UNIVERSITAT ROVIRA I VIRGILI
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Pere Duran Montgé
ISBN:978-84-691-9485-6/DL:T-24-2009

UNIVERSITAT ROVIRA I VIRGILI

IRTA RECERCA I TECNOLOGIA AGROALIMENTÀRIES

Efectes de la composició d'àcids grassos de la dieta sobre la deposició de grassa i àcids grassos en el porc

Effects of dietary fatty acid composition on pig fat and fatty acid depositon

Per **Pere Duran i Montgé,**

dirigida pel **Dr. Enric Esteve i Garcia**

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Enric Esteve Garcia, director del Departament de Nutrició Animal de l'IRTA-Mas de Bover

CERTIFICA QUE:

La Tesi Doctoral titulada "***Efecte de la composició d'àcids grassos de la dieta sobre la deposició de grassa i àcids grassos en el porc"*** presentada per Pere Duran Montgé, ha estat realitzada sota la seva direcció i compleix els requisit necessaris per a optar al títol de Doctor.

I perquè se'n prengui coneixement i tingui els efectes que correspongui, signo aquesta certificació.

Constantí, 2 de gener de 2008

Dr. Enric Esteve Garcia

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RESUM

És sabut que la grassa de la dieta produeix diferents efectes sobre el metabolisme segons la quantitat i composició d'AG. Els treballs de la bibliografia han demostrat que els PUFA disminueixen la deposició de greix, però aquest efecte en porcs encara no havia estat comprovat. Per tant, la hipòtesi del projecte va ser investigar si la composició del greix de la dieta modificava la deposició del greix en el porc. El treball experimental es va dividir en dues parts; primer es va feu una prova de digestibilitat amb l'objectiu de conèixer la disponibilitat real de la grassa de la dieta per després realitzar la segona part de la tesis, la prova d'engreix dels animals.
Els resultats de la prova de digestibilitat mostraren que tant la digestibilitat fecal i ileal de la grassa eren semblants, mentre que la digestibilitat dels AG canviava considerablement degut al procés de biohidrogenació que té lloc al cec i còlon de l'animal interferint en els resultats quan es mesurava a nivell fecal. Els resultats de digestibilitat ileal dels AG mostraren com al augmentar la cadena d'AG, la digestibilitat disminuïa i al augmentar el nombre d'insaturacions, la digestibilitat augmentava.
Amb les digestibilitats de les grasses es formularen les dietes d'engreix per tal de que totes tinguessin la mateixa quantitat de greix digestible, a més d'una última dieta dissenyada per tenir un contingut molt baix de greix. Setanta cinc verres creuades de 62 ± 5 kg de pes viu foren divididas en 7 grups a raó de 10 animals per tractament i alimentades amb una de les set dietes: una dieta que contenia un nivell molt baix de greix (NF) i sis dietes a base de ordi i soja amb un contingut de greix aproximat del 10%. Les grasses utilitzades foren sèu vacú (T), oli de gira-sol alt oleic (HOSF), oli de gira-sol (SFO), oli de llinosa (LO), mescla (B) (55% de seu, 35% d'oli de gira-sol i 10% d'oli de llinosa) i una dieta amb oli de peix (40% d'oli de peix i 60% d'oli de llinosa). Al principi de la prova 5 animals d'un pes aproximat de 62 kg de pes viu foren sacrificats per tal de mesurar-ne la composició corporal per tal de fer el balanç. Els porcs foren sacrificats a un pes final de 99.8 ± 8.5 kg. Immediatament després del sacrifici es van prendre mostres de sang per analitzar posteriorment, i mostres de fetge, múscul *semimebranosus* i greix subcutani pels analisis d'expressió gènica. Es varen prendre mesures de qualitat i rendiment de la canal per cada animal. La part esquerra de cada carcassa fou especejada en peces comercials, i el pernil, llom i ventresca foren disseccionats en magre, grassa subcutànea, grassa intramuscular i

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os. Es prengueren mostres de magre, grassa subcutànea i grassa intramuscular, fetge i grassa perirenal, i es conservaren fins al seu anàlisis de grassa i AG. La canal dreta fou reduïda a peces petites per poder-la picar. Les visceres foren dividides en visceres blanques i vermelles per picar-les posteriorment. Les visceres i canal es van picar per separat i se'n prengueren mostres per analitzar posteriorment.

Els resultats de creixement no mostraren diferències significatives de consum diari, creixement diari i pes final entre els tractaments si bé els animals alimentats amb B tendiren a ser més pesats que els alimentats amb FO. Els animals alimentats amb B mostraren una relació consum:creixement més baixa respecte els alimentats amb NF; mentre els altres tractaments mostraren valors entremitjos. Cal notar que els alimentats amb sèu o amb nivells alts de n-3 mostraren creixements una mica inferiors als altres,i els alimentats amb nivells alts de n-3 mostraren una relació consum/guany més alta que els altres. Aquests resultats, si bé no foren significatius excepte per a les dietes sense greix, suggereixen que poden haver-hi diferències de rendiment entre fonts de greix, i que podrien ser importants en condicions pràctiques.

Les anàlisis de laboratori mostraren que les diferències principals de composició d'AG foren degudes a la dieta subministrada, mentre que les diferències degudes al teixit foren menors tot i que es van observar patrons de deposició segons el tipus de teixit, en el sentit de un perfil més saturat en els dipòsits de greix més interns. En quan als resultats d'expressió gènica, es van observar importants efectes sobre la lipogènesis deguts a la composició dels teixits-dieta, tot i així els factors edat i teixit resultaren ser més importants sobre l'expressió gènica. Les correlacions entre hormones tiroidees i gens relacionats amb la síntesis de lípids en el teixit adipós i amb l'expressió de gens de síntesis lipídica a diferents edats, suggereixen que les hormones tiroidees podrien servir de connexió entre els AG de la dieta i els efectes observats en la deposició de greix.

Els canvis en el metabolisme lipídic degut a la grassa de la dieta van anar lligats amb canvis del contingut total de greix en el porc. La grassa total dels animals alimentats amb HOSF (26.7%) i SFO (26.7%) fou superior al dels animals alimentats amb T (22.9%) mostrant que les grasses saturades, en comptes de les PUFA, disminuïen la deposició de greix. També es va observar una tendència a menor deposició de greix en els alimentats amb nivells alts de n-3.

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El balanç d'AG en porcs alimentats amb la dieta NF mostrà que el ratio dels AG sintetitzats *de novo* en porcs fou 1.5/1/3 pels àcids palmític, steàric i oleic respectivament; i es veia modificada segons la composició del greix de la dieta. La proporció de PUFA depositats en relació amb els presents a la dieta depèn del seu nivell en la dieta i que en general disminueix al augmentar el nombre d'insaturacions de la cadena d'AG.

En quant als AG essencials, a nivells baixos de la dieta, el percentatge dipositat va ser alt, però va disminuir fins als voltants del 50 % quan el nivell a la dieta va ser relativament elevat (1 %). La conversió de 18:3n-3 a EPA va ser relativament baixa, i gairebé nul·la a DHA, fins i tot inferior a la obtinguda en animals alimentats amb una dieta rica en 18 2n-6.

ABSTRACT

Dietary fat is known to have different effects on metabolism depending on its amount and fatty acid composition. Data from bibliography have shown that in rodents and poultry PUFA decrease animal fat deposition, but in pigs this hypothesis has not yet been tested. Therefore, the hypothesis of the project was to investigate if the composition of dietary fat modifies fat deposition in the pig. The thesis project was divided in two parts; first, a digestibility trial in order to know the fat digestibilities used in the second part of the project, the growing trial.

Results from the digestibility trial showed that ileal and faecal digestibilities were similar, while FA digestibility differed considerably due FA biohydrogenation in the large intestine interfering in the measurement of FA digestibility at faecal level. Results of ileal digestibility showed that individual FA digestibility increased with increasing unsaturation, and decreased with FA length.

Using the measured fat digestibilities, six diets were formulated to have the same amount of digestible fat. Seventy-five crossbred gilts (62 ±5x kg LW average) were divided in 10 animals per treatment and were fed one of seven treatments: a very low fat diet formulated to contain a very low level of fat (NF) and six fat supplemented diets (10%) based on a barley-soybean meal. The supplemental fats were tallow (T), high-oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (B) (55% tallow, 35% sunflower oil, 10% linseed oil) and fish oil blend (FO) (40% fish oil, 60%

linseed oil). In addition 5 pigs were killed at 62 kg to measure body composition at the start of the balance period. Gilts were killed at 99.8±8.5 kg live weight. Immediately after slaughter blood samples were taken for later analysis, and samples from liver, *semimebranosus* and subcutaneous fat were taken for later analysis of mRNA abundance. Production and quality carcass parameters were recorded for each pig. The left side of each carcass was cut in comercial parts, and ham, loin and belly were dissected into lean, subcutaneous and intermuscular fat and bone. Samples of lean tissue, subcutaneous fat, intermuscular fat, liver and flare fat were removed, and stored until analysis of fat and FA. The right-hand side of each carcass was reduced into small pieces and stored frozen at -20˚C for subsequent grinding. Viscera were divided into red and white viscera and stored for subsequent grinding. Viscera and right-hand side carcasses were ground and mixed separately and samples were taken for subsequent analysis of fat and FA.

There were no differences in average daily feed intake, average daily gain and final body weight of gilts among dietary treatments. Gilts fed B tended to be heavier than gilts fed FO. Gilts fed B had lower feed:gain ratio than gilts fed NF with other dietary treatments being intermediate. It must be noted that gilts fed tallow or high levels of n-3 FA showed numerically lower gains than those fed the rest of fat sources, and gilts fed high levels of n-3 showed a higher feed/gain ratio than the rest. These results, although not significant except for the pigs fed NF, suggest that there could be differences in performance between the different sources of fat, and they could be important in practical conditions.

Laboratory analysis showed that main tissue FA profile modifications were due to dietary treatment although tissue fatty acid pattern depositions were observed.

Although tissue FA composition linked to dietary treatment produced important changes in lipogenic gene expression, tissue and age are more important factors determining mRNA contents of lipid metabolism related genes. Correlations between thyroid hormones and genes of fat synthesis in adipose tissue and also with expression of lipogenic genes at different ages, suggest that thyroid hormones could be a link between dietary FA and the observed changes in fat deposition.

Changes in fat metabolism due dietary fat also found to modify whole-body fat content. Whole-body fat content of HOSF (26.7%) and SFO (26.7%) fed animals was higher than T (22.9%) fed animals showing that saturated FA, but not PUFA,

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decreased fat deposition. Also a trend for reduced fat deposition was observed when high levels of n-3 were fed.

Whole body FA balance showed that the ratio of *de novo* FA synthesis ratio of pigs fed the very low fat diet was 1.5/1/3 for palmitic, stearic and oleic acids, respectively and it was modified by the dietary fat composition. The deposition of essential FA depended on dietary fat content and in general decreased with increasing FA length. Essential FA deposition was relatively high when dietary fat content was low whereas its deposition decreased when it was found in relatively high levels (>1 % from diet). Linolenic acid conversion to EPA was rather low and almost nul for DHA.

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INDEX

ACACA	acetyl CoA carboxylase
ACTH	adrenocorticotropic hormone
aFD	apparent faecal digestibility
aID	ileal digestibility
ATP	adenosine triphosphate
D6D	∆6-desaturase
DHA	docosahexaenoic acid
DM	dry matter
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acids
FAD	flavin adenine dinucleotide
FASN	fatty acid synthase
GC	gas chromatography
GH	growth hormone
HDL	high density lipoprotein
HNF	hepatic nuclear factor
HPRT1	hypoxanthine phosphoribosyltransferase
IDL	intermediate density lipoprotein
IGF	Insulin-like growth factors
LDL	low density lipoprotein
LT	longissimus thoracicus
LW	live weight
LXR	liver X receptor
mRNA	messenger ribonucleic acid

MUFA	monounsaturated fatty acids
NAD	nicotinamide adenine dinucleotide
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acids
RMSE	root mean square error
SCD	stearoyl CoA desaturase
SFA	saturated fatty acids
SM	semimembranosus
SREBP	sterol regulatory element-binding protein
T3	3,5,3'-triiodothyronine
T4	thyroxine
TNF	tumor necrosis factor
TSH	thyroid stimulating hormone
VLDL	very low density lipoprotein

BACKGROUND AND OBJECTIVES

Previous studies in broilers performed in IRTA showed that:

- Polyunsaturated fatty acids reduce fat deposition in separable fat depots with respect to monounsaturated and saturated fats.
- Polyunsaturated fatty acids produce lower abdominal fat deposition compared to saturated or monounsaturated fatty acids.
- An increase in the amount of dietary polyunsaturated fatty acids do not increse fat deposition in separable fat depots, while saturated fats do increase them.
- Polyunsaturated fatty acids reduce muscle fat cholesterol.
- Diets rich in polyunsaturated fatty acids improve feed to gain ratio.
- Dietary fatty acid profile modifies tissue fatty acid profile.

With these observations it was projected a new study in pigs with the following objectives:

- Determinate the ileal and faecal digestibility of fatty acids according to the fatty acid profile of the diet, in order to know the fatty acid availability in the growing trial.
- Determinate the composition of *de* novo synthesized fatty acids when the pig receives a diet without fat.
- Determinate if the fatty acid profile modifies the amount of deposited fat.

- Determinate if the fatty acid profile modifies the distribution of the fat: subcuteaneous, intramuscular, intermuscular, flare fat.
- Determinate if fatty acids are distibuted uniformely in the different tissues.
- To carry out a fatty acid balance to determine if some fatty acids are oxidyzed/deposited differently
- Determinate the deposition of essetial fatty acids and the conversion to their long-chain derivates (EPA and DHA)
- Determinate if the fatty acid profile modifies blood parameters and to study the expression of genes related to lipid metabolism.

INTRODUCTION

Lipid compounds

What are fats?

Although there is no precise definition, the term “fat” is generally applied to those foods or components of foods that are clearly fatty in nature, greasy in texture and immiscible with water (Gurr, 1984). The difference between fats and oils is basically the state at ambient temperature. Chemically, however, there is little distinction since the substances are all composed predominantly of esters (triacylglycerols or triglycerides) of glycerol with fatty acids (FA). Scientists use the more general term “lipid” to describe a chemically varied group of biological substances that are generally hydrophobic in nature and in many cases soluble in organic solvents (Smith, 2000). More accurate definitions are possible when lipids are considered from structural and biosynthetic perspective, and many classifications have been used over the years. But probably the most common classification of lipids is the division into “simple” and “complex” groups, with simple lipids being those yielding at most two types of products on hydrolysis (e.g., fatty acids , sterols, acylglycerols) and complex lipids (e.g., glycerophospholipids, glycosphingolipids) yielding three or more products on hydrolysis. This classification leads to 8 categories of lipids which in turn are divided in different subclasses (Fahy *et al.*, 2005) not mentioned in this work:

a) Fatty Acyls (example)

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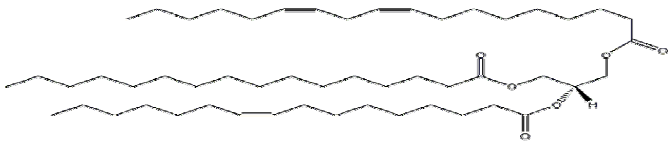
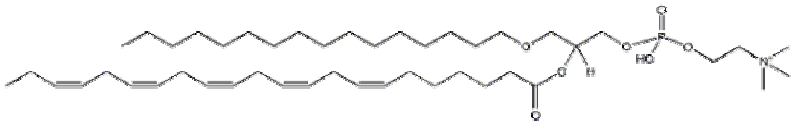
Fatty acid: Eicosapentaenoic acid (EPA)

b) Glycerolipids (example)



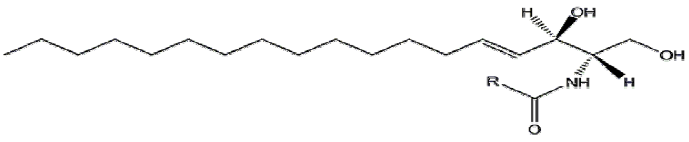
Triglyceride

c) Glycerophospholipids (example)



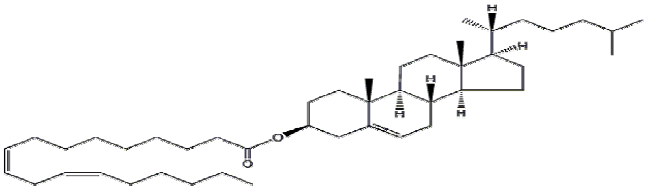
Glycerophosphocholines

d) Sphingolipids (example)



N-acylsphingosine (ceramide)

e) Sterol Lipids

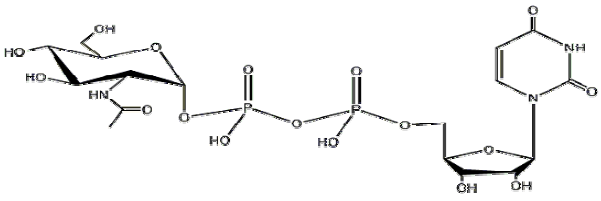


18:2 Cholesterol ester

f) Prenol Lipids

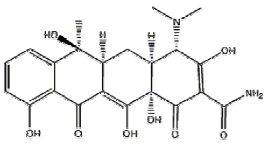
2E,6E-farnesol (Isoprenoids)

g) Saccharolipids



UDP-GlcNAc (Acylaminosugars)

h) Polyketides



Tetracycline (Aromatic polyketides)

Fats in live organisms

All before mentioned types of fats are differently distributed in live organisms and their functions and quantities differ greatly and sometimes their role is not limited to a single function.

Structural fats comprise mainly glycerophospholipids, glycolipids (glycerolipids) and sterol lipids which form the integral part of cell membranes acting as cell limits and mediating the cellular transport, intracellulary act as barriers between cell compartments and are also sites of production of many biochemical substances important in metabolism. Storage fats are mainly triacylglycerols (glycerolipids) and provide a high energy source in a low density compound. In animals, storage fat is a

long-term supply of energy stored in specialized cells, the fat cells, in adipose tissue. In plants, seeds store big amounts of oils prepared to supply energy to the plant embryo. The accumulation of fats in and their release from the adipose tissue are controlled by an interplay of dietary and hormonal factors. The fat molecules in membranes and in fat depots are present in large aggregates. Individual fat molecules may be released from either of these sources by specific enzyme processes and converted into metabolites with important functions. Examples are the prostaglandins from essential fatty acids, bile acids and steroid hormones from cholesterol. Even though fats are insoluble in water, they need to be transported between tissues in the body in the bloodstream, which in essentially an aqueous medium. This is solved by binding the fats to proteins in the form of lipoproteins.

Fats in feeds

The use fats in feeds

The main benefits of using fats in pig rations are first that fats serve as rich energy sources in feeds, and second they improve the physical properties of the final ration. Another benefit of adding fats in feeds is to supply essential FA, which are necessary to optimize performance mainly during the first growing stages of pig.

Fats are relatively inexpensive in terms of energy:price ratio meaning that its use will improve the benefits to producers. In this context, fats have a higher energy density what could lead to a higher energy intake during the growing period. This higher energy intake also could be linked to the fact that rations containing fats are more acceptable.

Fats assist in the formation of pellet and result in a less dusty product, helping to keep a cleaner ambient and atmosphere. However, there are physical limitations in the amount of fat that can be added to the ration, as it reduces pellet durability and makes the feed greasy.

Fat digestion and absorption

Fat is not soluble in the medium where it has to be digested and absorbed. This makes necessary a previous dispersion and transformation in more stable forms. Fat assimilation process is described by two phases: first, digestion or hydrolysis and second, absorption.

Fat digestion

Although the main part of digestion does not take place in stomach, a process of emulsion takes place in the stomach by means of gastric juices and the proteolytic action.

The main part of digestion takes place in the proximal part of the small intestine. Fat digestibility is lower than other nutrients such as proteins and carbohydrates because of insolubility in aqueous media. The bile, composed by detergent substances, is essential in the digestion process and absorption through the intestines because of its amphypatic properties. The bile, together with the pancreatic juices and stomach secretions, transforms drastically the physico-chemical properties of the emulsion originated in the stomach. The main components of bile are the bile salts with important emulsifying properties. Pigs secrete 1.8 liters of bile per day (Jorgensen *et al.*, 1992). Lipid digestion enzymes are from pancreatic origin, which are also composed by water and salts (as a buffer solution promoting the enzymatic action). Pancreatic enzymes with lipolytic action are lipases, cholesterol esterase, phospholipase A2. Bile salts favour lipid emulsification leading to the production of micelles that can be attacked by enzymes as the pancreatic lipase. In the action of the lipases, another protein called colipase is necessary which anchors the pancreatic lipase near the surface of the oil droplet despite the presence of bile salts that might otherwise displace it (Brindley, 1984). The bile salts, colipase and lipase are thought to interact to form a ternary complex, stabilizing the lipase and preventing its inactivation (Freeman, 1984).

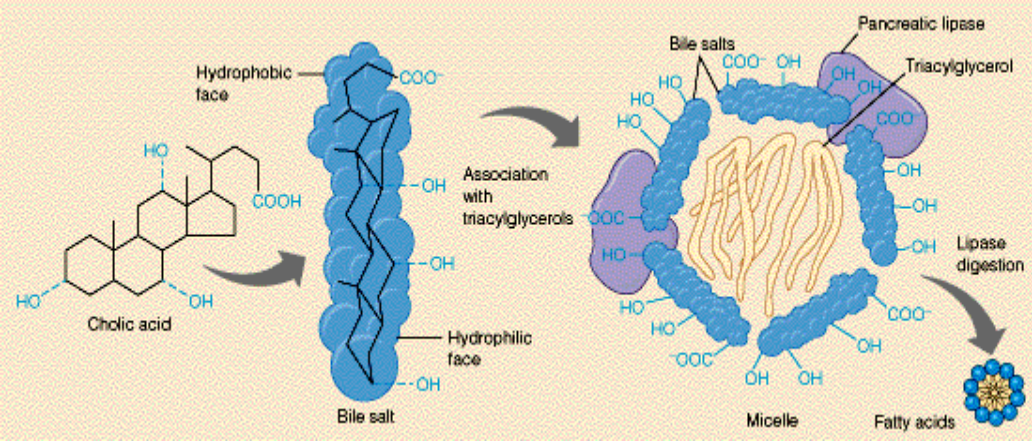


Figure 1: Bile salts emulsifying action in the intestines (Mathews *et al.*, 2002)

Factors that modulate the enzymatic action are pH, cofactors (e.g. calcium ion or colipase) and the amount of enzyme delivered during digestion, which depends on the amount of fat in diet (Reis de Souza *et al.*, 1992). Lipase hydrolyzes preferably FA found in positions 1 and 3 from triglyceride, and the reaction products are a mixture of glycerol, fatty acids, monoacylglycerol and diaacylglycerol (less than 10% of triglycerides are not hydrolyzed). Therefore, the process transforms fat in more polar derivates, which interact with the aqueous phase.

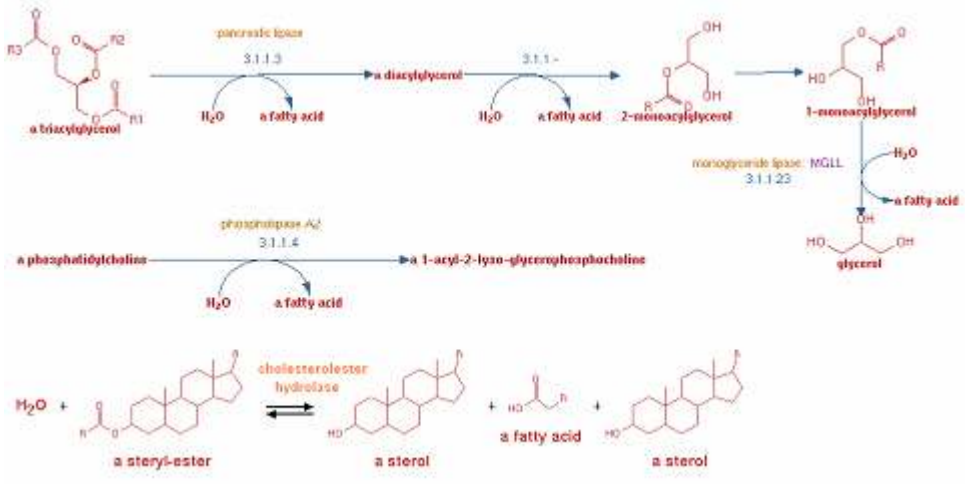


Figure 2: Hydrolysis of triacylglycerides, phosphatidylcholine and cholesterol ester (Karp, 2007)

Fat absorption

Glycerol, fatty acids, monoacylglycerol and other derivates as lisophosphoglycerol or cholesterol are absorbed by intestinal cells (enterocytes) from jejunum and ileum, where they are re-esterified producing triacylglycerides, phospholipids and cholesterol esters.

The digested lipids in the micellar phase are carried to the surface of the enterocytes where they can encounter two barriers for their absorption. The first is a layer of unstirred water at the surface of the microvillus membrane. This layer is thought to provide the major rate-limiting factor in the uptake process (Dietschy, 1998). It is believed that absorption across the second barrier, the lipid membrane of the microvillus, could occur when the micelles collide with it. Short-chain fatty acids are absorbed more rapidly than long-chain fatty acids because of their different solubility in the aqueous medium, meaning that the rate limiting factor in the absorption process is the diffusion in the cell surface. Long-chain FA are more hydrophobic, therefore they need to be associated to micelles through the aqueous medium from the cell surface and finally cross the cell membrane. Monoacylglycerole and lysophospholipids are practically all absorbed, whereas cholesterol is absorbed more slowly and not completely. Absorption of digestion products is independent from substrate content, but is decreased when the intestinal flux increases substantially (Freeman, 1984). At the jejunum level the main part of absorption of lipid hydrolysis products takes place (Sambrook, 1979). Bile salt absorption takes place at the distal ileum, where is redirected to the liver via portal vein, closing the so-called enterohepatic circulation. About 95% of the bile acids which are delivered to the duodenum will be recycled by way of the enterohepatic circulation.

Although the translocation process of FA and monoacylglyceroles has not been well elucidated, it is thought to consist in passive diffusion through the plasmatic cell membrane. Also it has been speculated about the possibility that this transport would be mediated by specific cell transporters (Lowe and Sacchettini, 1987), it seems that this transporter would not be essential during lipid absorption, at least in mouse, although it would play a role in lipid depot contents (Vassileva *et al.*, 2000).

Once lipids are absorbed by enterocytes, they move to the endoplasmic reticulum surface where they are reesterificated via the monoacylglycerole pathway and to a lesser extent by the alpha-glycerophosphate pathway. Then triglycerides are transferred inside the endoplasmic reticulum where they are incorporated to chylomicrons, which in turn are transferred to the bloodstream.

Non absorbed fats in the ileum are hydrolyzed in the colon by bacterian lipases delivering FA which will not be absorbed by the intestine, but are susceptible to be modified by intestinal flora. In addition, bacteria can synthesize FA from acetate, meaning that excreta FA composition will be different from dietary FA (Meunier *et al.*, 1989).

Definition of the term “digestibility”

The definitions related above, refer to digestibility as the breakdown of dietary nutrients to products ready to be absorbed by the intestinal tract. But in Animal Nutrition, digestion and absorption are just described by the term digestion. Generally, nutrient digestibility is defined as the nutrient ratio not present in the excreta and therefore is supposed to be absorbed (McDonald *et al.*, 1979).

Commonly, faecal digestibility or apparent digestibility is described as a digestibility coefficient, showing the digested percent of a specific nutrient. This value doesn’t

evaluate endogenous secretions and desquamations, bacterial modifications of nutrients…(Sambrook, 1979):

$$Apparent\ digestibility\ (\%) = \frac{fat\ ingested - fat\ excreted}{fat\ ingested} * 100$$

Because some endogenous or metabolic loss occurs due to endogenous secretions, due to loss of bile salts, cell desquamations, structural lipids from mucosa, the apparent digestibility of fat underestimates the true digestibility, which is calculated as (Jorgensen *et al.*, 1993):

$$True\ digestibility\ (\%) = \frac{fat\ ingested - (fat\ excreted + endogenous\ \ fat\ loss)}{fat\ ingested} * 100$$

Because fat is not absorbed beyond the ileum and avoiding the effects of intestinal microflora, a more accurate calculation can be performed at the ileum, named ileal digestibility:

$$Ileal\ digestibility\ (\%) = \frac{fat\ ingested - fat\ excreted\ ileum}{fat\ ingested} * 100$$

Different surgical techniques are described for collection of ileal digesta (Köhler *et al.*, 1991), in addition different excreta collection methods can be chosen (total collection, use of markers TiO_2, Cr_2O_3…). This broad spectrum of measuring techniques results in a wider array of methods for nutrient digestibility and some differences in their results.

Factors affecting fat digestibility

From the different digestibility trials found in the literature, it can be deduced that in generally digestibility is over 95% when fats are liquid at physiological temperature, and decreases rapidly when the melting point is over physiological temperature

(Laplace *et al.*, 1986). The following table shows FA digestibility of diets rich in a specific FA (therefore minimizing the effects of endogenous fats and microflora) (Table 1).

Table 1. Fatty acid digestibility of different rich fat diets

Fatty acid	g FA/kg diet	Faecal Digestibility%	Ileal Digestibility%
C8:0	9.8[a]	-	100
C10:0	7.5[a]	-	100
C12:0	36.3[a]	-	96.8
C14:0	19.2[b]	97.8	97.2
	19.2[a]	-	91.8
C16:0	21.7[b]	94.4	94.2
	25.7[e]	76.9	78.8
	33.5[d]	75.4	76.1
C16:1	4.6[d]	91.4	92.5
C18:0	18.6[d]	4.3	75.0
	12.3[e]	35.9	62.0
	2.6[b]	-10.4	36.3
C18:1 (n-9)	64.8[c]	99.2	95.3
	50.8[d]	86.9	85.7
	42.6[e]	97.0	95.0
	15.9[f]	97.6	95.2
C18:2 (n-6)	24.5[c]	99.4	93.9
	14.3[f]	99.5	94.0
C18:3 (n-3)	10.8[c]	98.8	97.2
	4.3[f]	97.2	98.2
C20:1 (n-9)	9.5[f]	96.8	94.9
C20:5 (n-3)	8.1[b]	99.9	98.1
C22:1 (n-9)	42.2[f]	84.7	79.4
C22:6 (n-3)	13.7[b]	94.9	96.8

a: Coconut oil (Jorgensen *et al.*, 2000)
b: Fish oil (Jorgensen *et al.*, 2000)
c: Rapeseed oil (Jorgensen *et al.*, 2000)
d: Animal fat (Jorgensen *et al.*, 1992)
e: Tallow (Ozimek *et al.*, 1984)
f: Rapeseed oil (Ozimek *et al.*, 1984)

From this table, it can be deduced that individual FA digestibility increases with increasing unsaturation, and decreases with FA length.

The observation that increasing unsaturation ratio of fats, fat and FA digestibility increase has been previously described by (Jorgensen and Fernández, 2000), and also Wiseman *et al.* (1990) and Powles *et al.* (1993) have observed that increasing the ratio of unsaturated FA/saturated FA, digestibility increases.

The position in the triglyceride also modifies digestibility as described by Averette Gatlin (2005), observing that FA digestibility from stearic acid found in position 2 from triglyceride had lower digestibility.

The FA length modifies fat and FA digestibility as described by Cera *et al.* (1989), although results in the literature are not conclusive (Jorgensen *et al.*, 2000).

Literature widely describes an increase in faecal fat digestibility as fat inclusion level in diet increases (Eeckhout and De Paepe, 1988, Wiseman *et al.*, 1990, Powles *et al.*, 1993 and 1994). A similar effect was found at ileal level (Jorgensen *et al.*, 1993). This is due a higher interference from endogenous secretions when fat inclusion is low (Jorgensen and Just, 1988).

Others factors modifying fat digestibility are weaning (Cera *et al.*, 1989): weaned pigs have lower digestibility, age (Le Goff and Noblet, 2001),type and protein content has modified fat digestibility in some experiments (Just, 1982, Li and Sauer, 1994) but not in others (Jorgensen *et al.*, 1996, Jorgensen and Fernández, 2000, Jorgensen *et al.*, 2000), while fiber decreases fat digestibility (Le Goff and Noblet, 2001).

Pig lipid metabolism

Introduction

Meat tissues are composed of five primary chemical constituents: water, proteins, lipids, carbohydrates and inorganic matter. In the work of Shields *et al.* (1983) it was found that at 109 kg live weight (LW), pig empty body composition was 51.0% water, 14.4% crude protein, 30.4% fat and 2.5% ash, whereas pork skeletal muscle separable lean (leg, loin and shoulder) composition is 72.3% water, 21.1% protein, 5.9% fat and 1.0% ash (USDA, 2006). At early stages, pig has a very low fat content and during its growth increases its protein content and bone and fat mass. While protein deposition is determined by its genetic capacity, fat deposition depends on energy intake. At birth, pig body fat content is 1.73% whereas at 145 kg LW its content can reach 41.1% mainly in separable fat depots (subcutaneous fat, intermuscular fat and flare fat). This increase is mainly at the expense of body water and during the main part of growth, whereas changes in protein and ash ratio are minimal. When animals are in a positive energy balance, FA are deposited in general without modification. Consequently, fatty acid composition will depend on dietary FA composition; diets rich in PUFA (linseed or soybean oil) will have high PUFA contents in fat, animals fed diets rich in MUFA will have high MUFA contents and diets rich in saturated FA will have hign saturated FA contents (Table 2). Animals fed low fat diets, will reflect *de novo* FA synthesis.

Animal adipose tissue is composed primarily of neutral lipids (triglycerides) and phospholipids that collectively range from 1.5% to 13% in muscle tissue. Lipids also exist as sterols and sterol esters (cholesterol components) and cerebrosides. Various lipid forms serve as an energy source for the cell, as structural and functional

component of the cell membrane, as insulation or protection for vital organs, and as solubilizing agents for certain hormones and vitamins (A, D, E, and K). Fats can be metabolized to yield 2.25 times more energy than carbohydrates or proteins and thus are an energy-dense nutrient.

Table 2. Back fat fatty acid composition (%) of pigs fed different fat added diets

	Fat free[a]	Linseed oil[b]	Olive oil[b]	Tallow[c]	Soy oil[c]
C14:0	1.4	0.9	1.2	1.4	1.2
C16:0	15.9	16.4	18.0	22.8	21.1
C16:1	6.5	1.2	1.7	-	-
C18:0	5.6	10.3	9.0	13.8	12.6
C18:1	62.4	33.3	51.5	44.0	34.8
C18:2	8.1	14.2	11.8	8.8	21.4
C18:3	-	19.0	1.5	1.0	2.3
C20:4	-	0.2	0.4	0.2	0.2
C20:5	-	0.2	0.02	-	-
C22:5	-	0.4	0.2	0.1	0.1
C22:6	-	0.08	0.09	-	-
SFA	23.0	28.1	28.7	38.7	35.2
MUFA	68.9	37.5	57.0	50.7	39.4
PUFA	8.1	34.4	14.3	10.6	25.4

[a] Madsen *et al.*(1992). Diet consisted of 85% sucrose, 10% tapioca meal, 5% potato starch. Casein was used as protein source and 1% sunflower oil was added in order to meet linoleic requirements.

[b] Nuernberg *et al.* (2005). Basal diet consisted of 20% barley, 23% wheat, 10% rye, soybean meal 15%, 4% rapeseed meal, 15% triticale, 7% wheat bran, 2% soybean oil and 1.5 soybean oil. Fat was added at 5% to basal diet

[c] Bee *et al.* (2002). Diet consisted of 42% wheat, 30% flaked potatoes, 12% soybean meal, 2.5% potato protein and 2% yeast. Fat was added at 5%

Lipoprotein circulation and lipoprotein lipase action

Lipoprotein metabolism

As in the digestive tract, lipids have to avoid the solubility problems in the aqueous medium which is the bloodstream. This is achieved by transporting them in lipoproteins, a biochemical assembly that contains both proteins and lipids.

Chylomicrons are the lipoproteins transporting fats from the enterocyte to the lymph vessels by exocytosis and these vessels are emptied into the vena cava. Then fats are transported to peripheral tissues (heart, muscles and adipose tissue). VLDL function is similar to chylomicrons, but instead of transporting triglycerides from enterocytes, they are from liver origin. Triglycerides from both types of lipoproteins are hydrolyzed to glycerol and FA in the surface of the tissue facing the blood vessel. Hydrolyzation is achieved by the activation of lipoprotein lipase by the apoprotein CII present in the lipoproteins (LaRosa *et al.*, 1970). Then FA migrate directly through the cell membrane or are linked with albumin and are transported to distant cells. Inside the cell, FA are catabolized, or in adipose cells are used to synthesize triglycerides. The glycerol returns to the liver where is used to synthesize glucose in the gluconeogenesis pathway. As a consequence of the hydrolysis, chylomicrons are transformed to chylomicron remnants and VLDL to IDL or LDL. Chylomicron remnants are taken up by the liver by receptor-mediated endocytosis, LDL and IDL are taken up by the liver LDL receptor.

The LDL are the main form of cholesterol transport to tissues and HDL are the main form of transport of the excess of cholesterol from tissues to the liver where it is excreted or metabolized.

Type and amount of dietary fat modifies pig plasma and lipoprotein lipid composition (Faidley *et al.*, 1990). In humans, dietary fat saturation plays a considerable role in modulating plasma cholesterol concentrations and determining the risk for coronary heart disease. Swine have been used as a model for many human conditions including type 1 (insulin-deficient) and type 2 (insulin-resistant) Diabetes Mellitus research because of their phenotypic similarities to humans including: cardiovascular anatomy and function, metabolism, lipoprotein profile, size, tendency to obesity, and omnivorous habits (Bellinger *et al.*, 2006). As in humans, in postprandial pigs, the amount and type of dietary fat has an effect on composition of lipoproteins (Luhman *et al.*, 1992). Saturated FA (SFA), specifically myristic and palmitic acids, are recognized as the single dietary factor that has the greatest negative effect incrising LDL cholesterol concentrations and therefore incrising cardiovascular desease incidence. In contrast, monounsaturated FA (MUFA) and PUFA have been shown to decrease plasma cholesterol concentrations in clinical studies (Nair *et al.*, 1996, Temme *et al.*, 1996, Kris-Etherton and Yu, 1997, Wijendran and Hayes, 2004).

Lipoprotein Lipase

Lipoprotein lipase appears to be the rate limiting step in the FA uptake from lipoproteins (Nilsson-Ehle *et al.*, 1980). Thus hydrolysis of triglyceride and uptake of FA by adipocytes in meat animals provides substrate for subsequent triglyceride accretion which, in turn, leads to an increase in adipocyte volume and, hence, an increase in adipose tissue mass accretion (Kris-Etherton and Etherton, 1982). Also it has been suggested that lipoprotein lipase is a good indicator of the rate of lipid deposition in swine (Allen *et al.*, 1976) and the enzyme probably is important for maintenance of the dynamics of lipid metabolism to provide FA to the adipose tissue. Because of these observations, factors that affect lipoprotein lipase activity are

important determinants of the FA taken up by adipocytes from the plasma lipoproteins and indirectly from the diet.

Different factors modulate lipoprotein lipase activity (Goldberg and Merkel, 2001) and regulate its gene expression (Merkel *et al.*, 2002). Among these factors triglycerides and FA composition would play its particular role. Saturated FA appear to be less preferred substrates than unsaturated FA. The 1 position in triglycerides and phospholipids is hydrolyzed in preference to the 2 position (Olivecrona, 1987). Linolenic acid, may also increase lipoprotein lipase gene transcription during adipocyte differentiation (Amri *et al.*, 1996).

Endocrine influences on fat metabolism

The endocrine system involves hormones of varied nature including glycoproteins and peptides, and steroids. From observations in fetal pigs (Ramsay *et al.*, 1987), it is assumed that central neural mechanisms do not significantly affect lipogenesis in adipose tissue and that metabolic effects occurring in adipose tissue are to the consequence of endocrinology changes. According to Mersmann (1986), adipose tissue lipogenic capacity may be regulated by different hormones, and probably the major anabolic hormone in nonruminant mammalian species is insulin, although, it has been suggested that the role of insulin in acute regulation of lipogenesis in swine adipose tissue is not clear. According to O'Hea *et al.* (1970) it is known that thyroid hormones and insulin regulate lipogenesis, but in pigs, insulin may be less important compared to other species.

Different hormones that are directly or indirectly associated with the metabolic control of fat metabolism are described below.

Growth hormone

Growth hormone (GH) is a protein consisting of 191 amino acid residues considered as the most important hormone regulating growth processes. It has anabolic and catabolic functions in the body. Possibly different parts of the same molecule play different roles in metabolism. As an anabolic agent it has an important role in controlling nutrient partitioning in the processes of growth and lactation. Contrarily, it can be lipolytic and diabetogenic and therefore clearly catabolic in its function.

In this context, in pigs selected for high backfat had lower GH contents compared to a breed selected for low backfat (Althen and Gerrits, 1976). The GH clearly antagonized lipid accretion by preadipocytes *in vitro* and did not affect directly preadipocyte differentiation but suppressed lipid deposition and lipogenic enzyme activities (Hausman and Martin, 1989). In growing pigs, GH increased the abundance of Insulin Growth Factor-1 (IGF-I) mRNA, inhibited *in vitro* glucose oxidation and lipogenesis in adipose tissue (Wolverton *et al.*, 1992) and the addition of dietary fat did no interfere in its action and did not affect IGF-1 mRNA contents.

Adrenocorticotropic hormone (ACTH or corticotropin)

ACTH is a polypeptide hormone produced and secreted by the pituitary gland. It plays an important role in the hypothalamic-pituitary-adrenal axis. ACTH stimulates the cortex of the adrenal gland and promotes the synthesis of corticosteroids, mainly glucocorticoids but also sex steroids. In mouse, but not in human, ACTH induces lipolysis (Kiwaki and Levine, 2003).

Catecholamines

Catecholamines are released by sympathetic nerves and by adrenal medulla and have important regulatory functions in the adaptation of the body to rest and stress

situations. The main catecholamines produced are epinephrine and norepinephrine and they act on a wide range of tissue cells, distant from sites of release. The main effect on fat cells is to stimulate lipolysis in order to induce thermogenesis.

Insulin-like growth factors (IGFs)

Somatomedins, named IGFs because they are structurally similar and have properties similar to those of insulin, were discovered as the “sulphate factors” because they regulate chondroitin sulphate formation in cartilage. Although several IGFs have been identified it is generally considered that IGF-1 and IGF-2 are the two most important somatomedins which provide the ultimate endocrine link in the chain of hormones regulating cellular growth. Frick *et al.* (2000) observed in rats that IGF-1 altered adipose tissue metabolism reducing the capacity of metabolize glucose. The IGF-1 stimulates porcine preadipocyte proliferation and differentiation in stromal-vascular cells and preadipocyte cells, and in stromal-vascular cells stimulates preadipocyte proliferation and enhances differentiation, in part by increasing the number of fat cell per cluster (Ramsay *et al.*, 1989).

Insulin

Insulin is clearly anabolic in different aspects: in its mode of action, influences the levels of others hormones, through its strong lipogenic properties, in affecting body composition. In many ways, insulin is referred as an antilipolytic hormone, but it can also influence lipolytic events.

The main action in adipose tissue is to inhibit the activity of the hormone-sensitive lipase, reducing the release of free FA and glycerol.

The differences between breeds of pigs, in body composition relative to insulin levels, is illustrated by the fat partitioning index proposed by Lister (1976). This index

separates adipose tissue into internal and subcutaneous components and the hypothesis that insulin levels are higher in fatter animals with a high fat partitioning index.

Glucagon

Glucagon is an important hormone involved in carbohydrate metabolism. It is produced by the pancreas, and is released when the glucose level in the blood is low (hypoglycemia), causing the liver to convert stored glycogen into glucose and release it into the bloodstream. The action of glucagon is thus opposite to that of insulin, which instructs the body's cells to take in glucose from the blood when body is satiated. Among different actions it induces liberation of free FA to bloodstream and it regulates the activity of lipogenic enzymes (Sul and Wang, 1998).

Thyroid hormones

Thyroid hormones exert actions in all tissues and affect many metabolic pathways. Their physiological actions can be divided into effects on cellular differentiation and development, and effects on metabolic pathways. Two principal metabolically active compounds are produced: thyroxine (T4) and 3,5,3'-triiodothyronine (T3). Although T4 is the predominant form secreted, T3 is probably the more active form, having both a wide distribution in tissues and a high affinity for nuclear binding sites.

Alteration in oxygen consumption is one of the signs defining thyroid hormone action. Thyroid hormones are important regulators of energy metabolism, and different mechanisms are involved in this action, one of them is to promote gene expression and enzyme activity of lipogenic genes as shown in rat liver (Roncari and Murthy, 1975, Dozin *et al.*, 1986).

Thyroid stimulating hormone (TSH) is the most sensitive and useful indicator of thyroid hormone levels.

Glucocorticoids

Glucocorticoids are a class of steroid hormones characterized by the ability to bind with the cortisol receptor and promotes similar effects. The name glucocorticoid derives from the observations that these hormones were involved in glucose metabolism. Glucocorticoids are catabolic in their action (Buttery, 1983) and it is likely that insulin acts to counteract their action (Odedra and Millward, 1982). Glucocorticoids can have a marked effect on body composition because in addition to their catabolic effects for proteins they can be strongly lipogenic in some species. In pigs the influence of hydrocortisone on adipose tissue development may be direct and mediated by development of glucocorticoid receptors (Hausman and Hausman, 1993).

Sex steroids

The androgen testosterone and oestrogens act as potent anabolic agents in the body. As such they have been used widely as exogenous stimulants, in particular for young cattle and sheep.

Testosterone is an extremely potent growth stimulant contributing to the superior growth rates of entire males, compared with castrated males in pigs, but also in different species. Adipose tissue contains the enzyme to aromatize testosterone and testosterone reduces fat deposition.

Prolactin

Prolactin is a peptide hormone primarily associated with lactation. In a similar mode of action to growth hormone, prolactin acts as a homeorhetic control and may alter the capacity for net protein accretion in muscle as well as altering the metabolism of other tissues, in particular adipose tissue.

Adipokines

In addition to the view of the adipose tissue as affected by hormonal regulation, it could be viewed as an organ due its secretory functions modifying hormone contents as previously shown in sex steroids. Some of these hormones are leptin, adiponectin, resistin and TNFα.

Leptin, although does not influence directly lipid metabolism, plays a role on food intake and therefore on lipid deposition. Cholecystokinin and glucagons are two important hormones affecting satiety but probably leptin is the most important on growth and tissue deposition. Leptin is a peptide produced by adipocytes which is considered to signal the magnitude of white adipose tissue deposits within the body. Receptors have been identified in several parts of the brain, notably the hypothalamus, but also in other tissues including the ovaries. In porcine adipose tissue can act on tissue lipid accretion by altering lipolysis and lipogenesis (Ramsay, 2001).

Resistin, a recently discovered hormone (Steppan *et al.*, 2001), is related to inflammatory function in man. Serum content of this hormone correlates well with its mRNA expression in human subcutaneous adipose tissue (Heilbronn *et al.*, 2004); therefore there is a link between resistin and adipose tissue mass, as for leptin.

Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and FA catabolism. Adiponectin is exclusively secreted from adipose tissue into the blood and is very abundant in plasma relative to many hormones. Levels of the hormone are inversely correlated with body mass index (Weyer *et al.*, 2001). The hormone plays a role in type 2 diabetes (Weyer *et al.*, 2001) and coronary artery disease (Hotta *et al.*, 2000). In pigs, adiponectin acts regulating lipid metabolism in the adipocyte, and may thus be a determinant of adipocyte size and overall lipid accumulation (Jacobi *et al.*, 2006).

Fat utilization

Fat oxidation

Under most circumstances dietary fat is deposited in fat depots, mostly unaltered because growing pigs in commercial conditions are in a positive energy balance as they are fed *ad libitum* (Chwalibog and Thorbek, 1995), whereas in nursing and weaned piglets, dietary fat oxidation rates are higher (Odle, 1997). Fatty acids are readily used as an oxidative energy source by many tissues of the body including skeletal muscle, liver, cardiac muscle, and adipose tissue. Mitochondrial oxidation of FA yields CO_2 and ketone bodies as products. Different studies have attempted to determine the oxidation rates of FA using different kinds of labeled FA in rodents and humans and they agree in that SFA are catabolized to a lesser extent than unsaturated FA (Jones *et al.*, 1985, Leyton *et al.*, 1987) and that the degree of oxidation of FA decreases as FA length increases (Bjorntor.P, 1968, Leyton *et al.*, 1987). In relation to unsaturated FA there are few disagreements and it seems that as unsaturation increases, oxidation increases (DeLany et al., 2000). It appears that different factors as feeding status, dietary fat level, developmental stage, FA

composition influence FA partitioning into deposition and oxidation (Ide *et al.*, 1996, Poumes-Ballihaut *et al.*, 2001, Iritani *et al.*, 2005). In the work in pigs of Chwalibog *et al.* (1992), they concluded that, based on data obtained in respiration chambers, all digestible dietary lipids were stored; therefore there was no oxidation of dietary fat. This observation is in contrast to the previous work of Flanzy *et al.* (1970) who observed a net oxidation for linoleic acid of 50%, and in a recent work of Kloareg *et al.* (2007) the oxidation rates for linolenic acid and linoleic acid of 69% and 60% respectively.

Long-PUFA synthesis

Polyunsaturated fatty acids (PUFA) from the n-3 and n-6 series are always of dietary origin, since pig does not synthesize them and they serve as substrates for synthesis of long-PUFA (Figure 3); linoleic acid to arachidonic acid and linolenic acid to EPA and DPA and, to a lesser extent, DHA, and to hormones and prostaglandins. In adult man, the apparent conversion of linolenic acid to EPA is limited (les than 8%) and is even less for DHA (less than 4%) (Burdge and Calder, 2005). In the work of Kloareg *et al.* (2007) more than one third of the digested linolenic acid was deposited as EPA, DPA and DHA, suggesting that its conversion to metabolites is more efficient in pigs than in man.

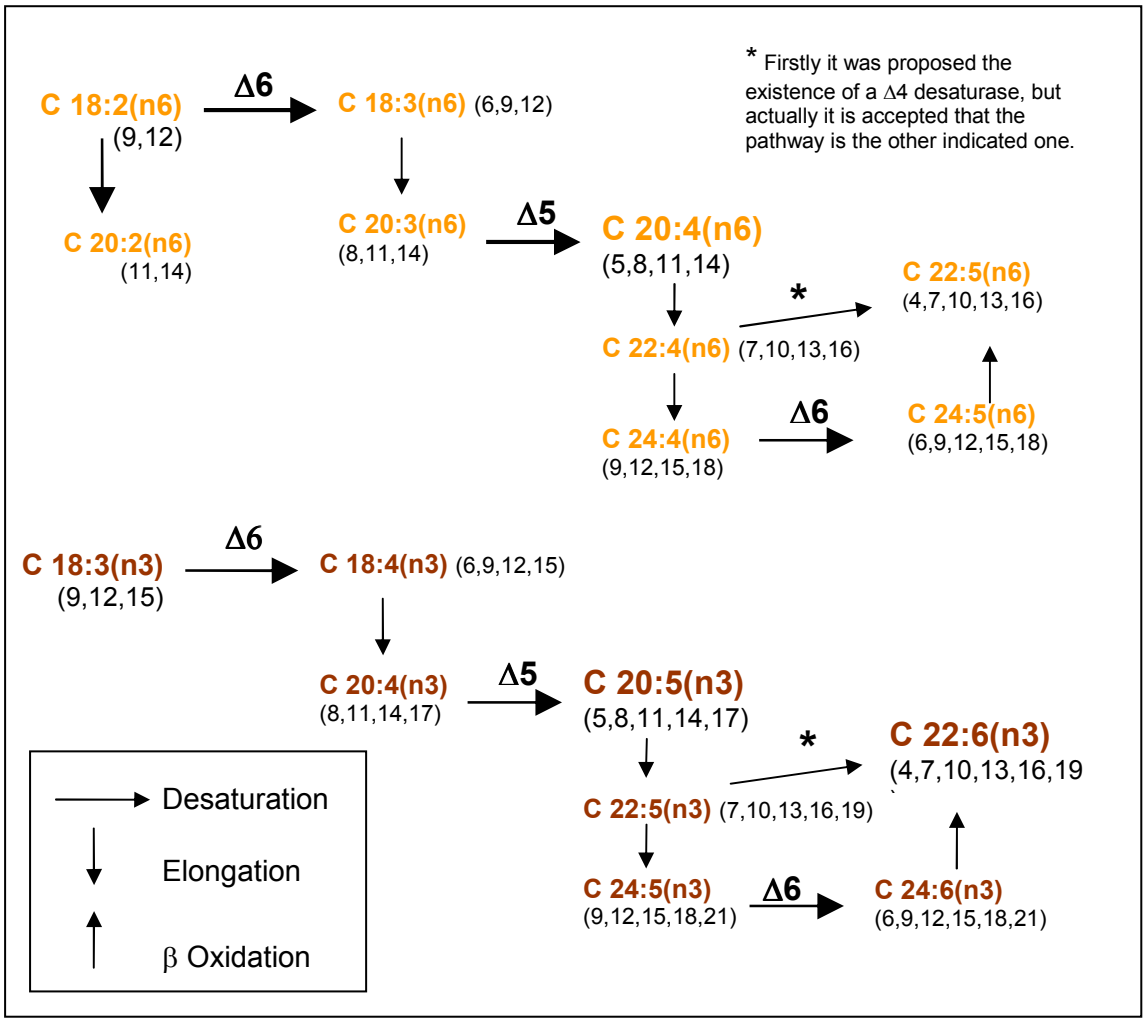


Figure 3. Synthesis pathways of long-PUFA in animals. In brackets it is indicated the position of the unsaturations in the in the FA chain.

De novo fat synthesis

Pigs can synthesise fats from carbohydrates or proteins, but in a positive energy balance they mostly use carbohydrates. In western countries pig diets tend to be quite low in fat content (2 to 4% of the diet) and rarely access the 10-15%. The consequence of feeding low-fat diets is that the organism must synthesize a considerable amount of FA, *de novo*. Glucose is metabolized via glycolysis to pyruvate, enters the mitochondrion, traverses the initial steps of the tricarboxylic acid cycle to citrate, exits the mitochondrion, is cleaved to acetyl-CoA by ATP-citrate lyase, is carboxylated to malononyl-CoA by acetyl-CoA carboxylase, and then is

polymerized to palmitic acid by fatty acid synthase. Much of the FA that is synthesized enters into the production of triacylglycerides. Glucose also provides most of glyceride-glycerol for triglyceride synthesis. More than 70% of the glucose carbon is in glyceride-FA in older growing pigs (O'Hea and Leveille, 1969).

Pig *de novo* fat synthesis largely takes place in the adipose tissue (O'Hea and Leveille, 1969, Bergen and Mersmann, 2005). In pigs, *de novo* FA synthesis leads to a ratio between C16:0/C18:0/C18:1 of 1.5/1/3 (Leat et al., 1964). Mersmann (1986) suggested that fatty acid synthase enzyme properties observed in swine liver would result in carbon incorporated for FA elongation rather than *de novo* synthesis. The factors affecting lipogenesis rates include age, cell size, sex and feed intake, and have been reviewed by Mersmann (1986) and will not be discussed here, just the effects on FA composition on lipogenesis will be discussed in the next chapters.

Transcription regulation of fat metabolism

The regulation of FA and triacylglycerol synthesis and catabolization is regulated at several steps, and involves transcriptional and posttranscriptional controls that respond to specific hormones, to metabolites derived from diet, to fasting and refeeding, to exercise-mediated energy expenditure. Important roles are played by transcription factors, proteins that bind to DNA and help to regulate the rate of transcription of specific genes. Some of these transcription factors that regulate the transcription of some enzymes of FA and triacylglycerol synthesis are described next with a summary table (Table 3).

Table 3. Hormone and fatty acid regulation of hepatic metabolism (Jump, 2002)

		Fatty acid regulated transcription factors					
	Insulin	PUFA	PPARα	HNF-4α	LXRα/β	SREBP1c	SREBP2
Glucose utilization							
L-PK	+	-	-	+			
Glucose storage	+						
Lipogenesis							
CL, ACACA, FASN	+	-			+	+	
Triglyceride synthesis							
GPAT	+				+	+	
Apolipoproteins							
Apo CII, CIII	+						
Fatty acid desaturation							
Δ5-desaturase		-					
Δ6-desaturase		-					
Δ9-desaturase (SCD)	+	-	+			+	
Cholesterol synthesis							+
Gluconeogenesis							
(PepCk)	-		+	+		-	
Fatty acid oxidation							
Mitochondrial (CPT1)	-	+	+				
Peroxisomal (AOX)		+	+				
Microsomal (CYP4A)		+	+	+			
VLDL secretion							
ApoB				+			
MTB	-					-	
Bile acid synthesis							
Chol 7α hydroxylase	-			+	+		
Chol 27 hydroxylase	-						
Ketogenesis							
Mitochondrial HMG CoA synthase	-		+				

ACACA, acetyl CoA carboxylase; AOX, acyl CoA oxidase; apoC, apolipoproteins CII and CIII; apolipoprotein B; CL, ATP-citrate lyase; CPT1, carnitine palmitoyl transferase I; CoA, coenzyme A; CYP4A, cytochrome P450 monooxygenase-4A; FASN, fatty acid synthase; GPAT, plycerol phosphate acyl transferase; HMG, hydroxymethulglutaryl; HNF-4α, hepatic nuclear factor-4α; L-PK, L-type pyruvate kinase; LXR, liver X receptor; MTP, microsomal transfer protein; PepCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferators activated receptor; PUFA polyunsaturated fatty acid; SCD1, stearoyl CoA desaturase; SREBP, sterol regulatory element binding protein; +, induce; -, repress

Sterol regulatory element-binding protein (SREBP)

SREBPs belong to the helix-loop-helix family of transcription factors. Different isoforms have been identified and SREBP-1c and SREBP-1a are important for the regulation of genes involved in lipid synthesis (Osborne, 2000). Transgenic mice overexpressing SREBP-1a develop fatty liver because of the induction of lipogenesis

and triglyceride synthesis. In isolated hepatocytes, dominant-negative SREBP-1c inhibits the glucose-induced stimulation of pyruvate kinase, fatty acid synthase and acetyl CoA carboxylase mRNA, showing that SREBP-1c is required for the glucose effect on lipogenesis (Foretz *et al.*, 1999).

Worgall *et al.* (1998) reported that SREBP-1 and SREBP-2 were targets of FA control in established cell lines. Subsequent reports established that only SREBP-1c was a major target of PUFA control in liver (Brown and Goldstein, 1997, Xu *et al.*, 1999). The principal mecanism for SREBP regulation of gene transcription involves control of its nuclear abundance, and n-3 PUFA enhance SREBP1c-mRNA decay (Xu *et al.*, 2001).

Liver X Receptor (LXR)

LXRα and β are nuclear receptors that are activated by oxysterols which decrease excess free cholesterol in cells by activating genes that control the rate of bile acid synthesis and cholesterol efflux. Promoter analysis indicate that the LXR response element in SREBP-1c promoter is not required for PUFA suppression of gene transcription. This observation (within others), coupled with the absence of PUFA suppression of many LXR-regulated genes *in* vivo, argue against LXRα as a target for PUFA control of gene transcription *in vivo* (Jump *et al.*, 2005).

Peroxisome Proliferator-activated receptor γ and α

PPAR plays a major role in the regulation of genes involved in glucose and lipid metabolism including FA transport, FA binding proteins, FA-CoA synthesis, microsomal, peroxisomal and mitochondrial oxidation, desaturation … PPARs bind DNA as heterodimer with Retinoid X receptor at direct promoters of these genes (Desvergne and Wahli, 1999).

The PPARγ is a nuclear transcription factor whose ligands include 15-deoxy-$\Delta^{12,14}$prostaglandin, FA and the glitazone drugs. As a part of its general effect in promoting the differentiation of adipocytes, PPARγ markedly upregulates the expression of most enzymes of lipogenesis and of triglyceride synthesis (Willson *et al.*, 2001).

The PPARα is a transcription factor that is activated by fibrates and by FA, particularly in fasted animals. When compared to fibrates, however, 20-carbon PUFA are weak PPARα activators (Desvergne and Wahli, 1999), although different FA are known to activate PPARα (Jump, 2002).

Hepatic nuclear factor-4α

A wide range of hepatic genes is controlled either directly or indirectly by HNF-4α. These include the genes encoding apolipoproteins and bile acid synthesis (Hayhurst *et al.*, 2001). HNF-4α bind fatty acyl CoA and FA (Dhe-Paganon *et al.*, 2002). According to Jump *et al.* (2005), additional studies are required to evaluate the role that FA, fatty acyl CoA play in the control of HNF-4α and its regulatory network *in vivo*.

Enzymatic regulation of fat metabolism

The main step in cellular fat metabolism regulation lies in the different cellular compartimentation of FA oxidation and synthesis allowing each process to be individually controlled and integrated with cellular requirements. Although the starting material of one process is the ending product of the other, the enzymatic equipment is also different.

Enzymatic regulation of fatty acid biosynthesis

The main pathway for *de novo* fatty acid synthesis occurs in the cytosol and is present in many tissues. The cofactors required are NADPH, ATP, Mn^{2+}, biotin and HCO_3^- (as source of CO_2). The Acetyl-CoA is the substrate and palmitate is the end product.

Production of Malonyl-CoA is the controlling step for the initiation of FA synthesis and is produced by the carboxylation acetyl-CoA in the presence of ATP and acetyl CoA carboxylase (ACACA). The ACACA has a requirement for the vitamin biotin and consist in multienzyme protein containing a variable number of identical subunits each containing different active and regulatory sites. ACACA is an allosteric enzyme and is activated by citrate, which increases in fed state.

In mammals, FA synthase complex is a dimer with identical monomers, each one containing the seven enzyme activities required for the synthesis of palmitate and an acyl carrier protein where the acyl group is linked during the synthesis. The aggregation of the multienzyme in a single polypeptide that is encoded by a single gene offers great efficiency and protect against interferences.

Enzymatic regulation of fatty acid oxidation

Fatty acid oxidation takes place in the mitochondria, each step produces an acyl-CoA derivate and ATP, and utilizes NAD^+ and FAD as coenzymes. $FADH_2$ and NADH will lead to the synthesis of ATP in respiratory chain of electrons. In animals the acetyl-CoA derivates are incorporated to the citric acid cycle or lead to the liver production of ketone bodies for transport acetyl-CoA derivates to extrahepatic organs in a more suitable compound. Both possibilities are intended for its degradation and production

of energy,although ketone bodies can be excreted through urine or lead to the spontaneous formation of CO_2 and acetone which are also excreted by the lungs.

In the presence of ATP and coenzyme A, FA can be converted to an active FA, Acyl CoA. Acyl CoA is converted to acylcarnitine in outer mitochondrial membrane, which is able to penetrate the inner mitochondrial membrane leading to its restauration to acyl CoA in the mitochondrial matrix. Carnitine palmitoyltranferase-I regulates the entry of acyl groups into the mitochondrial matrix, which is a crucial step prior to oxidation. Its activity is low in the fed state, and high in starvation, allowing FA oxidation to increase.

β-oxidation is the main pathway for FA oxidation, and two carbons are cleaved at each step starting form the carboxyl end. These two carbon units formed are acetyl CoA by means of a series of enzymes found i the mitochondrial matrix or inner membrane adjacent to the respiratory chain coupling the system with the phosphorylation of ADP to ATP. A modificated form of β-oxidation is found in peroxisomes and leads to the fomation of acetyl CoA and H_2O_2. The enzymes in peroxisomes are specialized in attacking long chain FA and the sequence ends in octanoyl CoA which is removed from peroxisomes.

In the liver, the production of ATP originated from the oxidation of FA is kept in a narrow range. When the level of serum free FA is raised, the amount of ATP generated must be regulated with the partitioning of acetyl CoA between ketogenesis (producing less energy) and the citric acid cycle (producing higher amounts of energy).

Comparison of lipid metabolism in different species

The general view that lipogenesis is similarly regulated in all tissues (liver and adipose tissue) comes from the situation in rodents, although some tissue selectivity can be observed in other species. For example, lipogenesis in birds is active exclusively in liver (Griffin and Hermier, 1988), whereas in pig adipose tissue is by far the main lipogenic organ (O'Hea and Leveille, 1969) with little contribution from liver. Knockout and transgenic rodents are useful to make explorations of the role or function of different genes related to lipid metabolism, commonly in obesity and atherosclerotic related diseases (Swanson *et al.*, 2004), therefore, rodents are extensively used for research in lipid metabolism. Domestic pigs, because of their similarity to humans in body size and their anatomical/physiological characteristics, including their innate tendency to overconsume food, have been used to study multiple aspects of atherosclerosis and cardiovascular disease (Mersmann, 1986). Another important factor in lipogenesis is the precursor for fatty acid synthesis; in species like birds, human and pigs, the preferred carbon source is glucose, whereas in other species like ruminants, whose digestive fermentation end product is acetate, and cats, that do not metabolize glucose well, acetate is used for FA synthesis (Table 4).

Table 4. Organ/tissue sites of de novo fatty acid synthesis or lipogenesis (DNL) and primary carbon precursor sources in various species (Bergen and Mersmann, 2005)

	Species								
Item	Humans (Primates)	Rodents (Rats, Mice)	Rabbits	Pigs	Dogs	Cats	Avian species	Cattle	Sheep/Goats
Primary DNL-site	Liver	Liver	Liver/ Adipose	Adipose	Adipose	Adipose	Liver	Adipose	Adipose
Secondary DNL-site	Adipose	Adipose			Liver	Liver		Mammary1	Mammary
Other	Mammary; Muscle	Mammary; Muscle	Mammary; Muscle	Mammary; Muscle	Mammary; Muscle	Mammary; Muscle	Mammary; Muscle	Mammary; Muscle	Mammary; Muscle
Precursor for DNL	Glucose	Glucose	Glucose/ Acetate3	Glucose	Acetate2/ Glucose	Acetate2	Glucose	Acetate3	Acetate3
Type of digestive system	Simple Stomach/ Intestine	Simple Stomach/ Intestine	Simple Stomach/ Intestine Hindgut fermentor	Simple Stomach/ Intestine	Simple Stomach/ Intestine	Simple Stomach/ Intestine	Simple Stomach/ Intestine	Ruminant foregut fermentor	Ruminant foregut fermentor

1 In lactating ruminants (especially dairy cows and goats), the mammary gland is a major site of DNL. All mammalian species exhibit mammary DNL during lactation.
2 Preferred carbon source in dogs and cats for adipose tissue DNL
species with foregut or hindgut fermentation, acetic acid is the principal (or only) precursor for DNL.

In addition, hormone roles are not the same for all species; rodent adipocytes respond dramatically to insulin with stimulation of anabolic and inhibition of catabolic lipid metabolism. In pigs, insulin may be less important compared to other species like rodents and birds (O'Hea *et al.*, 1970) and in general, ruminants are not very responsive to insulin, perhaps because glucose is only a minor substrate for FA synthesis. There is considerable stringency for the agonists and antagonists to stimulate or inhibit β Adrenergic receptors in pigs, whereas in rodents are stimulated or inhibited by a broad range of agonists and antagonists (Mersmann, 1995).

The regulatory role of SREBP-1c in lipogenesis arise from SREBP-1c null mice, SREBP-1c over-expression, and negative-dominant SREBP-1c expression in rodents, but such data are lacking in most species (Gondret *et al.*, 2001). In chicken liver, SREBP-1c and FASN transcripts and proteins are elevated co-ordinately, whereas in pigs, SREBP-1c and FASN transcripts and lipogenesis are elevated in adipose tissue (Gondret *et al.*, 2001). PPARα, controlling the oxidation related

metabolism by controlling enzymes as ACACA and mitochondrial carnitine palmitoyltransferase, are highly expressed in rodent liver and to a modest extent in adipose tissue. In contrast, in pig there appears to be a greater expression of PPARα in adipose tissue than in liver (Ding *et al.*, 2000).

FA regulation of fat metabolism in different species

Different studies in rodents give rise to the suggestion that the active molecule responsible for the action for dietary fat on lipid metabolism could be free FA themselves or their CoA derivates and that compounds arising from cyclo- or lipo-oxygenase pathways (prostanoids, leukotrienes) are probably not important in the regulation of gene expression by FA (Pegorier *et al.*, 2004). As previously described, FA (saturated FA and PUFA) and some eicosanoids are potent ligands of PPAR (Desvergne and Wahli, 1999). LXRs indirectly regulate the expression of lipogenic genes such as FASN, through the regulation of SREBP-1c gene transcription and PUFA may potentially induce LXRα levels in cells, while inhibiting LXRα binding activating ligands, for example oxysterols (Jump, 2002). Fatty acids and cholesterol induce changes in the nuclear abundance of SREBP transcripts by two mechanisms: 1) The increase in cholesterol, due to PUFA treatment, induces the inhibition of proteolytic process in endoplasmatic reticulum membrane and thus a decrease in the abundance of SREBP in the nucleus; and 2) PUFA decreases the transcription of SREBP-1a and 1c (Pegorier *et al.*, 2004). Overall, these observations in transcript regulators could be related to lower fat deposition in rats (Shimomura *et al.*, 1990) fed diets rich in PUFA compared to more saturated fats.

What has been observed in birds is a reduction in abdominal and total carcass fat in birds fed PUFA compared to more saturated fats (Sanz *et al.*, 2000, Crespo and Esteve-Garcia, 2001 and 2002b and 2002a), possibly due to a higher oxidation rate in birds fed PUFA diets.

In pigs, dietary fat inclusion inhibits lipogenesis as observed in different studies (Allee *et al.*, 1971a, Mersmann *et al.*, 1984). This agrees with the higher lipogenic rate observed by Smith *et al.* (1996) in cultured adipocytes isolated from pigs fed a cornstarch diet as compared to pigs fed a 10% fat added diet.

Allee *et al.* (1971b) demonstrated that 10% of corn oil or beef tallow added to diets had the same effects on suppressing lipogenesis in porcine adipose tissue. Smith *et al.* (1996) tested *in vitro* lipogenesis in cultured adipocytes from piglets previously fed with different dietary treatments differing in their FA source. They observed that the stearic acid enriched diet resulted in lower lipogenesis compared to a linoleic acid enriched diet and attributed this finding to the lower absorption of stearic acid. These findings are in contrast to those of Hsu *et al.* (2004) who found no differences in mRNA contents of FASN neither in adipose tissue, nor in the liver in weaned pigs fed 2% of tallow or DHA oil in diet.

In vivo experiments with pigs (Hsu *et al.*, 2004, Liu *et al.*, 2005) have shown that dietary fish oil, rich in long-PUFA lowered SREBP1 expression in the liver.

Fats in pig meat

The influence of fat on meat quality

Pig fat composition is the main factor for meat composition as practically there are no differences in the aminoacidic composition between different pig breeds. Therefore it is important to know the factors that define pig fat composition.

It is important to improve animal production but actually there is an increasing interest on meat composition since producers until consumer are interested in specific quality characteristics for each type of meat product. The slaughter-house and producers are interested in a high meat and carcass production and a good carcass conformation, not too much fat and a white fat. The butcher would be more interested in meat colour, in a meat that does not lose water, its texture, its shelf life, intermuscular fat disposition and quantity … The meat processor will be interested in different characteristics depending on the kind of product is going to produce, but in general the focuss is on pH, and intramuscular fat content, fat composition (high content in oleic acid), color stability, special odors, an optimal texture for grinding. Finally, the consumer will look for a product with a good aspect and color stability, lean, tasty, juicy, good odor, a long shelf life and also healthy (free of additives, contaminants, saturated fats …).

In some of these factors fat plays its role: color stability and shelf life depends on meat oxidative stability which in turn depends on fat oxidation status, taste depends on fat composition and origin, fat firmness depends on FA composition … All these factors will be briefly reviewed.

The primary factors defining animal fat texture and melting point are the content in stearic acid and linoleic acid (Wood *et al.*, 2003). In this context, SFA and MUFA are

positively correlated and PUFA negatively correlated with pork flavour (Cameron and Enser, 1991). The difference in the degree of unsaturation of lipids and FA in different feeding groups has been suggested to produce different flavour characteristics (Larick and Turner, 1990), Campo *et al.* (2003) found that oleic acid was characterized by oily odour, linoleic acid by cooking oil odour and linolenic acid by fishy and linseed odours. The ability of unsaturated FA, especially those with more than two double bonds, to rapidly oxidize, is important in regulating shelf life of meat (rancidity and colour deterioration). All these undesired fat/meat characteristics are related to the type of feeding:

- Restricted feed leads to a higher linoleic fat content due a less *de novo* fat synthesis (SFA and MUFA).
- Feeds with high fat content mean also a diminution of endogenous fat and a higher contribution of diteary fats containing linolenic acid.
- The type of fat contained in feeds is basic as it defines the final linoleic fat content and fat quality in tissues (Warnants *et al.*, 1996). Diets with soybean oil, sunflower oil are rich in linoleic acid or diets with linseed oil rich in linolenic acid will lead to fat tissues with high PUFA contents (Courboulay *et al.*, 1999, Wiseman *et al.*, 2000, Hoz *et al.*, 2003).

Some of these deleterious factors can be improved by the addition of additives as copper and Vitamin E (Lauridsen *et al.*, 1999), copper increasing tissue MUFA content (Thompson *et al.*, 1973) and Vitamin E improving fat firmness (Isabel *et al.*, 2003). Feeding unsaturated fats during the early stages of pigs and change to more SFA before slaughter (Wiseman and Agunbiade, 1998, Warnants *et al.*, 1999).

In any case, the best alternative is not to increase PUFA dietary content in order to avoid high PUFA tissue content. For a firm fat and oxidative stability the limit

proposed in linoleic acid tissue content is 15% (Enser, 1983, Wood, 1983) and iodine value < 70 (Barton-Gade, 1987) although upper limits in non processed meat can be tolerated (Warnants et al., 1996)

Fats and Pork as part of the human diet

Regarding to the e-journal Feed International, during 2005 there were 182 worldwide launches of products derived from meat, milk or eggs with claim of extra omega-3. This total jumped to 278 in 2006, due in part to more introductions from the meat sector. This interest in omega-3 products is seen in both North America and in Europe, although tendencies are reported also in Asia. This interest is based on the demonstrated findings that consumption of omega-3, specially eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) are known to have important functions during gestation (Allen and Harris, 2001), they are a basic constituent of cell brain (Birch et al., 1998), they play a role in the immune function (Miles and Calder, 1998), retina function (Birch et al., 1998), signal transduction (Bazan, 2003), gene regulation (Jump and Clarke, 1999), and have protective properties against cancer (Hardman, 2002) and specially on coronary heart disease (Wijendran and Hayes, 2004).

Although most studies claiming for omega-3 benefits are based on fish oil benefits, the meat industry is pushing to introduce its meat healthy products with the argument that there is a low consumption in western countries of fish products. Meaning that instead of eating fish directly, this could be consumed first by animals and second, meat would be eaten by consumers. The interest from science in producing meat with omega-3 has reached its top in examples as the genetically modified pigs which

express the delta-12-desaturase and therefore are able to synthesize n-3 FA (Saeki *et al.*, 2004). Another possibility, also related to GMO is to produce seeds plants able to synthesize long omega-3 FA by the introduction of genes from sea microalgae (Qi *et al.*, 2004). Probably, at this moment where pig industry has more potential in growing with their health benefits is in the use of oils from seeds. Currently, there is meat labeled as omega-3 fortified with linolenic acid, although this is still not very common. This source of healthy fats, although not containing long-chain omega-3 PUFA, improves the n-6/n-3 ratio, a parameter that also is used to establish some index of idoneity of nutritional quality of fat in terms of human health. Typical western countries diet have an n-6/n-3 ratio closed to 30 value, whereas is recommended to reduce it to 5 or less. A ratio of 30 means that for each 30 g of linoleic and linoleic acid derivates (n-6 FA) there is an ingestion of 1 g of linolenic and linolenic acid derivates, including long-chain omega-3 PUFA (n-3 FA). This recommendation is based on the fact that the ratio at the time when human genetic code was established was 1-2:1 (Simopoulos, 1994).

EXPERIMENTAL WORK

Ileal and faecal fat digestibility

Introduction

As described in the main introduction, different studies have determined fat digestibility in the growing pig. However, most of these studies experimented with fat levels similar to those used in comercial conditions (4-6%). In addition, they have used fat sources commonly used in commercial conditions or in the region or country where the experiments were performed. For these reasons it was assumed necessary to perform a digestibility trial with the objective of measuring the ileal and faecal digestibility of fatty acids according to the fatty acid profile of the diet, in order to formulate diets which would have the same amount of digestible fat. Also, to study the difference between ileal and faecal digestibility, to determine the effects of the large intestine on undigested fat.

Paper 1: Fat and fatty acid digestibility of different fat sources in growing pigs

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Fat and fatty acid digestibility of different fat sources in growing pigs ☆

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Abstract

The effect of fat source on fat and fatty acid (FA) apparent faecal (aFD) and ileal digestibility (aID) was studied in growing pigs. Faecal and ileal digestibilities were measured, using titanium dioxide as inert marker, in intact and ileo-rectally anastomosed pigs, respectively. Five different fat sources, added at 10% to a barley based diet (B), were tested: tallow (T), high oleic sunflower oil (HOSF), sunflower oil (SO), linseed oil (LO) and a fat blend (FB; 5.5% T, 3.5% SO and 1% LO of diet).

Except for B and T, fat aFD and aID were relatively similar among diets and site of measurements and the same was observed when it was obtained from the sum of FA aFD. However fat and sum of FA aID varied according to dietary FA composition and it was inversely related with the saturated FA content, due to the lowest aID of palmitic and stearic. aID of linoleic in SO and linolenic in LO were higher than the respective FA of the other diets and no differences were observed for oleic acid. The aFD of the unsaturated FA was higher than the corresponding aID values, despite similar fat aID and aFD. This together with the lower (in some cases negative) aFD of stearic acid suggest that there is biohydrogenation of unsaturated FA in the hindgut. Measurement at the end of the ileum should give a better estimation of digestibility of fat and FA than at the faecal level.

Keywords: Pig; Fat; Fatty acids; Digestibility

1. Introduction

Fats and oils are important dietary ingredients due to their high energy value, and their fatty acid (FA) pattern is reflected in that of animal products. Faecal digestibility of fat and individual FA is commonly used, but, unlike ileal digestibility, it can be confused by microflora biohydrogenation of unsaturated FA in the hindgut (Reis De Souza et al., 1995; Jørgensen et al., 2000).

This study aims to investigate the influence of FA composition of different dietary fats and oils on their faecal and ileal digestibility in the growing pig.

2. Materials and methods

The working protocol was approved by the IRTA's Ethical Committee.

The study was carried out in two different trials corresponding to faecal (aFD) and ileal digestibility (aID), respectively. Two groups of 12 male pigs (initial bodyweight

Abbreviations: aFD, apparent faecal digestibility; aID, apparent ileal digestibility; B, Basal diet; FA, fatty acid; FB, fat blend diet; HOSF, high oleic sunflower oil diet; LO, linseed oil diet; SO, sunflower oil diet; T, tallow diet.

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Table 1
Fat content and fatty acid profile of diets used in ileal trial [a]

Diets	Basal [b]	Tallow [c]	High oleic sunflower oil	Sunflower oil	Linseed oil	Fat blend
Crude fat (g/100 g)	2.0	11.9	12.0	11.7	11.7	12.0
Sum fatty acids (g/100 g)	1.86	8.33	8.70	9.89	9.32	8.65
Fatty acids (%)						
Myristic (C14:0)	0.6	3.2	0.2	0.2	0.2	1.6
Palmitic (C16:0)	21.5	25.6	7.3	9.4	8.5	18.4
Palmitoleic (C16:1)	0.2	2.4	0.1	0.1	0.1	2.1
Stearic (C18:0)	1.9	18.0	3.6	3.8	3.5	10.3
Oleic (C18:1)	12.8	31.0	65.7	27.3	17.8	23.5
Linoleic (C18:2)	54.3	12.3	18.6	55.2	22.4	20.0
Linolenic (C18:3)	4.6	1.3	0.9	0.8	45.6	19.6

[a] The chemical characterisation of the diets used in the faecal trial was very similar and is not shown.
[b] Basal diet is a barley-based diet supplemented with a mineral and vitamin premix.
[c] In trial 1 12% tallow was included in the diet instead of 10%, crude fat content for that diet in trial 1 was of 13.3%.

of 40 kg in trial 1 and 50 kg in trial 2) were used. In trial 2, pigs were surgically prepared with an ileo-rectal anastomosis (Laplace et al., 1985).

Six diets were tested: a basal diet (B) consisting of barley and a vitamin and mineral premix, and five diets with the addition of 10% tallow (T), sunflower oil

Table 2
Apparent faecal and ileal digestibility of organic matter, crude fat and individual fatty acids[1]

Diets[2]	Basal	Tallow	High oleic sunflower oil	Sunflower oil	Linseed oil	Fat blend	SE[3]
Apparent faecal digestibility (%)							
Organic matter	82.2^{b}	83.3^{ab}	82.4^{b}	83.8^{a}	82.9^{ab}	82.7^{ab}	0.65
Crude fat	29.4	86.5^{a}	84.7^{b}	85.5^{b}	85.0^{b}	85.4^{b}	0.41
Supplemental fat[4]		96.3^{a}	96.2^{a}	95.9^{a}	95.9^{a}	96.4^{a}	0.49
Fatty acids							
Myristic (C14:0)	5.4	94.2^{a}	39.6	47.3	33.0	90.7^{b}	0.38
Palmitic (C16:0)	37.4^{e}	84.7^{a}	61.6^{d}	75.2^{b}	71.3^{c}	82.5^{a}	1.50
Palmitoleic (C16:1)	78.0	98.0^{a}	89.0	94.4	97.2	98.4^{a}	0.18
Stearic (C18:0)	−1127	64.1^{a}	−58.9^{b}	−44.5^{b}	−42.0^{b}	47.6^{a}	8.90
Oleic (C18:1)	73.3^{c}	95.9^{a}	95.7^{a}	95.7^{a}	94.2^{b}	94.7^{ab}	1.04
Linoleic (C18:2)	88.4^{d}	91.2^{c}	93.5^{b}	97.2^{a}	93.8^{b}	92.6^{bc}	1.00
Linolenic (C18:3)	90.9	94.4	89.4	93.4	98.6^{a}	98.5^{a}	0.12
Sum of fatty acids	43.8^{d}	84.1^{c}	84.5^{c}	87.8^{a}	87.6^{ab}	85.5^{bc}	1.29
Apparent ileal digestibility(%)							
Organic matter	78.1	78.0	78.3	79.5	78.2	77.1	0.81
Crude fat	47.6	78.4^{b}	84.4^{a}	83.8^{a}	85.6^{a}	84.5^{a}	1.39
Supplemental fat[4]		83.7^{b}	90.8^{a}	90.4^{a}	92.6^{a}	91.1^{a}	1.63
Fatty acids							
Myristic (C14:0)	83.7	84.6^{a}	83.9	85.3	79.0	87.8^{a}	1.86
Palmitic (C16:0)	74.6^{b}	64.3^{b}	77.6^{b}	84.8^{a}	78.1^{ab}	73.3^{a}	2.42
Palmitoleic (C16:1)	73.2	94.9^{b}	82.9	88.2	82.8	97.1^{a}	1.05
Stearic (C18:0)	55.3	51.2^{b}	72.5^{a}	80.8^{a}	74.9^{a}	59.0^{b}	3.38
Oleic (C18:1)	73.9^{b}	88.6^{a}	89.2^{a}	89.6^{a}	89.4^{a}	91.1^{a}	1.83
Linoleic (C18:2)	90.0^{dc}	87.2^{d}	89.2^{dc}	96.4^{a}	94.7^{ab}	92.5^{bc}	1.43
Linolenic (C18:3)	91.9	88.7	80.6	88.4	98.9^{a}	96.8^{b}	0.26
Sum of fatty acids	83.1^{c}	74.9^{d}	86.9^{b}	91.9^{a}	93.2^{a}	85.5^{bc}	1.70

[1]No significant influence of period and animal was observed. Fatty acid digestibilities were compared only in cases where levels were above 0.14% of the diet, values superindexed with a letter. Means within a row with different letters are significantly different ($P<0.05$).
[2]For faecal digestibility a 12% of tallow was included in the diet instead of 10%.
[3]SE (PooledStandard Error) values correspond solely to the treatments compared which are indicated by letter superscripts.
[4]Corrected subtracting the contribution of basal fat content.

(SFO), high oleic sunflower oil (HOSF), linseed oil (LO) or a fat blend (FB; 5.5% T, 3.5% SO, 1% LO) at the expense of barley. All diets were supplemented with 0.5% of titanium dioxide as inert marker. In trial 1, tallow was included at 12% as a lower digestibility was presumed. Pigs were fed *ad libitum* in trial 1 and at 2.4 times maintenance in trial 2. All pigs were randomly assigned to one of the six treatments according to a double 6×6 Latin square design. Each period consisted of 5 days of adaptation followed by 2 days of digesta collection. Digesta were collected twice a day and frozen immediately.

Dietary nutrients were analysed according to AOAC procedures (AOAC, 1990) and titanium dioxide according to Short et al. (1996). Lipids were extracted according to Folch et al. (1957) and FA determined by gas chromatography using nonadecanoic acid as internal standard. Data were analyzed as a double Latin square and means were compared by Duncan's test. Fatty acid digestibilities were compared only in cases where levels were above 0.14% of the diet. Arc sine transformations were used to have homogeneous variances.

3. Results and discussion

Diet composition (Table 1) shows that the contents of the main FA varied considerably according to the FA composition of the supplemented fats and oils. Digestibility measurements of the two trials are presented in Table 2. Organic matter digestibility was similar between diets and it was higher at the faecal (83%±2.2) than at the ileal (78%±2.8) level. Fat-rich diets had a similar crude fat aFD (85%±1.6), which was much higher than that of B (29%±11.7), suggesting that the structural lipids in barley may have a lower digestibility than lipids of supplemented fats and oils. The higher impact of endogenous fat secretion on fat aFD of low fat diets (Jørgensen and Fernández, 2000) may also explain these differences. Supplemental fat digestibility of T was not different from the other fat-supplemented diets, as reported by Jørgensen et al (2000), possibly due to the effect of the microflora in the hindgut. Except for T, fat aID was very similar between diets; this is also true for supplemented fat digestibility.

aFD of all FA was inferior in B and, saturated FA (myristic, palmitic and stearic acids) were more digestible in T and FB than in oil-supplemented diets. In oil-supplemented diets no major differences were observed in aFD of oleic acid and, in general aFD of polyunsaturated FA (linoleic and linolenic) was higher than in animal fat diets. Except for B, faecal digestibility of the sum of FA was slightly higher for SO or LO and similar to aFD of crude fat. Except for T and FB, that displayed the highest stearic concentrations, aFD of this FA was negative suggesting that microflora could increase its level in faecal content, at the expense of PUFA (Jørgensen et al., 2000). Sum of FA aID was lower in T, even aID of saturated FA (palmitic and stearic) was lower for T, which has a high content in these FA. Like for aFD, only small differences were observed in oleic acid aID. SO linoleic aID and LO linolenic aID were higher than the respective FA aID of the other diets. FB had a relatively low aID of saturated FA in contrast with PUFA. This diet is interesting because it contained relatively similar amounts of the main FA and confirmed the decrease in FA digestibility as carbon chain length of FA increased and the increase in digestibility as unsaturation of the FA increased observed in the other diets. aID of the sum of FA in oil-supplemented diets was higher than that of crude fat, but these values were similar for animal fat. Differences between sum of FA and crude fat digestibilities could be caused by different solvent extraction of lipids (Christie, 1982). In general, unsaturated FA aID was lower than corresponding aFD and the contrary was observed for saturated FA. The low aID of the sum of FA in T is thus explained by its high saturated FA content and their lower aID. LO and SO had the highest digestibilities due to their higher linolenic and linoleic acid contents, respectively, while SFHO had an intermediate digestibility. Although the influence of endogenous FA (Jørgensen et al., 1992a) cannot be discarded, our results fully agree with other studies where biohydrogenation of unsaturated FA was observed (Jørgensen et al., 1992b; Reis De Souza et al., 1995; Jørgensen et al., 2000). It can be hypothesised that oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids reaching the hindgut are, at least in part, saturated by the microflora and converted into stearic acid (C18:0), which would explain the lower digestibility coefficients (in some cases negative) at the faecal than at the ileal level observed for this FA.

4. Conclusions

FA digestibilities differ whether they are measured at the ileal or the faecal level, probably as a result of biohydrogenation of unsaturated FA into stearic acid by the hindgut microflora. Measuring digestibility of individual FA at the end of the ileum should give a better estimation of the nutritive value of fat sources. Individual FA digestibility increases with increasing unsaturation, and decreases with FA length. Therefore, digestibility of fat sources is a function of their FA content.

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References

AOAC, 1990. Official Methods of Analysis, 15th Ed. AOAC, Arlington, USA. 1230 pp.

Christie, W.W., 1982. Lipid Analysis, 2nd ed. Pergamon Press, Oxford. 207 pp.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Jørgensen, H., Fernández, J.A., 2000. Chemical composition and energy value of different fat sources for growing pigs. Acta Agric. Scand. Sect. A Anim. Sci. 50, 129–136.

Jørgensen, H., Jakobsen, K., Eggum, B.O., 1992a. Excretion of biliary fat and fatty acids in growing pigs. J. Anim. Feed Sci. 1, 139–149.

Jørgensen, H., Jakobsen, K., Eggum, B.O., 1992b. The influence of different protein, fat and mineral levels on the digestibility of fat and fatty acids measured at the terminal ileum and in faeces of growing pigs. Acta Agric. Scand. Sect. A Anim. Sci. 42, 177–184.

Jørgensen, H., Gabert, V.M., Hedemann, M.S., Jensen, S.K., 2000. Digestion of fat does not differ in growing pigs fed diets conteining fish oil, rapeseed oil or coconut oil. J. Nutr. 130, 852–857.

Laplace, J.P., Darcy-Vrillon, B., Picard, M., 1985. Evaluation de la disponibilité de acides aminés: choix raisonné d'une méthode. J. Rech. Porc. Fr. 17, 353–370.

Reis De Souza, T., Peiniau, J., Mounier, A., Aumaitre, A., 1995. Effect of addition of tallow and lecithin in the diet of weanling piglets on the apparent total tract and ileal digestibility of fat and fatty acids. Anim. Feed Sci. Technol. 55, 77–91.

Short, F.J., Gorton, P., Wiseman, J., Boorman, K.N., 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59, 215–221.

Effects of dietary fatty acid composition on pig fat and fatty acid depositon and *de novo* fat synthesis

Introduction

This part included the main efforts from this work although it just included one growing trial. The proposed objectives from this were:

- Determinate if the fat source modifies growing parameters.
- Determinate the composition of *de* novo synthesized fatty acids when the pig receives a diet without fat.
- Determinate if the fatty acid profile modifies the amount of deposited fat.
- Determinate if the fatty acid profile modifies the distribution of the fat: subcuteaneous, intramuscular, intermuscular, flare fat.
- Determinate if fatty acids are distibuted uniformely in the different tissues.
- To carry out a fatty acid balance to determine if some fatty acids are oxidyzed/deposited differently
- Determinate the deposition of essetial fatty acids and the conversion to their long-chain derivates (EPA and DHA)
- Determinate if the fatty acid profile modifies blood parameters and to study the expression of genes related to lipid metabolism.

The data obtained from the analysed parameters and samples obtained was divided in different manuscripts. **Paper 2** reports the growing parameters and carcass analysis including the dissection results and carcass analysis and includes the objective “Determinate if the fat source modifies growing parameters” and part of the objective “Determinate if the fatty acid profile modifies the distribution of the fat: subcuteaneous, intramuscular, intermuscular, flare fat”

Papers 3 and 4 contained the data which intended to study the last hypotheses: “Determinate if the fatty acid profile modifies some biochemical parameters related to the regulation of lipid metabolism lipídic” and it was divided into two papers due the large amount of data generated, number 3 with the idea to explain the results on gene experssion as a function of dietary treatment/composition and paper 4 with the idea of gene expression as an indicator of certain metabolic processes, and it also included the data from thyroid hormone blood contents.

Paper 5 attempts to answer the main hypothesis of this project: “Determinate if the fatty acid profile modifies the amount of deposited fat”, but also includes other objectives as “Determinate the composition of *de* novo synthesized fatty acids when the pig receives a diet without fat” and “To carry out a fatty acid balance to determine if some fatty acids are oxidyzed/deposited preferently”.

Paper 6 probably contains the main part of the results obtained from the fatty acid analysis. Although previous studies reported analysis from different tissues in pigs as affected by different dietary FA compositions, this was done more extensively in terms of tissues and fat sources and due the large amounts of data generated, only the main FA are reported. The objectives achieved in this paper are: “Determinate if fatty acids are pattern distributed in pig”, “Determinate the fatty acid profile of each fraction” and part of the objective “Determinate if the fatty acid profile modifies the distribution of the fat: subcuteaneous, intramuscular, intermuscular, flare fat”.

Paper 2: Effect of source of dietary fat on swine carcass fat content and distribution

Submitted to *Meat Science*

Effect of source of dietary fat on swine carcass fat content and distribution

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ABSTRACT

Seventy gilts were used to determine the effect of adding 10% of different fat sources (tallow: T, high oleic sunflower oil: HOSF, sunflower oil: SFO, linseed oil: LO, fat blend: FB, oil blend: OB) vs. feeding a diet with no fat added (NF) on carcass fat content and distribution. Gilts fed HOSF and SFO showed higher numerical values for flare fat and fat content in carcass and major cuts compared with T, LO, FB and OB. Ground carcass composition of SFO showed higher fat and lower lean than T. Gilts fed NF showed higher feed:gain and loin fat than FB, and had higher flare fat, loin intermuscular fat and fat:lean than T. Belly from NF had lower lean and higher intermuscular fat and fat:lean than other diets except for HOSF. Fat source had minor effects on carcass fat, while NF resulted in carcasses and major cuts with higher fat content.

Key Words: Dietary fat source; Swine; Carcass; Dissection

INTRODUCTION

The pork industry has made significant efforts to decrease total amount of fat deposited in pigs through genetics, management and nutrition. At the same time extensive research has been dedicated towards changing the fatty acid composition of animal products to better match dietary recommendations for the human diet. Several authors support that the response of animal adipose tissue to dietary fatty acids (FAs) is dependent upon the fatty acid profile of the diet. Clarke (2000) reviewed differences in metabolism between saturated and polyunsaturated fatty acids in studies conducted with rats and concluded that diets rich in *n*-6 and particularly *n*-3 fatty acids suppress hepatic lipogenesis,

reduce hepatic triglyceride output, enhance ketogenesis, induce fatty acid oxidation in liver and muscle resulting in decreased fat deposition. Sanz, Flores, Pérez de Ayala, and López-Bote (1999) showed that feeding 10% of sunflower oil to broiler chickens reduced abdominal fat pad without affecting feed intake compared with tallow. In addition, Crespo and Esteve-Garcia (2001) found that diets rich in polyunsaturated fatty acids reduce abdominal fat deposition in broilers compared with saturated or monounsaturated fats. Crespo and Esteve-Garcia (2002a) also found that dietary polyunsaturated fatty acids decrease fat deposition in separable fat depots but not in the remainder carcass.

It is well known that tissue fatty acid composition in pigs closely resembles the fatty acid composition of the diet. However, there is limited research on the effect of dietary fat sources on total fat and fat distribution in different depots of swine carcasses. The objective of this study was to determine the effect of adding 10% of different dietary fat sources (beef tallow, high-oleic sunflower oil, sunflower oil, linseed oil, fat blend: tallow, sunflower oil, linseed oil, and oil blend: fish oil, linseed oil) on performance, carcass characteristics, and carcass fat content and distribution of crossbred gilts. The amount of dietary fat may also influence fat deposition in swine. Dugan, Aalhus, Lien, Schaefer, and Kramer (2001) demonstrated that barrows produced leaner carcasses when fed 5 versus 2% canola oil as a consequence of improved feed efficiency, more lean and less subcutaneous fat in primal cuts of barrows fed 5% canola oil. The effect of feeding a diet with no fat added versus adding 10% of different fat sources on carcass fat content and distribution was also evaluated.

MATERIALS AND METHODS

Animals and Diets

Experimental procedures were approved by IRTA ethical committee. Crossbred gilts (Duroc x Landrace) were fed a barley-corn-soybean meal based diet during a pre-experimental period. Five gilts were slaughtered at the beginning of the study as a reference value for carcass composition. Seventy gilts (61.8±5.2 kg live weight) were assigned to one of seven dietary treatments in two replicates, with 5 animals per treatment in each replicate. Diets were formulated to meet NRC (1998) requirements and assigned randomly by animal weight. Six fat supplemented (10%) diets were based on barley and soybean meal and a semi-synthetic diet was formulated using ingredients to contain no fat (Table 1). The experimental fat supplemented diets (10% fat) were formulated in order to supply an equal amount of digestible fat based on previous measurements (Duran-Montgé, Lizardo, Torrallardona, & Esteve-Garcia, 2007). Six fat sources were selected to achieve diets with different fatty acid composition. Thus, beef tallow (T) fat source was included to have a diet rich in saturated fatty acids while high-oleic sunflower oil (HOSF), sunflower oil (SFO), and linseed oil (LO) were selected to have diets rich in oleic, linoleic and linolenic acids, respectively. Fat Blend (FB) diet was formulated to have similar content of the main fatty acids (palmitic, stearic, oleic, linoleic and linolenic), and oil blend (OB) diet was selected to have a high content of long-chain polyunsaturated fatty acids because of the elevated levels of these fatty acids in fish oil and linseed oil. Animals were fed *ad libitum* individually with free access to water. Animal weight and feed intake were recorded at the beginning, at three weeks

and at the end of the trail. Diet composition and nutrient content are shown in Table 1, and fatty acid content of the seven diets is presented in Table 2.

Dietary Fat Content and Fatty Acid Composition

Feed lipids were extracted following the procedure of Folch, Lees, and Stanley (1957), converted to fatty acid methyl esters using BF_3 and methanolic KOH (Morrison & Smith, 1964) and analyzed using GC (Agilent 6890N, Wilmington, DE, USA) equipped with an automatic injector, using C19:0 as internal standard.

Carcass Measurements

Pigs were transported to the slaughter facilities of IRTA-Monells approximately 14 h before slaughter and were held off feed and had access to water during lairage. Gilts (99.8±8.5 kg live weight) were humanely harvested using CO_2 stunning following standard procedures of an officially inspected facility. Carcass and flare fat weights and fat and muscle depths were recorded for each carcass within 1 h post-mortem using the Fat-O-Meat'er probe (SFK Technology, Denmark). Muscle and fat depth were measured both at the last rib level and between the 3rd and 4th thoracic ribs counting from the last one (60 mm from the mid-line), and percent carcass lean was predicted using the Spanish official equation (Gispert & Diestre, 1994). The left side of each carcass was commercially cut and the main cuts (ham, loin, shoulder, belly, tenderloin, and other fat cuts: belly trimmings plus jowl) were weighed to obtain cut percentages. Ham, loin and belly were dissected into lean, subcutaneous and intermuscular fat and bone following the procedure of Walstra and Merkus

(1995). Muscle samples of *Longissimus thoracis* and *Semimembranosus* muscles were removed, vacuum packaged and stored at -20˚C until analysis of fat content. The right side of each carcass was reduced into small pieces and stored frozen at -20˚C for subsequent grinding. Right side carcasses were ground three times through different plates (5 eyes, 8 mm and 3 mm plates) using an industrial grinder with 160 mm head (Grinder Cato-PA160, Sabadell, Spain). Subsequent to grinding side carcasses were homogenized using a mixer during 1 minute and samples (500 g) collected for proximate analysis.

Ground Carcass Composition

Ground carcass samples were analyzed for lipid content by chloroform-methanol extraction (Folch et al., 1957), crude protein was determined following the Dumas method using a nitrogen/protein analyzer FP528 (Leco, St. Joseph, MI; AOAC, 2000 method 968.06), water by freeze-drying using a freeze-drier (Liodelta Telstar, Terrassa, Spain) and ash content was determined by ashing the samples in a muffle oven at 525 ºC for 3 h (AOAC, 1990 method 923.03).

Intramuscular Fat

Intramuscular fat content was determined in *Longissimus thoracis* and *Semimembranosus* muscles using Near Infrared Transmittance (NIT, Infratec® 1265, Tecator, Höganäs, Sweden) (Gispert, Valero, Oliver, & Diestre, 1997).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with replication in the model. Least square means differences between

dietary treatments were assessed by the Tukey-Kramer test. Initial live weight and carcass weight were included in the model as a covariate for live animal and carcass data, respectively. Significance was determined at $P < 0.05$, and differences of $P \geq 0.05$ to $P \leq 0.10$ are discussed as trends.

RESULTS AND DISCUSSION

Dietary fat content and fatty acid composition

Fatty acid content and composition of the experimental diets are shown in Table 2. The NF diet had a low content of all FA, whereas T diet was characterized by high content of saturated fatty acids (myristic, palmitic and stearic). High oleic sunflower oil diet was rich in oleic and monounsaturated fatty acids, SFO had a high content of linoleic acid, polyunsaturated and *n*-6 fatty acids, and LO showed high values of linolenic acid, polyunsaturated and *n*-3 fatty acids. Oil blend diet showed high content of EPA, DHA, polyunsaturated and *n*-3 fatty acids, while FB showed intermediate FA content in major fatty acids among dietary treatments. Fatty acid ratios also reflected differences between dietary FAs with the PUFA:SFA ratio being high for LO and SFO followed by OB and NF, then HOSF and FB, and lowest for T. The ratio of *n*-6:*n*-3 FAs was highest for SFO followed by HOSF, then NF, T and FB, and lowest for LO and OB.

Animal performance

As shown in Table 3, there were no differences ($P > 0.05$) in average daily feed intake, average daily gain and final live weight of gilts among dietary treatments. Gilts fed FB tended ($P = 0.098$) to be heavier than gilts fed OB. Gilts fed FB had

an improved ($P < 0.05$) feed:gain ratio over gilts fed NF (0.42 kg feed kg^{-1}gain) with other dietary treatments being intermediate. Addition of fat/oil in the diet has previously been found to improve feed efficiency (Pettigrew & Moser, 1991; Smith, Tokach, O'Quinn, Nelssen, & Goodband, 1999). De la Llata, Dritz, Tokach, Goodband, Nelssen, and Loughin (2001) indicated that adding 6% fat to corn-soybean meal diets consistently improved feed efficiency of barrows and gilts in all phases of growth under commercial conditions. Allee, Romsos, Leveille, and Baker (1972) found that addition (10%) of fat to a low-fat diet (1%), regardless of the source, resulted in an increase in daily gain and gain:feed ratio. Dugan et al. (2001) found leaner carcasses as a consequence of improved feed efficiency, more lean and less subcutaneous fat in primal cuts of barrows fed 5 *vs*. 2% canola oil, but no differences in feed efficiency between treatments were found in gilts. However, taking into account dietary energy (Table 1) consumption in this study, feed efficiency was higher for NF compared with diets with fat added, with LO and OB showing lower efficiency values in terms of dietary energy conversion relative to other diets. Results from this study were similar in animal performace among dietary treatments. However, LO and OB showed numerically higher feed:gain ratio and lower average daily gain which would indicate a lower efficiency of fats with a high content in *n*-3 fatty acids.

Carcass characteristics and major primal cuts

Carcass characteristics and carcass joints of gilts fed the different experimental diets are shown in Table 4. Carcasses from pigs slaughtered at 100 kg live weight showed higher killing out percentage, and increased flare fat and fat and

loin depth compared with animals slaughtered at 60 kg live weight. Landgraf et al. (2006) also reported increases in dressing percentage with increasing pig live weight from 75.9 to 77.5% and from 77.5 to 85.2% at 60 to 90 kg and 90 to 120 kg, respectively. Killing out percentage of gilt carcasses fed SFO tended to be greater (P = 0.084) than OB, while there were no differences (P > 0.05) among gilts fed T, HOSF, SFO, LO and FB. Carcass weight, fat depths measured at the last rib and between the 3rd and 4th last ribs, muscle depth measured between the 3rd and 4th last ribs, and estimated lean percentage did not differ (P > 0.05) among dietary fat sources. However, fat depths (last rib and 3-4 last rib) and flare fat % of HOSF and SFO fed gilts were similar to NF and numerically higher (P > 0.05) than T with values from LO, FB and OB being intermediate.

Previous studies evaluating the effect of source of dietary fat on swine carcass characteristics have shown that the type of fat does not influence carcass traits. Mitchaothai et al. (2007) evaluated the performance and carcass characteristics of growing-finishing pigs fed 5% beef tallow or sunflower oil. Final body weight, average daily feed intake, average daily gain, feed:gain ratio, and carcass traits (back fat thickness, fat:lean ratio) did not differ between pigs for the two fat supplements. Nuernberg et al. (2005) also found that carcass composition (backfat thickness, lean percentage and muscle area) did not differ between oil supplements (5% olive or linseed oil) in growing-finishing gilts and barrows. These results agree with Bee, Gebert, and Messikommer (2002) who reported that dietary fat source (5% tallow or soy oil) had no effect on growth performance (weight gain, feed intake and gain:feed) or carcass traits (lean %, loin, shoulder, ham and belly %, omental fat %, subcutaneous fat %, 13th-rib fat

depth) in swine. Rossi and Corino (2002) evaluated the effect of supplementing barrows with tallow, corn oil or rapeseed oil during growing (3%) and finishing (2.5%), and reported that growth performance and carcass characteristics were not influenced by dietary fat. Although treatments HOSF and SFO showed higher numerical values for fat depth compared with T, carcass data from the current work also show that dietary fat source do not alter carcass characteristics.

Killing out percentage was higher ($P < 0.05$) and tended to be greater ($P = 0.052$) for gilts fed NF compared with OB and FB, respectively. Fat depth measured between the 3rd and 4th last ribs from gilts fed NF tended to be higher ($P = 0.072$) than T. Flare fat percentage was higher ($P < 0.05$) for gilts fed NF relative to T with other treatments being intermediate. These results disagree with data reviewed by Pettigrew and Moser (1991) indicating that added fat in growing-finishing pig diets generally increased carcass fatness. Miller et al. (1990) evaluated control vs. 10% of animal fat, safflower oil, sunflower oil or canola oil in the diet, and found that last-rib fat thickness was increased with the supplemental dietary fat or oils. Allee et al. (1972) compared addition of 10% dietary fat differing widely in FA composition (corn oil, lard, coconut oil, and tallow) with a control diet (1% fat). Feeding 10% dietary fat resulted in increased body fat as measured by backfat thickness, perirenal fat and fat trim, while pigs fed the control diet had greater percent of the carcass in lean cuts and tended to have larger *longissimus* muscle area. Stahly and Cromwell (1979), and Stahly, Cromwell, and Overfield (1981) also found that carcass backfat thickness and fat percentage were increased in pigs fed 5% tallow fat compared with 0% fat. Other authors reported no effect of fat addition in the diet on

carcass characteristics. De la Llata et al. (2001) indicated that adding 6% fat (choice white grease) to corn-soybean meal diets did not affect carcass yield, backfat or loin depth, or lean percentage. Smith et al. (1999) and Williams, Cline, Schinckel, and Jones (1994) did not observe changes in carcass characteristics with increasing dietary fat in finishing pigs. Results from carcass data in the present work show an effect of dietary fat level on carcass composition, indicating that diets with no fat added may result in carcasses with more fat compared with 10% addition of dietary tallow.

Weight and percentage of ham, shoulder, belly, tenderloin and other fat cuts did not differ ($P > 0.05$) among dietary treatments. Loin weight from gilts fed SFO was higher ($P < 0.05$), and loin from gilts fed T tended to be heavier ($P = 0.073$) compared with FB. Gilts fed SFO tended to show greater ($P = 0.088$) loin percentage compared with gilts fed FB. Loin weight and percentage from gilts fed NF were higher ($P < 0.05$) compared with gilts fed FB. Percentage of other fat cuts from gilts fed HOSF tended to be higher ($P = 0.086$) than gilts fed NF. Dietary fat source or fat level did not affect weight or percentage of major primal cuts except for the loin which was lighter for gilts fed FB.

Dissection of major primal cuts

Joint dissections of ham, loin and belly into lean, subcutaneous and intermuscular fat and bone are presented in Table 5. Percentage of lean and bone decreased whereas subcutaneous and intermuscular fat percentage and fat:lean ratio increased in major cuts from 60 to 100 kg LW, except for intermuscular fat percent in the ham which did not show a significant increase with increasing carcass weight. D'Souza, Pethick, Dunshea, Suster, Pluske,

and Mullan (2004) also indicated that the fat to lean muscle ratio in female pigs increased significantly for the loin, belly and the ham primal cuts with animal age.

Results from joint dissections showed that weight and percentage of lean, subcutaneous and intermuscular fat and bone, lean:bone and fat:lean ratios from the ham did not differ ($P > 0.05$) among dietary treatments. Lean weight from the loin was higher ($P < 0.05$) for gilts fed T compared with HOSF and FB, which did not differ. There were no differences in lean weight from the loin among other dietary treatments. Gilts fed NF showed higher subcutaneous fat weight from the loin than FB and higher ($P < 0.05$) intermuscular fat weight than T, LO and FB, which did not differ. Intermuscular fat weight from the loin tended to be higher ($P = 0.099$) for gilts fed NF than OB. Other dietary treatments showed similar values for subcutaneous and intermuscular fat from the loin. Fat:lean ratio of NF was higher compared with T and tended to be higher compared with LO and FB ($P = 0.074$ and $P = 0.054$, respectively). Opposite results were reported by Allee et al. (1972) who evaluated pigs fed diets containing 10% fat which tended to have more fat in the *longissimus* muscle than those fed the control diet. Bone weight and lean:bone ratio from the loin did not differ among dietary treatments. Dugan, Aalhus, Robertson, Rolland, and Larsen (2004) found no differences in body cavity fat or intermuscular fat due to fat/oil type (canola oil vs. tallow) or level (2 vs. 5%). In addition, no main effects of fat/oil type were found in their study for the bone content of lean cuts.

Dissection data presented as percentage showed that lean from the loin was lower for gilts fed NF compared with T and tended to be lower ($P = 0.065$) than gilts fed LO. Percentage of loin intermuscular fat from gilts fed NF was greater

($P < 0.05$) than T, LO and FB, which did not differ. There were no differences among treatments in percentage of subcutaneous fat and bone from the loin. Percentage of separable fat (subcutaneous and intermuscular fat) was higher ($P < 0.05$) for NF compared with T. Loin dissection results disagree with Kouba, Enser, Whittington, Nute, and Wood (2003) that found no effect of feeding a control vs. a linseed (6%) diet in foreloin tissue composition in gilts.

Results from belly dissection showed lower lean weight for NF compared with T, SFO, LO, FB and OB, which did not differ. Weight of intermuscular fat and fat:lean ratio were higher for NF compared with all fat added treatments except for HOSF. There were no differences in weight of subcutaneous fat, bone and lean:bone ratio among dietary treatments. However, subcutaneous fat weight from the belly tended to be higher for HOSF fed gilts compared with T and LO ($P = 0.098$ and $P = 0.064$, respectively).

Dissection data presented as percentage showed that lean from the belly of gilts fed HOSF was lower than LO and tended to be lower ($P = 0.056$) than T fed gilts. Lean percentage from the belly was lower for gilts fed NF compared with LO, T, SFO, FB, and OB, which did not differ. Percentage of subcutaneous fat from gilts fed HOSF was higher ($P < 0.05$) than LO and tended to be higher ($P = 0.081$) compared with OB fed gilts. Intermuscular fat percentage from gilts fed NF was greater than all other treatments, which did not differ. Percentage of separable fat (subcutaneous plus intermuscular) from the belly was higher for NF compared with fat added treatments except for HOSF. There were no differences in bone percentage among dietary treatments.

It has been shown in rats and broilers that the distribution of dietary fat within adipose tissues depends on the fatty acid profile. Soriguer et al. (2002) reported

that diets rich in SFAs induce a relative increase in the amount of intra-abdominal adipose tissue in rats. In broiler chickens, dietary supplementation with 10% corn-oil-fed birds were leaner than tallow-fed birds (Brue & Latshaw, 1985). Sanz et al. (1999) fed broilers 10% tallow or sunflower oil and found feeding sunflower oil reduced abdominal fat pad. Crespo and Esteve-Garcia (2002a, 2002b) found that broilers fed linseed oil had less abdominal fat than those fed tallow showing that PUFAs reduce fat deposition in separable fat depots with respect to MUFAs and SFAs. Results from dissection of main cuts in gilts from this study do not appear to support the same pattern of fat deposition and distribution according to dietary fatty acid profile reported in rats and broilers. In the current work, T supplemented diet with highest content of saturated fatty acids showed lower numerical values for carcass fat measurements and dissection data compared with other dietary fat sources, and significantly leaner carcasses than gilts fed a diet with no fat added. Other results from the present research found that dietary T reduced whole animal fat content compared with SFO and HOSF. In addition, dietary tallow showed lower expression of lipogenic enzymes in adipose tissue and reduced triiodothyronine (T3) hormone contents in blood (unpublished data). Gilts fed HOSF with a high content in monounsaturated fatty acids had higher numerical values of fat content in carcass and major cuts compared with gilts fed diets predominant in polyunsaturated fatty acids such as LO, FB and OB. In contrast to results reported in rats and broilers, diets rich in polyunsaturated fatty acids did not show reduced fat in the belly compared with diets rich in saturated fatty acids.

Ground carcass composition and intramuscular fat

Ground carcass composition and intramuscular fat (IMF) percentage of *Longissimus thoracis* and *Semimembranosus* muscles are shown in Table 6. Animals slaughtered at 100 kg live weight showed lower moisture and ash, similar percentage of protein, and higher fat percentage compared with reference animals slaughtered at the beginning of the trial (60 kg live weight) showing typical growth trend of different tissues. Carcass content of moisture and ash did not differ ($P > 0.05$) among dietary treatments. However, carcass moisture from gilts fed SFO tended to be lower than T ($P = 0.078$) and FB ($P = 0.086$). Carcasses from gilts fed SFO showed higher fat content and lower protein content than T (4.3% higher and 1.2% lower, respectively), and tended ($P = 0.075$) to have more fat than FB (3.5% higher), with other treatments being intermediate.

Gilts slaughtered at 60 kg LW showed similar percentage of intramuscular fat in *Longissimus thoracis* and lower percent in *Semimembranosus* muscle compared with animals slaughtered at 100 kg LW with high animal variation in IMF percentage (*RMSE* = 0.427 and 1.055 for LT and SM, respectively). D'Souza et al. (2004) indicated that IMF levels in the loin (m. *longissimus*) remained constant in female pigs between 16 (68.7 kg LW) and 25 (104.6 kg LW) weeks of age (from 2.77 to 2.88 %), while IMF levels in the ham (m. *biceps femoris*) increased significantly with age (from 2.63 to 2.88 %). Although there were no differences ($P > 0.05$) in IMF content of LT and SM muscles from gilts fed the different experimental diets, animals fed HOSF showed the highest numerical values for IMF in both muscles. Rhee, Davidson, Knabe, Cross, Ziprin, and Rhee (1988) fed a control or a similar diet containing 12% high-oleic

sunflower oil, and found no differences between the groups in marbling scores for gilts. Other authors reported reductions in marbling when supplementing with different fat sources. Addition of canola oil (0, 5 or 10%) resulted in improvements in rate of gain and feed efficiency without affecting backfat thickness or *longissimus* muscle area but resulted in reductions in loin marbling (Myer, Lamkey, Walker, Brendemuhl, & Combs, 1992). Miller et al. (1990) studied the effect of feeding control vs. 10% of animal fat, safflower oil, sunflower oil or canola oil in the diet on swine carcass and meat quality traits. No differences existed for marbling scores between control and animal fat or safflower oil diets. However, pigs supplemented with sunflower or canola oil had lower marbling scores. Results of the present trial suggest that dietary fat level or composition do not alter intramuscular fat, although the numerically higher IMF level of gilts fed HOSF suggest a minor effect, but the variability in this parameter appears to be large.

CONCLUSIONS

Among the fat supplemented diets gilts fed diets rich in monounsaturated fatty acids (high oleic sunflower oil and sunflower oil) showed higher numerical values for flare fat and fat content in carcass and major cuts compared with gilts fed diets rich in saturated (tallow) and polyunsaturated fatty acids (linseed oil, fat blend and oil blend). Ground carcass composition of sunflower oil fed gilts showed higher fat and lower lean content than tallow. Gilts fed a diet with no fat added showed higher feed:gain ratio than fat blend, higher flare fat and carcass fat depth than tallow, and higher subcutaneous and intermuscular fat and

fat:lean ratio in loin and belly compared with gilts fed 10% of other fat sources except for high oleic sunflower oil. Although the fatty acid composition of the diet has major effects on tissue fatty acid composition, source of dietary fat in this study showed minor effects on animal performance, carcass characteristics and carcass fat content and distribution. Feeding a diet with no fat added may result in carcasses and major cuts with a higher fat content compared with diets including high levels of fat addition.

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REFERENCES

Allee, G.L., Romsos, D.R., Leveille, G.A., & Baker, D.H. (1972). Lipongenesis and enzymatic activity in pig adipose tissue as influenced by source of dietary fat. *Journal of Animal Science*, 35, 41-47.

AOAC (1990). Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Washington, D.C.

Bee, G., Gebert, S., & Messikommer, R. (2002). Effect of dietary energy supply and fat source on the fatty acid pattern of adipose and lean tissues and lipogenesis in the pig. *Journal of Animal Science*, 80, 1564-1574.

Brue, R.N., & Latshaw, J.D. (1985). Energy utilization by the broiler chicken as affected by various fats and fat levels. *Poultry Science*, 64, 2119-2130.

Clarke, S.D. (2000). Polyunsaturated fatty acid regulation of gene transcription: A mechanism to improve energy balance and insulin resistance. *British Journal of Nutrition*, 83, S59-S66.

Crespo, N., & Esteve-Garcia, E. (2001). Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poultry Science*, 80, 71-78.

Crespo, N., & Esteve-Garcia, E. (2002a). Dietary polyunsaturated fatty acids decrease fat deposition in separable fat depots but not in the remainder carcass. *Poultry Science*, 81, 512-518.

Crespo, N., & Esteve-Garcia, E. (2002b). Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. *Poultry Science*, 81, 1533-1542.

De la Llata, M., Dritz, S.S., Tokach, M.D., Goodband, R.D., Nelssen, J.L., & Loughin, T.M. (2001). Effects of dietary fat on growth performance and carcass characteristics of growing-finishing pigs reared in a commercial environment. *Journal of Animal Science*, 79, 2643-2650.

D'Souza, D.N., Pethick, D.W., Dunshea, F.R., Suster, D., Pluske, J.R., & Mullan, B.P. (2004). The pattern of fat and lean muscle tissue deposition differs in the different pork primal cuts of female pigs during the finisher growth phase. *Livestock Production Science*, 91, 1-8.

Dugan, M.E.R., Aalhus, J.L., Lien, K.A., Schaefer, A.L., & Kramer, J.K.G. (2001). Effects of feeding different levels of conjugated linoleic acid and total oil to pigs on live animal performance and carcass composition. *Canadian Journal of Animal Science*, 81, 505-510.

Dugan, M.E.R., Aalhus, J.L., Robertson, W.M., Rolland, D.C., & Larsen, I.L. (2004). Practical dietary levels of canola oil and tallow have differing effects on

gilt and barrow performance and carcass composition. *Canadian Journal of Animal Science*, 84, 661-671.

Duran-Montgé, P., Lizardo, R., Torrallardona, D., & Esteve-Garcia, E. (2007). Fat and fatty acid digestibilility of different fat sources in growing pigs. *Livestock Science*, 109, 66-69.

Folch, J., Lees, M., & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226, 497-509.

Gispert, M., & Diestre, A. (1994). Classement des carcasses de porc en Espagne: un pas vers l'harmonization communautaire. *Tecniporc*,17(2), 29–32.

Gispert, M., Valero, A., Oliver, M. A., & Diestre, A. (1997). Problemas asociados a la falta de grasa en canales porcinas. *Eurocarne*, 61, 27–32.

Kouba, M., Enser, M., Whittington, F.M., Nute, G.R., & Wood, J.D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *Journal of Animal Science*, 81, 1967-1979.

Landgraf, S., Susenbeth, A., Knap, P.W., Looft, H., Plastow, G.S., Kalm, E., & Roehe, R. (2006). Developments of carcass cuts, organs, body tissues and chemical body composition during growth of pigs. *Animal Science*, 82, 889-899.

Miller, M.F., Shackelford, S.D., Hayden, K.D., & Reagan, J.O. (1990). Determination of the alteration in fatty acid profiles, sensory characteristics and carcass traïts of swine fed elevated levels of monounsaturated fats in the diet. *Journal of Animal Science*, 68, 1624-1631.

Mitchaotai, J., Yuangklang, C., Wittayakun, S., Vasupen, K., Wongsutthavas, S., Srenanul, P., Hovenier, R., Everts, H., & Beynen, A.C. (2007). Effect of

dietary fat type on meat quality and fatty acid composition of various tissues in growing–Wnishing swine. *Meat Science*, 76, 95–101.

Morrison, W.R., & Smith, L.M. (1964). Preparation of fatty acid methyl esters + dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research*, 5, 600-608.

Myer, R.O., Lamkey, J.W., Walker, W.R., Brendemuhl, J.H., & Combs, G.E. (1992). Performance and carcass characteristics of swine when fed diets containing canola oil and added copper to alter the unsaturated:saturated ratio of pork fat. *Journal of Animal Science*, 70, 1417-1423.

Nuernberg, K., Fischer, K., Nuernberg, G., Kuechenmeister, U., Klosowska, D., Eliminowska-Wenda, G., Fiedler, I., & Ender, K. (2005). Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science*, 70, 63-74.

NRC. 1998. Nutrient Requirements for Swine. 10th ed. Natl. Acad. Press, Washington, DC.

Pettigrew, J.E., & Moser, R.L. (1991). Fat in swine nutrition. In E.R. Miller, D.E. Ullrey, and A.J. Lewis, *Swine nutrition* (pp. 133-145). Butterworth-Heinemann, Toronto, ON.

Rhee, K.S., Davidson, T.L., Knabe, D.A., Cross, H.R., Ziprin, Y.A., & Rhee, K.C. (1988). Effect of dietary high-oleic sunflower oil on pork carcass traits and fatty acid profiles of raw tissues. *Meat Science*, 24, 249-260.

Rossi, R., & Corino, C. (2002). Influence of long-term nutrition with different dietary fats on fatty acid composition of heavy pigs backfat. *Italian Journal of Animal Science*, 1, 7-16.

Sanz, M., Flores, A., Pérez de Ayala, P., & López-Bote, C.J. (1999). Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. *British Poultry Science*, 40, 95-101.

Shimomura, Y., Tamura, T., & Suzuki, M. (1990). Less body-fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. *Journal of Nutrition*, 120:1291-1296.

Smith, J.W., II, Tokach, M.D., O'Quinn, P.R., Nelssen, J.L., & Goodband, R.D. (1999). Effects of dietary energy density and lysine:calorie ratio on growth performance and carcass characteristics of growing-finishing pigs. *Journal of Animal Science*, 77, 3007-3015.

Soriguer, F., Moreno, F., Rojo-Martínez, G., Cardona, F., Tinahones, F., Gómez-Zumaquero, J.M., García-Fuentes, E., & Morcillo, S. (2002). Redistribution of abdominal fat after a period of food restriction in rats is related to the type of dietary fat. *British Journal of Nutrition*, 89, 115-122.

Stahly, T.S., & Cromwell, G.L. (1979). Effect of environmental temperature and dietary fat supplementation on the performance and carcass characteristics of growing and finishing swine. *Journal of Animal Science*, 49, 1478-1488.

Stahly, T.S., Cromwell, G.L., & Overfield, J.R. (1981). Interactive effects of season of year and dietary fat supplementation, lysine source and lysine level on the performance of swine. *Journal of Animal Science*, 53, 1269-1277.

Walstra, P., & Merkus, G.S.M. (1996). Procedure for assessment of the lean meat percentage as a consequence of the new EU reference dissection method in pig carcass classification. Report ID-DLO 96.014, Zeist, The Netherlands, 22pp.

Williams, N.H., Cline, T.R., Schinckel, A.P., & Jones, D.J. (1994). The impact of ractopamine, energy intake, and dietary fat on finisher pig growth performance and carcass merit. *Journal of Animal Science*, 72, 3152-3162.

Table 1. Ingredient and chemical composition of experimental diets.

	NF	T	HOSF	SFO	LO	FB	FO
Diet formulation (%)							
Barley		62.5	64.2	63.7	64.1	63.8	63.7
Soybean meal 44%		24.1	23.7	23.8	23.7	23.8	23.8
Wheat starch	70.0						
Soybean protein isolated	14.0						
Sugar beet pulp	10.1						
Molasses	4.0						
Tallow		10.97				5.45	
High oleic sunflower oil			9.58				
Sunflower oil				9.97		3.47	
Linseed oil					9.68	0.99	5.80
Fish oil							3.87
L-lysine HCl		0.01	0.02	0.02	0.02	0.02	0.02
DL- methyonine	0.04						
Dicalcium Phosphate	0.2	0.28	0.27	0.27	0.27	0.27	0.27
Calcium carbonate	1.08	1.61	1.61	1.61	1.61	1.61	1.91
Sodium Chloride	0.15	0.22	0.22	0.22	0.22	0.22	0.22
Mineral/Vitamin complex[a]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Nutrient and energy content							
Dry Matter (%)	98.0	98.0	98.1	97.9	97.6	97.9	97.9
GE[b] (Kcal Kg-1)	3.82	4.47	4.50	4.54	4.46	4.54	4.46
Crude Protein (%)	14.1	15.6	15.1	15.5	15.9	15.8	16.1
Crude fat	0.32	12.7	12.6	13.4	11.6	11.6	11.5
Ash (%)	2.93	5.03	4.83	4.82	5.09	5.11	5.16

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

[a] One kg of feed contains: Vitamin A: 5000 IU; Vitamin D_3: 1000 IU; Vitamin E: 15 mg; Vitamin B_1: 1,3 mg; Vitamin B_2: 3,5 mg; Vitamin B_{12}: 0.025 mg; Vitamin B_6: 1,5 mg; Calcium pantothenate: 10 mg; Nicotinic acid: 15 mg; Biotin: 0,1 mg; Folic acid: 0,6 mg; Vitamin K_3: 2 mg; Fe: 80 mg; Cu: 6 mg; Co: 0,75 mg; Zn: 60 mg; Mn: 30 mg; I: 0,75 mg; Se: 0,10 mg; Ethoxiquin: 0,15 mg.

[b] GE=Gross energy

Table 2. Fatty acid content and composition of experimental diets (% of dietary FA).

	NF	T	HOSF	SFO	LO	B	FO
C14:0	ND	3.34	0.12	0.13	0.09	1.72	1.53
C14:1	ND	0.59	ND	ND	ND	0.24	0.02
C16:0	0.55	28.7	7.87	11.2	8.21	20.0	12.9
C16:1 n-7	ND	2.37	0.19	0.11	0.08	1.35	1.81
C18:0	0.15	22.8	4.25	5.56	3.64	12.8	4.07
C18:1n-9 trans	0.06	0.23	0.10	0.27	0.06	0.27	0.10
C18:1 n-7	ND	3.03	ND	0.22	0.06	1.05	ND
C18:1 n-9 cis	0.8	34.8	86.8	32.5	20.7	29.5	19.0
C18:1n-11	0.05	1.23	0.86	0.82	0.78	1.20	1.44
C18:2 n-6	2.0	14.20	21.5	73.3	26.2	26.0	21.5
C18:3 n-3	0.26	1.90	1.29	1.26	47.1	18.9	31.2
C20:0	ND	0.13	0.40	0.43	0.19	0.24	0.26
C22:0	ND	0.07	1.15	1.02	0.20	0.23	0.24
C20:5	ND	ND	ND	ND	ND	0.02	3.88
C22:6	ND	ND	ND	ND	ND	0.04	12.3
C24:0	ND	0.03	0.42	0.31	0.15	0.05	0.13
Sum of FA	4.0	117	126	128	108	115	115
SFA*	0.6	57.2	14.3	18.8	12.6	36.0	20.0
MUFA*	0.7	36.2	87.4	33.2	21.1	30.5	20.5
PUFA*	1.6	16.1	22.8	74.5	73.4	45.2	70.8
PUFA/SFA*	2.7	0.3	1.6	4.0	5.8	1.3	3.5
n-6 FA*	1.4	14.2	21.5	73.3	26.2	26.1	22.5
n-3 FA*	0.2	1.9	1.3	1.3	47.2	19.0	48.3
n-6/n-3 ratio	7.0	7.5	16.5	56.4	0.60	1.4	0.5

*FA: fatty acids, SFA Saturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA

Table 3. Average daily gain, final live weight, average daily feed intake and feed:gain ratio of gilts fed seven diets.

	NF	T	HOSF	SFO	LO	FB	OB	*RMSE*[†]
ADFI*, kg d^{-1}	3.01	2.94	2.89	2.86	2.90	3.06	2.84	*0.283*
ADG*, kg d^{-1}	0.95	1.04	1.03	1.01	0.96	1.10	0.93	*0.133*
FLW*, kg	98.0	101.4	101.0	100.2	98.4	103.6	97.3	*5.08*
Feed:Gain	3.21[a]	2.83[ab]	2.85[ab]	2.84[ab]	3.02[ab]	2.79[b]	3.05[ab]	*0.301*

Treatments: NF: no fat, T: tallow, HOSF: high oleic sunflower oil, SFO: sunflower oil, LO: linseed oil, FB: fat blend (tallow, sunflower oil, linseed oil), and OB: oil blend (fish oil, linseed oil).

Means within the same raw with different letters differ ($P < 0.05$).

[†]RMSE: Root Mean Square Error.

* ADFI: average daily feed intake, ADG: average daily gain, FLW: final live weight.

Table 4: Carcass parameters of gilts and weight and percentage of major primal cuts.

Carcass parameters	60 kg	NF	T	HOSF	SFO	LO	FB	OB	RMSE[†]
Carcass weight, kg	46.3	79.6	78.1	79.0	76.6	75.0	79.4	73.7	6.66
Killing out, %	75.2	80.0[a]	78.8[ab]	78.4[ab]	79.3[ab]	78.5[ab]	78.2[ab]	77.5[b]	1.38
Flare fat, %	1.13	1.95[a]	1.50[b]	1.84[ab]	1.83[ab]	1.62[ab]	1.62[ab]	1.71[ab]	0.313
Last rib fat depth, mm	12.3	16.2	13.5	15.7	15.9	14.3	14.0	14.9	2.30
Fat depth 3-4 l.r.*	12.9	18.6	15.1	17.9	17.9	16.3	16.1	16.7	2.64
Muscle depth 3-4 l.r.*	34.2	52.3	51.0	49.8	49.7	50.8	51.7	49.2	4.01
Carcass lean, %*	-	53.5	56.3	53.6	53.6	55.3	55.6	54.6	2.40
Weight of major primal cuts, kg									
Ham	5.48	9.11	9.18	9.09	9.05	9.24	9.20	9.04	0.298
Loin	3.93	7.06[a]	6.86[ab]	6.64[ab]	6.90[a]	6.71[ab]	6.42[b]	6.74[ab]	0.344
Shoulder	3.08	4.98	5.19	5.10	5.07	5.05	5.15	5.14	0.205
Belly	1.99	3.63	3.40	3.69	3.70	3.55	3.67	3.54	0.257
Tenderloin	0.28	0.45	0.46	0.45	0.46	0.47	0.47	0.46	0.037
Other fat cuts[δ]	1.78	2.87	2.97	3.16	3.08	2.98	3.12	2.99	0.236
Percentage of major primal cuts									
Ham	24.34	24.43	24.50	24.38	24.17	24.67	24.65	24.35	0.802
Loin	17.44	18.84 a	18.29 ab	17.69[ab]	18.42 ab	17.94 ab	17.23[b]	18.04 ab	0.931
Shoulder	13.65	13.40	13.86	13.70	13.54	13.54	13.77	13.84	0.529
Belly	8.81	9.69	9.08	9.84	9.88	9.50	9.82	9.49	0.694
Tenderloin	1.26	1.21	1.22	1.22	1.22	1.25	1.27	1.24	0.098
Other fat cuts[δ]	7.90	7.70	7.92	8.48	8.19	7.98	8.34	8.08	0.599

60 kg: animals slaughtered at the beginning of the trial.

Treatments: NF: no fat, T: tallow, HOSF: high oleic sunflower oil, SFO: sunflower oil, LO: linseed oil, FB: fat blend (tallow, sunflower oil, linseed oil), and OB: oil blend (fish oil, linseed oil).

Means within the same raw with different letters differ ($P < 0.05$) among dietary treatments. [†]RMSE: Root Mean-Square Error.

[δ]Other fat cuts: belly trimmings plus jowl.

Table 5. Ham, loin and belly dissection into lean, subcutaneous and intermuscular fat and bone of gilt carcasses.

Ham dissection, kg	60 kg	NF	T	HOSF	SFO	LO	FB	OB	RMSE†
Lean	3.36	5.91	6.13	5.84	5.86	6.10	6.03	5.90	0.301
Subcutaneous Fat*	1.02	2.01	1.89	2.07	2.05	1.98	2.00	2.00	0.202
Intermuscular Fat	0.25	0.44	0.41	0.40	0.39	0.39	0.40	0.43	0.089
Fat:Lean	0.34	0.41	0.38	0.42	0.42	0.39	0.40	0.41	0.050
Bone	0.49	0.75	0.76	0.79	0.73	0.78	0.78	0.75	0.056
Lean:Bone	6.85	7.50	7.74	7.14	7.68	7.49	7.47	7.51	0.580
Loin dissection, kg									
Lean	1.91	3.50^{ab}	3.74^{a}	3.38^{b}	3.51^{ab}	3.63^{ab}	3.45^{b}	3.48^{ab}	0.210
Subcutaneous Fat*	1.07	2.33^{a}	2.06^{ab}	2.16^{ab}	2.27^{ab}	2.02^{ab}	1.91^{b}	2.13^{ab}	0.287
Intermuscular Fat	0.19	0.48^{a}	0.31^{b}	0.39^{ab}	0.39^{ab}	0.33^{b}	0.32^{b}	0.36^{ab}	0.090
Fat:Lean	0.59	0.80^{a}	0.63^{b}	0.75^{ab}	0.76^{ab}	0.66^{ab}	0.65^{ab}	0.71^{ab}	0.105
Bone	0.51	0.75	0.76	0.72	0.73	0.73	0.74	0.77	0.069
Lean:Bone	3.82	4.41	4.58	4.32	4.38	4.56	4.19	4.16	0.442
Belly dissection, kg									
Lean	0.93	1.50^{a}	1.66^{b}	1.63^{ab}	1.74^{b}	1.74^{b}	1.77^{b}	1.69^{b}	0.117
Subcutaneous Fat*	0.44	0.96	0.89	1.04	0.98	0.87	0.93	0.88	0.120
Intermuscular Fat	0.31	0.90^{a}	0.58^{b}	0.73^{ab}	0.68^{b}	0.65^{b}	0.69^{b}	0.69^{b}	0.128
Fat:Lean	0.76	1.24^{a}	0.88^{bc}	1.08^{ab}	0.95^{bc}	0.87^{c}	0.92^{bc}	0.93^{bc}	0.141
Bone	0.21	0.27	0.28	0.29	0.30	0.28	0.28	0.29	0.033
Lean:Bone	4.44	5.11	5.70	5.44	5.67	5.78	5.88	5.58	0.708
Ham dissection, %									
Lean	67.89	64.98	66.80	64.34	64.89	65.96	65.59	65.29	2.223
Subcutaneous Fat*	18.57	21.98	20.52	22.67	22.68	21.41	21.64	21.61	2.156
Intermuscular Fat	4.60	4.79	4.43	4.32	4.35	4.17	4.30	4.84	1.005
Sub.+Inter. Fat	23.14	26.77	24.95	26.99	27.02	25.58	25.94	26.44	2.419
Bone	8.94	8.26	8.25	8.68	8.09	8.46	8.47	8.27	0.596
Loin dissection, %									
Lean	55.02	49.95^{a}	54.67^{b}	51.45^{ab}	50.99^{ab}	54.13^{ab}	53.77^{ab}	51.89^{ab}	3.108
Subcutaneous Fat*	27.21	32.63	29.73	31.98	32.84	30.05	29.82	31.36	3.393
Intermuscular Fat	4.90	6.78^{a}	4.54^{b}	5.69^{ab}	5.58^{ab}	4.91^{b}	4.96^{b}	5.34^{ab}	1.177
Sub.+Inter. Fat	31.73	39.41^{a}	34.28^{b}	37.67^{ab}	38.42^{ab}	34.95^{ab}	34.78^{ab}	36.69^{ab}	3.485
Bone	12.87	10.65	11.05	10.88	10.59	10.92	11.45	11.41	1.008
Belly dissection, %									
Lean	51.33	41.70^{a}	48.96^{bc}	44.88^{ac}	47.21^{bc}	49.28^{b}	48.32^{bc}	47.86^{bc}	2.943
Subcutaneous Fat*	22.27	26.45^{ab}	25.74^{ab}	27.94^{b}	26.42^{ab}	24.48^{a}	25.45^{ab}	24.86^{ab}	2.281
Intermuscular Fat	15.64	24.25^{a}	17.16^{b}	19.33^{b}	18.27^{b}	18.21^{b}	18.47^{b}	19.14^{b}	2.664
Sub.+Inter. Fat	38.04	50.71^{a}	42.90^{ab}	47.26^{b}	44.68^{b}	42.69^{b}	43.92^{b}	44.01^{b}	3.300
Bone	10.75	7.59	8.14	7.86	8.10	8.03	7.76	8.13	1.050

60 kg: animals slaughtered at the beginning of the trial.
Treatments: NF: no fat, T: tallow, HOSF: high oleic sunflower oil, SFO: sunflower oil, LO: linseed oil, FB: fat blend (tallow, sunflower oil, linseed oil), and OB: oil blend (fish oil, linseed oil).
Means within the same raw with different letters differ ($P < 0.05$) among dietary treatments.
†RMSE: Root Mean-Square Error.
*Includes skin.

Table 6: Ground carcass composition and intramuscular fat content of *Longissimus thoracis* and *Semimembranosus* muscles of gilts.

	60 kg	NF	T	HOSF	SFO	LO	FB	OB	RMSE†
Ground carcass composition, %									
Moisture	58.8	52.3	53.8	52.7	51.1	53.0	53.8	52.2	2.14
Fat	19.7	27.0^{ab}	24.9^{b}	27.1^{ab}	29.2^{a}	26.1^{ab}	25.7^{ab}	26.7^{ab}	2.65
Protein	17.9	17.4^{ab}	17.9^{b}	17.2^{ab}	16.7^{a}	17.7^{ab}	17.4^{ab}	17.5^{ab}	0.85
Ash	3.55	3.38	3.37	3.04	3.07	3.28	3.14	3.49	0.379
Intramuscular fat, % (NIT)*									
Longissimus thoracis	1.61	1.58	1.53	1.65	1.50	1.36	1.46	1.62	0.427
Semimembranosus	2.24	2.42	2.87	3.30	2.60	2.56	2.76	2.63	1.055

60 kg: animals slaughtered at the beginning of the trial.

Treatments: NF: no fat, T: tallow, HOSF: high oleic sunflower oil, SFO: sunflower oil, LO: linseed oil, FB: fat blend (tallow, sunflower oil, linseed oil), and OB: oil blend (fish oil, linseed oil).

Means within the same raw with different letters differ ($P < 0.05$) among dietary treatments.

†RMSE: Root Mean-Square Error.

*Near Infrared Transmittance.

Paper 3: Dietary fat source and level affect metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues

Submitted to *Animal*

UNIVERSITAT ROVIRA I VIRGILI
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I ÀCIDS GRASSOS EN EL PORC
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Dietary fat source and level affect metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues

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ABSTRACT

Little is known on pig gene expression related to dietary fatty acids and most work have been conducted in rodents to study metabolism of fat. The aim of this study was to investigate how dietary fats regulate fat metabolism of pigs in different tissues. Fifty-six crossbred gilts (62 ±5.x kg BW) were divided in 8 animals per treatment. Pigs were fed one of seven treatments: a semi-synthetic diet formulated to contain a very low level of fat (NF) and six fat supplemented diets (ca 10%.) based on barley and soybean meal. The supplemental fats were tallow (T), high-oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (B) (55% tallow, 35% sunflower oil, 10% linseed oil) and fish oil blend (FO) (40% fish oil, 60% linseed oil). Pigs were slaughtered at 100 kg BW and autopsies from liver, adipose tissue and muscle semimembranousus were collected for qPCR. The mRNA abundances of genes related to lipogenesis were modified due to dietary treatments in both liver (SREBP1, acetyl CoA carboxylase and stearoyl CoA desaturase) and adipose tissue (fatty acid synthase, acetyl CoA carboxylase and stearoyl CoA desaturase), but were not changed in semimembranousus muscle. In the liver, the mRNA abundances of genes encoding lipogenic enzymes were highest in pigs fed high oleic sunflower oil and lowest in pigs fed fish oil. In adipose tissue, the mRNA abundances were highest in pigs fed the NF diet and lowest in pigs fed tallow, which is rich in saturated FA. The study demonstrated that dietary FA stimulate lipogenic enzyme gene expression differently in liver, fat and muscles tissues.

KEYWORDS: Pig; Dietary fat; gene expression; lipogenesis

INTRODUCTION

Considerable attention has been paid to effects of dietary fats in modulating lipid metabolism (Bortz *et al.*, 1963, Allee *et al.*, 1971). Pig *de novo* fat synthesis largely takes place in the adipose tissue (O'Hea and Leveille, 1969, Bergen and Mersmann, 2005). Deposition and mobilization of fat depots is regulated at several levels. As for other metabolites, fat oxidation and synthesis occurs in different cellular compartments and these processes are directly controlled by the level and activity of the enzymes involved. Enzyme activity may be regulated at transcription, translational or post-translation level. During the last decade it has been recognized that specific dietary lipids have unique biological activities which are due to their stimulatory/inhibitory effects on transcription of genes encoding enzymes involved in fat metabolism (Jump, 2002).

Regulation of FA metabolism is done through changes in transcription, mRNA processing, mRNA stability or activity of several transcription factors like the peroxisome proliferator activated receptor (PPAR) family (involved in FA oxidation) (Lee *et al.*, 1995, Kersten *et al.*, 1999) and sterol regulatory element binding proteins (SREBP) 1 (involved in FA synthesis) (Kim and Spiegelman, 1996, Yahagi *et al.*, 1999). These two transcription factors are central for the regulation of expression of several key enzymes involved in pathways controlling fat metabolism (Jump, 2002).

The main focus on effects of dietary FA on transcription of lipogenic genes has been at hepatic level (Azain, 2004) since the liver is the most important organ regulating FA metabolism in mouse and human (Bergen and Mersmann, 2005). However, in pigs, adipose tissue is the most important organ in fat synthesis (O'Hea and Leveille, 1969). Little is known about effects of dietary FA on regulating gene transcription in pigs, and existing studies only have been performed on liver (Theil and Lauridsen,

2007), muscle and adipose tissue of weaning pigs and porcine adypocites (Hsu *et al.*, 2004, Liu *et al.*, 2005a). This study was therefore conducted to investigate the effect of diets with different FA composition on the transcription of genes involved in fat metabolism in adipose tissue, liver and semimembranousus muscle in growing pigs during the fattening period.

MATERIAL AND METHODS

Animals and diets

Sixty-one crossbred female pigs (Duroc ♂x Landrace ♀) were fed a barley-corn-soybean meal-based diet during a 4-week pre-experimental period. Five gilts were slaughtered at the beginning of the trial (null animals), whereas 56 gilts (61.8 ±5.2 kg BW) were randomly selected and assigned to one of seven dietary treatments (8 animals per treatment). Treatments were assigned randomly by animal weight and litter. The experiment comprised three periods with 3, 3 and 2 pigs per treatment, respectively. Pigs had access to feed and water *ad libitum* and feed consumption was measured individually. Gilts were slaughtered at an average BW of 99.8 ±8.5kg. Samples from liver, neck backfat (adipose tissue) and *semimembranosus* muscle were taken after slaughter and immediately frozen in liquid nitrogen and then stored at -75°C until analysis. Experimental procedures were approved by IRTA's ethical committee.

Seven diets were formulated to meet NRC (1998) requirements (Table1). Six fat supplemented diets (containing 10-11% fat in total) were based on barley and soybean meal. Additionally, a semi- synthetic diet was formulated to contain no fat because traditional diets with no fat addition always contain around 2% of dietary fat. Fat was weighed and added into the mixer (1000L capacity) and mixed with the rest

of the feed for 5 min. Different fat sources were selected to obtain different FA composition of the diets: tallow (T), high-oleic sunflower oil (SFHO), sunflower oil (SFO), linseed oil (LO), blend (55% tallow, 35% sunflower oil, 10% linseed oil) (B) and fish oil blend (40% fish oil, 60% linseed oil) (FO). The T diet was intended to have a high content in saturated FA (SFA), the HOSF diet was intended to have a high level in oleic acid, the SFO diet was intended to have a high content in linoleic acid (FA from the n-6 series), LO was intended to have a high content in linolenic acid (FA from the n-3 series). The B diet was intended to have a similar content in the main FA (palmitic, stearic, oleic, linoleic, and linolenic acids). The FO diet was intended to have a high content in long-PUFA. Oils and fats were for food or feed quality grade. The fat supplemented diets were formulated in order to supply equal amounts of digestible fat based on previous measurements (Duran-Montgé *et al.*, 2007).

Chemical analyses

Diets were analyzed for dry matter content (DM), crude protein, energy and FA. The dry matter content and energy were determined according to AOAC (1990), and crude protein by Dumas (AOAC, 2000). For FA determination lipids were extracted by the Folch method (Folch et al., 1957) and then transmethylated with BF_3 and methanolic KOH (Morrison and Smith, 1964). Diet composition and nutrient content are shown in Table 1 and FA profile of the seven diets in Table 2. Fatty acid contents were determined using an automated GC (Hewlett Packard 6890, USA) equipped with an automatic injector, using C19:0 as internal standard.

Table 1. Diet composition and nutrient content

	NF	T	HOSF	SFO	LO	FB	FO
Diet formulation (%)							
Barley		62.5	64.2	63.7	64.1	63.8	63.7
Soybean meal 44%		24.1	23.7	23.8	23.7	23.8	23.8
Wheat starch	70.0						
Soybean protein isolated	14.0						
Sugar beet pulp	10.1						
Molasses	4.0						
Tallow		10.97				5.45	
High oleic sunflower oil			9.58				
Sunflower oil				9.97		3.47	
Linseed oil					9.68	0.99	5.80
Fish oil							3.87
L-lysine HCl		0.01	0.02	0.02	0.02	0.02	0.02
DL- methyonine	0.04						
Dicalcium Phosphate	0.2	0.28	0.27	0.27	0.27	0.27	0.27
Calcium carbonate	1.08	1.61	1.61	1.61	1.61	1.61	1.91
Sodium Chloride	0.15	0.22	0.22	0.22	0.22	0.22	0.22
Mineral/Vitamin complex[a]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Nutrient and energy content							
Dry Matter (%)	98.0	98.0	98.1	97.9	97.6	97.9	97.9
GE[b] (Kcal Kg-1)	3.82	4.47	4.50	4.54	4.46	4.54	4.46
Crude Protein (%)	14.1	15.6	15.1	15.5	15.9	15.8	16.1
Crude fat	0.32	12.7	12.6	13.4	11.6	11.6	11.5
Ash (%)	2.93	5.03	4.83	4.82	5.09	5.11	5.16

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

[a] One kg of feed contains: Vitamin A: 5000 IU; Vitamin D_3: 1000 IU; Vitamin E: 15 mg; Vitamin B_1: 1,3 mg; Vitamin B_2: 3,5 mg; Vitamin B_{12}: 0.025 mg; Vitamin B_6: 1,5 mg; Calcium pantothenate: 10 mg; Nicotinic acid: 15 mg; Biotin: 0,1 mg; Folic acid: 0,6 mg; Vitamin K_3: 2 mg; Fe: 80 mg; Cu: 6 mg; Co: 0,75 mg; Zn: 60 mg; Mn: 30 mg; I: 0,75 mg; Se: 0,10 mg; Ethoxiquin: 0,15 mg.

[b] GE=Gross energy

Abundance of mRNA's

Approximately 30 mg of frozen liver or *semimembranosus* muscle tissues or 100 mg of backfat were homogenized in a tube containing TriReagent (Molecular Research

Center, 5645 Montgomery Rd., Cincinnati, Ohio 45212, USA), and then BCP (Molecular Research Center, 5645 Montgomery Rd., Cincinnati, Ohio 45212, USA) was added in order to separate the upper phase containing the RNA upon centrifugation of the homogenate. RNA was precipitated in isopropanol and the precipitate was washed twice in ethanol 75%. Finally, the pellet was resuspended in 20 μl RNAse free water (100μl in liver samples).

Table 2. Fatty acid content of experimental diets (mg/g)

	NF	T	HOSF	SFO	LO	B	FO
C14:0	ND	3.34	0.12	0.13	0.09	1.72	1.53
C14:1	ND	0.59	ND	ND	ND	0.24	0.02
C16:0	0.55	28.7	7.87	11.2	8.21	20.0	12.9
C16:1 n-7	ND	2.37	0.19	0.11	0.08	1.35	1.81
C18:0	0.15	22.8	4.25	5.56	3.64	12.8	4.07
C18:1n-9 trans	0.06	0.23	0.10	0.27	0.06	0.27	0.10
C18:1 n-7	ND	3.03	ND	0.22	0.06	1.05	ND
C18:1 n-9 cis	0.8	34.8	86.8	32.5	20.7	29.5	19.0
C18:1n-11	0.05	1.23	0.86	0.82	0.78	1.20	1.44
C18:2 n-6	2.0	14.20	21.5	73.3	26.2	26.0	21.5
C18:3 n-3	0.26	1.90	1.29	1.26	47.1	18.9	31.2
C20:0	ND	0.13	0.40	0.43	0.19	0.24	0.26
C22:0	ND	0.07	1.15	1.02	0.20	0.23	0.24
C20:5	ND	ND	ND	ND	ND	0.02	3.88
C22:6	ND	ND	ND	ND	ND	0.04	12.3
C24:0	ND	0.03	0.42	0.31	0.15	0.05	0.13
Sum of FA	4.0	117	126	128	108	115	115
SFA*	0.6	57.2	14.3	18.8	12.6	36.0	20.0
MUFA*	0.7	36.2	87.4	33.2	21.1	30.5	20.5
PUFA*	1.6	16.1	22.8	74.5	73.4	45.2	70.8
PUFA/SFA*	2.7	0.3	1.6	4.0	5.8	1.3	3.5
n-6 FA*	1.4	14.2	21.5	73.3	26.2	26.1	22.5
n-3 FA*	0.2	1.9	1.3	1.3	47.2	19.0	48.3
n-6/n-3 ratio	7.0	7.5	16.5	56.4	0.60	1.4	0.5

*FA: fatty acids, SFA Saturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA

Table 3. Accession numbers, amplicon location (span of exons), amplicon length, range of Ct values of different tissues and slope of standard curve of the analyzed genes

Gene	Accession Number	Amplicon Location	Amplicon Length (bp)	Range Ct in liver,adipose tissue & muscle	Slope of std. curve
ACACA	AF175308	44-45	133	27-31, 26-29, 28-32	-3.36
FASN	AY954688	4-5	108	29-34, 23-28, 29-34	-3.60
SREBP-1	NM_214157	5-6	114	25-30, 25-29, 26-29	-3.52
PPAR α	AF228696	8-9	70	24-29, 29-32, 28-31	-3.72
SCD	AY487829	4-5	95	26-32, 20-26, 27-33	-3.45
D6D	AY512561	3-4	72	23-29, 29-33, 29-32	-3.41
HPRT1	NM001032376	4-6	95	25-28, 29-32, 29-32	-3.64

Acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), sterol regulatory element binding protein-1 (SREBP1), peroxisome proliferator activated receptor α (PPAR-α), stearoyl CoA desaturase (SCD), Δ6-desaturase (D6D), HPRT1: Hypoxanthine phosphoribosyltransferase

Purified RNA was reverse-transcribed with oligo-dT and random primers using Superscript III RNAse H reverse transcriptase kit (Invitrogen, Taastrup, Denmark) according to the manufacturer's protocol.

Reverse-transcribed material (1µL) was amplified with TaqMan Universal PCR Master Mix (Applied Biosystems, Stockholm, Sweden) using primer pairs specific for each gene and signal was detected quantitatively by SYBR Green (SREBP1 and PPARα), or probes labeled with carboxyfluorescein (FAM) on the 5´ end. Two different types of probes were used; locked nucleic acid probes (Human probe Library, Roche Applied Science, Denmark) were used to quantify Acetyl CoA Carboxylase (ACACA) (human probe #13), Fatty Acid Synthase (FASN) (#9), Stearoyl CoA Desaturase (SCD) (#82) and hypoxanthine phosphoribosyltransferase (HPRT1) (#22), whereas Delta 6 Desaturase (D6D) was detected using a minor

groove binding probe. Primers and probes were designed by using Primer Express Version 2.0 software (Applied Biosystems, Stockholm, Sweden), and HPRT1 was used as endogenous control (housekeeping gene). The design of primers for SREBP1 did not allow discrimination between SREBP-1a and SREBP-1c. Details of primer/probe design and runs of real time RT-PCR are given in Table 3. No amplification was found in ribonuclease free water and in samples containing genomic pig DNA. For RT-PCR, 40 cycles were used at 95 ºC for 15 s, and 60 ºC for 60 s. The response was quantified as the number of PCR cycles required to reach a certain threshold, and samples were analyzed in duplicates. The oligonucleotide sequences of forward primer, probe (in case it was used) and reverse primer for the studied genes were as follows:

ACACA: 5'-atgtttcggcagtccctgat, 5'-ctctgcct and 5'-tgtggaccagctgaccttga,

FASN: 5'-cgtgggctacagcatgatag, 5'-catcacca and 5'-gaggagcaggccgtgtctat,

SREBP1: 5'-cggacggctcacaatgc and 5'-gacggcggatttattcagctt,

PPARα: 5'-catcctcgcgggaaagg and 5'-ggccatacacagtgtctccatgt,

SCD: 5'-gccgagaagctggtgatgtt, 5'-cagaggag and 5'-cagcaataccagggcacgat and

D6D: 5'-gacggccttcatccttgct, 5'-cctctcaggcccaggctgggtg and

5'- acagagagatggccgtaatcgt.

HPRT1: 5'-cagtcaacgggcgatataaaagta, 5'-tggtggag, 5'-ccagtgtcaattatatcttcaacaatcaa

Calculations and statistics

Data to evaluate mRNA quantities were obtained as Ct values (the cycle number at which logarithmic plots cross a calculated threshold line) according to the manufacturer's guidelines, and used to determine ΔCt values (ΔCt =Ct of the target gene – Ct of the housekeeping gene). The ΔCt data of each target gene were

analyzed separately for each tissue using the MIXED procedure of SAS (Littell et al., 1996). Data were expressed relative to NF diet by calculating ΔΔCt values (ΔCt of treatment – ΔCt of NF diet) and results were converted to expression levels according to Pfaffl (2001) by taking into account the PCR efficiencies. The following statistical model was applied separately for each tissue:

$$X_{ijk} = \mu + \alpha_i + \beta_j + \chi_{ij} + U_{jk} + \varepsilon_{ijk}$$

where y is the observation (e.g. ΔCt values) in the j'th period from the k'th litter, μ is the overall mean, α_i is a fixed treatment effect, β_j is a fixed effect of period, χ_{ij} is the interaction effect treatment x period, U_{jk} is the sow x period random effect , and ε the residual errors. The treatment*period interaction was not significant for any of the variables, and therefore only main effects are reported.

Significance levels of the correlate coefficient are based on Fischer (1925)

RESULTS

Fatty acids in diets

The NF diet had a low content in all FA, whereas the fat-supplemented diets had high contents of specific FA; the T diet was characterized as having high contents of saturated FA's (palmitic and stearic FA), HOSF, SFO and LO diets were rich in oleic, linoleic and linolenic acids, respectively, the B diet had similar content of palmitic, stearic, linoleic and linolenic FA (Table 2) and the FO diet was rich in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Gene Expression related to treatments in the different tissues

Transcription of genes was analyzed separately for each type of tissue. Within a tissue, the housekeeping gene (HPRT1) was not affected by treatment (liver $p=0.59$, adipose tissue $p=0.88$, muscle $p=0.48$). Hence, the HPRT1 was considered a suitable housekeeping gene.

In liver (Table 4) the dietary treatments affected the transcription of ACACA ($p=0.014$), SREBP1 ($p<0.001$) and SCD ($p<0.001$). For these three genes, hepatic transcription was higher (all $p\leq0.03$) in pigs fed HOSF than in those fed FO, NF, B and T. The lowest mRNA abundance was observed in pigs fed FO.

In adipose tissue (Table 4) ACACA, FASN and SCD were affected by dietary treatment ($p<0.01$, $p<0.0001$, $p<0.01$ respectively). The mRNA abundance of the FASN and SCD was much higher for the NF diet when compared to he other diets, and ACACA mRNA abundance was also higher for the NF diet compared to the T, HOSF, B and FO diets. For ACACA and FASN the lowest mRNA abundances were observed in T, whereas for SCD the lowest mRNA abundance was observed in pigs

fed LO. No differences were observed in the mRNA abundance of SREBP1, PPAR-α and D6D (p=0.70, p=0.79, p=0.86 respectively).

None of the selected target genes were affected by dietary fat source in the *semimembranosus* muscle (Table 4) (all p>0.05).

Table 4. Liver, adipose tissue and muscle mRNA abundances[1].

	NF	T	HOSF	SFO	LO	B	FO	*P*
Liver								
ACACA	1.0^{bc}	1.00^{bc}	1.49^{a}	1.16^{ab}	1.07^{bc}	1.03^{bc}	0.85^{c}	*0.014*
FASN	1.0	0.72	1.15	1.20	0.69	0.87	0.49	*0.25*
SREBP1	1.0^{c}	1.31^{bc}	2.06^{a}	1.52^{abc}	1.46^{abc}	1.09^{c}	0.61^{d}	*0.0002*
PPAR-α	1.0	1.06	1.03	0.88	1.06	0.91	0.86	*0.86*
SCD	1.0^{cd}	1.56^{bc}	2.89^{a}	2.22^{ab}	1.80^{abc}	1.16^{cd}	0.64^{d}	*0.0004*
D6D	1.0	1.52	1.52	2.00	1.04	0.95	0.70	*0.11*
Adipose Tissue								
ACACA	1.0^{a}	0.65^{d}	0.66^{cd}	0.84^{ab}	0.84^{abc}	0.75^{bcd}	0.69^{bcd}	*0.0049*
FASN	1.0^{a}	0.30^{d}	0.39^{bcd}	0.53^{b}	0.33^{cd}	0.38^{bcd}	0.42^{bcd}	*<0.0001*
SREBP1	1.0	0.83	0.94	0.93	0.91	1.08	0.85	*0.70*
PPAR-α	1.0	1.08	1.16	1.13	1.01	0.94	0.96	*0.79*
SCD	1.0^{a}	0.43^{b}	0.41^{bc}	0.54^{b}	0.22^{c}	0.43^{b}	0.38^{bc}	*0.0031*
D6D	1.0	1.17	1.28	1.15	1.20	0.96	1.04	*0.86*
Muscle								
ACC	1.00	1.43	1.15	1.26	1.24	0.98	1.35	*0.38*
FAS	1.00	1.18	1.26	1.05	1.07	0.70	1.55	*0.59*
SREBP	1.00	1.38	0.85	1.22	1.27	1.11	1.07	*0.34*
PPAR-α	1.00	1.13	1.10	1.37	0.99	0.86	1.01	*0.23*
SCD	1.00	2.11	1.15	0.83	0.98	0.75	1.28	*0.12*
D6D	1.00	1.62	1.09	1.43	1.05	0.83	1.42	*0.09*

[1]Means within are row with different letter are significantly different (p<0.05)

Acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), Sterol regulatory element binding protein-1 (SREBP1), Peroxisome proliferator activated receptor α (PPAR-α), Stearoyl CoA desaturase (SCD), Δ6-desaturase (D6D), of pigs fed either a diet with no fat (NF) or a diet with 10% of fat from different sources: tallow (T), high oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (55% tallow, 35% sunflower oil, 10% linseed oil) (B) or fish oil blend (40% fish oil, 60% linseed oil) (FO). The mRNA's abundances are expressed relative to the NF diet.

Correlations between gene expression on dietary FA composition (Table 5) showed that MUFA content correlated positively (P<0.05) with ACACA, SREBP1 and SCD in the liver and n3 content correlated negatively with SCD (P<0.1), irrespective of the inclusion of the NF diet. Other correlations were observed in the liver, but most of them included MUFA. In adipose tissue negative correlations were observed when NF diet was included between unsaturated FA and SCD (P<0.05) and FAS (P<0.1), and SFA correlated negatively with ACACA (P<0.1). When NF diet was not included, other correlations were observed; n6 content and n6/n3 ratio correlated positively with adipose tissue FASN (P<0.05).

Table 5. Correlations coefficients (r) between diet FA composition and gene expression of genes that were affected by treatments in the liver (Liv) and adipose tissue (AdT).

Treat	ACACA Liv	FASN Liv	SREBP1 Liv	SCD Liv	ACACA AdT	FASN AdT	SREBP1 AdT	SCD AdT
n6	NS	NS	NS	NS	NS	NS(0.88)	NS	NS
n3	NS	-0.78(-0.77*)	NS	NS	NS	NS	NS	NS(0.75*)
n6/n3	NS	0.72*(0.76*)	NS	NS	NS	NS(0.81)	NS	NS
PUFA	NS	NS	NS	NS	NS(0.79*)	NS	NS	NS
MUFA	0.86(0.91)	NS	0.79(0.79*)	0.81(0.78*)	NS	NS	NS	NS
UFA	NS	NS	NS	NS	NS	-0.71*(NS)	NS	-0.79(NS)
SFA	NS	NS	NS	NS	-0.67*(NS)	NS	NS	NS
PUFA/SFA	NS	NS	NS	NS	NS(0.79*)	NS	NS	NS
MUFA/SFA	0.93(0.92)	NS	0.80(0.79*)	0.81(0.81)	NS	NS	NS	NS
MUFA/PUFA	0.76(0.75)	NS	0.71*(NS)	NS	NS	NS	NS	NS

Acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), Sterol regulatory element binding protein-1 (SREBP1), Stearoyl CoA desaturase (SCD).
Significant values were P<0.05 (* when P<0.1). Not significant (NS).
In brackets, data when NF diet was excluded from comparisons.

DISCUSSION

The mRNA abundance in the liver

Fats and especially PUFA are inhibitors of hepatic lipogenesis in rats (Wilson et al., 1986). Different studies (Brown and Goldstein, 1997, Xu *et al.*, 1999) suggest that SREBP1c regulates the expression of several lipogenic genes involved in the synthesis of FA. *In vivo* experiments with different species such as rats (Xu et al., 2002) and pigs (Hsu *et al.*, 2004, Liu *et al.*, 2005b) have shown that dietary fish oil, rich in long-PUFA lowered SREBP1 expression in the liver, similarly to the results of the present experiment in which pigs fed the FO diet, characterized with the highest contents in very long PUFA, clearly showed the lowest SREBP1 expression. In the present experiment pigs fed FO diet also tended to show the lowest ACACA and SCD mRNA abundances possibly caused by inhibition of lipogenic genes by the low SREBP1 expression. In contrast, pigs fed the HOSF diet showed the highest expression of SREBP1, ACACA and SCD, suggesting that the high oleic acid content of this diet indeed stimulated the expression of SREBP1, as observed in rats (Xu *et al.*, 1999). Pigs fed with a diet that did no include fat (NF diet) received a higher fraction of energy from carbohydrate, but this did not increase the expression of genes related to lipogenesis as SREBP1, ACACA or SCD genes compared to fat added diets. Experiments with other species have shown the influence of PUFA on expression of SREBP1c (Blake and Clarke, 1990, Mater *et al.*, 1999, Xu *et al.*, 1999) brought about by a decrease in the mature form of SREBP-1 and consequently a decreased expression of lipogenic genes (Xu *et al.*, 1999). In our study, pigs fed the diets rich in n-6 (SFO) and n-3 (LO) FA did not show low expression of lipogenic genes in the liver when compared to pigs fed higher a SFA level (T diet). Our results are similar to those of (Theil and Lauridsen, 2007) in which pigs of 56 days of age

fed with sunflower oil, diet rich in n-6 FA, had similar expression levels of lipogenic genes as pigs fed animal fat, rich in SFA. The SCD is involved in the transformation of stearic acid into oleic acid and its mRNA is reduced in rat liver by PUFA (Lochsen et al., 1997), but not by stearic and oleic acids. Sessler et al. (1996) suggested that this action was caused by a reduced mRNA stability. These findings indicate that this FA conversion is not inhibited by its reaction products as shown by the fact that the diet rich in oleic acid (HOSF diet) did not reduce SCD expression. Different studies (Lee *et al.*, 1995, Leone *et al.*, 1999) report that the increase in lipid oxidation due to FA is mediated by PPARα since FA bind to PPARα and act as a transcription promoters, which lead to changes in expression of genes involved in lipid metabolism and storage. In contrast to SREBP1, no changes are reported in PPARα expression caused exclusively by dietary FA (Pegorier *et al.*, 2004, Jump *et al.*, 2005). In agreement with this, we did not observe changes in PPARα mRNA abundance in response to dietary treatments neither in the liver, nor in adipose or muscle tissues. Although not significant (P=0.11), animals fed with very long PUFA (FO diet) tended to have a lower expression of D6D and SFO pigs a higher expression of this gene. This observation agrees with the findings of Tang *et al.* (2003) in rats where fish oil decreased D6D expression but for unknown reasons, the results observed with SFO show an opposite trend to that observed by Tang *et al.* (2003) using safflower oil, which is also high in n-6 PUFA.

mRNA abundance in adipose tissue

In previous reports, the effects of different FA types were tested to investigate their effects on lipogenesis; Allee *et al.* (1971) demonstrated that 10% of corn oil or beef tallow added to diets had the same effects on suppressing lipogenesis in porcine

adipose tissue. Smith *et al.* (1996) tested *in vitro* lipogenesis in cultured adipocytes from piglets previously fed with different dietary treatments differing in their FA source. They observed that the C18:0 enriched diet resulted in lower lipogenesis compared to a C18:2 enriched diet and attributed this finding to the lower absorption of C18:0. Under most circumstances fat is deposited in fat depots without modifications of the FA's because growing pigs in commercial conditions are in a positive energy balance and diets contain large amounts of carbohydrates in the form of starch which are used for *de novo* synthesis of lipids. In species like chickens, rodents or human, liver is the main organ for *de novo* synthesis, whereas in pigs this mainly takes place in adipose tissue (O'Hea and Leveille, 1969). In contrast to the liver, pigs fed with the no fat added diet (NF diet) showed the highest mRNA abundance in adipose tissue of genes involved in the synthesis of stearic acid (ACACA and FASN) and also a gene involved in FA desaturation (SCD). These results agree with the higher lipogenic rate observed by Smith *et al.* (1996) in cultured adipocytes isolated from pigs fed a cornstarch diet as compared to pigs fed a 10% fat added diet, and it also agrees with the findings of Mersmann *et al.* (1984) who measured adipose tissue lipogenesis comparing carbohydrate and fat as energy source. In the present study, the higher SCD mRNA abundance in adipose tissue with the NF diet showed a different response pattern to what was observed in the liver. In addition pigs fed with high oleic acid contents (HOSF diet) did not show the highest SCD expression as observed for the liver. In rodents (Blake and Clarke, 1990, Iritani *et al.*, 1998) diets containing high levels of PUFA result in lower lipogenesis than when fed diets containing high levels of saturated or monounsaturated FA and this is similar in broilers where sunflower oil (a PUFA rich diet) lowers fatty acid synthesis (Sanz et al., 2000). Different studies with pigs report

higher lipogenic rates in animals fed unsaturated FA than SFA (Waterman *et al.*, 1975, Freire *et al.*, 1998, Kouba and Mourot, 1998, Kouba *et al.*, 1999). According to Chilliard (1993) and Azain (2004), in species like rodents and poultry where the liver is the primary site of lipogenesis, unsaturated FA are more inhibitory on FA synthesis than saturated ones; in species where adipose tissue is the primary site of lipogenesis, like pigs, SFA are equivalent (or more potently) inhibiting lipogenesis than unsaturated FA. This is confirmed in the present experiment, as animals fed the diet with the highest contents in SFA (T diet) tended to decrease the mRNA abundance of FASN, ACACA and SDC relative to other diets. In contrast to the abundance of liver SREBP1 mRNA, adipose tissue showed no differences between treatments, suggesting that fat synthesis in pig adipose tissue could be regulated in a different manner than in the liver. Thus, our experiment indicates that the responses to dietary fat sources are tissue specific, which is consistent with findings by Kouba and Mourot (1998), and Waterman *et al.* (1975). Pigs fed the T diet, which had a high content in SFA, showed the lowest ACACA and FASN mRNA abundance and pigs fed SFO diet, rich in linoleic acid, showed the highest expression of those genes among fat added diets; meaning that SFA inhibit expression of these genes; other unsaturated fats resulted in intermediate values. These findings are in contrast to those of Hsu *et al.* (2004) who found no differences in mRNA contents of FASN neither in adipose tissue, nor in the liver in weaned pigs fed 2% of tallow or DHA oil in diet. In spite of these differences, both studies agree in the sense that liver showed lower SREBP1 mRNA abundances in pigs fed DHA than tallow. The different mRNA abundance observed in liver but not in adipose tissue agrees with the work of Ding *et al.* (2003). Liu *et al.* (2005b) also found differences in the liver but not in adipose tissue when feeding pigs with 10% algal DHA for 2 days and they suggested that it

was due to the inability to increase DHA content in adipose tissue in that short period of time. A possible explanation to the observed tissue differences in these different studies related to SREBP1 could be the previously mentioned specie/tissue specificity.

In contrast to other tissues, *semimembranosus* muscle was not affected by any of the selected target genes. It is possible that this was related to the secondary role of muscles in lipid reservoir depots.

In the liver, FASN expression was reduced by dietary n3 FA as shown by correlation results, meaning that although FASN was not affect by dietary treatments, linolenic acid in diet could exert an effect on FASN liver expression. Correlations showed that MUFA (or absence of other metabolically active FA) could exert an effect on liver gene expression, relative to lipogenic genes. In liver these correlations were significant irrespective of the inclusion of the NF treatment, but in adipose tissue correlations differed when this diet was included. This suggests that dietary effects on liver gene expression are due to FA composition, while in adipose tissue effects may also depend on the fat level. The correlation between FASN expression in adipose tissue and n6 dietary content and n6/n3 ratio suggests that linoleic acid in diet could exert a positive action adipose FA synthesis.

CONCLUSIONS

Results of the present study show that dietary FA profile modifies mRNA abundances of genes encoding lipogenic enzymes and that these dietary effects are tissue specific. Adipose tissue is the main organ for FA synthesis and our results suggest that the effects of the different FA on this tissue are different from those found in the

liver and in *semimembranousus* muscle. In liver FO diet rich in long-PUFA decreased mRNA of lipogenic genes whereas in adipose tissue, the main reductions in lipogenic enzyme mRNA's were observed in pigs fed T, which is rich in SFA. None of the selected target genes were affected by dietary fat source in semimembranousus muscle.

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REFERENCES

Allee GL, Baker DH and Leveille GA 1971. Influence of level of dietary fat on adipose tissue lipogenesis and enzymatic activity in pig. Journal of Animal Science 33, 1248

AOAC 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Washinton DC.

AOAC 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washinton DC.

Azain MJ 2004. Role of fatty acids in adipocyte growth and development. Journal of Animal Science 82, 916-924.

Bergen WG and Mersmann HJ 2005. Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. Journal of Nutrition 135, 2499-2502.

Blake WL and Clarke SD 1990. Suppression of rat hepatic Fatty-Acid Synthase and S-14 gene-transcription by dietary polyunsaturated fat. Journal of Nutrition 120, 1727-1729.

Bortz W, Chaikoff IL and Abraham S 1963. Localization of block in lipogenesis resulting from feeding fat. Journal of biological chemistry 238, 1266-&.

Brown MS and Goldstein JL 1997. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89, 331-340.

Chilliard Y 1993. Dietary-fat and adipose-tissue metabolism in ruminants, pigs, and rodents - a Review. Journal of Dairy Science 76, 3897-3931.

Ding ST, Lapillone A, Heird WC and Mersmann HJ 2003. Dietary fat has effects on fatty acid metabolism transcript concentrations in pigs. Journal Animal Science 81, 423-431.

Duran-Montgé P, Lizardo R, Torrallardona D and Esteve-Garcia E 2007. Fat and fatty acid digestibility of different fat sources in growing pigs. Livestock Science 109, 66-69.

Fisher RA 1925. Table VA in Statistical Methods for Research Workers. Oliver & Boyd, Edinburgh.

Folch J, Lees M and Stanley GHS 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry 226, 497-509.

Freire JPB, Mourot J, Cunha LF, Almeida JAA and Aumaitre A 1998. Effect of the source of dietary fat on postweaning lipogenesis in lean and fat pigs. Annals of Nutrition and Metabolism 42, 90-95.

Hsu JM, Wang PH, Liu BH and Ding ST 2004. The effect of dietary docosahexaenoic acid on the expression of porcine lipid metabolism-related genes. Journal of Animal Science 82, 683-689.

Iritani N, Komiya M, Fukuda H and Sugimoto T 1998. Lipogenic enzyme gene expression is quickly suppressed in rats by a small amount of exogenous polyunsaturated fatty acids. Journal of Nutrition 128, 967-972.

Jump DB 2002. Dietary polyunsaturated fatty acids and regulation of gene transcription. Current opinion in lipidology 13, 155-164.

Jump DB, Botolin D, Wang Y, Xu JH, Christian B and Demeure O 2005. Fatty acid regulation of hepatic gene transcription. Journal of Nutrition 135, 2503-2506.

Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B and Wrahli W 1999. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. Journal of Clinical Investigation 103, 1489-1498.

Kim JB and Spiegelman BM 1996. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes & Development 10, 1096-1107.

Kouba M and Mourot J 1998. Effect of a high linoleic acid diet on Delta 9-desaturase activity, lipogenesis and lipid composition of pig subcutaneous adipose tissue. Reproduction Nutrition Development 38, 31-37.

Kouba M, Bonneau M and Noble RC 1999. Realtive development of subcutaneous, intermuscular, and kidney fat in growing pigs with different body composition. Journal of Animal Science 77, 622-629.

Lee SST, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez Salguero PM, Westphal H and Gonzalez FJ 1995. Targeted disruption of the alpha-isoform of the Peroxisome Proliferator-Activated Receptor gene in mice results in abolishment of the pleiotropic effects of Peroxisome Proliferators. Molecular and Cellular Biology 15, 3012-3022.

Leone TC, Weinheimer CJ and Kelly DP 1999. A critical role for the peroxisome proliferator-activated receptor alpha (PPAR alpha) in the cellular fasting response: The PPAR alpha-null mouse as a model of fatty acid oxidation disorders. Proceedings Of The National Academy Of Sciences Of The United States Of America 96, 7473-7478.

Littell RC, Milliken GA, Stroup WW and Wolfinger RD 1996. SAS System for mixed models. In SAS Inst. Inc., Cary, NC.

Liu BH, Kuo CF, Wang BH and Ding ST 2005a. Effect of docosahexaenoic acid and arachidonic acid on the expression of adipocyte determination and differentiation-dependent factor 1 in differentiating porcine adipocytes. Journal of Animal Science 83, 1516-1525.

Liu BH, Wang YC, Kuo CF, Cheng WM, Shen TF and Ding ST 2005b. The effects of docosahexaenoic acid oil and soybean oil on the expression of lipid metabolism related mRNA in pigs. Asian-Australasian Journal of Animal Sciences 18, 1451-1456.

Lochsen T, Ormstad H, Braud H, Brodal B, Christiansen EN and Osmundsen H 1997. Effects of fish oil and n-3 fatty acids on the regulation of Delta(9) fatty acid desaturase mRNA and -activity in rat liver. Journal of Nutritional Biochemistry 8, 408-413.

Mater MK, Thelen AP, Pan DA and Jump DB 1999. Sterol response element-binding protein 1c (SREBP1c) is involved in the polyunsaturated fatty acid suppression of hepatic S14 gene transcription. Journal of biological chemistry 274, 32725-32732.

Mersmann HJ, Pond WG and Yen JT 1984. Use of carbohydrate and fat as energy source by obese and lean swine. Journal of Animal Science 58, 894901.

Morrison WR and Smith LM 1964. Preparation of fatty acid methyl esters + dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5, 600-&.

O'Hea EK and Leveille GA 1969. Significance of adipose tissue and liver as sites of fatty acid synthesis in pig and efficiency of utilization of various substrates for lipogenesis. Journal of Nutrition 99, 338-&.

Pegorier JP, Le May C and Girard J 2004. Control of gene expression by fatty acids. Journal of Nutrition 134, 2444S-2449S.

Pfaffl MW 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 29.

Sanz M, Lopez-Bote CJ, Menoyo D and Bautista JM 2000. Abdominal fat deposition and fatty acid synthesis are lower and beta-oxidation is higher in broiler chickens fed diets containing unsatuarated rather than saturated fat. Journal of Nutrition 130, 3034-3037.

Sessler AM, Kaur N, Palta JP and Ntambi JM 1996. Regulation of Stearoyl-CoA Desaturase 1 mRNA stability by polyunsaturated fatty acids in 3T3-L1 adipocytes. Journal of biological chemistry 271, 29854-29858.

Smith DR, Knabe DA and Smith TB 1996. Depression of lipogenesis in swine adipose tissue by specific dietary fatty acids. Journal of Animal Science 74, 975-983.

Tang CR, Cho HP, Nakamura MT and Clarke SD 2003. Regulation of human Delta-6 desaturase gene transcription: identification of a functional direct repeat-1 element. Journal of Lipid Research 44, 686-695.

Theil PK and Lauridsen C 2007. Interactions between dietary fatty acids and hepatic gene expression in livers of pigs during the weaning period. Livestock Science 108, 26-29.

Waterman RA, Romsos DR, Tsai AC, Miller ER and Leveille GA 1975. Influence of dietary safflower oil and tallow on growth, plasma-lipids and lipogenesis in rats, pigs and chicks. Proceedings of the Society for Experimental Biology and Medicine 150, 347-351.

Wilson MD, Hays RD and Clarke SD 1986. Inhibition of liver lipogenesis by dietary polyunsaturated fat in severely diabetic rats. Journal of Nutrition 116, 1511-1518.

Xu J, Nakamura MT, Cho HP and Clarke SD 1999. Sterol Regulatory Element Binding Protein-1 expression is suppressed by dietary polyunsaturated fatty acids - A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. Journal of biological chemistry 274, 23577-23583.

Xu J, Cho H, O'Malley S, Park JHY and Clarke SD 2002. Dietary polyunsaturated fats regulate rat liver sterol regulatory element binding proteins-1 and-2 in three distinct stages and by different mechanisms. Journal of Nutrition 132, 3333-3339.

Yahagi N, Shimano H, Hasty AH, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Shiomoiri F, Ohashi K, Osuga J-i, Harada K, Gotoda T, Nagai R, Ishibashi S and Yamada N 1999. A crucial role of Sterol Regulatory Element-binding Protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. Journal of biological chemistry 274, 35840-35844.

UNIVERSITAT ROVIRA I VIRGILI
EFECTES DE LA COMPOSICIÓ D'ÀCIDS GRASSOS DE LA DIETA SOBRE LA DEPOSICIÓ DE GREIX
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Paper 4: Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulation factors in liver and adipose tissue but not in semimembranosus muscle of pigs during the fattening period

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Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulation factors in liver and adipose tissue but not in *semimembranosus* muscle of pigs during the fattening period

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ABSTRACT

It has been shown previously that lipid metabolism is regulated by fatty acids and that thyroid hormones are important regulators of energy metabolism. The effects of age, dietary fat level, dietary FA profile on thyroid hormone levels and expression of lipogenic genes and tissue FA composition were studied. Sixty-one crossbred gilts (62 ±5.x kg BW average) were either slaughtered at the beginning of the trial (n=5) or fed one of seven diets (n=8 pigs per diet): a semi-synthetic diet formulated to contain a very low level of fat (NF) and six diets based on a barley-soybean meal supplemented with approx 10% fat of different origin. The supplemental fats were tallow (T), high-oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (B) (55% tallow, 35% sunflower oil, 10% linseed oil) and fish oil blend (FO) (40% fish oil, 60% linseed oil). In general, the dietary FA profiles modified the FA composition of tissues. The study further showed that pigs fed the NF diet had the highest free and total triiodothyronine (T3) values followed by pigs fed SFO. The 60 kg pigs showed higher total T3 hormone content than pigs weighing 100 kg. Correlations between thyroid hormones and genes of fat synthesis in adipose tissue (acetyl CoA carboxylase, fatty acid synthase and stearoyl CoA desaturase) and the large differences in expression of lipogenic genes at different ages (60 and 100 kg BW), suggest a role of thyroid hormones, in particular T3, in regulating whole animal fat metabolism and these effects were brought about by altered expression of lipogenic genes. In liver, SREBP1 mRNA contents showed effects of dietary treatment and correlation with acetyl CoA carboxylase and stearoyl CoA desaturase, whereas in adipose tissue SREBP1 showed no correlations with other lipogenic genes. Age and tissue specificity showed greater influence on mRNA abundance of genes related with lipid metabolism than diet and tissue fatty acid composition. In the

pig adipose tissue there is greater fatty acid synthesis as indicated by the higher expression of lipogenic genes in adipose tissue than in the liver.

KEYWORDS: pig; tissue; fatty acid composition; age; gene expression; lipogenesis; thyroid hormones

INTRODUCTION

Factors affecting pig lipid metabolism include diet and feeding regimen (1981), sex (Mersmann, 1984), age (Scott *et al.*, 1981), breed (Mersmann *et al.*, 1984). The main sites for fat synthesis in animals are liver and adipose tissue, but important species differences exist; in rodents and birds, liver is the main organ for FA synthesis, but in pig, *de novo* fat synthesis largely takes place in the adipose tissue (O'Hea and Leveille, 1969, Bergen and Mersmann, 2005).

PPARs belong to the steroid hormone nuclear superfamily of ligand-activated transcription factors. Different isoforms of PPARs have been identified and PPARα is a subtype implicated in regulating genes involved in lipid oxidation and n-3 and n-6 FA are some of their natural ligands. SREBPs belong to the helix-loop-helix family of transcription factors. Different isoforms have been identified and SREBP-1c and SREBP-1a are important for the regulation of genes involved in lipid synthesis. Evidence from *in vitro* (Worgall *et al.*, 1998) and *in vivo* (Xu *et al.*, 1999) experiments show that PUFA have a role in inhibiting maturation of SREBP at post-translational level, thereby repressing lipogenic gene expression.

Interactions between diets and the genome are effected both via hormones and directly by dietary components. For instance, it is known that thyroid hormones and insulin regulate lipogenesis, but in pigs, insulin may be less important compared to

other species (O'Hea *et al.*, 1970). Thyroid hormones are important regulators of energy metabolism, and different mechanisms are involved in this action, one of them is to promote expression of lipogenic genes and encoded enzymes as shown in rat liver (Roncari and Murthy, 1975, Dozin *et al.*, 1986). Dietary fat inhibits triiodothyronine (T3) promotion of rat liver lipogenic enzymes (Clarke and Hembree, 1990) while PUFA increase total T3 hormone compared to animals fed animal fat (Takeuchi *et al.*, 1995, Ferrini *et al.*, 2007).

The present experiment was therefore conducted to investigate the relationships between age, tissues, dietary fatty acids, thyroid hormones, and expression of genes involved in lipid metabolism.

MATERIAL AND METHODS

Animals and diets

Sixty-one crossbred female pigs (Duroc ♂x Landrace ♀) were fed a barley-corn-soybean meal-based diet during a 4-week pre-experimental period. Five gilts were slaughtered at the beginning of the trial (null animals), whereas 56 gilts (61.8 ±5.2 kg BW) were randomly selected and assigned to one of seven dietary treatments (8 animals per treatment). Treatments were assigned randomly by animal weight and litter. The experiment comprised three periods with 3, 3 and 2 pigs per treatment, respectively. Pigs had access to feed and water *ad libitum* and feed consumption was measured individually. Gilts were slaughtered at an average BW of 99.8 ±8.5kg kg. Samples from liver, neck backfat (adipose tissue) and *semimembranosus* tissues were taken after slaughter and immediately frozen in liquid nitrogen and then stored at -75°C until analysis. Experimental procedures were approved by IRTA's ethical committee.

Seven diets were formulated to meet NRC (1998) requirements. Six fat supplemented diets (containing 10-11% fat in total) were based on barley and soybean meal. Additionally, a semi- synthetic diet was formulated to contain no fat because traditional diets with no fat addition always contain around 2% of dietary fat. Different fat sources were selected to obtain different FA composition of the diets: tallow (T), high-oleic sunflower oil (SFHO), sunflower oil (SFO), linseed oil (LO), blend (55% tallow, 35% sunflower oil, 10% linseed oil) (B) and fish oil blend (40% fish oil, 60% linseed oil) (FO). The T diet was intended to have a high content in SFA, the HOSF diet was intended to have a high level in oleic acid, the SFO diet was intended to have a high content in linoleic acid (FA from the n-6 series), LO was intended to have a high content in linolenic acid (FA from the n-3 series). The B diet was intended to have a similar content in the main FA (palmitic, stearic, oleic, linoleic, and linolenic acids). The FO diet was intended to have a high content in long-PUFA. Oils and fats were for food or feed quality grade. The fat supplemented diets were formulated in order to supply equal amounts of digestible fat based on previous measurements (Duran-Montgé *et al.*, 2007a).

Composition of diets and tissues, and analyses of blood thyroid hormones

Analysis procedures and dietary dry matter content (DM), crude protein, energy, fat and FA profile are described in the work of Duran-Montgé *et al.* (2007c). For FA determination in tissues, lipids were extracted by the Folch method (Folch *et al.*, 1957) and then transmethylated with BF_3 and methanolic KOH (Morrison and Smith, 1964). Fatty acid contents were determined using an automated GC (Hewlett Packard 6890, USA) equipped with an automatic injector, using C19:0 as internal

standard. Analysis of thyroid hormones was performed with RIA kitsCoat-count total t3, Coat-count total t4 Coat-count free t4 from DPC Los Angeles, California.

Abundance of mRNA's

The mRNa extraction and purification procedures, probes and details of primer/probe design and runs of realtime RT-PCR are described in the work of Duran-Montgé *et al.* (2007c).

Calculations and statistics

Data to evaluate mRNA quantities was obtained as Ct values (the cycle number at which logarithmic plots cross a calculated threshold line) according to the manufacturer's guidelines, and used to determine ΔCt values (ΔCt =Ct of the target gene – Ct of the housekeeping gene). The ΔCt data of each target gene was analyzed separately for each tissue using the MIXED procedure of SAS (Littell *et al.*, 1996). Data was expressed relative to NF diet by calculating ΔΔCt values (ΔCt of treatment – ΔCt of NF diet) and converting results into expression levels according to Pfaffl (2001) by taking into account the PCR efficiencies. The following statistical model was applied separately for each tissue:

$$X_{ijk} = \mu + \alpha_i + \beta_j + \chi_{ij} + U_{jk} + \varepsilon_{ijk}$$

where X is the observation (e.g. ΔCt values) in the j'th period from the k'th litter, μ is the overall mean, α_i is a fixed treatment effect, β_j is a fixed effect of period, χ_{ij} is the interaction effect treatment x period, U_{jk} is the sow x period random effect , and ε the residual errors. The treatment*period interaction was not significant for any of the variables, and therefore only main effects are reported.

The model for age comparisons was:

$$Y_{jkl} = \mu + \delta_l + U_{jk} + \varepsilon_{jkl}$$

where Y is the observations (e.g. . ΔCt values) at l=60 or 100 kg live weight, μ is the overall mean, U_{jk} is the sow x period random effect, and ε the residual errors.

The model for comparisons between tissues was:

$$Z_{ijklm} = \mu + \gamma_m + \alpha_i + \beta_j + \lambda + U_{jk} + \varepsilon_{ijklm}$$

where Z is the observations (e.g. ΔCt values) in the i'th treatment, j'th period from the k'th litter, μ is the overall mean, γ_m is a fixed tissue effect, α_i is a fixed treatment effect, β_j is a fixed effect of period, λ is Log10 of the RNA sample content, U_{jk} is the sow x period random effect, and ε the residual errors. The treatment*period interaction was initially included in the model but it was found not to be significant for any of the variables, and therefore was removed from the model.

Significance levels of the correlate coefficient are base on Fischer (1925)

RESULTS

Fatty acids in diets

NF diet had a low content in all FA, whereas the fat-supplemented diets had high contents of specific FA; the T diet was characterized as having high contents of saturated FA (SFA; mainly palmitic and stearic FA), HOSF, SFO and LO diets were rich in oleic, linoleic and linolenic acids, respectively, the B diet had similar content of palmitic, stearic, linoleic and linolenic acids and the FO diet was rich in long PUFA (Duran-Montgé *et al.*, 2007c).

Table 1. Content (mg FA/g sample) of n-6 and n-3 FA, SFA, MUFA, PUFA in the liver, adipose tissue and muscle.

	60kg	NF	T	HOSF	SFO	LO	B	FO	*S.E.*
Liver									
C 18:2 n-6	4.64	4.95^{c}	5.18^{c}	5.15^{c}	10.0^{a}	6.80^{b}	6.53^{b}	4.31^{c}	0.428
C 18:3 n-6	0.08	0.14^{bc}	0.15^{b}	0.15^{b}	0.30^{a}	0.06^{cd}	0.08^{bcd}	0.04^{d}	*0.041*
C 18:3 n-3	0.17	0.21^{c}	0.22^{c}	0.14^{c}	0.16^{c}	3.68^{a}	1.53^{b}	2.07^{b}	*0.310*
C 20:2 n-6	0.22	0.36^{a}	0.27^{b}	0.31^{b}	0.37^{a}	0.19^{c}	0.18^{c}	0.12^{d}	*0.019*
C 20:3 n-6	0.24	0.25^{ab}	0.27^{a}	0.21^{bc}	0.18^{c}	0.19^{c}	0.25^{ab}	0.17^{c}	*0.020*
C 20:4 n-6	5.66	6.16^{b}	6.45^{b}	7.68^{a}	7.54^{a}	2.70^{e}	4.44^{c}	3.56^{d}	*0.200*
C 20:3 n-3	0.02	ND^{e}	0.01^{d}	0.02^{d}	ND^{e}	0.35^{a}	0.13^{c}	0.24^{b}	*0.014*
C 20:5 n-3	0.23	0.27^{d}	0.50^{d}	0.18^{d}	0.09^{d}	4.08^{b}	2.03^{c}	5.23^{a}	*0.205*
C 22:4 n-6	0.32	0.36^{b}	0.21^{d}	0.30^{c}	0.40^{a}	0.04^{f}	0.09^{e}	0.05^{f}	*0.020*
C 22:5 n-3	0.94	0.86^{b}	1.00^{b}	0.53^{c}	0.36^{d}	1.21^{a}	1.37^{a}	0.97^{b}	*0.075*
C 22:6 n-3	0.91	1.13^{b}	1.28^{b}	1.21^{b}	0.61^{c}	0.57^{c}	0.94^{bc}	5.19^{a}	*0.198*
Total n-6 FA	11.2	12.3^{c}	12.8^{bc}	13.9^{b}	18.9^{a}	10.2^{d}	11.8^{c}	8.39^{e}	*0.639*
Total n-3 FA	2.29	2.40^{de}	2.96^{d}	2.09^{de}	1.22^{e}	9.84^{b}	5.97^{c}	13.59^{a}	*0.630*
n6/n3	4.87	5.42^{c}	4.59^{c}	6.99^{b}	15.2^{a}	1.49^{de}	2.37^{d}	0.90^{e}	0.501
Total SFA	12.0	15.0	13.3	12.5	13.0	13.2	12.9	13.5	*0.705*
Total MUFA	4.90	9.49^{a}	6.75^{b}	8.82^{a}	5.05^{bc}	4.25^{c}	5.44^{bc}	3.92^{c}	*0.839*
Total PUFA	13.5	14.6^{d}	15.6^{cd}	15.7^{cd}	19.9^{ab}	19.8^{ab}	17.5^{bc}	21.8^{a}	*1.067*
Adipose tissue									
C 18:2 n-6	88.7	63.3^{d}	86.8^{c}	83.9^{c}	218^{a}	100^{b}	103^{b}	86.2^{c}	*4.35*
C 18:3 n-6	0.21	ND^{c}	ND^{c}	0.24^{b}	0.45^{a}	ND^{c}	0.20^{b}	0.18^{b}	*0.031*
C 18:3 n-3	7.44	5.2^{d}	9.2^{d}	5.8^{d}	6.3^{d}	102^{a}	41.5^{c}	61.7^{b}	*1.51*
C 20:2 n-6	5.19	4.11^{c}	4.85^{bc}	4.96^{b}	12.3^{a}	5.58^{b}	5.32^{b}	4.98^{b}	*0.261*
C 20:3 n-6	0.80	0.61^{d}	0.85^{b}	0.77^{b}	1.29^{a}	0.59^{d}	0.73^{c}	0.73^{c}	*0.037*
C 20:4 n-6	2.52	1.92^{c}	2.47^{b}	2.53^{b}	3.60^{a}	1.73^{c}	2.03^{c}	2.69^{b}	*0.110*
C 20:3 n-3	1.20	1.02^{d}	1.38^{d}	0.95^{d}	1.04^{d}	12.7^{a}	5.21^{c}	7.32^{b}	*0.301*
C 20:5 n-3	ND	ND	ND	ND	ND	1.57^{b}	0.83^{c}	5.99^{a}	*0.118*
C 22:4 n-6	0.87	0.67^{cd}	0.78^{bc}	0.80^{b}	1.19^{a}	0.60^{d}	0.63^{d}	0.74^{c}	*0.036*
C 22:5 n-3	1.21	0.75^{d}	1.52^{c}	0.82^{d}	1.32^{c}	2.77^{b}	2.34^{b}	7.42^{a}	*0.159*
C 22:6 n-3	1.65	1.02^{c}	1.94^{bc}	1.25^{bc}	2.44^{b}	1.40^{bc}	1.60^{bc}	23.4^{a}	*0.47*
Total n-6 FA	97.5	70.6^{e}	97.5^{cd}	93.0^{d}	237^{a}	109^{bc}	112^{b}	95.9^{d}	*4.63*
Total n-3 FA	11.5	8.0^{d}	14.1^{d}	8.9^{d}	11.2^{d}	120^{a}	51.5^{c}	106^{b}	*2.08*
n-6/n-3	8.48	8.87^{c}	7.08^{d}	10.8^{b}	21.6^{a}	0.90^{f}	2.09^{e}	0.86^{f}	*0.33*
Total SFA	289	324^{a}	286^{b}	231^{d}	269b^{c}	256^{cd}	276^{bc}	256^{cd}	*9.8*
Total MUFA	343	439^{a}	447^{a}	471^{a}	371^{bc}	335^{c}	374^{b}	341^{bc}	*13*
Total PUFA	109	78.6^{f}	112^{e}	102^{e}	248^{a}	229^{b}	164^{d}	202^{c}	*5.2*
Muscle									
C 18:2 n-6	2.81	2.79^{c}	4.55^{bc}	3.83^{bc}	10.53^{a}	4.75^{bc}	5.36^{b}	4.11^{bc}	*0.770*
C 18:3 n-6	0.01	0.03	0.04	0.03	0.04	0.03	0.04	0.03	*0.005*
C 18:3 n-3	0.12	0.16^{c}	0.36^{c}	0.16^{c}	0.21^{c}	3.87^{a}	1.87^{b}	2.37^{b}	*0.230*
C 20:2 n-6	0.11	0.13^{b}	0.19^{b}	0.16^{b}	0.39^{a}	0.17^{b}	0.19^{b}	0.15^{b}	*0.036*
C 20:3 n-6	0.08	0.09^{ab}	0.10^{a}	0.09^{bc}	0.10^{ab}	0.07^{c}	0.09^{ab}	0.09^{bc}	*0.006*
C 20:4 n-6	0.78	0.89^{ab}	0.82^{bc}	0.95^{ab}	1.02^{a}	0.63^{d}	0.73^{cd}	0.83^{bc}	*0.050*
C 20:3 n-3	0.02	0.03^{c}	0.06^{c}	0.03^{c}	0.04^{c}	0.41^{a}	0.21^{b}	0.25^{b}	*0.026*
C 20:5 n-3	0.05	0.05^{d}	0.08^{d}	0.06^{d}	0.02^{d}	0.35^{b}	0.21^{c}	0.83^{a}	*0.028*
C 22:4 n-6	0.10	0.09^{b}	0.09^{b}	0.09^{b}	0.12^{a}	0.04^{d}	0.06^{c}	0.04^{d}	*0.005*
C 22:5 n-3	0.13	0.14^{d}	0.19^{c}	0.13^{d}	0.12^{d}	0.28^{b}	0.28^{b}	0.36^{a}	*0.019*
C 22:6 n-3	0.09	0.11^{b}	0.13^{b}	0.13^{b}	0.09^{b}	0.08^{b}	0.12^{b}	1.25^{a}	*0.046*
Total n-6 FA	3.81	4.01^{c}	5.92^{bc}	5.10^{bc}	12.2^{a}	5.69^{bc}	6.48^{b}	5.24^{bc}	*0.829*
Total n-3 FA	0.41	0.50^{c}	0.81^{c}	0.51^{c}	0.48^{c}	5.00^{a}	2.69^{b}	5.05^{a}	*0.311*
n-6/n-3	9.25	8.98^{c}	7.83^{d}	11.3^{b}	26.3^{a}	1.33^{f}	2.57^{e}	1.11^{f}	*0.402*
Total SFA	6.63	11.7	14.4	10.3	12.5	10.6	13.5	10.8	*1.47*
Total MUFA	7.56	17.4	22.1	18.2	16.0	13.6	18.2	13.6	*2.13*
Total PUFA	4.22	4.46^{d}	6.68^{cd}	5.53^{d}	12.6^{a}	10.7^{ab}	9.13^{bc}	10.2^{ab}	*1.00*

[1]Means within are row with different letter are significantly different ($p<0.05$). No comparisons were performed with 60 kg BW animals.

NF: No fat diet, T: Tallow diet, HOSF: High oleic Sunflower oil diet, LO: Linseed oil diet, B: Blend, FO: Fish oil (40% fish oil, 60% linseed oil) FA: fatty acids, SFA: Saturated FA MUFA: monounsaturated FA, PUFA: polyunsaturated FA,

Fatty acids in tissues

In liver, the FA content reflected the dietary differences to a great extent (Table 1). Linoleic acid and n-6 FA (18:3 n-6, 20:2 n-6, 20:4 n-6 and 22:4 n-6 FA) were high in SFO fed animals. Linolenic acid was high in LO fed animals and EPA and DHA were high in FO fed animals. The n-6/n-3 ratio differed greatly between treatments, the highest value (15.2) was observed in SFO fed animals and the lowest (0.90) in FO fed animals. No differences were observed in SFA content; but the MUFA content was high in HOSF and NF fed animals; and the PUFA content was high in FO, SFO and LO fed animals, and low in NF fed animals.

In adipose tissue n-6 FA were high in SFO fed animals. Proportion of linolenic acid was high in LO fed animals, and EPA and DHA were high in FO fed animals. The n-6/n-3 ratio in adipose tissue showed similar trends as in the liver. However, the proportion of SFA was high in NF and low in HOSF fed animals; MUFA were high in NF, T and HOSF fed animals; PUFA were high in SFO and low in NF fed animals.

As observed in other tissues n-6 FA content in muscle was high in SFO fed animals, linolenic acid was high in LO fed animals and EPA and DHA high in FO fed animals. Furthermore, the n-6/n-3 ratio was high in SFO and low in LO and FO fed animals, but differences were lower in muscle tissue as compared to liver and adipose tissues. No differences were observed in SFA and MUFA content in the muscle whereas PUFA were higher in SFO fed animals.

Gene Expression related to treatments in different tissues

As previously described, the housekeeping gene was considered suitable for the study of the different studied tissues (Duran-Montgé *et al.*, 2007c).

Dietary treatment modified mRNA's content of ACACA, SREBP1 and SCD in the liver (Fig. 1)and FASN, PPAR-α and D6D in adipose tissue (Fig. 2), but not in the muscle (Fig 3) (Duran-Montgé *et al.*, 2007c)

In adipose tissue FASN mRNA abundances correlated to ACACA ($r = 0.84$, $P<0.05$) and SCD ($r = 0.95$, $P<0.05$) mRNA abundance. Also, a significant correlation was observed between PPARα and D6D ($r= 0.83$, $P<0.05$).

Comparison between tissues (Table 2) showed that FASN ($P<0.0001$), ACACA ($P<0.0001$), SCD ($P<0.0001$) and SREBP1 ($P=0.0059$) were more expressed in adipose tissue than in *semimembranosus* and liver and PPARα and D6D were more expressed in liver than in the other tissues studied ($P<0.0001$).

Gene expression at different weights (age)

Developmental changes in fat metabolism were tested by comparing transcription of genes at 60 and 100 kg BW. In animals of 100 kg BW, the liver gene expression of target genes was higher for ACACA, SREBP1, D6D and PPARα, whereas SCD and FASN mRNA abundances were comparable to that of 60 kg BW pigs (Table 3). In adipose tissue ACACA, FASN and SCD mRNA abundance was lower in pigs of 100 kg BW and not different for SREBP1, PPARα and D6D. Similarly, transcriptions of ACACA, FASN and SCD in muscle tissue were lower in 100 kg BW pigs, but also D6D and SREBP1 tended to be lower. In contrast, PPARα transcription in muscle was higher in 100 kg BW pigs than in 60 kg pigs.

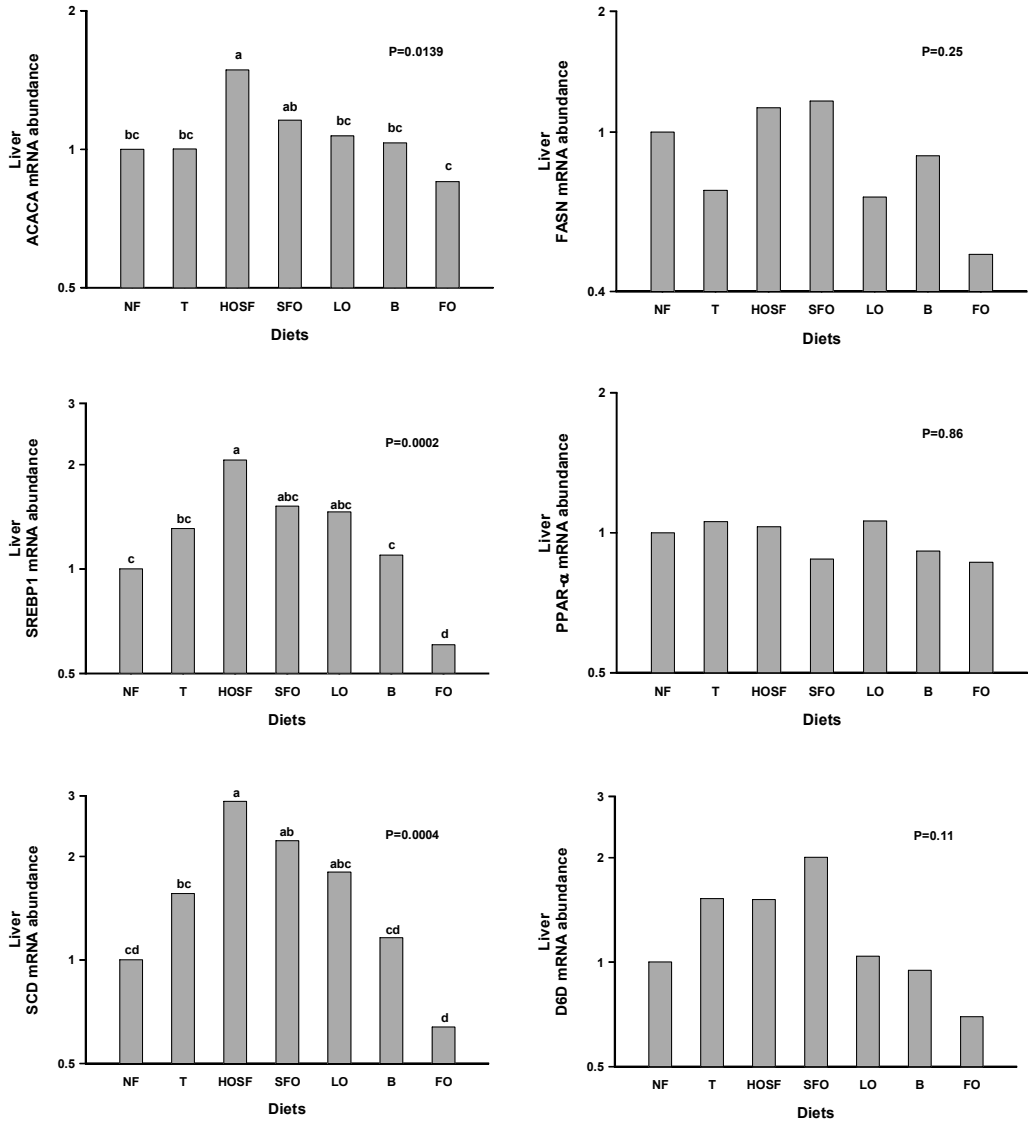

Figure 1. Liver mRNA abundance of acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), sterol regulatory element binding protein-1 (SREBP1), peroxisome proliferator activated receptor α (PPAR-α), stearoyl CoA desaturase (SCD), Δ6-desaturase (D6D), of pigs fed either a diet with no fat (NF) or a diet with 10% of fat from different sources: tallow (T), high oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (55% tallow, 35% sunflower oil, 10% linseed oil) (B) or fish oil blend (40% fish oil, 60% linseed oil) (FO). The mRNA's abundances are expressed relative to the NF diet.

Thyroid hormones in blood

The NF diet showed the highest free and total triiodothyronine (T3) values followed by pigs fed SFO (Table 4). Animals fed T showed the lowest free T3 contents in blood. 60 kg BW animals showed higher total T3 hormone content than 100 kg BW. Free T3 correlated with FASN ($r = 0.90$, $P<0.05$), ACACA ($r = 0.83$, $P<0.05$) and SCD ($r = 0.79$, $P<0.05$) and total T3 correlated with FASN ($r = 0.88$, $P<0.05$), ACACA ($r = 0.76$, $P<0.05$) and SCD ($r = 0.90$, $P<0.05$) in adipose tissue.

Dietary treatments did not affect plasma Thyroxine (T4) levels ($p>0.05$), and no correlations between T4 and transcription levels were found.

DISCUSSION

Tissue fatty acid composition

Dietary FA can be incorporated into tissues to a large extent (Leat *et al.*, 1964). A large proportion of energy originated from carbohydrates could be used for de novo synthesis of fatty acids. Still, tissue composition showed considerably that of the fats supplemented in the diet. These differences caused by dietary manipulation had different consequences in each tissue although, in general, tendencies were similar. Liver had a relatively high content of n-6 and n-3 derivates compared to other tissues meaning that liver was important for PUFA metabolism as supported by the high hepatic expression of D6D, while adipose tissue was the tissue that reflected to greater extent dietary manipulations in terms of total tissue FA content as demonstrated by the dietary differences observed in SFA, MUFA and PUFA contents. Although dietary modifications were observed in muscle, these differences between dietary treatments were not as high as in liver or adipose tissue and within treatment variability was higher. Interestingly, animals fed the NF diet showed high

contents of two n-6 derivates (C20:2 n-6 and C20:3 n-6) in liver although this diet had the lowest contents in this FA series. In agreement with other reports (Kouba *et al.*, 2003, Nuernberg *et al.*, 2005), animals fed diets with high contents of linolenic acid (LO diet) did not increase DHA levels. In addition, animals fed LO had low arachidonic acid contents. Nuernberg *et al.* (2005) suggested that this decrease could be a result of the competition for the same enzymes in their elongation and desaturation metabolism and linolenic acid would have more affinity for these enzymes than linoleic acid.

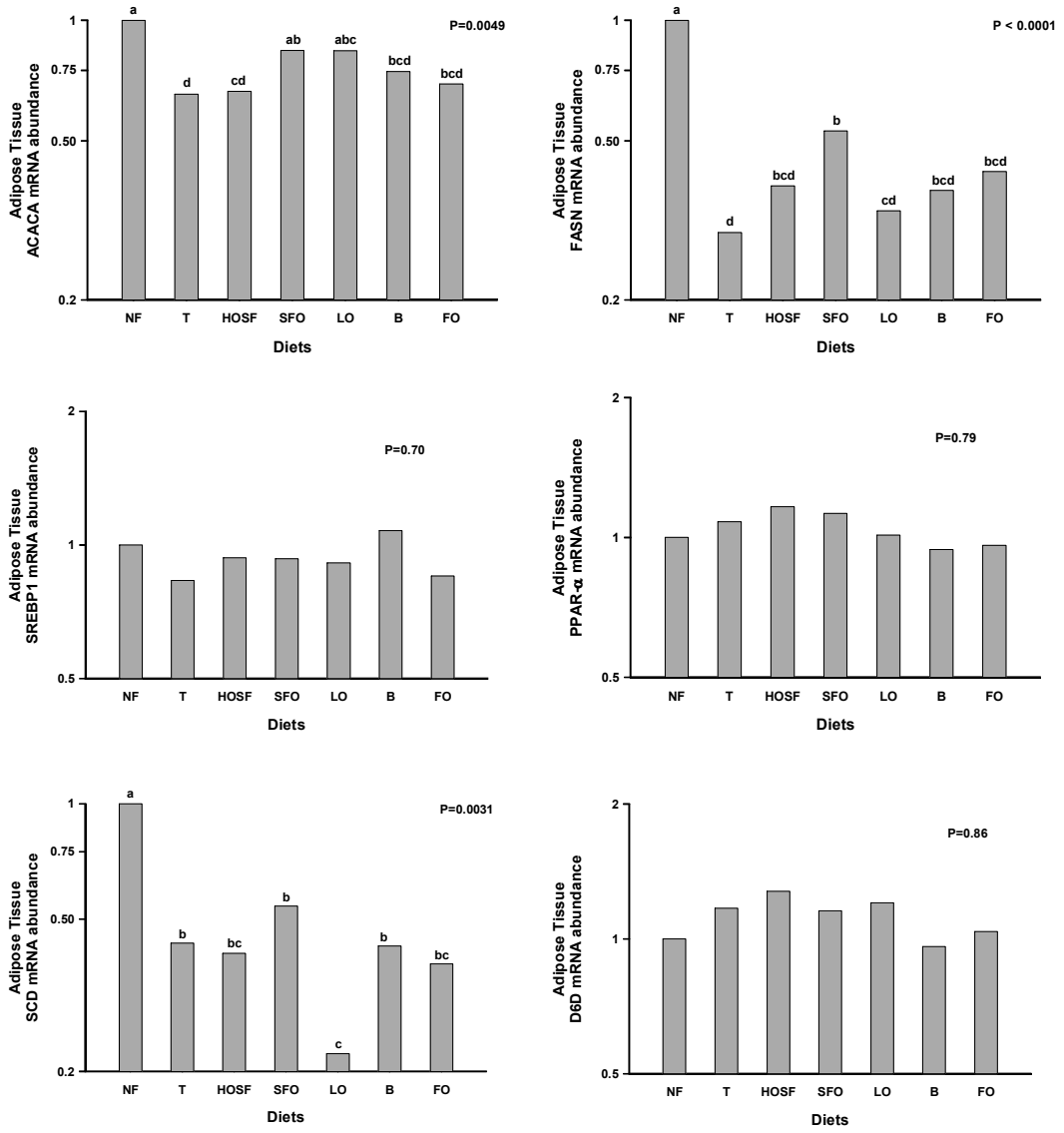

Figure 2. Adipose tissue mRNA abundance of acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), sterol regulatory element binding protein-1 (SREBP1), peroxisome proliferator activated receptor α (PPAR-α), stearoyl CoA desaturase (SCD), Δ6-desaturase (D6D), of pigs fed either a diet with no fat (NF) or a diet with 10% of fat from different sources: tallow (T), high oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (55% tallow, 35% sunflower oil, 10% linseed oil) (B) or fish oil blend (40% fish oil, 60% linseed oil) (FO). The mRNA's abundances are expressed relative to the NF diet.

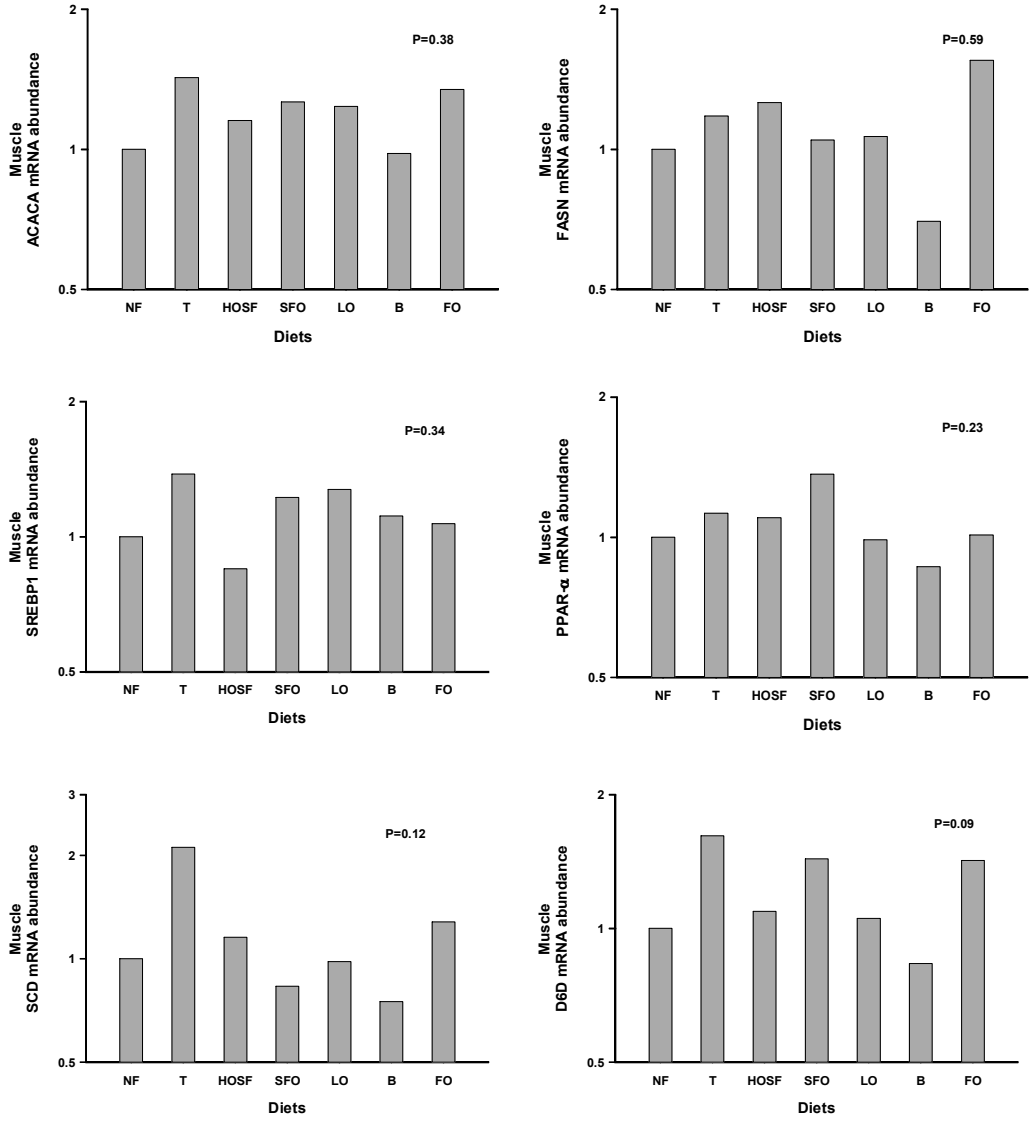

Figure 3. Muscle (*semimembranosus*) mRNA abundance of acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), sterol regulatory element binding protein-1 (SREBP1), peroxisome proliferator activated receptor α (PPAR-α), stearoyl CoA desaturase (SCD), Δ6-desaturase (D6D), of pigs fed either a diet with no fat (NF) or a diet with 10% of fat from different sources: tallow (T), high oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (55% tallow, 35% sunflower oil, 10% linseed oil) (B) or fish oil blend (40% fish oil, 60% linseed oil) (FO). The mRNA's abundances are expressed relative to the NF diet.

Table 2. Gene Ct values in the studied tissues[1]

	Adipose Tissue	Liver	Semimembranosus	S.E.
ACACA	27.5[a]	29.2[b]	30.3[c]	0.11
FASN	25.7[a]	31.8[b]	31.5[b]	0.16
SREBP1	26.8[a]	27.6[b]	27.4[b]	0.14
PPAR	30.4[c]	27.2[a]	29.6[b]	0.14
SCD	23.2[a]	29.9[b]	30.9[c]	0.19
D6D	30.1[b]	26.0[a]	30.0[b]	0.14

[1]Ct values are LSMeans Squares. Means within are row with different letter are significantly different ($p<0.05$)

Fat metabolism in different organs

Expression of lipogenic genes varied considerably among tissues, as indicated by the different Ct values (Table 2; 1 unit change on the Ct scale corresponds to a twofold change in gene expression when the PCR efficiency is 100%) and emphasises that different tissues have varying functions related to fat metabolism. This is in agreement with O'Hea and Leveille (1969). The high expression of lipogenic genes (SCD, ACACA, SREBP and FASN) in adipose tissue was expected because in pigs, FA are preferentially synthesized *de novo* in this tissue rather than in the liver. D6D enzyme is the main step in the synthesis of long-PUFA; this gene was preferentially expressed in liver which can be expected as this is the main organ synthesizing long-PUFA. This is also supported by the high content of long-PUFA and their intermediates in the liver. PPARα acts as a transcription factor of enzymes related to FA oxidation; this gene was more expressed in liver than in adipose and muscle tissues meaning that the liver probably exerts a pivotal function in the oxidation of FA. The high hepatic expression of D6D compared to other tissues likely

explains the high levels of EPA and Arachidonic acid in this tissue, emphasizing the importance of this key enzyme in the synthesis of long-PUFA derivates (Brenner, 1971). It is interesting to note that PPARα and D6D were more expressed in the liver than in adipose tissue while the opposite was observed for FASN and SCD expression, suggesting that liver would be the preferential site for long chain PUFA synthesis as described by Scott and Bazan (1989) and pig adipose tissue would be the main site for *de novo* fat synthesis. Changes in adipose tissue lipogenesis are probably the main cause for the differences in whole body fat content observed in this study (Duran-Montgé *et al.*, 2007b)

Fat metabolism at different age

Expression of lipogenic genes varied with age (and/or weight) in the present study. Scott (1981) indicated a plateau in carbon flux and lipogenic enzyme activities in pig adipose tissue followed by a decline in activities with increased animal age. In the report of Scott (1981) the maximum lipogenic rates and ACACA activities corresponded to 60 kg BW which was then decreased at 85 and 100 kg BW. These findings are supported by the present study, since expression of lipogenic genes were greater at 60 kg BW compared to 100 kg BW. The greater expression of ACACA and SREBP1 found in the liver in the present study suggest that the liver could be responsible for the increased lipogenesis during the fattening period. Chwalibog and Thorbek (2000) also found that oxidation of fat

decreased during the fattening period at high and low feeding levels, and that pigs fed high feeding level did not oxidise fat from 60 to 100 kg BW.

Contrasting to muscle and adipose tissue, differences between ages in the liver showed higher ACACA, SREBP1 and SCD mRNA abundance in 100 kg pigs,

suggesting that adipose tissue loses capacity for lipogenesis on per gram of tissue basis. And it emphasises that lipogenesis is regulated differently in the liver and in adipose tissue and furthermore, that the importance of the organs concerning whole animal fat metabolism changes over time.

Changes in dietary fat composition had different impact on expression of genes related to lipid metabolism, depending on the tissues. The greatest transcription of ACACA, SREBP1 and SCD observed in liver for pigs fed HOSF, SFO and LO, intermediate in pigs fed NF, T and B and lower in pigs fed the FO diet, is indicative of an increased fat biosynthetic fat metabolism in HOSF, SFO and LO; probably of FA elongation metabolism as there was a higher expression of ACACA in these treatments (indicative of synthesis of malonyl-CoA, which is essential in the elongation process). Further, a high expression of D6D was observed in the liver (indicative of unsaturation of long chain PUFA) and no modification of FASN expression due to treatments and its expression was low in the liver, indicating low *de novo* FA synthesis. In contrast, animals fed FO (rich in long chain PUFA) responded with a depressed FA synthesis/conversion in the liver, probably because synthesis of more long chain FA was not required.

In adipose tissue, transcription of ACACA, FASN and SCD was greatest for pigs fed NF diet. The higher expression of these genes related to *de novo* FA synthesis in NF fed animals responds to necessity of an increased fat synthesis due to the low fat supply from dietary origin. The lowest transcription of ACACA and FASN in adipose tissue observed in pigs fed T, suggest that saturated FA inhibitit pig *de novo* fat synthesis. This observation is supported by the observation of lower whole body fat content in tallow fed animals from the present study (Duran-Montgé *et al.*, 2007b) and in other studies, where lower carcass fat content was estimated, also in tallow

fed pigs compared to PUFA (Mourot *et al.*, 1991, Mourot *et al.*, 1994). In contrast to the liver, no dietary effects on SREBP in adipose tissue were found.

The above mentioned differences due to dietary treatment are clearly tissue dependent. In liver, SREBP is claimed to be the link for FA regulation of gene expression, whereas in adipose tissue, other mechanisms than differential gene expression may be involved.

Table 3. Relative mRNA abundance of genes related to lipid metabolism in 100 kg BW pigs compared to 60kg BW (relative mRNA abundance=1)(P value)

	60kgBW	ACACA	FASN	SREBP1	PPARα	SCD	D6D
Liver	1	2.60 ***	0.92(0.81)	2.54 (**)	4.52 ***	1.99 (0.13)	4.00 **
Adipose fat	1	0.56 ***	0.36 ***	1.02 (0.92)	1.13 (0.43)	0.42 *	0.82 (0.4)
Muscle	1	0.59 **	0.26 ***	0.68 (0.071)	1.42 *	0.22 ***	0.63 (0.054)

Acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), sterol regulatory element binding protein-1 (SREBP1), peroxisome proliferator activated receptor α (PPAR-α), stearoyl CoA desaturase (SCD), Δ6-desaturase (D6D) in liver, adipose tissue and muscle (*semimembranosus*).
* ($p<0.05$), ** ($p<0.01$), ***($p<0.001$)

PUFA (n-6 and n-3 FA) and especially long PUFA which are abundantly found in fish oil are known to be potent regulators of gene expression (Xu *et al.*, 2002, Jump *et al.*, 2005). The increase in D6D could be expected considering its role in the synthesis of C18:3 (n-6) and long chain PUFA like C20:4(n-6), which likely explained the positive correlation with the tissue content of these two FA (unpublished). FASN correlation with EPA could be expected as EPA is known to reduce liver mRNA abundances of lipogenic genes in rodents (Iritani *et al.*, 1998, Jump *et al.*, 2005). As previously reported (Theil and Lauridsen, 2007), the n-6/n-3 ratio could exert an effect on D6D gene expression in liver as shown by their correlation (unpublished), clearly observed

in the present study in FO fed animals, whose n-6/n-3 ratio in liver was very low due to the high tissue content in long-PUFA from dietary origin. This would suggest that animals fed fish oil would have high tissue content of long-PUFA which makes synthesis of long-PUFA unnecessary.

The correlation between SCD mRNA and SFA contents in adipose tissue (unpublished) can be explained by the fact that SFA are substrates for SCD enzyme (Bloomfield and Bloch, 1960). Finally we studied correlations between mRNA abundances of the different studied genes. Some of these correlations can be expected, because expression of these genes would be coordinated (Xu *et al.*, 1999), and transcription factors as SREBP1 regulate the mRNA abundances of lipogenic genes. SCD can be regulated by SREBP-1c (Miyazaki *et al.*, 2001) and FASN is known to be regulated by SREBP1 isoforms (Shimano *et al.*, 1996, Shimano *et al.*, 1997, Horton *et al.*, 1998). In the liver, genes that responded to dietary treatment seemed to be coordinated as there were positive correlations between mRNA abundances for these genes (SREBP1, ACACA and SCD). Matsuzaka *et al.*(2002) have described what they call dual regulation of D6D in mouse liver by PPARα and SREBP. Although we have not observed statistically significant correlations in the liver between D6D and PPARα or SREBP, it should be noted that a significant correlation was observed between PPARα and D6D in adipose tissue.

Thyroid hormone in blood

Thyroid hormones are known to increase expression and enzyme protein content of rat liver lipogenic genes (Roncari and Murthy, 1975, Dozin *et al.*, 1986) and nuclear response elements to thyroid hormones have been identified in the FASN gene in humans and chickens (Xiong *et al.*, 1998) which would be responsible for this action.

Table 4. Thyroid hormones values in blood[1]

	60kg	NF	T	HOSF	SFO	LO	B	FO	*S.E.*
T3 free (pg/ml)	1.76	1.82^{a}	1.44^{c}	1.55^{bc}	1.72^{ab}	1.54^{bc}	1.49^{c}	1.59^{bc}	*0.08*
T3 total (ng/dl)	58.6^{x}	52.4^{a}	44.0^{bc}	40.3^{c}	47.5^{ab}	40.7^{c}	40.0^{c}	41.3^{c}	*2.3*
T4 free (ng/dl)	1.07	1.00	1.08	0.97	1.03	0.94	1.00	1.02	*0.05*
T4 total (μg/dl)	4.22	4.38	3.91	3.85	4.12	3.95	3.79	4.47	*0.26*

[1]Results are LSMeans Squares. Means within are row with different letter are significantly different ($p<0.05$). Comparisons with 60 kg BW were performed separately with all animals at 100 kg BW
NF: No fat diet, T: Tallow diet, HOSF: High oleic Sunflower oil diet, LO: Linseed oil diet, B: Blend, FO: Fish oil (40% fish oil, 60% linseed oil)

Dietary fat inhibits T3 promotion of rat liver lipogenic enzymes (Clarke and Hembree, 1990); specifically this action could be done by unesterified long-PUFA inhibiting binding of thyroid hormone to the nuclear receptor (Inoue *et al.*, 1989). In broiler chickens (Ferrini *et al.*, 2007) and in rats (Takeuchi *et al.*, 1995) PUFA increase total T3 hormone while body fat deposition decreases compared to animals fed animal fat; these reports also observed higher lipoprotein lipase activity in animals fed animal fat. According to our results, the higher free T3 hormone in blood is compatible with enhanced lipogenesis in adipose tissue. Thus, the higher total T3 content observed in 60 kg BW animals goes in parallel with the higher mRNA contents of lipogenic enzymes and that is also true for the high contents of free T3 observed in NF fed animals followed by SFO fed animals. We consider the present results to support the hypothesis that higher free T3 hormone in blood is compatible with enhanced lipogenesis as ACACA (Zhang *et al.*, 2003) and FASN (Xiong *et al.*, 1998) DNA sequences contain T3 response elements that could explain the correlation observed in our study between mRNA abundances of ACACA and FASN, and T3 free hormone content in blood. The reasons for the differential effect of fat type on blood

thyroid hormone are unknown. A possible explanation is a decline in the conversion rate of T4 to T3 hormone, rather than a decline in their secretion from the thyroid gland (Chopra *et al.*, 1985).

CONCLUSIONS

Results of the present study show that changes in mRNA abundances of genes related to lipogenesis in liver and adipose tissue, but not in muscle could be partly driven by T3 hormone signaling, as suggested by the observed correlations between hormones and genes of fat synthesis (SCD, ACACA, FASN), and additionally, the large differences observed with regard to live weights (60 and 100 kg) in expression of lipogenic genes in adipose tissue accompanied by differences in T3 hormone. Age and tissue specificity caused larger differences in values of mRNA abundance of genes related to lipid metabolism than did fatty acid composition of diets and tissues. Results from this experiment indicate that adipose tissue is the organ for *de novo* FA synthesis in pig, and the liver is the primary organ for long-PUFA synthesis. The relative importance of these organs changes as animal grow as demonstrated by the changes in gene expression at different stages.

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REFERENCES

Bergen WG and Mersmann HJ 2005. Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. Journal of Nutrition 135, 2499-2502.

Bloomfield DK and Bloch K 1960. Formation of Delta-9-Unsaturated fatty acids. Journal of biological chemistry 235, 337-345.

Brenner R 1971. The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. Lipids 6, 567-575.

Clarke SD and Hembree J 1990. Inhibition of triiodothyronines induction of rat-liver lipogenic enzymes by dietary-fat. Journal of Nutrition 120, 625-630.

Chopra IJ, Huang TS, Beredo A, Solomon DH, Chua Teco GN and Mead JF 1985. Evidence for an inhibitor of extrathyroidal conversion of thyroxine to 3,5,3'-triiodothyronine in sera of patients with nonthyroidal illnesses. J Clin Endocrinol Metab 60, 666-672.

Chwalibog A and Thorbek G 2000. Estimation of net nutrient oxidation and lipogenesis in growing pigs Archives of Animal Nutrition 53, 253-271

Dozin B, Magnuson MA and Nikodem VM 1986. Thyroid hormone regulation of malic enzyme synthesis. Dual tissue- specific control. J. Biol. Chem. 261, 10290-10292.

Duran-Montgé P, Lizardo R, Torrallardona D and Esteve-Garcia E 2007a. Fat and fatty acid digestibility of different fat sources in growing pigs. Livestock Science 109, 66-69.

Duran-Montgé P, Realini C, Lizardo R and Esteve-Garcia E 2007b. Dietary tallow reduces whole pig fat compared to other dietary oils by means of reduced lipogenesis. Animal (unpublished work).

Duran-Montgé P, Theil P, Lauridsen C and Esteve-Garcia E 2007c. Dietary fat source and level affect metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. Animal (unpublished work).

Ferrini G, Menoyo D, Esteve-Garcia E, Baucells MD and Barroeta AC 2007. Effect of dietary fat saturation on lipid metabolim in broiler chickens. In Universitat Autónoma de Barcelona, unpublished work.

Fisher RA 1925. Table VA in Statistical Methods for Research Workers. Oliver & Boyd, Edinburgh.

Folch J, Lees M and Stanley GHS 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry 226, 497-509.

Horton JD, Shimomura I, Brown MS, Hammer RE, Goldstein JL and Shimano H 1998. Activation of cholesterol synthesis in preference to fatty acid synthesis in Liver and adipose tissue of transgenic mice overproducing Sterol Regulatory Element-binding Protein-2. J. Clin. Invest. 101, 2331-2339.

Inoue A, Yamamoto N, Morisawa Y, Uchimoto T, Yukioka M and Morisawa S 1989. Unesterified long-chain fatty-acids inhibit Thyroid-Hormone Binding to the Nuclear Receptor - solubilized receptor and the receptor in cultured-cells. European Journal of Biochemistry 183, 565-572.

Iritani N, Komiya M, Fukuda H and Sugimoto T 1998. Lipogenic enzyme gene expression is quickly suppressed in rats by a small amount of exogenous polyunsaturated fatty acids. Journal of Nutrition 128, 967-972.

Jump DB, Botolin D, Wang Y, Xu JH, Christian B and Demeure O 2005. Fatty acid regulation of hepatic gene transcription. Journal of Nutrition 135, 2503-2506.

Kouba M, Enser M, Whittington FM, Nute GR and Wood JD 2003. Effect of high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. Journal of Animal Science 81, 1967-1979.

Leat WMF, Cuthbert.A, Howard AN and Gresham GA 1964. Studies on pigs reared on semi-synthetic diets containing no fat beef tallow and maize oil - Composition of carcass and fatty acid composition of various depot fats. Journal of Agricultural Science 63, 311-&.

Littell RC, Milliken GA, Stroup WW and Wolfinger RD 1996. SAS System for mixed models. In SAS Inst. Inc., Cary, NC.

Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Yoshikawa T, Hasty AH, Tamura Y, Osuga J, Okazaki H, Iizuka Y, Takahashi A, Sone H, Gotoda T, Ishibashi S and Yamada N 2002. Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPAR alpha. Journal of Lipid Research 43, 107-114.

Mersmann HJ 1984. Effect of sex on lipogenic activity in swine adipose tissue. Journal of Animal Science 58, 600-604.

Mersmann HJ, Pond WG and Yen JT 1984. Use of carbohydrate and fat as energy source by obese and lean swine. Journal of Animal Science 58, 894901.

Mersmann HJ, Allen CD, Chai EY, Brown LJ and Fogg TJ 1981. Factors influencing the lipogenic rate in swine adipose tissue. Journal of Animal Science 52.

Miyazaki M, Kim YC and Ntambi JM 2001. A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. Journal of Lipid Research 42, 1018-1024.

Morrison WR and Smith LM 1964. Preparation of fatty acid methyl esters + dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5, 600-&.

Mourot J, Peiniau P and Mounier A 1994. Effect of dietary linoleic-acid on lipogenesis in adipose-tissue of pig. Reproduction Nutrition Development 34, 213-220.

Mourot J, Chauvel J, Le Denmat M, Mounier A and Peiniau P 1991. Linoleic acid level in the diet of pigs: effects on fat deposition and on meat linoleic acid oxydation during storage. Journées Rech. Porcine en France 23, 357-364.

Nuernberg K, Fischer K, Nuernberg G, Kuechenmeister U, Klosowska D, Eliminowska-Wenda G, Fiedler I and Ender K 2005. Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. In Meat Science, pp. 63-74.

O'Hea EK and Leveille GA 1969. Significance of adipose tissue and liver as sites of fatty acid synthesis in pig and efficiency of utilization of various substrates for lipogenesis. Journal of Nutrition 99, 338-&.

O'Hea EK, Leveille GA and Sugahara M 1970. Lipogenesis and enzyme activity in pig adipose tissue as influenced by dietary protein and fat. International Journal of Biochemistry 1, 173-&.

Pfaffl MW 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 29.

Roncari DA and Murthy VK 1975. Effects of thyroid hormones on enzymes involved in fatty acid and glycerolipid synthesis. J. Biol. Chem. 250, 4134-4138.

Scott BL and Bazan NG 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. Proceedings Of The National Academy Of Sciences Of The United States Of America 86, 2903-2907.

Scott RA, Cornelius SG and Mersmann HJ 1981. Effects of age on lipogenesis an lipolysis in lean and obese swine. Journal of Animal Science 52, 505-511.

Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS and Goldstein JL 1996. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J. Clin. Invest. 98, 1575-1584.

Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS and Goldstein JL 1997. Isoform 1c of Sterol Regulatory Element Binding Protein is less active than

isoform 1a in livers of transgenic mice and in cultured cells. J. Clin. Invest. 99, 846-854.

Takeuchi H, Matsuo T, Tokuyama K and Suzuki M 1995. Serum triiodothyronine concentration and Na+,K+-ATPase activity in liver and skeletal muscle are influenced by dietary fat type in rats. Journal of Nutrition 125, 2364-2369.

Theil P and Lauridsen C 2007. Interactions between dietary fatty acids and hepatic gene expression of pigs during the weaning period. Livestock Science Unpublished work.

Worgall TS, Sturley SL, Seo T, Osborne TF and Deckelbaum RJ 1998. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. Journal of biological chemistry 273, 25537-25540.

Xiong S, Chirala S, Hsu M and Wakil S 1998. Identification of thyroid hormone response elements in the human fatty acid synthase promoter. Proceedings Of The National Academy Of Sciences Of The United States Of America 95, 12260-12265.

Xu J, Nakamura MT, Cho HP and Clarke SD 1999. Sterol Regulatory Element Binding Protein-1 expression is suppressed by dietary polyunsaturated fatty acids - A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. Journal of biological chemistry 274, 23577-23583.

Xu J, Cho H, O'Malley S, Park JHY and Clarke SD 2002. Dietary polyunsaturated fats regulate rat liver sterol regulatory element binding proteins-1 and-2 in three distinct stages and by different mechanisms. Journal of Nutrition 132, 3333-3339.

Zhang YQ, Yin LY and Hillgartner FB 2003. SREBP-1 integrates the actions of thyroid hormone, insulin, cAMP, and medium-chain fatty acids on ACC alpha transcription in hepatocytes. Journal of Lipid Research 44, 356-368.

UNIVERSITAT ROVIRA I VIRGILI
EFECTES DE LA COMPOSICIÓ D'ÀCIDS GRASSOS DE LA DIETA SOBRE LA DEPOSICIÓ DE GREIX
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Paper 5: Fatty acid tissue distribution in pigs fed different dietary fat sources

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Fatty acid tissue distribution in pigs fed different dietary fat sources

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ABSTRACT

Dietary fat is a key factor on meat physico-chemical properties and in recent years there is an increasing interest on meat FA composition due its implication on human health. The objectives of the experiment were to study the differential tissue FA partitioning and the effect of dietary fat composition on tissue FA composition.

Seventy-five crossbred gilts (62 ±5.x kg BW average) were divided in 10 animals per treatment. Pigs were fed one of seven treatments: a semi-synthetic diet formulated to contain a very low level of fat (NF) and six fat supplemented diets (10%) based on a barley-soybean meal. The supplemental fats were tallow (T), high-oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (B) (55% tallow, 35% sunflower oil, 10% linseed oil) and fish oil blend (FO) (40% fish oil, 60% linseed oil).

Tissue fatty acid pattern depositions were observed. The highest saturated fatty acid contents were observed in flare fat, followed by intermuscular fat ; subcutaneous fat showed the lowest value. Monounsaturated fatty acids showed the opposite pattern, subcutaneous fat showed the highest contents, intermuscular fat intermediate contents and flare fat the lowest. PUFA were preferentially deposited in intramuscular fat.

Intramuscular fat was less susceptible to treatment modification. Important tissue FA modifications were observed due to dietary treatments, mainly in diets rich in PUFA. The SFA were high in NF and low in SFHO and SFO fed animals, MUFA were high in SFHO and low in SFO, LO and FO fed animals. Pigs fed LO and FB showed detectable levels of EPA which seemed to depend on the linolenic content of the diet. The only effective way to increase tissue DHA contents was to add DHA in the diet as showed the results of FO fed animals. PUFA were differentially deposited depending on its FA type.

INTRODUCTION

Dietary fat and its influence on pig fat depots has been studied for many years. First as an important energy source, second because its implications in meat characteristics, and more recently years because its fatty acid (FA) composition has important implications for human health. Not only saturated FA (SFA) and type of SFA are important, but also n-6:n-3 ratio (ratio between linoleic and linolenic acids ingested) is a risk factor in coronary diseases (Simopoulos, 2001) and cancer (Larsson *et al.*, 2004), especially long n-3 poly unsaturated FA (PUFA), the most active FA.

Pig fat is distributed mainly in 4 fat depots: subcutaneous fat, intermuscular fat, intramuscular fat and visceral fat. Several studies have shown that dietary fat modifies tissue fat composition, especially diets rich in PUFA. Tissue FA composition is not modified to the same extent in the different locations and different depots could be involved and it seems, as observed by different authors (Dean and Hilditch, 1933, Monziols *et al.*, 2007), that the increase in saturation of the backfat goes in parallel with the increase in body temperature. Different studies have focused on the effects of dietary FA composition on different fat depots (Brooks, 1971, Eder *et al.*, 2001) but not an extense study has been performed on a wide range of fatty diets and fat depots. For this reason the objective of the present study was to determine the effects of dietary FA composition and tissue specificity on FA profile of different fat depots.

MATERIAL AND METHODS

Animals and diets

Seventy-five crossbred female pigs (Duroc ♂x Landrace ♀) were fed a barley-corn-soybean meal-based diet during a 4-week pre-experimental period. Five gilts were slaughtered at the beginning of the trial, whereas 70 gilts (61.8 ±5.2 kg BW) were randomly selected and assigned to one of seven dietary treatments (10 animals per treatment). Treatments were assigned randomly by animal weight and litter. The experiment comprised three groups of 3, 2, 3 and 2 pigs per treatment, respectively. Pigs had *ad libitum* access to feed and water and pigs were penned individually. Gilts were slaughtered at an average BW of 99.8 ±8.5kg. Intramuscular, intermuscular and subcutaneous samples from *longissimus thoracicus* (LT) and *semimembranosus* (SM) tissues, and intermuscular and subcutaneous samples from belly (B) were taken after slaughter, airless packed and frozen at -20°C until analysis. Experimental procedures were approved by IRTA's ethical committee.

Diets were formulated to meet NRC (1998) requirements are described in the work of Duran-Montgé *et al.* (Duran-Montgé *et al.*, 2007b). Six fat supplemented diets (containing 10-11% fat in total) were based on barley and soybean meal. Additionally, a semi- synthetic diet was formulated using "purified" ingredients to contain no fat because traditional diets with no fat addition always contain around 2% of dietary fat. Different fat sources were selected to obtain different FA composition of the diets: tallow (T), high-oleic sunflower oil (SFHO), sunflower oil (SFO), linseed oil (LO), fat blend (55% tallow, 35% sunflower oil, 10% linseed oil) (FB) and fish oil blend (40% fish oil, 60% linseed oil) (FO). Oils and fats were for food or feed quality grade. As previously described, NF diet was formulated to contain a very low fat level. The T diet was intended to have a high content in SFA, the HOSF diet was

intended to have a high level in oleic acid, the SFO diet was intended to have a high content in linoleic acid, LO was intended to have a high content in linolenic acid. The FB diet was intended to have a similar content in the main FA (palmitic, stearic, oleic, linoleic, and linolenic acids). The FO diet was intended to have a high content in long-PUFA. The amount of fats included in diets were slightly different in order to supply equal amounts of digestible fat based on results observed previously (Duran-Montgé *et al.*, 2007a).

Analysis

Analysis procedures and dietary dry matter content (DM), crude protein, energy, fat and FA profile are described in the work of Duran-Montgé *et al.* (2007b). For FA determination in tissues, lipids were extracted by the Folch method (Folch et al., 1957) and then transmethylated with BF_3 and methanolic KOH (Morrison and Smith, 1964). Fatty acid contents were determined using an automated GC (Hewlett Packard 6890, USA) equipped with an automatic injector, using C19:0 as internal standard.

Statistics

The following statistical model was applied for a preliminary examination of results

$$X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \chi_{ik} + \varepsilon_{ijk}$$

where X is the observations (FA profile) in the j'th period from the k'th tissue, μ is the overall mean, α_i is a fixed dietary treatment effect, β_j is a fixed effect of period, γ_k is a fixed tissue effect, χ_{ik} is the interaction effect treatment x tissue. This model gave 10 (tissues) x 7 (treatments) = 2415 comparisons per each FA which was considered impractical, but was interesting to observe the contribution of each parameter to the

final observation and evaluate the treatment x tissue interaction. The specific weight of each factor was calculated as described by Little et al. (1981).

In order to compare tissue differences and treatment differences separately after finding that the period and tissue x treatment effects were minimal compared to tissue effects and treatment effects , the models used were:

$$Y_i = \mu + \alpha_i + \varepsilon_i$$

where Y is the observations (FA profile), μ is the overall mean, α_i is a fixed treatment effect, and ε the residual errors.

$$Z_k = \mu + \gamma_k + \varepsilon_k$$

where Z is the observations (FA profile), μ is the overall mean, γ_k is a fixed tissue effect, and ε the residual errors. Individual comparisons with the Duncan test were performed when P value < 0.05.

RESULTS AND DISCUSSION

Effect of tissue

Palimitic acid (Table 1)was high in the three subcutaneous fat depots although also were found low contents in other fat depots (intermuscular and intramuscular fat). In flare fat depot, palmitic acid was higher than other tissues except in SFHO and SFO fed animals that showed high contents in *longissimus thoracicus* (LT), and LO fed animals that were high LT intermuscular fat. The lowest values were found. In general, intermuscular palmitic acid contents were not different from other tissue intermuscular fats; the same was true for subcutaneous fat.

Stearic acid (Table 2) was high in flare fat and as for palmitic acid, was low in subcutaneous fat compared to intramuscular and intermuscular fats from the same

tissue. Stearic acid content in subcutaneous fat was not similar between LT, SM and belly as in palmitic acid (the same was true for intermuscular and intramuscular fat).

Table 1. Palmitic acid (C16:0) profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	24.3^{yzu}	$26.3^{a\ y}$	$22.4^{b\ yz}$	$17.7^{e\ z}$	$17.7^{e\ z}$	$19.1^{de\ xy}$	$21.0^{c\ yzu}$	$19.8^{cd\ yz}$	*0.95*	*20.6^{u}*
SM SC	23.5^{zu}	$24.1^{a\ z}$	$20.9^{b\ uv}$	$18.1^{d\ yz}$	$18.3^{d\ z}$	$18.6^{cd\ xy}$	$20.5^{b\ zu}$	$19.3^{c\ z}$	*0.98*	*20.0^{v}*
B SC	23.0^{zuv}	$23.8^{a\ z}$	$21.3^{b\ zuv}$	$17.9^{e\ yz}$	$17.9^{e\ z}$	$18.7^{de\ xy}$	$20.3^{bc\ u}$	$19.2^{cd\ z}$	*0.82*	*19.9^{v}*
LT Inter	25.5^{y}	$27.6^{a\ x}$	$23.4^{b\ y}$	$19.1^{d\ yz}$	$19.0^{d\ xyz}$	$20.4^{cd\ x}$	$22.4^{cb\ xy}$	$21.9^{bc\ x}$	*1.37*	*22.0^{y}*
SM Inter	24.5^{yz}	$25.6^{a\ y}$	$22.4^{b\ yz}$	$18.4^{c\ yz}$	$18.6^{c\ yz}$	$18.3^{c\ y}$	$22.0^{b\ yz}$	$19.2^{c\ z}$	*1.82*	*20.7^{u}*
B Inter	24.4^{yzu}	$25.5^{a\ y}$	$23.3^{b\ y}$	$19.2^{d\ yz}$	$18.8^{d\ yz}$	$18.8^{d\ xy}$	$21.5^{bc\ yzu}$	$20.7^{cd\ xy}$	*1.28*	*21.1^{zu}*
LT	22.6^{uv}	$23.3^{a\ z}$	$22.2^{ab\ yzu}$	$21.6^{bc\ x}$	$20.5^{cd\ x}$	$19.9^{d\ xy}$	$21.9^{b\ yz}$	$20.6^{cd\ y}$	*0.79*	*21.4^{zy}*
SM	21.3^{v}	$21.9^{a\ u}$	$20.6^{b\ v}$	$18.2^{c\ yz}$	$18.1^{c\ z}$	$18.2^{c\ y}$	$20.2^{b\ u}$	$18.8^{c\ z}$	*0.90*	*19.4^{v}*
Flare Fat	27.4^{x}	$28.4^{a\ x}$	$24.6^{b\ x}$	$20.2^{d\ xy}$	$20.0^{d\ xy}$	$20.4^{d\ x}$	$23.8^{b\ x}$	$21.9^{c\ x}$	*1.13*	*22.7^{x}*
RMSE	*1.19*	*0.94*	*1.00*	*1.54*	*1.25*	*1.34*	*1.09*	*0.96*		
Treat.mean	25.2^{a}	22.3^{b}	18.8^{e}	18.8^{e}	19.1^{e}	21.6^{c}	20.1^{d}			

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean
Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

In our study, as previously observed by Monziols *et al.* (2007), saturated FA (SFA) (Table 3), including palmitic and stearic acids, showed a pattern in their distribution: the highest contents were found in flare fat, intermuscular fat showed intermediate values and subcutaneous fat had the lowest contents. This observation is in line of the hypothesis by Dean and Hilditch (1933) and Monziols *et al.* (2007) that the increase in saturation of the backfat goes in parallel with the increase in body temperature. The SFA were high in flare fat and low in intramuscular fat and

subcutaneous fat (except for LT subcutaneous fat showing some intermediate values). These observations agree with the observations of Marcello *et al.* (1983) but are in contrast to the work of Miller *et al.* (1990) were no differences between subcutaneous and intramuscular fat were detected. Intramuscular SFA contents had less treatment variability suggesting that it does not follow the same pattern. Also it may have different proportions of phospholipids and TG than other tissues. The FA composition of the polar and neutral lipids of muscle is far less susceptible to the type of dietary fat than that of the triglycerides of adipose tissue (Warnants *et al.*, 1996, Eder *et al.*, 2001)

Table 2. Stearic acid (C18:0) profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	15.0 ^{y}	16.5 $^{a\ z}$	12.7 $^{b\ yz}$	10.0 $^{d\ yz}$	11.0$^{cd\ yz}$	12.4 $^{bc\ yz}$	12.7 $^{b\ z}$	13.4 $^{b\ z}$	*1.02*	20.6^{u}
SM SC	12.3 ^{u}	12.2 $^{a\ vw}$	10.4 $^{b\ u}$	8.62 $^{c\ u}$	10.2 $^{b\ zu}$	10.7 $^{b\ zu}$	11.1 $^{b\ uv}$	10.8 $^{b\ wg}$	*0.97*	20.0^{v}
B SC	12.2 ^{u}	12.9 $^{a\ v}$	10.4 $^{b\ u}$	8.37 $^{c\ u}$	9.59 $^{bc\ u}$	10.5 $^{b\ zu}$	11.1 $^{b\ uv}$	11.0 $^{b\ vwg}$	*1.07*	19.9^{v}
LT Inter	15.8^{y}	18.0 $^{a\ y}$	13.9 $^{b\ y}$	11.2 $^{c\ y}$	12.1 $^{c\ y}$	13.8 $^{b\ y}$	14.2 $^{b\ y}$	14.6 $^{b\ y}$	*0.93*	22.0^{y}
SM Inter	13.4 ^{u}	14.4 $^{a\ u}$	12.7 $^{b\ yz}$	9.50 $^{c\ zu}$	11.3 $^{b\ yz}$	11.3 $^{b\ zu}$	12.7 $^{b\ z}$	11.9 $^{b\ uvw}$	*1.27*	20.7^{u}
B Inter	13.9 ^{z}	14.8 $^{a\ u}$	12.5$^{ab\ yz}$	10.2 $^{c\ yz}$	11.3$^{bc\ yz}$	11.8 $^{bc\ zu}$	12.9 $^{ab\ z}$	12.9 $^{ab\ zu}$	*1.46*	21.1^{zu}
LT	13.4 ^{z}	12.5 $^{a\ v}$	11.3$^{bc\ zu}$	11.1 $^{c\ y}$	11.4$^{bc\ yz}$	12.2 $^{ab\ yzu}$	11.9$^{abc\ zu}$	12.1 $^{ab\ uv}$	*0.61*	21.4^{zy}
SM	11.7^{u}	11.0 $^{a\ w}$	10.2 $^{b\ u}$	8.96 $^{c\ zu}$	10.1 $^{b\ zu}$	10.3 $^{ab\ u}$	10.5 $^{ab\ v}$	10.3 $^{ab\ g}$	*0.70*	19.4^{v}
Flare Fat	19.6 ^{x}	20.8 $^{a\ x}$	17.7 $^{b\ x}$	13.1 $^{e\ x}$	14.8 $^{d\ x}$	15.8 $^{cd\ x}$	17.1 $^{bc\ x}$	16.4 $^{bc\ x}$	*1.47*	22.7^{x}
RMSE	*0.69*	*1.13*	*1.21*	*0.92*	*1.02*	*1.45*	*1.05*	*0.88*		
Treat.mean	14.2	14.7^{a}	12.5^{b}	10.0^{e}	11.4^{d}	12.1^{c}	12.7^{b}	12.5^{b}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean

Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

MUFA (Table 5), that corresponded mainly to oleic acid (Table 4), was high in subcutaneous fat and belly intermuscular fat. Except in SFHO fed animals that showed the lowest value in the LT intramuscular fat, the lowest contents were observed in flare fat. The MUFA tissue distribution showed the opposite pattern to that observed in SFA: subcutaneous fat showed the highest contents, intermuscular fat intermediate contents and flare fat the lowest contents. As in SFA, intramuscular fat showed a narrower range of variability compared to other fat depots and therefore was less susceptible to treatment modification. **Table 3**. Saturated fatty acids profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	41.6 ^{z}	44.8 $^{a\,z}$	37.8 $^{b\,z}$	29.4 $^{e\,zu}$	30.3 $^{e\,zu}$	33.1 $^{d\,zu}$	35.7 $^{c\,zu}$	35.1 $^{c\,yz}$	*1.29*	35.2^{c}
SM SC	38.2 ^{u}	38.4 $^{a\,v}$	33.7 $^{b\,uv}$	28.5 $^{e\,u}$	30.2 $^{d\,zu}$	31.2 $^{cd\,uv}$	33.9 $^{b\,uv}$	32.1 $^{c\,uv}$	*1.62*	32.6^{d}
B SC	37.4 ^{u}	38.7 $^{a\,v}$	34.3 $^{b\,uv}$	28.1 $^{f\,u}$	29.2 $^{ef\,u}$	31.0 $^{de\,uv}$	33.5$^{bc\,uv}$	32.3 $^{cd\,uv}$	*1.25*	32.4^{d}
LT Inter	43.6 ^{y}	47.6 $^{a\,y}$	40.0 $^{b\,y}$	32.0 $^{d\,xyz}$	32.7 $^{d\,y}$	35.9 $^{c\,xy}$	38.9 $^{b\,y}$	38.7 $^{b\,x}$	*1.80*	38.0^{b}
SM Inter	40.3 ^{z}	42.1 $^{a\,u}$	37.7 $^{b\,z}$	29.7 $^{d\,zu}$	31.7$^{cd\,yz}$	31.4 $^{cd\,uv}$	36.9 $^{b\,yz}$	33.1 $^{c\,zu}$	*2.63*	34.7^{c}
B Inter	40.9 ^{z}	42.7 $^{a\,zu}$	38.7 $^{b\,yz}$	31.5 $^{d\,yzu}$	32.1 $^{d\,yz}$	32.5 $^{cd\,zuv}$	36.9 $^{b\,yz}$	35.9 $^{bc\,y}$	*2.43*	35.8^{c}
LT	38.0 ^{u}	37.5 $^{a\,v}$	35.7 $^{b\,u}$	34.6 $^{bc\,xy}$	33.7 $^{c\,y}$	33.8 $^{c\,yz}$	35.8 $^{b\,zu}$	34.6 $^{bc\,yz}$	*1.12*	35.0^{c}
SM	34.8 ^{v}	34.7 $^{a\,w}$	32.9 $^{b\,v}$	28.8 $^{d\,zu}$	29.9$^{cd\,zu}$	30.1 $^{cd\,v}$	32.6 $^{b\,v}$	30.8 $^{c\,v}$	*1.39*	31.4^{e}
Flare Fat	49.5 ^{x}	51.3 $^{a\,x}$	45.3 $^{b\,x}$	35.2 $^{f\,x}$	36.6 $^{ef\,x}$	38.0 $^{e\,x}$	43.3 $^{c\,x}$	40.6 $^{d\,x}$	*2.18*	41.5^{a}
RMSE	*1.25*	*1.92*	*1.52*	*2.37*	*1.73*	*1.84*	*2.06*	*1.63*		
Treat.mean	42.0^{a}	37.4^{b}	30.7^{f}	32.0^{e}	33.0^{d}	36.5^{b}	34.6^{c}			

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean

Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

Table 4. Oleic acid (C18:1) profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	38.3 ^{x}	42.1 $^{b\,xyz}$	44.2 $^{b\,xy}$	54.0 $^{a\,x}$	34.2 $^{d\,y}$	34.2 $^{d\,xyz}$	38.0 $^{c\,xy}$	32.9 $^{d\,yz}$	*1.75*	39.9^{b}
SM SC	40.4 ^{x}	45.0 $^{b\,x}$	46.1 $^{b\,x}$	53.7 $^{a\,x}$	37.4 $^{d\,x}$	36.2 $^{d\,x}$	40.0 $^{c\,x}$	37.0 $^{d\,x}$	*1.87*	42.2^{a}
B SC	40.3 ^{x}	44.9 $^{b\,x}$	45.6 $^{b\,x}$	53.7 $^{a\,x}$	37.5 $^{cd\,x}$	35.2 $^{d\,xy}$	39.0 $^{c\,xy}$	35.2 $^{d\,xy}$	*2.22*	41.6^{a}
LT Inter	38.1 ^{x}	40.5 $^{b\,z}$	42.2 $^{b\,yz}$	52.0 $^{a\,xy}$	32.8 $^{d\,y}$	31.9 $^{d\,zu}$	36.4 $^{c\,yz}$	31.0 $^{d\,z}$	*2.00*	38.1^{c}
SM Inter	38.4 ^{x}	41.9 $^{b\,yz}$	41.0 $^{b\,z}$	50.4 $^{a\,yz}$	32.6 $^{d\,y}$	31.5 $^{d\,zu}$	36.3 $^{c\,yz}$	32.5 $^{d\,yz}$	*2.13*	38.0^{c}
B Inter	40.0 ^{x}	44.1 $^{b\,xy}$	44.2 $^{b\,xy}$	52.3 $^{a\,xy}$	34.3 $^{d\,y}$	32.8 $^{d\,yzu}$	38.0 $^{c\,xy}$	33.0 $^{d\,yz}$	*1.77*	39.8^{b}
LT	34.7 ^{y}	37.5 $^{bc\,u}$	40.5 $^{ab\,z}$	43.2 $^{a\,u}$	33.1 $^{de\,y}$	30.1 $^{e\,u}$	34.9 $^{cd\,z}$	31.3 $^{de\,z}$	*2.55*	36.0^{d}
SM	34.8 ^{y}	42.9 $^{b\,xyz}$	43.3 $^{b\,xyz}$	47.5 $^{a\,z}$	33.3 $^{d\,y}$	33.0 $^{d\,yzu}$	37.5 $^{c\,xyz}$	32.9 $^{d\,yz}$	*2.99*	38.8^{c}
Flare Fat	32.6 ^{y}	37.1 $^{b\,u}$	37.4 $^{b\,u}$	48.9 $^{a\,z}$	27.9 $^{d\,z}$	26.1 $^{e\,v}$	30.8 $^{c\,u}$	25.4 $^{e\,u}$	*1.65*	33.3^{e}
RMSE	*2.16*	*2.16*	*2.31*	*2.14*	*2.13*	*2.30*	*1.95*	*2.23*		
Treat.mean	37.5	41.9^{b}	42.7^{b}	50.9^{a}	33.6^{d}	32.3^{e}	36.7^{c}	32.4^{e}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean
Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

Linoleic acid (Table 6) was high in intramuscular fat in the 60 kg animals, NF, T and LO fed animals, and also was high in SM intermuscular fat in T, SFHO, SFO, LO and FO fed animals Apparently, no tissue pattern was observed in linoleic content and when given a specific fat added diet, and in contrast to the results of Villegas *et al.* (1973) linoleic acid was not preferentially deposited in the backfat rather than in perirenal fat. Linolenic highest values (Table 7) varied depending on the treatment, but the lowest values were observed in intramuscular fat and the highest in flare fat. Apparently, there was no FA pattern distribution for linolenic acid.

Arachidonic acid (Table 8) showed a clear differential distribution as it was high in intramuscular fat and low and not significantly different from other tissues in all other fat depots.

Eicosapentaenoic acid (EPA) (Table 9) was present in all tissues of animals fed, LO, FB or FO. In other treatment fed animals, just was detected in the intramuscular fat. EPA present in LT and SM is probably from endogenous synthesis (elongation of linolenic acid) as it was not present in diets (except FO fed animals).

Table 5. Monounsaturated fatty acids profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	44.5 ^{xy}	48.5 ^{byz}	50.8 ^{bxy}	57.8 ^{axy}	37.9 ^{dy}	38.1 ^{dxyz}	43.1 ^{cxy}	37.9 ^{dyz}	*1.97*	44.9b
SM SC	47.4 ^{x}	52.2 ^{bx}	53.1 ^{bx}	58.8 ^{ax}	42.0 ^{dx}	40.9 ^{dx}	45.8 ^{cx}	42.6 ^{dx}	*2.05*	48.0^{a}
B SC	47.1 ^{x}	51.4 ^{bxy}	52.5 ^{bx}	58.2 ^{ax}	41.9 ^{cdx}	39.6 ^{dxy}	44.2 ^{cxy}	40.6 ^{cdxy}	*2.56*	46.9^{a}
LT Inter	44.1 ^{xy}	46.1 ^{bz}	48.5 ^{byz}	55.4 ^{ayzu}	36.2 ^{dy}	35.4 ^{dz}	41.1 ^{cy}	35.7 ^{dz}	*2.06*	42.6^{c}
SM Inter	45.2 ^{xy}	48.5 ^{byz}	47.5 ^{bz}	55.0 ^{azu}	36.7 ^{dy}	35.7 ^{dz}	41.6 ^{cy}	37.5 ^{dyz}	*2.23*	43.2^{c}
B Inter	46.4 ^{x}	50.1 ^{bxy}	50.1^{bxyz}	56.5 ^{axyz}	37.9 ^{dy}	36.3 ^{dyz}	43.0 ^{cxy}	37.6 ^{dyz}	*1.96*	44.5^{b}
LT	41.9 ^{y}	45.5 ^{abz}	48.8 ^{ayz}	49.5 ^{aw}	38.4 ^{cdy}	35.6 ^{dz}	42.0 ^{bcy}	37.2 ^{dz}	*2.71*	42.6^{c}
SM	42.0 ^{y}	51.2 ^{axy}	50.7 ^{axy}	53.1 ^{auv}	38.1 ^{cy}	38.2 ^{cxyz}	44.0 ^{bxy}	38.9 ^{cyz}	*3.18*	45.0^{b}
Flare Fat	37.6 ^{z}	42.1 ^{bu}	42.7 ^{bu}	51.8 ^{avw}	30.6 ^{dz}	28.9 ^{eu}	34.8 ^{cz}	29.3 ^{deu}	*1.77*	37.1^{d}
RMSE	*2.62*	*2.32*	*2.31*	*1.87*	*2.43*	*2.61*	*2.29*	*2.44*		
Trestment	44.0	48.6^{b}	49.3^{b}	55.4^{a}	37.6^{d}	36.5^{e}	42.1^{c}	37.5^{d}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean
Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

As EPA, docosahexaenoic acid (DHA) (Table 10) was high in intramuscular fat, compared to other fat depots. The lowest contents were found in belly intermuscular fat and LT subcutaneous fat. Quantities were in general greater than for EPA in all

studied tissues. In addition, DHA was detected in all tissues and treatments except in LT subcutaneous fat of NF fed animals.

PUFA (Table 11) were preferentially deposited in intramuscular fat. Observing other reservoir fat depots, they did not show any specific pattern and differences among subcutaneous fat depots were observed and also differences among intermuscular fat depots were observed.

In diets with a relatively high content in linolenic acid (LO, FB and FO diets), the n6/n3 ratio (Table 12) was high in LT, whereas in animals fed with other diets the highest values were observed in LT subcutaneous fat, were the wider fat depot layers are observed.

Table 6. Linoleic acid (C18:2n-6) profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	11.6 ^{y}	5.63 $^{d z}$	9.24 $^{c yz}$	11.0 $^{bc yz}$	28.6 $^{a xy}$	12.3 $^{b yz}$	13.1 ^{b}	11.1 $^{bc y}$	*1.65*	13.0^{uz}
SM SC	11.6 ^{y}	7.54 $^{e y}$	9.69$^{d xyz}$	10.4 $^{cd z}$	24.4 $^{a zu}$	12.2 $^{b yz}$	12.9 ^{b}	10.8 $^{c y}$	*1.06*	12.6^{uz}
B SC	12.9 ^{xy}	8.32 $^{e y}$	10.5$^{d xyz}$	11.8 $^{cd xyz}$	25.7$^{a yzu}$	13.4 $^{bc xyz}$	13.9 ^{b}	12.4 $^{bcd xy}$	*1.30*	13.7^{yz}
LT Inter	10.2 ^{y}	5.15 $^{d z}$	9.26 $^{c yz}$	10.8 $^{bc yz}$	28.3$^{a xyz}$	11.7 $^{b z}$	12.3 ^{b}	10.7 $^{bc y}$	*1.54*	12.6^{uz}
SM Inter	11.8 ^{y}	7.62 $^{d y}$	11.9 $^{c x}$	13.0 $^{bc x}$	28.4 $^{a xy}$	15.2 $^{b x}$	13.9 ^{bc}	13.2 $^{bc x}$	*2.23*	14.8^{x}
B Inter	10.5 ^{y}	6.08 $^{e z}$	8.81 $^{d z}$	10.3 $^{cd z}$	27.2$^{a xyz}$	12.3 $^{b yz}$	12.3 ^{b}	11.1 $^{bc y}$	*1.17*	12.6^{uz}
LT	12.5 ^{xy}	9.69 $^{c x}$	9.75$^{c xyz}$	10.6 $^{c yz}$	22.1 $^{a u}$	14.1 $^{b xy}$	12.7 ^{bc}	11.6 $^{bc xy}$	*1.97*	12.9^{zu}
SM	15.0 ^{x}	8.77 $^{d xy}$	11.1 $^{c xy}$	12.3 $^{bc xy}$	26.1$^{a xyz}$	14.4 $^{b xy}$	13.7 ^{b}	12.3 $^{bc xy}$	*2.09*	14.1^{xy}
Flare Fat	10.7 ^{y}	5.38 $^{e z}$	9.59 $^{d yz}$	11.1 $^{c yz}$	29.9 $^{a x}$	13.5 $^{b xyz}$	13.4 ^{b}	11.8 $^{c xy}$	*1.60*	13.5^{yz}
RMSE	*2.03*	*1.05*	*1.56*	*1.26*	*2.89*	*1.64*	*1.47*	*1.31*		
Treat.mean	11.9	7.04^{e}	10.0^{d}	11.2^{c}	26.8^{a}	13.3^{b}	13.1^{b}	11.7^{c}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean

Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

Table 7. Linolenic acid (C18:3n-3) profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*p*	Tis.Mean
LT SC	0.84 ^{x}	0.38 ^{fyz}	0.87 ^{dxy}	0.63 ^{eyz}	0.73 ^{dex}	13.5 ^{az}	5.94 ^{cxy}	9.30 ^{bxy}	***	1.97^{b}
SM SC	0.98 ^{x}	0.61 ^{fx}	1.06 ^{dx}	0.69 ^{ey}	0.70 ^{ex}	12.3 ^{azu}	5.16 ^{cy}	7.74 ^{bzu}	***	2.04^{a}
B SC	1.03 ^{x}	0.65 ^{ex}	1.01 ^{dxy}	0.74 ^{exy}	0.77 ^{ex}	13.0 ^{az}	6.08 ^{cxy}	8.71 ^{byz}	***	2.21^{a}
LT Inter	0.82 ^{x}	0.39 ^{fyz}	0.92 ^{dxy}	0.65 ^{ey}	0.65 ^{ex}	14.1 ^{ayz}	5.82 ^{cxy}	9.09 ^{bxyz}	***	1.96^{b}
SM Inter	0.97 ^{x}	0.59 ^{fx}	1.08 ^{dx}	0.87 ^{dex}	0.82 ^{ex}	14.3 ^{ayz}	5.33 ^{cy}	9.21 ^{bxy}	***	2.27^{a}
B Inter	0.83 ^{x}	0.44 ^{fyz}	0.87 ^{dxy}	0.63 ^{eyz}	0.70 ^{ex}	16.0 ^{axy}	5.94 ^{cxy}	9.15 ^{bxyz}	***	2.04^{b}
LT	0.51 ^{y}	0.37 ^{ez}	0.65 ^{dz}	0.43 ^{eu}	0.42 ^{ey}	8.49 ^{av}	3.41 ^{cu}	5.34 ^{bv}	***	1.31^{d}
SM	0.62 ^{y}	0.39 ^{fyz}	0.82 ^{dy}	0.51 ^{ezu}	0.51 ^{ey}	10.9 ^{au}	4.43 ^{cz}	6.75 ^{bu}	***	1.61^{c}
Flare Fat	0.85 x	0.46 ^{fy}	1.01 ^{dxy}	0.70 ^{ey}	0.72 ^{ex}	16.8 ^{ax}	6.50 ^{cx}	10.7 ^{bx}	***	2.24^{a}
P	***	***	*	***	***	***	***	***		
Treat.mean	0.83	0.47^{f}	0.92^{d}	0.64^{e}	0.65^{e}	13.0^{a}	5.25^{c}	8.24^{b}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean
Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

Effect of fat source

Fat source had a higher impact on FA composition than tissue.

Palmitic acid was high in NF fed pigs and low in SFHO, SFO, LO fed animals (diets with the lowest palmitic contents). As palmitic acid, stearic acid was high in NF fed animals and low in SFHO fed animals. Palmitic and stearic acids could be either from dietary or endogenous origin. In NF fed animals, palmitic and stearic are largely synthesized *de novo* and they are not diluted with PUFA from dietary origin as no fat was added in NF diet. The SFA showed similar trends as palmitic and stearic acids, they were high in NF fed animals and low in SFHO and SFO fed animals. In contrast to previous works (Koch *et al.*, 1968) SFA showed important variations in their

contents probably due extreme FA compositions used in the present experiment as in the work of Brooks (1971).

Oleic acid was high in SFHO fed animals (diet with the highest oleic acid content) and low in SFO, LO and FO fed animals. Interestingly, although FB diet had lower oleic acid content than SFO, FB fed animals had higher oleic acid tissue contents than SFO. Probably this higher oleic acid content is

Table 9. Eicosapentaenoic acid (C20:5n-3) profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*p*	Tis.Mean
LT SC	ND	ND	ND	ND	ND	0.14 $^{b\,u}$	0.07 $^{c\,yzu}$	0.68 $^{a\,u}$	***	NC
SM SC	ND	ND	ND	ND	ND	0.19 $^{b\,zu}$	0.10 $^{c\,yz}$	0.74 $^{a\,zu}$	***	NC
B SC	ND	ND	ND	ND	ND	0.14 $^{b\,u}$	0.08 $^{c\,yzu}$	0.64 $^{a\,u}$	***	NC
LT Inter	ND	ND	ND	ND	ND	0.14 $^{b\,u}$	0.06 $^{c\,u}$	0.65 $^{a\,u}$	***	NC
SM Inter	0.04 ^{y}	ND	0.03 $^{d\,y}$	ND	ND	0.20 $^{b\,z}$	0.09 $^{c\,yzu}$	0.87 $^{a\,yz}$	***	0.07^{b}
B Inter	ND	ND	ND	ND	ND	0.18 $^{b\,zu}$	0.07 $^{c\,zu}$	0.69 $^{a\,u}$	***	NC
LT	0.22 ^{x}	0.21 ^{d}	0.23 $^{d\,x}$	0.11 ^{e}	0.06 ^{f}	1.39 $^{b\,x}$	0.68 $^{c\,x}$	2.52 $^{a\,x}$	***	0.36^{a}
SM	0.24 ^{x}	0.15 ^{de}	0.17 $^{d\,x}$	0.11 ^{e}	0.06 ^{f}	1.01 $^{b\,y}$	0.52 $^{c\,x}$	2.38 $^{a\,x}$	***	0.30^{a}
Flare Fat	ND	ND	ND	ND	ND	0.23 $^{b\,z}$	0.11 $^{c\,y}$	0.99 $^{a\,y}$	***	NC
P	*	ns	***	ns	ns	***	***	***		
Treat.mean	NC	NC	NC	NC	NC	0.28^{B}	0.14^{C}	1.03^{a}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean

Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

ND: Not detected; NC: Not calculated

Table 10. Docosahexaenoic acid (C22:6n-3) profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*p*	Tis.Mean
LT SC	0.11 ^{z}	ND	0.06$^{b\ v}$	0.06 $^{b\ v}$	0.11 $^{b\ z}$	0.06 $^{b\ v}$	0.08 $^{b\ uv}$	2.78$^{a\ yzu}$	***	0.11^{d}
SM SC	0.23 ^{y}	0.12 $^{e\ y}$	0.22$^{b\ yz}$	0.15 $^{de\ yz}$	0.25 $^{b\ x}$	0.16 $^{cd\ yz}$	0.20 $^{bc\ xyz}$	2.90$^{a\ xyzu}$	***	0.26^{b}
B SC	0.12 ^{z}	0.06$^{d\ yz}$	0.10$^{c\ yz}$	0.12 $^{c\ yz}$	0.23 $^{b\ xy}$	0.13 $^{c\ yz}$	0.14 $^{c\ zu}$	2.34 $^{a\ u}$	***	0.18^{c}
LT Inter	0.10 ^{z}	0.05$^{c\ yz}$	0.09 $^{bc\ zu}$	0.10$^{bc\ zu}$	0.18 b ^{xyz}	0.10 $^{bc\ zu}$	0.12 $^{bc\ zuv}$	2.51 $^{a\ zu}$	***	0.16^{c}
SM Inter	0.12 ^{z}	0.08$^{c\ yz}$	0.13 $^{b\ zu}$	0.10$^{bc\ zu}$	0.11 $^{bc\ yz}$	0.11 $^{bc\ zu}$	0.14 $^{b\ zu}$	2.85$^{a\ xyzu}$	***	0.18^{c}
B Inter	0.10 ^{z}	0.03 $^{c\ z}$	0.08 $^{bc\ uv}$	0.07$^{bc\ uv}$	0.17 $^{b\ xyz}$	0.07 $^{bc\ uv}$	0.07 $^{bc\ v}$	2.63 $^{a\ zu}$	***	0.12^{d}
LT	0.39 ^{x}	0.47 $^{b\ x}$	0.31 $^{bc\ x}$	0.31 $^{bc\ x}$	0.23 $^{c\ xy}$	0.28 $^{bc\ x}$	0.32 $^{bc\ x}$	3.26$^{a\ xyz}$	***	0.44^{a}
SM	0.47 ^{x}	0.32 $^{b\ x}$	0.27 $^{bc\ x}$	0.36 $^{b\ x}$	0.21 $^{c\ xyz}$	0.22 $^{c\ xy}$	0.27 $^{bc\ xy}$	3.57 $^{a\ xy}$	***	0.38^{a}
Flare Fat	0.25 ^{y}	0.03 $^{d\ z}$	0.13 $^{c\ y}$	0.16 $^{bc\ y}$	0.24 $^{b\ x}$	0.15 $^{bc\ yz}$	0.17 $^{bc\ yz}$	3.68 $^{a\ x}$	***	0.21^{c}
P	***	***	***	***	*	***	***	***		
Treat.mean	0.21	0.08^{e}	0.14^{d}	0.13^{d}	0.18^{b}	0.13^{d}	0.15^{b}	2.80^{a}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean
Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).
ND: Not detected

linked to a higher SFA dietary content in FB fed animals that could be desaturated to oleic acid (Bloomfield and Bloch, 1960). MUFA contents were parallel to those observed in oleic acid contents, therefore MUFA were high in SFHO fed animals and low in SFO, LO and FO fed animals.

Linoleic acid was high in SFO fed animals (diet rich in linolenic acid) and low in NF fed animals (diet with no added fat), while other diets showed intermediate values depending on their dietary linoleic content. Different authors have found that if fats with a high proportion of linoleic acid as in sunflower oil, soy oil or corn oil are replaced with fats containing high levels of MUFA or SFA such as tallow, olive oil or rapeseed oil, the concentration of linoleic acid in tissue decline in favour of oleic acid,

whereas the other FA remain more or less constant (Koch *et al.*, 1968, Brooks, 1971, Wiseman and Agunbiade, 1998). In addition to these observations, Eder *et al.* (2001) pointed out that the concentration of linoleic acid in the triglycerides is apparently determined only by the dietary linoleic acid intake. The different linoleic acid accretion observed in pigs fed FO and T suggested that some variations in the linoleic acid deposition could by affected by the presence of other dietary FA since these animals received the same levels of linoleic acid. In addition to this observation, it seems that the whole dietary fat content also exerts an effect on tissue linoleic acid content; in the work of Warnants *et al.* (1999) similar levels of dietary linoleic acid were used during the finishing phase (6 weeks before slaughter), to those of the present experiment diets (SFHO, LO, FB an FO), but in the present work linoleic acid was a minor constituent compared to other FA. In the work of Warnants *et al.* (1999), linoleic acid was more efficiently deposited reaching levels of 17% in backfat carcass, whereas in the present work, althought similar linoleic acid levels, linoleic acid reached the maximum levels in belly subcutaneous fat (13.9%). In the present work, when linoleic and linolenic acid increased oleic acid was mainly replaced by these FA and second by SFA, meaning that SFA are less susceptible to modification. Linolenic acid was high in LO fed animals (diet rich in linolenic acid) and low in NF fed animals (diet with no added fat), while other diets showed intermediate values depending on its dietary linolenic content. Linolenic acid levels in meat samples (*M. longissimus thoracis and semimembranosus*) in FO and LO fed pigs probably exceed levels (Van Oeckel *et al.*, 1996) that would not affect the sensory properties of meat. Arachidonic acid was high in SFO fed animals and low in other diets, except FO fed animals that showed intermediate values probably due it was present in diet in low levels (data not shown). The increase in arachidonic acid in FO fed pigs contrast to the previous

observations of an arachidonic-lowering effect of dietary fish oil (Leskanich *et al.*, 1997). Linseed oil fed animals (FB and LO fed animals) showed reduced arachidonic acid levels compared to other fat added diets in some of the studied tissues. Nuernberg *et al.* (2005) suggested that this decrease could be a result of the competition for the same enzymes in their elongation and desaturation metabolism.

EPA was high in FO fed animals (diet rich in EPA), LO and FB showed lower values and in pigs fed the other diets this FA was not detected (except in 60kg animals and T intermuscular SM). DHA was high in FO fed animals. Except in FO fed animals, EPA and DHA present in tissues would originate from elongation of linolenic acid. Pigs fed LO and FB showed detectable levels of EPA which seemed to depend on its linolenic dietary content (linolenic content in LO doubled FB linolenic content, consequently EPA content in LO fed animals tissues doubled FB fed animals tissues). Although DHA is at the end of the same synthesis pathway as EPA, the only effective way to increase tissue DHA contents was to add DHA in the diet as showed the results of FO fed animals. Different studies report the increase in docosapentaenoic acid and EPA, but no increase in DHA contents due a high linolenic acid dietary content (Nuernberg *et al.*, 2005).

Table 11. Polyunsaturated fatty acid profile (% of detected FA)

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	13.8 ^{y}	6.76 ^{eu}	11.4 ^{dz}	12.8 ^{dz}	31.7 ^{axy}	28.8 ^{bxy}	21.2 ^{c}	27.0 ^{bxy}	*1.81*	19.9^{d}
SM SC	14.4 ^{y}	9.37 ^{ez}	13.2 ^{dyz}	12.6 ^{dz}	27.8 ^{ay}	27.9 ^{ay}	20.4 ^{c}	25.4 ^{by}	*1.62*	19.5^{d}
B SC	15.5 ^{y}	9.94 ^{dz}	13.2 ^{cyz}	13.7 ^{cyz}	28.9 ^{axy}	29.4 ^{axy}	22.3 ^{b}	27.1 ^{axy}	*2.04*	20.7^{cd}
LT Inter	12.3 ^{y}	6.26 ^{eu}	11.5 ^{dz}	12.6 ^{dz}	31.1 ^{axy}	28.7 ^{bxy}	20.0 ^{c}	25.6 ^{by}	*3.45*	19.4^{d}
SM Inter	14.5 ^{y}	9.31 ^{ez}	14.8 ^{dxy}	15.3 ^{dy}	31.7 ^{abxy}	32.9 ^{ax}	21.5 ^{c}	29.4 ^{bxy}	*3.67*	22.1^{b}
B Inter	12.7 ^{y}	7.17 ^{eu}	11.1 ^{dz}	12.0 ^{dz}	30.0 ^{axy}	31.2 ^{axy}	20.2 ^{c}	26.4 ^{bxy}	*2.10*	19.7^{d}
LT	20.1 ^{x}	17.0 ^{bcx}	15.5 ^{cxy}	15.9 ^{cy}	27.8 ^{ay}	30.5 ^{axy}	22.2 ^{b}	28.3 ^{axy}	*2.97*	22.4^{b}
SM	23.1 ^{x}	14.1 ^{dy}	16.4 ^{cdx}	18.1 ^{cx}	32.0 ^{axy}	31.7 ^{axy}	23.4 ^{b}	30.3 ^{ax}	*2.17*	23.6^{a}
Flare Fat	12.9 ^{y}	6.58 ^{eu}	11.9 ^{dz}	13.0 ^{dz}	32.8 ^{ax}	33.1 ^{ax}	21.9 ^{c}	30.1 ^{bx}	*2.26*	21.4^{bc}
RMSE	*2.98*	*1.65*	*2.03*	*1.58*	*3.38*	*3.22*	*2.55*	*3.05*		
Treat.mean	15.5	9.44^{e}	13.3^{d}	13.9^{d}	30.4^{a}	30.5^{a}	21.3^{c}	27.9^{b}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean

Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different ($P<0.05$).

The effects of diet on content of PUFA in the intramuscular fat were not as pronounced as those found for subcutaneous fat, and this difference may be related to the lower deposition rate in the intramuscular fat tissue (Nguyen *et al.*, 2003) and to the presence of more structural lipids in intramuscular fat (containing elevated amounts of PUFA not susceptible to be easily modified), compared with other fat depots (Warnants *et al.*, 1999). Also according to other studies, *longissimus thoracicus* muscle is less sensitive to PUFA incorporation than belly (Leszczynski *et*

al., 1992) and *longissimus thoracicus* subcutaneous fat (Koch *et al.*, 1968). PUFA were high in SFO and LO fed animals and low in NF fed animals. Interestingly, FO fed animals showed in some of the tissues a significantly lower content than in pigs fed SFO although the diet only differed in PUFA by 3.7 mg/kg diet. This differential PUFA deposition may have two explanations, one is that PUFA could have a preferential site of deposition depending on its dietary composition and PUFA would have been deposited in a non studied tissue in FO fed animals, and second PUFA from FO fed animals could be more oxidized than PUFA from SFO fed animals. A whole animal FA balance performed in this trial (data not published), showed that EPA and DHA from FO diet are more oxidized than linoleic or linolenic acids from other PUFA diets, and this would explain the differences in PUFA deposition depending on dietary treatment. The n6/n3 is a parameter that evaluates nutritional quality of fat in terms of human health (Simopoulos, 2001). This ratio was high in SFO fed animals, which would not make it interesting according to current recommendations for a healthy diet, while the opposite can be said about the diet that contained linseed oil (LO, FB and FO fed animals) as observed by different studies (Hoz *et al.*, 2003, Nuernberg *et al.*, 2005) .

CONCLUSIONS

A pattern in tissue FA distribution was confirmed as SFA were preferentially depositesd in interior fat depots compared to more external fat depots. Intramuscular fat was less susceptible to treatment modification. The main tissue FA differences were due to dietary PUFA. Ingestion of linseed oil increases EPA tissue contents, whereas the only effective way to increase tissue DHA contents was to add DHA in

the diet. SFA were less susceptible to modification than MUFA when PUFAs were supplemented in the diet.

Table 12. Ratio n6/n3

	60kg	NF	T	SFHO	SFO	LO	FB	FO	*RMSE*	Tis.Mean
LT SC	11.0^{x}	12.6$^{b\,x}$	8.74^{c}	14.2$^{b\,x}$	30.3$^{a\,x}$	0.85$^{d\,zuvw}$	2.00$^{d\,uv}$	0.84$^{d\,uv}$	*2.20*	9.95a
SM SC	8.41^{z}	8.9$^{c\,u}$	7.22^{d}	11.0$^{b\,z}$	21.7$^{a\,u}$	0.92$^{f\,zuv}$	2.18$^{e\,zu}$	0.91$^{f\,zu}$	*1.03*	7.58^{d}
B SC	9.79^{y}	10.9$^{b\,yz}$	8.70^{c}	12.5$^{b\,xyz}$	22.5$^{a\,zu}$	0.96$^{d\,zu}$	2.08$^{d\,uv}$	1.01$^{d\,yz}$	*1.43*	8.37^{c}
LT Inter	9.96^{y}	9.7$^{c\,zu}$	8.66^{c}	12.9$^{b\,xy}$	27.5$^{a\,xy}$	0.78$^{d\,uvw}$	1.91$^{d\,uv}$	0.84$^{d\,uv}$	*1.43*	8.89^{bc}
SM Inter	9.09^{yz}	10.2$^{bc\,zu}$	9.29^{c}	11.8$^{b\,yz}$	27.1$^{a\,xyz}$	1.02$^{d\,z}$	2.37$^{d\,yz}$	0.99$^{d\,yz}$	*2.14*	9.02^{ab}
B Inter	9.47^{y}	11.9$^{b\,x}$	8.44^{c}	12.9$^{b\,xy}$	25.6$^{a\,xyzu}$	0.72$^{d\,w}$	1.88$^{d\,v}$	0.84$^{d\,uv}$	*1.71*	8.86^{bc}
LT	9.34^{y}	8.8$^{c\,u}$	7.40^{d}	11.4$^{b\,yz}$	23.6$^{a\,yzu}$	1.43$^{f\,x}$	2.81$^{e\,x}$	1.22$^{f\,x}$	*0.83*	8.11^{cd}
SM	9.45^{y}	9.0$^{c\,u}$	7.84^{d}	11.1$^{b\,z}$	26.2$^{a\,xyzu}$	1.22$^{f\,y}$	2.56$^{e\,xy}$	1.08$^{f\,y}$	*1.24*	8.47^{c}
Flare Fat	8.51^{z}	9.3$^{c\,u}$	7.47^{d}	11.4$^{b\,yz}$	26.2$^{a\,xyzu}$	0.75$^{e\,vw}$	1.88$^{e\,v}$	0.75$^{e\,v}$	*1.83*	8.28^{c}
RMSE	*0.57*	*1.11*	*1.74*	*1.17*	*3.40*	*0.14*	*0.21*	*0.08*		
Treat.mean	9.4	10.0^{c}	8.20^{d}	12.0^{b}	25.6^{a}	1.10^{f}	2.33^{e}	1.07^{f}		

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil), LT SC: longissimus thoracicus subcutaneous fat, SM SC: s*emimembranosus* subcutaneous fat, B SC: belly subcutaneous fat, LT Inter: longissimus thoracicus intermuscular fat, SM Inter: s*emimembranosus* intermuscular fat, B Inter: belly intermuscular fat, LT: longissimus thoracicus intramuscular fat, SM: s*emimembranosus* intramuscular fat. Treat.mean: treatment mean, Tis.mean: tissue mean
Diets with different letter in row (a, b, c, d, …) or column (x, y, z, u, …) were significantly different (P<0.05).

REFERENCES

Bloomfield DK and Bloch K 1960. Formation of Delta-9-Unsaturated fatty acids. Journal of biological chemistry 235, 337-345.

Brooks CC 1971. Fatty acid composition of pork lipids as affected by basal diet, fat source and fat level. Journal Animal Science 33, 1224-1231.

Dean HK and Hilditch TP 1933. The body fats of the pig. III. The influence of body temperature on the composition of depot fats. Biochemical Journal 27, 1950-1961.

Duran-Montgé P, Lizardo R, Torrallardona D and Esteve-Garcia E 2007a. Fat and fatty acid digestibility of different fat sources in growing pigs. Livestock Science 109, 66-69.

Duran-Montgé P, Theil P, Lauridsen C and Esteve-Garcia E 2007b. Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulation factors in liver and adipose tissue but not in semimembranosus muscle of pigs during the fattening period. Animal (unpublished work).

Eder K, Nonn H and Kluge H 2001. The fatty acid composition of lipids from muscle and adipose tissues of pigs fed various oil mixtures differing in their ratio between oleic acid and linoleic acid. European Journal of Lipid Science and Technology 103, 668-676.

Folch J, Lees M and Stanley GHS 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry 226, 497-509.

Hoz L, Lopez-Bote CJ, Cambero MI, D'Arrigo M, Pin C, Santos C and Ordonez JA 2003. Effect of dietary linseed oil and [alpha]-tocopherol on pork tenderloin (Psoas major) muscle. Meat Science 65, 1039-1044.

Koch DE, Pearson AM, Magee WT, Hoefer JA and Schweigert BS 1968. Effect of diet on the fatty acid composition of pork fat. Journal Animal Science 27, 360-365.

Larsson SC, Kumlin M, Ingelman-Sundberg M and Wolk A 2004. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 79, 935-945.

Leskanich CO, Matthews KR, Warkup CC, Noble RC and Hazzledine M 1997. The effect of dietary oil containing (n-3) fatty acids on the fatty acid, physicochemical, and organoleptic characteristics of pig meat and fat. Journal of Animal Science 75, 673-683.

Leszczynski DE, Pikul J, Easter RA, McKeith FK, McLaren DG, Novakofski J, Bechtel PJ and Jewell DE 1992. Characterization of lipid in loin and bacon from finishing pigs fed full-fat soybeans or tallow. Journal of Animal Science 70, 2175-2181.

Marchello MJ, Cook NK, Slanger WD, Johnson VK, Fischer AG and Dinusson WE 1983. Fatty-acid composition of lean and fat tissue of swine fed various dietary levels of sunflower seed. Journal of Food Science 48, 1331-1334.

Miller MF, Shackelford SD, Hayden KD and O RJ 1990. Determination of the alteration in fatty acid profiles, sensory characteristics and carcass traits of swine fed elevated levels of monounsaturated fats in the diet. Journal Animal Science 68:1624-1631.

Monziols M, Bonneau M, Davenel A and Kouba M 2007. Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. Meat Science 76, 54-60.

Morrison WR and Smith LM 1964. Preparation of fatty acid methyl esters + dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5, 600-&.

Nguyen LQ, Nuijens MCGA, Everts H, Salden N and Beynen AC 2003. Mathematical relationships between the intake of n-6 and n-3 polyunsaturated fatty acids and their contents in adipose tissue of growing pigs. Meat Science 65, 1399-1406.

Nuernberg K, Fischer K, Nuernberg G, Kuechenmeister U, Klosowska D, Eliminowska-Wenda G, Fiedler I and Ender K 2005. Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. In Meat Science, pp. 63-74.

Simopoulos AP 2001. The mediterranean diets: What is so special about the diet of Greece? The scientific evidence. Journal of Nutrition 131, 3065S-3073.

Van Oeckel MJ, Casteels M, Warnants N, Van Damme L and Boucque CV 1996. Omega-3 fatty acids in pig nutrition: Implications for the intrinsic and sensory quality of the meat. Meat Science 44, 55-63.

Villegas FJ, Hedrick HB, Veum TL, McFate KL and Bailey ME 1973. Effect of diet and breed on fatty acid composition of porcine adipose tissue. Journal Animal Science 36, 663-668.

Warnants N, Van Oeckel MJ and Boucque CV 1996. Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. Meat Science 44, 125-144.

Warnants N, Van Oeckel MJ and Boucqué CV 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. Journal of Animal Science 77, 2478-2490.

Wiseman J and Agunbiade JA 1998. The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. Livestock Production Science 54, 217-227.

Paper 6: Dietary tallow reduces whole pig fat compared to other dietary oils by means of reduced lipogenesis

Submitted to *Animal*

Dietary tallow reduces whole pig fat compared to other dietary oils by means of reduced lipogenesis

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ABSTRACT

Dietary fatty acid composition has shown to influence fat deposition in rodents and chickens. The objectives of the experiment were to study the effect of dietary fat composition on whole pig FA composition and to perform a whole-body balance in order to study *de novo* FA synthesis and the fate of essential FA.

Seventy-five crossbred gilts (62 ±5x kg BW average) were divided in 10 animals per treatment. Pigs were fed one of seven treatments: a semi-synthetic diet formulated to contain a very low level of fat (NF) and six fat supplemented diets (10%) based on a barley-soybean meal. The supplemental fats were tallow (T), high-oleic sunflower oil (HOSF), sunflower oil (SFO), linseed oil (LO), blend (B) (55% tallow, 35% sunflower oil, 10% linseed oil) and fish oil blend (FO) (40% fish oil, 60% linseed oil). In addition 5 pigs were killed at 60 kg to measure body composition at the start of the balance period. Carcass and viscera fat and fatty acid composition were determined in order to perform a balance of pigs growing from 60 to approximately 100 kg BW. Viscera and body FA composition reflected dietary treatments; red viscera showed higher long PUFA content. Whole-body fat content of HOSF (26.7%) and SFO (26.7%) fed animals was higher than T (22.9%) fed animals. The ratio of *de novo* FA synthesis ratio of pigs fed the very low fat diet was 1.5/1/3 for palmitic, stearic and oleic acids, respectively. The different diets containing important amounts of PUFA (SFO,LO and FO) showed deposition ratios of linoleic and linolenic acid between 73.2% and 64.8%, and 64.1% and 72.9% respectively whereas deposition rates of arachidonic, eicosapentaenoic and docosahexaenoic acids were 33.6%, 47.9% and 48.9% respectively. EPA and ARA tissue content increased in the diets containing high

levels of linolenic and linoleic acids respectively, but the diet containing high linolenic acid did not increase DHA deposition.

INTRODUCTION

Different factors determine FA composition in slaughter pigs. In practical conditions of positive energy balance, dietary FA composition has a strong influence, depending on the level of dietary fat. *De novo* FA synthesis depends on different factors, mainly dietary fat level, and finally FA mobilization, although the latter is not important in practical conditions because pigs are in a positive energy balance (Chwalibog and Thorbek, 1995). Another source of body fat is originated from the transformation of carbohydrate into fat. Pigs reared on semisynthetic diets with no fat in the diet synthesized approximately 55% of oleic and palmitoleic acids and 45% of palmitic and stearic acids (Leat *et al.*, 1964, Flanzy *et al.*, 1970). However, these measurements were conducted with animals which differed in many respects with the current commercial breeds used nowadays, such as body composition, growth rate, and feed efficiency. Polyunsaturated fatty acids (PUFA) from the n-3 and n-6 series are always of dietary origin, since pig does not synthesize them and they serve as substrates for synthesis of long-PUFA as arachidonic acid, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Long-PUFA are known to have important functions during gestation (Allen and Harris, 2001), they are a basic constituent of cell brain (Birch *et al.*, 1998), they play a role in the immune function (Miles and Calder, 1998), retina function (Birch *et al.*, 1998), signal transduction (Bazan, 2003), gene regulation (Jump and Clarke, 1999), and have protective properties against coronary heart disease (Wijendran and Hayes, 2004) and cancer (Hardman, 2002). Due to their important functions, the conversion of linoleic and linolenic acids into

their long-PUFA derivates is a matter of interest. In a whole-body balance analysis of linoleic and linolenic acids, the difference between their intake and the sum of their faecal excretion and accumulation (including their respective long-PUFA) equals the disappearance or oxidation (Cunnane and Anderson, 1997b, Bazinet *et al.*, 2003) and the use of tracers is not needed; partitioning of these FA can be performed quantitatively, and conversion to long-PUFA can be determined; in contrast saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), can be synthesized by the organism, and it is necessary to use radioactively labeled FA for their traceability (Murphy, 2006).

Very few experiments have analyzed pig carcass composition, and according to our knowledge there are even less reports on whole-body fat and FA composition (Cunnane and Anderson, 1997b, Poumes-Ballihaut *et al.*, 2001, Crespo and Esteve-Garcia, 2002b) and on whole pig (Metz and Dekker, 1981, Bazinet *et al.*, 2003, Kloareg *et al.*, 2006). Studies on rats (Shimomura *et al.*, 1990) and poultry (Crespo and Esteve-Garcia, 2002a) have shown that PUFA reduce fat deposition, but there are no similar works in pigs. Most pig studies have focused on meat and subcutaneous FA composition as it is an important factor related to meat quality. Therefore, fat and FA composition on carcass and viscera was determined in order to achieve the objectives of the experiment which are to perform a whole-body balance for the study of the *de novo* FA synthesis and the fate of essential FA, and to study the effect of dietary fat composition on whole animal fat.

MATERIAL AND METHODS

Animals and diets

Seventy-five crossbred female pigs (Duroc ♂x Landrace ♀) were fed a barley-corn-soybean meal-based diet during a 4-week pre-experimental period. Gilts (61.8 ±5.2 kg BW) were divided in 10 animals per treatment in four separated periods/series of 3, 2, 2, and 3 gilts per treatment. Seven diets formulated to meet NRC (1998) requirements except for linoleic levels in NF diet were assigned randomly by animal weight and origin. Six fat supplemented (10%) diets were based on barley and soybean meal (Duran-Montgé *et al.*, 2008b). Additionally, a semi- synthetic diet was formulated using “purified” ingredients to contain no fat because traditional diets with no fat addition always contain around 2% of dietary fat. Fat was weighed and added into the mixer (1000L capacity) and mixed with the rest of the feed for 5 min. The fat level was higher than practical commercial levels in order to detect differences between dietary treatments.

Different fat sources were selected to provide different fatty acid composition in the diets: tallow (T), high-oleic sunflower oil (SFHO), sunflower oil (SFO), linseed oil (LO), blend (55% tallow, 35% sunflower oil, 10% linseed oil) (B) and fish oil blend (40% fish oil, 60% linseed oil) (FO). The NF diet was included to the experiment in order to determine the *de novo* FA synthesis in a diet containing very low fat. Pigs were fed individually *ad libitum*. The fat supplemented diets were formulated in order to supply equal amounts of digestible fat based on previous measurements (Duran-Montgé *et al.*, 2007). Feed consumption was measured individually.

Gilts were slaughtered at an average live weight of 99.8 ±8.5kg using CO_2 stunning. Five gilts were slaughtered at the beginning of the trial, at abouty 60 kg live body

weight. After slaughter each animal was divided into white viscera, red viscera and carcass. White viscera included all the digestive tract (esophagus, stomach, intestines and rectum) without intestinal contents; red viscera included brain, spinal cord, liver, trachea, lungs, kidneys, bladder, pancreas, heart, trachea, and other small organs). Viscera were weighted and immediately frozen at -20ºC. After 24 hours from slaughter the left side carcass was weighed, cut into head, loin, belly, ham and shoulders, and frozen (-20ºC). The frozen carcass cuts were cut into small pieces and ground using a meat grinder (Grinder Cato-PA160, Sabadell, Spain) followed by a mixer; the same procedure was followed for the white and red viscera. Experimental procedures were approved by IRTA ethical committee.

Analysis

Analysis procedures and dietary dry matter content (DM), crude protein, energy, fat and FA profile are described in the work of Duran-Montgé *et al.*(2008b). For FA determination in tissues, lipids were extracted by the Folch method (Folch *et al.*, 1957) and then transmethylated with BF_3 and methanolic KOH (Morrison and Smith, 1964). Fatty acid contents were determined using an automated GC (Hewlett Packard 6890, USA) equipped with an automatic injector, using C19:0 as internal standard.

Calculations and Statistics

Whole animal composition was calculated as the sum of the three compartment (red visceras, white visceras and carcass) composition, and viscera is calculated from red visceras and white visceras compositions.

The statistical model for this study was

$$y_{ijk} = \mu + \alpha_i + \beta_j + U_{jk} + \varepsilon_{ijk}$$

where y is the overall i treatment effect observation in a j period from a k litter, μ is the overall mean, α_i is a fixed treatment effect treatment, β_j is a fixed effect of period. U_{jk} contains the sow x period random effects variable, and e contains the residual errors. Treatment comparisons were done between FA profiles. For comparisons between linolenic, docosahexaenoic and eicosahexaenoic acids logarithmic transformations were used due constant coefficient of variation (Snedecor and cochran).

Whole fat or FA balance was calculated as the difference between the composition at slaughter at 100 kg and sum of the digested fat or FA and the mean composition of the animals slaughtered at 60 kg. The FA net synthesis was calculated as:

Net synthesis= $Q_f - Q_0$ - Dig

The deposition ratio of digested FA was calculated as:

$$Deposition\ Ratio = \frac{(Q_f - Q_0)}{Dig} \times 100$$

Where:

Q_0 = FA content at the beginning of the trial (n-3 or n-6 series in Deposition Ratio).

Q_f = FA content at the end of the experiment (n-3 or n-6 series in Deposition Ratio).

Dig = Amount of digested FA during the experiment (n-3 or n-6 series in Deposition Ratio).

Deposition of long PUFA was calculated as for linoleic and linolenic acid and it was assumed that there was no conversion of linolenic acid coming from diet or previously present in the animal. Disappearance ratio of EPA and DHA was used to correct the disappearance of linolenic acid in animals fed the FO diet (Cunnane and Anderson, 1997b).

Table 1. Fatty acid profile and fat content (%) of pig carcass

	60kg	NF	T	HOSF	SFO	LO	B	FO	StdErr
Fat content	19.3	26.0	24.4	26.8	27.5	25.3	25.8	25.6	0.77
C 14:0	1.33	1.27^{b}	1.41^{a}	1.10^{cd}	1.02^{e}	1.02^{de}	1.27^{b}	1.16^{c}	0.027
C 16:0	23.8	24.4^{a}	21.7^{b}	18.4^{e}	17.9^{e}	18.1^{e}	20.9^{c}	19.2^{d}	0.261
C 16:1	2.58	2.35^{a}	2.36^{a}	1.54^{d}	1.32^{e}	1.43^{de}	1.94^{b}	1.73^{c}	0.065
C 18:0	13.2	14.1^{a}	11.7^{b}	9.33^{d}	10.8^{cd}	11.2^{bc}	11.8^{b}	11.5^{b}	0.23
C 18:1 n-9 trans	0.19	0.16^{c}	0.28^{a}	0.13^{cd}	0.12^{d}	0.12^{d}	0.19^{b}	0.13^{d}	0.008
C 18:1 n-9 cis	39.7	44.3^{b}	44.3^{b}	52.2^{a}	34.9^{d}	33.7^{e}	38.8^{c}	34.1^{de}	0.42
C 18:1 n-11 cis	3.10	3.11^{a}	2.89^{b}	1.97^{d}	1.85^{e}	2.02^{d}	2.53^{c}	2.45^{c}	0.059
C 18:2 n-6	11.4	6.62^{f}	10.5^{e}	11.6^{d}	27.5^{a}	13.3^{b}	12.9^{b}	11.7^{d}	0.35
C 18:3 n-3	0.88	0.47^{f}	0.93^{d}	0.72^{e}	0.70^{ef}	14.18^{a}	5.42^{c}	8.86^{b}	-
C 20:0	0.24	0.23^{a}	0.17^{cd}	0.16^{d}	0.18^{bc}	0.18^{bc}	0.18^{bc}	0.19^{b}	0.006
C 20:1 n-9	0.93	0.98^{a}	0.88^{b}	1.00^{a}	0.74^{c}	0.66^{d}	0.79^{c}	0.87^{b}	0.021
C 20:2 n-6	0.63	0.43^{e}	0.53^{d}	0.57^{cd}	1.31^{a}	0.63^{b}	0.61^{bc}	0.58^{bcd}	0.020
C 20:3 n-6	0.12	0.076^{e}	0.12^{b}	0.11^{c}	0.16^{a}	0.085^{d}	0.098^{c}	0.10^{c}	0.003
C 20:4 n-6	0.53	0.35^{d}	0.44^{c}	0.49^{b}	0.55^{a}	0.33^{d}	0.34^{d}	0.47^{bc}	0.015
C 20:3 n-3	0.15	0.10^{d}	0.16^{d}	0.11^{d}	0.11^{d}	1.60^{a}	0.67^{c}	0.92^{b}	0.029
C 20:5 n-3	0.02	ND	0.032^{d}	ND	ND	0.269^{b}	0.125^{c}	0.928^{a}	-
C 22:4 n-6	0.16	0.10^{d}	0.11^{c}	0.13^{b}	0.16^{a}	0.074^{f}	0.088^{e}	0.10^{d}	0.004
C 22:5 n-3	0.20	0.12^{f}	0.21^{d}	0.14^{ef}	0.17^{e}	0.43^{b}	0.36^{c}	1.02^{a}	0.014
C 22:6 n-3	0.17	0.057^{d}	0.11^{c}	0.11^{bc}	0.15^{b}	0.10^{c}	0.14^{bc}	3.11^{a}	-
SFA	39.2	40.6^{a}	35.7^{b}	29.3^{d}	30.3^{d}	31.0^{d}	34.7^{b}	32.8^{c}	0.37
MUFA	46.5	51.0^{b}	50.9^{b}	56.7^{a}	38.8^{de}	37.9^{e}	44.2^{c}	39.3^{d}	0.46
PUFA	14.3	8.3^{e}	13.5^{d}	14.0^{d}	30.8^{a}	31.1^{a}	20.9^{c}	27.9^{b}	0.45
n-6/n-3 ratio	9.08	10.2^{c}	8.45^{d}	11.9^{b}	26.6^{a}	0.87^{f}	2.11^{e}	0.88^{f}	-

Means within a row with different letters are significantly different (P<0.05).

ND: Under limit of detection

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Table 2. Fatty acid profile and fat content (%) of whole pig

	60kg	NF	T	HOSF	SFO	LO	B	FO	StdErr
Fat content	18.2	25.4^{ab}	22.9^{c}	26.7^{a}	26.7^{a}	24.4^{abc}	24.2^{bc}	24.7^{abc}	0.76
C 14:0	1.34	1.29^{b}	1.42^{a}	1.15^{c}	1.05^{d}	1.03^{d}	1.29^{b}	1.17^{c}	0.026
C 16:0	23.9	24.8^{a}	21.8^{b}	19.2^{d}	18.2^{e}	18.3^{e}	21.0^{c}	19.5^{d}	0.25
C 16:1	2.52	1.70^{c}	1.90^{b}	1.40^{de}	1.31^{e}	1.54^{cd}	2.26^{a}	2.32^{a}	0.066
C 18:0	13.6	14.6^{a}	12.2^{b}	9.86^{d}	11.3^{c}	11.8^{bc}	12.2^{b}	12.0^{bc}	0.26
C 18:1 n-9 trans	0.19	0.17^{bc}	0.28^{a}	0.16^{bcd}	0.13^{de}	0.12^{e}	0.18^{b}	0.15^{cde}	0.011
C 18:1 n-9 cis	39.15	43.7^{b}	43.4^{b}	51.0^{a}	34.5^{d}	33.1^{e}	38.3^{c}	33.4^{de}	0.41
C 18:1 n-11 cis	3.04	3.05^{a}	2.78^{b}	1.94^{d}	1.84^{d}	1.97^{d}	2.49^{c}	2.40^{c}	0.06
C 18:2 n-6	11.27	6.44^{d}	10.63^{c}	11.38^{c}	27.10^{a}	13.24^{b}	12.86^{b}	11.58^{c}	0.33
C 18:3 n-3	0.87	0.46^{f}	0.95^{d}	0.71^{e}	0.70^{e}	13.92^{a}	5.43^{c}	8.83^{b}	
C 20:0	0.24	0.23^{a}	0.17^{c}	0.17^{c}	0.18^{bc}	0.18^{bc}	0.18^{bc}	0.20^{b}	0.006
C 20:1 n-9	0.92	0.96^{a}	0.86^{b}	0.97^{a}	0.72^{d}	0.65^{e}	0.78^{c}	0.86^{b}	0.018
C 20:2 n-6	0.62	0.41^{e}	0.53^{d}	0.56^{cd}	1.27^{a}	0.63^{b}	0.60^{bc}	0.57^{bcd}	0.019
C 20:3 n-6	0.13	0.08^{e}	0.12^{b}	0.11^{c}	0.15^{a}	0.09^{d}	0.10^{c}	0.11^{c}	0.003
C 20:4 n-6	0.65	0.39^{c}	0.53^{b}	0.54^{b}	0.61^{a}	0.35^{c}	0.39^{c}	0.51^{b}	0.017
C 20:3 n-3	0.15	0.10^{d}	0.15^{d}	0.11^{d}	0.10^{d}	1.57^{a}	0.66^{c}	0.91^{b}	0.029
C 20:5 n-3	0.03	ND	0.04^{d}	ND	ND	0.30^{b}	0.14^{c}	0.96^{a}	
C 22:4 n-6	0.17	0.10^{c}	0.12^{b}	0.13^{b}	0.17^{a}	0.07^{e}	0.09^{d}	0.10^{c}	0.004
C 22:5 n-3	0.21	0.12^{f}	0.22^{d}	0.14^{ef}	0.17^{e}	0.44^{b}	0.37^{c}	1.01^{a}	0.015
C 22:6 n-3	0.19	0.07^{d}	0.14^{bc}	0.12^{c}	0.17^{b}	0.12^{c}	0.16^{bc}	3.14^{a}	
SFA	39.8	41.5^{a}	36.5^{b}	30.7^{e}	31.0^{e}	31.8^{e}	35.4^{c}	33.6^{d}	0.37
MUFA	43.3	50.3^{b}	49.8^{b}	55.5^{a}	38.5^{d}	37.2^{d}	43.7^{c}	38.6^{d}	0.46
PUFA	14.3	8.2^{e}	13.7^{d}	13.8^{d}	30.5^{a}	31.0^{a}	20.9^{c}	27.8^{b}	0.45
n-6/n-3 ratio	8.92	10.0^{c}	8.21^{d}	11.6^{b}	25.4^{a}	0.88^{f}	2.11^{e}	0.88^{f}	

Means within a row with different letters are significantly different (P<0.05).

ND: Under limit of detectionNF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

RESULTS AND DISCUSSION

Palmitic and stearic acid carcass (Table 1) and whole animal (Table 2) contents were high in NF fed animals probably because there is higher contribution of FA from *de novo* synthesis. Oleic acid can be *de novo* synthesized, the highest percentage content was found in HOSF, diet with a high content in oleic acid; therefore higher amounts of this FA can deposited when it is from exogenous origin than if it was only from endogenous origin as in NF fed pigs. N-6 and n-3 series FA are only from dietary origin since animals are not able to desaturate n-6 and n-3 carbons. As expected, the highest contents in these FA were found in animals fed diets rich in these FA; the highest linoleic content was observed in SFO fed animals, and the highest linolenic content was observed in animals fed LO. A higher dietary impact on composition was observed in SFO fed pigs than in LO fed pigs, probably due a higher content of linoleic acid in SFO diet and a higher content in linoleic acid in pigs at 60 kg BW.

EPA and DHA were high in FO fed animals as diet was high in these FA. Diets with a high content in linolenic (LO and B diets) were able to increase EPA content but not DHA as showed our results.

Differences in content of minor FA were comparing between treatments. Although its content was low compared to other FA, it was appreciated a small content of C18:1 n-9 trans, specially in tallow fed animals (T and B treatments) because this FA is typically detected in beef fats. Cis-vaccenic acid (C18:1 n-11 cis) was high in NF fed animals compared to other treatments, suggesting that this FA is from *de novo* synthesis. FA form n-6 series are synthesized by elongation of linoleic acid and FA from n-3 are synthesized from linolenic as it is demonstrated by the high contents observed in animals fed whether linoleic or linolenic acid. C20:0 is probably from *de*

novo synthesis as the highest content was observed in NF fed animals. C20:1 n-9 cis probably is synthesized by elongation of oleic acid as the highest content was observed in HOSF fed animals.

Carcass and whole-body FA compositions did not show noticeable differences and probably the most significant difference between carcass and viscera composition was that arachidonic percentage contents in viscera (Table 3) doubled the contents in carcass because of high contents observed in red viscera (unpublished data). SFA were higher in viscera than carcass, which is consistent with the assumption that degree of saturation increases as body temperature increases (Dean and Hilditch, 1933).

Carcass fat content did not show statistical differences when comparing dietary treatments (P=0.07), but in whole-body fat (as in viscera) content differences were statistically significant and showed that HOSF (26.7%) and SFO (26.7%) fed animals had higher fat deposition than T (22.9%) fed animals. This is in parallel from independent observations showing, a positive and significant correlation between carcass fat content and energy carcass content (unpublished data). In addition fat tissue dissection and fat depth were numerically lower in tallow fed compared to other fat added diets (unpublished data). Gene expression (Duran-Montgé et al.) of lipogenic enzymes also showed reduced expression in tallow fed animals. A comparable result was previously found by Mourot et al. (1991) first in pigs fed two different diets comparable in FA profile to T and SFO but in a lower fat level between 35 and 100 kg BW and second using different levels of dietary linoleic acid (Mourot *et al.*, 1994) but in these experiments carcass fat content was estimated (Desmoulin *et al.*, 1988), not measured directly as in the present experiment. Also in the work of Dugan *et al.* (2004) it was found that tallow fed barrows had less subcutaneous fat

than canola oil fed barrows. These results contrast to what was previously found in species different from pigs; Shimomura (1990) and Matsuo (2002) found that rats fed a diet rich linoleic acid had lower body fat accumulation than rats fed tallow, in chickens PUFA decreased fat deposition in separable fat depots (Crespo and Esteve-Garcia, 2002a). As suggested by Azain (2004) and Chilliard (1993) it seems that in species like rodents and poultry where the liver is the primary site of lipogenesis, unsaturated FA are more inhibitory on FA synthesis than saturated ones, in species where adipose tissue is the primary site of lipogenesis, like pigs, SFA are equivalent (or more potent) inhibiting lipogenesis than unsaturated FA. To our knowledge this is the first time of an observation in this line and gives another meaning to the pig as model for animal fat metabolism.

Balance (Table 4) was performed using the weight at the beginning of the trial and weight before slaughter, and using for estimation the digested fat and FA. Feed consumption was recorded and digested fat and FA were calculated using the results of a previous experiment (Duran-Montgé *et al.*, 2007).

As it has been demonstrated dietary fat inclusion reduces *de novo* fat synthesis (Mersmann *et al.*, 1984, Smith *et al.*, 1996). NF diet had a very low fat and FA content; therefore exogenous fat did not interfere in *de novo* fat synthesis. Results showed that the approximate ratio of FA synthesis between C16:0/C18:0/C18:1 was 1.5/1/3 agreeing with previous findings (Leat *et al.*, 1964) and the assumption/hypothesis of Lizardo *et al.* (2002).

Observing the other fat added diets is not possible to determine *de novo* fat synthesis as exogenous FA and its catabolitzation makes impossible to calculate endogenous synthesis. Dietary balances in fat added diets showed that when diet was rich in a specific FA then it was catabolitzed preferentially; T was rich in stearic acid, therefore

it shows the lowest net synthesis value, the same happens in HOSF fed animals and oleic acid, SFO and linoleic acid, LO and linolenic and FO and long PUFA (EPA, DHA and Arachidonic acid). It is noticeable the low yo no veo ninguna y tu has dicho antes que no la puedes estimar net synthesis of SFA observed in T fed animals what could be compensated with a high synthesis of oleic acid, in contrast animals fed with more unsaturated diets (HOSF, SFO, LO, B, FO) showed higher net synthesis of SFA but lower (but not significant) oleic acid net synthesis.

Observing diets were no long PUFA were added we can estimate the endogenous synthesis from theirs precursors. Delta-6-desaturase is know to be the rate limiting step of long PUFA synthesis (Huang *et al.*, 1991, Yamazaki *et al.*, 1992) and it can be regulated by dietary FA (Cho *et al.*, 1999). In previous studies with pigs it has been shown that there is little formation of DHA levels from its precursors in the diet, whereas EPA increases linearly from its precursors in diet (Blank *et al.*, 2002, James *et al.*, 2003). Other studies with human subjects also have attempted to increase DHA tissue content and they conclude that the conversion from its precursors is minimum. In the present experiment arachidonic acid was highly synthesized (59g) in animals fed SFO diet (diet rich in linoleic acid) compared to other dietary treatments and EPA was synthesized (70g) when animals were fed LO diet. Although these diets showed high net synthesis, they showed the lowest conversion rate from their eighteen-carbon precursor as the delta-6 desaturase is regulated by PUFA (Tang *et al.*, 2003). Interestingly when feeding LO diet (linolenic acid diet), DHA was not more synthesized than in other dietary treatments.

Data obtained from the balance can be transformed into Deposition Rate for linoleic and linolenic as these FA cannot be *de novo* synthesized. Different studies have attempted to determine the deposition rates or its inverse, oxidation rates, of FA

using different kinds of labeled FA in rodents and humans and they agree in that SFA are deposited to a higher extent than unsaturated FA (Jones *et al.*, 1985, Leyton *et al.*, 1987) and that the degree of deposition of FA increases as FA length increases (Bjorntor.P, 1968, Leyton *et al.*, 1987). In relation to unsaturated FA there are few disagreements and it seems that as unsaturation increases, deposition decreases (DeLany *et al.*, 2000). It appears that different factors as feeding status, dietary fat level, FA composition influence FA partitioning into deposition and oxidation (Flanzy *et al.*, 1970, Ide *et al.*, 1996, Poumes-Ballihaut *et al.*, 2001, Iritani *et al.*, 2005) and the present results suggest that diet FA composition influenced FA deposition. In pigs grown between 45 and 100 kg live weight, Flanzy (1970) measured linoleic acid deposition rates bettween 57 and 65% depending on dietary fat source. Our results on linoleic and linolenic acid deposition rates were clearly higher than in rats. Cunnane and Anderson (1997b) measured an approximate deposition of 22% of linoleic acid and Poumes-Ballihaut et al. (2001) 46% meanwhile in pig we measured values between 74% and 65% in diets rich in linoleic acid (SFO, LO, B and FO), they measured an approximate deposition of 12% and 40% respectively in linolenic acid, meanwhile in pigs we measured ratios between 64% and 63% approximately. Diets rich in PUFA showed lower PUFA deposition rates and in general deposition decreased as unsaturation degree increased (EPA ≈ DHA < linolenic < linoleic) which agrees with previous reports (Cunnane and Anderson, 1997b).

Table 3. Fatty acid profile and fat content (%) of pig viscera.

	60kg	NF	T	HOSF	SFO	LO	B	FO	StdErr
Fat content	11.2	21.0^{a}	16.8^{b}	20.3^{a}	20.6^{a}	17.4^{b}	17.0^{b}	17.7^{b}	0.82
C 14:0	1.38	1.40^{b}	1.61^{a}	1.24^{cd}	1.15^{d}	1.15^{d}	1.41^{b}	1.31^{c}	0.031
C 16:0	25.8	28.0^{a}	24.2^{b}	21.9^{d}	21.2^{d}	21.2^{d}	23.3^{bc}	22.3^{cd}	0.420
C 16:1	1.82	1.86^{a}	1.79^{a}	1.01^{c}	0.91^{c}	0.96^{c}	1.45^{b}	1.30^{b}	0.059
C 18:0	18.4	19.9^{a}	17.0^{b}	14.5^{c}	16.1^{bc}	17.3^{b}	17.0^{b}	17.4^{b}	0.566
C 18:1 n-9 trans	0.25	0.33^{a}	0.40^{a}	0.35^{a}	0.18^{b}	0.18^{b}	0.27^{ab}	0.28^{ab}	0.047
C 18:1 n-9 cis	32.9	37.0^{b}	36.2^{b}	44.8^{a}	28.3^{d}	26.9^{de}	31.7^{c}	26.3^{e}	0.63
C 18:1 n-11 cis	2.34	2.27^{a}	2.16^{b}	1.40^{d}	1.34^{d}	1.45^{d}	1.91^{c}	1.83^{c}	0.051
C 18:2 n-6	10.28	5.06^{e}	10.0^{d}	10.1^{cd}	25.8^{a}	11.9^{bc}	12.0^{b}	10.5^{bcd}	0.56
C 18:3 n-3	0.76	0.37^{f}	0.92^{d}	0.61^{e}	0.61^{e}	13.3^{a}	5.29^{c}	8.56^{b}	
C 20:0	0.27	0.27^{a}	0.21^{bc}	0.20^{c}	0.23^{bc}	0.24^{b}	0.22^{bc}	0.23^{b}	0.009
C 20:1 n-9	0.77	0.78^{a}	0.66^{b}	0.80^{a}	0.55^{c}	0.54^{c}	0.62^{b}	0.66^{b}	0.019
C 20:2 n-6	0.57	0.35^{c}	0.45^{b}	0.49^{b}	1.05^{a}	0.52^{b}	0.52^{b}	0.46^{b}	0.029
C 20:3 n-6	0.20	0.11^{b}	0.16^{a}	0.13^{ab}	0.16^{a}	0.12^{b}	0.16^{a}	0.12^{b}	0.011
C 20:4 n-6	2.01	0.94^{bc}	1.41^{a}	1.09^{bc}	1.13^{b}	0.60^{d}	0.93^{bc}	0.86^{cd}	0.089
C 20:3 n-3	0.14	0.08^{d}	0.12^{d}	0.07^{d}	0.08^{d}	1.21^{a}	0.54^{c}	0.72^{b}	0.023
C 20:5 n-3	0.09	0.05^{e}	0.12^{d}	0.06^{e}	0.06^{e}	0.64^{b}	0.37^{c}	1.39^{a}	
C 22:4 n-6	0.30	0.11^{c}	0.17^{b}	0.16^{b}	0.19^{a}	0.07^{d}	0.10^{cd}	0.10^{cd}	0.009
C 22:5 n-3	0.33	0.13^{d}	0.28^{c}	0.13^{d}	0.09^{d}	0.48^{b}	0.45^{b}	1.00^{a}	0.024
C 22:6 n-3	0.45	0.20^{d}	0.35^{b}	0.24^{cd}	0.21^{d}	0.21^{d}	0.29^{bc}	3.2^{a}	
SFA	46.7	50.2^{a}	44.0^{b}	38.4^{d}	39.2^{d}	40.6^{cd}	42.8^{bc}	42.0^{bc}	0.91
MUFA	38.1	42.4^{b}	41.9^{b}	48.6^{a}	31.3^{d}	30.1^{d}	36.3^{c}	30.6^{d}	0.71
PUFA	15.2	7.45^{d}	14.2^{c}	13.1^{c}	29.4^{a}	29.2^{a}	20.9^{b}	27.4^{a}	0.82
n-6/n-3 ratio	7.45	7.82^{c}	6.81^{d}	10.8^{b}	26.9^{a}	0.84^{f}	1.98^{e}	0.81^{f}	

Means within a row with different letters are significantly different (P<0.05).

ND: Under limit of detection

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Table 4. Fatty acid balance during pig growth from 60 to 100 kg BW (kg of FA). Disappearance ratio (%) of n-6 and n-3 FA

	NF	T	HOSF	SFO	LO	B	FO	StdErr
Balance of not essential FA (kg of FA)[1]								
C16:0	3.192^{a}	0.164^{d}	1.574^{b}	0.942^{c}	0.849^{c}	0.647^{cd}	0.750^{c}	0.170
C18:0	1.990^{a}	0.030^{d}	0.686^{bc}	0.822^{bc}	0.832^{b}	0.497^{c}	0.916^{b}	0.112
C18:1	6.018^{a}	1.920^{b}	-0.080^{c}	1.224^{b}	1.158^{b}	1.594^{b}	1.530^{b}	0.327
Balance of n-6 and n-3 FA (kg of FA)[1]								
C18:2 n-6	-0.048^{a}	-0.277^{a}	-0.550^{b}	-2.410^{d}	-1.020^{c}	-1.020^{c}	-0.820^{c}	0.079
C20:4 n-6	0.004^{d}	0.025^{c}	0.042^{b}	0.059^{a}	-0.010^{e}	-0.001^{de}	-0.044^{e}	0.003
C18:3 n-3	-0.029^{a}	-0.063^{a}	-0.083^{a}	-0.033^{a}	-2.304^{d}	-1.011^{b}	-1.529^{c}	0.090
C20:5 n-3	-0.007^{a}	-0.002^{a}	-0.014^{a}	-0.007^{a}	0.070^{a}	0.009^{a}	-1.587^{b}	0.033
C22:6 n-3	-0.005^{a}	0.007^{a}	0.002^{a}	0.021^{a}	0.015^{a}	0.002^{a}	-1.147^{b}	0.024
Deposition ratio (%) of n-6 and n-3 FA[2]								
C18:2 n-6(%)		89.1^{c}	82.0^{bc}	73.2^{abc}	64.8^{a}	66.5^{ab}	64.9^{a}	5.2
C20:4 n-6 (%)							33.6	
C18:3 n-3(%)		88.7^{bc}	99.0^{c}	106.7^{c}	64.1^{ab}	62.9^{a}	63.2^{a}	8.2
C20:5 n-3 (%)							47.9	
C22:6 n-3 (%)							48.9	

[1] Positive values in the balance mean positive net synthesis; negative values mean oxidation or negative net synthesis. Balance is calculated as detailed in Material and Methods.

[2] Deposition ratio is calculated as detailed in Material and Methods.

Means within a row with different letters are significantly different (P<0.05).

As *in vivo* and *in vitro* radiotracer methodology has shown that deposition rate of long-PUFA is high compared to other FA (Leyton *et al.*, 1987, Madsen *et al.*, 1998), Poumes-Ballihaut et al. (2001) considered deposition rates of DHA (36%) in a whole-body FA balance unexpected; similarly to this result, the present experiment and in agreement with DeLany et al. (2000) who fed humans continuously with a diet

containing PUFA and long-PUFA, deposition rate of FA decreased with degree of unsaturation. It seems that *in vitro studies* cannot reproduce exactly the nutritional status of whole animal and *in vivo* studies using bolus of radioactive tracers cannot reproduce a continuous dietary status, and for these reasons some results on long-PUFA deposition rates must be interpreted with caution. The results of the present experiment suggest that diets with low content in a given FA showed higher deposition differences between animals and finally the estimates had higher standard errors; this could explain the values above 100% observed in linolenic-SFO (106.7% of deposition). As observed by Cunnane and Anderson (1997a) it seems that linoleic acid is more preserved in linoleic-deficient rats than in the fat added diets. Interestingly, in T fed animals linoleic and linolenic acid deposition rates were higher than in diets with a high content in these FA. It seems that when animals are fed low contents in essential FA these are deposition (not oxidized) and when the level is high then they are metabolized. It is important to notice that although T showed higher deposition rates of PUFA than diets rich in these FA, it showed lower net fat synthesis and also the lowest whole animal fat content. With all the presented data we hypothesize that variation in the FA balance and the whole animal fat content caused by dietary fat is mainly due lipogenesis, not due oxidation. That could explain the previously commented species differences in fat metabolism (Azain, 2004).

CONCLUSIONS

Under the conditions of the experiment viscera and carcass composition reflect dietary fat treatments. Dietary tallow lowers whole animal fat content as compared to sunflower oil and high oleic sunflower oil, in parallel with lower expression of

lipogenic enzymes in adipose tissue (Duran-Montgé *et al.*, 2008b) and reduced triiodothyronine contents in blood (Duran-Montgé *et al.*, 2008a).

When the diet contains very little fat, ratio of endogenous FA synthesis is 1’5:1:3 (palmitic,stearic and oleic acids) and when fat is added this values vary depending on FA diet composition. Deposition rate of essential FA seems to decrease with FA unsaturation. When no EPA and DHA were added to diet, high linolenic acid contents in diet just can increase EPA whole animal contents, but not DHA.

REFERENCES

Allen KGD and Harris MA 2001. The role of n-3 fatty acids in gestation and parturition. Experimental Biology and Medicine 226, 498-506.

Azain MJ 2004. Role of fatty acids in adipocyte growth and development. Journal of Animal Science 82, 916-924.

Bazan NG 2003. Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor. Journal of Lipid Research 44, 2221-2233.

Bazinet RP, McMillan EG, Seebaransingh R, Hayes AM and Cunnane SC 2003. Whole-body beta-oxidation of 18 : 2 omega 6 and 18 : 3 omega 3 in the pig varies markedly with weaning strategy and dietary 18 : 3 omega 3. Journal of Lipid Research 44, 314-319.

Birch EE, Hoffman DR, Uauy R, Birch DG and Prestidge C 1998. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatric Research 44, 201-209.

Bjorntor.P 1968. Rates of Oxidation of Different Fatty Acids by Isolated Rat Liver Mitochondria. Journal of Biological Chemistry 243, 2130-&.

Blank C, Neumann MA, Makrides M and Gibson RA 2002. Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio. Journal of Lipid Research 43, 1537-1543.

Crespo N and Esteve-Garcia E 2002a. Dietary polyunsaturated fatty acids decrease fat deposition in separable fat depots but no in the remainder carcass. Poultry Science 81:512-518.

Crespo N and Esteve-Garcia E 2002b. Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. Poultry Science 81:1533-1542.

Cunnane SC and Anderson MJ 1997a. Pure linoleate deficiency in the rat: Influence on growth, accumulation of n-6 polyunsaturates, and [1-C-14]linoleate oxidation. Journal of Lipid Research 38, 805-812.

Cunnane SC and Anderson MJ 1997b. The majority of dietary linoleate in growing rats is beta-oxidized or stored in visceral fat. Journal of Nutrition 127, 146-152.

Chilliard Y 1993. Dietary-fat and adipose-tissue metabolism in ruminants, pigs, and rodents - a Review. Journal of Dairy Science 76, 3897-3931.

Cho HP, Nakamura M and Clarke SD 1999. Cloning, expression, and fatty acid regulation of the human Delta-5 desaturase. Journal of biological chemistry 274, 37335-37339.

Chwalibog A and Thorbek G 1995. Quantitative partition of protein carbohydrate and fat pools in growing pigs. Archives of Animal Nutrition 48, 53-61.

Dean HK and Hilditch TP 1933. The body fats of the pig. III. The influence of body temperature on the composition of depot fats. Biochemical Journal 27, 1950-1961.

DeLany JP, Windhauser MM, Champagne CM and Bray GA 2000. Differential oxidation of individual dietary fatty acids in humans. American Journal of Clinical Nutrition 72, 905-911.

Desmoulin B, Ecolan P and Bonneau M 1988. Estimation de la composition tissulaire des carcasses de porcs. INRA Prod. Anim. 1, 59-64.

Dugan MER, Aalhus JL, Roertson WM, Rolland DC and Larsen IL 2004. Practical dietary levels of canola oil and tallow have differing effects on gilt and barrow performance and carcass composition. Canadian Journal of Animal Science 84, 661-671.

Duran-Montgé P, Lizardo R, Torrallardona D and Esteve-Garcia E 2007. Fat and fatty acid digestibility of diffrent fat sources in growing pigs. Livestock Science.

Duran-Montgé P, Theil P, Lauridsen C and Esteve-Garcia E 2008a. Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulation factors in liver and adipose tissue but not in semimembranosus muscle of pigs during the fattening period. Animal (unpublished work).

Duran-Montgé P, Theil P, Lauridsen C and Esteve-Garcia E 2008b. Dietary fat source and level affect metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. Animal (unpublished work).

Flanzy J, François AC and Rérat A 1970. Utilisation metabolique des acides gras chez le porc. Ann. Biol. Bioch. Biophys. 10 (4):603-620.

Folch J, Lees M and Stanley GHS 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry 226, 497-509.

Hardman WE 2002. Omega-3 fatty acids to augment cancer therapy. Journal of Nutrition 132, 3508-3512.

Huang YS, Smith RS, Redden PR, Cantrill RC and Horrobin DF 1991. Modification of liver fatty-acid metabolism in mice by n-3 and n-6 Delta-6-Desaturase substrates and products. Biochimica et Biophysica Acta 1082, 319-327.

Ide T, Murata M and Sugano M 1996. Stimulation of the activities of hepatic fatty acid oxidation enzymes by dietary fat rich in alpha-linolenic acid in rats. Journal of Lipid Research 37, 448-463.

Iritani N, Kimura T, Fukuda H and Sugimoto T 2005. Differences in labelled triolein turnover after oral administration between liver and adipose tissue of rats. British Journal of nutrition 93, 53-58.

James MJ, Ursin VM and Cleland LG 2003. Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. American Journal of Clinical Nutrition 77, 1140-1145.

Jones PJH, Pencharz PB and Clandinin MT 1985. Whole-body oxidation of dietary fatty-acids - Implications for energy-utilization. American Journal of Clinical Nutrition 42, 769-777.

Jump DB and Clarke SD 1999. Regulation of gene expression by dietary fat. Annual Review of Nutrition 19, 63-90.

Kloareg M, Noblet J and Van Milgen J 2006. Estimation of whole body lipid mass in finishing pigs. Animal Science 82, 241-251.

Leat WMF, Cuthbert.A, Howard AN and Gresham GA 1964. Studies on pigs reared on semi-synthetic diets containing no fat beef tallow and maize oil - Composition of

carcass and fatty acid composition of various depot fats. Journal of Agricultural Science 63, 311-&.

Leyton J, Drury PJ and Crawford MA 1987. Differential oxidation of saturated and unsaturated fatty-acids in vivo in the rat. British Journal of nutrition 57, 383-393.

Lizardo R, Milgen Jv, Mourot J, Noblet J and Bonneau M 2002. A nutritional model of fatty acid composition in the growing-finishing pig. Livestock Production Science 75, 167-182.

Madsen L, Froyland L, Dyroy E, Helland K and Berge RK 1998. Docosahexaenoic and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation. Journal of Lipid Research 39, 583-593.

Matsuo T, Takeuchi H, Suzuki H and Suzuki M 2002. Body fat accumulation is greater in rats fed a beef tallow diet than in rats fed a safflower or soybean oil diet. Asia Pacific Journal of Clinical Nutrition 11, 302-308.

Mersmann HJ, Pond WG and Yen JT 1984. Use of carbohydrate and fat as energy source by obese and lean swine. Journal of Animal Science 58, 894901.

Metz SHM and Dekker RA 1981. The contribution of fat mobilization to the regulation of fat deposition in growing large white and piétrain pigs. Animal Production 33, 149-157.

Miles EA and Calder PC 1998. Modulation of immune function by dietary fatty acids. Proceedings of the nutrition society 57, 277-292.

Morrison WR and Smith LM 1964. Preparation of fatty acid methyl esters + dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5, 600-&.

Mourot J, Peiniau P and Mounier A 1994. Effect of dietary linoleic-acid on lipogenesis in adipose-tissue of pig. Reproduction Nutrition Development 34, 213-220.

Mourot J, Chauvel J, Le Denmat M, Mounier A and Peiniau P 1991. Linoleic acid level in the diet of pigs: effects on fat deposition and on meat linoleic acid oxydation during storage. Journées Rech. Porcine en France 23, 357-364.

Murphy BD 2006. Stable isotope methods for the in vivo measurement of lipogenesis and triglyceride metabolism. Journal of Animal Science 84, E94-E104.

Poumes-Ballihaut C, Langelier B, Houlier F, Alessandri JM, Durand G, Latge C and Guesnet P 2001. Comparative bioavailability of dietary alpha-linolenic and docosahexaenoic acids in the growing rat. Lipids 36, 793-800.

Shimomura Y, Tamura T and Suzuki M 1990. Less body-fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. Journal of Nutrition 120, 1291-1296.

Smith DR, Knabe DA and Smith TB 1996. Depression of lipogenesis in swine adipose tissue by specific dietary fatty acids. Journal of Animal Science 74, 975-983.

Tang CR, Cho HP, Nakamura MT and Clarke SD 2003. Regulation of human Delta-6 desaturase gene transcription: identification of a functional direct repeat-1 element. Journal of Lipid Research 44, 686-695.

Wijendran V and Hayes KC 2004. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annual Review of Nutrition 24, 597-615.

Yamazaki K, Fujikawa M, Hamazaki T, Yano S and Shono T 1992. Comparison of the conversion rates of alpha-linolenic acid (18-3(n-3)) and stearidonic acid (18-4(n-3)) to longer polyunsaturated fatty-acids in rats. Biochimica et Biophysica Acta 1123, 18-26.

DISCUSSION

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The main objective of the thesis project was to study the effect of type of dietary fat on pig fat deposition. In order to know the amount of digestible fat administered to the pigs, and to adjust the formulation of the experimental diets to the same level of digestible fat, a balance trial was performed to measure ileal and faecal digestibilities. The next step was to perform the growing trial in which the animals from the *de novo* fat synthesis trial and the animals from the fat fed trial were grown between 60 kg LW and 100 kg LW. In the first group of animals the diet was composed of ingredients with very low or null fat content, and for the group of animals fed diets rich in different fats, the same fats from the digestibility trial were used, and also an additional treatment was added, the fish oil blend.

Paper 1: Ileal and faecal fat digestibility

As described in the paper, digestibility results were consistent with previous studies. The necessity for this study was to obtain digestibility values with the used fat sourses and with similar levels in order to obtain reliable results for the next trials from this PhD thesis. Although results from the ileal and faecal digestibility trials were consistent, maybe other digestibility protocols (p.e. total faeces collection, T-canula) would improve the precision of the experiment. because the anastomosis procedure requires two different trials for the ileal and faecal collections, adding another source of variability when comparing ileal and faecal results. In addition the use of markers adds another source of error in the analytical determination of the marker, and the variability in the distribution of the marker in the chyme. It is interesting to note that both Folch fat extraction method (Folch *et al.*, 1957) and the Soxhlet method with previous acid hydrolysis (AOAC, 1990) were used in the faecal experiment (the first

method was not published). Comparison of results showed that they were similar, but more variability was observed with the Folch method. Also, the higher variability observed in the Folch method, probably was due a lower sample amount used for the analysis, therefore increasing the error.

Papers 2,3,4,5,6: Effects of dietary fatty acid composition on pig fat and fatty acid depositon and de novo fat synthesis

For this trial, that included the *de novo* experiment and the different fats fed experiment , a Duroc x Landrace breed fed high amounts of fat was used. Duroc is known to show high intramuscular fat deposition. The reason of using Duroc genetics was to increase the amount of intramuscular fat in order to improve the response in this important parameter. This was also the reason for using diets with a high fat content to maximize treatment differences. Still considerable variability was observed, possibly due to differences in the genetic origin of the experimental animals, which were of different progenies.

In the performance results, no differences were observed between animals fed different fats but a poorer efficiency in feed conversion index was observed in NF fed animals compared to other fat added treatments.

Pig FA composition

In analytical procedures for the growing trial, both extraction methods (Folch and Soxhlet) were used (the latest not shown) as in the digestibility experiment. With the extraction by the Soxhlet method, we observed that animals fed diets rich in PUFA showed lower carcass fat content. We first thought the difference was real: lower fat

deposition in animals fed PUFA; however, subsequent analysis showed that it was the extraction by Soxhlet, that probably did not completely extract PUFA fats (more polar). These conclusions were derived from the observation that results with the Folch method differed from those of the Soxhlet extraction, and second and important, the sum of the different carcass components (water, fat, protein and ashes) was considerably short of 100% in animals fed PUFA. Therefore, the extraction for meat was the Folch method, which is the commonly used for FA analysis.

As diets had extreme FA compositions, these differences were finally observed in tissue sample composition (intramuscular fat, subcutaneous fat, intermuscular fat, flare fat, liver, viscerae and carcass). Differences between tissues were the mentioned in previous studies (Brooks, 1971, Eder *et al.*, 2001), but in the present experiment were extensively studied in different pig parts (ham, belly and loin). As suggested in this work, tissue differences could have two explanations; first that the increase in saturation of the fat goes in parallel with the increase in body temperature (Monziols *et al.*, 2007), and second FA composition depends on tissue function in the organism (liver as the main site for long-PUFA synthesis, subcutaneous fat as a reservoir organ and in muscles fat works as structural fat in cell membrane).

Although fat showed differences between tissues, differences between treatments were more important by far. Carcass FA composition showed extreme values due extreme diet FA compositions. Animals fed with the no fat diet (NF) showed the lowest PUFA content and that the synthesis of *de novo* FA is aproximately 1.5/1/3 for palmitic, stearic and oleic acids as observed in previous studies (Kloareg *et al.*, 2007). These values are probably the lowest found in bibliography observed in pigs rendered in commercial conditions. Animals fed high oleic sunflower oil showed the

highest contents in oleic acid. Pigs fed diets rich in linoleic and linolenic, showed high tissue contents in these FA, In high PUFA fed pigs it was possible to observe high deposition levels of these essential FA in meat, carcass or whole animal. Also it is interesting to point out that our results showed that when PUFA are added at low levels (0-1% diet) its deposition in the whole animal is high whereas when it is added at higher levels they are extensively catabolized and that this oxidation would depend on their unsaturation level. This last observation also was corroborated by the oxidation results of fish oil fed pigs. Diets rich in PUFA (lionoleic and linolenic) also allowed the determination of endogenous long-PUFA synthesis from their precursor. These results showed that DHA levels are not increased in meat by increasing the levels of its precursors. Fish oil blend treatment was characterized by showing an extreme FA composition (diet with a high content in polyunsaturated fatty acids).

It is interesting to note that the fat of NF fed pigs was very consistent in texture. Whereas FA compositions of animals fed HOSF diet showed values that could be interesting for cured meat products (although it had slightly high PUFA contents), animals fed diets rich in PUFA were not practical for meat products.

Pig fat content

As it was mentioned before, the main objective of this study was to determine if the type of dietary fat would modify the amount of deposited fat. The answer to this question is affirmative,although different from what has been observed in rodents and birds: Several parameters support this hypothesis: First results showed a lower (numerically but not statistically) fat depth measured at the last rib and between the 3rd and 4th ribs in animals fed beef tallow, suggestive of lower fat content. Second, results from carcass fat content showed again that animals fed with tallow had a

tendency (not significant) in having a lower fat content. Finally when we obtained the results of viscerae fat content, the tendencies observed in carcass became significant at whole animal level; therefore we had more reliable results that were also confirmed by the gene expression analysis (lower expression of lipogenic genes) and the dissection also showed a lower (not significant) amount of dissected fat in tallow fed pigs.

We did not find a response in the amount of intramuscular fat. A high variability was observed in the results of this parameter.

Observations on lipid metabolism modifications

Results on gene expression clearly showed differences between liver and adipose tissue, and its specific functions: Adipose tissue as the main site for *de novo* FA synthesis, and liver as the main site for synthesis of long chain polyunsaturated FA from its precursors.

Results on thyroid hormones also were correlated with the amounts of deposited fat due type of dietary fat and with animals at different weight.

Also a wide range of blood parameters were analyzed (insulin, glucose, triglycerides, non esterified FA, lipoproteins …) giving no noticeable results, in part due the high inter-animal variability.

One explanation to the observed differences between pig, and bird and rodents metabolism is the site of *de novo* fat synthesis. The amount of SREBP1 seems to be modified in pigs, rodents and birds by dietary FA composition in liver, the main site for *de novo* synthesis, but not in pig. Whereas pig adipose tissue, seems refractive to SREBP1 interference by dietary FA composition and therefore the effects observed

in birds and rodents are distinct from that in pigs. In pigs, the effects observed on SREBP1 just would lead to a regulation in the synthesis of long chain PUFA.

The present work showed that saturated FA decrease pig fat deposition by a reduction in expression of lipogenic enzymes but can not explain the molecular basis of this observation, for this reason, further studies are needed to uderstand why saturated FA and not PUFA descrease fat deposition.

CONCLUSIONS

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Measuring ileal digestibility of individual fatty acids rather than faecal should give a more precise estimation of the true availability of fatty acids presumably due to biohydrogenation which may take place in large intestine.
Individual fatty acid digestibility increases with increasing unsaturation, and decreases with fatty acids length.

Dietary fat caused effects on animal performance, as shown by the differences in the feed to gain ratio between animals fed no fat and those fed high level of fat: Differences between fats, although not significant in this experiment, could be important in practical conditions, especially in diets containing high levels of n-3 fatty acids.

Although there were no important treatment differences in fat depots and lean weights, it is interesting to observe that the diet containing a very low level of fat did not reduce fat deposition, and in some fat depots increased the fat content compared to other fat added diets.

A pattern in tissue fatty acid distribution was observed as saturated fatty acids were preferentially deposited in the inner fat depots compared to more external fat depots. Intramuscular fat was less susceptible to modification by the composition of dietary fat.

When the diet contains very little fat, ratio of endogenous fatty acid synthesis is 1’5:1:3 (palmitic, stearic and oleic acids) and when fat is added this values vary depending on fatty acid diet composition:
When palmitic and stearic acid, or oleic acids are added to diet, these fatty acids are increased in tissue contents, but the most noticeable differences are observed when PUFA are included to diet (linoleic, linolenic acids, EPA and DHA).

When PUFA were supplemented to diet, saturated fatty acids are less susceptible to modification than MUFA because PUFA tend to replace MUFA when is deposited.

Linolenic acid conversion to EPA was rather low and almost negligible for DHA, meaning that the only effective way to increase DHA tissue contents is by its direct inclusion to diet.

Deposition rate of very long chain PUFA seems to decrease with unsaturation degree as suggested by the lower deposition of DHA and EPA compared to PUFA.

Net synthesis and degradation of fatty acids is modified by dietary fat composition, as to compensate an unbalanced dietary fatty acid composition. .

Dietary fatty acid profile modifies mRNA abundances of genes encoding lipogenic enzymes and these dietary effects are tissue specific.

In liver Fish oil diet rich in long-PUFA decreased mRNA of lipogenic genes whereas in adipose tissue, the main reductions in lipogenic enzyme mRNA's were observed in pigs fed Tallow, which is rich in saturated fatty acids.

Changes in mRNA abundances of genes related to lipogenesis in liver and adipose tissue could be partly driven by T3 hormone signaling, as suggested by the correlations observed between hormones and genes of fat synthesis (Stearoyl CoA desaturase, Acetyl CoA carboxilase, Fatty acid synthase) in animals fed different diets and animals at different weights.

Age and tissue specificity showed larger differences in values of mRNA abundance of genes related to lipid metabolism than did fatty acid composition of diets and tissues, and probably these differences are due their tissue specific function: Adipose tissue is the organ for *de novo* fatty acid synthesis in pig, and the liver is the primary organ for long-PUFA synthesis.

The relative importance of liver and adipose tissue changes as the animal grows as demonstrated by the changes in gene expression at different weights.

Dietary tallow lowers whole animal fat content as compared to sunflower oil and high oleic sunflower oil, in parallel with lower expression of lipogenic enzymes in adipose tissue and reduced triiodothyronine contents in blood. This suggests that the level of this hormone is affected by the composition of dietary fat, and in turn may be one of the signals regulating fat deposition. Comparing the fat deposition of pigs fed very little fat and those fed tallow or n-3 fatty acids, it is suggested that some fatty acids may affect fat deposition.

CONCLUSIONS

CITED REFERENCES

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REFERENCES

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Althen TG and Gerrits RJ 1976. Pituitary and Serum Growth Hormone Levels in Duroc and Yorkshire Swine Genetically selected for High and Low Backfat. Journal Animal Science 42, 1490-1497.

Allee GL, Baker DH and Leveille GA 1971a. Influence of Level of Dietary Fat on Adipose Tissue Lipogenesis and Enzymatic Activity in the Pig. Journal Animal Science 33, 1248-1254.

Allee GL, Baker DH and Leveille GA 1971b. Influence of level of dietary fat on adipose tissue lipogenesis and enzymatic activity in pig. Journal of Animal Science 33, 1248-&.

Allen CE, Beitz DC, Cramer DA and Kauffman RG 1976. Biology of fat in meat animals. North Central Regional Research Publication No. 234, University of Wisconsin, Madison.

Allen KGD and Harris MA 2001. The role of n-3 fatty acids in gestation and parturition. Experimental Biology and Medicine 226, 498-506.

Amri EZ, Teboul L, Vannier C, Grimaldi PA and Ailhaud G 1996. Fatty acids regulate the expression of lipoprotein lipase gene and activity in preadipose and adipose cells. Biochemical Journal 314, 541-546.

AOAC 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Washinton DC.

Averette Gatlin L, See MT and Odle J 2005. Effects of chemical hydrogenation of supplemental fat on relative apparent lipid digestibility in finishing swine. Journal of Animal Science 83, 1890-1898.

Barton-Gade PA 1987. Meat and fat quality in boars, castrates and gilts. Livestock Production Science 16, 187-196.

Bazan NG 2003. Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor. Journal of Lipid Research 44, 2221-2233.

Bee G, Gebert S and Messikommer R 2002. Effect of dietary energy supply and fat source on the fatty acid pattern of adipose and lean tissues and lipogenesis in the pig. Journal Animal Science 80:1564-1574.

Bellinger DA, Merricks EP and Nichols TC 2006. Swine models of type 2 diabetes mellitus: Insulin resistance, glucose tolerance, and cardiovascular complications. Ilar Journal 47, 243-258.

Bergen WG and Mersmann HJ 2005. Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. Journal of Nutrition 135, 2499-2502.

Birch EE, Hoffman DR, Uauy R, Birch DG and Prestidge C 1998. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatric Research 44, 201-209.

Bjorntor.P 1968. Rates of Oxidation of Different Fatty Acids by Isolated Rat Liver Mitochondria. Journal of biological chemistry 243, 2130-&.

Brindley DN 1984. Digestion, absorption and transport of fats: General principles. Fats in Animal Nutrition, Butterworths, 85-103.

Brooks CC 1971. Fatty acid composition of pork lipids as affected by basal diet, fat source and fat level. Journal Animal Science 33, 1224-1231.

Brown MS and Goldstein JL 1997. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89, 331-340.

Burdge GC and Calder PC 2005. Conversion of alfa-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reproduction Nutrition Development 45, 581-597.

Buttery PJ 1983. Hormonal-Control of Protein Deposition in Animals. Proceedings of the nutrition society 42, 137-148.

Cameron ND and Enser MB 1991. Fatty acid composition of lipid in Longissimus dorsi muscle of Duroc and British Landrace pigs and its relationship with eating quality. Meat Science 29, 295-307.

Campo MM, Nute GR, Wood JD, Elmore SJ, Mottram DS and Enser M 2003. Modelling the effect of fatty acids in odour development of cooked meat in vitro: part I--sensory perception. Meat Science 63, 367-375.

Cera KR, Mahan DC and Reinhart GA 1989. Apparent fat digestibilities and performance responses of postweaning swine fed diets supplemented with coconut oil, corn oil or tallow. Journal Animal and Feed Sciences 67, 2040-2047.

Courboulay V, Riaublanc A, Gandemer G, Davenel A, Granier R and Bouyssière M 1999. Dietary fatty acid and carcass lean content. What effect on the quality of backfat? In Journées Rech. Porcine en France, pp. 287-299.

Crespo N and Esteve-Garcia E 2001. Dietary Fatty Acid Modifies Abdominal Fat Deposition in Broiler Chickens. Poultry Science 80:71-78.

Crespo N and Esteve-Garcia E 2002a. Dietary polyunsaturated fatty acids decrease fat deposition in separable fat depots but no in the remainder carcass. Poultry Science 81:512-518.

Crespo N and Esteve-Garcia E 2002b. Dietary Linseed oil produces lower Abdominal fat deposition but higher de novo fatty acid synthesis in broiler chickens. Poultry Science 81:1555-1562.

Chwalibog A and Thorbek G 1995. Quantitative partition of protein carbohydrate and fat pools in growing pigs. Archives of Animal Nutrition 48, 53-61.

Chwalibog A, Jakobsen K, Henckel S and Thorbek G 1992. Estimation of quantitative oxidation and fat retention from carbohydrate, protein and fat in growing pigs. J. Anim. Physiol. a. Anim. Nutr. 68, 123-135.

DeLany JP, Windhauser MM, Champagne CM and Bray GA 2000. Differential oxidation of individual dietary fatty acids in humans. American Journal of Clinical Nutrition 72, 905-911.

Desvergne B and Wahli W 1999. Peroxisome proliferator-activated receptors: Nuclear control of metabolism. Endocrine Reviews 20, 649-688.

Dhe-Paganon S, Duda K, Iwamoto M, Chi Y-I and Shoelson SE 2002. Crystal Structure of the HNF4alpha Ligand Binding Domain in Complex with Endogenous Fatty Acid Ligand. J. Biol. Chem. 277, 37973-37976.

Dietschy JM 1998. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. Journal of Nutrition 128, 444S-448S.

Ding ST, Schinckel AP, Weber TE and Mersmann HJ 2000. Expression of porcine transcription factors and genes related to fatty acid metabolism in different tissues and genetic populations. Journal of Animal Science 78, 2127-2134.

Dozin B, Magnuson MA and Nikodem VM 1986. Thyroid hormone regulation of malic enzyme synthesis. Dual tissue- specific control. J. Biol. Chem. 261, 10290-10292.

Eder K, Nonn H and Kluge H 2001. The fatty acid composition of lipids from muscle and adipose tissues of pigs fed various oil mixtures differing in their ratio between oleic acid and linoleic acid. European Journal of Lipid Science and Technology 103, 668-676.

Eeckhout W and De Paepe M 1988. La digestibilité de l'huile de soja et/ou suif présents en diverses proportions et divers pourcentages d'incorporation dans un aliment simple pour porcelets / Soybean oil digestibility on diferent dietary compositions in piglets. Revue de l'Agriculture Brussells 41, 1455-1465.

Enser M 1983. Workshop CEC Progr. Coord. Res. Anim. Husb. In Brussels, p. 53.

Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH, Murphy RC, Raetz CRH, Russell DW, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze MS, White SH, Witztum JL and Dennis EA 2005. A comprehensive classification system for lipids. European Journal of Lipid Science and Technology 107, 337-364.

Faidley TD, Luhman CM, Galloway ST, Foley MK and Beitz DC 1990. Effect of Dietary-Fat Source on Lipoprotein Composition and Plasma-Lipid Concentrations in Pigs. Journal of Nutrition 120, 1126-1133.

Flanzy J, François AC and Rérat A 1970. Utilisation metabolique des acides gras chez le porc. Ann. Biol. Bioch. Biophys. 10 (4):603-620.

Folch J, Lees M and Stanley GHS 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry 226, 497-509.

Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard C, le Liepvre X, Berthelier-Lubrano C, Spiegelman B, Kim JB, Ferre P and Foufelle F 1999. ADD1/SREBP-1c Is Required in the Activation of Hepatic Lipogenic Gene Expression by Glucose. Mol. Cell. Biol. 19, 3760-3768.

Freeman CP 1984. The digestion, absorption and transport of fats - Non-ruminants. Fats in Animal Nutrition, Butterworths, 105-122.

Frick F, Oscarsson J, Vikman-Adolfsson K, Ottosson M, Yoshida N and Eden S 2000. Different effects of IGF-I on insulin-stimulated glucose uptake in adipose tissue and skeletal muscle. Am J Physiol Endocrinol Metab 278, E729-737.

Goldberg IJ and Merkel M 2001. Lipoprotein lipase: Physiology, biochemistry, and molecular biology. Frontiers in Bioscience 6, D388-D405.

Gondret F, Ferre P and Dugail I 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. Journal of Lipid Research 42, 106-113.

Griffin HD and Hermier D 1988. Plasma lipoprotein metabolism and fattening in poultry. In:Leaness in domestic birds. Butterworths, London.

Gurr MI 1984. Role of fats in food and Nutrition. Elsevier, London and New York.

Hardman WE 2002. Omega-3 fatty acids to augment cancer therapy. Journal of Nutrition 132, 3508-3512.

Hausman GJ and Martin RJ 1989. The influence of human growth hormone on preadipocyte development in serum-supplemented and serum-free cultures of stromal-vascular cells from pig adipose tissue. Domestic Animal Endocrinology 6, 331-337.

Hausman GJ and Hausman DB 1993. Endocrine regulation of porcine adipose tissue development: Cellular and metabolic aspects. In Growth of the pig. CAB International, Illinois.

Hayhurst GP, Lee YH, Lambert G, Ward JM and Gonzalez FJ 2001. Hepatocyte nuclear factor 4 alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. Molecular and Cellular Biology 21, 1393-1403.

Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E and Smith SR 2004. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. Journal of Clinical Endocrinology and Metabolism 89, 1844-1848.

Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T and Matsuzawa Y 2000. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arteriosclerosis Thrombosis and Vascular Biology 20, 1595-1599.

Hoz L, Lopez-Bote CJ, Cambero MI, D'Arrigo M, Pin C, Santos C and Ordonez JA 2003. Effect of dietary linseed oil and [alpha]-tocopherol on pork tenderloin (Psoas major) muscle. Meat Science 65, 1039-1044.

Hsu JM, Wang PH, Liu BH and Ding ST 2004. The effect of dietary docosahexaenoic acid on the expression of porcine lipid metabolism-related genes. Journal of Animal Science 82, 683-689.

Ide T, Murata M and Sugano M 1996. Stimulation of the activities of hepatic fatty acid oxidation enzymes by dietary fat rich in alpha-linolenic acid in rats. Journal of Lipid Research 37, 448-463.

Iritani N, Kimura T, Fukuda H and Sugimoto T 2005. Differences in labelled triolein turnover after oral administration between liver and adipose tissue of rats. British Journal of nutrition 93, 53-58.

Isabel B, Lopez-Bote CJ, de la Hoz L, Timon M, Garcia C and Ruiz J 2003. Effects of feeding elevated concentrations of monounsaturated fatty acids and vitamin E to swine on characteristics of dry cured hams. Meat Science 64, 475-482.

Jacobi S, Gabler N and Spurlock M 2006. Adiponectin regulates the expression of genes associated with fatty acid synthesis and oxidation in primary pig adipocytes. FASEB J. 20, A167-d-168.

Jones PJH, Pencharz PB and Clandinin MT 1985. Whole-body oxidation of dietary fatty-acids - Implications for energy-utilization. American Journal of Clinical Nutrition 42, 769-777.

Jorgensen H and Just A 1988. Effect of different dietary components on site of absorption/site of disappearance of nutrients. In Digestive physiology in the pig. EAAP, Jablona, pp. 230-239.

Jorgensen H and Fernández JA 2000. Chemical composition and energy value of different fat sources for growing pigs. Acta Agriculturae Scandinavica Section a Animal Science 50, 129-136.

Jorgensen H, Jakobsen K and Eggum BO 1992. Excretion of biliary fat and fatty acids in growing pigs. Journal Animal and Feed Sciences 1, 139-149.

Jorgensen H, Jakobsen K and Eggum BO 1993. Determination of endogenous fat and fatty acids at the terminal ileum and on faeces in growing pigs. Acta Agriculturae Scandinavica Section a Animal Science 43, 101-106.

Jorgensen H, Jensen SK and Eggum BO 1996. The influence of rapeseed oil on digestibility, energy metabolism and tissue fatty acid composition in pigs. Acta Agriculturae Scandinavica Section a Animal Science 46, 65-75.

Jorgensen H, Gabert VM, Hedemann MS and Jensen SK 2000. Digestion of fat does not differ in growing pigs fed diets conteining fish oil, rapeseed oil or coconut oil. Journal of Nutrition 130, 852-857.

Jump DB 2002. Dietary polyunsaturated fatty acids and regulation of gene transcription. Current opinion in lipidology 13, 155-164.

Jump DB and Clarke SD 1999. Regulation of gene expression by dietary fat. Annual Review of Nutrition 19, 63-90.

Jump DB, Botolin D, Wang Y, Xu JH, Christian B and Demeure O 2005. Fatty acid regulation of hepatic gene transcription. Journal of Nutrition 135, 2503-2506.

Just A 1982. The influence of crude fiber from cereals on the net energy value of diets for growth in pigs. Livestock Production Science 9, 569-580.

Karp PD 2007. BioCyc Database Collection. In SRI International.

Kiwaki K and Levine JA 2003. Differential effects of adrenocorticotropic hormone on human and mouse adipose tissue. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 173, 675-678.

Kloareg M, Noblet J and van Milgen J 2007. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. British Journal of nutrition 97, 35-44.

Köhler T, Mosenthin R, Hartog LAd, Huisman J and Verstegen MWA 1991. Adaptative effects of ileo-rectal anastomosis on digestion in pigs. Digestive physiology in the pig. EAAP. Wageningen, 349-355.

Kris-Etherton PM and Etherton TD 1982. The Role of Lipoproteins in Lipid Metabolism of Meat Animals. Journal Animal Science 55, 804-817.

Kris-Etherton PM and Yu S 1997. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr 65, 1628S-1644.

Laplace JP, Corring T, Rérat A and Demarme Y 1986. Le porc et son élevage, bases scientifiques et techniques. Maloine, Paris.

Larick DK and Turner BE 1990. Flavor Characteristics of Forage- and Grain-Fed Beef as Influenced by Phospholipid and Fatty Acid Compositional Differences. Journal of Food Science 55, 312-317.

LaRosa JC, Levy RI, Herbert P, Lux SE and Fredrickson DS 1970. A specific apoprotein activator for lipoprotein lipase. Biochemical and Biophysical Research Communications 41, 57-62.

Lauridsen C, Nielsen JH, Henckel P and Sorensen MT 1999. Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E, and copper. Journal Animal Science 77, 105-115.

Le Goff G and Noblet J 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. Journal of Animal Science 79, 2418-2427.

Leat WMF, Cuthbert.A, Howard AN and Gresham GA 1964. Studies on pigs reared on semi-synthetic diets containing no fat beef tallow and maize oil - Composition of carcass and fatty acid composition of various depot fats. Journal of Agricultural Science 63, 311-&.

Leyton J, Drury PJ and Crawford MA 1987. Differential oxidation of saturated and unsaturated fatty-acids in vivo in the rat. British Journal of nutrition 57, 383-393.

Li S and Sauer WC 1994. The effect of dietary fat content on amino acid digestibility in young pigs. Journal of Animal Science 72, 1737-1743.

Lister D 1976. Effects of Nutrition and Genetics on Composition of Body. Proceedings of the nutrition society 35, 351-356.

Liu BH, Wang YC, Kuo CF, Cheng WM, Shen TF and Ding ST 2005. The effects of docosahexaenoic acid oil and soybean oil on the expression of lipid metabolism related mRNA in pigs. Asian-Australasian Journal of Animal Sciences 18, 1451-1456.

Lowe JB and Sacchettini JC 1987. Expression of rat intestinal fatty acid-binding protein in E coli. Journal of biological chemistry 262, 5931-5937.

Luhman CM, Faidley TD and Beitz DC 1992. Postprandial Lipoprotein Composition in Pigs Fed Diets Differing in Type and Amount of Dietary-Fat. Journal of Nutrition 122, 120-127.

Madsen A, Jakobsen K and Mortensen HP 1992. Influence of dietary fat on carcass fat quality in pigs. Acta Agric. Scand. Anim Sci. 42:120-225.

Mathews CK, van Holde KE and Ahern KG 2002. Bioquímica. Pearson Educación, Madrid.

McDonald P, Edwards RA and Greenhalgh JFD 1979. Valoración de los alimentos: (A) Digestibilidad. Nutrición animal, Ed. ACRIBIA, 184-197.

Merkel M, Eckel RH and Goldberg IJ 2002. Lipoprotein lipase: genetics, lipid uptake, and regulation. Journal of Lipid Research 43, 1997-2006.

Mersmann HJ 1986. Swine in Cardiovascular disease. CRC Press, Inc., Boca Raton, Florida.

Mersmann HJ 1995. Species Variation in Mechanisms for Modulation of Growth by Beta-Adrenergic Receptors. Journal of Nutrition 125, 1777S-1782.

Mersmann HJ, Pond WG and Yen JT 1984. Use of carbohydrate and fat as energy source by obese and lean swine. Journal of Animal Science 58, 894901.

Meunier P, Minaire Y and Lambert R 1989. Bacteriologie du colon. La Digestion. SIMEP 2e Edition, 120-131.

Miles EA and Calder PC 1998. Modulation of immune function by dietary fatty acids. Proceedings of the nutrition society 57, 277-292.

Monziols M, Bonneau M, Davenel A and Kouba M 2007. Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. Meat Science 76, 54-60.

Nair SSD, Leitch JW, Falconer J and Garg ML 1996. Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action. Journal of Nutrition 127, 383-393.

Nilsson-Ehle P, Garfinkel AS and Schotz MC 1980. Lipolytic Enzymes and Plasma-Lipoprotein Metabolism. Annual Review of Biochemistry 49, 667-693.

Nuernberg K, Fischer K, Nuernberg G, Kuechenmeister U, Klosowska D, Eliminowska-Wenda G, Fiedler I and Ender K 2005. Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. In Meat Science, pp. 63-74.

O'Hea EK and Leveille GA 1969. Significance of adipose tissue and liver as sites of fatty acid synthesis in pig and efficiency of utilization of various substrates for lipogenesis. Journal of Nutrition 99, 338-&.

O'Hea EK, Leveille GA and Sugahara M 1970. Lipogenesis and enzyme activity in pig adipose tissue as influenced by dietary protein and fat. International Journal of Biochemistry 1, 173-&.

Odedra BR and Millward DJ 1982. Effect of Corticosterone Treatment on Muscle Protein-Turnover in Adrenalectomized Rats and Diabetic Rats Maintained on Insulin. Biochemical Journal 204, 663-672.

Odle J 1997. New Insights into the Utilization of Medium-Chain Triglycerides by the Neonate: Observations from a Piglet Model. Journal of Nutrition 127, 1061-1067.

Olivecrona T 1987. Lipoprotein lipase from milk - the model enzyme in lipoprotein lipase research. In Lipoprotein Lipase. J. Borensztajn, Evener: Chicago.

Osborne TF 2000. Sterol regulatory element-binding proteins (SREBPs): Key regulators of nutritional homeostasis and insulin action. Journal of biological chemistry 275, 32379-32382.

Ozimek L, Sauer WC, Kozikowski W and Jorgensen H 1984. The ileal and fecal digestibility of fat and fatty acids in pigs fed semi-purified diets containing beef tallow or rapeseed oil. Agriculture & Forestry Bulletin, 14-16.

Pegorier JP, Le May C and Girard J 2004. Control of gene expression by fatty acids. Journal of Nutrition 134, 2444S-2449S.

Poumes-Ballihaut C, Langelier B, Houlier F, Alessandri JM, Durand G, Latge C and Guesnet P 2001. Comparative bioavailability of dietary alpha-linolenic and docosahexaenoic acids in the growing rat. Lipids 36, 793-800.

Powles J, Wiseman J, Cole DJA and Hardy B 1993. Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Animal Production 57, 137-146.

Powles J, Wiseman J, Cole DJA and Hardy B 1994. Effect of chemical structure of fats upon their apparent digestible energy value when given to young pigs. Animal Production 58, 411-417.

Qi B, Fraser T, Mugford S, Dobson G, Sayanova O, Butler J, Napier JA, Stobart AK and Lazarus CM 2004. Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nat Biotech 22, 739-745.

Ramsay TG 2001. Porcine leptin alters insulin inhibition of lipolysis in porcine adipocytes in vitro. Journal of Animal Science 79, 653-657.

Ramsay TG, Hausman GJ and Martin RJ 1987. Central Endocrine Regulation of the Development of Hormone Responses in Porcine Fetal Adipose Tissue. Journal Animal Science 64, 745-751.

Ramsay TG, White ME and Wolverton CK 1989. Insulin-Like Growth Factor 1 Induction of Differentiation of Porcine Preadipocytes. Journal Animal Science 67, 2452-2459.

Reis de Souza T, Peiniau J, Lebreton B and Giboulot A 1992. Effect of mixed, sparyed fat and emulsifying agents on total and ileal apparent digestibilities of lipids in piglet. Journées Rech. Porcine en France 24, 167-172.

Roncari DA and Murthy VK 1975. Effects of thyroid hormones on enzymes involved in fatty acid and glycerolipid synthesis. J. Biol. Chem. 250, 4134-4138.

Saeki K, Matsumoto K, Kinoshita M, Suzuki I, Mikami Y, Hirabayashi M, Kashiwazaki N, Hosoi Y, Murata N and Iritani A 2004. Functional expressio of a delta-12 fatty acid

desaturase gene from spinach in transgenic pigs. Proceedings of the National Academy of Sciences of the USA 101, 6361-61366.

Sambrook IE 1979. Studies on digestion and absorption in the intestines of growing pigs. British Journal of nutrition 42, 279-287.

Sanz M, Lopez-Bote CJ, Menoyo D and Bautista JM 2000. Abdominal fat deposition and fatty acid synthesis are lower and beta-oxidation is higher in broiler chickens fed diets containing unsatuarated rather than saturated fat. Journal of Nutrition 130, 3034-3037.

Shields RG, Jr., Mahan DC and Graham PL 1983. Changes in Swine Body Composition from Birth to 145 Kg. Journal Animal Science 57, 43-54.

Shimomura Y, Tamura T and Suzuki M 1990. Less body-fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. Journal of Nutrition 120, 1291-1296.

Simopoulos AP 1994. Fatty acids In *Functional foods*. New York: Chapman and Hall.

Smith A 2000. Oxford Dictionary of Biochemistry and Molecular Biology. Oxford University press, Oxford.

Smith DR, Knabe DA and Smith TB 1996. Depression of lipogenesis in swine adipose tissue by specific dietary fatty acids. Journal of Animal Science 74, 975-983.

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS and Lazar MA 2001. The hormone resistin links obesity to diabetes. Nature 409, 307-312.

Sul HS and Wang D 1998. Nutritional and hormonal regulation of enzymes in fat synthesis: Studies of fatty acid synthase and mitochondrial glycerol-3-phosphate acyltransferase gene transcription. Annual Review of Nutrition 18, 331-351.

Swanson KS, Mazur MJ, Vashisht K, Rund LA, Beever JE, Counter CM and Schook LB 2004. Genomics and Clinical Medicine: Rationale for Creating and Effectively Evaluating Animal Models. Experimental Biology and Medicine 229, 866-875.

Temme EH, Mensink RP and Hornstra G 1996. Comparison of the effects of diets enriched in lauric, palmitic, or oleic acids on serum lipids and lipoproteins in healthy women and men. Am J Clin Nutr 63, 897-903.

Thompson EH, Allen CE and Meade RJ 1973. Influence of Copper on Stearic Acid Desaturation and Fatty Acid Composition in the Pig. Journal Animal Science 36, 868-873.

USDA 2006. USDA National Nutrient Database for Standard Reference. In U.S. Department of Agriculture.

Vassileva G, Huwyler L, Poirier K, Agellon LB and Toth MJ 2000. The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice. FASEB Journal 14, 2040-2046.

Warnants N, Van Oeckel MJ and Boucque CV 1996. Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. Meat Science 44, 125-144.

Warnants N, Van Oeckel MJ and Boucqué CV 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. Journal of Animal Science 77, 2478-2490.

Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE and Tataranni PA 2001. Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. Journal of Clinical Endocrinology and Metabolism 86, 1930-1935.

Wijendran V and Hayes KC 2004. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annual Review of Nutrition 24, 597-615.

Willson TM, Lambert MH and Kliewer SA 2001. Peroxisome proliferator-activated receptor gamma and metabolic disease. Annual Review of Biochemistry 70, 341-367.

Wiseman J and Agunbiade JA 1998. The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. Livestock Production Science 54, 217-227.

Wiseman J, Cole DJA and Hardy B 1990. The dietary energy values of soya-bean oil, tallow and their blends for growing/finishing pigs. Animal Production 50, 513-518.

Wiseman J, Redshaw MS, Jagger S, Nute GR and Wood JD 2000. Influence of type and dietary rate of inclusion of oil on meat quality of finishing pigs. Animal Science 70, 307-315.

Wolverton CK, Azain MJ, Duffy JY, White ME and Ramsay TG 1992. Influence of somatotropin on lipid metabolism and IGF gene expression in porcine adipose tissue. Am J Physiol Endocrinol Metab 263, E637-645.

Wood JD 1983. Workshop CEC Progr. Coord. Res. Anim. Husb. In Brussels, p. 9.

Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR and Enser M 2003. Effects of fatty acids on meat quality: a review. Meat Science 66, 21-32.

Worgall TS, Sturley SL, Seo T, Osborne TF and Deckelbaum RJ 1998. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. Journal of biological chemistry 273, 25537-25540.

Xu J, Nakamura MT, Cho HP and Clarke SD 1999. Sterol Regulatory Element Binding Protein-1 expression is suppressed by dietary polyunsaturated fatty acids - A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. Journal of biological chemistry 274, 23577-23583.

Xu J, Teran-Garcia M, Park JHY, Nakamura MT and Clarke SD 2001. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. Journal of biological chemistry 276, 9800-9807.

Annexes

Annexe 1. Weight means of the carcass cuts according a comercial cut in pigs fed different diets at 100 kg live weight.

	60KgLW	NF	T	HOSF	SFO	LO	FB	FO
Flare fat	469	1454	1065	1284	1289	1086	1201	1119
Brain	112	126	122	129	123	124	133	122
White viscera	4091	5422	5707	5664	5512	5429	5878	5706
Intestinal cont.	1369	1147	2040	2134	1968	2166	2323	1864
Red viscera	3430	4615	4659	4661	4607	4945	4960	4844
Blood	2636	3679	3889	3721	3807	4078	4023	3983
Ham	5484	9382	9282	8952	8964	8977	9442	8629
Loin	3929	7291	6934	6458	6802	6450	6617	6346
Shoulder	3076	5122	5238	5011	5030	4921	5270	4935
Belly	1986	3736	3434	3546	3651	3422	3764	3351
Tenderloin	283	464	465	444	457	459	484	445
Back claw	1161	1846	1841	1806	1770	1770	1920	1754
Front loin	2086	3561	3505	3200	3341	3374	3372	3192
Front claw	774	1118	1173	1126	1098	1110	1147	1092
Belly trimmings	763	1228	1327	1390	1418	1261	1439	1275
DAVENT	595	963	981	984	879	910	969	865
Head	1975	2920	2965	2938	2902	2865	3058	2851
Ham trimmings	421	772	697	735	757	732	794	724

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Annexe 2. *Longissimus dorsi* intermuscular fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW	NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.09	0.08	0.05	0.06	0.06	0.05	0.06	0.06
C 12:0	0.09	0.09	0.08	0.08	0.07	0.07	0.08	0.08
C 14:0	1.38	1.37	1.61	1.08	1.01	1.07	1.37	1.33
C 16:0	25.5	27.6	23.4	19.1	19.0	20.4	22.4	21.9
C 16:1 n-7	1.98	1.91	2.05	0.99	0.99	1.02	1.54	1.53
C 18:0	15.8	18.0	13.9	11.2	12.1	13.8	14.2	14.6
C 18:1 n-9 trans	0.41	0.31	0.47	0.10	0.18	0.22	0.20	0.22
C 18:1 n-9 cis	38.1	40.5	42.2	52.0	32.8	31.9	36.4	31.0
C 18:1 n-11 cis	2.57	2.33	2.35	1.32	1.48	1.54	1.99	2.03
C 18:2 n-6	10.2	5.1	9.3	10.8	28.3	11.7	12.3	10.7
C 18:3 n-6	0.01	ND	0.03	0.02	0.06	ND	ND	0.02
C 18:3 n-3	0.83	0.39	0.92	0.65	0.65	14.13	5.83	9.15
C 20:0	0.23	0.27	0.17	0.20	0.19	0.21	0.21	0.21
C 20:1 n-9 cis	0.89	0.96	0.82	1.01	0.70	0.70	0.78	0.86
C 20:2 n-6 cis	0.57	0.31	0.45	0.49	1.17	0.55	0.53	0.49
C 20:3 n-6	0.08	0.04	0.07	0.06	0.11	0.04	0.06	0.07
C 20:4 n-6	0.24	0.13	0.20	0.22	0.32	0.11	0.13	0.23
C 20:3 n-3 cis	0.12	0.07	0.11	0.08	0.08	1.50	0.61	0.76
C 20:5 n-3 cis	ND	ND	ND	ND	ND	0.14	0.07	0.65
C 22:4 n-6	0.07	0.04	0.05	0.06	0.09	0.02	0.03	0.05
C 22:5 n-3	0.10	0.09	0.12	0.08	0.14	0.29	0.22	0.80
C 22:6 n-3 cis	0.11	0.05	0.09	0.10	0.24	0.12	0.14	2.55
Saturated FA	43.6	47.6	40.0	32.0	32.7	35.9	38.9	38.7
MUFA	44.1	46.1	48.5	55.4	36.2	35.4	41.1	35.7
PUFA	12.3	6.3	11.5	12.6	31.1	28.7	20.0	25.6

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

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Annexe 3. *Semimembranosus* intermuscular fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.11	0.09	0.07	0.06	0.06	0.06	0.07	0.06
C 12:0	0.10	0.09	0.08	0.08	0.07	0.07	0.08	0.07
C 14:0	1.45	1.39	1.53	1.10	1.05	1.03	1.37	1.16
C 16:0	24.5	25.6	22.4	18.4	18.6	18.3	22.0	19.2
C 16:1 n-7	2.64	2.48	2.16	1.45	1.22	1.35	1.78	1.66
C 18:0	13.4	14.4	12.7	9.5	11.3	11.3	12.7	11.9
C 18:1 n-9 trans	0.21	0.24	0.45	0.22	0.29	0.19	0.22	0.16
C 18:1 n-9 cis	38.4	41.9	41.0	50.4	32.6	31.5	36.3	32.5
C 18:1 n-11 cis	3.04	2.98	2.55	1.89	1.76	1.94	2.34	2.31
C 18:2 n-6	11.8	7.6	11.9	13.0	28.4	15.2	13.9	13.2
C 18:3 n-6	0.04	0.03	0.05	0.05	0.08	0.05	0.06	0.06
C 18:3 n-3	0.98	0.60	1.11	0.88	0.83	14.52	5.40	9.25
C 20:0	0.21	0.22	0.17	0.15	0.17	0.17	0.18	0.18
C 20:1 n-9	0.81	0.82	0.74	0.89	0.65	0.63	0.72	0.78
C 20:2 n-6	0.57	0.42	0.59	0.56	1.21	0.64	0.61	0.57
C 20:3 n-6	0.12	0.07	0.11	0.10	0.17	0.09	0.09	0.11
C 20:4 n-6	0.38	0.24	0.30	0.35	0.44	0.27	0.28	0.41
C 20:3 n-3	0.15	0.11	0.16	0.12	0.12	1.31	0.55	0.78
C 20:5 n-3	ND	ND	0.03	ND	ND	0.20	0.09	0.87
C 22:4 n-6	0.14	0.08	0.10	0.11	0.14	0.08	0.09	0.12
C 22:5 n-3	0.14	0.07	0.14	0.10	0.08	0.33	0.26	0.93
C 22:6 n-3	0.13	0.09	0.10	0.11	0.12	0.12	0.14	2.95
Saturated FA	40.3	42.1	37.7	29.7	31.7	31.4	36.9	33.1
MUFAS	45.2	48.5	47.5	55.0	36.7	35.7	41.6	37.5
PUFA	14.5	9.3	14.8	15.3	31.7	32.9	21.5	29.4

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

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Annexe 4. Belly intermuscular fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.23	0.24	0.17	0.17	0.19	0.16	0.17	0.18
C 12:0	0.23	0.23	0.16	0.18	0.20	0.18	0.18	0.16
C 14:0	1.50	1.47	1.64	1.22	1.15	1.12	1.40	1.31
C 16:0	24.4	25.5	23.3	19.2	18.8	18.8	21.5	20.7
C 16:1 n-7	2.52	2.31	2.33	1.30	1.22	1.18	1.73	1.59
C 18:0	13.9	14.8	12.5	10.2	11.3	11.8	12.9	12.9
C 18:1 n-9 trans	0.17	0.14	0.31	0.20	0.11	0.09	0.22	0.12
C 18:1 n-9 cis	40.0	44.1	44.2	52.3	34.3	32.8	38.0	33.0
C 18:1 n-11 cis	2.82	2.63	2.38	1.63	1.63	1.55	2.14	2.04
C 18:2 n-6	10.5	6.1	8.8	10.3	27.2	12.3	12.3	11.1
C 18:3 n-6	ND	ND	ND	ND	0.06	ND	ND	ND
C 18:3 n-3	0.85	0.44	0.87	0.63	0.71	16.07	5.95	9.30
C 20:0	0.21	0.21	0.18	0.18	0.16	0.16	0.20	0.21
C 20:1 n-9	0.88	0.93	0.80	0.95	0.65	0.61	0.78	0.83
C 20:2 n-6	0.52	0.32	0.37	0.47	1.05	0.50	0.51	0.47
C 20:3 n-6	0.08	0.04	0.06	0.06	0.11	0.04	0.06	0.07
C 20:4 n-6	0.27	0.18	0.21	0.26	0.39	0.13	0.16	0.26
C 20:3 n-3	0.14	0.09	0.12	0.09	0.09	1.66	0.67	0.84
C 20:5 n-3	ND	ND	ND	ND	ND	0.18	0.07	0.71
C 22:4 n-6	0.08	ND	0.04	0.05	0.09	ND	0.04	0.05
C 22:5 n-3	0.11	0.05	0.10	0.08	0.13	0.27	0.24	0.86
C 22:6 n-3	0.11	0.04	0.08	0.07	0.20	0.07	0.10	2.72
Saturated FA	40.9	42.7	38.7	31.5	32.1	32.5	36.9	35.9
MUFAS	46.4	50.1	50.1	56.5	37.9	36.3	43.0	37.6
PUFA	12.7	7.2	11.1	12.0	30.0	31.2	20.2	26.4

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Annexe 5. Flare fat intermuscular fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

GPR	60 kg LW NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.10	0.08	0.06	0.07	0.06	0.07	0.08	0.07
C 12:0	0.09	0.08	0.08	0.08	0.07	0.07	0.09	0.08
C 14:0	1.47	1.36	1.69	1.18	1.11	1.14	1.47	1.35
C 16:0	27.4	28.4	24.6	20.2	20.0	20.4	23.8	21.9
C 16:1 n-7	1.77	1.68	1.85	0.82	0.75	0.82	1.33	1.29
C 18:0	19.6	20.8	17.7	13.1	14.8	15.8	17.1	16.4
C 18:1 n-9 trans	0.18	0.29	0.26	0.14	0.15	0.12	0.19	0.12
C 18:1 n-9 cis	32.6	37.1	37.4	48.9	27.9	26.1	30.8	25.4
C 18:1 n-11 cis	2.16	2.06	2.07	1.20	1.18	1.28	1.70	1.72
C 18:2 n-6	10.7	5.4	9.6	11.1	29.9	13.5	13.4	11.8
C 18:3 n-6	ND	ND	ND	ND	0.08	ND	ND	0.04
C 18:3 n-3	0.86	0.47	1.02	0.71	0.73	16.86	6.52	10.81
C 20:0	0.32	0.30	0.21	0.20	0.21	0.23	0.23	0.23
C 20:1 n-9	0.81	0.81	0.64	0.77	0.54	0.51	0.59	0.69
C 20:2 n-6	0.46	0.28	0.35	0.41	1.02	0.47	0.45	0.41
C 20:3 n-6	0.07	0.03	0.07	0.07	0.12	0.05	0.07	0.07
C 20:4 n-6	0.28	0.17	0.22	0.27	0.39	0.16	0.20	0.32
C 20:3 n-3	0.10	0.07	0.10	0.07	0.08	1.31	0.52	0.76
C 20:5 n-3	ND	ND	ND	ND	ND	0.23	0.11	0.99
C 22:4 n-6	0.07	0.06	0.07	0.08	0.12	0.03	0.05	0.08
C 22:5 n-3	0.14	0.05	0.15	0.11	0.14	0.35	0.28	1.02
C 22:6 n-3	0.26	0.05	0.14	0.17	0.26	0.16	0.18	3.71
Saturated FA	49.5	51.3	45.3	35.2	36.6	38.0	43.3	40.6
MUFAS	37.6	42.1	42.7	51.8	30.6	28.9	34.8	29.3
PUFA	12.9	6.6	11.9	13.0	32.8	33.1	21.9	30.1

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

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Annexe 6. *Longissimus dorsi* intramuscular fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW	NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.15	0.16	0.12	0.14	0.14	0.13	0.13	0.12
C 12:0	0.09	0.09	0.10	0.10	0.09	0.11	0.10	0.09
C 14:0	1.07	1.12	1.30	1.17	1.08	0.96	1.19	1.07
C 16:0	22.6	23.3	22.2	21.6	20.5	19.9	21.9	20.6
C 16:1 n-7	2.48	3.21	3.00	2.34	1.98	1.77	2.50	2.02
C 18:0	13.4	12.5	11.3	11.1	11.4	12.2	11.9	12.1
C 18:1 n-9 trans	0.28	0.21	0.28	0.15	0.18	0.23	0.31	0.18
C 18:1 n-9 cis	34.7	37.5	40.5	43.2	33.1	30.1	34.9	31.3
C 18:1 n-11 cis	3.58	3.94	3.58	2.95	2.60	2.61	3.22	2.94
C 18:2 n-6	12.5	9.7	9.8	10.6	22.1	14.1	12.7	11.6
C 18:3 n-6	0.11	0.10	0.08	0.06	0.07	0.05	0.06	0.05
C 18:3 n-3	0.52	0.37	0.66	0.43	0.43	8.52	3.41	5.38
C 20:0	0.17	0.14	0.15	0.17	0.15	0.15	0.15	0.16
C 20:1 n-9	0.63	0.54	0.62	0.76	0.53	0.50	0.54	0.58
C 20:2 n-6	0.60	0.49	0.42	0.44	0.74	0.46	0.41	0.41
C 20:3 n-6	0.45	0.42	0.31	0.28	0.26	0.31	0.31	0.30
C 20:4 n-6	3.87	4.07	2.62	2.95	3.14	2.89	2.61	2.93
C 20:3 n-3	0.10	0.06	0.09	0.06	0.06	0.98	0.40	0.56
C 20:5 n-3	0.23	0.21	0.24	0.11	0.07	1.46	0.71	2.58
C 22:4 n-6	0.57	0.45	0.28	0.29	0.36	0.18	0.20	0.12
C 22:5 n-3	0.71	0.60	0.55	0.37	0.35	1.24	0.93	0.97
C 22:6 n-3	0.41	0.50	0.34	0.31	0.24	0.28	0.36	3.29
Saturated FA	38.0	37.5	35.7	34.6	33.7	33.8	35.8	34.6
MUFAS	41.9	45.5	48.8	49.5	38.4	35.6	42.0	37.2
PUFA	20.1	17.0	15.5	15.9	27.8	30.5	22.2	28.3

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

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Annexe 7. *Longissimus dorsi* subcutaneous fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW	NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.07	0.06	0.04	0.04	0.04	0.04	0.04	0.04
C 12:0	0.08	0.08	0.07	0.06	0.06	0.06	0.07	0.06
C 14:0	1.28	1.30	1.51	0.95	0.93	0.97	1.23	1.12
C 16:0	24.3	26.3	22.4	17.7	17.7	19.1	21.0	19.8
C 16:1 n-7	1.91	1.75	2.05	0.94	1.00	1.14	1.48	1.34
C 18:0	15.0	16.5	12.7	10.0	11.0	12.4	12.7	13.4
C 18:1 n-9 trans	0.48	0.71	0.45	0.14	0.23	0.20	0.50	0.28
C 18:1 n-9 cis	38.3	42.1	44.2	54.0	34.2	34.2	38.0	32.9
C 18:1 n-11 cis	2.61	2.41	2.53	1.44	1.61	1.71	2.09	2.12
C 18:2 n-6	11.6	5.6	9.2	11.0	28.6	12.3	13.1	11.1
C 18:3 n-6	0.03	ND	0.03	0.03	0.07	0.02	0.03	0.03
C 18:3 n-3	0.85	0.39	0.88	0.64	0.73	13.50	5.96	9.31
C 20:0	0.28	0.28	0.20	0.22	0.21	0.23	0.20	0.24
C 20:1 n-9	1.06	1.10	0.96	1.20	0.83	0.81	0.84	1.05
C 20:2 n-6	0.69	0.38	0.44	0.58	1.44	0.63	0.64	0.63
C 20:3 n-6	0.08	0.04	0.08	0.07	0.14	0.04	0.07	0.08
C 20:4 n-6	0.23	0.16	0.21	0.23	0.34	0.13	0.17	0.26
C 20:3 n-3	0.15	0.09	0.13	0.10	0.11	1.69	0.75	1.05
C 20:5 n-3	ND	ND	ND	ND	ND	0.14	0.08	0.68
C 22:4 n-6	0.07	0.04	0.05	0.06	0.11	0.03	0.04	0.06
C 22:5 n-3	0.07	ND	0.10	0.05	0.06	0.22	0.24	0.91
C 22:6 n-3	0.09	ND	0.06	0.06	0.13	0.06	0.08	2.79
Saturated FA	41.6	44.8	37.8	29.4	30.3	33.1	35.7	35.1
MUFAS	44.5	48.5	50.8	57.8	37.9	38.1	43.1	37.9
PUFA	13.8	6.7	11.4	12.8	31.7	28.8	21.2	27.0

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Annexe 8. *Semimembranosus* intramuscular fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW	NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.10	0.12	0.09	0.08	0.09	0.09	0.10	0.09
C 12:0	0.08	0.08	0.08	0.07	0.07	0.07	0.08	0.07
C 14:0	1.03	1.14	1.29	0.96	0.97	0.97	1.17	1.05
C 16:0	21.3	21.9	20.6	18.2	18.1	18.2	20.2	18.8
C 16:1 n-7	2.65	3.26	2.73	1.86	1.63	1.80	2.41	2.08
C 18:0	11.7	11.0	10.2	9.0	10.1	10.3	10.5	10.3
C 18:1 n-9 trans	0.14	0.15	0.26	0.13	0.12	0.12	0.19	0.13
C 18:1 n-9 cis	34.8	42.9	43.3	47.5	33.3	33.0	37.5	32.9
C 18:1 n-11 cis	3.76	4.20	3.48	2.74	2.44	2.65	3.12	3.02
C 18:2 n-6	15.0	8.8	11.1	12.3	26.1	14.4	13.7	12.3
C 18:3 n-6	ND	0.07	0.09	0.09	0.10	0.09	0.09	0.08
C 18:3 n-3	0.62	0.40	0.83	0.52	0.51	10.94	4.48	6.79
C 20:0	0.14	0.21	0.19	0.18	0.21	0.19	0.21	0.19
C 20:1 n-9	0.60	0.66	0.68	0.80	0.59	0.56	0.63	0.65
C 20:2 n-6	0.62	0.41	0.47	0.53	0.96	0.50	0.48	0.45
C 20:3 n-6	0.44	0.29	0.26	0.28	0.26	0.23	0.24	0.25
C 20:4 n-6	4.25	2.86	2.03	3.09	3.03	2.07	2.00	2.44
C 20:3 n-3	0.13	0.09	0.13	0.10	0.09	1.15	0.50	0.70
C 20:5 n-3	0.26	0.16	0.18	0.11	0.06	1.08	0.57	2.44
C 22:4 n-6	0.54	0.30	0.22	0.28	0.33	0.13	0.17	0.13
C 22:5 n-3	0.73	0.44	0.44	0.40	0.32	0.88	0.75	1.00
C 22:6 n-3	0.49	0.33	0.28	0.37	0.22	0.24	0.29	3.62
Saturated FA	34.8	34.7	32.9	28.8	29.9	30.1	32.6	30.8
MUFAS	42.0	51.2	50.7	53.1	38.1	38.2	44.0	38.9
PUFA	23.1	14.1	16.4	18.1	32.0	31.7	23.4	30.3

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Annexe 9. *Semimembranosus* subcutaneous fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.09	0.09	0.06	0.06	0.06	0.07	0.07	0.06
C 12:0	0.08	0.08	0.07	0.07	0.06	0.07	0.07	0.06
C 14:0	1.43	1.40	1.48	1.17	1.13	1.16	1.38	1.23
C 16:0	23.5	24.1	20.9	18.1	18.3	18.6	20.6	19.3
C 16:1 n-7	2.58	2.43	2.39	1.52	1.35	1.49	1.92	1.76
C 18:0	12.3	12.2	10.4	8.6	10.2	10.7	11.1	10.8
C 18:1 n-9 trans	0.30	0.36	0.37	0.26	0.23	0.17	0.30	0.17
C 18:1 n-9 cis	40.4	45.0	46.1	53.7	37.4	36.2	40.0	37.0
C 18:1 n-11 cis	3.19	3.25	3.05	2.11	2.02	2.13	2.55	2.53
C 18:2 n-6	11.6	7.5	9.7	10.4	24.4	12.2	12.8	10.8
C 18:3 n-6	0.04	ND	ND	0.03	0.05	ND	0.03	0.02
C 18:3 n-3	0.98	0.61	1.08	0.70	0.71	12.39	5.18	7.77
C 20:0	0.24	0.23	0.20	0.20	0.21	0.22	0.20	0.22
C 20:1 n-9	0.98	1.05	1.06	1.24	0.94	0.82	0.89	0.97
C 20:2 n-6	0.67	0.49	0.58	0.62	1.38	0.68	0.66	0.63
C 20:3 n-6	0.10	0.07	0.10	0.10	0.14	0.07	0.09	0.09
C 20:4 n-6	0.33	0.23	0.30	0.32	0.41	0.21	0.25	0.34
C 20:3 n-3	0.15	0.12	0.16	0.11	0.12	1.54	0.65	0.92
C 20:5 n-3	ND	ND	ND	ND	ND	0.19	0.10	0.75
C 22:4 n-6	0.11	0.08	0.09	0.10	0.13	0.07	0.08	0.09
C 22:5 n-3	0.16	0.09	0.18	0.10	0.14	0.34	0.29	0.93
C 22:6 n-3	0.24	0.13	0.23	0.15	0.26	0.17	0.19	2.92
Saturated FA	38.2	38.4	33.7	28.5	30.2	31.2	33.9	32.1
MUFAS	47.4	52.2	53.1	58.8	42.0	40.9	45.8	42.6
PUFA	14.4	9.4	13.2	12.6	27.8	27.9	20.4	25.4

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Annexe 10. Belly subcutaneous fat fatty acid compositon in pigs fed different diets at 100 kg live weight.

	60 kg LW	NF	T	HOSF	SFO	LO	FB	FO
C 10:0	0.09	0.09	0.05	0.07	0.06	0.06	0.06	0.06
C 12:0	0.08	0.09	0.08	0.07	0.07	0.07	0.07	0.07
C 14:0	1.36	1.35	1.62	1.19	1.08	1.10	1.34	1.25
C 16:0	23.0	23.8	21.3	17.9	17.9	18.7	20.3	19.2
C 16:1 n-7	2.56	2.37	2.62	1.45	1.45	1.50	1.85	1.80
C 18:0	12.2	12.9	10.4	8.4	9.6	10.5	11.1	11.0
C 18:1 n-9 trans	0.21	0.17	0.33	0.14	0.16	0.13	0.21	0.15
C 18:1 n-9 cis	40.3	44.9	45.6	53.7	37.5	35.2	39.0	35.2
C 18:1 n-11 cis	3.04	2.93	2.84	1.81	2.00	2.02	2.30	2.46
C 18:2 n-6	12.9	8.3	10.5	11.8	25.7	13.4	13.9	12.4
C 18:3 n-6	0.03	ND	ND	ND	0.05	0.02	0.02	0.03
C 18:3 n-3	1.03	0.66	1.02	0.74	0.77	13.12	6.12	8.80
C 20:0	0.21	0.20	0.16	0.16	0.17	0.19	0.17	0.19
C 20:1 n-9	0.90	0.94	0.87	1.04	0.79	0.71	0.74	0.91
C 20:2 n-6	0.68	0.50	0.51	0.58	1.29	0.65	0.61	0.62
C 20:3 n-6	0.09	0.04	0.09	0.07	0.13	0.06	0.07	0.08
C 20:4 n-6	0.26	0.21	0.22	0.23	0.34	0.15	0.17	0.27
C 20:3 n-3	0.16	0.12	0.14	0.11	0.11	1.51	0.71	0.88
C 20:5 n-3	ND	ND	ND	ND	ND	0.14	0.08	0.65
C 22:4 n-6	0.07	0.05	0.05	0.06	0.09	0.04	0.04	0.06
C 22:5 n-3	0.12	0.07	0.12	0.09	0.12	0.20	0.24	0.84
C 22:6 n-3	0.12	0.06	0.10	0.12	0.24	0.13	0.14	2.36
Saturated FA	37.4	38.7	34.3	28.1	29.2	31.0	33.5	32.3
MUFAS	47.1	51.4	52.5	58.2	41.9	39.6	44.2	40.6
PUFA	15.5	9.9	13.2	13.7	28.9	29.4	22.3	27.1

Values are means with no statistical analysis performed.

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

Annexe 11. Blood parametres in pigs fed different diets at 100 kg live weight

	NF	T	HOSF	SFO	LO	FB	FO
Fasting period							
Non Esterified FA (mmol/L)	0.45	0.53	0.52	0.59	0.47	0.60	0.43
Glucose (mg/dL)	121	121	145	132	125	121	133
Triglycerides (mg/dL)	45.8^{a}	70.8^{b}	61.0^{ab}	63.4^{ab}	65.7^{b}	81.7^{b}	68.5^{b}
Choleterol (mg/dL)	110	116	118	111	107	107	110
insulin (microg/L)	0.069	0.017	0.005	0.027	0.063	0.029	0.006
HDL (mmol/L)	1.07^{ab}	1.20^{a}	1.20^{a}	1.14^{ab}	1.04^{ab}	1.11^{ab}	1.00^{b}
LDL(mmol/L)	1.61	1.63	1.73	1.66	1.74	1.64	1.75
After meal							
Non Esterified FA (mmol/L)	0.05^{d}	0.15^{bc}	0.16^{abc}	0.17^{ab}	0.19^{a}	0.13c	0.15^{bc}
Glucose (mg/dL)	104	99	94	89	104	97	95
Triglycerides (mg/dL)	18.9^{c}	48.3^{a}	34.7^{b}	35.7^{ab}	42.3^{ab}	41.8^{ab}	39.9^{ab}
Choleterol (mg/dL)	101^{ab}	115^{a}	94^{b}	104^{ab}	94^{b}	103^{ab}	100^{ab}

NF: No fat, T: Tallow, SFHO: High-oleic sunflower oil, SFO: Sunflower oil, LO: linseed oil, FB: fat blend (55% tallow, 35% sunflower oil, 15% linseed oil), FO: Fish oil blend (40 fish oil, 60 % linseed oil)

The statistical analysis performed was the same as in papers 2 and 3 (Mixed procedure of SAS)

Means within a row with different letters are significantly different (P<0.05).