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Fatty acid composition of several muscles and adipose tissues of pigs fed n-3 PUFA rich diets

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ABSTRACT

During two months, sixty Pietrain × (Landrace × Large White) finishing pigs (50.7 to 115.2 kg live weight) received diets containing various levels of C18:3n-3 from linseed and C22:6n-3 from *Schizochytrium* microalgae to increase the content of these fatty acids (FA) in their lean and fat tissues. Samples of tissues have been extracted from the carcass at the slaughterhouse. Tissues of pigs fed linseed had the highest C18:3n-3 and C20:3n-3 contents, while the C20:4, C20:5 and C22:6n-3 contents increased in tissues with microalgae diets. Diaphragm was fatter, but contained less monounsaturated FA, total n-6 and n-3 polyunsaturated FA (PUFA) than *longissimus thoracis et lumborum* and *semimembranosus* muscles due to their different roles. The leaf fat was the most saturated and monounsaturated tissue, regardless of the diet. Adipose tissues located in extremities contained more n-3 and n-6 PUFA than adipose tissues located in the middle of the carcass. This study showed the existence of a PUFA gradient depending on tissue location.

1. Introduction

The European n-3 polyunsaturated fatty acid (PUFA) consumption is inferior to recommendations of public health which advise consuming one n-3 PUFA for five n-6 PUFA (Anses, 2011). The n-3 PUFA are involved in many body functions such as vision and brain development and consuming too few of them can cause health problems such as cardiovascular disease and metabolic disorders (Hu et al., 2002; Oomen et al., 2000; Schmidt, Skou, Christensen, & Dyerberg, 2000). For this reason, consumers must rebalance their PUFA consumption ratio by consuming more n-3 PUFA, including 2 g of α-linolenic acid (ALA), 250 mg of eicosapentaenoic (EPA) and 250 mg of docosahexaenoic (DHA) acids per day (Anses, 2011).

The fatty acid (FA) composition in tissues reflecting dietary FA, animals fed with n-3 PUFA could serve as a source of these FA for humans (Mourot & Hermier, 2001). This solution allows people not to change their consumption habits and consist in feeding animal with plants rich in n-3 PUFA, which is often flax or rape. However, these two plants provide the precursor of n-3 PUFA but not its derivatives. Despite humans own desaturases which allows synthesizing long chain PUFA derivatives from ALA, the conversion rate is very low since it is < 8% for EPA (Brenna, 2002; Goyens, Spilker, Zock, Katan, & Mensink, 2006; Igarashi, Ma, Chang, Bell, & Rapoport, 2007; Plourde & Cunnane, 2007) and < 4% for DHA (Brenna, 2002; Burdge & Calder, 2005). It was showed in human that the n-3 and n-6 PUFA families compete for desaturases which are enzymes involved in PUFA synthesis (Lavialle & Layé, 2010). It was also demonstrated that ALA is in competition with C24:5n-3 for the Δ6-desaturase (Portolesi, Powell, & Gibson, 2007) explaining the low conversion rate. Consequently, innovative source of n-3 PUFA such as microalgae have been used in diets to supply these deficient FA to the animal. (Baéza et al., 2015a, 2015b; Sardi, Martelli, Lambertini, Parisini, & Mordenti, 2006).

The FA composition also depends on location of tissues in the carcass (Kloareg, Noblet, & Van Milgen, 2007; Monziols, Bonneau, Davenel, & Kouba, 2007). In France, except for certain parts of the carcass, the entire pig is consumed as fresh meat (30%) or is transformed (70%) without changing its FA profile (Guillevic, Kouba, & Mourot, 2009a). It is important to know whether certain tissues capture or synthesize healthier FA than others. The aim of this study was to enrich several tissues of pigs with n-3 PUFA by feeding linseed and microalgae and to compare their FA composition.

2. Materials and methods

2.1. Animals and diets

Animals used in this experiment have been reared and slaughtered at the INRA experimental site in France in compliance with French

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regulations for the human care and use of animals in research. Certificate of authorization to experiment on living animals has been delivered by the National Research Agency to Jacques Mourot for Agralid project (ANR 12-ALID-003) using ministerial experiment reference number 02279.

Sixty growing-finishing crossbred [(Large White × Landrace) sows × Pietrain boar] male pigs have been assigned to five equal groups according to their initial liveweight (LW). Pigs have been reared at an initial LW of 50.7 ± 2.7 kg from 14 to 22 weeks of age and received feed and water *ad libitum* in individual crates on concrete flooring. Each group has been fed with one of five experimental diets including a control diet containing soybean and palm oil (SP), dehulled and extruded linseed (EL; TRADILIN®, Valorex, Combourtillé, France), mix of 75% dehulled and extruded linseed and 25% DHA rich microalgae (3EL/MAG) or a mix of 50% dehulled and extruded linseed and 50% microalgae (EL/MAG). The fifth diet contained only the DHA rich microalgae (MAG; DHA Gold®, *Schizochytrium* sp., DSM, Belgium) as the source of dietary lipids. The five diets respectively contained 0.55, 4.23, 3.22, 2.43 and 0.69 g of ALA and 0.01, 0.00, 1.72, 3.34 and 7.03 g of DHA per kg of feed. Diets contained 3% fat on average, 17.5% protein on average and have been supplemented with wheat to be isoenergetic on a net energy basis (Sauvant, Perez, & Tran, 2004). Diets have been also supplemented with 40 ppm of α-tocopherol acetate to prevent PUFA oxidation (Table 1).

Since diets followed nutritional recommendations for pigs, amino acid contents did not limit growth. Animal feed consumption has been measured twice a week throughout the rearing phase by subtracting feed refusal from distributed feed quantities. Animals have been weighed once a week in the morning before feed distribution. Before the end of the experiment, one pig on the EL diet has been removed from the experiment due to health problems.

2.2. Slaughter and tissues sample collection

Pigs have been stunned using electronarcosis and slaughtered by bleeding at 115.2 ± 7.5 kg LW after a 16 hour fasting period. After evisceration, the liver, heart and kidneys have been removed from the carcass and weighed. The carcass has been then cut in half lengthwise and weighed to obtain the total hot weight. Approximatively 100 g ± 30 g of each tissue have been sampled to determine the FA composition. The *longissimus thoracis et lumborum* muscle (LTL) and the subcutaneous backfat (SCB) have been sampled at the third-to-last rib on the right side of the carcass. The belly has also been sampled on the right side of the carcass at the same level of LTL and SCB. Other tissues have been sampled along the carcass: subcutaneous adipose tissue in the neck (SCN) and ham (SCH) (near the tail), internal adipose tissue between the *gracilis* and *semitendinosus* muscles in the ham (IH), some leaf fat, the *semimembranosus* (SM) muscle and the diaphragm. Tissue samples have been individually packed and frozen at − 20 °C until analysis.

2.3. Chemical analysis of tissues

Lipids of feed and tissues have been cold extracted using chloroform/methanol (v/v 2:1) (Folch, Lees, & Sloane Stanley, 1957). The FA methyl esters have been saponified with a methanolic sodium hydroxide solution in the presence of C17:0 as the internal FA standard. They have been then methylated with boron trifluoride (BF3) (Morrison & Smith, 1964). FA methyl esters have been recovered with a pentane and distilled water solution and analyzed *via* gas chromatography (7890 GC system, Agilent Technologies, USA). The chromatograph was equipped with a 0.25 mm × 30 m capillary column made from polysiloxane polymer filled with dimethylpolysiloxane and 50% of cyanopropylphenyl in stationary phase. The temperature treatment began at 150 °C and increased 4 °C per minute up to 220 °C, where it was maintained for 10.5 min. Injector and flame-ionization detector temperatures were kept constant at 220 and 280 °C, respectively. Hydrogen was the carrier gas. Retention times and peaks were determined using the chromatography software ChemStation Agilent. Identities of peaks have been verified by comparing peaks to the retention time of standard FA methyl esters. Results were expressed as percentages of the total FA to compare tissues composition.

Table 1
Composition (% fresh) and analysis of experimental diets.

	SP	EL	3EL/MAG	EL/MAG	MAG
Wheat	24.4	22.8	20.4	22.2	21.6
Maize	24.4	22.8	20.4	22.2	21.6
Barley	24.4	22.8	20.4	22.2	21.6
Soya meal 48	17.9	18.7	17.0	18.2	17.8
Wheat bran	2.5	2.6	12.1	5.1	7.5
Palm oil	0.2	–	–	–	–
Soya oil	0.2	–	–	–	–
Dehulled and extruded linseed	–	4.1	3.1	2.0	–
Microalgae	–	–	0.9	1.9	3.7
Cane molasses	3.0	3.0	3.0	3.0	3.0
L-Lysine HCL	0.3	0.3	0.3	0.3	0.3
L-Threonine	0.1	0.1	0.1	0.1	0.1
L-Tryptophan	0.0	0.0	0.0	0.0	0.0
DL-Methionine	0.1	0.1	0.1	0.1	0.1
Salt	0.5	0.5	0.5	0.5	0.5
Calcium carbonate	0.5	0.5	0.6	0.6	0.6
Dicalcium phosphate	1.0	1.0	0.9	1.0	0.9
Premix	0.7	0.7	0.7	0.7	0.7
Chemical analysis					
Dry matter (%)	84.7	85.5	85.4	85.4	86.4
Gross energy (MJ/kg DM)	18.3	18.4	18.9	18.5	18.6
Gross protein (g/kg DM)	19.4	20.4	19.9	18.9	19.4
Total fat (% DM)	2.6	3.0	3.3	3.9	4.5
Fatty acids (g/kg of feed)					
C16:0	2.53	2.37	3.12	4.15	5.62
C18:1n-9	3.55	3.54	3.23	3.52	2.85
C18:2n-6	8.30	8.57	8.30	8.35	8.45
C18:3n-3	0.55	4.23	3.22	2.43	0.69
C22:5n-6	0.00	0.00	0.62	1.19	2.51
C22:6n-3	0.01	0.00	1.72	3.34	7.03
SFA	3.03	3.01	4.07	5.58	7.54
MUFA	3.81	3.76	3.49	3.86	3.13
PUFA	8.92	12.84	14.05	15.68	19.27
n-6 PUFA	8.31	8.57	8.99	9.69	11.24
n-3 PUFA	0.61	4.26	5.05	5.97	8.02
n-6/n-3	13.65	2.01	1.78	1.62	1.40

SP, soyabean-palm diet, EL, dehulled and extruded linseed diet; 3EL/MAG, 75% dehulled and extruded linseed + 25% of DHA rich microalgae diet; EL/MAG, 50% linseed + 50% microalgae; MAG, DHA rich microalgae diet; SFA, saturated fatty acid is the sum of C10:0, C12:0, C14:0 C15:0, C16:0, C18:0, C20:0, C22:0 and C24:0; MUFA, monounsaturated fatty acid is the sum of C16:1n-9, C16:1n-7, C18:1n-9trans, C18:1n-9cis, C18:1n-7, C20:1n-9, C22:1n-11, C22:1n-9 and C24:1; PUFA, polyunsaturated fatty acid is the sum of C18:2n-6trans, C18:2n-6cis, C18:3n-6, C18:3n-3, C18:4n-3, C20:2, C20:4n-6, C20:3n-3, C20:4n-3, C20:5n-3, C22:4n-6, C22:5n-6, C22:5n-3 and C22:6n-3; n-6 PUFA is the sum of C18:2n-6trans, C18:2n-6cis, C18:3n-6, C20:3n-6, C20:4n-6, C22:4n-6 and C22:5n-6; n-3 PUFA is the sum of C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:5n-3 and C22:6n-3.

2.4. Statistical analysis

Data was analyzed using a one-way ANOVA type III (PROC GLM) procedure in SAS® 9.4 with the diet and the tissue as the main factor. Factors were studied separately. To compare tissues, multiple-comparison was applied regarding a Bonferroni-test. Results have been presented as least-squares means by diet or tissue.

3. Results

3.1. Total lipid content in tissues

Dietary FA had an effect on total lipid content in IH ($P < 0.001$), but not on lipid content in other tissues. The diaphragm (6% lipid

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