chromatographic parameters were identical to those described above. Identification of each FA was confirmed by comparing their mass spectra to the computer library of the GC/MS database Wiley 275. L and NBS75 K. L.

Table 2
Effect of genetic selection on pig on-farm performance and carcass characteristics.

Trait	Group		SEM
	C	S[1]	
Live measurements at 26 wk			
Age, d	179.5	174.5	
Body weight, kg	105.8	102.3	2.7
Backfat thickness[2], mm	15.6[a]	13.1[b]	0.7
Loin thickness[2], mm	44.2	44.1	0.6
Live measurements at 29 wk			
Age, d	196.5	191.5	
Body weight, kg	117.1	112.9	2.8
Backfat thickness[2], mm	18.6[a]	14.7[b]	0.8
Loin thickness[2], mm	47.8	47.6	0.9
Carcass measurements at 30 wk			
Age, d	200.5	195.5	
Carcass weight, kg	88.3	85.1	2.2
Backfat thickness[2], mm	21.7[a]	18.4[b]	0.7
Loin thickness[2], mm	46.0	49.3	1.4
Lean content[2], %	45.5[a]	49.3[b]	0.9
Intramuscular fat, % of DM[3]	12.1	11.1	0.8

[a, b]Means within a row with different superscripts differ ($P<0.05$). $n=15$ for control and selected groups.
[1]Pigs in the selected group (S) were selected for decreased backfat thickness at constant intramuscular fat content. (C) is the control (unselected) group.
[2]Ultrasonic backfat and loin thickness live measurements were determined at 5 cm off the midline at the position of the last rib using the Piglog technology (SFK-Technology, Herlev, Denmark) while in the carcass they were measured at 6 cm off the midline between the third and fourth last ribs using AutoFOM automatic scanner (SFK-Technology).
[3]Intramuscular fat was determined in the *gluteus medius* muscle and was expressed in % of dry matter (DM).