Universitat Autònoma de Barcelona

USE OF RE-ESTERIFIED OILS IN PIG AND BROILER CHICKEN DIETS

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SOTA LA DIRECCIÓ DELS DOCTORS:

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Que la memòria titulada "**Use of re-esterified oils in pig and broiler chicken diets**", presentada per Ester Vilarrasa Gustems amb la finalitat d'optar al grau de Doctor en Veterinària, ha estat realitzada sota la seva direcció i, considerant-la acabada, autoritzen la seva presentació perquè sigui jutjada per la comissió corresponent.

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"No val la pena arribar a la meta si un no ha gaudit del viatge."

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Resum

La incorporació de matèries grasses és una pràctica habitual en la formulació de pinsos per a porcs i pollastres. Els olis àcids són subproductes de la indústria de refinació d'olis, donant lloc a matèries primeres sostenibles i interessants des del punt de vista econòmic. Ara bé, el seu elevat contingut en àcids grassos (**AG**) lliures, fa que el seu valor nutritiu sigui inferior al del seu corresponent oli natiu. Podem neutralitzar els AG lliures dels olis àcids a través de la seva esterificació amb glicerol (subproducte de la indústria del biodièsel), obtenint, com a producte final d'aquest procés, els olis re-esterificats. La diferent estructura molecular dels olis re-esterificats pot millorar el seu valor nutritiu i, en particular, l'absorció dels AG saturats. Per tot plegat, l'objectiu global d'aquesta tesi va ser investigar l'ús potencial dels olis re-esterificats en l'alimentació de porcs i pollastres.

La caracterització dels greixos (*Capítol 3, 4 i 5*) va mostrar que els olis re-esterificats presentaven la mateixa composició en AG que la dels seus corresponents olis natius, però una major proporció d'AG saturats situats a la posició *sn*-2 de les molècules d'acilglicerols, i una major quantitat de mono- i diglicèrids (amb els AG principalment esterificats a les posicions *sn*-1,3), el que va donar lloc a un menor contingut en energia bruta.

Als tres primers experiments (*Capítol 3, 4 i 5*), es va comparar l'ús de dos olis re-esterificats de palma (amb baix i alt contingut en mono- i diglicèrids) amb el del seu corresponent oli àcid (control negatiu) i oli natiu (control positiu). D'una banda, no es van observar diferències en els coeficients d'absorció dels AG entre els animals alimentats amb l'oli natiu i els alimentats amb l'oli àcid de palma (excepte en pollets de primera edat). Per tant, els olis àcids de palma poden considerar-se una font de greix alternativa en l'alimentació d'animals monogàstrics, sempre que tinguin < 60% d'AG lliures i < 3% d'humitat, impureses i fracció insaponificable. D'altra banda, els animals alimentats amb olis re-esterificats de palma van assolir coeficients d'absorció similars o inclús superiors als dels animals alimentats amb el seu corresponent oli natiu, encara que es van observar algunes peculiaritats en funció de l'espècie i de l'estat fisiològic dels animals.

En pollastres (*Capítol 5*), el grau de saturació del greix va exercir un major impacte en l'absorció aparent dels AG que l'estructura molecular del greix. Per aquest motiu, en l'últim experiment (*Capítol 6*) es va investigar quina seria la millor estratègia nutricional (olis re-esterificats purs o mescles) per obtenir la millor eficiència en termes

d'absorció aparent dels AG. La substitució de l'oli re-esterificat de palma per oli re-esterificat de soja va millorar l'absorció aparent dels AG totals, encara que no es va observar sinergisme entre els dos olis re-esterificats.

Una altra troballa interessant va ser que, independentment de la font de greix, de l'espècie i de l'estat fisiològic dels animals, la fracció lipídica eliminada per les femtes i les excretes estava principalment constituïda per AG lliures, el que suggereix que el factor limitant del procés d'absorció del greix de la dieta no és el procés d'hidròlisi lipídica, sinó el procés de solubilització micel·lar.

Per tot això, podem considerar que, des d'un punt de vista nutricional, els olis re-esterificats són una bona font de greix alternativa per ser utilitzats en l'alimentació d'animals monogàstrics. Això no obstant, i degut a que el procés d'esterificació suposa un cost afegit, la seva viabilitat econòmica dependrà del preu diferencial entre l'oli natiu i l'oli àcid.

Resumen

La incorporación de materias grasas es una práctica habitual en la formulación de piensos para cerdos y pollos. Los aceites ácidos son subproductos de la industria de refinación de aceites, resultando en materias primas sostenibles e interesantes desde el punto de vista económico. Ahora bien, su elevado contenido en ácidos grasos (**AG**) libres, hace que su valor nutritivo sea inferior al de su correspondiente aceites nativo. Podemos neutralizar los AG libres de los aceites ácidos a través de su esterificación con glicerol (subproducto de la industria del biodiesel), obteniendo, como producto final de este proceso, los aceites re-esterificados. La distinta estructura molecular de los aceites re-esterificados puede mejorar su valor nutritivo y, en particular, la absorción de los AG saturados. Por todo ello, el objetivo global de esta tesis fue investigar el uso potencial de los aceites re-esterificados en la alimentación de cerdos y pollos.

La caracterización de las grasas (*Capítulo 3, 4 y 5*) mostró que los aceites re-esterificados presentaban la misma composición en AG que la de sus correspondientes aceites nativos, pero una mayor proporción de AG saturados situados en la posición *sn*-2 de las moléculas de acilgliceroles, y una mayor cantidad de mono- y diglicéridos (con los AG principalmente esterificados en las posiciones *sn*-1,3), lo que dio lugar a un menor contenido en energía bruta.

En los tres primeros experimentos (*Capítulo 3, 4 y 5)*, se comparó el uso de dos aceites re-esterificados de palma (con bajo y alto contenido en mono- y diglicéridos) con el de su correspondiente aceite ácido (control negativo) y aceite nativo (control positivo). Por un lado, no se observaron diferencias en los coeficientes de absorción de los AG entre los animales alimentados con el aceite nativo y los alimentados con el aceite ácido de palma (excepto en pollitos de primera edad). Por lo tanto, los aceites ácidos de palma pueden considerarse una fuente de grasa alternativa en la alimentación de animales monogástricos, siempre que tengan < 60% de AG libres y < 3% de humedad, impurezas y fracción insaponificable. Por otro lado, los animales alimentados con aceites re-esterificados de palma alcanzaron coeficientes de absorción similares o incluso superiores a los de los animales alimentados con su correspondiente aceite nativo, aunque se observaron algunas peculiaridades en función de la especie y del estado fisiológico de los animales.

En pollos (*Capítulo 5*), el grado de saturación de la grasa ejerció un mayor impacto en la absorción aparente de los AG que la estructura molecular de la grasa. Por esta razón, en el último experimento (*Capítulo 6*) se investigó cual sería la mejor

estrategia nutricional (aceites re-esterificados puros o mezclas) para obtener la mejor eficiencia en términos de absorción aparente de los AG. La sustitución del aceite re-esterificado de palma por aceite re-esterificado de soja mejoró la absorción aparente de los AG totales en pollos, aunque no se observó sinergismo entre los dos aceites re-esterificados.

Otro hallazgo interesante fue que, independientemente de la fuente de grasa, de la especie y del estado fisiológico de los animales, la fracción lipídica eliminada por las heces y las excretas estuvo principalmente constituida por AG libres, lo que sugiere que el factor limitante del proceso de absorción de la grasa dietética no es el proceso de hidrólisis lipídica, sino el proceso de solubilización micelar.

Por todo ello, podemos considerar que, desde un punto de vista nutricional, los aceites re-esterificados son una buena fuente de grasa alternativa para ser utilizados en la alimentación de animales monogástricos. Sin embargo, y debido a que el proceso de esterificación supone un coste añadido, su viabilidad económica dependerá del precio diferencial entre el aceite nativo y el aceite ácido.

Summary

The addition of fat sources in pig and broiler-chicken diets is a common practice. Acid oils are by-products from oil refining industry, resulting in sustainable and economically interesting raw materials. However, they have a lower nutritive value than their corresponding native oil, due to their high free fatty acid (**FA**) content. We can neutralize the free FA content of acid oils through their esterification with glycerol (by-product from biodiesel industry). Re-esterified oils are the end products of this process. The different molecular structure of re-esterified oils may enhance the apparent absorption of saturated FA and, consequently, their overall nutritive value. Therefore, the global aim of this thesis was to investigate the potential use of re-esterified oils in pig and broiler-chicken diets.

Fat characterization (*Chapter 3, 4 and 5*) showed how re-esterified oils had the same FA composition as their corresponding native oils, but a greater proportion of saturated FA located at the acylglycerol *sn*-2 position and a greater amount of mono- and diacylglycerols (with FA mainly esterified at the *sn*-1,3 positions), which resulted in a lower gross energy content.

In the first three experiments (*Chapter 3, 4 and 5*), the use of two re-esterified oils (with a low and a high mono- and diacylglycerol content) was compared with that of their corresponding acid (negative control) and native (positive control) oils. On one hand, no differences in FA apparent absorption coefficients were observed between animals fed native or acid palm oils (except in young broiler chickens). Thus, acid palm oils can be considered interesting alternative fat sources to be used in monogastric-animal diets, provided they have < 60% of free FA and < 3% of moisture, impurities, and unsaponifiable matter. On the other hand, animals fed re-esterified palm oils achieved similar or even better apparent absorption coefficients than those fed their corresponding native oil, although some peculiarities were observed according to the species and the physiological state of the animals.

In broiler chickens (*Chapter 5*), the fat degree of saturation exerted a greater impact on FA apparent absorption than did the fat molecular structure. For this reason, in the last experiment (*Chapter 6*), the best nutritional strategy (pure re-esterified oils or blends of re-esterified oils) was investigated, in order to obtain the best efficiency in terms of FA apparent absorption. The addition of re-esterified soybean oil in replacement of re-esterified palm oil in broiler chicken diets improved the total FA apparent absorption, although no synergism was observed between re-esterified oils.

Another interesting finding was that, regardless of the dietary fat source, the species, and the physiological sate of the animals, fat lost in feces or excreta was mainly composed of free FA, which suggests that micelle formation, and not fat hydrolysis, is the rate-limiting step of fat absorption.

Taken together, from a nutritional point of view, re-esterified oils are good, alternative fat sources to be used in monogastric-animal diets. However, considering that the esterification process represents an additional cost, the economic viability of re-esterified oils will depend on the price differential between native and acid oils.

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AME	apparent metabolizable energy
BW	body weight
CM	chylomicron
DAG	diacylglycerol
DE	digestible energy
FA	fatty acid
FCR	feed conversion ration
FFA	free fatty acid
G:F	gain-to-feed ratio
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
LDL	low-density lipoprotein
MAG	monoacylglycerol
MUFA	monounsaturated fatty acid
NMR	nuclear magnetic resonance
PA	acid palm oil
PEH	re-esterified palm oil high in mono- and diacylglycerols
PEL	re-esterified palm oil low in mono- and diacylglycerols
PN	native palm oil
PUFA	polyunsaturated fatty acid
SA	acid soybean oil
SEH	re-esterified soybean oil high in mono- and diacylglycerols
SEL	re-esterified soybean oil low in mono- and diacylglycerols
SFA	saturated fatty acid
SN	native soybean oil
TAG	triacylglycerol
UFA	unsaturated fatty acid
UFA:SFA	unsaturated-to-saturated fatty acid ratio
VLDL	very-low-density lipoprotein

CHAPTER 1

General introduction

The following literature review highlights the importance of fat in animal nutrition and details the composition, the availability and the price of different fat sources. Afterwards, fat digestion, absorption, and metabolism processes are described, to finish describing in detail the factors that directly affect these processes.

1.1. Why do we include fat in monogastric animal diets?

Cereals are the most important dietary energy sources for animal nutrition. However, in feed manufacturing, fats have been increasingly used in line with the improvements in the productive potential of several species. Fats raise the energetic density of feeds, satisfying the high energy demands of the animals, and resulting in a better animal performance. Fats have the highest caloric value because fatty acids (**FA**) are chemically more reduced than the carbon atoms found in carbohydrates. Therefore, the oxidation of lipids releases more than twice as much energy as carbohydrates (9 kcal/g *vs*. 4 kcal/g). Nevertheless, fats are not only the most concentrated sources of energy, but also those with the most variable nutritive value, due to their extremely diverse chemical structure (NRC, 1994; NRC, 2012).

Besides the use of fat as energy source, its addition to feed has many other important functions:

a) Nutritional aspects:

- Supplies essential FA. Diets deficient in these FA will cause metabolic disorders, since essential FA have influence on many important physiological processes, including lipid metabolism, cell division and differentiation, and immune function and inflammation (Cunnane, 1984; Whitehead, 1984).
- Facilitates the absorption of fat-soluble vitamins (A, D, E, and K).
- Slows the passage rate of digesta through the gastrointestinal tract and, as a consequence, improves the absorption of other nutrients. In several studies, greater concentration of dietary lipids has been shown to improve the digestibility of amino acids by increased time of contact with absorptive cells (Cervantes-Pahm and Stein, 2008), and the digestibility of dietary fiber by allowing more time for microbial fermentation (Washburn, 1991). This is referred to as the extra-caloric effect of fat.
- Increases feed efficiency, because of their lower heat increment in comparison with other ingredients (Cho and Kim, 2012). In addition, the

lower heat increment of fat reduces the endogenous heat production, which can be advantageous in warm climates where feed intake is compromised due to caloric stress (Stahly and Cromwell, 1979; Coffey et al., 1982).

b) *Technological aspects:*
   - Diminishes pulverulence, because of their ability to bind dust.
   - Reduces particle separation in mash diets.
   - Lubricates machinery during pelleting, improving their yield and lifetime.

c) *Other aspects:*
   - Increases palatability, because it improves feed texture and organoleptic properties of feed.
   - Lubricates food bolus during mastication and swallowing.
   - Affects meat quality (Madsen et al., 1992), since dietary FA strongly influence the FA composition of the end-product.

For feed and meat producers, the priority is to use high quality fat sources at competitive prices, without health risk and ensuring the quality and safety of the final products.

1.2. What are oils and fats?

Lipids are generally defined as a group of naturally occurring compounds insoluble in water, but soluble in organic substances such as benzene, ether and chloroform. They include a diverse range of compounds, like FA and their derivatives, carotenoids, terpenes, steroids and bile acids. It is beyond the scope of this thesis to describe all classes of lipids, so main emphasis has been given on those lipid classes that are present in dietary oils and fats.

Fats and oils used in animal feeds consist predominantly of glyceryl esters of FA, with some nonglyceridic materials present in small quantities. The terms fats and oils are used interchangeably, and the choice of terms is usually based on the physical state of the material at ambient temperature and tradition. Generally, fats appear solid at ambient temperatures and oils appear liquid. The physical properties of fats and oils are determined by the length and saturation degree of the carbonic chain of FA. Based on the aliphatic-chain length, FA are divided into different classes: short-chain FA contain less than 6 carbon atoms, medium-chain FA contain FA with 6 to 12 carbon

atoms, long-chain FA contain FA with 14 to 20 carbon atoms, and very-long-chain FA contain more than 20 carbon atoms. The number of double bonds normally ranges from 0 to 6 in the most common dietary FA. Dependent on the number of double bonds, FA can be divided into saturated fatty acids (**SFA**; absence of double bonds) and unsaturated fatty acids (**UFA**). Normally, double bonds are of *cis*- configuration, although some FA with *trans*- double bonds are known. The UFA can be further divided into monounsaturated fatty acids (**MUFA**; presence of one double bond) and polyunsaturated fatty acids (**PUFA**; presence of more than one double bond). Polyunsaturated fatty acids, in turn, are also divided into families dependent on the position of the first double bond in the carbon chain counted from the methyl end of the FA: the ω-3 or n-3 family is based on α-linolenic acid, the ω-6 or n-6 family is based on linoleic acid, whereas the ω-9 or n-9 family is based on oleic acid. Names and formulas of common FA are presented in **Table 1.1**.

Table 1.1. Nomenclature and chemical notation of the most common fatty acids

Systematic nomenclature	Trivial nomenclature	Notation
Octanoic acid	Caprylic acid	C8:0
Decanoic acid	Capric acid	C10:0
Dodecanoic acid	Lauric acid	C12:0
Tetradecanoic acid	Myristic acid	C14:0
Hexadecanoic acid	Palmitic acid	C16:0
cis-9-hexadecenoic acid	Palmitoleic acid	C16:1 n-9
Octadecanoic acid	Stearic acid	C18:0
cis-9-octadecenoic acid	Oleic acid	C18:1 n-9
cis-11-octadecenoic acid	Vaccenic acid	C18:1 n-7
All-*cis*-9,12-octadecadienoic acid	Linoleic acid	C18:2 n-6
All-*cis*-9,12,15-octadecatrienoic acid	α-Linolenic acid	C18:3 n-3
All-*cis*-6,9,12-octadecatrienoic acid	γ-Linolenic acid	C18:3 n-6
Eicosenoic acid	Arachidic acid	C20:0
cis-11-eicosenoic acid	Gondoic acid	C20:1 n-9
All-*cis*-11, 14-eicosadienoic acid	Eicosadienoic acid	C20:2 n-6
All-*cis*-8,8,11,14-eicosatetraenoic acid	Arachidonic acid	C20:4 n-6
All-*cis*-5,8,11,14,17-eicosapentaenoic acid	Eicosapentaenoic acid	C20:5 n-3
All-*cis*-4,7,10,13,16,19-docosahexaenoic acid	Docosahexaenoic acid	C22:6 n-3

1.2.1. Native oils

Native oils are mainly composed of triacylglycerols (**TAG**; usually > 95%), accompanied by lower levels of diacylglycerols (**DAG**), monoacylglycerols (**MAG**) and free fatty acids (**FFA**), and minor lipid components such as phospholipids and

glycolipids. A TAG consists of a glycerol backbone to which three FA are acylated, each with its own chain length and degree of saturation. The three hydroxyl groups are numbered by the stereospecific numbering system, designating the FA to the position *sn*-1, *sn*-2, and *sn*-3 (**Figure 1.1**).

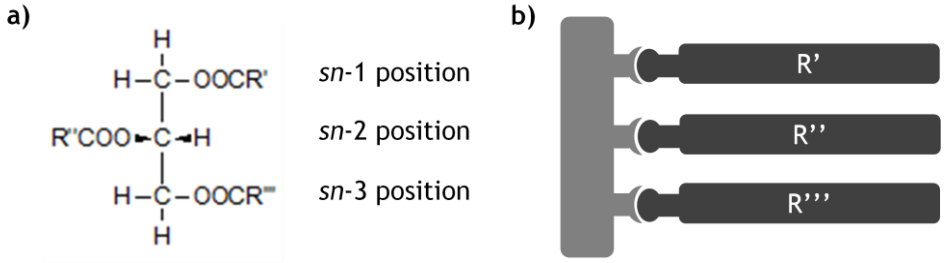

Figure 1.1. Stereochemical configuration (a) and schematic representation (b) of a triacylglycerol molecule. R', R'', and R''' are the fatty acids acylated at the sn-1, sn-2, and sn-3 positions, respectively, according to the stereochemical nomenclature.

The possible number of different TAG including enantiomers is n^3, where n is the number of FA present. Considering that many dietary fats and oils often have five or more major FA, the number of potential individual TAG becomes enormous. However, the actual number of TAG molecules is smaller than the theoretical number due to preferential acylation of FA to particular positions and in particular molecular species (Small, 1991; Mu and Høy, 2004; Berry, 2009). **Table 1.2.** shows the primary TAG species, and the FA composition and distribution of some major native oils and fats. The proportion of a particular FA that is located at the *sn*-2 position is presented in brackets. When the distribution is random, 33% of a particular FA is present at each position. A percentage of, for example, 10 for palmitic acid in palm oil means that 10% of all palmitic acid in palm oil is present at the *sn*-2 position. This implies that 90% of palmitic acid is esterified at either the *sn*-1 or *sn*-3 positions. Generally, linoleic acid from vegetable oils is predominantly positioned at the *sn*-2 position, while the proportion of palmitic acid at the *sn*-2 position is low. However, the FA positional distribution in animal fats is different. Palmitic acid in tallow is fairly evenly distributed, while in lard this FA is the predominant FA in the *sn*-2 position.

Table 1.2. Primary triacylglycerol species (Small, 1991; Mu and Høy, 2004; Berry, 2009), fatty acid composition (% of total fatty acids), and fatty acid positional distribution (% of each fatty acid that is located at the sn-2 position of acylglycerol molecules; presented in brackets; Berry, 2009) of some of the most common native oils and fats

Fat source	Major TAG species[1]	Fatty acids (*sn*-2 %)				
		C16:0	C18:0	C18:1 n-9	C18:2 n-6	C18:3 n-3
Vegetable oil						
Palm	POP, POO, POL	45 (10)	4 (18)	38 (60)	10 (60)	tr (-)
Soybean	LLL, LLO, LLP	11 (6)	4 (3)	22 (31)	54 (43)	8 (29)
Rapeseed	OOO, LOO, OOLn	5 (11)	2 (11)	57 (30)	22 (47)	10 (44)
Sunflower	LLL, OLL, LOO	7 (20)	4 (21)	22 (33)	65 (35)	tr (-)
Animal fat						
Tallow	POO, POP, POS	27 (28)	19 (14)	35 (52)	5 (85)	tr (-)
Lard	SPO, OPL, OPO	27 (81)	17 (7)	39 (11)	11 (12)	tr (-)

TAG = triacylglycerols

[1] Abbreviations used to describe acyl chains of triacylglycerols: P = palmitic (C16:0); S = stearic (C18:0); O = oleic (C18:1 n-9); L = linoleic (C18:2 n-6); Ln = linolenic (C18:3 n-3).

Concerning the stock availability of crude native oils, the global annual production of the main vegetable oils is about 174.9 Mt (2014/15 forecast; USDA, 2014). **Figure 1.2** shows the production levels in 1994/95 through 2014/15 (forecast) for the four major vegetable oils. Over this 20-year period, the production of these four oils together has increased by 97.1 Mt. This has come from palm oil (47.5 Mt), soybean oil (26.4 Mt), rapeseed oil (16.0 Mt), and sunflower oil (7.2 Mt). The graph shows clearly that the production of palm oil has exceeded that of soybean oil since 2004/05 and that these two oils are increasingly dominant in the market, representing 63% of the total world vegetable oil production. Indonesia, Malaysia and Thailand are the major palm oil producing countries, and the Malaysian price of palm oil on 2013/14 was 612 €/t (USDA, 2014). On the other hand, USA, China, Argentina and Brazil are the main soybean oil producers, and the Rotterdam price of soybean oil on 2013/14 was 722 €/t (USDA, 2014).

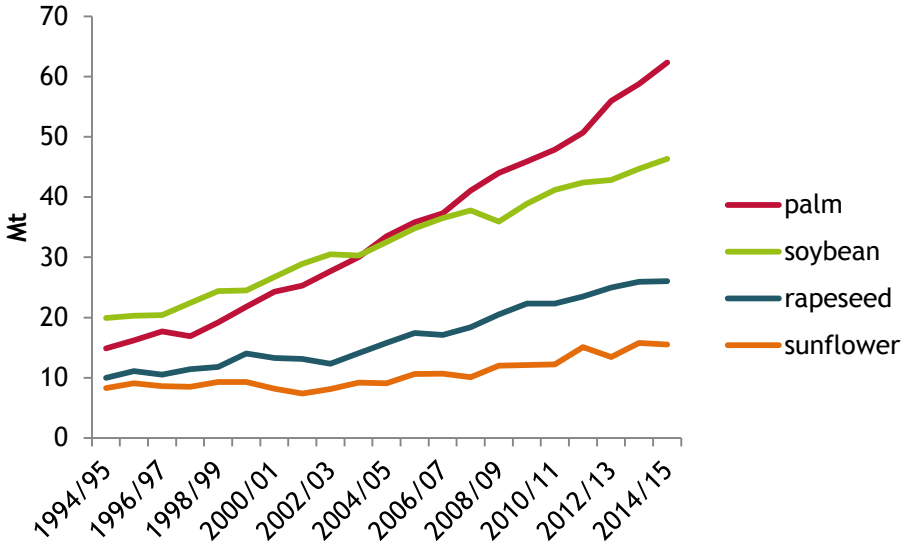

Figure 1.2. Global production (Mt) of the four major vegetable oils (palm, soybean, rapeseed, and sunflower) over 20 years between 1994/95 and 2014/15 (forecast) (USDA, 2014).

1.2.2. Fatty by-products – Acid oils

"Fatty by-products" are defined as those fatty substances which directly derive from primary industrial transformation processes of crude native oils. Acid oils are by-products obtained from the refining process of crude native oils. The aim of the refining process is to reduce the FFA content and to remove other gross impurities such as phospholipids, oxidized materials, colored materials, metals and undesirable flavors present in crude native oils (**Figure 1.3**). The most common method of refining is by treating the fat or oil with an alkali solution, i.e. chemical refining. This results in a large reduction of FFA through their conversion into water-soluble soaps. During this process, a similar proportion of TAG is emulsified, so that the end product contains similar proportions of FFA and TAG. In contrast, oils with a low phospholipid content, such as palm oil, may undergo vacuum distillation, i.e. physical refining. This process makes use of the lower boiling point of the FFA when compared to the boiling point of TAG. From both refining processes, valuable by-products (acid oils) are obtained. The main difference between acid oils coming from both refining processes is their FFA content. Thus, acid oils coming from chemical refining have a lower FFA content (>50%) than those coming from physical refining (>90%). In a previous European project (ref. FP6 FOOD-CT-2004-007020), several samples of acid oils coming from both chemical and physical refining processes were analyzed. Besides the FFA content,

differences corresponding to composition, oxidation level, and presence of contaminants were also observed (Nuchi et al., 2009).

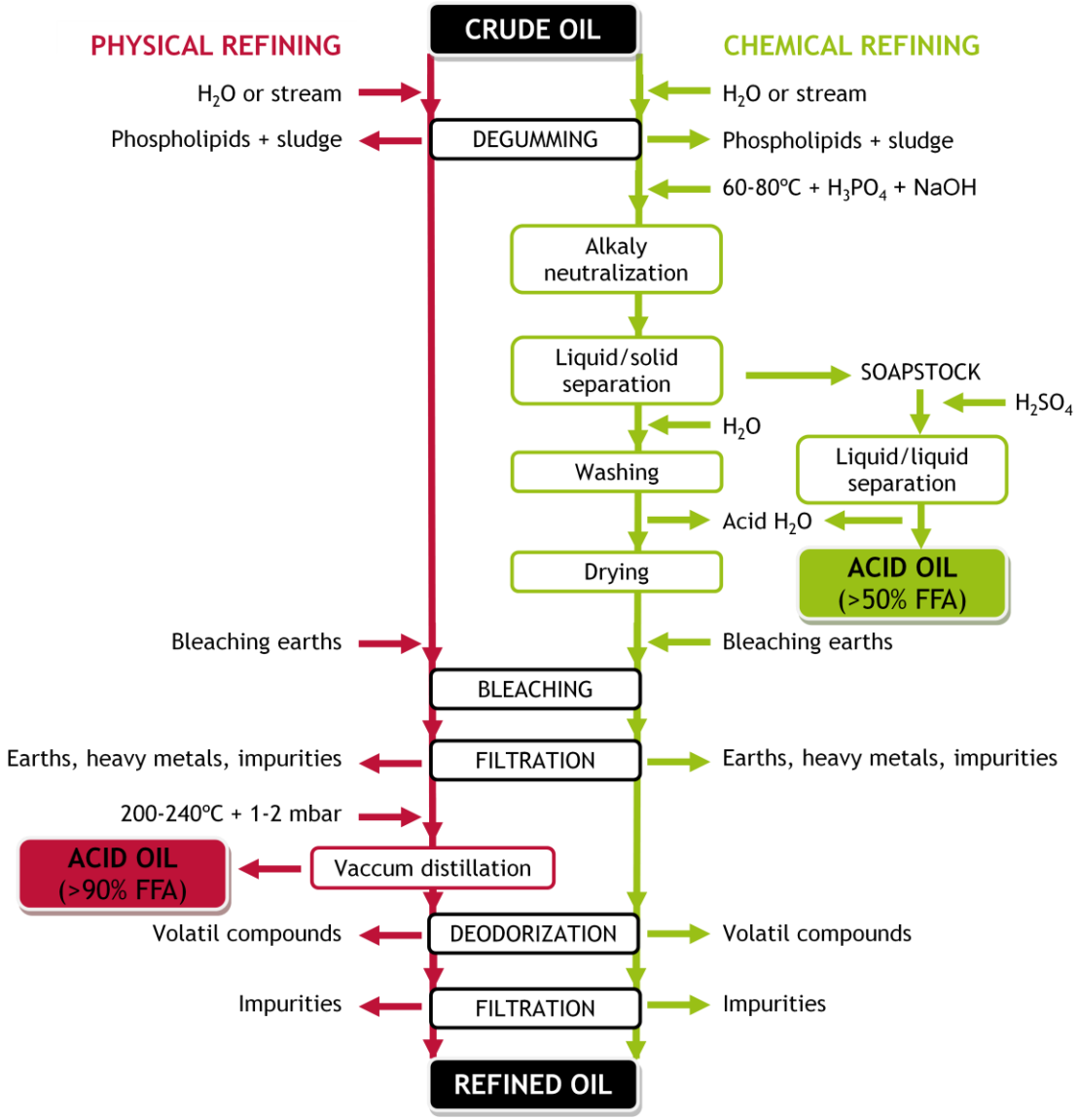

Figure 1.3. Scheme of the physical and chemical refining processes of crude oils.
FFA = free fatty acids.

It is difficult to estimate the world acid oil stock availability, since not all crude oils are refined (animal nutrition and industrial use). To calculate the approximate amount of acid oils obtained from the refining processes, the initial FFA content of crude oils (typically ranging between 1 to 5% of FFA) and the percentage of crude oil which flows during the refining process into the FFA (1% and 0.1% for each 1% of FFA in the chemical and physical refining process, respectively) must be considered (Parini and Cantini, 2009). Therefore, during the refining process, acid oils are generated in

amounts normally ranging between 1.1 to 10% of the total processed oil. These acid oils, as by-products, are traded at a discount relative to crude or refined native oils. In either case, the crucial point that decides whether acid oils are profitable is the price differential between the native oil and the acid oil, which fluctuates. For example, according to the Malaysian Palm Oil Board, before October 2009, the discount between acid and native palm oil typically exceeded 150 €/t, and it was as high as 500 €/t in May 2008. However, since November 2009, the price differential between acid and native palm oils narrowed. In early 2010 the discount of acid with respect to native palm oil was less than 75 €/t (Cheah, 2010). To our knowledge, there are no official statistics on stock availability and prices of acid oils.

Acid oils are economically interesting alternatives to native oils. Furthermore, the reintroduction of these by-products into the food chain via the animal production step would contribute to get more environmentally friendly production systems. However, it is well known that acid oils have a lower energetic value than their corresponding native oils.

1.2.3. Technical lipids – Re-esterified oils

"Technical lipids" are defined as fat sources obtained through secondary technological processes, which acquire special characteristics for specific nutritional purposes. Today, lipid modification strategies in the food industry include fractionation, hydrogenation, and interesterification. It is beyond the scope of this thesis to describe all these procedures. Only special emphasis has been given to interesterification, sometimes also called esterification, because this is the process used to obtain re-esterified oils; our object of study.

The interesterification reaction involves the rearrangement of FA within and between acylglycerol molecules, so the molecular structure of native oils is changed. However, this process does not change the degree of saturation or the isomeric state of FA, as they only shift from one position to another (Lida et al., 2002). The basic objective in modifying fats is to take advantage of how fat is digested, and change the molecular structure of acylglycerol molecules in such a way as to increase the absorption of products resulting from fat digestion. Another goal is to modify the

melting and crystallization behavior of dietary fats for their use in the food industry. In fact, the interesterification technique has been widely adopted as a substitute of partial hydrogenation of fats, because it increases the melting point of fats without leading to the generation of SFA and *trans* FA, which have been reported to have detrimental effects on human health (Destaillats et al., 2007).

According to the catalyst used in the reaction, two types of interesterification reactions are available:

a) *Chemical interesterification* (relatively uncontrolled) produces a complete randomization of acyl groups in acylglycerol molecules by using chemical catalysts (most often sodium methoxide), although under particular conditions, the reaction can also be carried out avoiding the use of a catalyst (Feltes et al., 2013). For example, chemical interesterification of native palm oil increases the proportion of palmitic acid at the *sn*-2 position. However, in lard, this interesterification decreases the proportion of palmitic acid at the middle position (Renaud et al., 1995).

b) *Enzymatic interesterification* (highly controlled) produces a specific positional distribution of FA in acylglycerol molecules by using lipases with regio-specificity. With this technique it is possible to design specific structured lipids for specific nutritional purposes, such as a human breast milk fat substitute, which has a very similar FA composition and distribution to human milk fat (Betapol™).

Nowadays, chemical rather than enzymatic interesterification is preferred because of the lower investment and production costs involved, since chemical catalysts are much less expensive than lipases. However, it must be taken into account that the high temperatures applied for the chemical interesterification process may lead to oil oxidation, resulting in the development of off-flavors and a dark color (Feltes et al., 2013). To date, only high value-added fat products have been processed through enzymatic esterification.

In addition, depending on the nature of the substrates, there are three types of reactions associated with interesterification:

a) *Transesterification* (TAG-TAG). Exchange of acid radicals from one ester to another.

b) *Acidolysis* (FFA-TAG). Reaction of fatty esters with an acid (usually FFA).

c) *Alcoholysis* (Alcohol-TAG). Reaction between fat and alcohol. The alcoholysis of TAG with glycerol is called glycerolysis and is widely used for MAG and DAG production, although TAG molecules can also be obtained.

MAG and DAG have one and two FA esterified to the glycerol backbone, respectively (**Figure 1.4**).

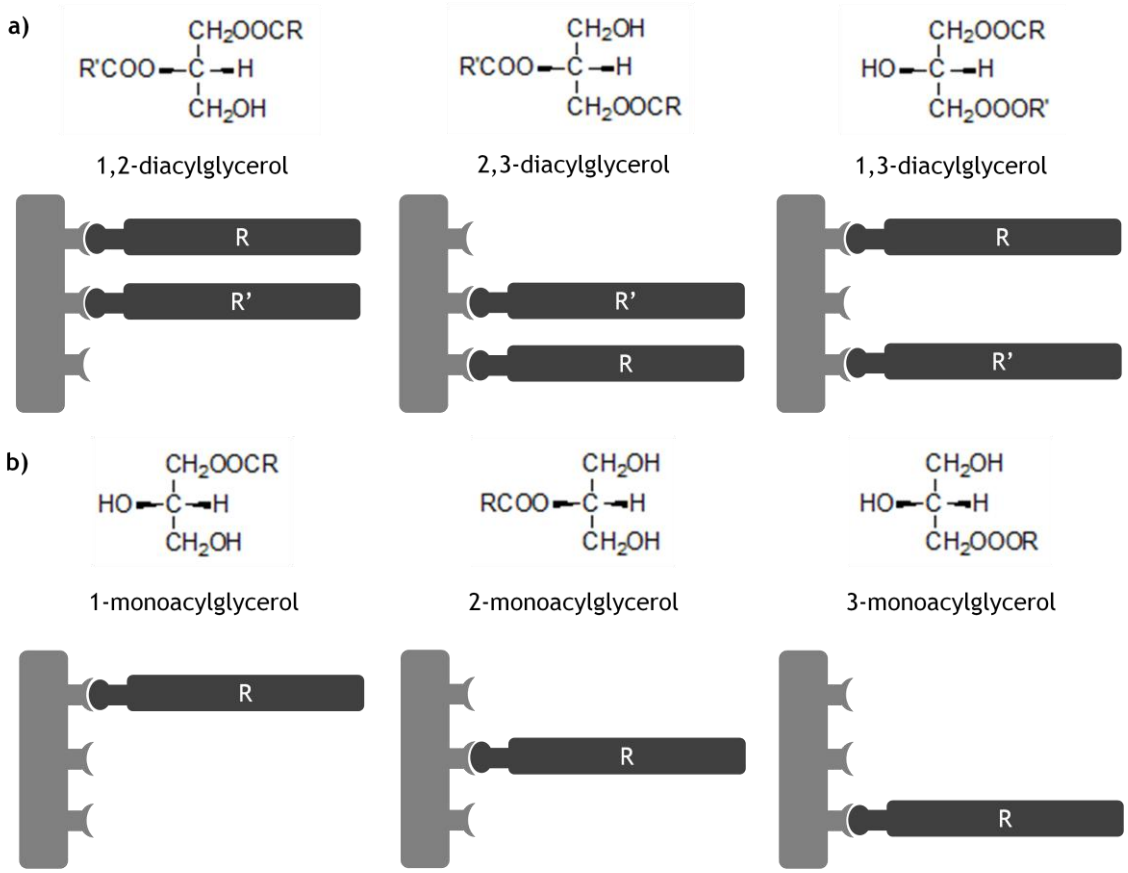

Figure 1.4. Stereochemical configuration and schematic representation of diacylglycerol (a) and monoacylglycerol (b) molecules.

Due to their amphiphilic properties, MAG and DAG are anionic oil-water emulsifiers. They rarely occur in large quantities in nature, but are primarily intermediate products of TAG digestion. Although the positional isomers of DAG and MAG that occur in the process of TAG digestion are 1(3),2-DAG and 2-MAG, a substantial fraction of DAG and MAG in re-esterified oils are present as 1,3-DAG and 1(3)-MAG, due to acyl migration. This is because primary esters (*sn*-1 and *sn*-3) are thermodynamically more stable than the secondary ester (*sn*-2) (Crossley et al., 1959; Lo et al., 2008).

The glycerol molecule needed for the glycerolysis reaction is another low cost by-product obtained from biodiesel production. Glycerol is a trihydroxy sugar alcohol that is an intermediate in the carbohydrate and lipid metabolisms. The term "glycerol" is only applicable to the pure chemical compound, while the term "glycerin" normally applies to the commercial product, after removing salts, methanol, and FFA. In general, biodiesel production generates 10% crude glycerin (Quispe et al., 2013). As biodiesel production increases, so does production of the primary by-product. A world production of 1.2 Mt of glycerin was forecasted for the year 2012 (Feltes et al., 2013). From the 1970s until the year 2004, high-purity glycerin had a stable price between 900 and 1,400 €/t. Recently, however, the price of crude glycerin has fallen to about 60-80 €/t, due to the glut in the glycerol market (Johnson and Taconi, 2007; Quispe et al., 2013).

Re-esterified oils, our object of study, are obtained through a modification of the glycerolysis reaction. They are produced using, as raw materials, acid oils (rich in FFA) instead of native oils (rich in TAG). As a consequence, re-esterified oils are obtained through a direct esterification of glycerol with FFA (**Figure 1.5**). This is a way of trying to give added value to acid oils and to re-introduce these by-products to the feed industry, reducing the amount of residues and contributing to more sustainable feed manufacturing systems. If the price differential between native and acid oils is high, then, the cost of the esterification process (about 100 €/t; personal communication) can be compensated by the lower costs of the raw materials. In this case, re-esterified oils could be competitive with regard to crude native oils (Parini and Cantini, 2009).

Back to the manufacturing process, one important drawback of the esterification reaction is the low miscibility between the substrates (the system comprises a hydrophobic oil phase and a hydrophilic glycerin phase). However, as the reaction proceeds, the MAG and DAG obtained may act as emulsifiers of the hydrophobic and hydrophilic phases, improving the contact between both substrates and therefore enhancing the conversion rates. The amount of MAG, DAG, and TAG obtained in the final product can be adjusted during the reaction design, by setting the stoichiometric glycerol-to-FA ratio (**Figure 1.5**; Parini and Cantini, 2009). The reaction is generally carried out at 190-250ºC in a stirred reactor under a residual pressure of 1-3 mm Hg. The reaction is an equilibrium that can be shifted towards the end products by removing the water from the product. Thus, water is continuously removed from the reaction vessel and glycerol is condensed and refluxed into the reactor by means of a hot condenser, set at a temperature of approximately 80-100ºC (**Figure 1.6**).

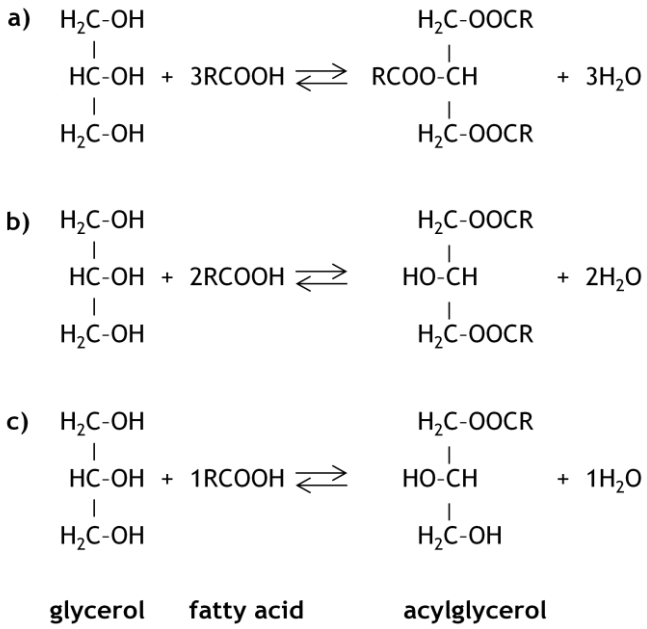

Figure 1.5. Esterification reaction. Formation of triacylglycerols (a), diacylglycerols (b), and monoacylglycerols (c) from glycerol and free fatty acids.

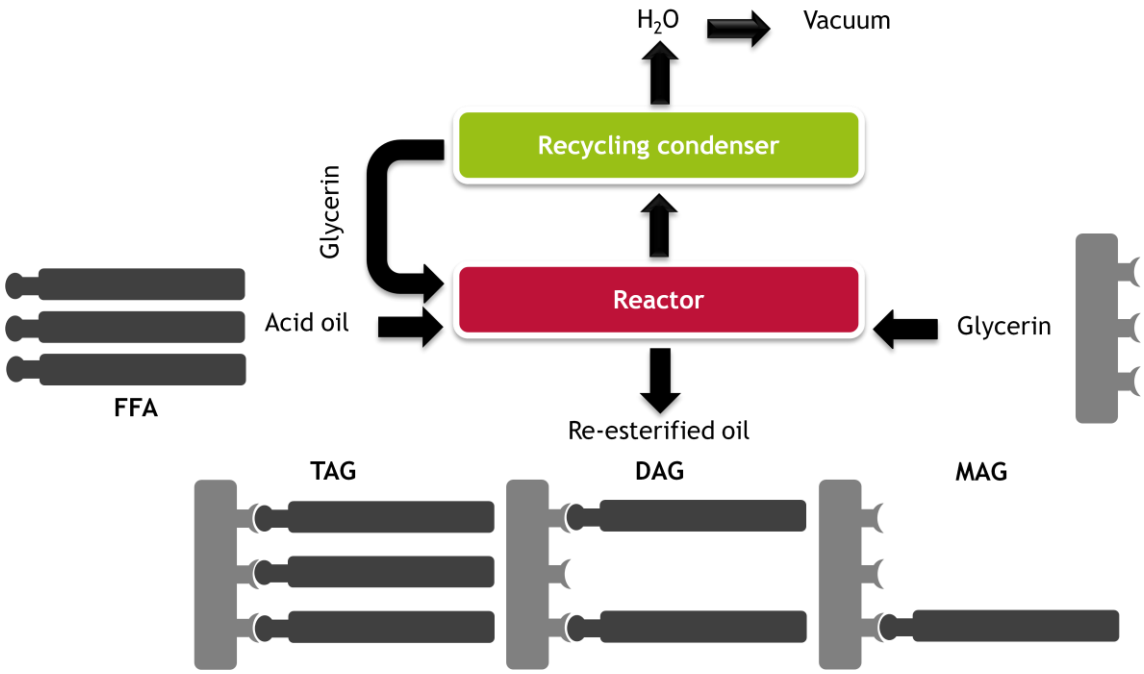

Figure 1.6. Scheme of the esterification process.

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; TAG = triacylglycerols.

The purpose of this project is whether it is possible to give added value to acid oils, through direct esterification of FFA with glycerol.

1.3. Fat digestion, absorption and metabolism

In this section, the most relevant processes that take place during digestion, absorption and metabolism of fat are described. Much of the knowledge of these processes has been derived from studies carried out with rats and humans. Although the broad evidence available suggests that the basic physico-chemical mechanisms involved in these processes are similar in all monogastric species, we have tried to mention the peculiarities related to pigs and broiler chickens. Mammals and birds, despite having many similarities with regard to lipid digestion, absorption and metabolism processes, show some differences which seem to come, basically, from their different anatomical structures: birds have a muscular stomach (the gizzard) and have almost no lymphatic system. These anatomical differences will condition some differences in lipid digestion and transport processes.

1.3.1. Digestion process

Fats and oils are not water soluble, meaning that they are not able to pass through the unstirred-water layer that surrounds the surface of the small intestine. Therefore, the overall process of fat digestion (**Figure 1.7)** is designed to convert fats into more polar derivatives through three sequential steps:

a) The dispersion of bulk fat globules into finely divided emulsion particles.

b) The enzymatic hydrolysis of FA esters by specific lipases.

c) The aqueous dispersion of lipolytic products in bile-salt micelles.

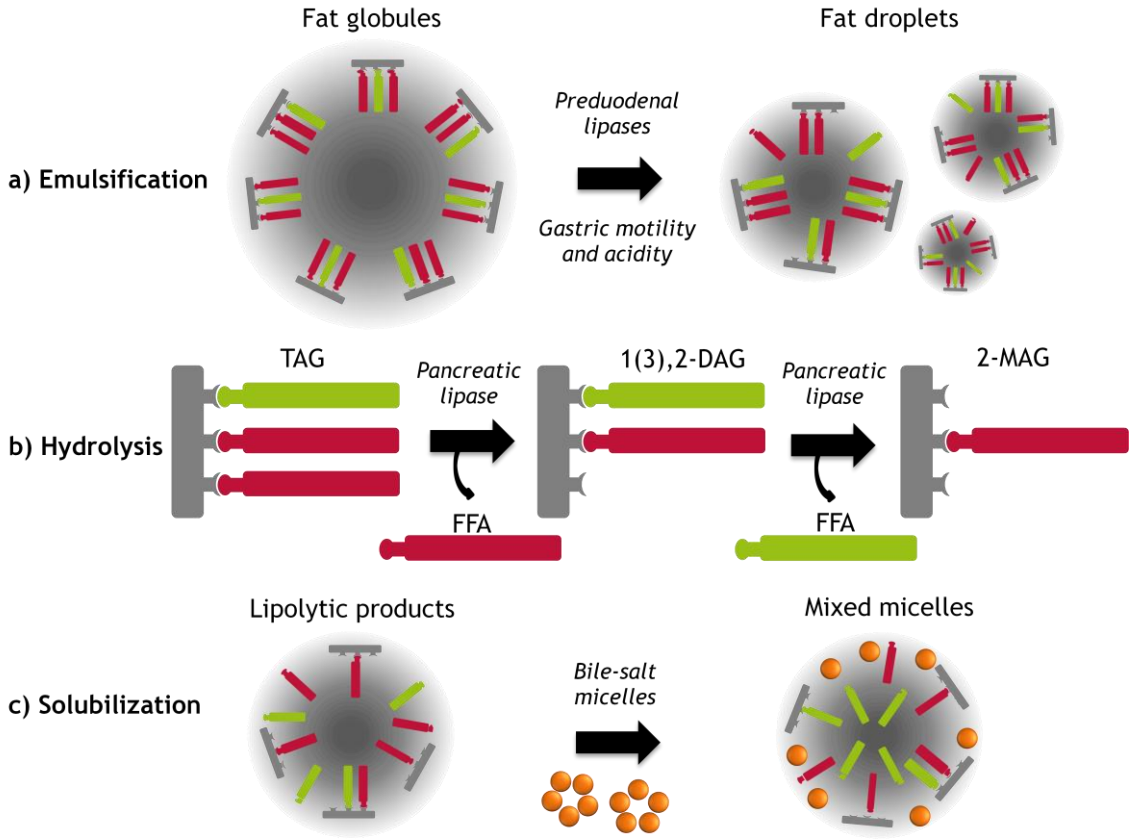

Figure 1.7. Diagrammatic representation of fat digestion in the gastrointestinal tract, through three sequential steps: a) emulsification, b) hydrolysis, and c) solubilization. *DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; TAG = triacylglycerols*

Initially, the large fat globules, with a relatively small surface-to-volume ratio, are emulsified. In the gizzard or stomach (depending on species), fats are warmed to body temperature and the lipid globules are broken down into droplets thanks to gastric motility and acidity. This promotes the action of pre-duodenal lipases, resulting in partial digestion of lipids. Lingual lipase and gastric lipase are two enzymes secreted from the serous glands and the gastric mucosa, respectively, which are important in the hydrolysis of fat in the stomach. While lingual lipases are important in some species (i.e. rats and mice; Hamosh and Scow, 1973), in pigs, enzymatic digestion of fat is started by gastric lipases (Jensen et al., 1997). To the best of our knowledge, there are no reports describing lipases of the upper gastrointestinal tract in birds. Pre-duodenal lipases have a low optimal pH (2.5-6.5), preferentially cleave FA located at the *sn*-3 position of the TAG molecules, and hydrolyze TAG that contain medium-chain FA faster than those containing long-chain FA.

Pre-duodenal lipases are rapidly inactivated by pancreatic trypsin and therefore cease to be active when the food bolus passes into the duodenum. As food arrives in the small intestine, the secretion of sodium bicarbonate neutralizes the acidic food bolus, raising the pH between 5.0 and 7.5. This brusque change in pH causes an abrupt change in the physical behavior of FFA in the crude emulsion. They become partly charged (ionized), migrate to the interface of the emulsion particle, and commence limited "spontaneous emulsification" (Salentinig et al., 2011). On the other hand, the presence of food in the duodenum stimulates the secretion of cholecystokinin, which, in turn, induces the secretion of biliary and pancreatic juices. Bile salts help to stabilize the emulsion droplets for the adsorption of the lipase-colipase complex (Salentinig et al., 2011). Pancreatic lipase is an interface-active enzyme that acts at the oil/water interfaces of the emulsion droplets, and is secreted in an active form. This enzyme specifically hydrolyzes the primary (*sn*-1 and *sn*-3) ester bonds of TAG to give 2-MAG and the corresponding FFA (Mattson and Beck, 1956). While pure pancreatic lipase works inefficiently in a bile salt-lipid mixture, lipase present in pancreatic juice hydrolyzes TAG extremely efficiently. This apparent discrepancy led to the discovery of the cofactor called colipase. This cofactor is synthesized and secreted by the pancreas as pro-colipase and is activated to colipase in the small intestine by trypsin hydrolysis (Borgström et al., 1979). Colipase protects pancreatic lipase from denaturation and anchors it to the lipid-water interphase of the droplet, permitting the access of lipase to the inner core of TAG. When this cofactor is not present, bile salts inhibit lipase activity, probably by displacing it from the surface of the emulsified lipid droplets (Borgström, 1975). Thus, pancreatic lipase, colipase, and bile salts act synergistically in the lipid hydrolytic reactions of the upper small intestine.

As a result of the combined activities of pancreatic enzymes, the primary products of lipolysis are 2-MAG and FFA, with 1(3),2-DAG and glycerol as minor components. The aqueous solubility of these products is relatively low and, therefore, they are removed from the water-oil interface by incorporation into bile-salt micelles and transported to the site of fat absorption. The first compounds which are spontaneously incorporated into bile-salt micelles are MAG, medium-chain FA, long-chain UFA, and phospholipids, which have amphiphilic properties. The entry of these compounds expands the mixed micelle, generating a hydrophobic core that acts as liquid crystals with the ability to solubilize other more water-insoluble products, such as long-chain SFA, DAG, fat-soluble vitamins and cholesteryl esters (Krogdahl, 1985) . These assemblies diffuse through the unstirred-water layer to the brush-border membrane of the enterocytes, where they are absorbed.

1.3.2. Absorption process

Lipid absorption takes place at the apical part of the plasma membrane of the epithelial cells lining the gut. In common with other monogastric species, the major intestinal site of fat absorption in the pig and the chick is the jejunum (Freeman, 1984). In the fowl, however, uptake of lipids has also been shown to occur in the duodenum (Hurwitz et al., 1973). This proximal lipid absorption appears to be associated with a reflux or 'antiperistaltic' activity observed in this region (Krogdahl, 1985).

Although the precise mechanism by which the lipolytic products pass from the mixed micelles into the mucosal cells has not yet been determined, the leading theory suggests that the disruption of micelles is necessary before fat absorption. Once micelles dissociate, the lipolytic products are incorporated in the external half of the lipid bilayer of the brush border membrane (Schulthess et al., 1994). Subsequently, these compounds are assumed to diffuse either passively across the membrane or alternatively in a facilitated, protein-mediated process. Depending on the aqueous solubility of the lipolytic products, they are absorbed as follows:

a) 2-MAG and short- and medium-chain FA are taken up mainly by passive diffusion across the membrane driven by a concentration gradient (Schulthess et al., 1994; Trotter et al., 1996; Ho and Storch, 2001).

b) Long-chain FA enter inside the cells both by simple diffusion or by an active process mediated by binding and carrier proteins (Ho and Storch, 2001).

After lipid absorption, bile salts remain in the intestinal lumen. Since bile salts are not absorbed in the upper small intestine, they are continuously re-utilized for subsequent micelle formation. Most of them are absorbed in the distal ileum by an active transport system and return to the liver, where they are recycled. A passive absorptive mechanism also exists in the jejunum for undissociated bile acids. However, because under normal physiological conditions bile salts in this region of the intestine are conjugated, little passive absorption occurs in the jejunum (Freeman, 1984). In contrast to mammalian species, the active absorption mechanism for bile salts appears to be more extensively located throughout the length of the small intestine in birds. Thus, similar rates of bile salt absorption occur in both the jejunum and ileum (Hurwitz et al., 1973).

Undigested acylglycerols (TAG and DAG) and some FFA, due to their low or null solubility in bile salt solution, remain as insoluble fat droplets in the gut aqueous medium and they are finally lost in feces or excreta (da Costa, 2003).

1.3.3. Metabolism process

Fat metabolism includes the mucosal reassembly of the absorbed lipolytic products into lipoprotein particles, their distribution and storage in body fat reserves, as well as fat mobilization (lipolysis) and *de novo* fat synthesis (lipogenesis). Because fat metabolism is an extensive field, this section only gives a brief overview of fat metabolism and how it might be affected by dietary fat.

1.3.3.1. Mucosal triacylglycerol re-synthesis

Once in the enterocytes, the subsequent fate of FA depends on their chain-length. Short- and medium-chain FA pass from the mucosal cells directly without re-esterification and bound to albumin to the portal blood. However, 2-MAG and FA with more than 12 carbon atoms are bound to intracellular FA-binding proteins, transferred to the endoplasmic reticulum (Ockner and Manning, 1974), and re-esterified into TAG (Sethi et al., 1993).

The re-esterification process can occur in two different ways either the MAG pathway or the glycerol-3-phosphate pathway (**Figure 1.8**). In the enterocytes, when 2-MAG are available, the MAG pathway is the predominant one, re-synthesizing as much as 80-100% of the TAG, because 2-MAG inhibits the glycerol-3-phosphate pathway (Lehner and Kuksis, 1996). However, in absence of MAG, the glycerol-3-phosphate pathway becomes the major pathway for the formation of TAG. Therefore, when feeding dietary fats with high amounts of FFA (i.e. acid oils) or 1,3-DAG and 1(3)-MAG (i.e. re-esterified oils), it is expected that the glycerol-3-phosphate pathway increases their importance. However, it has been suggested that a deficit of 2-MAG can limit the re-synthesis of TAG in the enterocytes, and FA can escape directly into the portal vein (Murase et al., 2002).

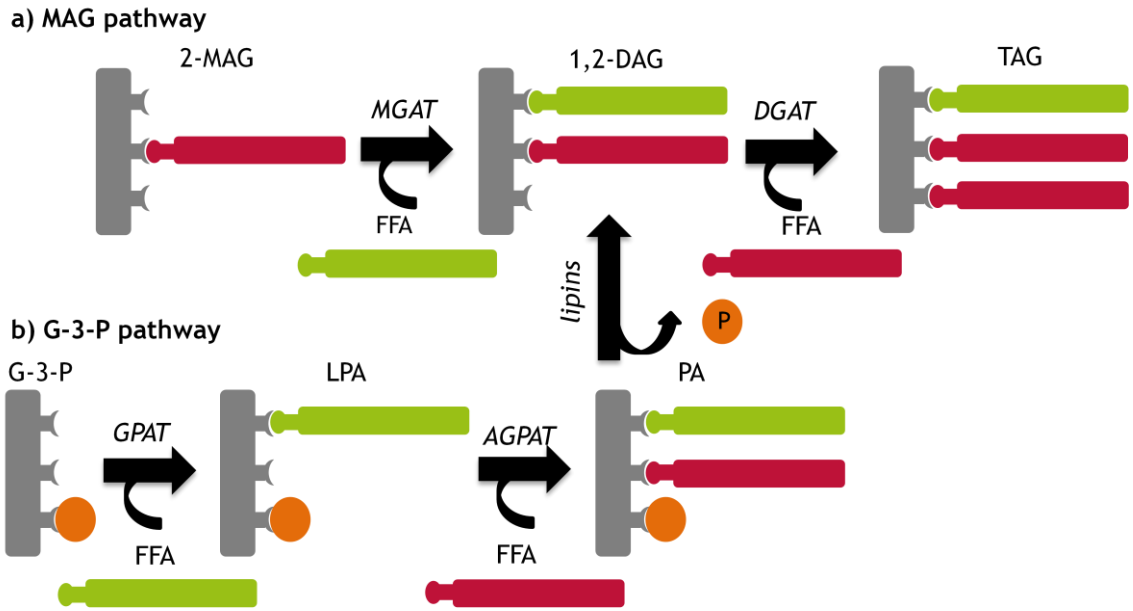

Figure 1.8. Scheme of mucosal triacylglycerol re-synthesis through a) monoacylglycerol pathway or b) glycerol-3-phosphate pathway.

AGAPAT = 1-acylglycerol-3-phosphate O-acyltransferase; DAG = diacylglycerols; DGAT = diacylglycerol acyltransferase; FFA = free fatty acids; G-3-P = glycerol-3-phosphate; GPAT = glycerol-3-phosphate O-acyltransferase; LPA = lysophosphatidic acid; MAG = monoacylglycerols; MGAT = monoacylglycerol acyltransferase; PA = phosphatidic acid; TAG = triacylglycerol

1.3.3.2. Transport

The newly re-synthesized TAG are transported to the Golgi apparatus where they are re-packaged into lipoproteins. These particles contain a core of TAG and cholesteryl esters covered by a surface layer of amphiphilic compounds such as phospholipids, cholesterol, and apolipoproteins (Sethi et al., 1993). It is in this form that re-esterified TAG are stable enough to be transported from the intestinal mucosal cells to the systemic circulation (aqueous environment). In mammals, these lipoproteins are transported to the general circulation via lymph. This is why they are called chylomicrons (**CM**) (Sethi et al., 1993). In contrast, because the intestinal lymphatic system is poorly developed in birds, lipoproteins are secreted directly into the portal venous system and, therefore, they are termed portomicrons (Krogdahl, 1985). Although portomicrons pass through the liver before they reach the rest of the circulation, these particles are too large to go through the cellular sieve of the hepatic capillary bed and be metabolized (Hermier, 1997). Therefore, for simplicity, in the remaining text, the term "chilomicron" will refer to both chylomicrons and

portomicrons. **Figure 1.9** shows the scheme for the transport of TAG and cholesterol in blood.

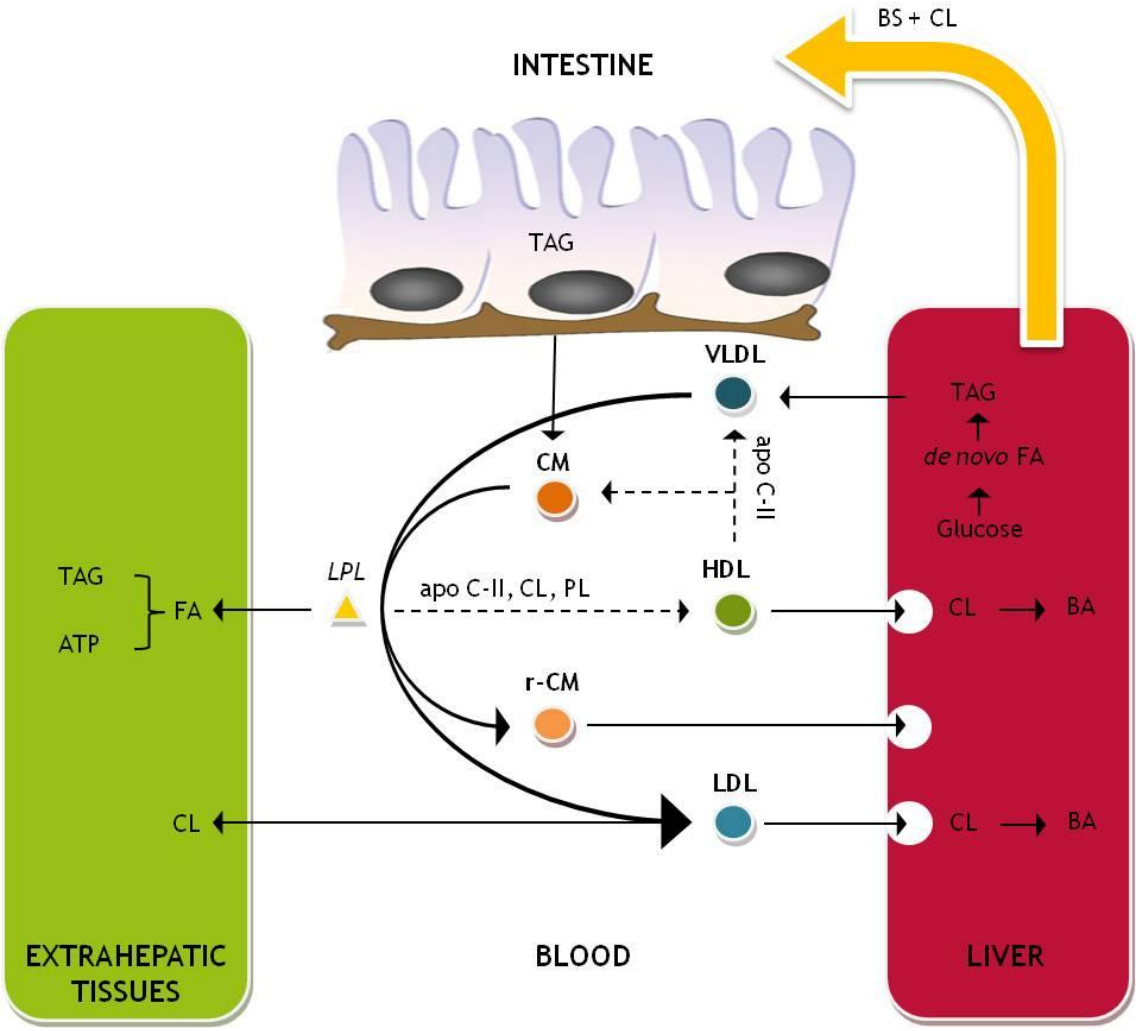

Figure 1.9. Scheme of the transport of triacylglycerols and cholesterol in blood.
The classes of lipoproteins shown are: CM = chilomicrons (in birds they are called portomicrons); r-CM = remnant chilomicrons; VLDL = very-low-density lipoproteins; LDL = low-density lipoproteins; HDL = high-density lipoproteins. The enzyme involved in the process of lipoprotein metabolism: LPL = lipoprotein lipase. The lipid compounds involved in the lipid metabolism process: TAG = triacylglycerols; FA = fatty acids; CL = cholesterol; BA = bile acids; BS = bile salts; PL = phospholipids.

The function of very-low-density lipoproteins (**VLDL**) is similar to that of CM, but instead of transporting TAG from exogenous origin (diet), they are from endogenous origin (synthesized in the liver). Thus, during fasting, VLDL are the major lipoproteins, however, CM become the major lipoproteins following fat feeding (Mu

and Høy, 2004). Once both lipoproteins have acquired apolipoprotein C-II from the high-density lipoprotein (**HDL**) fraction, they are capable of activating lipoprotein lipase; the key enzyme for the hydrolysis of TAG. Lipoprotein lipase is synthesized mainly in adipose tissue and skeletal muscle, and exhibits its function at the vascular endothelium where it is attached (Sethi et al., 1993). Lipoprotein lipase, like pancreatic lipase, also preferentially hydrolyses FA in the *sn*-1,3 positions of TAG (Nilsson-Ehle et al., 1973). Hepatic lipase is the second major enzyme in the vascular bed responsible for TAG and phospholipid hydrolysis (Nilsson-Ehle et al., 1980). Hepatic lipase is like lipoprotein lipase, but does not require apolipoprotein C-II for activation (Kirchmair et al., 1995).

The FA released by lipoprotein lipase are rapidly transported across the endothelium, where they are re-esterified to TAG for energy storage (mainly in adipose tissue) or oxidized for energy production (mainly in muscle). The activity of lipoprotein lipase depends on the metabolic state of the organism. Thus, lipoprotein lipase activity in adipose tissue appears to be stimulated by insulin (fed state). Conversely, skeletal muscle lipoprotein lipase appears to be inhibited by insulin, so that increased skeletal muscle lipoprotein lipase activity occurs during the post-absorptive period, when insulin concentrations have returned to fasting levels (Cryer et al., 1976).

The fate of 2-MAG is not exactly known, but it is believed that they are removed quickly from the circulation after further hydrolysis by lipoprotein lipase or monoglyceride hydrolase. Alternatively, MAG may be transported to the liver attached to albumin or in the core of the remnant CM (Zock et al., 1996). It could be argued that if 2-MAG were taken up into the tissue, then it could be expected that the *sn*-2 FA would be retained in the tissue. However, whereas pigs consume diets with a high PUFA content at the *sn*-2 position of TAG, deposit fat with a high proportion of palmitic acid at the *sn*-2 position and linoleic acid content at the *sn*-1,3 positions. This strongly suggests that 2-MAG are hydrolyzed.

As CM and VLDL are delipidated, their diameter is reduced and their density increases, resulting in the formation of remnant CM and low-density lipoproteins (**LDL**), respectively (Nilsson-Ehle et al., 1980). The surface materials (phospholipids, cholesterol and apolipoprotein C-II) are transferred to HDL to maintain the stability of the lipoprotein particles. The transfer of apolipoprotein C-II results in cessation of further TAG removal by lipoprotein lipase (Sethi et al., 1993). High-density lipoproteins, which are assembled in the plasma, transport cholesterol from peripheral tissues to the liver and act as an acceptor/donor of apolipoproteins to other lipoproteins (da Costa, 2003).

In the liver, specific remnant receptors are activated, and the remnant CM and LDL are taken into hepatic cells by endocytosis. Before endocytosis, LDL particles deliver cholesterol to hepatic and extrahepatic tissues. In the liver, cholesterol is converted to bile acids or directly incorporated into the bile and excreted into the intestine to facilitate lipid absorption (da Costa, 2003).

1.3.3.3. Deposition

Once in the blood, dietary FA can be oxidized to yield ATP (energy source) or deposited as energy storage, according to the physiological state of the animals. Chickens and pigs, under practical conditions, are generally in a positive energy balance, as they are fed *ad libitum*. Thus, in the postprandial period, the FA released from CM by lipoprotein lipase may flow into adipocytes for esterification and storage as TAG. Consequently, body fat is expected to be a reflection of the dietary FA composition. However, it is important to consider the contribution of FA from *de novo* synthesis and to which extent dietary FA are further elongated and desaturated, or oxidized.

1.3.3.4. *De novo* fatty acid synthesis

The general view that lipogenesis is similarly regulated in all tissues (liver and adipose tissue) comes from the scenario found in rodents, although some tissue selectivity has been observed in other species. For example, lipogenesis in birds is active exclusively in the liver, whereas in pig the adipose tissue is by far the main lipogenic organ (Bergen and Mersmann, 2005).

The rate of *de novo* formation of long-chain FA is rapid in well-fed animals, especially when the diet has little or no fat, since the presence of fat in the diet inhibits the synthesis of fat from carbohydrates (Lehner and Kuksis, 1996). This could be because the efficiency of carbohydrates and dietary fat for body fat synthesis has been reported to be 75% and 90%, respectively (Freeman, 1983).

Without going into details, the biosynthesis of long-chain SFA occurs in the cytoplasm of cells. The addition of two carbon units to a two-carbon acetyl primer results in the formation of the common even-chain FA. The two enzyme complexes involved in FA biosynthesis are acetyl-CoA carboxylase and FA synthetase (Enser, 1984).

1.3.3.5. Fatty acid elongation and desaturation

The major end-product of the FA synthesis is palmitic acid (C16:0), which in turn can be elongated to stearic acid (C18:0). Cell membranes, however, require UFA to maintain their structure, fluidity and functions (Enser, 1984). The first double bond introduced into a saturated acyl chain is generally catalyzed by the enzyme Δ9-desaturase, which is universally present in both plants and animals. This enzyme results in the conversion of stearic acid (C18:0) to oleic acid (C18:1 n-9). The other double bonds are also introduced by further oxidative desaturation. Animal systems, however, cannot introduce double bonds beyond the Δ-9 position (measured from the methyl end of the FA) (Lehner and Kuksis, 1996). For this reason, linoleic (n-6 family) and α-linolenic (n-3 family) acids are recognized as metabolically essential. The C20 and C22 members of each family are metabolically linked with the first (C18) member by a series of enzymatically catalyzed and controlled desaturation and elongation processes. Animals are not generally able to interconvert n-6 and n-3 FA and, therefore, it is important to consume dietary n-6 and n-3 FA in an appropriate ratio. An excess of either one of them will lead to inflammatory disorders (distorted balance between the synthesis of active thromboxanes and prostaglandins) (Lehner and Kuksis, 1996). In any case, linoleic acid is the only essential FA whose diet requirement has been demonstrated in pigs (NRC, 2012) and broiler chickens (NRC, 1994).

1.3.3.6. β-oxidation

In the fasting state, FA are released into the circulation via the hydrolysis of adipose tissue by hormone-sensitive lipase activity (Coppack et al., 1994). While in mammals adrenaline and noradrenaline are the main hormones that stimulate hormone-sensitive lipase, in birds the main activator is glucagon (Langin et al., 1996). Then, FA are transported bound to albumin until they reach the tissues where the oxidation occurs. Fatty acid β-oxidation is the process by which FA are broken down to produce energy. Fatty acids primarily enter inside the cell via FA protein transporters on the cell surface. Once inside, FA must be activated to acyl-CoA. Fatty acyl-CoA synthase adds a CoA group to the FA. Carnitine palmitoyltransferase 1 then converts the long chain acyl-CoA to long chain acylcarnitine. The FA moiety is transported by carnitine translocase across the inner mitochondrial membrane. Carnitine palmitoyltransferase 2 then converts the long chain acylcarnitine back to long chain acyl-CoA. The long chain acyl-CoA can then enter the FA β-oxidation pathway, where

two-carbon units are removed from the FA carboxyl end during every round of the cycle. During this enzymatic breakdown, energy-rich molecules are formed (NADH, $FADH_2$), which are used by the electron transport chain to produce ATP. The acetyl-CoA derivates resulting from this process are incorporated into the citric acid cycle or are used by the liver to form ketone bodies, which produce less energy.

1.4. Factors affecting fat digestion, absorption and metabolism

Fats destined for inclusion in animal diets have an extremely variable chemical structure. It is not surprising, therefore, that the physico-chemical properties of fat may have important consequences on fat digestion, absorption and metabolism processes. The amount of energy that an animal can obtain from dietary fat mainly depends on the fat absorption, which is directly related to the relative efficiency of products of fat digestion to form micelles in the intestinal lumen. Thus, higher fat absorption will result in more available energy.

There have been numerous studies designed to estimate the dietary energy value of fats in broiler chickens and pigs, and it has been reported that the two chemical variables of most importance are the degree of saturation of FA and the FFA content (Wiseman et al., 1998). In any case, several other factors (related and not related to dietary fat) can have important implications on fat digestion, absorption and metabolism processes.

1.4.1. Factors related to dietary fat

1.4.1.1. Fatty acid composition

1.4.1.1.1. Fatty acid chain length

The chain length of a FA is an important determinant of fat digestion and absorption processes. The hydrolysis of TAG by pre-duodenal lipases and pancreatic lipase is affected by the chain length of FA located at the *sn*-1,3 positions, because these lipolytic enzymes are much more active on TAG with medium-chain FA than on those with long-chain FA (Greenberger et al., 1966). In addition, it is currently thought that the rate of absorption of FA is negatively related to chain length. Thus, the shorter the chain, the easier the absorption, since medium-chain FA are less insoluble in water

than are long-chain SFA. Therefore, medium-chain FA can diffuse directly into enterocytes, even without the presence of bile salts, as they are able to cross the unstirred water layer lining the gut (Greenberger et al., 1966; Caliari et al., 1996). On the other hand, free long-chain SFA, due to their high individual melting points above body temperature (**Table 1.3**), form micelles less readily (Berry and Sanders, 2005) and have a strong tendency to form insoluble soaps with divalent cations, such as calcium and magnesium, at the alkaline pH of the small intestine (Renaud et al., 1995; Lien et al., 1997).

Table 1.3. Melting point of fatty acids according their chain length (Azain, 2001).

Notation	Melting point, °C
C8:0	16.5
C10:0	31.4
C12:0	44.0
C14:0	58.0
C16:0	63.0
C18:0	71.5
C20:0	75.4

The chain length of FA also affects the transport and metabolism processes. Once inside the enterocytes, medium-chain FA can be dispersed relatively easily in water, they are poorly re-esterified to TAG and instead, they are readily transported through the enterocyte basolateral membrane as complexes with albumin via the portal vein to the liver, where they are rapidly metabolized (Kiyasu et al., 1952). In contrast, long-chain FA are re-esterified to TAG inside enterocytes, packaged into CM particles, and transported via the lymphatic system (via portal vein in birds) until they reach the general circulation.

Fatty acids also seem to influence plasma cholesterol concentrations differently, depending on their chain length. For instance, stearic acid (C18:0) has been reported to be neutral with respect to serum cholesterol, as opposed to the hypercholesterolemic properties observed for palmitic (C16:0), myristic (C14:0), and lauric (C12:0) acids (Grande et al., 1970; Denke and Grundy, 1992).

Finally, the utilization of different FA as energy source is also dependent on FA chain length. The reason why medium-chain FA are preferentially oxidized over long-chain FA is because they can diffuse passively into the mitochondria for oxidation without the presence of L-carnitine, which sometimes may be the rate-limiting step of energy metabolism (Heo et al., 2002; Noguchi et al., 2002). As a general rule, the

oxidation rate of FA increases as the chain length of the FA decreases (DeLany et al., 2000; Leyton et al., 2000). For that reason (shunting FA towards oxidation rather than storage), recent studies have confirmed the potential of medium-chain FA to reduce body weight (**BW**) and particularly body fat in humans and rodents (Geliebter et al., 1983; St-Onge et al., 2003).

1.4.1.1.2. Fatty acid saturation degree

In general, it is well established that digestibility increases as double bonds are introduced (Doreau and Chilliard, 1997; Cho and Kim, 2012), although the differences in digestibility between PUFA and MUFA with a chain length of 18 carbon atoms are low. The lower digestibility of SFA may be attributed to their apolarity and high individual melting points (**Table 1.4**). When double bonds are introduced in the carbon chain of FA, melting point is lowered. The melting point is lowered further when two or more double bonds are introduced.

Table 1.4. Melting point of fatty acids according their degree of saturation (Azain, 2001).

Notation	Melting point, °C
C16:0	63.0
C16:1 n-9	1.5
C18:0	71.5
C18:1 n-9	16.3
C18:2 n-6	-5.0
C18:3 n-3	-11.3

Pancreatic lipase is known to operate only on oil-water interface, so that high-melting TAG might not be hydrolyzed under normal reaction conditions (Powles et al., 1993; Singh et al., 2009). Deschodt-Lanckman et al. (1971) reported a twofold-greater response of pancreatic lipase to unsaturated dietary fats than to saturated fats. On the other hand, free SFA are less rapidly incorporated into micelles, which makes them rely on an adequate presence of bile salts and UFA for efficient emulsification (Polin et al., 1980; Stahly, 1984). In addition, the transport of FA through the cytosol of the absorptive cells seems to be influenced by a FA-binding protein, which has a greater affinity towards UFA than SFA (Ockner and Manning, 1974). This may be the reason why long-chain UFA are absorbed in the proximal portion of the small intestine, while long-chain SFA are more likely to be absorbed in the distal portion (Ockner et al.,

1972). Moreover, it must be also considered that long-chain SFA have a greater ability to form insoluble soaps with divalent cations in the gut than have long-chain UFA. In turn, calcium soaps of palmitic and stearic acids are 10-20 times less soluble than the calcium soaps of oleic and linoleic acids (Denke et al., 1993).

Considering lipid metabolism, numerous studies have shown that saturated fats increase circulating concentrations of serum TAG and cholesterol, and their replacement by polyunsaturated or monounsaturated oils has the opposite effects in human and rodents (McNamara, 1992). Weintraub et al. (1988) and Zampelas et al. (1998) suggested that the faster clearance of circulating TAG in men fed unsaturated fat sources can be related to a greater hormonal activation of lipoprotein lipase, which has been associated to a lower risk of atherosclerosis.

In addition, various studies have attempted to determine the oxidation rates of FA using different kinds of labeled FA in rodents and humans (DeLany et al., 2000; Leyton et al., 2000). These authors agreed that FA are more readily catabolized when they are unsaturated and when their double bonds are closer to the methyl end of the aliphatic chain, which could explain the differences found in fat deposition. Thus, it has been observed that PUFA-rich diets reduce abdominal fat and total body fat compared to SFA-rich diets in rats and chickens (Shimomura et al., 1990; Sanz et al., 1999; Crespo and Esteve-Garcia, 2001; Ferrini et al., 2008; Wongsuthavas et al., 2011; González-Ortiz et al., 2013).

1.4.1.1.3. Interactions between fatty acids

As already mentioned, the digestion of long-chain FA is affected by their individual degree of saturation, but also by the unsaturated-to-saturated FA ratio of the overall fat. In this regard, Renner and Hill (1961) observed how both stearic and palmitic acids were virtually unutilized by the chick when fed singly in the diet. However, when these SFA were fed in a mixture of FA such as hydrolyzed lard or beef tallow, absorption was significantly improved, particularly of palmitic acid. This improved utilization may have been due to the presence of UFA in the FFA mixtures. Because long-chain UFA have a greater ability to form mixed micelles than have long-chain SFA, their presence may increase the capacity of mixed micelles to take up SFA in the core and, therefore, improve their absorption. This interaction is frequently referred to as the phenomenon of 'synergism'. Therefore, the inclusion of a relatively unsaturated fat to a relatively saturated one could increase the overall digestibility of the fat blend, above the predicted numerical value obtained for the two individual fats

(Ketels and de Groote, 1989). However, whilst synergism between individual FA differing in the degree of saturation is accepted, it is not so clear if there is synergism between fats of different unsaturated-to-saturated FA ratios (Wiseman et al., 1990).

1.4.1.2. Fat molecular structure

1.4.1.2.1. Fatty acid positional distribution within acylglycerol molecules

Numerous literature reviews have focused on the physiological importance that the FA positional distribution within acylglycerol molecules exerts on fat digestion, absorption and metabolism processes (Small, 1991; Bracco, 1994; Decker, 1996; Hunter, 2001; Berry and Sanders, 2005; Mu and Porsgaard, 2005; Linderborg and Kallio, 2005; Mu, 2006; Karupaiah and Sundram, 2007; Berry, 2009; Innis, 2011; Michalski et al., 2013). However, this information is not commonly included in feed composition databases (e.g. INRA, 2004; FEDNA, 2010; CVB, 2011), probably due to the cost and complexity of the analysis, and the lack of knowledge.

As already mentioned, pancreatic lipase specifically hydrolyses FA in the *sn*-1 and *sn*-3 positions of TAG (Mattson and Beck, 1956). Consequently, the positional distribution of FA in dietary TAG will determine whether FA will be absorbed as 2-MAG or as FFA. 2-MAG are well absorbed regardless of their constituent FA, because their hydrophilic character makes them easily incorporated into mixed micelles (Schulthess et al., 1994; Ho and Storch, 2001). However, free long-chain SFA, as already mentioned, are not well absorbed from the lumen, in part because of their melting points substantially above body temperature and their strong tendency to form insoluble soaps with divalent cations, such as calcium and magnesium at the alkaline pH of the small intestine. Indeed, Renaud et al. (1995) when feeding rats with diets containing either native palm oil (90% palmitic acid at the *sn*-1 and *sn*-3 positions), lard (81% palmitic acid at the *sn*-2 position) or their interesterified counterparts (33% palmitic acid at each position), found that the fecal excretion of palmitic acid was greater in the diets that contained high amounts of palmitic acid at the *sn*-1 and *sn*3 positions (native palm oil and interesterified lard). Similar results have been observed in rats (Tomarelli et al., 1968; Lien et al., 1997), human newborn infants (Filer et al., 1969; Carnielli et al., 1995a), and broiler chickens (Renner and Hill, 1961; Lin and Chiang, 2010).

Regarding lipid metabolism, the FA positional distribution of TAG re-synthesized in the mucosal cells may also have consequences on blood lipid parameters, since approximately 75% of the *sn*-2 dietary FA are conserved through the digestion and absorption processes (Kubow, 1996). Redgrave et al. (1988) demonstrated that SFA in the *sn*-2 position always resulted in a slower TAG lipolysis and clearance of the remnant CM compared to SFA in the *sn*-1,3 positions. They attributed this phenomenon to the formation of a saturated 2-MAG which conferred a rigid structure to the CM surface, expelling lipoprotein lipase. In agreement with this hypothesis, some studies have shown that feeding fats rich in SFA at the *sn*-2 position results in a more pronounced postprandial lipaemia, because SFA in the *sn*-2 position may be absorbed more efficiently (as 2-MAG) and cleared from circulation more slowly than SFA in the *sn*-1,3 positions (Kubow, 1996). Delayed remnant-CM clearance from the blood stream has been strongly associated with atherosclerotic lesions in susceptible species such as rabbits, due to the increased exposure of the aortic tissue to these FA and possibly to increased fat deposition (Kritchevsky et al., 2000). Related to this, it has been found in human studies that hypercholesterolemic animal fats, such as lard, contain mainly SFA in the *sn*-2 position. In contrast, palm oil (only 10% of total palmitic acid in the *sn*-2 position) has been shown to be less hypercholesterolemic than predicted from its high palmitic acid content (Zhang et al., 1997; Ladeia et al., 2008; Fattore and Fanelli, 2013).

Due to the rearrangement of FA during the esterification process, re-esterified oils are expected to show a higher proportion of long-chain SFA at the sn-2 position of acylglycerol molecules, which can be advantageous in terms of fat digestion. Saturated FA located at the sn-2 position of acylglycerol molecules will be more easily absorbed as 2-MAG than as FFA.

1.4.1.2.2. Mono- and diacylglycerol content

Little attention has been paid to the nutritional characteristics of MAG and DAG, since they have only been recognized as intermediates in the process of TAG digestion. As already mentioned, during fat digestion, TAG are hydrolyzed by 1,3-specific lipases to 1,2-DAG and 2,3-DAG, which are further hydrolyzed to 2-MAG and FFA. These are

the normal end products of TAG digestion that are absorbed by intestinal mucosal cells and used for reconstruction of circulating CM TAG. In contrast, 1,3-DAG and 1(3)-MAG are the main positional isomers of fat sources rich in MAG and DAG. Compared to TAG, these acylglycerol molecules may exert a differential effect on fat digestion, absorption, and metabolism processes, as reviewed by Rudkowska et al. (2005). On one hand, 1,3-DAG and 1(3)-MAG are probably completely hydrolyzed to glycerol and FFA (Murata et al., 1994; Watanabe et al., 1997a; Kondo et al., 2003; Martin et al., 2014). Before their complete hydrolysis, however, MAG and DAG, due to their amphiphilic properties, may contribute to improve intraluminal solubilization and, therefore, enhance lipase action, micelle formation and enterocyte uptake of the dietary lipid fraction (Martin et al., 2014). Very few studies have focused on the potential beneficial effects of MAG and DAG on fat absorption. In the study of Garrett and Young (1975), the efficacy of oleic acid in enhancing palmitic acid absorption was compared with that of 2-MAG of oleic acid (mono-olein). Both oleic acid and mono-olein progressively increased the absorption of palmitic acid. However, at lower ratios, mono-olein was up to five times as effective, while at higher ratios, mono-olein was only twice as effective as oleic acid. Although oleic acid readily forms mixed micelles with bile salts in which SFA are then solubilized, the MAG of this FA appear to be more effective in this regard than the FFA, as the former has higher amphiphilic properties than the latter. In this sense, Freeman (1984) observed how higher concentrations of MAG decreased the critical bile-salt micelle concentration.

The reduced 2-MAG supply in the intestinal epithelium when 1,3-DAG and 1(3)-MAG are offered, may lead to altered lipid metabolism. On one hand, because 2-MAG cannot be supplied in sufficient quantity during the absorption of 1,3-DAG and 1(3)-MAG, TAG re-synthesis in enterocytes may proceed *via* de glycerol-3-phosphate pathway, which is less active than the 2-MAG pathway (Tada, 2004; Yanagita et al., 2004). This may explain why replacement of TAG by DAG can lower serum TAG concentration in the fasted state (Hara et al., 1993; Yamamoto et al., 2001) and in the postprandial state (Murata et al., 1994; Watanabe et al., 1997b; Taguchi et al., 2000; Yanagita et al., 2004). As a consequence, some authors have suggested that greater amounts of FFA are released into the portal circulation rather than being incorporated into CM (Watanabe et al., 1997a; Tada, 2004). This hepatic exposure to non-esterified FA by increasing DAG intake may lead to greater β-oxidation by the liver than that after TAG intake (Murata et al., 1997; Watanabe et al., 1997a). As a consequence, studies in both animals and humans have shown satiety (Kamphuis et al., 2003) and reduced

body fat accumulation (Watanabe et al., 1997a; Nagao et al., 2000; Maki et al., 2002) after intake of DAG oil as compared to ordinary TAG oil.

The esterification process can be developed until different levels, resulting in different final products, which can differ in the proportions of TAG, DAG and MAG molecules. The presence of DAG and MAG molecules in re-esterified oils can increase the absorption of free SFA present in the gut, as a matter of their emulsifying effect.

1.4.1.2.3. Free fatty acid content

Because FFA are produced during the natural digestion process, one would think that a dietary supply of already hydrolyzed fat would be beneficial in terms of fat utilization. However, several studies have shown that the digestibility of FFA in comparison with TAG is lower in pigs (Powles et al., 1993; 1994) and broiler chickens (Sklan, 1979; Atteh and Leeson, 1985; Wiseman and Salvador, 1991; Blanch et al., 1995; Vilà and Esteve-Garcia, 1996a), which suggests that TAG or some of their breakdown products may be required for absorption of FFA. On one hand, MAG are important in mixed micelles because, due to their swelling ability, facilitate the incorporation of hydrophobic FFA in the core of mixed micelles (Freeman, 1984). On the other hand, it has been observed that the endogenous secretion of bile salts is stimulated by the presence of TAG and 2-MAG in the small intestine, but not by FFA (Sklan, 1979). As a consequence, animals fed acid oils have a more reduced emulsification and, therefore, absorption of lipids than those fed native oils.

Nevertheless, as mentioned before, the negative effects of dietary FFA on energy utilization is related to their saturation degree (Wiseman and Salvador, 1991). Vilà and Esteve-Garcia (1996a) found that in three-week-old broiler chickens, dietary saturated FFA (i.e., palmitic acid and stearic acid) decreased the digestibility and the metabolizable energy value of the added dietary fat, whereas dietary unsaturated FFA (i.e., oleic and linoleic acid) did not. It is well known that the acid group of FFA reacts with ionized minerals, such as calcium and magnesium, forming soaps. However, Atteh and Leeson (1984, 1985a) showed how most of the calcium soaps of dietary UFA were absorbed by the bird as opposed to calcium soaps formed with SFA, due to their higher solubility and lower melting points. Thus, it has been suggested that the negative effect

of FFA is restricted to young animals with immature digestive systems fed saturated FFA (Wiseman and Salvador, 1991).

No studies focused on the effects of dietary fats rich in FFA on lipid metabolism have been found.

1.4.1.3. Other compounds present in fat sources

Besides TAG, DAG, MAG and FFA molecules, fat sources (mainly by-products and technical lipids) may also content undesirable substances, such as water, impurities, unsaponifiable compounds (sterols, alcohols…), peroxides, secondary oxidation products, polymers, foreign substances, and toxics in variable amounts. In a previous European project (ref. FP6 FOOD-CT-2004-007020), focused on the quality and safety of feeding fats obtained from waste or by-products from the food chain, levels of contamination and oxidation of several fat sources were analyzed in depth. Detailed information about this project can be found at the Feeding Fats Safety website (htttp://www.ub.edu/feedfat/).

The presence of high amounts of these compounds in fat sources can exert negative effects on their nutritive value (either reducing the gross energy content of the fat or impairing the fat digestion and absorption processes) or even harm the health of the animals and the quality of the food produced. Vilà and Esteve-Garcia (1996b) reported that as the content of non-elutable material of dietary fat sources increased, the apparent metabolizable energy of fats decreased in broiler chickens.

1.4.2. Other factors

1.4.2.1. Animal physiology

Factors such as animal age has a great influence on fat absorption. The ability to digest lipids is not fully developed in very young animals, because many of the physiological functions necessary for lipid digestion are immature at hatch/birth and continue to develop for several weeks. Thus, the absorption of fat improves with increasing age (Krogdahl, 1985; Gu and Li, 2003).

In young birds, the utilization of dietary fats is limited because they have a reduced capacity to produce and secret bile salts and a less efficient bile salt recycling (Soede, 2005). In this sense, several studies have reported that bile salts appear to be

effective in improving the absorption of saturated-type fats, especially in young chicks (Gomez and Polin, 1976; Polin et al., 1980).

In piglets, on the other hand, it is clear that lipase activity is low from birth to the first week of age, suggesting that pancreatic lipase is the limiting factor of fat digestion (Jensen et al., 1997; Gu and Li, 2003). However, research has shown that newly weaned pigs are less capable of utilizing fat than suckling pigs. Whereas suckling pigs digest the fat from sow's milk very efficiently (about 96%; Lindemann et al., 1986), the digestibility of fat in weaning pigs declines to 65-80%, according to the dietary fat source (Cera et al., 1988). Thus, weaning influences the physiological responses of pigs. The gastrointestinal system has to adapt to the considerable changes in the physicochemical properties of feed, as well as to a changes in the pattern of intake.

The health status of the animals also influences the efficiency of fat digestion. It is often observed that fat digestibility is disturbed most severely during and after (sub)-clinical cases of infectious diseases affecting the intestinal epithelium. Besides the damage of the intestinal epithelial cells, the modification of the ratio between crypts and depths of the villi also affects fat absorption. The release of bile from the gall bladder into the intestine is affected principally by the action of the hormone cholecystokinin. The release of this hormone is mediated by sensors located in the crypts of the intestinal mucosa. As a result of an infectious challenge, the crypt depth increases and, therefore, there is a lack of stimulation of the cholecystokinin cells. Consequently, bile salt secretion in the intestinal lumen decreases and micelle formation takes place to a lower extent, impairing fat absorption (Soede, 2005).

1.4.2.2. Diet composition

Several dietary factors other than dietary fat may also affect fat absorption. Diets high in cereals (barley, wheat, and rye) are recognized to have relatively high proportions of indigestible carbohydrates known as non-starch polysaccharides. These non-starch polysaccharides induce increased intestinal viscosity, which impairs the digestibility of all dietary nutrients, including fat, by interfering with the diffusion of pancreatic enzymes, target substrates and the end products of the digestion process (Soede, 2005). In this respect, intestinal viscosity was found to interfere with the digestibility of SFA more dramatically than with the digestibility of UFA (Smulikowska and Mieczkowska, 1996). The addition of enzymes for the breakdown of water-soluble non-starch polysaccharides in wheat, barley and rye based diets clearly improved the digestibility of fat (Smulikowska and Mieczkowska, 1996; Dänicke et al., 2000).

Moreover, several studies have indicated that by including viscous water-soluble non-starch polysaccharides in the diet, the microbial activity in the small intestine is increased markedly. As a result, deconjugation of bile salts may occur, making fat emulsification less effective (Langhout et al., 2000).

Impaired fat digestibility is also associated with higher dietary mineral contents. Free FA available in the digesta, long-chain SFA in particular, are submissive to interact with divalent elements to form soaps. If insoluble soaps are formed with calcium and magnesium, there is the possibility that both the FA and the mineral content will be unavailable by the animals (Whitehead et al., 1971; Atteh and Leeson, 1984; Lin and Chiang, 2010).

CHAPTER 2

Background, hypotheses, and objectives

Among the ingredients used in animal diets, fats and oils are the most concentrated sources of energy. Usually they provide about 15 and 30% of the energy of compound feeds. The search for new alternative fat sources and the improvement of the energy efficiency of these raw materials is of great practical interest for feed producers.

This PhD dissertation is part of a project (ref. AGL2010-22008-C02) that emerged from a previous European project (ref. FP6 FOOD-CT-2004-007020), focused on the quality and safety of feeding fats obtained from waste or by-products from the food chain (more information about this project can be found at the Feeding Fats Safety website; htttp://www.ub.edu/feedfat/). As a result of this project, some new lines of research were opened. Vegetable acid oils, from chemical and physical refining, are economically interesting alternatives for feed and meat producers. Nevertheless, the origin and the quality of these fatty by-products are quite variable, and their nutritive value is typically lower than their corresponding native oils, because of their high free fatty acid (**FFA**) content.

The purpose of this thesis is, therefore, to give added value to these acid oils. It is possible to neutralize the FFA content of these by-products through esterification of FFA with glycerol (both economically interesting by-products from oil refining and biodiesel industries, respectively). Re-esterified oils, the end products of this process, have different physico-chemical and nutritional properties that can provide valuable benefits in animal nutrition, apart from the environmental benefits associated with their use.

The literature review has shown that the nutritional value of fats does not only depend on their FA composition, but is also affected by the arrangement of their components (molecular structure). Re-esterified oils can improve the absorption of saturated fatty acids (**SFA**), due to their higher proportion of long-chain SFA at the *sn*-2 position of acylglycerol molecules. Furthermore, the presence of different proportions of mono- (**MAG**) and diacylglycerols (**DAG**) can also improve the absorption of free SFA present in the gut, through their emulsifying effect. We therefore hypothesize that re-esterified oils can be used in monogastric-animal diets, providing a greater nutritive value than their corresponding acid oil and also than their analogous native oil. Re-esterified oils would therefore be interesting, alternative fat sources for feed producers.

To date, very little is known about the use of these re-esterified oils in monogastric-animal diets. Lipid nutrient requirements, digestive physiology, and metabolism are similar but not identical among monogastric animals and, consequently, the impact to animal performance and health may vary. Thus, the global

aim of this thesis is to **investigate the potential use of re-esterified oils in pig and broiler chicken diets.**

The specific objectives are:

- To characterize the molecular structure of re-esterified oils.

- To assess whether changes in the fatty acid (**FA**) positional distribution and acylglycerol composition of re-esterified oils induce significant changes in the dietary energy value, the individual FA apparent absorption coefficients, and the acylglycerol and FFA composition of feces and excreta in pigs and broiler chickens at different physiological stages.

- To test the effects of feeding re-esterified oils on growth performance and carcass fat depots, both in terms of quantity and quality, in pigs and broiler chickens.

- To establish which would be the best nutritional strategy (pure re-esterified oils or blends of re-esterified oils with different degrees of saturation), in order to obtain the most favorable nutrient efficiency in terms of dietary energy value, individual FA apparent absorption coefficients and growth performance, depending on the physiological stage of broiler chickens, and ensuring the quality of the final product.

To assess these objectives, four different trials were performed. In the first three experiments, the use of two re-esterified oils (with a low and a high MAG and DAG content) was compared with that of their corresponding acid (negative control) and native (positive control) oils in weaning-piglet (*Chapter 3*), fattening-pig (*Chapter 4*), and broiler chicken (*Chapter 5*) diets. In the last experiment, blends of re-esterified oils with different degrees of saturation were used in broiler chicken diets, in order to establish the best nutritional strategy to be used in feed formulation (*Chapter 6*).

CHAPTER 3

Use of re-esterified palm oils, differing in their molecular structure, in weaning-piglet diets

Use of re-esterified palm oils, differing in their molecular structure, in weaning-piglet diets

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Submitted to Animal

3.1. Abstract

Re-esterified oils are new fat sources obtained from chemical esterification of acid oils with glycerin (both economically interesting by-products from oil refining and biodiesel industries, respectively). The different fatty acid positional distribution and acylglycerol composition of re-esterified oils may enhance the apparent absorption of saturated fatty acids and, thus, their overall nutritive value. The aim of the present study was to investigate the potential use of re-esterified palm oils, in comparison with their corresponding acid and native oils, and also with an unsaturated fat source in weaning-piglet diets, studying their effects on fatty acid apparent absorption, acylglycerol and free fatty acid composition of feces, and growth performance. One-hundred and twenty weaning piglets (average weight of 8.50 ± 1.778 kg) were blocked by initial body weight and randomly assigned to one of the five dietary treatments, resulting in four piglets per pen and six replicates per treatment. Dietary treatments were the result of a basal diet supplemented with 10% (as-fed basis) of native soybean oil (SN), native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH). Results from the digestibility balance showed that SN reached the greatest total fatty acid apparent absorption, and statistically different from PN, PA, and PEL ($P < 0.05$). There were no statistical differences among palm-oil sources ($P > 0.05$), but PEH achieved the greatest total fatty acid apparent absorption, which did not differ from SN ($P = 0.63$). Animals fed PA and PN showed similar apparent absorption coefficients ($P > 0.05$), despite the high free fatty acid content of the former. The acylglycerol and free fatty acid composition of feces was mainly composed of free fatty acids, which suggests that the lack of bile salts must be considered the limiting factor of fat absorption in weaning piglets. There were no significant differences in growth performance ($P > 0.05$). We conclude that PEH oil can be used in weaning-piglet diets as a good, alternative fat source, showing a similar total fatty acid apparent absorption to that of a SN oil.

3.2. Introduction

Nursing piglets digest fat from sow's milk very efficiently (Cho and Kim, 2012). However, fat digestibility decreases during the first weeks post-weaning (Cera et al., 1988). The particular fatty acid (**FA**) positional distribution of sow's milk seems to aid fat utilization. About 60% of palmitic acid in sow's milk is esterified at the acylglycerol

sn-2 position (Innis et al., 1993). In contrast, palmitic acid in vegetable oils is predominately esterified at the *sn*-1,3 positions (Mattson and Volpenhein, 1963). Thus, whereas the intraluminal hydrolysis of fat from sow's milk mainly results in the formation of 2-monopalmitin, the hydrolysis of vegetable oils produces free palmitic acid (Innis et al., 1997), which is poorly absorbed due to its tendency to form insoluble soaps with divalent cations in the gut (Small, 1991).

Re-esterified oils are obtained by reacting acid oils with glycerin. On one hand, these technical fats are expected to have an increased proportion of saturated fatty acids (**SFA**) located at the acylglycerol *sn*-2 position, with respect to their corresponding native oil, thus having a FA positional distribution more similar to that of sow's milk. On the other hand, these re-esterified oils may also have greater proportions of mono- (**MAG**) and diacylglycerols (**DAG**) These amphiphilic molecules can act as emulsifying agents, able to enhance lipase activity and FA incorporation into mixed micelles. It was hypothesized that the expected different molecular structure of re-esterified oils may enhance the SFA apparent absorption and, thus, their overall nutritive value, especially in young animals. The aim of the present study was to investigate the potential use of re-esterified palm oils, differing in their molecular structure, in comparison with their corresponding acid and native oils, and also with an unsaturated fat source in weaning-piglet diets, studying their effects on FA apparent absorption, acylglycerol and free fatty acid (**FFA**) composition of feces, and growth performance.

3.3. Materials and methods

3.3.1. Experimental fats

Experimental fats were supplied by SILO S.p.a. (Florence, Italy). Re-esterified palm oils were produced using, as raw materials, acid palm oil (a by-product obtained from the refining process of crude palm oil, with a high FFA content) and glycerin (a by-product obtained from the methylation process applied for biodiesel production), which were processed in a reactor for 4-6 hours, under high vacuum conditions (1-3 mm Hg), at temperatures between 190-250^{o}C, and without chemical catalysts. According to the stoichiometric proportion of FFA and glycerol, fats with the same FA profile, but with a different FA positional distribution, and triacylglycerol (**TAG**), DAG and MAG proportions were obtained (**Table 3.1**).

Table 3.1. Chemical analyses of the experimental fats[1]

Item		SN oil	PN oil	PA oil	PEL oil	PEH oil
Moisture, %		0.07	0.14	0.45	0.04	0.07
Impurities, %		<0.50	<0.50	<0.50	0.50	<0.50
Unsaponifiable matter, %		0.71	0.30	1.95	2.54	1.30
Fatty acid composition and distribution, %						
C16:0	Total	11.7	44.9	47.5	47.8	47.9
	sn-2 %[2]	18.8	9.68	5.33	19.9	16.7
C18:0	Total	3.50	4.50	4.60	4.74	4.76
	sn-2 %[2]	12.7	12.2	11.1	28.7	20.3
C18:1 n-9	Total	28.1	38.1	35.6	35.3	35.5
	sn-2 %[2]	33.8	47.2	9.61	25.6	24.0
C18:2 n-6	Total	49.3	9.84	8.59	7.99	8.18
	sn-2 %[2]	38.7	53.7	15.3	30.3	25.3
C18:3 n-3	Total	5.35	0.27	0.27	0.23	0.17
	sn-2 %[2]	27.6	54.7	24.7	34.6	19.6
Minor fatty acids		2.09	2.43	3.38	3.92	3.52
SFA	Total	15.2	50.7	54.2	55.1	54.7
	sn-2 %[2]	18.0	10.0	5.83	20.4	16.9
MUFA	Total	30.1	39.2	36.9	36.7	36.9
	sn-2 %[2]	32.8	46.6	9.66	25.7	23.8
PUFA	Total	54.7	10.1	8.86	8.22	8.35
	sn-2 %[2]	37.6	53.9	15.6	30.4	25.2
Acylglycerol composition, %						
TAG		97.0	75.9	12.9	48.0	26.3
DAG	Total	1.73	15.5	21.8	40.1	50.8
	1(3),2-DAG %[3]	33.3	26.5	32.3	29.7	32.6
MAG	Total	-	0.79	9.12	10.9	22.9
	2-MAG %[4]	-	25.0	10.7	9.09	8.29
FFA		1.31	7.85	56.2	1.07	-
Glycerol-to-fatty acid ratio[5], mol/mol		0.33	0.33	0.22	0.46	0.55
Gross energy, kcal/kg		9,446	9,305	9,361	9,171	8,991

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; TAG = triacylglycerols.

[1] Native soybean oil (SN oil), native palm oil (PN oil), acid palm oil (PA oil), re-esterified palm oil low in mono- and diacylglycerols (PEL oil), and re-esterified palm oil high in mono- and diacylglycerols (PEH oil).

[2] Fatty acid positional distribution, expressed as the proportion of each fatty acid located at the *sn*-2 position of the acylglycerol molecules, calculated as follows: *sn*-2 % = (*sn*-2 / Total) × *a* × 100, where *sn*-2 is the FA composition at the *sn*-2 position (converted to mol%), Total is the total FA composition of the fat (converted to mol%), and *a* is the ratio between the moles of FA located at the *sn*-2 position and the moles of total FA. *a* was 0.30, 0.28, 0.08, 0.23, and 0.20 for SN, PN, PA, PEL, and PEH oils, respectively.

[3] The proportion of 1(3),2-DAG *vs.* 1,3-DAG.

[4] The proportion of 2-MAG *vs.* 1(3)-MAG.

[5] Estimated calculation based on the values of the acylglycerol and FFA composition.

Oil samples were analyzed in triplicate. Moisture (Method 926.12 of the AOAC International, 2005), impurities (ISO 663:2007), and unsaponifiable matter (Method 933.08 of the AOAC International, 2005) content was determined as a quality control.

The acylglycerol composition of experimental fats was analyzed according to the ISO 18395:2005, in which TAG, DAG, MAG, and FFA are separated according to their molecular size. Briefly, a solution of approximately 10 mg of oil/mL of tetrahydrofurane was injected into an Agilent 1100 series HPLC chromatograph (Agilent Technologies; Santa Clara, CA, USA) equipped with a refractive index detector and two Styragel columns (Styragel HR 1 and Styragel HR 0.5) of 30 cm × 0.78 cm i.d., filled with a spherical styrene divinylbenzene copolymer of 5 μm particle size (Water Associates; Milford, MA, USA) connected in series. The mobile phase consisted of tetrahydrofurane. The acylglycerol molecules were quantified by internal normalization. Moreover, given the potential importance of different positional isomers of MAG and DAG molecules in the digestion and absorption processes, we also analyzed the experimental fats by high-resolution ^{1}H nuclear magnetic resonance (**NMR**) spectroscopy. Thus, 2-MAG were distinguished from 1(3)-MAG, and 1(3),2-DAG from 1,3-DAG species by area integration of the individual resonances corresponding to the central CH at the *sn*-2 position in each type of compound. These species can be detected in the area covering 5.3-3.8 ppm, clearly differentiating the H2 protons belonging to 1(3),2-DAG (5.05 ppm), 1,3-DAG (4.03 ppm), 2-MAG (4.88 ppm) and 1(3)-MAG (3.89 ppm) derivatives (**Figure 3.1**). The degree of unsaturation and the chain length of FA do not influence the chemical shift values (Sacchi et al., 1997). Briefly, oil samples (about 6 mg) were dissolved in deuterated chloroform and placed into a 5-mm-diameter NMR tubes. Conventional one-dimensional ^{1}H NMR spectra were collected under routine conditions on a Bruker 600 MHz spectrometer (Bruker; Billerica, MA, USA), equipped with a triple-channel TXI probe. All experiments were recorded at 298 K, using a recycle delay of 3 s and 4 scans per sample. After Fourier transformation and base-line correction, the areas of the selected H2 proton signals of the spectrum were quantified by area integration.

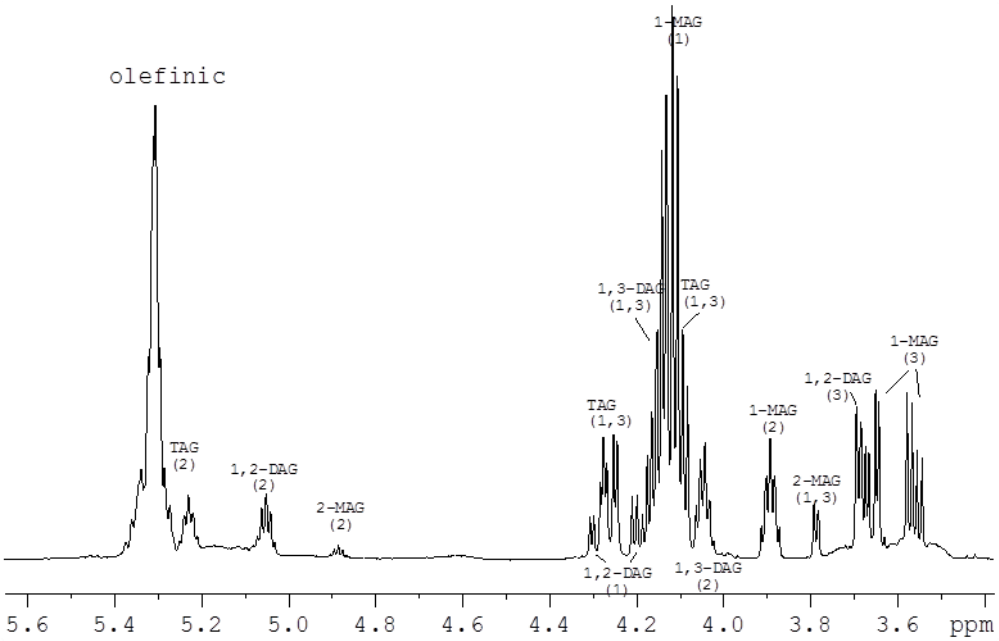

Figure 3.1. Expanded area of the 1H nuclear magnetic ressonance spectrum of a fat sample, with the 1H chemical shift assignment of the glycerol signals corresponding to the differently substituted glycerol units.

The total FA composition of experimental fats was determined by gas chromatography, according to the methylation method described by Guardiola et al. (1994). Briefly, 50 mg of oil were methylated with sodium methoxide (0.5 N), followed by boron trifluoride (20 wt% in methanol), and FA methyl esters were extracted with n-hexane. Subsequently, FA methyl esters were analyzed using an Agilent 4890D gas chromatograph (Agilent Technologies; Santa Clara, CA, USA), equipped with a flame ionization detector and a polar capillary column (SP-2380, 60 m x 0.25 mm i.d., 0.2 μm from Supelco; Bellefonte, PA, USA). Helium was used as the carrier gas. Fatty acid methyl esters were identified by matching their retention times with those of their relative standards (Supelco 37 component FAME Mix, Sigma-Aldrich Co.; St. Louis, MO, USA) and quantified by internal normalization.

The FA composition at the *sn*-2 position of the acylglycerol molecules was determined by the EU official method (Commission Regulation (EEC) No. 2568/91 – Annex VII). Briefly, the fat was hydrolyzed by pancreatic lipase (EC 3.1.1.3 from porcine pancreas Type II, Sigma-Aldrich Co.; St. Louis, MO, USA) to selectively cleave the ester bonds at the *sn*-1,3 positions. 2-MAG were isolated by thin-layer chromatography using silica gel plates (Merck; Darmstadt, Germany), impregnated in boric acid (5 wt% in methanol). The 2-monoolein and 1-monoolein standards (Sigma-Aldrich Co.; St. Louis, MO, USA) were spotted for identifying the 2-MAG zone spot. The developing solvent

was a mixture of chloroform/acetone (90:10, by vol). The zone spot was visualized under UV light after being sprayed with 0.2 wt% of 2,7-dichlorofluorescein in methanol. Then, 2-MAG were scraped, and the FA composition of 2-MAG was determined as described above. Finally, to assess the distribution of each FA within the three positions of the acylglycerol molecules, a modification of the formula suggested by Mattson and Volpenhein (1961) was used. These authors calculated the proportion of each FA that is located at the *sn*-2 position of the acylglycerol molecules (*sn*-2 %), applying the following formula:

$$sn\text{-}2\ \% = (sn\text{-}2\ /\ Total) \times a \times 100,$$

where *sn*-2 is the FA composition at the *sn*-2 position (converted to mol%), *Total* is the total FA composition of the fat (converted to mol%), and *a* is the ratio between the moles of FA located at the *sn*-2 position and the moles of total FA. Thus, in the original formula (Mattson and Volpenhein, 1961), *a* was equal to 0.33, since it was designed for native oils that are mainly constituted by TAG. In our study, however, experimental fats were a mixture of TAG, DAG, MAG, and FFA molecules. For this reason, *a* was calculated from the acylglycerol composition of the fat, and the average molecular weight (according to the total FA composition of the fat) and the glycerol-to-FA ratio for each molecular species. These calculations were also used to obtain an estimation of the global glycerol-to-FA ratio of our experimental fats (**Table 3.1**).

Finally, combustion energies of the experimental fats were measured by an adiabatic bomb calorimeter (IKA-Kalorimeter system C4000; Staufen, Germany).

3.3.2. Animals and diets

The study was performed at the experimental farm of *IRTA Mas de Bover* (Constantí, Tarragona, Spain). The experimental procedure received the prior approval from the Ethical Committee of the same institution. All animal housing and husbandry conformed to the European Union Guidelines (2010/63/EU).

A total of 120 male and female crossbred weaning piglets ([Landrace × Duroc] × Pietrain) were obtained from the swine herd of the same experimental station. Piglets were weaned at 26 days of age and received a commercial feed for 5 days. Then, piglets (average weight of 8.50 ± 1.778 kg) were blocked by initial body weight (**BW**) (six blocks), and randomly assigned to one of the five dietary treatments. Animals were housed in pens of four animals, avoiding that two piglets of the same maternal origin were allocated at the same pen. The gender of the animals was not taken into account for the allocation of the animals. Throughout the study, feed and water were supplied

ad libitum, and animals were raised under controlled conditions of light and temperature.

The feeding program consisted of two diets (in mash form): a pre-starter diet (from day 0 to 14) and a starter diet (from day 14 to 29), that were formulated to meet or exceed NRC (1998) requirements and to minimize basal fat levels. Pre-starter diets were supplemented with 1% of Celatom (Jesús Riesgo; Madrid, Spain) to increase the amount of HCl-insoluble ash, as an inert digestibility marker. The five dietary treatments were the results of including 10% (as-fed basis) of one of the following experimental fats to the basal diet: native soybean oil (**SN**), native palm oil (**PN**), acid palm oil (**PA**), re-esterified palm oil low in MAG and DAG (**PEL**), or re-esterified palm oil high in MAG and DAG (**PEH**). The composition of the experimental diets is presented in **Table 3.2**.

Analytical determinations of feeds were performed according to the methods of AOAC International (2005): dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fat (Method 2003.05), and crude fiber (Method 962.09). Gross energy was determined as described previously for experimental fats, and HCl insoluble ash of pre-starter feeds was determined following the method of McCarthy et al. (1974). Lipids from feeds were extracted with chloroform/methanol (2:1, by vol), according to the Folch et al. (1974) procedure. Ten milligrams of nonadecanoic acid (C19:0, Sigma-Aldrich Chemical Co.; St. Louis, MO, USA) were added as an internal standard before processing. The FA content was analyzed following the method of Morrison and Smith (1964). Briefly, lipids were methylated with boron trifluoride (20 wt% in methanol) and methanolic KOH (0.5 *N*). Subsequently, the FA methyl esters were extracted with n-hexane and submitted to gas chromatography (Agilent 6890N gas chromatograph, equipped with a flame ionization detector, and a fused-silica capillary column [DB23; 30 m × 0.25 mm i.d. and 0.25 μm film thickness] from Agilent Technologies; Santa Clara, CA, USA). Helium was used as the carrier gas. Fatty acid methyl esters were identified by matching their retention times with those of their relative standards (Supelco 37 component FAME Mix, Sigma-Aldrich Co.; St. Louis, MO, USA). Peak areas were integrated and converted to concentration by comparison with the internal standard-peak area. The macronutrient and the FA composition of the experimental diets are presented in **Table 3.3**.

Table 3.2. Ingredient composition of the experimental diets (as-fed basis)

Ingredients, %	Pre-starter diet (from 0 to 14 days)	Starter diet (from 14 to 29 days)
Wheat	20.00	14.45
Corn	17.28	10.00
Barley	13.21	27.60
Sweet whey	10.97	6.86
Soybean meal 44%	10.00	27.85
Experimental fats[1]	10.00	10.00
HP 300[2]	8.00	-
Potato protein	5.33	-
Dicalcium phosphate	1.64	1.25
Celatom[3]	1.00	-
Sodium bicarbonate	0.77	-
Calcium carbonate	0.53	0.78
Vitamin and mineral premix[4]	0.40	0.40
L-Lysine	0.44	0.30
DL-Methionine	0.24	0.14
L-Threonine	0.14	0.10
L-Tryptophan	0.02	-
Ethoxyquin 66%	0.02	0.02
Sodium chloride	-	0.25

[1] Native soybean oil (SN oil), native palm oil (PN oil), acid palm oil (PA oil), re-esterified palm oil low in mono- and diacylglycerols (PEL oil), or re-esterified palm oil high in mono- and diacylglycerols (PEH oil).

[2] Hamlet Protein; Horsens, Denmark.

[3] Jesús Riesgo; Madrid, Spain.

[4] Provides per kg of feed: vitamin A (from retinol), 10000 IU; vitamin D_3 (from cholecalciferol), 2000 IU; vitamin E (from alfa-tocopherol), 25 mg; thiamine, 1.5 mg; riboflavin, 3.5 mg; pyridoxine, 2.4 mg; cobalamine, 20 µg; calcium pantothenate, 14 mg; nicotinic acid, 20 mg; biotin, 50 µg; folic acid, 0.5 mg; menadione, 1.5 mg; Ca, 0.21 g; Mg, 57 mg; Fe (from $FeSO_4 \cdot 7H_2O$), 120 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 150 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$), 0.6 mg; Zn (from ZnO), 110 mg; Mn (from MnO), 60 mg; I (from $Ca(I_2O_3)_2$), 0.75 mg; Se (from Na_2SeO_3), 0.37 mg; ethoxyquin, 3.33 mg.

Table 3.3. Analyzed[1] macronutrient content and fatty acid composition of the experimental diets[2]

	Pre-starter diets (from 0 to 14 days)					Starter diets (from 14 to 29 days)				
Item	SN	PN	PA	PEL	PEH	SN	PN	PA	PEL	PEH
Macronutrient content, %										
Dry matter	91.3	91.1	91.1	91.4	91.4	91.6	91.4	91.3	91.4	91.5
Crude protein	20.3	19.6	20.0	20.2	20.5	20.2	20.4	20.7	20.5	20.3
Crude fat	10.7	10.7	11.8	11.3	11.4	11.5	11.3	11.0	11.4	10.1
Crude fiber	2.62	2.44	2.86	2.43	2.48	3.95	3.78	3.90	3.85	3.76
Ash	6.17	6.09	6.22	6.15	6.09	5.16	5.17	5.15	5.27	5.23
Gross energy, kcal/kg	4,392	4,378	4,383	4,364	4,346	4,434	4,420	4,426	4,407	4,389
Fatty acid composition, %										
C16:0	12.7	38.9	42.0	41.1	42.0	11.7	38.4	41.8	41.2	41.3
C18:0	3.30	4.20	4.39	4.46	4.39	3.15	4.03	4.28	4.38	4.26
C18:1 n-9	25.5	35.1	33.4	33.1	33.4	25.1	34.7	32.9	32.8	32.9
C18:2 n-6	49.7	17.5	15.7	16.2	15.7	50.8	18.2	16.3	16.6	16.7
C18:3 n-3	5.38	0.91	0.86	0.91	0.86	5.83	1.12	1.04	1.08	1.03
Minor fatty acids	3.41	3.31	3.67	4.22	3.67	3.38	3.45	3.77	4.01	3.80
SFA	17.5	45.2	49.0	48.7	48.8	16.4	44.7	48.7	48.5	48.3
MUFA	27.4	36.4	34.5	34.2	34.5	27.0	35.9	34.0	33.8	34.0
PUFA	55.1	18.4	16.6	17.1	16.7	56.6	19.3	17.3	17.7	17.7

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

[1] All samples were analyzed at least in duplicate.

[2] Diets with 10% (as-fed basis) of native soybean oil (SN), native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

3.3.3. Controls and sampling

The amount of feed and BW of the animals were recorded at the beginning, on day 14 and at the end of the trial, to calculate average daily feed intake (**ADFI**), average daily gain (**ADG**), and gain-to-feed ratio (**G:F**) for each period and for the overall study.

From day 6 to 10, a balance study was undertaken. Feces were collected once a day by rectal stimulation and were immediately frozen at -20°C. Feces were pooled within a pen, freeze-dried, ground, and kept at 5°C until further analyses. Fecal samples were analyzed by the same methods as those described for feeds (HCl-insoluble ash, gross energy, and FA content) to determine the digestible energy of the diets and the apparent absorption of FA. The apparent absorption coefficient of a particular nutrient (X) was calculated as follows:

% apparent absorption of X = $\{1 - [(F_X / F_M) / (D_X / D_M)]\} \times 100$,

where F_X is the concentration of a particular nutrient in feces, F_M is the concentration of the inert marker in feces, D_X is the concentration of a particular nutrient in the diet, and D_M is the concentration of the inert marker in the diet. The digestible energy was calculated from the product of energy apparent absorption and its corresponding feed gross energy.

The acylglycerol and FFA composition of feces was analyzed according to ISO 18395:2005, as described for experimental fats. Before analysis, fat was extracted from feces with diethyl ether after acidification with HCl 1 *N*. The acylglycerol and FFA molecules were quantified by internal normalization. Finally, these values were expressed as grams per 100 g of fat intake, based on values of fat apparent absorption.

3.3.4. Statistical analysis

Normality of the data and homogeneity of the variance were verified. The experiment was designed as a randomized complete block design with six blocks of initial BW and five dietary treatments. All data were subjected to ANOVA using the GLM procedure of SAS (version 9.2, SAS Inst. Inc.; Cary, NC, USA). Differences between treatment means were tested using Tukey's correction for multiple comparisons. The experimental unit was de pen, so there were six replicates per dietary treatment. Results in tables are reported as least square means and differences were considered significant at $P < 0.05$.

3.4. Results and discussion

3.4.1. Characterization of experimental fats

The chemical analyses of the experimental fats are presented in **Table 3.1**. Whereas SN oil was mainly composed of linoleic (49.3%) and oleic (28.1%) acids, palm oils were high in palmitic (47.0 ± 1.43%) and oleic (36.1 ± 1.32%) acids. Little variability was observed in the FA composition of palm oils.

The specific distribution of FA within acylglycerol molecules found in native oils was in agreement with that reported by Mattson and Volpenhein (1963). In both SN and PN oils, whereas SFA were preferentially esterified at the *sn*-1,3 positions, unsaturated fatty acids (**UFA**) were preferentially esterified at the *sn*-2 position. The chemical esterification process involved a certain redistribution of FA within acylglycerol molecules. Although this process did not result in a complete random distribution of FA (i.e., 33% of each FA should appear in the *sn*-2 position), re-esterified palm oils showed a greater percentage of SFA located at the *sn*-2 position and, therefore, a lower percentage of MUFA and PUFA located at the *sn*-2 position, when compared with their corresponding PN oil. The reason for the lower proportion of *sn*-2 FA in PEH oil rather than in PEL oil was the greater 1(3)-MAG and 1,3-DAG content of the former. In this sense, PA oil, due to its high FFA content, showed the lowest proportions of *sn*-2 FA.

Regarding fat acylglycerol and FFA composition, native oils, considered as positive controls, were mainly composed of TAG, although PN oil showed a lower TAG and a greater DAG and FFA contents than did SN oil. Acid palm oil, as a negative control, showed a high FFA content, so its glycerol-to-FA ratio decreased. Re-esterified oils showed variable amounts of TAG, DAG and MAG, and low levels of FFA. PEH oil showed a greater amount of MAG and DAG, and a lower amount of TAG than did PEL oil. The different acylglycerol and FFA composition observed in experimental fats was closely related to their glycerol-to-FA ratio and, in turn, to their subsequent gross energy content. Given that the average heat of combustion of palm FA (9,455 kcal/kg) is more than twice that of glycerol (4,346 kcal/kg), the increased glycerol-to-FA ratio of PEH oil resulted in a lower gross-energy content. On the other hand, in both re-esterified oils, 1(3)-MAG and 1,3-DAG were the major positional isomers.

Finally, indices of lipid quality showed low levels of moisture, impurities and unsaponifiable matter for native oils. However, PA oil, PEH oil, and especially PEL oil showed a greater unsaponifiable matter content.

3.4.2. Digestibility balance

The effects of dietary fat sources on the digestible energy of the diets and the apparent absorption of individual FA are presented in **Table 3.4**. As expected, UFA were better absorbed than were SFA, and stearic acid was less well absorbed than was palmitic acid, because as unsaturation increases, digestibility is increased, and as the FA chain length increases, digestibility is reduced (Doreau and Chilliard, 1997). In this sense, SN, due to its high UFA content, showed the greatest total FA apparent absorption and, therefore, the greatest digestible energy. The more unsaturated the fat, the greatest the digestible energy, as has been observed by several authors with young pigs (Cera et al., 1988; Powles et al., 1994) and also with growing/finishing pigs (Wiseman et al., 1990; Powles et al., 1993). However, SN treatment also showed the lowest SFA apparent absorption, in particular, of stearic acid. The negative values of stearic acid apparent absorption may be caused by endogenous losses (fat secretion during the passage of digesta and fat synthesis in the hind gut), but, above all, by the biohydrogenation of UFA of the C18-family into stearic acid by the microflora of the hind gut (Carlson and Bayley, 1968; Duran-Montgé et al., 2007). Therefore, hind-gut fermentation might contribute to changes in FA composition of feces and, consequently, influence digestibility coefficients of individual FA. Compared to apparent ileal digestibility, apparent fecal digestibility underestimates the apparent absorption of stearic acid and overestimates the apparent absorption of UFA of the C18-family, but the total FA apparent absorption is not affected (Jørgensen et al., 1992).

No differences were found between PN and PA treatments ($P > 0.05$), unlike what was expected. In contrast with our results, Powles et al. (1994) reported a progressive linear decline in the apparent absorption of both saturated and unsaturated fat sources with increasing FFA content in young pigs. However, in agreement with our results, DeRouchey et al. (2004) examined the effects of different levels of FFA in choice white grease (up to 53%) and found that the concentration of FFA had no effect on fat apparent absorption in weaning piglets. Both our PA oil and the maximum level of FFA used by DeRouchey et al. (2004) did not exceed 60% of FFA and, therefore, the rest of acylglycerol molecules present in the oil could have provided enough MAG for the formation of mixed micelles. Thus, acid oils are an economically interesting alternative to their corresponding native oils, provided they have a moderate amount of FFA and a low percentage of moisture, impurities and unsaponifiable matter.

Table 3.4. Digestible energy of the diets (kcal/kg) and individual fatty acid apparent absorption coefficients (%) in weaning piglets fed different dietary fat sources

	Dietary treatments[1]						
Item	SN	PN	PA	PEL	PEH	RMSE[2]	*P*-values
DE, kcal/kg	3,830[a]	3,698[ab]	3,721[ab]	3,681[b]	3,741[ab]	77.0	*
Total fatty acids	77.7[a]	65.4[b]	65.6[b]	65.9[b]	72.6[ab]	6.27	**
SFA	23.5[b]	45.6[a]	47.9[a]	49.8[a]	58.5[a]	10.63	***
MUFA	74.5	78.8	80.1	78.2	84.0	6.63	NS
PUFA	96.6[a]	87.1[b]	88.0[b]	86.9[b]	90.4[b]	2.23	***
C16:0	60.7	54.1	52.4	57.0	64.6	7.82	NS
C18:0	-120.4[b]	-28.6[a]	3.4[a]	-21.1[a]	1.5[a]	29.97	***
C18:1 n-9	91.4	85.6	86.1	85.6	89.5	3.44	NS
C18:2 n-6	96.6[a]	87.2[b]	88.0[b]	86.9[b]	90.5[b]	2.21	***
C18:3 n-3	96.7[a]	85.1[c]	87.3[bc]	86.3[bc]	89.8[b]	2.48	***

DE = digestible energy; MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; RMSE = root mean square error; SFA = saturated fatty acids.

[1] Diets with 10% (as-fed basis) of native soybean oil (SN), native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

[2] n = 6.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Regarding re-esterified oils, although no statistically significant differences were found among palm oil treatments, PEH showed the greatest total FA apparent absorption, which was not statistically different from SN ($P = 0.63$). This is possibly due to its high MAG and DAG content. Jones et al. (1992) observed that the addition of distilled MAG to tallow increased the digestibility of total FA and long-chain SFA in weanling pigs, due to their emulsifying effect. Nevertheless, the greater *sn*-2 palmitic acid content of PEL oil did not enhance the apparent absorption of this long-chain SFA, when compared with that of PN and PA treatments ($P > 0.05$). In contrast with our results, greater fat absorption has been found in human newborn infants (Filer et al., 1969; Carnielli et al., 1995b) and rats (Tomarelli et al., 1968; Renaud et al., 1995; Lien et al., 1997) fed TAG with palmitic acid esterified at the *sn*-2 rather than at the *sn*-1,3 positions. It is possible that the lack of improvement observed for the total FA apparent absorption in PEL may be due to the lower palmitic acid content at the *sn*-2 position of this oil (19.9%) when compared with that of fats used in the studies cited above (from 33 to 84%).

3.4.3. Acylglycerol and free fatty acid composition of feces

The products of fat digestion in feces were analyzed to better understand how the fat molecular structure affects the digestion and absorption processes. The acylglycerol and FFA composition of feces (in grams per 100 g of fat intake) is shown in **Table 3.5**. Feces contained low levels of TAG, DAG and MAG. This means that TAG and DAG were almost completely hydrolyzed along the gastrointestinal tract, and MAG were absorbed due to their hydrophilic character (2-MAG and some 1(3)-MAG) or completely hydrolyzed to glycerol and FFA (mainly 1(3)-MAG). Consequently, FFA constituted the major lipid fraction in feces for all treatments, suggesting that the lack of bile salts is the main limiting factor of fat absorption in weaning piglets. In agreement with our results, several authors have attributed the decreased fat apparent absorption in weaning piglets to insufficient secretion of bile (Jones et al., 1992), although others have suggested a decreased secretion of lipases due to the adaptation of the pancreas to the new diet (Lindemann et al., 1986; Jensen et al., 1997).

Table 3.5 Acylglycerol and free fatty acid composition of feces (g/100 g of fat intake) of weaning piglets fed different dietary fat sources

	Dietary treatments[1]						
Item	SN	PN	PA	PEL	PEH	RMSE[2]	*P*-values
TAG	1.02	0.95	1.06	1.08	1.10	0.148	NS
DAG	4.22	3.98	4.17	4.11	4.09	0.529	NS
MAG	2.01^{ab}	1.49^{b}	2.05^{ab}	2.30^{a}	1.87^{ab}	0.367	*
FFA	15.7^{b}	18.8^{ab}	22.2^{a}	20.0^{ab}	16.8^{b}	3.03	*

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; NS = not significant; RMSE = root mean square error; TAG = triacylglycerols.

[1] Diets with 10% (as-fed basis) of native soybean oil (SN), native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

[2] n = 6.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Regarding differences among dietary treatments, animals fed PA excreted a greater amount of FFA than did those fed SN and PEH diets ($P < 0.05$). Small differences were also found for MAG content. Animals fed PEL excreted a greater MAG content than those fed PN ($P = 0.008$). In contrast with the findings of Simoes (1985),who reported a greater response of lipase to unsaturated fats than to saturated

fats in growing pigs, our data did not show a lower excretion of TAG, DAG and MAG by animals fed SN treatment in comparison with those fed palm oil sources ($P > 0.05$), ruling out the possibility of an increased hydrolytic activity when unsaturated fat sources are added.

3.4.4. Growth performance

The effects of dietary fat sources on growth performance are presented in **Table 3.6**. Growth performance of the piglets was unaffected by added dietary fat sources ($P > 0.05$), although G:F showed a close numerical relationship with total FA apparent absorption values. Thus, for the whole experimental period, the best G:F were found for animals fed SN and, among P treatments, animals fed PEH were those that performed the best. In agreement with our results, several authors have also consistently reported limited, if any, growth response during the first 2- to 3-week post-weaning when various supplemental fat sources were added to diets (Lawrence and Maxwell, 1983; Cera et al., 1988; Jung et al., 2003).

Table 3.6. Growth performance of weaning piglets fed different dietary fat sources

Item	Dietary treatments[1]					RMSE[2]	*P*-values
	SN	PN	PA	PEL	PEH		
From 0 to 14 days							
ADFI, g	407	435	431	405	410	50.0	NS
ADG, g	320	317	328	292	327	37.5	NS
G:F, g/g	0.78	0.73	0.78	0.72	0.76	0.055	NS
BW at 14 days, kg	13.0	13.0	13.1	12.6	13.1	0.52	NS
From 14 to 29 days							
ADFI, g	754	798	817	819	797	54.3	NS
ADG, g	511	518	506	535	536	28.4	NS
G:F, g/g	0.67	0.65	0.62	0.66	0.67	0.034	NS
BW at 29 days, kg	20.1	20.8	20.7	20.6	21.1	0.91	NS
From 0 to 29 days							
ADFI, g	587	623	631	619	610	46.6	NS
ADG, g	420	421	419	417	435	24.0	NS
G:F, g/g	0.71	0.68	0.68	0.68	0.70	0.032	NS

ADFI = average daily feed intake; ADG = average daily gain; BW = body weight; G:F = gain-to-feed ratio; NS = not significant; RMSE = root mean square error.

[1] Diets with 10% (as-fed basis) of native soybean oil (SN), native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

[2] n = 6.

Among dietary treatments, SN was the treatment that reached the greatest FA apparent absorption, due to its high unsaturated FA content. Although no differences were found among palm-oil sources, PEH achieved the best total FA apparent absorption coefficient, and was not different from SN, probably due to the emulsifying effect of MAG and DAG. In conclusion, PEH oil can be used in weaning-piglet diets as a good, alternative fat source to its corresponding PN and PA oils.

CHAPTER 4

Use of re-esterified palm oils, differing in their molecular structure, in fattening-pig diets

Use of re-esterified palm oils, differing in their molecular structure, in fattening-pig diets

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4.1. Abstract

Re-esterified oils are new fat sources obtained from the chemical esterification of acid oils with glycerin (both economically interesting by-products from oil refining and biodiesel industries, respectively). The different fatty acid positional distribution and acylglycerol composition of re-esterified oils may enhance the apparent absorption of saturated fatty acids and, therefore, their overall nutritive value, which might lead to an increased deposition of saturated fatty acids. The aim of the present study was to investigate the potential use of re-esterified palm oils, in comparison with their corresponding acid and native oils in fattening-pig diets, studying their effects on fatty-acid apparent absorption, acylglycerol and free fatty acid composition of feces, growth performance, carcass-fat depots, and fatty-acid composition of backfat. Seventy-two crossbred boars and gilts (average weight of 24.7 ± 2.55 kg) were blocked by gender and initial body weight, housed in adjacent individual boxes, and fed one of the four dietary treatments, which were the result of a basal diet supplemented with 4% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH). Regarding results from the digestibility balance, PA and PN showed similar apparent absorption coefficients ($P > 0.05$), despite the high, free fatty acid content of the former. However, re-esterified palm oils (both PEL and PEH) showed a higher apparent absorption of total fatty acids than did their corresponding native and acid oils ($P < 0.001$), mainly due to the increased apparent absorption of saturated fatty acids ($P < 0.001$). This resulted in a greater feed efficiency and an increased deposition of saturated fatty acids in backfat of pigs fed PEH, when compared to those fed PA ($P < 0.05$), although no differences were found for carcass-fat depots ($P > 0.05$). We conclude that PEH oil can be used in fattening-pig diets as a better alternative fat source than PA oil.

4.2. Introduction

Palm oil is one of the few vegetable oils rich in saturated fatty acids (**SFA**). Thus, its use in fattening-pig diets is attractive because it may be associated with a positive influence on fat firmness (Wiseman and Agunbiade, 1998). However, its high SFA content impairs its nutritive value (Powles et al., 1993). Besides the importance of the fat degree of saturation, the fat molecular structure also plays an important role in the nutritive value of fats. Given the specificity of pancreatic lipase for the hydrolysis of

fatty acids (**FA**) located at the *sn*-1,3 positions (Mattson and Beck, 1956), SFA are better absorbed if they are located at the *sn*-2 position, because they are absorbed as 2-monoacylglycerols, instead of as free SFA. Free SFA are highly hydrophobic and have a great ability to form insoluble soaps with divalent cations in the gut (Small, 1991). This fact might become more relevant with the use of acid palm oils (by-products from oil-refining industry), which are rich in free SFA.

The chemical esterification of acid palm oil with glycerin (another economically interesting by-product from biodiesel industry) results in the formation of re-esterified palm oils. These alternative fat sources are expected to show an increased proportion of SFA located at the acylglycerol *sn*-2 position, and a higher amount of mono- (**MAG**) and diacylglycerol (**DAG**) molecules than their corresponding native oil. Therefore, it was hypothesized that the different molecular structure of re-esterified palm oils may enhance the SFA apparent absorption and, thus, their overall nutritive value, which might lead to an increased deposition of SFA. The aim of the present study was to investigate the potential use of re-esterified palm oils, differing in their acylglycerol structure, in comparison with their corresponding acid and native oils in fattening-pig diets, studying their effects on FA apparent absorption, acylglycerol and free fatty acid (**FFA**) composition of feces, growth performance, carcass-fat depots, and FA composition of backfat.

4.3. Materials and methods

4.3.1. Experimental fats

Experimental fats were supplied by SILO S.p.a. (Florence, Italy). Re-esterified palm oils were produced using, as raw materials, acid palm oil (a by-product obtained from the refining process of crude palm oil, with a high FFA content) and glycerin (a by-product obtained from the methylation process applied for biodiesel production), which were processed under high vacuum conditions (1-3 mm Hg), for 4-6 hours, at 190-250^{o}C, and without chemical catalysts. According to the stoichiometric proportion of FFA and glycerol, fats with the same FA composition, but with different FA positional distribution, and triacylglycerol (**TAG**), DAG and MAG proportions were obtained (**Table 4.1**).

Table 4.1. Chemical analyses of the experimental fats[1]

Item		PN oil	PA oil	PEL oil	PEH oil
Moisture, %		0.10	0.17	0.36	1.13
Impurities, %		<0.50	<0.50	0.50	<0.50
Unsaponifiable matter, %		0.58	1.14	1.92	1.78
Fatty acid composition and distribution, %					
C16:0	Total	43.4	45.0	45.1	46.4
	sn-2 %[2]	8.53	6.81	17.5	11.1
C18:0	Total	4.34	4.50	4.54	4.57
	sn-2 %[2]	5.33	8.74	19.5	15.1
C18:1 n-9	Total	39.3	37.7	37.9	37.2
	sn-2 %[2]	52.2	11.3	24.8	19.1
C18:2 n-6	Total	10.1	9.66	9.19	8.59
	sn-2 %[2]	59.3	11.2	24.8	19.0
Minor fatty acids		2.89	3.23	3.30	3.26
SFA	Total	49.1	50.8	51.1	52.5
	sn-2 %[2]	8.33	6.92	17.6	11.6
MUFA	Total	40.5	39.1	39.4	38.7
	sn-2 %[2]	51.4	11.4	24.8	19.1
PUFA	Total	10.4	10.1	9.49	8.79
	sn-2 %[2]	58.4	11.2	24.8	19.0
Acylglycerol and FFA composition, %					
TAG		87.5	16.3	41.9	11.8
DAG	Total	7.72	22.3	44.3	45.8
	1(3),2-DAG %[3]	26.7	31.8	27.1	31.3
MAG	Total	0.70	8.26	12.6	42.4
	2-MAG %[4]	40.0	8.85	7.25	7.74
FFA		4.04	53.2	1.22	0.00
Glycerol-to-fatty acid ratio[5], mol/mol		0.34	0.23	0.47	0.67
Gross energy, kcal/kg		9359	9178	9118	8641

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; TAG = triacylglycerols.

[1] Native palm oil (PN oil), acid palm oil (PA oil), re-esterified palm oil low in mono- and diacylglycerols (PEL oil), and re-esterified palm oil high in mono- and diacylglycerols (PEH oil).

[2] Fatty acid positional distribution, expressed as the proportion of each fatty acid located at the *sn*-2 position of the acylglycerol molecules, calculated as follows: *sn*-2 % = (*sn*-2 / Total) × *a* × 100, where *sn*-2 is the FA composition at the *sn*-2 position (converted to mol%), Total is the total FA composition of the fat (converted to mol%), and *a* is the ratio between the moles of FA located at the *sn*-2 position and the moles of total FA. *a* was 0.30, 0.09, 0.21, and 0.15 for PN, PA, PEL, and PEH oils, respectively.

[3] The proportion of 1(3),2-DAG *vs.* 1,3-DAG.

[4] The proportion of 2-MAG *vs.* 1(3)-MAG.

[5] Estimated calculation based on the values of the acylglycerol and FFA composition.

Oil samples were analyzed in triplicate for moisture (Method 926.12 of the AOAC International, 2005), impurities (ISO 663:2007), unsaponifiable matter (Method 933.08 of the AOAC International, 2005), acylglycerol and FFA composition (ISO 18395:2005), MAG and DAG positional isomers (Sacchi et al., 1997), total FA composition (Guardiola et al., 1994), *sn*-2 FA composition (Commission Regulation (EEC) No. 2568/91 – Annex VII), and gross energy content (IKA-Kalorimeter system C2000 basic; Staufen, Germany), as described in more detail in *Chapter 3*.

4.3.2. Animals and diets

The study was performed at the experimental farm of *IRTA Mas de Bover* (Constantí, Tarragona, Spain). The experimental procedure received the prior approval from the Ethical Committee of the same institution. All animal housing and husbandry conformed to the European Union Guidelines (2010/63/EU).

A total of 72 crossbred ([Landrace × Duroc] × Pietrain) boars and gilts, with an initial BW of 24.7 ± 2.55 kg, were obtained from the swine herd of the same experimental station, blocked by gender and initial body weight (**BW**) (nine blocks of BW for each gender), and randomly assigned to one of the four dietary treatments. Animals were housed in adjacent individual boxes, had free access to feed and water, and were raised under controlled conditions of light and temperature.

The experiment was planned to cover the BW range from ~25 to 100 kg of BW. For this purpose, the feeding program consisted of three diets (in pelleted form): a starter diet (from ~25 to 50 kg of BW; from 0 to 35 days of the experiment), a grower diet (from ~50 to 75 kg of BW; from 35 to 70 days of the experiment) and a finisher diet (from ~75 to 100 kg of BW; from 70 to 100 days of the experiment). Basal diets were formulated to meet or exceed NRC (2012) requirements and to minimize basal fat levels. One-half of the starter diet was supplemented with 1% of Celatom (Jesús Riesgo; Madrid, Spain) to increase the amount of HCl-insoluble ash as an inert digestibility marker and, thus, perform the digestibility balance. The four dietary treatments were the results of including 4% (as-fed basis) of one of the following experimental fats in the basal diets: native palm oil (**PN**), acid palm oil (**PA**), re-esterified palm oil low in MAG and DAG (**PEL**), or re-esterified palm oil high in MAG and DAG (**PEH**). The composition of experimental diets is presented in **Table 4.2**.

Table 4.2. Ingredient composition of the experimental diets (as-fed basis)

	Starter diet (from 0 to 35 days)		Grower diet (from 35 to 70 days)	Finisher diet (from 70 to 100 days)
Ingredients (%)	With marker	Without marker		
Wheat	38.44	29.68	22.00	17.61
Barley	30.00	39.97	52.22	59.51
Soybean meal 44%	15.40	14.85	10.43	7.62
Experimental fats[1]	4.00	4.00	4.00	4.00
Biscuit meal	4.00	4.00	4.00	4.00
Sunflower meal	3.69	4.00	4.00	4.00
Dicalcium phosphate	1.12	1.10	0.93	0.82
Celatom[2]	1.00	-	-	-
Calcium carbonate	0.71	0.77	0.71	0.70
Sodium bicarbonate	0.55	0.54	0.67	0.76
Vitamin and mineral premix[3]	0.40	0.40	0.40	0.40
L-Lysine	0.44	0.43	0.41	0.39
L-Threonine	0.16	0.15	0.16	0.14
DL-Methionine	0.11	0.10	0.09	0.06
Ethoxyquin 66%	0.02	0.02	0.02	0.02

[1] Native palm oil (PN oil), acid palm oil (PA oil), re-esterified palm oil low in mono- and diacylglycerols (PEL oil), or re-esterified palm oil high in mono- and diacylglycerols (PEH oil).
[2] Jesús Riesgo; Madrid, Spain.
[3] Provides per kg of feed: vitamin A (from retinol), 5500 IU; vitamin D_3 (from cholecalciferol), 1100 IU; vitamin E (from alfa-tocopherol), 7 mg; thiamine, 0.5 mg; riboflavin, 1.4 mg; pyridoxine, 1.0 mg; cobalamine, 0.008 mg; calcium pantothenate, 5.6 mg; nicotinic acid, 8 mg; menadione, 0.5 mg; Ca, 0.72 g; Mg, 57 mg; Fe (from $FeSO_4 \cdot 7H_2O$), 80 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 10 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$), 0.4 mg; Zn (from ZnO), 100 mg; Mn (from MnO), 40 mg; I (from $Ca(I_2O_3)_2$), 0.5 mg; Se (from Na_2SeO_3), 0.25 mg; ethoxyquin, 2.67 mg.

Analytical determinations of feeds were performed according to the methods of AOAC International (2005): dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fat (Method 2003.05), and crude fiber (Method 962.09). Gross energy was determined as described previously for experimental fats and HCl-insoluble ash of pre-starter feeds was determined following the method of McCarthy et al. (1974). Lipids from feeds were extracted with chloroform/methanol (2:1, by vol) according to the Folch et al. (1974) procedure. Nonadecanoic acid (C19:0, Sigma-Aldrich Chemical Co.; St. Louis, MO, USA) was added as an internal standard prior to processing. The FA content was analyzed following the method of Morrison and Smith (1964). The macronutrient and the FA composition of the experimental diets are presented in **Table 4.3**.

Table 4.3. Analyzed[1] macronutrient content and fatty acid composition of the experimental diets[2]

	Starter diet (from 0 to 35 days)								Grower diet (from 35 to 70 days)				Finisher diet (from 70 to 100 days)			
	With marker				Without marker											
Item	PN	PA	PEL	PEH	PN	PA	PEL	PEH	PN	PA	PEL	PEH	PN	PA	PEL	PEH
Macronutrient content, %																
Dry matter	88.8	88.4	88.7	88.5	88.4	88.3	88.3	88.2	88.9	88.2	89.0	88.7	89.8	89.7	89.6	89.7
Crude protein	15.8	16.3	16.0	16.1	16.3	16.5	16.3	16.3	15.3	15.3	15.0	15.4	14.7	14.9	14.3	14.4
Crude fat	6.13	6.08	5.89	6.04	5.88	5.90	5.95	6.04	5.95	6.20	6.01	5.70	6.03	6.35	6.04	6.18
Crude fiber	3.88	3.84	3.97	3.79	4.12	3.79	3.78	3.76	4.36	4.38	4.51	4.07	4.49	4.03	4.13	4.20
Ash	5.63	5.59	5.52	5.60	4.77	4.70	4.82	4.63	4.39	4.32	4.48	4.39	4.32	4.40	4.24	4.42
Gross energy, kcal/kg	4,047	4,040	4,037	4,018	4,087	4,080	4,078	4,059	4,090	4,083	4,081	4,061	4,139	4,132	4,129	4,110
Fatty acid composition, %																
C16:0	32.9	33.5	34.3	34.2	33.5	34.7	33.7	33.9	33.5	33.4	34.0	33.7	32.7	32.6	33.0	31.4
C18:0	3.37	3.45	3.41	3.48	3.56	3.50	3.38	3.38	3.23	3.35	3.37	3.32	3.24	3.32	3.26	3.15
C18:1 n-9	36.0	34.1	33.5	33.6	35.3	34.4	35.2	34.0	34.5	34.4	34.3	33.4	36.4	36.3	36.1	35.9
C18:2 n-6	23.3	24.5	24.3	24.2	23.3	23.0	23.2	24.2	24.3	24.4	23.9	25.0	23.3	23.3	23.2	25.0
C18:3 n-3	1.62	1.82	1.73	1.70	1.56	1.71	1.66	1.73	1.70	1.82	1.73	1.81	1.65	1.78	1.70	1.87
Minor fatty acids	2.79	2.68	2.77	2.78	2.79	2.67	2.82	2.80	2.72	2.64	2.65	2.74	2.73	2.67	2.73	2.69
SFA	37.5	38.2	39.1	39.0	38.3	39.4	38.4	38.6	38.1	38.0	38.7	38.3	37.2	37.1	37.6	35.8
MUFA	37.4	35.5	34.9	35.0	36.7	35.8	36.7	35.4	35.9	35.8	35.6	34.8	37.8	37.7	37.5	37.3
PUFA	25.0	26.3	26.1	26.0	24.9	24.7	24.9	26.0	26.0	26.2	25.7	26.9	25.0	25.2	24.9	26.9

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

[1] All samples were analyzed, at least, in duplicate.

[2] Diets with 4% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

4.3.3. Controls and sampling

The amount of feed and BW of the animals were recorded at the beginning and at the end of each phase (0, 35, 70 and 100 days of the experiment), which enabled the calculation of average daily feed intake (**ADFI**), average daily gain (**ADG**), and gain-to-feed ratio (**G:F**) for each period and for the overall study.

From day 6 to 9, a digestibility balance was undertaken. Feces were collected once a day by rectal stimulation and were immediately frozen at -20ºC. On the last day of the balance, feces from each animal were pooled, homogenized, and a representative sample was freeze-dried, ground, and kept at 5ºC until further analyses. Fecal samples were analyzed by the same methods as those described for feeds (HCl-insoluble ash, gross energy, and FA content) to determine the digestible energy of the diets and the apparent absorption of FA. The apparent absorption coefficient of a particular nutrient (X) was calculated as follows:

% apparent absorption of X = $\{1 - [(F_X / F_M) / (D_X / D_M)]\} \times 100$,

where F_X is the concentration of a particular nutrient in feces, F_M is the concentration of the inert marker in feces, D_X is the concentration of a particular nutrient in the diet, and D_M is the concentration of the inert marker in the diet. The digestible energy was calculated from the product of energy apparent absorption and its corresponding feed gross energy.

The acylglycerol and FFA composition of feces was analyzed according to ISO 18395:2005. Prior to analysis, fat was extracted from feces with diethyl ether after acidification with HCl 1 *N*. The acylglycerol and FFA molecules were quantified by internal normalization. Finally, these values were expressed as grams per 100 g of fat intake, based on values of fat apparent absorption.

At the end of the experimental period, three boars and three gilts of each treatment (those with BW close to the treatment average weight) were transported to the *IRTA* experimental abattoir (Monells, Girona, Spain). Animals were fasted (deprived of feed but not water) for approximately 16 h. The following morning, they were reweighed to obtain the fasted live weight, stunned using 85% CO_2 for 120 s, and killed by exsanguination. The carcasses were then scalded, mechanically dehaired before evisceration, split along the mid line, and weighed. Fat and muscle thickness were measured using the Fat-O-Meat'er probe (Carometec A/S; Soeborg, Denmark) between the third- and the fourth-last ribs at 6 cm to the midline. Values obtained were used to calculate the lean meat percentage according to the Spanish official equation (Font-i-Furnols and Gispert, 2009). The liver and perirenal fat were weighed, and were

expressed in absolute values and as a percentage of the live animal weight. Backfat samples (from the dorsal midline overlaying the *M. longissimus dorsi*) were taken, vacuum packed, and stored at -20ºC until chemical analyses. The FA composition of backfat samples was analyzed following the same method as that described for feed and feces.

4.3.4. Statistical analysis

Normality of the data and homogeneity of the variance were verified. The experiment was designed as a randomized complete block design with nine blocks of initial BW within sexes and four dietary treatments. All data were subjected to ANOVA using the GLM procedure of SAS (version 9.2, SAS Inst. Inc.; Cary, NC, USA). For carcass-fat depots and FA composition of backfat, the model only included dietary treatment and sex as fixed effects. Differences between treatment means were tested using Tukey's correction for multiple comparisons. Individual pig data served as the experimental unit, so there were 18 replicates per dietary treatment. For carcass-fat depots and FA composition of backfat, there were only six observations per dietary treatment. For perirenal fat and liver weights, the BW before slaughter was included as a covariate in the model. Results in tables are reported as least square means and differences were considered significant at $P < 0.05$.

4.4. Results and discussion

4.4.1. Characterization of experimental fats

The chemical analyses of the experimental fats are presented in **Table 4.1**. Experimental fats showed a similar FA composition, indicating that the esterification of acid palm oil with glycerin did not substantially modify the FA composition of fat. More than 80% of the total FA was composed of palmitic (45.0 ± 1.23%) and oleic (38.0 ± 0.65%) acids.

Regarding the fat acylglycerol and FFA composition, PN oil, considered the positive control, was mainly composed of TAG. Acid palm oil, as a negative control, showed a high FFA content. Re-esterified oils contained 45.1 ± 1.06% of DAG, almost no FFA, and variable amounts of TAG and MAG. Re-esterified palm oil high in MAG and DAG showed a higher amount of MAG (42.4% vs. 12.6%) and a lower amount of

TAG (11.8% vs. 42.4%) than did PEL oil. In both re-esterified oils, 1(3)-MAG and 1,3-DAG were the major positional isomers.

The FA positional distribution within acylglycerol molecules found in PN oil was in agreement with that reported by Mattson and Volpenhein (1963), SFA being preferentially esterified at the *sn*-1,3 positions and unsaturated FA at the *sn*-2 position, since FA distribution in vegetable oils is genetically determined. However, the chemical esterification process does not allow a selective esterification of FA in the different glycerol positions. Therefore, compared to PN oil, re-esterified palm oils showed a certain redistribution of FA within acylglycerol molecules. Although this process did not result in a complete random distribution of FA (i.e., 33% of each FA should appear in the *sn*-2 position), re-esterified palm oils showed a higher percentage of SFA and, therefore, a lower percentage of MUFA and PUFA located at the *sn*-2 position, when compared with their corresponding PN oil. The reason for the lower proportion of *sn*-2 FA in PEH oil than in PEL was the higher 1(3)-MAG content of the former. In this sense, PA oil, due to its high FFA content, showed the lowest proportions of *sn*-2 FA.

Finally, indices of lipid quality showed low levels of moisture, impurities, and unsaponifiable matter for PN and PA oils, but yielded values above 2.5% for re-esterified palm oils.

4.4.2. Digestibility balance

The effects of dietary fat sources on the digestible energy of the diets and the apparent absorption of individual FA are presented in **Table 4.4**. As expected, unsaturated FA were better absorbed than were SFA, and stearic acid was less well absorbed than was palmitic acid. According to Doreau and Chilliard (1997), as unsaturation increases, digestibility is increased, and as FA chain-length increases, digestibility is reduced. However, it must be taken into account that the apparent fecal absorption of individual FA is affected by microflora biohydrogenation of unsaturated FA in the hindgut, resulting in an underestimation of the apparent absorption of stearic acid and an overestimation of the apparent absorption of unsaturated FA of the C18-family (Duran-Montgé et al., 2007). In any case, the total FA apparent absorption is not affected (Jørgensen et al., 1992)

Contrary to what was expected, no differences in FA apparent absorption were found between PN and PA treatments ($P > 0.05$). The same results were found by DeRouchey et al. (2004), who examined the effects of different levels of FFA in choice white grease in weaning piglets. In contrast, Powles et al. (1993) reported that the FFA

content of fats appeared to be one of the major determinants of the digestible energy values of fats when given to growing/finishing pigs. The reason of these inconsistent results may be related to the FFA content. Whereas our PA oil and the choice white grease used by DeRouchey et al. (2004) had a FFA content below 60%, the level of FFA used by Powles et al. (1993) was of 80%. Thus, acid oils may be an economically interesting alternative to native oils, provided they have a moderate amount of FFA and a low percentage of moisture, impurities, and unsaponifiable matter, as we have also observed in weaning piglets (*Chapter 3*).

Table 4.4. Digestible energy of the diets (kcal/kg) and individual fatty acid apparent absorption coefficients (%) in fattening pigs fed different dietary fat sources

	Dietary treatments[1]					
Item	PN	PA	PEL	PEH	RMSE[2]	*P*-values
DE, kcal/kg	3,441	3,438	3,470	3,469	74.0	NS
Total fatty acids	84.3^{b}	85.1^{b}	88.7^{a}	88.9^{a}	2.78	***
SFA	69.1^{b}	70.9^{b}	81.2^{a}	80.8^{a}	5.99	***
MUFA	92.9^{b}	93.4^{ab}	94.2^{a}	94.4^{a}	1.26	**
PUFA	94.3	94.6	94.4	94.1	1.50	NS
C16:0	78.0^{b}	78.7^{b}	87.7^{a}	89.2^{a}	4.62	***
C18:0	-2.69	4.77	18.2	9.32	26.63	NS
C18:1 n-9	96.0^{b}	96.3^{ab}	96.8^{a}	97.0^{a}	0.89	**
C18:2 n-6	94.9	95.0	94.8	94.6	1.52	NS
C18:3 n-3	94.2	94.8	94.1	93.9	1.38	NS

DE = digestible energy; MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; RMSE = root mean square error; SFA = saturated fatty acids.
[1] Diets with 4% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).
[2] n = 18.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Concerning the use of re-esterified palm oils, animals fed both PEL and PEH diets showed a higher total FA apparent absorption, compared with those fed their corresponding native (PN) and acid (PA) oil-enriched diets ($P < 0.001$). These differences were mainly given by the enhanced SFA apparent absorption observed in both PEL and PEH treatments ($P < 0.001$), although small differences were also found for the apparent absorption of monounsaturated FA ($P = 0.003$). Nevertheless, these differences were not enough to be reflected in the feed digestible energy ($P > 0.05$), probably because fat only accounted for 14% of feed gross energy.

The improved SFA apparent absorption observed in re-esterified palm oils may be related to their higher content of SFA located at the *sn*-2 position, and also to their higher amount of MAG and DAG molecules (**Table 4.1**). The importance of the FA positional distribution within acylglycerol molecules on fat absorption has already been demonstrated by several authors (Carnielli et al., 1995b; Renaud et al., 1995; Lien et al., 1997). For example, although lard and palm oil have similar levels of SFA, Renaud et al. (1995) reported that rats fed with lard showed a higher total FA apparent absorption than did those fed with palm oil. In lard, the majority of palmitic acid is esterified at the *sn*-2 position, whereas in palm oil this SFA is mainly located at the *sn*-1,3 positions (Mattson and Lutton, 1958). As a consequence, palmitic acid is easily absorbed as 2-MAG in lard or poorly absorbed as FFA in palm oil. In any case, it is important to note that our levels of *sn*-2 SFA were not as high as were those used in the studies cited above (from 33 to 84% of palmitic acid located at the *sn*-2 position). On the other hand, the importance of the presence of MAG and DAG molecules in dietary fats has been less studied. However, due to their amphiphilic properties, they may act as emulsifiers and might thus contribute towards improved digestion of fat. In this sense, Jones et al. (1992) observed that the addition of distilled MAG to tallow increased the digestibility of total FA and long-chain SFA in weaning pigs.

4.4.3. Acylglycerol and free fatty acid composition of feces

The acylglycerol and FFA composition of feces (in grams per 100 g of fat intake) is shown in **Table 4.5**. Consistent with the results observed in weaning piglets (*Chapter 3*), feces contained low levels of TAG, DAG and MAG. This means that TAG and DAG were almost completely hydrolyzed along the gastrointestinal tract, and MAG were absorbed due to their hydrophilic character [2-MAG and some 1(3)-MAG] or completely hydrolyzed to glycerol and FFA [mainly 1(3)-MAG]. Free FA constituted the major lipid fraction in feces for all treatments, suggesting that the formation of mixed micelles may be the rate-limiting step of fat absorption in fattening pigs, as has also been observed in weaning piglets (Jones et al., 1992, *Chapter 3*), but less severely.

Regarding dietary treatments, differences were only observed for FFA content. Animals fed PEL and PEH excreted a lower amount of FFA than did those fed PN and PA ($P > 0.05$). The amount of FFA excreted per 100 g of fat intake paralleled the results of SFA apparent absorption, suggesting that the emulsification of SFA is the most

critical step in fat utilization, due to their strong tendency to form insoluble soaps with calcium and magnesium, at the alkaline pH of the small intestine (Renaud et al., 1995; Lien et al., 1997).

Table 4.5. Acylglycerol and FFA composition of feces (g/100 g of fat intake) of fattening pigs fed different dietary fat sources

	Dietary treatments1					
Item	PN	PA	PEL	PEH	RMSE[2]	P-values
TAG	0.73	0.76	0.81	0.81	0.293	NS
DAG	4.45	4.17	4.38	4.41	1.183	NS
MAG	2.58	2.76	2.18	2.14	0.750	NS
FFA	14.8[a]	14.8[a]	12.1[b]	12.0[b]	3.08	**

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; NS = not significant; RMSE = root mean square error; TAG = triacylglycerols.
[1] Diets with 4% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).
[2] n = 18.
* P < 0.05; ** P < 0.01; *** P < 0.001.
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

4.4.4. Growth performance and carcass-fat depots

The effects of dietary fat sources on growth performance are presented in **Table 4.6**. Differences were only found for the G:F in the starter period and in the overall experiment. Animals fed PEH showed a higher G:F than did those fed PA ($P = 0.001$), and PN and PEL showed intermediate values. Thus, for the overall period, to gain 1 kg of BW, animals from the PA treatment needed to consume 10.7% more feed than did those from PEH ($P < 0.001$), probably related to the lower coefficient of total FA apparent absorption reported in animals fed PA.

In comparing our results with those of the literature, Scheeder et al. (2003) reported that the FA positional distribution of dietary fats did not affect growth performance in growing-finishing pigs. In relation to the fat acylglycerol composition, Murata et al. (1997), Taguchi et al. (2001), and Murase et al. (2005) also did not find differences in the performance of rats fed DAG or TAG oil-enriched diets. In contrast, Meng et al. (2004) reported that the weight gain was lower in rats fed a DAG oil-rich diet, while Kamphuis et al. (2003) observed a lesser feeling of hunger and appetite in women fed a DAG oil-rich diet.

Table 4.6. Growth performance of fattening pigs fed different dietary fat sources

Item	Dietary treatments[1]				RMSE[2]	*P*-values
	PN	PA	PEL	PEH		
From 0 to 35 days						
ADFI, g	1,449	1,504	1,438	1,438	148.7	NS
ADG, g	707	691	690	727	105.2	NS
G:F, g/g	0.48^{ab}	0.46^{b}	0.49^{ab}	0.51^{a}	0.044	*
BW at 35 days, kg	49.5	48.9	49.0	50.1	3.66	NS
From 35 to 70 days						
ADFI, g	2,024	2,023	1,963	1,987	203.8	NS
ADG, g	820	795	809	823	92.7	NS
G:F, g/g	0.40	0.39	0.41	0.42	0.030	NS
BW at 70 days, kg	78.1	76.8	77.3	79.0	5.91	NS
From 70 to 100 days						
ADFI, g	2,702	2,591	2,592	2,577	380.9	NS
ADG, g	1,031	935	936	996	145.7	NS
G:F, g/g	0.39	0.37	0.36	0.39	0.056	NS
BW at 100 days, kg	109	105	105	109	8.5	NS
From 0 to 100 days						
ADFI, g	2,022	2,045	1,966	1,972	167.8	NS
ADG, g	844	793	806	842	85.4	NS
G:F, g/g	0.41^{ab}	0.39^{b}	0.41^{ab}	0.43^{a}	0.027	**

ADFI = average daily feed intake; ADG = average daily gain; BW = body weight; G:F = gain-to-feed ratio; NS = not significant; RMSE = root mean square error.

[1] Diets with 4% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

[2] n = 18.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

The effects of dietary fat sources on carcass yield and carcass-fat depots are presented in **Table 4.7**. Carcass yield, backfat thickness, muscle depth, carcass lean percentage, and liver and perirenal fat proportions were not affected by dietary fat sources ($P > 0.05$). Regarding the FA positional distribution of dietary fat, Ponnampalam et al. (2011) observed how animals fed lard-enriched diets showed a greater external adipose tissue thickness than did those fed native palm oil, which has a lower *sn*-2 SFA content. In relation to the fat acylglycerol composition, Murase et al. (2002) and Meng et al. (2004) reported a reduced accumulation of fat in visceral adipose and subcutaneous tissue, and a reduced fat content in the liver of animals fed 1,3-DAG-enriched diets, as compared to those fed TAG-enriched diets of the same FA composition. The presence of dietary 1,3-DAG has been shown to increase β-oxidation of FA in the liver (Murata et al., 1997) and in the small intestine (Murase et al., 2002).

We might not have encountered significant differences in fat depots of animals fed re-esterified palm oils because the effect of MAG and DAG might have counteracted the effect of *sn*-2 SFA.

Table 4.7. Carcass yield and carcass fat depots of fattening pigs fed different dietary fat sources

Item		Dietary treatments[1]				RMSE[2]	*P*-values
		PN	PA	PEL	PEH		
Carcass yield, %		80.1	80.2	81.1	80.0	1.36	NS
Backfat thickness[3], mm		15.8	14.8	15.8	16.0	2.41	NS
Muscle depth[3], mm		60.0	58.0	58.3	58.3	5.08	NS
Lean percentage[4], %		61.4	62.0	61.1	61.0	2.33	NS
Perirenal fat,	kg	0.86	0.80	0.97	1.00	0.284	NS
	g/kg BW	7.87	7.23	9.12	9.08	2.599	NS
Liver,	kg	2.06	2.17	2.01	2.28	0.341	NS
	g/kg BW	18.8	19.4	18.3	21.0	3.05	NS

RMSE = root mean square error; NS = not significant.

[1] Diets with 4% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

[2] n = 6.

[3] Measurement made between the 3rd and 4th last ribs at 6 cm to the carcass midline with Fat-O-Meat'er (Carometec A/S; Soeborg, Denmark).

[4] Calculated from backfat thickness and muscle depth between 3rd and 4th last ribs using the Spanish official equation (Lean percentage, % = 66.91 – 0.895 × backfat thickness + 0.144 × muscle depth; Font-i-Furnols and Gispert, 2009).

4.4.5. Fatty acid composition of backfat

The effect of dietary fat sources on FA composition of backfat is presented in **Table 4.8**. The FA composition of backfat was a clear reflection of the FA composition of diets, as has been extensively reviewed by Madsen et al. (1992). However, although finisher diets showed a very similar FA composition, PEH animals deposited more SFA than did PN and PA ones ($P < 0.05$), which in turn resulted in a lower unsaturated-to-saturated FA ratio ($P < 0.05$). This difference may be related to the higher SFA apparent absorption observed for PEH treatment, although a higher SFA content should have also been expected in animals from PEL treatment. In agreement with our results, Smink et al. (2008) demonstrated that the feeding of randomized palm oil instead of native palm oil significantly raised the palmitic acid content of breast meat and abdominal fat in broiler chickens. Ponnampalam et al. (2011) also observed that weaning piglets fed diets with chemically or enzymatically modified palm oil deposited

more SFA in the subcutaneous adipose tissue than did those fed a diet with native palm oil. In contrast, Innis et al. (1996) and Scheeder et al. (2003) did not find differences in the FA composition of adipose tissue of pigs fed inter-esterified and native fats.

Table 4.8. Fatty acid composition (%) of backfat of fattening pigs fed different dietary fat sources

	Dietary treatments[1]					
Item	PN	PA	PEL	PEH	RMSE[2]	*P*-values
C14:0	1.13	1.11	1.04	1.11	0.115	NS
C16:0	23.6^{ab}	23.2^{b}	23.7^{ab}	24.7^{a}	0.80	*
C16:1	1.53	1.50	1.49	1.58	0.188	NS
C18:0	11.3	11.7	12.4	12.8	0.99	NS
C18:1 n-9	42.8	43.1	43.3	42.2	1.37	NS
C18:1 n-7	1.71	1.77	1.73	1.76	0.146	NS
C18:2 n-6	14.8	13.9	13.1	12.5	1.52	NS
C18:3 n-3	0.77	0.79	0.71	0.69	0.10	NS
C20:1 n-9	0.63	0.70	0.75	0.76	0.111	NS
C20:2 n-6	0.50	0.53	0.51	0.47	0.047	NS
Minor fatty acids	1.10	1.44	1.09	1.31	0.275	NS
SFA	36.7^{b}	36.8^{b}	37.7^{ab}	39.4^{a}	1.59	*
MUFA	47.0	47.8	47.8	46.8	1.59	NS
PUFA	16.3	15.5	14.5	13.9	1.66	NS
UFA:SFA	1.73^{a}	1.73^{a}	1.65^{ab}	1.54^{b}	0.114	*

MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; RMSE = root mean square error; SFA = saturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio.

[1] Diets with 4% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), or re-esterified palm oil high in mono- and diacylglycerols (PEH).

[2] n = 6.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Feeding fattening pigs with diets supplemented with re-esterified palm oils resulted in a higher total FA apparent absorption, mainly due to the increased absorption of SFA, than did their corresponding native and acid oils. This resulted in a greater feed efficiency and an increased SFA deposition in backfat of pigs fed PEH when compared to those fed PA. Thus, we conclude that, from a productive point of view, re-esterified palm oils, especially the one with a higher MAG and DAG

content, can be used in fattening-pig diets as better alternative fat sources than their corresponding acid oil.

CHAPTER 5

Use of re-esterified oils, differing in their degree of saturation and molecular structure, in broiler chicken diets

Use of re-esterified oils, differing in their degree of saturation and molecular structure, in broiler chicken diets

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5.1. Abstract

Re-esterified oils are new fat sources obtained from the chemical esterification of acid oils with glycerin (both economically interesting by-products from oil refining and biodiesel industries, respectively). The different fatty acid positional distribution and acylglycerol composition of re-esterified oils may enhance the apparent absorption of saturated fatty acids and, therefore, their overall nutritive value, which might lead to an increased deposition of saturated fatty acids. The aim of the present study was to investigate the potential use of re-esterified oils, differing in their degree of saturation and molecular structure, in comparison with their corresponding acid and native oils in broiler chicken diets, studying their effects on fatty-acid apparent absorption, acylglycerol and free fatty acid composition of feces, growth performance, carcass fat depots, and fatty-acid composition of abdominal adipose tissue. For this purpose, 144 one-day-old female broiler chickens (average weight of 42.8 ± 2.02 g) were randomly distributed in 48 cages. Birds were fed a basal diet supplemented with 6% (as-fed basis) of: native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), re-esterified palm oil high in mono- and diacylglycerols (PEH), native soybean oil (SN), acid soybean oil (SA), re-esterified soybean oil low in mono- and diacylglycerols (SEL), or re-esterified soybean oil high in mono- and diacylglycerols (SEH), which resulted in a 2 × 4 factorial arrangement. Digestibility balances showed that the fat degree of saturation, in general, exerted a greater impact than did the fat molecular structure. The dietary utilization of S sources was higher than was that of P ones. However, the increased *sn*-2 saturated fatty acid content of EL oils, in the starter period, and also the increased mono- and diacylglycerol content of EH oils, in the grower-finisher period, exerted favorable effects on the apparent absorption of saturated fatty acids, especially in those birds fed re-esterified palm oils. The excreta acylglycerol and free fatty acid composition was mainly composed of free fatty acids, and their amount almost paralleled the results observed for the apparent absorption of saturated fatty acids. For growth performance, birds fed S exhibited better feed conversion ratios and lower abdominal fat-pad weights than did those fed P. The fatty acid composition of abdominal adipose tissue was also mainly affected by the degree of saturation of dietary fat sources. We concluded that re-esterified oils, mainly from P sources, can be used in broiler chicken diets as alternative fat sources, showing similar or even higher total fatty acid apparent absorption than do their corresponding native and acid oils, with small changes in abdominal adipose tissue fatty acid composition.

5.2. Introduction

The use of supplemental fats in poultry diets is a widespread practice because of their high energy value. For this reason, the search for new, quality fat sources at competitive prices is of great practical interest. Acid oils, a by-product obtained from the oil refining industry, usually containing a high proportion of free fatty acids (**FFA**) and little triacylglycerols (**TAG**), are an economically interesting alternative. However, the absorption of fatty acids (**FA**), especially the saturated ones, has been reported to be much lower when FA are in free form than when they are part of TAG (Young, 1961; Wiseman and Salvador, 1991; Vilà and Esteve-Garcia, 1996a). Free FA need a higher amount of bile acids to be incorporated into mixed micelles than monoacylglycerols (**MAG**), because the former are more hydrophobic and have a higher ability to form insoluble soaps with divalent cations in the aqueous media of the intestine than have the latter (Small, 1991).

It has been hypothesized that the esterification of FFA present in acid oils with glycerol (another by-product from biodiesel industry) would improve the nutritive value of acid oils, especially in young animals fed saturated fat sources, due to the expected increased amount of *sn*-2 SFA and MAG and diacylglycerol (**DAG**) proportions that can be achieved in re-esterified oils. The FA positional distribution within acylglycerol molecules becomes important when the specificity of pancreatic lipase is considered. Pancreatic lipase preferentially hydrolyzes FA located at the *sn*-1,3 positions of acylglycerol molecules (Mattson and Beck, 1956). For this reason, long-chain SFA are better absorbed if situated in the *sn*-2 position than if located at the *sn*-1,3 positions of the acylglycerol molecules (Small, 1991; Bracco, 1994; Decker, 1996; Karupaiah and Sundram, 2007), because they are absorbed as 2-MAG, instead as FFA. Regarding the fat acylglycerol composition, until now MAG and DAG molecules have only been recognized as intermediates in the process of TAG digestion. However, they are amphiphilic molecules, able to act as emulsifying agents, and thus enhance the FA incorporation into mixed micelles.

As there is limited information about the use of these technical fats in poultry nutrition, the aim of the present study was to investigate the potential use of re-esterified oils, differing in their degree of saturation and molecular structure, in comparison with their corresponding acid (negative control) and native (positive control) oils in broiler chicken diets, studying their effects on FA apparent absorption, excreta acylglycerol and FFA composition, growth performance, carcass fat depots, and FA composition of abdominal adipose tissue.

5.3. Materials and methods

5.3.1. Experimental fats

Experimental fats were supplied by SILO S.p.a. (Florence, Italy). Re-esterified oils were produced using, as raw materials, palm or soybean acid oils (by-products obtained from the refining process of crude oils, with a high FFA content) and glycerine (a by-product obtained from the methylation process applied for the biodiesel production), which were processed in a reactor during 4-6 h under high vacuum conditions (1-3 mm Hg), without chemical catalysts, and setting the temperature around 190-250°C. According to the stoichiometric proportion of FFA and glycerol, fats with the same FA profile, but with a different FA positional distribution, and TAG, DAG and MAG proportions were obtained (**Table 5.1**).

Oil samples were analyzed in triplicate for moisture (Method 926.12 of the AOAC International, 2005), impurities (ISO 663:2007), unsaponifiable matter (Method 933.08 of the AOAC International, 2005), acylglycerol and FFA composition (ISO 18395:2005), MAG and DAG positional isomers (Sacchi et al., 1997), total FA composition (Guardiola et al., 1994), *sn*-2 FA composition (Commission Regulation (EEC) No. 2568/91 – Annex VII), and gross energy content (IKA-Kalorimeter system C4000; Staufen, Germany), as described in more detail in C*hapter 3*.

5.3.2. Animals and diets

The study was performed at the animal experimental facilities of the *Servei de Granges i Camps Experimentals* (Universitat Autònoma de Barcelona; Bellaterra, Barcelona, Spain). The experimental procedure received the prior approval from the Animal Protocol Review Committee of the same institution. All animal housing and husbandry conformed to the European Union Guidelines (2010/63/EU).

A total of 144 one-day-old female broiler chickens of the Ross 308 strain were obtained from a commercial hatchery (Terra-Avant S.A.; Anglès, Girona, Spain), where birds with extreme weights were discarded. On arrival, chicks were wing-banded, weighed (initial body weight (**BW**), 42.8 ± 2.02 g), and randomly assigned to one of the eight dietary treatments, with three chicks per cage and six cages per dietary treatment. Birds were housed in wire-floor cages with excreta collection trays. Throughout the study, feed and water were supplied *ad libitum*, and animals were raised under controlled conditions of light and temperature, as recommended by the breeder.

Table 5.1. Chemical analyses of the experimental fats[1]

Item		PN oil	PA oil	PEL oil	PEH oil	SN oil	SA oil	SEL oil	SHE oil
Moisture, %		0.14	0.35	0.02	0.34	0.05	0.08	0.09	0.10
Impurities, %		<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Unsaponifiable matter, %		0.30	1.59	1.80	1.55	0.27	0.18	0.29	0.30
Fatty acid composition and distribution, %									
C16:0	Total	41.9	45.0	40.1	40.2	10.7	10.8	11.4	10.9
	sn-2 %[2]	7.89	12.4	20.3	15.3	1.59	1.70	4.36	9.00
C18:0	Total	4.63	5.82	8.46	8.57	4.42	4.39	4.15	3.93
	sn-2 %[2]	9.35	15.5	24.3	18.2	1.59	1.30	4.83	12.1
C18:1 n-9	Total	36.4	37.1	39.5	39.5	21.2	22.6	21.3	22.1
	sn-2 %[2]	49.6	10.0	31.7	16.8	34.1	15.1	28.6	19.4
C18:2 n-6	Total	11.5	8.57	8.05	7.74	54.2	53.4	53.6	54.5
	sn-2 %[2]	64.4	16.0	34.6	16.6	43.0	19.4	37.3	21.5
C18:3 n-3	Total	0.56	0.26	0.18	0.15	7.68	6.72	7.83	6.75
	sn-2 %[2]	39.3	38.5	33.5	21.7	30.8	15.2	28.7	19.8
Minor fatty acids		5.02	3.18	3.69	3.80	1.79	2.18	1.76	1.82
SFA	Total	48.1	52.7	50.9	51.2	15.2	15.5	15.6	15.0
	sn-2 %[2]	8.36	12.7	20.5	15.3	1.69	1.71	4.61	9.82
MUFA	Total	38.2	38.4	40.8	40.9	22.9	24.4	23.0	23.8
	sn-2 %[2]	48.0	9.88	31.7	16.8	32.2	14.2	27.3	19.2
PUFA	Total	13.7	8.84	8.24	7.89	61.9	60.1	61.4	61.2
	sn-2 %[2]	62.7	16.7	34.6	16.7	41.4	19.0	36.2	21.3
Acylglycerol and FFA composition, %									
TAG		84.5	29.8	58.8	22.0	98.2	45.0	78.6	34.6
DAG	Total	10.3	11.7	33.9	48.9	0.78	0.00	11.5	36.0
	1(3),2-DAG %[3]	28.6	39.1	32.5	26.7	50.0	0.00	29.2	28.9
MAG	Total	0.42	2.61	5.55	27.9	0.27	0.00	8.90	28.1
	2-MAG %[4]	33.3	11.8	14.3	7.26	50.0	0.00	7.41	6.63
FFA		4.79	55.8	1.75	1.17	0.75	55.0	0.95	1.32
Glycerol-to-fatty acid ratio[5], mol/mol		0.34	0.17	0.41	0.58	0.33	0.15	0.40	0.56
Gross energy, kcal/kg		9,305	9,361	9,328	8,917	9,465	9,437	9,280	8,955

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; TAG = triacylglycerols.

[1] Native palm oil (PN oil), acid palm oil (PA oil), re-esterified palm oil low in MAG and DAG (PEL oil), re-esterified palm oil high in MAG and DAG (PEH oil), native soybean oil (SN oil), acid soybean oil (SA oil), re-esterified soybean oil low in MAG and DAG (SEL oil), and re-esterified soybean oil high in MAG and DAG (SEH oil).

[2] Fatty acid positional distribution, expressed as the proportion of each fatty acid located at the *sn*-2 position of acylglycerol molecules, calculated as follows: *sn*-2 % = (*sn*-2 / Total) × *a* × 100, where *sn*-2 is the FA composition at the *sn*-2 position (converted to mol%), Total is the total FA composition of the fat (converted to mol%), and *a* is the ratio between the moles of FA located at the *sn*-2 position and the moles of total FA. *a* was 0.30, 0.12, 0.26, 0.16, 0.33, 0.15, 0.29, and 0.19 for PN, PA, PEL, PEH, SN, SA, SEL, and SEH oils, respectively.

[3] The proportion of 1(3),2-DAG vs. 1,3-DAG.

[4] The proportion of 2-MAG vs. 1(3)-MAG.

[5] Estimated calculation based on the values of the acylglycerol and FFA composition.

Birds received a starter feed (in mash form) until day 20 and a grower-finisher feed (in pelleted form) from day 20 to 40. The wheat- and soybean-meal-based diets were formulated to meet or exceed FEDNA requirements (2008), and to minimize basal fat levels. The eight dietary treatments were the result of including 6% (as-fed basis) of one of the following experimental fats to the basal diet: native palm oil (**PN**), acid palm oil (**PA**), re-esterified palm oil low in MAG and DAG (**PEL**), re-esterified palm oil high in MAG and DAG (**PEH**), native soybean oil (**SN**), acid soybean oil (**SA**), re-esterified soybean oil low in MAG and DAG (**SEL**), or re-esterified soybean oil high in MAG and DAG (**SEH**).

The eight experimental diets resulted in a 2 × 4 factorial arrangement, with two levels of fat saturation degree [palm oil (P) and soybean oil (S)] and four types of fat molecular structure [native oil (N, as a positive control), acid oil (A, as a negative control), re-esterified oil low in MAG and DAG (EL), and re-esterified oil high in MAG and DAG (EH)]. The composition of the experimental diets is presented in **Table 5.2**.

Analytical determinations of feeds were performed according to the methods of the AOAC International (2005): dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fat (Method 2003.05), and crude fiber (Method 962.09). Gross energy was determined as described previously for fats, and the FA content was analyzed following the method of Sukhija and Palmquist (1988). Briefly, samples (about 100 mg) were incubated with methanolic chloride, and a known amount of nonadecanoic acid (C19:0, Sigma-Aldrich Chemical Co.; St. Louis, MO, USA) was added as an internal standard. Then, the FA methyl esters were extracted with toluene and submitted to gas chromatography (Agilent 6890 gas chromatograph, equipped with a flame ionization detector, and a polar capillary column [DB23, 60 m x 0.32 mm i.d., 0.25 μm] from Agilent Technologies; Santa Clara, CA, USA). Helium was used as the carrier gas. Fatty acid methyl esters were identified by matching their retention times with those of their relative standards (Supelco 37 component FAME Mix, Sigma-Aldrich Co.; St. Louis, MO, USA). Peak areas were integrated and converted to concentration by comparison with the internal standard-peak area. The macronutrient and FA composition of experimental diets are presented in **Table 5.3.**

Table 5.2. Ingredient composition of the experimental diets (as-fed basis)

Ingredients, %	Starter diet (from 0 to 20 days)	Grower-finisher diet (from 20 to 40 days)
Wheat	55.30	62.86
Soybean meal 48%	31.44	26.05
Experimental fats[1]	6.00	6.00
Sunflower meal	3.31	-
Dicalcium phosphate	1.74	1.40
Calcium carbonate	0.93	2.56
Sodium chloride	0.40	0.35
Vitamin and mineral premix[2]	0.30	0.30
DL-Methionine	0.23	0.19
L-Lysine	0.22	0.18
L-Threonine	0.05	0.04
Enzyme supplement[3]	0.05	0.05
Ethoxyquin 66%	0.02	0.02
Choline chloride	0.01	-

[1] Native palm oil (PN oil), acid palm oil (PA oil), re-esterified palm oil low in MAG and DAG (PEL oil), re-esterified palm oil high in MAG and DAG (PEH oil), native soybean oil (SN oil), acid soybean oil (SA oil), re-esterified soybean oil low in MAG and DAG (SEL oil), and re-esterified soybean oil high in MAG and DAG (SHE oil).

[2] Provides per kg of feed: vitamin A (from retinol), 13,500 IU; vitamin D_3 (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; thiamine, 3 mg; vitamin riboflavin, 9 mg; pyridoxine, 4.5 mg; cobalamine, 16.5 µg; menadione, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from $FeSO_4 \cdot 7H_2O$), 54 mg; I (from $Ca(I_2O_3)_2$), 1.2 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$), 0.6 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na_2SeO_3), 0.18 mg; Mo (from $(NH_4)_6Mo_7O_{24}$), 1.2 mg.

[3] Provides per kg of feed: ß-glucanase 350 IU; xylanase 1,125 IU.

Table 5.3. Analyzed[1] macronutrient content and fatty acid composition of the experimental diets[2]

	Starter diets (from 0 to 20 days)								Grower-finisher diets (from 20 to 40 days)							
Item	PN	PA	PEL	PEH	SN	SA	SEL	SEH	PN	PA	PEL	PEH	SN	SA	SEL	SEH
Macronutrient content, %																
Dry matter	89.5	90.0	89.9	89.9	90.0	90.0	90.0	89.8	87.4	88.4	88.0	88.2	88.9	88.2	88.6	88.1
Crude protein	21.0	20.8	20.1	20.9	20.9	19.8	20.5	20.4	19.4	19.5	19.5	19.1	19.2	19.4	19.2	19.5
Crude fat	8.10	8.33	8.35	8.37	8.34	8.54	8.42	7.98	6.92	7.08	7.41	6.89	7.54	7.35	7.23	6.78
Crude fiber	3.73	3.79	3.65	3.74	3.57	3.72	3.82	3.54	3.77	3.56	3.86	3.80	3.61	3.75	3.63	3.48
Ash	6.03	5.90	6.02	6.05	6.06	5.97	5.80	5.80	5.15	5.22	5.18	5.24	5.28	5.13	5.24	5.09
Gross energy, kcal/kg	4,263	4,266	4,264	4,240	4,272	4,271	4,261	4,242	4,142	4,145	4,143	4,118	4,151	4,150	4,140	4,121
Fatty acid composition, %																
C16:0	30.9	34.1	29.9	29.7	12.3	12.2	12.7	13.0	33.2	33.3	32.7	32.5	12.5	12.5	12.8	13.3
C18:0	4.14	5.02	6.73	7.01	4.05	4.31	3.97	3.86	4.01	6.27	6.67	6.63	3.83	3.90	3.62	3.76
C18:1 n-9	30.9	31.5	32.1	32.2	21.1	21.9	21.2	21.3	31.4	32.7	33.1	32.7	20.5	21.5	20.7	20.8
C18:2 n-6	26.0	23.2	24.1	25.0	52.3	51.6	51.7	51.8	23.2	21.3	21.1	21.9	52.8	52.0	52.5	52.2
C18:3 n-3	2.26	1.97	2.04	2.18	6.74	6.36	6.97	6.36	1.65	1.44	1.43	1.56	6.72	6.25	6.97	6.37
Minor fatty acids	5.79	4.30	5.11	3.84	3.54	3.68	3.46	3.69	6.55	5.02	4.95	4.72	3.65	3.86	3.39	3.64
SFA	37.8	42.1	40.3	39.0	18.2	18.4	18.4	18.8	40.1	43.3	43.1	42.6	18.4	18.6	18.2	19.0
MUFA	32.7	32.8	33.6	33.8	22.8	23.7	22.9	23.0	33.3	34.0	34.4	34.0	22.1	23.2	22.3	22.4
PUFA	29.5	25.2	26.1	27.2	59.0	57.9	58.7	58.1	26.6	22.7	22.5	23.4	59.5	58.2	59.5	58.5
UFA:SFA	1.65	1.38	1.48	1.56	4.49	4.43	4.43	4.31	1.49	1.31	1.32	1.35	4.43	4.38	4.49	4.26

DAG = diacylglycerols; MAG = monoacylglycerols; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; UFA = unsaturated fatty acids.

[1] All samples were analyzed at least in duplicate.

[2] Diets with 6% (as-fed basis) of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in MAG and DAG (PEL), re-esterified palm oil high in MAG and DAG (PEH), native soybean oil (SN), acid soybean oil (SA), re-esterified soybean oil low in MAG and DAG (SEL), or re-esterified soybean oil high in MAG and DAG (SEH).

5.3.3. Controls and sampling

Feed consumption and BW were measured weekly to calculate the average daily feed intake (**ADFI**), the average daily gain (**ADG**), and the feed conversion ratio (**FCR**) throughout the experiment.

Two digestibility balances were carried out using the total-excreta-collection method (Bourdillon et al., 1990). Excreta were collected in the starter period from day 8 to 12 and in the growing-finishing period from day 34 to 36. On the last day of both balances, feed consumption was measured and total excreta were collected, weighed and homogenized, and a representative sample was frozen at -20ºC. Contaminants such as feed, feathers, down, and scales were removed. The excreta samples were freeze-dried, ground and kept at 5ºC until further analyses. Excreta samples were analyzed by the same methods as those described for feeds, to determine the apparent absorption of FA and the apparent metabolizable energy (**AME**) of the diets. The apparent absorption coefficient of a particular nutrient (X) was calculated as follows:

% apparent absorption of X = [(X ingested – X excreted) / X ingested] × 100.

In the case of the AME, the apparent absorption coefficient of gross energy was multiplied by its corresponding feed gross energy.

The acylglycerol and FFA composition of excreta was analyzed according to ISO 18395:2005. Prior to analysis, fat was extracted from excreta with diethyl ether after acidification with HCl 1N. The acylglycerol and FFA molecules were quantified by internal normalization. Finally, these values were expressed as grams per 100 g of fat intake, based on the values of fat apparent absorption obtained for each digestibility balance.

At the end of the experimental period, the 40-day-old broiler chickens were fasted for 3 h, stunned, slaughtered, bled, plucked, and chilled at 4ºC for 12 h in a local slaughterhouse (Gimave S.A.; Ripollet, Barcelona, Spain). Carcasses (total BW excluding blood and feathers) were weighed, and the liver and abdominal fat pad (from proventriculus surrounding the gizzard down to the cloaca) for each bird were removed and weighed. Abdominal fat pad and liver weights were expressed in absolute values and as a percentage of carcass weight. A representative sample of the abdominal fat pad was taken and frozen at -20ºC. The FA composition of abdominal adipose tissue was determined by the method of Carrapiso et al. (2000).

5.3.4. Statistical analysis

Normality of the data and homogeneity of the variance were verified. All data were subjected to a two-way ANOVA using the GLM procedure of SAS (version 9.2, SAS Institute Inc.; Cary, NC, USA). The models included the fat degree of saturation (P, S) and the fat molecular structure (N, A, EL, EH) as main factors, and the two-way interaction. Differences between treatment means were tested using Tukey's correction for multiple comparisons. The cage served as the experimental unit, so that there were six replicates per treatment. For abdominal fat pad and liver weights, the broiler carcass weight was included as a covariate in the model, in order to correct these variables for variations not related to dietary treatment effect. Results in tables are reported as least square means and differences were considered significant at $P < 0.05$.

5.4. Results and discussion

5.4.1. Characterization of experimental fats

The chemical analyses of the experimental fats are presented in **Table 5.1**. Palm oils were high in palmitic (41.8 ± 2.29%) and oleic (38.1 ± 1.61%) acids, and soybean oils in linoleic (53.9 ± 0.51%) and oleic (21.8 ± 0.67%) acids. The average unsaturated-to-saturated FA proportions were 49:51 wt/wt and 85:15 wt/wt for P and S oils, respectively. Little variability was observed among oils of the same degree of saturation.

The specific distribution of FA within the acylglycerol molecules found in N oils was in agreement with that reported by Mattson and Volpenhein (1963). Saturated fatty acids (**SFA**) were preferentially esterified at the *sn*-1,3 positions, and unsaturated fatty acids (**UFA**) at the *sn*-2 position in N oils. The chemical esterification process involved a certain redistribution of FA in the acylglycerol molecules. Although this process did not result in a complete random distribution of FA (i.e., 33% of each FA should appear in the *sn*-2 position), re-esterified oils showed a higher percentage of SFA located at the *sn*-2 position and, therefore, a lower percentage of mono- (**MUFA**) and polyunsaturated fatty acids (**PUFA**) located at this position, when compared with their corresponding N oils. The reason why, in most cases, EH oils showed a lower proportion of *sn*-2 FA than did EL oils was due to their higher 1(3)-MAG and 1,3-DAG content, as will be discussed in more detail below. In this sense, A oils, due to their high FFA content, showed low proportions of *sn*-2 FA.

Regarding acylglycerol and FFA composition, native oils (N), as positive controls, were mainly composed of TAG (91.4 ± 9.69%), whereas acid oils (A) from chemical refining, as negative controls, had a high content of FFA (55.4 ± 0.57%). However, in general, PN and PA oils showed a lower TAG and a higher DAG, MAG, and FFA content than did SN and SA oils, respectively. Re-esterified oils showed variable amounts of TAG, DAG, and MAG, and low levels of FFA. Broadly speaking, EH oils showed a higher amount of MAG and DAG, and a lower amount of TAG than did EL oils. Although EL and EH oils of a different degree of saturation had almost an identical glycerol-to-FA ratio (PEL: 0.41 mol/mol vs. SEL: 0.40 mol/mol; PEH: 0.58 mol/mol vs. SEH: 0.56 mol/mol), their acylglycerol composition was quite different. Re-esterified palm oils showed a lower TAG content and a higher DAG content than did re-esterified soybean oils, which could be related to the differences observed in the acylglycerol and FFA composition of A oils, from which they originated. On the other hand, in all re-esterified oils, 1(3)-MAG and 1,3-DAG were the major positional isomers, which, in terms of fat absorption, are not expected to be as well-absorbed as are 2-MAG and 1(3),2-DAG, because pancreatic lipase specifically hydrolyzes the external *sn*-1,3 positions.

5.4.2. Digestibility balances

The effects of dietary fat sources on the AME of the diets and the apparent absorption of individual FA in both starter (from 8 to 12 days) and grower-finisher (from 34 to 36 days) periods are presented in **Table 5.4**. Two ages were employed, as it is well known that young birds are less able to utilize fats than older birds (Krogdahl, 1985; Wiseman and Salvador, 1991).

In general, when the interaction component of the model was not significant, the factor degree of saturation showed a greater effect on FA apparent absorption than did the factor molecular structure. However, when the interaction between both factors was significant, the magnitude of the difference observed among P treatments was greater than it was among S treatments.

Table 5.4. Apparent metabolizable energy of the diets (kcal/kg) and individual fatty acid apparent absorption coefficients (%) in broiler chickens fed different dietary fat sources[1]

	Dietary treatments[2]								Degree of saturation[3]		Molecular structure[4]					*P*-values		
	P				S											Degree of	Molecular	
Item	N	A	EL	EH	N	A	EL	EH	P	S	N	A	EL	EH	RMSE	saturation	structure	Interaction
From 8 to 12 days																		
AME	3,102	3,018	3,126	3,099	3,228	3,188	3,217	3,177	3,086	3,202	3,165^{a}	3,103^{b}	3,172^{a}	3,138^{ab}	53.3	***	*	NS
Total FA	74.1	67.8	77.1	70.8	85.7	82.3	83.5	79.0	72.4	82.6	79.9^{a}	75.1^{b}	80.3^{a}	74.9^{b}	4.04	***	***	NS
SFA	58.7^{bc}	50.6^{c}	66.8^{ab}	58.6^{bc}	77.8^{a}	73.2^{a}	74.3^{a}	68.9^{ab}	58.6	73.5	68.2	61.9	70.5	63.8	6.05	***	**	*
MUFA	82.0	77.3	82.9	77.0	84.6	80.8	82.1	77.0	79.8	81.1	83.3^{a}	79.1^{ab}	82.5^{a}	77.0^{b}	4.01	NS	**	NS
PUFA	79.7	75.8	80.3	74.4	87.8	85.0	86.1	82.1	77.5	85.2	83.8^{a}	80.4^{ab}	83.2^{a}	78.3^{b}	3.21	***	***	NS
C16:0	64.5^{cd}	56.0^{d}	71.4^{bc}	65.2^{cd}	81.7^{a}	78.0^{ab}	79.2^{ab}	75.2^{ab}	64.2	78.5	73.1	67.0	75.3	70.2	5.23	***	**	**
C18:0	49.5^{ef}	45.5^{f}	61.3^{cde}	55.6^{def}	78.4^{a}	72.7^{abc}	73.8^{ab}	65.8^{bcd}	53.0	72.7	64.0	59.1	67.6	60.7	6.64	***	*	**
C18:1 n-9	82.4	77.8	83.4	77.6	85.1	81.3	82.6	77.6	80.3	81.6	83.8^{a}	79.5^{ab}	83.0^{a}	77.6^{b}	3.96	NS	**	NS
C18:2 n-6	79.3	75.9	80.3	74.6	87.6	84.7	85.7	81.8	77.5	85.0	83.4^{a}	80.3^{ab}	83.0^{a}	78.2^{b}	3.18	***	***	NS
C18:3 n-3	79.4	76.6	80.2	75.2	89.9	87.1	88.7	84.6	77.8	87.6	84.7^{a}	81.8^{ab}	84.4^{a}	79.9^{b}	2.77	***	***	NS
From 34 to 36 days																		
AME	2,953	2,902	3,017	2,936	3,044	2,974	3,008	3,015	2,952	3,010	2,998	2,938	3,012	2,976	88.9	*	NS	NS
Total FA	74.9^{c}	76.4^{c}	80.5^{bc}	80.6^{abc}	86.4^{a}	80.0^{bc}	82.8^{ab}	83.7^{ab}	78.1	83.2	80.7	78.2	81.6	82.1	3.18	***	*	**
SFA	64.7^{c}	70.3^{bc}	75.8^{ab}	76.3^{ab}	80.5^{a}	73.9^{ab}	76.4^{ab}	78.5^{a}	71.8	77.3	72.6	72.1	76.1	77.4	4.42	***	*	***
MUFA	82.6^{ab}	81.9^{ab}	85.2^{a}	84.8^{a}	85.9^{a}	78.9^{b}	82.0^{ab}	82.5^{ab}	83.6	82.3	84.2	80.4	83.6	83.6	3.03	NS	*	*
PUFA	80.6	79.9	82.4	82.5	88.4	82.4	85.0	85.8	81.4	85.4	84.5^{a}	81.1^{b}	83.7^{ab}	84.1^{a}	2.65	***	*	NS
C16:0	65.4^{c}	70.4^{bc}	76.3^{ab}	76.8^{ab}	81.7^{a}	75.2^{ab}	78.2^{a}	79.9^{a}	72.2	78.7	73.6	72.8	77.3	78.3	4.15	***	**	***
C18:0	54.4^{c}	64.1^{bc}	70.4^{ab}	71.9^{ab}	77.5^{a}	69.2^{ab}	72.2^{ab}	75.2^{ab}	65.2	73.5	66.0	66.6	71.3	73.5	6.10	***	*	***
C18:1 n-9	83.0^{ab}	82.2^{ab}	85.5^{a}	85.0^{a}	86.1^{a}	79.3^{b}	82.3^{ab}	82.9^{ab}	83.9	82.6	84.5	80.7	83.9	84.0	3.01	NS	*	*
C18:2 n-6	80.5	79.9	82.4	82.4	88.2	82.1	84.7	85.5	81.3	85.1	84.3^{a}	81.0^{b}	83.6^{ab}	83.9^{ab}	2.69	***	*	NS
C18:3 n-3	81.3	80.2	81.8	83.6	90.5	84.7	87.3	88.0	81.7	87.6	85.9^{a}	82.5^{b}	84.5^{ab}	85.8^{a}	2.48	***	**	NS

AME = apparent metabolizable energy; DAG = diacylglycerols; FA = fatty acids; MAG = monoacylglycerols; MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; RMSE = root mean square error; SFA = saturated fatty acids.

[1] Diets with 6% (as-fed basis) of native palm (PN), acid palm oil (PA), re-esterified palm oil low in MAG and DAG (PEL), re-esterified palm oil high in MAG and DAG (PEH), native soybean oil (SN), acid soybean oil (SA), re-esterified soybean oil low in MAG and DAG (SEL), or re-esterified soybean oil high in MAG and DAG (SEH).

[2] n = 6; [3] n = 24; [4] n = 12

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a-f] Values within a row with different superscripts differ significantly at $P < 0.05$.

Concerning the fat degree of saturation effect, the dietary utilization (AME) of treatments containing S was much higher than were for those containing P ($P < 0.05$), as it is well known that chicks can better use FA from fat sources that are rich in UFA than from fats that are rich in SFA (Wiseman et al., 1991). However, the magnitude of the difference observed for AME between P and S sources in the grower-finisher period was only half of that in the starter period, mainly due to the improvement of the absorption of SFA in P treatments.

For the fat molecular structure effect, treatments containing N (the positive controls) showed higher AME values than did those containing A (the negative controls) in young birds ($P = 0.035$), the difference being more pronounced in treatments containing P. Because FFA are produced in the natural digestion process, it could be expected that a dietary supply of already hydrolyzed FFA would be beneficial in terms of fat utilization. However, several authors (Young, 1961; Wiseman and Salvador, 1991) have seen a reduction in the AME of hydrolyzed fats when compared with the AME of the neutral oil from which they originated. In addition, these reports indicated that the reduction in dietary energy value following hydrolysis was more pronounced with saturated than with unsaturated fats.

Regarding re-esterified oils, EL and EH diets showed different responses for the starter than for the grower-finisher period. In the starter period, EL diets showed a higher nutritive value (AME) than did A ($P = 0.019$) and not different from N ($P = 0.99$), mainly due to improved SFA apparent absorption. Nevertheless, the magnitude of the difference was much higher in P sources than it was in S ones, as indicated by the significance of the interaction for SFA apparent absorption ($P = 0.012$). Thus, although no differences were found among S sources ($P > 0.05$), PEL achieved higher SFA apparent absorption values than did PA ($P < 0.001$), and not different from S treatments ($P > 0.05$). In the grower-finisher period, PEL treatment even achieved higher SFA apparent absorption values than did PN ($P = 0.002$), and also not different from S sources ($P > 0.05$). The high SFA apparent absorption obtained by PEL can be due to its lower FFA content and its higher *sn*-2 SFA content, when compared with their corresponding acid and native oils. The chemical esterification reaction decreased the amount of FFA (from 55.8% in PA oil and 4.79% in PN oil to 1.75% in PEL oil) and raised the fraction of SFA at the *sn*-2 position (from 12.7 mol% in PA oil and 8.36 mol% in PN oil to 20.5 mol% in PEL oil). Thus, the greater absorption of SFA in PEL would result from the greater content of 2-monopalmitin and the lower content of free palmitic acid in the intestine after hydrolysis. Consistent with our results, previous studies in rats (Tomarelli et al., 1968; Renaud et al., 1995; Lien et al., 1997), human

newborn infants (Filer et al., 1969) and broiler chickens (Renner and Hill, 1961; Lin and Chiang, 2010) also found that SFA present at the *sn*-2 position of dietary acylglycerols were more readily absorbed than were SFA at the *sn*-1,3 positions.

In the starter period, EH treatments did not enhance the nutritive value (AME) of their corresponding A treatment from which they originated (P = 1.00), despite their markedly higher MAG and DAG content. Assuming that the most limiting process in fat digestion is micelle formation, it could be expected that amphiphilic molecules (consisting of a hydrophilic and a hydrophobic part) could contribute to fat digestibility of added feed fats, especially to the poorly absorbed SFA in young broiler chickens. However, our results suggest that high MAG and DAG content does not improve the absorption of SFA in young broiler chickens. Related to this, Taguchi et al. (2001) reported that the fecal excretion of FA after feeding rats of 5 weeks of age with a DAG enriched diet was almost the same as with the TAG enriched diet. The lack of improvement could be related to the high proportions of 1(3)-MAG and 1,3-DAG species present in EH oils. In young broiler chickens, the assimilation of dietary fats is limited because they have a reduced capacity to produce and secrete bile salts (Soede, 2005). Thus, dietary fats would remain longer in the intestinal lumen, which could lead to a complete hydrolysis of 1(3)-MAG and 1,3-DAG to glycerol and FFA, impairing micelle formation and fat absorption. However, in older birds, PEH achieved higher SFA apparent absorption values than did PN (P = 0.001) and not different from S treatments (P > 0.05), suggesting that MAG and DAG during the grower-finisher period are not completely hydrolyzed and, as a consequence, their emulsifying properties improve the incorporation rate of SFA into mixed micelles.

5.4.3. Acylglycerol and free fatty acid composition of excreta

The digestion products of fat in excreta were analyzed to better understand how the fat molecular structure affects the digestion and absorption processes. The acylglycerol and FFA composition of excreta (g/100 g of fat intake) in both starter (from 8 to 12 days) and grower-finisher (from 34 to 36 days) periods is shown in **Table 5.5**. TAG, DAG, and MAG were low in excreta of both periods. This means that TAG and DAG were almost completely hydrolyzed along the gastrointestinal tract, and MAG were absorbed due to their hydrophilic character [2-MAG and some 1(3)-MAG] or completely hydrolyzed to glycerol and FFA [mainly 1(3)-MAG].

Table 5.5. Acylglycerol and free fatty acid composition of excreta (g/100 g of fat intake) of broiler chickens fed different dietary fat sources[1]

	Dietary treatments[2]								Degree of saturation[3]		Molecular structure[4]					*P*-values		
	P				S											Degree of	Molecular	
Item	N	A	EL	EH	N	A	EL	EH	P	S	N	A	EL	EH	RMSE	saturation	structure	Interaction
From 8 to 12 days																		
TAG	2.21	3.05	2.47	3.12	2.59	2.69	2.41	2.84	2.71	2.63	2.40	2.87	2.44	2.98	0.616	NS	NS	NS
DAG	2.30	3.06	2.31	3.16	2.21	2.60	2.32	2.78	2.70	2.48	2.26^{c}	2.83^{ab}	2.31^{bc}	2.97^{a}	0.472	NS	***	NS
MAG	1.49	2.20	1.71	1.98	1.01	1.28	1.12	1.09	1.84	1.13	1.25^{b}	1.74^{a}	1.41^{ab}	1.54^{ab}	0.369	***	*	NS
FFA	20.5^{b}	28.9^{a}	16.2^{bc}	19.9^{b}	10.6^{c}	14.6^{bc}	12.5^{c}	15.2^{bc}	21.4	13.2	15.6	21.7	14.3	17.5	3.73	***	***	**
From 34 to 36 days																		
TAG	2.69	2.58	2.34	2.39	1.85	1.99	2.29	2.29	2.50	2.11	2.27	2.29	2.32	2.34	0.400	**	NS	NS
DAG	3.33	3.59	2.90	3.25	2.46	3.12	3.07	3.08	3.27	2.93	2.89	3.36	2.99	3.17	0.603	NS	NS	NS
MAG	1.98	1.95	1.71	1.72	1.38	1.83	1.61	1.72	1.84	1.64	1.68	1.89	1.66	1.72	0.390	NS	NS	NS
FFA	18.1^{a}	16.4^{ab}	13.8^{abc}	12.8^{bc}	9.10^{c}	13.9^{abc}	12.3^{bc}	11.7^{bc}	15.3	11.7	13.6	15.1	13.0	12.3	2.62	***	NS	**

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; NS = not significant; RMSE = root mean square error; TAG = triacylglycerols.

[1] Diets with 6% (as-fed basis) of native palm (PN), acid palm oil (PA), re-esterified palm oil low in MAG and DAG (PEL), re-esterified palm oil high in MAG and DAG (PEH), native soybean oil (SN), acid soybean oil (SA), re-esterified soybean oil low in MAG and DAG (SEL), or re-esterified soybean oil high in MAG and DAG (SEH).

[2] n = 6; [3] n = 24; [4] n = 12

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a-c] Values within a row with different superscripts differ significantly at $P < 0.05$.

Thus, regardless of the treatment, fat lost in excreta was mainly composed of FFA, which agrees with the results found by Sklan (1979).This suggests that in broiler chickens, especially at early ages, the main limiting factor of fat absorption is the emulsifying effect of bile salts, rather than the hydrolytic activity of pancreatic lipase, as was also indicated by Soede (2005). The amount of TAG, DAG and MAG molecules in excreta remained almost constant in both periods, while in the grower-finisher period FFA content decreased considerably, which explains the improved fat absorption observed in older broiler chickens, possibly due to improved bile secretion. Thus, most TAG, DAG and MAG molecules may probably come from endogenous losses or from dietary fat, in case they have melting points above the chicken's body temperature.

Main differences among treatments were observed for FFA. In this case, the interaction component of the model was significant (P = 0.004 and P = 0.002 for starter and grower-finisher periods, respectively), and the amount of FFA excreted per 100 g of fat intake almost paralleled the results of SFA apparent absorption, suggesting that the emulsification of SFA is the most critical step in fat absorption. Regarding TAG, DAG and MAG fractions, smaller differences were observed. For the fat degree of saturation, higher excretion of MAG (in the starter period, $P < 0.001$) and TAG (in the grower-finisher period, P = 0.002) were observed in P sources, which probably corresponds to acylglycerol molecules rich in SFA, and therefore with melting points above the chicken's body temperature, hindering the digestion and absorption processes. For the fat molecular structure, only minor differences were observed for DAG and MAG in the starter period.

5.4.4. Growth performance and carcass fat depots

The effects of dietary fat sources on growth performance in both starter (from 0 to 20 days) and grower-finisher (from 20 to 40 days) periods are presented in **Table 5.6**. No significant interactions were observed at any feeding period ($P > 0.05$), but several differences were detected for the fat degree of saturation. Regardless of the period, FCR were lower for birds fed S than were for those fed P ($P < 0.05$), probably due to their higher ability to absorb fat, as has also been seen in several studies (Pinchasov and Nir, 1992; Zollitsch et al., 1997; Crespo and Esteve-Garcia, 2001). During the starter period, the improved FCR in chicks fed S was due to the greater ADG (P = 0.043). In the grower-finisher period and the overall experiment, however, birds fed P consumed a greater amount of feed (P = 0.017) to gain the same weight (P = 0.39), than did those fed S.

Table 5.6. Growth performance and carcass fat depots of broiler chickens fed different dietary fat sources[1]

Item		Degree of saturation[2]		Molecular structure[3]				RMSE	*P*-values		
		P	S	N	A	EL	EH		Degree of saturation	Molecular structure	Interaction
From 0 to 20 days											
ADFI, g		49.9	50.3	51.0	48.9	49.5	50.9	2.87	NS	NS	NS
ADG, g		36.2	37.8	37.4	36.0	36.9	37.7	2.15	*	NS	NS
FCR, g/g		1.38	1.33	1.37	1.36	1.35	1.35	0.045	**	NS	NS
BW at 20 days, g		767	798	790	762	780	797	42.9	*	NS	NS
From 20 to 40 days											
ADFI, g		173	166	170	170	165	174	8.19	*	NS	NS
ADG, g		89.0	90.4	89.9	89.0	88.0	91.9	4.34	NS	NS	NS
FCR, g/g		1.95	1.84	1.89	1.91	1.88	1.90	0.061	***	NS	NS
BW at 40 d, g		2,548	2,605	2,588	2,542	2,541	2,635	114.8	NS	NS	NS
From 0 to 40 days											
ADFI, g		112	108	111	109	107	112	4.74	*	NS	NS
ADG, g/d per bird		62.6	64.1	63.6	62.5	62.5	64.8	2.87	NS	NS	NS
FCR, g/g		1.78	1.69	1.74	1.76	1.72	1.74	0.043	***	NS	NS
Carcass fat depots											
Abdominal fat,	g	65.2	55.8	59.2	60.3	61.3	61.3	8.28	***	NS	NS
	%	2.82	2.44	2.57	2.63	2.59	2.73	0.387	**	NS	NS
Liver,	g	51.9	49.1	54.0	48.4	49.3	50.4	5.25	NS	NS	NS
	%	2.26	2.14	2.35	2.11	2.13	2.21	0.221	NS	NS	NS

ADFI = average daily feed intake; ADG = average daily gain; BW = body weight; FCR = feed conversion ratio; NS = not significant; RMSE = root mean square error.

[1] Diets with 6% (as-fed basis) of native palm (PN), acid palm oil (PA), re-esterified palm oil low in MAG and DAG (PEL), re-esterified palm oil high in MAG and DAG (PEH), native soybean oil (SN), acid soybean oil (SA), re-esterified soybean oil low in MAG and DAG (SEL), or re-esterified soybean oil high in MAG and DAG (SEH).

[2] n = 24; [3] n = 12

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a-b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Boilers try to consume the amount of feed that covers their energy requirements (NRC, 1994). Therefore, differences in feed intake compensated differences in dietary AME, leading to an overall similar AME intake in both P and S groups ($P > 0.05$).

For the fat molecular structure, no differences among birds fed N and A diets were detected ($P > 0.05$), in agreement with the results of Young (1961), and Vilà and Esteve-Garcia (1996a). Birds fed re-esterified oil-enriched diets did not show differences among other treatments ($P > 0.05$). Concerning the effect of the FA positional distribution, Smink et al. (2008) and Lin and Chiang (2010) did not show differences in performance between broiler chickens fed high palmitic acid content at the TAG *sn*-2 position and those fed low palmitic acid content at the TAG *sn*-2 position. In contrast, Innis et al. (1997) reported a higher weight gain per liter of formula intake in piglets fed randomized oils than in those fed native oils. Regarding the fat acylglycerol composition, Murata et al. (1997), Taguchi et al. (2001), and Murase et al. (2005) did not find differences in performance of rats fed DAG or TAG oil-enriched diets. In contrast, Meng et al. (2004) reported that the weight gain was lower in rats fed a DAG oil-rich diet, and Kamphuis et al. (2003) observed a lesser feeling of hunger and appetite in women fed a DAG oil-rich diet.

The effects of dietary fat sources on abdominal fat pad and liver weights are presented in **Table 5.6**. Despite no differences in final BW, differences for carcass fat depots were observed for the fat degree of saturation. Broiler chickens fed P deposited a greater amount of abdominal fat than did those fed S ($P < 0.001$). The higher AME of S diets could be expected to cause a higher fat deposition. However, several studies (Sanz et al., 1999; Crespo and Esteve-Garcia, 2001, 2002a; Ferrini et al., 2008) have shown that dietary PUFA, compared to SFA, result in a lower abdominal fat depot in broiler chickens, which seems to be related to an increased rate of lipid catabolism and lower rate of FA synthesis. The same trend was observed for liver weight ($P = 0.08$). Crespo and Esteve-Garcia (2002a) and Ferrini et al. (2010) did not find differences in liver weight (both in absolute and relative basis) among animals fed saturated and unsaturated fat sources, but they did find differences in the liver lipid content. For the fat molecular structure effect, no differences were found for carcass fat depots. Regarding the fat FA positional distribution effect, Ponnampalam et al. (2011) found that animals fed lard-enriched diets (high *sn*-2 SFA) showed a greater external adipose tissue thickness than did those fed native palm oil (low *sn*-2 SFA). In relation to the fat acylglycerol composition, Murase et al., (2002) and Meng et al. (2004) reported a reduced accumulation of fat in visceral adipose and subcutaneous tissue, and a reduced fat content in the liver of rodents fed 1,3-DAG-enriched diets, as compared to those fed

TAG of the same FA composition. The presence of dietary 1,3-DAG has been shown to increase β-oxidation of FA in the liver (Murata et al., 1997) and in the small intestine (Murase et al., 2002). We might not have encountered significant differences in fat depots of broiler chickens fed re-esterified palm oils because the effect of MAG and DAG might have counteracted the effect of *sn*-2 SFA.

5.4.5. Fatty acid composition of abdominal adipose tissue

The effect of dietary fat sources on FA composition of abdominal adipose tissue is presented in **Table 5.7**. The FA composition of abdominal adipose tissue was a clear reflection of the dietary FA profile, which is consistent with the findings of other researchers (Pinchasov and Nir, 1992; Ferrini et al., 2008). Thus, as expected, animals fed P showed a higher SFA and MUFA content, and a lower PUFA content ($P < 0.001$) than did those fed S. However, the magnitude of the difference for the UFA-to-SFA ratio was markedly lower for the fat deposited (P: 2.00 ± 0.031 wt/wt and S: 2.90 ± 0.046 wt/wt) than was for the fat absorbed (P: 1.59 ± 0.039 wt/wt and S: 4.82 ± 0.038 wt/wt), due to the importance of maintaining the UFA-to-SFA ratio within a narrow range to maintain the fluidity of the cell membranes, as suggested by Villaverde et al. (2006).

Concerning the fat molecular structure effect, a slightly but significantly lower MUFA content was observed in abdominal adipose tissue of broilers fed N and EL, when compared with those fed A and EH ($P < 0.05$). PUFA levels were almost inversely related to those of MUFA. For the SFA content of abdominal adipose tissue, the interaction between the fat degree of saturation and the fat molecular structure was found to be significant ($P = 0.019$). Whereas the SFA content of abdominal adipose tissue was lower and not different among animals fed S sources, animals fed PEL showed a higher SFA content than did those fed PN ($P = 0.029$). This difference seems to be attributable to the higher dietary SFA content (3%) and the higher SFA apparent absorption (11%) of PEL when compared with PN. The study of Smink et al. (2008) also demonstrated that the increased absorption of SFA in randomized palm oils, especially of palmitic acid, caused higher SFA deposition.

Table 5.7. Fatty acid composition (%) of abdominal adipose tissue of broiler chickens fed different dietary fat sources[1]

	Dietary treatments[2]								Degree of saturation[3]		Molecular structure[4]					*P*-values		
	P				S											Degree of	Molecular	
Item	N	A	EL	EH	N	A	EL	EH	P	S	N	A	EL	EH	RMSE	saturation	structure	Interaction
C14:0	0.79^{b}	0.81^{ab}	0.87^{a}	0.82^{ab}	0.36^{c}	0.42^{c}	0.37^{c}	0.41^{c}	0.82	0.39	0.58	0.62	0.62	0.61	0.038	***	*	*
C16:0	26.4^{a}	26.7^{a}	28.0^{a}	27.0^{a}	18.8^{c}	20.0^{bc}	19.3^{bc}	20.7^{b}	27.0	19.7	22.6	23.3	23.6	23.8	1.01	***	*	*
C16:1 n-9	5.31	6.22	5.00	6.20	3.08	3.63	3.27	4.08	5.68	3.51	4.20^{b}	4.92^{a}	4.13^{b}	5.14^{a}	0.624	***	***	NS
C18:0	4.93^{ab}	4.79^{b}	5.76^{a}	5.07^{ab}	5.30^{ab}	5.48^{ab}	5.32^{ab}	5.27^{ab}	5.14	5.34	5.11	5.14	5.54	5.17	0.491	NS	NS	*
C18:1 n-9	44.1	45.5	44.2	45.3	32.4	34.5	32.3	34.3	44.8	33.4	38.3^{b}	40.0^{a}	38.2^{b}	39.8^{a}	1.30	***	**	NS
C18:1 n-7	2.07	2.34	2.20	2.28	1.87	1.91	1.86	2.01	2.22	1.91	1.97^{b}	2.13^{ab}	2.03^{ab}	2.14^{a}	0.159	***	*	NS
C18:2 n-6	13.7	11.5	11.8	11.1	32.6	29.1	31.9	28.5	12.0	30.5	23.1^{a}	20.3^{b}	21.8^{a}	19.8^{b}	1.40	***	***	NS
C18:3 n-3	0.96^{c}	0.75^{c}	0.76^{c}	0.74^{c}	4.02^{a}	3.46^{b}	4.14^{a}	3.38^{b}	0.80	3.75	2.49	2.11	2.45	2.06	0.146	***	***	***
C20:1 n-9	0.43^{a}	0.38^{b}	0.35^{bc}	0.36^{bc}	0.32^{c}	0.32^{c}	0.31^{c}	0.31^{c}	0.38	0.31	0.37	0.35	0.33	0.33	0.026	***	***	*
Minor FA	1.36	1.12	1.11	1.07	1.27	1.17	1.36	1.16	1.16	1.24	1.31^{a}	1.14^{ab}	1.23^{ab}	1.11^{b}	0.160	NS	*	NS
SFA	32.5^{b}	32.8^{ab}	35.2^{a}	33.4^{ab}	24.8^{c}	26.2^{c}	25.3^{c}	26.6^{c}	33.5	25.7	28.6	29.5	30.2	30.0	1.38	***	*	*
MUFA	52.1	54.6	51.9	54.4	37.8	40.5	37.8	40.8	53.2	39.2	44.9^{b}	47.5^{a}	44.8^{b}	47.6^{a}	1.64	***	***	NS
PUFA	15.5^{c}	12.6^{cd}	12.9^{cd}	12.2^{d}	37.5^{a}	33.3^{b}	36.9^{a}	32.6^{b}	13.3	35.1	26.5	23.0	24.9	22.4	1.60	***	***	*
UFA:SFA	2.09^{b}	2.06^{b}	1.85^{b}	2.00^{b}	3.05^{a}	2.81^{a}	2.97^{a}	2.77^{a}	2.00	2.90	2.57	2.44	2.41	2.38	0.173	***	NS	*

FA = fatty acids; MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; RMSE = root mean square error; SFA = saturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio.

[1] Diets with 6% (as-fed basis) of native palm (PN), acid palm oil (PA), re-esterified palm oil low in MAG and DAG (PEL), re-esterified palm oil high in MAG and DAG (PEH), native soybean oil (SN), acid soybean oil (SA), re-esterified soybean oil low in MAG and DAG (SEL), or re-esterified soybean oil high in MAG and DAG (SEH).

[2] n = 6; [3] n = 24; [4] n = 12

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a-d] Values within a row with different superscripts differ significantly at $P < 0.05$.

In general, the fat degree of saturation exerted a greater impact on FA apparent absorption, growth performance, carcass fat depots, and FA composition of abdominal adipose tissue than did the fat molecular structure. However, the increased fat sn-2 SFA content, in the starter period, and also the increased MAG and DAG content of re-esterified palm oils, in the grower-finisher period, exerted a favorable effect on the SFA apparent absorption. Thus, re-esterified oils can be used in broiler chicken diets as alternative fat sources, showing similar or even higher total FA apparent absorption results than do their corresponding native oils, with small changes in abdominal adipose tissue FA composition.

CHAPTER 6

Combination of re-esterified oils, differing in their degree of saturation, in broiler chicken diets

Combination of re-esterified oils, differing in their degree of saturation, in broiler chicken diets

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6.1. Abstract

Re-esterified oils contain higher proportions of mono- and diacylglycerols, and also higher proportions of saturated fatty acids (SFA) at the *sn*-2 position of acylglycerol molecules than does a native oil with the same degree of saturation, which enhances the apparent absorption of SFA. Moreover, as happens with native oils, their nutritive value could be further improved by blending re-esterified oils of extreme degrees of saturation. Therefore, the aim of the current study was to assess the effect of increasing the dietary unsaturated-to-saturated-fatty-acid ratio (UFA:SFA), by adding re-esterified soybean oil in replacement of re-esterified palm oil, on fatty acid (FA) apparent absorption and its consequences on growth performance, carcass fat depots and FA composition of abdominal adipose tissue. For this purpose, 120 one-day-old female broiler chickens (average weight of 46.5 ± 3.21 g) were randomly distributed in 30 cages. The two pure re-esterified oils, together with three re-esterified oil blends, were included in the basal diet at 6% (as-fed basis). The increasing dietary UFA:SFA resulted in an improved total FA apparent absorption (linear effect for the starter period, P = 0.001; quadratic effect for the grower-finisher period, P = 0.006) and, therefore, an improved feed conversion ratio for the overall period (linear effect, P = 0.003). In the starter period, the improved fat absorption was due to the growing presence of linoleic acid and the enhanced absorption of SFA, mono- and polyunsaturated FA (associative effects among FA; $P < 0.05$). In the growing-finishing period, however, the absorption of mono- and polyunsaturated FA was not affected ($P > 0.05$). The UFA:SFA of the abdominal adipose tissue varied in the same direction, but to a lesser extent than in that of the diet. Whilst the deposited-to-absorbed ratio of polyunsaturated FA remained relatively constant as the dietary UFA:SFA increased, the deposited-to-absorbed ratio of SFA increased, and that of monounsaturated FA decreased. Taken together, the addition of re-esterified soybean oil in replacement of re-esterified palm oil improved fat absorption, but no synergism was observed between re-esterified oils.

6.2. Introduction

From results of *Chapter 3*, re-esterified oils, obtained from the union of two low-cost by-products (glycerin and acid oils, derived from biodiesel and oil-refining industries, respectively), are interesting alternative fat sources to be used in broiler chicken diets. These technical fats are characterized by a greater *sn*-2 saturated fatty

acid (**SFA**) content, and mono- (**MAG**) and diacylglycerol (**DAG**) proportions when compared with their corresponding native oils. The different molecular structure of re-esterified oils exerted favorable effects on SFA apparent absorption, resulting in a similar or even a higher total fatty acid (**FA**) apparent absorption than did their corresponding native oils. In any case, the authors of this study observed how, in general, the fat degree of saturation exerted a greater impact on FA apparent absorption than did the fat molecular structure. For this reason, the combination of re-esterified oils, differing in their degree of saturation, could be beneficial in terms of fat utilization, as occurs in native oils (Sibbald et al., 1960; Lewis and Payne, 1966). Since long-chain unsaturated fatty acids (**UFA**) have a greater ability to form mixed micelles than do SFA, their presence has been shown to increase the capacity of mixed micelles to take up SFA in the core and, therefore, improve their absorption (Ketels and de Groote, 1989).

However, an excess of dietary UFA at the end of the rearing period could have a negative impact on carcass quality. Given that the FA composition of dietary fats has a direct influence on the FA composition of carcass fat depots (Pinchasov and Nir, 1992; Hrdinka et al., 1996; Bavelaar and Beynen, 2003; González-Ortiz et al., 2013), and that UFA decrease the firmness or consistency of the fat (Hrdinka et al., 1996; Zollitsch et al., 1997) and increase its susceptibility to oxidation (Cortinas et al., 2005), more saturated fat sources are recommended to be added to finisher diets. This is why the proportions between saturated and unsaturated fat sources are recommended to be adjusted throughout the rearing cycle according to the requirements of the producer.

Therefore, the aim of the current study was to assess the effect of increasing the dietary unsaturated-to-saturated-FA ratio (**UFA:SFA**), by adding re-esterified soybean oil in replacement of re-esterified palm oil, on FA apparent absorption and its consequences on growth performance, carcass fat depots and FA composition of abdominal adipose tissue.

6.3. Materials and methods

6.3.1. Experimental fats

Experimental fats were supplied by SILO S.p.a. (Florence, Italy).

Table 6.1. Chemical analyses of the experimental fats

Item		Re-esterified palm oil	Re-esterified soybean oil
Moisture, %		0.01	0.51
Impurities, %		<0.5	<0.5
Unsaponifiable matter, %		0.44	2.29
Fatty acid composition and distribution, %			
C16:0	Total	46.6	10.3
	sn-2 %[1]	18.9	11.4
C18:0	Total	4.77	3.65
	sn-2 %[1]	26.2	13.9
C18:1 n-9	Total	35.7	37.5
	sn-2 %[1]	27.8	23.9
C18:2 n-6	Total	8.30	40.4
	sn-2 %[1]	30.1	28.8
C18:3 n-3	Total	0.24	4.81
	sn-2 %[1]	29.1	24.0
Minor fatty acids		4.38	3.32
SFA	Total	54.1	14.1
	sn-2 %[1]	18.9	12.1
MUFA	Total	37.4	40.7
	sn-2 %[1]	27.8	23.6
PUFA	Total	8.54	45.2
	sn-2 %[1]	30.1	28.3
Acylglycerol composition, %			
TAG		54.4	55.0
DAG	Total	38.6	34.4
	1(3),2-DAG %[2]	21.8	30.2
MAG	Total	5.83	9.12
	2-MAG %[3]	10.3	8.90
FFA		1.18	1.55
Glycerol-to-fatty acid ratio[4], mol/mol		0.42	0.44
Gross energy, kcal/kg		9,223	9,222

DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; TAG = triacylglycerols.

[1] Fatty acid positional distribution expressed as the proportion of each fatty acid located at the *sn*-2 position of acylglycerol molecules, calculated as follows: *sn*-2 % = (*sn*-2 / Total) × *a* × 100, where *sn*-2 is the FA composition at the *sn*-2 position (converted to mol%), Total is the total FA composition of the fat (converted to mol%), and *a* is the ratio between the moles of FA located at the *sn*-2 position and the moles of total FA. *a* was calculated to be 0.23 and 0.24 for re-esterified palm and soybean oils, respectively.

[2] The proportion of 1(3),2-DAG *vs*. 1,3-DAG.

[3] The proportion of 2-MAG *vs*. 1(3)-MAG.

[4] Estimated calculation based on the values of the acylglycerol composition.

Re-esterified oils (**Table 6.1**) were produced using, as raw materials, palm or soybean acid oils (by-products obtained from the refining process of crude oils, with a high FFA content) and glycerin (a by-product obtained from the methylation process applied for the biodiesel production), which were processed in a reactor for 4-6 h, under high-vacuum conditions (1-3 mmHg), at temperatures between 190-250ºC, and without chemical catalysts.

Oil samples were analyzed in triplicate for moisture (Method 926.12 of the AOAC International, 2005), impurities (ISO 663:2007), unsaponifiable matter (Method 933.08 of the AOAC International, 2005), acylglycerol composition (ISO 18395:2005), positional isomers of mono- and diacylglycerols (Sacchi et al., 1997), total FA composition (Guardiola et al., 1994), *sn*-2 FA composition (Commission Regulation (EEC) No. 2568/91 – Annex VII), and gross energy content (IKA-Kalorimeter system C4000; Staufen, Germany), as described in more detail in *Chapter 3*.

6.3.2. Animals and diets

The trial was performed at the animal experimental facilities of the *Servei de Granges i Camps Experimentals* (Universitat Autònoma de Barcelona; Bellaterra, Barcelona, Spain). The experimental procedure received the prior approval from the Animal Protocol Review Committee of the same institution. All animal housing and husbandry conformed to the European Union Guidelines (2010/63/EU).

A total of 120 one-day-old female broiler chickens of the Ross 308 strain were obtained from a commercial hatchery (Pondex SAU; Juneda, Lleida, Spain). On arrival, chicks were wing-banded, weighed (initial body weight (**BW**), 46.5 ± 3.21 g), and randomly assigned to one of the five dietary treatments, with four chicks per cage and six cages per treatment. Birds were housed in wire-floor cages with excreta collection trays. Throughout the study, feed and water were supplied for *ad libitum* consumption, and animals were raised under controlled conditions of light and temperature, as recommended by the breeder.

The birds received a starter feed (in mash form) until day 20 and a grower-finisher feed (in pelleted form) between day 20 to 41. The wheat- and soybean-meal-based diets were formulated to meet or exceed FEDNA (2008) requirements and to minimize basal fat levels. The composition of experimental diets is presented in **Table 6.2**. Re-esterified palm and soybean oils were selected, as they represented extremes of

saturation likely to be encountered in the practical formulation of diets for broiler chickens.

Table 6.2. Ingredient composition of the experimental diets (as-fed basis)

Ingredients, %	Starter diet (from 0 to 20 days)	Grower-finisher diet (from 20 to 41 days)
Wheat	51.37	44.80
Soybean meal 48%	38.58	27.71
Barley	-	18.26
Experimental fats[1]	6.00	6.00
Dicalcium phosphate	1.69	1.33
Calcium carbonate	1.30	0.86
Sodium chloride	0.40	0.35
Vitamin and mineral premix[2]	0.30	0.30
DL-Methionine	0.23	0.18
L-Lysine	0.07	0.11
L-Threonine	-	0.02
Enzyme supplement[3]	0.05	0.05
Ethoxyquin 66%	0.02	0.02

[1] The five oil blends consisted of re-esterified palm oil blended with re-esterified soybean oil in the following proportions: 100:0, 75:25, 50:50, 25:75, and 0:100.

[2] Provides per kg of feed: vitamin A (from retinol), 13,500 IU; vitamin D_3 (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 4.5 mg; cobalamine, 16.5 µg; menadione, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from $FeSO_4 \cdot 7H_2O$), 54 mg; I (from $Ca(I_2O_3)_2$), 1.2 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$), 0.6 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na_2SeO_3), 0.18 mg; Mo (from $(NH_4)_6Mo_7O_{24}$), 1.2 mg.

[3] Provides per kg of feed: ß-glucanase 350 IU; xylanase 1,125 IU.

The two pure re-esterified oils were blended in the proportions shown in **Table 6.3** to produce five experimental diets varying in the UFA:SFA ratio (1.21, 1.60, 2.25, 3.25, and 5.09 for starter diets, and 1.18, 1.58, 2.16, 3.17, and 4.94 for grower-finisher diets). The two pure re-esterified oils, together with the three re-esterified oil blends, were included in the basal diet at 6% (as-fed basis).

Analytical determinations of feeds were performed according to the methods of the AOAC International (2005): dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fat (Method 2003.05), and crude fiber (Method 962.09). Gross energy was determined as described previously for fats, and the FA content was analyzed following the method of Sukhija and Palmquist (1988), adding nonadecanoic acid (C19:0, Sigma-Aldrich Chemical Co.; St. Louis, MO), as an internal standard. The macronutrient and FA composition of the experimental diets are presented in **Table 6.3**.

Table 6.3. Proportions of re-esterified oils used in oil blends, and analyzed[1] macronutrient content and fatty acid composition of experimental diets

	Starter diets (from 0 to 20 days)					Grower-finisher diets (from 20 to 41 days)				
Item	P	75P:25S	50P:50S	25P:75S	S	P	75P:25S	50P:50S	25P:75S	S
Proportion of re-esterified oils used in oil blends, %										
Re-esterified palm oil	100	75	50	25	-	100	75	50	25	-
Re-esterified soybean oil	-	25	50	75	100	-	25	50	75	100
Macronutrient content, %										
Dry matter	90.9	90.9	90.9	90.9	90.8	89.1	89.2	89.4	89.3	89.8
Crude protein	24.6	25.0	25.7	24.6	24.3	19.8	20.0	20.8	20.3	20.2
Crude fat	7.54	7.14	7.25	7.50	7.39	7.64	7.19	7.38	7.24	7.40
Crude fiber	2.81	2.73	2.82	3.07	2.75	2.81	2.74	2.72	2.86	2.68
Ash	7.18	7.24	7.52	7.40	6.89	5.35	5.20	5.47	5.03	5.23
Gross energy, kcal/kg	4,249	4,250	4,219	4,211	4,226	4,200	4,208	4,218	4,197	4,216
Fatty acid composition, %										
C16:0	38.5	32.5	26.0	19.2	12.3	39.3	32.9	26.4	19.8	12.9
C18:0	4.56	4.27	4.10	3.87	3.61	4.47	4.16	3.96	3.71	3.45
C18:1 n-9	30.7	30.7	31.1	31.3	31.4	30.5	30.4	30.7	30.9	31.0
C18:2 n-6	21.4	27.1	33.2	39.1	44.7	21.1	27.2	33.0	39.3	44.9
C18:3 n-3	1.90	2.77	3.76	4.61	5.37	1.73	2.63	3.50	4.37	5.27
Minor fatty acids	2.91	2.64	1.93	1.90	1.64	2.88	2.62	2.38	1.85	1.61
SFA	45.2	38.4	30.8	23.5	16.4	45.9	38.8	31.6	24.0	16.8
MUFA	31.5	31.7	32.3	32.7	33.5	31.2	31.4	31.9	32.3	33.0
PUFA	23.3	29.9	36.9	43.8	50.1	22.9	29.8	36.5	43.7	50.2
UFA:SFA	1.21	1.60	2.25	3.25	5.09	1.18	1.58	2.16	3.17	4.94

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; UFA = unsaturated fatty acids.

[1] All samples were analyzed at least in duplicate.

6.3.3. Controls and sampling

Feed consumption and BW were measured weekly to calculate average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**) throughout the experiment.

Two digestibility balances were carried out using the total-excreta-collection method (Bourdillon et al., 1990). Excreta were collected in the starter period from day 7 to 10 and in the growing-finishing period from day 36 to 38. On the last day of the balance, feed consumption was measured and total excreta was collected, weighed and homogenized, and a representative sample was frozen at -20ºC. Contaminants such as feed, feathers, down, and scales were removed. Then, the excreta samples were freeze-dried, ground and kept at 5ºC until further analysis. Excreta samples were analyzed by the same methods as those described for feeds, to determine the apparent absorption of FA and the apparent metabolizable energy (**AME**) of the diets. The apparent absorption coefficients of the nutrients were calculated as the difference between the amount ingested and the amount excreted, expressed as the percentage of the amount ingested. The AME was calculated from the product of energy apparent absorption and its corresponding feed gross energy.

At the end of the experimental period, the 41-day-old broiler chickens were fasted for 3 h, stunned, slaughtered, bled, plucked, and chilled at 4ºC for 12 h in a local slaughterhouse (Gimave S.A.; Ripollet, Barcelona, Spain). Carcasses (total BW excluding blood and feathers) were weighed, and the liver and abdominal fat pad (from the proventriculus surrounding the gizzard down to the cloaca) for each bird were removed and weighed. The percentages of the abdominal fat pad and liver were expressed as the percentage of carcass weight. A representative sample of the abdominal fat pad was taken and frozen at -20 ºC. The FA composition of the abdominal fat pad was determined by the method of Carrapiso et al. (2000).

6.3.4. Statistical analysis

Normality of the data and homogeneity of the variance were verified. All data were subjected to a one-way ANOVA using the GLM procedure of SAS (version 9.2, SAS Institute Inc.; Cary, NC, USA). All data were subjected to orthogonal polynomial contrasts to examine whether responses to increasing dietary UFA:SFA ratios (variability as a result of feeding different proportions of re-esterified palm and soybean oils) were linear or quadratic. The linear and quadratic trends were studied for

not equally-spaced levels. Prediction equations were obtained using the REG procedure of the same statistical package. The cage served as the experimental unit, so that there were six experimental units per treatment. For abdominal fat pad and liver weights, the broiler carcass weight was included as a covariate in the model, to correct these variables for variations not related to the dietary treatment effect. Results in the tables are reported as least square means and differences were considered significant at $P < 0.05$.

6.4. Results and discussion

6.4.1. Experimental fats and diets

Results from the chemical analyses of the experimental fats are presented in **Table 6.1**. Re-esterified palm and soybean oils had similar levels of monounsaturated fatty acids (**MUFA**; 39.1 ± 2.33%), but they differed in the content of SFA and polyunsaturated fatty acids (**PUFA**). Whereas re-esterified palm oil was mainly composed of palmitic acid (46.6%), re-esterified soybean oil was mainly composed of linoleic acid (40.4%). The FA composition of re-esterified palm oil was very similar to that of palm native oils (FEDNA, 2010), but the FA composition of re-esterified soybean oil showed a higher oleic acid content and a lower linoleic acid content than did that of soybean native oils (according to FEDNA, 2010, the oleic and linoleic acid contents of soybean native oils are 22% and 54%, respectively). In any case, they showed very different degrees of saturation, expressed as the UFA:SFA, which ranged from 0.85 to 6.09 for re-esterified palm and soybean oils, respectively. However, the UFA:SFA range of the diets was slightly narrower (**Table 6.3**; from 1.21 to 5.09 for starter diets and from 1.18 to 4.94 for grower-finisher diets), due to the contribution of the lipid content of the basal diet.

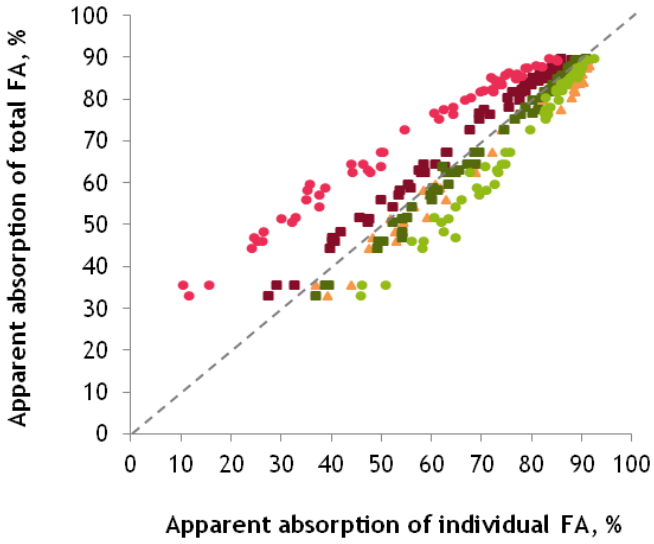
Concerning the FA positional distribution, only small differences were observed between both experimental fats. Whereas PUFA were almost randomly distributed among all three positions of the acylglycerol molecules, SFA were mainly located at the *sn*-1,3 positions. This FA positional distribution of re-esterified oils was slightly different from that of their corresponding native oils, in which PUFA are predominantly located at the *sn*-2 position and SFA at the *sn*-1,3 positions (Mattson and Volpenhein, 1963).

For the acylglycerol composition, both re-esterified oils showed a similar pattern: a high amount of triacylglycerols (**TAG**) (54.7 ± 0.42%), a moderate level of DAG (36.5

± 2.97%), and a small amount of MAG (7.48 ± 2.326%). Thus, both oils showed a similar glycerol-to-FA ratio (0.43 ± 0.014 mol/mol) and an identical gross-energy content (9,223 ± 0.7 kcal/kg). These values contrast with those of native oils, which are almost entirely composed of TAG, with a glycerol-to-FA ratio of 0.33 mol/mol.

6.4.2. Digestibility balances

The effects of increasing the dietary UFA:SFA by blends of re-esterified oils on the apparent absorption coefficients in both starter (7-10 days) and grower-finisher (36-38 days) periods are presented in **Table 6.4**. As expected, the absorption of long-chain UFA was substantially higher when compared with that of long-chain SFA, as has been previously reported by several authors using native oils (Renner and Hill, 1961; Young and Garrett, 1963). **Figure 6.1** shows the relationship between the absorption of individual and total FA coming from diets supplemented with blends of re-esterified oils.



Independent variable	Equation	R^2	*P*-value
■ C16:0	y = 0.96 ± 0.010x + 6.97 ± 0.719	0.993	***
● C18:0	y = 0.75 ± 0.011x + 28.1 ± 0.691	0.987	***
▲ C18:1 n-9	y = 1.01 ± 0.018x - 4.09 ± 1.374	0.982	***
■ C18:2 n-6	y = 1.07 ± 0.011x - 7.20 ± 0.814	0.994	***
● C18:3 n-3	y = 1.31 ± 0.023x - 30.5 ± 1.80	0.982	***

Figure 6.1. Relationship between the absorption of individual and total fatty acids (FA) in broiler chickens fed re-esterified oil blends. **** P < 0.001*

Table 6.4. Apparent metabolizable energy of the diets (kcal/kg) and individual fatty acid apparent absorption coefficients (%) in broiler chickens fed different dietary re-esterified oil blends

	Dietary treatments[1]						
Item	P	75P:25S	50P:50S	25P:75S	S	RMSE[2]	*P*-values[3]
From 7 to 10 d							
AME, kcal/kg	2,891	3,014	2,929	3,051	3,079	103.1	L**
Total fatty acids	47.5	55.8	51.6	67.0	66.9	10.30	L**
SFA	41.1	49.8	44.8	59.9	57.2	11.35	L*
MUFA	53.9	59.3	53.0	68.5	67.4	10.76	L*
PUFA	51.2	59.9	56.0	69.7	69.7	9.28	L***
C16:0	41.8	51.2	48.0	63.5	62.3	10.71	L**
C18:0	27.3	37.3	32.1	49.6	49.5	12.98	L**
C18:1 n-9	54.7	60.1	54.0	69.3	67.9	10.61	L*
C18:2 n-6	50.7	59.2	55.0	69.0	69.0	9.41	L**
C18:3 n-3	57.2	66.5	64.6	76.3	76.0	7.96	L***
From 36 to 38 d							
AME, kcal/kg	3,022	3,077	3,085	3,081	3,035	109.6	NS
Total fatty acids	81.6	84.3	85.2	87.8	85.6	2.75	L*; Q**
SFA	75.8	79.7	80.4	85.3	80.3	3.76	L*; Q***
MUFA	88.5	88.6	88.3	89.4	86.8	2.28	NS
PUFA	84.1	85.9	86.7	87.9	86.6	2.29	NS
C16:0	76.3	80.5	81.5	86.4	82.0	3.47	L**; Q***
C18:0	69.9	74.1	74.2	81.9	78.0	4.77	L**; Q**
C18:1 n-9	88.6	88.8	88.5	89.5	87.0	2.25	NS
C18:2 n-6	84.0	85.7	86.5	87.7	86.4	2.31	NS
C18:3 n-3	84.8	87.4	88.5	89.8	88.6	2.03	L**; Q**

AME = apparent metabolizable energy; MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; RMSE = root mean square error; SFA = saturated fatty acids.

[1] Diets with 6% (as-fed basis) of blends of re-esterified palm oil with re-esterified soybean oil (P:S; 0:100, 75:25, 50:50, 25:75, and 0:100) corresponded to dietary unsaturated-to-saturated fatty acid ratios of 1.21, 1.60, 2.25, 3.25, and 5.09 for starter period and 1.18, 1.58, 2.16, 3.17, and 4.94 for grower-finisher period, respectively.

[2] n = 6

[3] The orthogonal polynomial contrasts were performed according to the dietary unsaturated-to-saturated fatty acid ratios. L = linear; Q = quadratic.

*P < 0.05; **P < 0.01; ***P < 0.001.

For the linear regression analysis, we pooled data from the two digestibility balances. As expected, the absorption of individual FA was directly related to the absorption of total FA. The slopes of the equations showed how, as the FA chain-length increased, the contribution of individual FA to total FA apparent absorption was reduced (0.956 and 0.753 for C16:0 and C18:0, respectively). In contrast, as the number of double bonds increased, the contribution of individual FA to total FA

apparent absorption also increased (0.753, 1.01, 1.07, and 1.31 for C18:0, C18:1 n-9, C18:2 n-6, and C18:3 n-3, respectively). The slope of the equations also shows the interactions that exist among FA. Thus, a slope above 1 (linoleic and linolenic acids; $P < 0.05$) would indicate a synergistic effect, and a slope below 1 (palmitic and stearic acids; $P < 0.05$) would indicate an antagonistic effect. The results indicate that PUFA, due to their emulsifying capacities, can promote the incorporation of SFA into mixed micelles and, therefore, improve the total FA apparent absorption, as has already been described by several authors (Young and Garrett, 1963; Garrett and Young, 1975; Krogdahl, 1985; Wiseman and Lessire, 1987; Ketels and de Groote, 1989). In contrast, SFA, due to their apolarity and high melting points, may also exert an antagonistic effect on UFA apparent absorption, as Wiseman and Lessire (1987) and Ketels and de Groote (1989) also suggested.

Regarding the effect of age on FA apparent absorption, the coefficients achieved in the starter period were lower than were those found in the grower-finisher period, since the capacity of young birds to secrete bile salts is limited (Krogdahl, 1985). In any case, in both young and adult broiler chickens, the absorbability of total FA increased with increasing inclusion rates of re-esterified soybean oil in substitution of re-esterified palm oil.

In the starter period, the total FA apparent absorption varied linearly ($P = 0.001$) from 47.5 to 66.9% with an increase in the dietary UFA:SFA from 1.21 to 5.09, which means that young birds were not able to achieve the biological maximum of fat absorption, probably due to the lack of bile salts (Krogdahl, 1985). The simple linear regression analysis yielded the following equation $y = 0.20 \pm 0.05x + 47.7 \pm 3.30$ ($R^2 = 0.306$, $P < 0.001$), where y is the total FA apparent absorption and x is the percentage of re-esterified soybean oil in the replacement of re-esterified palm oil (at a 6% inclusion level in feed). Thus, the maximum total FA apparent absorption coefficient was achieved for the most unsaturated diet (100% re-esterified soybean oil). The greater absorbability of total FA due to the increasing UFA:SFA was, in part, due to the growing presence of linoleic acid, but also due to the significant improvement in the absorbability of SFA (linear effect, $P = 0.013$), as has also been reported by several authors using native oils or pure FA (Young and Garrett, 1963; Garrett and Young, 1975; Wiseman and Lessire, 1987; Ketels and de Groote, 1989). In addition, the increasing dietary UFA:SFA also exerted a significant improvement in the absorbability of MUFA and PUFA (linear effect, $P = 0.016$ and $P < 0.001$ for MUFA and PUFA, respectively), as also reported Wiseman and Lessire (1987) and Ketels and de Groote (1989). These data support the results of **Figure 6.1**, confirming that the presence of

UFA improves SFA apparent absorption, but the presence of SFA may also impair the absorption of MUFA and PUFA in young broiler chickens, with a limited bile concentration.

In the grower-finisher period, the total FA apparent absorption was also improved with the increase of the dietary UFA:SFA, but to a lesser extent (compared to the starter period, the magnitude of the difference was reduced nearly five times; from 81.6 to 85.6% with an increase in the dietary UFA:SFA from 1.18 to 4.98), and the increase was quadratic (P = 0.006). The best-fit prediction equation for values of total FA apparent absorption, as a function of the percentage of replacement of re-esterified palm oil by re-esterified soybean oil (at a 6% inclusion level in feed) was: $y = -0.001 \pm 0.0005x^2 + 0.14 \pm 0.050x + 81.5 \pm 1.05$ (R^2 = 0.291, P = 0.004). In this case, the maximum total FA apparent absorption coefficient was achieved for a fat blend of 25% of re-esterified palm oil mixed with 75% of re-esterified soybean oil, which corresponded to a dietary UFA:SFA of 3.17. This UFA:SFA was slightly lower than was that found by Ketels and de Groote (1989), who reported that fat digestibility reached a near asymptotical maximum at a dietary UFA:SFA of 4 or more. This lower UFA:SFA may be due to the beneficial effects that the increased *sn*-2 SFA content, and MAG and DAG proportions of re-esterified oils exert on fat absorption, when compared with native oils. As in the starter period, the increasing dietary UFA:SFA exerted a favorable effect in the apparent absorption of SFA (quadratic effect, P = 0.001), but no differences were found for MUFA and PUFA ($P > 0.05$), suggesting that UFA apparent absorption in adults was not influenced by composition of the fat mixtures, in agreement with the results of Wiseman and Lessire (1987).

Although several authors have found synergism between native fats of different degrees of saturation (Sibbald et al., 1960; Lewis and Payne, 1966), when we added re-esterified soybean oil to re-esterified palm oil, the total FA apparent absorption coefficients of the mixtures (determined) did not reach significantly greater values than did the sum of the means of the component parts (calculated) ($P > 0.05$). In this sense, although we found associative effects among FA, we failed to observe synergistic interactions between re-esterified oils, in agreement with the results of Wiseman and Lessire (1987). This could be due to neutralization of the synergistic and antagonistic effects observed among FA.

6.4.3. Growth performance and carcass fat depots

The effects of increasing the dietary UFA:SFA by blends of re-esterified oils on growth performance in both starter (0-20 days) and grower-finisher (20-40 days) periods are presented in **Table 6.5**.

Table 6.5. Growth performance and carcass fat depots of broiler chickens fed different dietary re-esterified oil blends

Item		Dietary treatments[1]					RMSE[2]	*P*-values[3]
		P	75P:25S	50P:50S	25P:75S	S		
From 0 to 20 days								
ADFI, g		49.3	49.3	50.0	48.2	46.2	2.29	NS
ADG, g		32.9	34.2	34.7	34.9	32.4	2.47	NS
FCR, g/g		1.50	1.45	1.44	1.38	1.44	0.073	NS
BW at 20 days, g		704	735	739	747	695	55.9	NS
From 20 to 41 days								
ADFI, g		168	165	169	168	164	4.8	NS
ADG, g		92.1	92.7	93.5	92.9	93.5	2.94	NS
FCR, g/g		1.83	1.78	1.81	1.80	1.75	0.044	NS
BW at 41 days, g		2,631	2,682	2,703	2,692	2,657	67.3	NS
From 0 to 41 days								
ADFI, g		110	109	111	109	106	2.94	NS
ADG, g		63.2	64.2	64.8	64.6	63.7	1.60	NS
FCR, g/g		1.75	1.69	1.71	1.69	1.67	0.031	L**
Carcass fat depots								
Abdominal fat,	g	53.9	56.8	55.8	59.0	52.1	6.58	NS
	%	2.21	2.40	2.36	2.48	2.21	0.272	NS
Liver,	g	50.8	50.5	52.0	52.8	50.8	2.81	NS
	%	2.16	2.12	2.17	2.23	2.14	0.113	NS

ADFI = average daily feed intake; ADG = average daily gain; BW = body weight; FCR = feed conversion ratio; NS = not significant; RMSE = root mean square error.

[1] Diets with 6% (as-fed basis) of blends of re-esterified palm oil with re-esterified soybean oil (P:S; 0:100, 75:25, 50:50, 25:75, and 0:100) corresponded to dietary unsaturated-to-saturated fatty acid ratios of 1.21, 1.60, 2.25, 3.25, and 5.09 for starter period and 1.18, 1.58, 2.16, 3.17, and 4.94 for grower-finisher period, respectively.

[2] n = 6.

[3] The orthogonal polynomial contrasts were performed according to the dietary unsaturated-to-saturated fatty acid ratios. L = linear.

*P < 0.05; **P < 0.01; ***P < 0.001.

In the overall period, FCR was improved significantly (linear effect, P = 0.003) with the increase of the dietary UFA:SFA, as has also been observed by several authors (Pinchasov and Nir, 1992; Zollitsch et al., 1997; Dvorin et al., 1998; Dänicke et al., 2000; Crespo and Esteve-Garcia, 2001; Wongsuthavas et al., 2008), using native oils.

The reason for the lower FCR in broiler chickens fed more unsaturated re-esterified-oil blends was probably the higher total FA apparent absorption observed in both starter and grower-finisher periods. However, in this experiment, feed intake and body weight gain were not significantly ($P > 0.05$) affected by the dietary UFA:SFA, neither in the starter nor in the grower-finisher period, in agreement with the results of other authors (Pinchasov and Nir, 1992; Sanz et al., 1999, 2000a, b; Newman et al., 2002), using native oils.

The effects of increasing the dietary UFA:SFA by blends of re-esterified oils on carcass fat depots are presented in **Table 6.5**. The abdominal fat pad and liver weights were not significantly affected by the dietary fat degree of saturation ($P > 0.05$). Pinchasov and Nir (1992) and Dvorin et al. (1998) also found no differences in the abdominal fat pad weight of broiler chickens fed native fat sources with extreme degrees of saturation. In contrast, several other studies (Sanz et al., 1999, 2000a, b; Crespo and Esteve-Garcia, 2002a, b; Newman et al., 2002; Ferrini et al., 2008; González-Ortiz et al., 2013) have found lower abdominal fat-pad weights in broiler chickens fed unsaturated native fat sources in comparison with those fed saturated ones. However, most of them used more saturated fat sources (such as tallow, with a higher stearic acid content) and more unsaturated fat sources (such as linseed oil or sunflower oil, with a higher linolenic and linoleic acid content, respectively), than the re-esterified oils used in this study (**Table 6.1**), which could explain why we did not find differences. Moreover, in contrast to native fats, our re-esterified oils showed an important content of 1,3-diacylglycerols, which have been involved with anti-obesity effects in rodents, due to their ability to increase the β-oxidation of FA in the liver and in the intestine (Murase et al., 2002; Meng et al., 2004), which may dilute the effects of the dietary degree of saturation.

6.4.4. Fatty acid composition of abdominal adipose tissue

The effects of increasing the dietary UFA:SFA by blends of re-esterified palm and soybean oils on FA composition of abdominal adipose tissue are presented in **Table 6.6**. As the dietary UFA:SFA increased, the deposition of PUFA increased (quadratic effect, $P < 0.001$), and that of SFA and MUFA decreased (linear effect, $P < 0.001$).

Table 6.6. Fatty acid composition (%) of abdominal adipose tissue of broiler chickens fed different dietary re-esterified oil blends

	Dietary treatments[1]						
Item	P	75P:25S	50P:50S	25P:75S	S	RMSE[2]	*P*-values[3]
C14:0	0.91	0.79	0.66	0.54	0.39	0.032	L***; Q***
C16:0	29.4	27.9	25.7	23.1	19.6	0.73	L***; Q**
C16:1 n-9	5.41	5.08	4.39	4.48	3.38	0.529	L***
C18:0	4.93	5.12	5.41	5.04	5.48	0.370	NS
C18:1 n-9	43.6	41.8	41.1	40.0	39.7	1.15	L***; Q**
C18:1 n-7	1.98	2.01	1.99	2.13	2.09	0.139	NS
C18:2 n-6	11.2	14.4	17.4	20.8	24.9	0.93	L***; Q***
C18:3 n-3	0.88	1.31	1.78	2.26	2.83	0.086	L***; Q***
C20:1 n-9	0.32	0.33	0.35	0.37	0.41	0.017	L***
Minor fatty acids	1.28	1.28	1.24	1.21	1.20	0.115	NS
SFA	35.9	34.4	32.3	29.1	25.8	0.87	L***; Q**
MUFA	51.6	49.5	48.1	47.3	45.8	1.35	L***; Q*
PUFA	12.4	16.1	19.6	23.6	28.4	1.05	L***; Q***
UFA:SFA	1.79	1.90	2.10	2.44	2.88	0.088	L***

MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; RMSE = root mean square error; SFA = saturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio.

[1] Diets with 6% (as-fed basis) of blends of re-esterified palm oil with re-esterified soybean oil (P:S; 0:100, 75:25, 50:50, 25:75, and 0:100) corresponded to dietary unsaturated-to-saturated fatty acid ratios of 1.18, 1.58, 2.16, 3.17, and 4.94, respectively.

[2] n = 6

[3] The orthogonal polynomial contrasts were performed according to the dietary unsaturated-to-saturated fatty acid ratios. L = linear; Q = quadratic.

*P < 0.05; **P < 0.01; ***P < 0.001.

In **Table 6.7**, linear regression equations between dietary and deposited FA (% of total FA) are presented. It can be observed that the magnitude of the difference found among treatments for SFA and PUFA was higher in diets than that found in abdominal adipose tissue, although MUFA behaved the opposite. Therefore, variations in the dietary UFA:SFA of 3.76 units only resulted in 1.09 units of difference for the abdominal adipose tissue UFA:SFA, indicating the existence of a physiological mechanism that maintains the UFA:SFA of body fat inside a relatively narrow range, which was also stated by Villaverde et al. (2006). Furthermore, the slopes of the regression lines show that PUFA are more easily modified than SFA in the abdominal adipose tissue. In this sense, it is interesting to note the similarity of the slope of the regression line obtained for PUFA in the present study (0.58± 0.021) to those observed by Beynen et al. (1980), and Waldroup and Waldroup (2005) with men (0.54) and broiler chickens (0.55), respectively.

Table 6.7. Relationship between dietary and deposited fatty acid composition (% of total fatty acids) in broiler chickens fed re-esterified oil blends

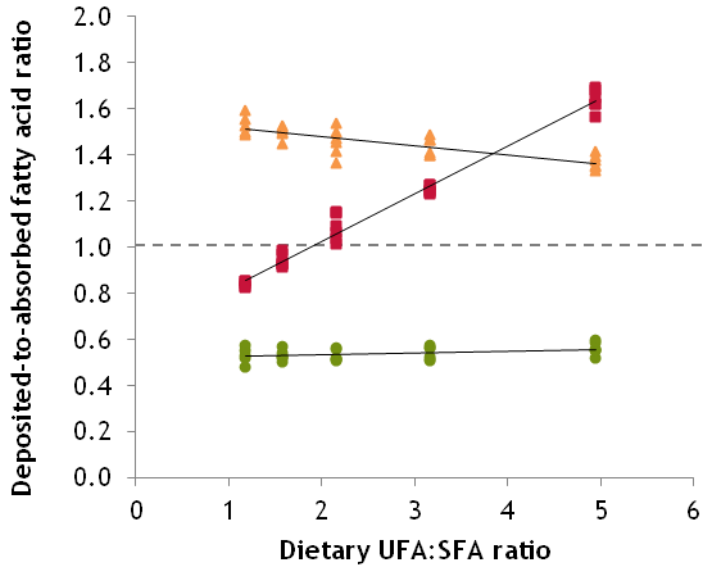
Item	Dietary Δ, %	Deposited Δ, %	Equation[1]	R^2	*P*-value
C16:0	26.4	9.86	y = 0.38 ± 0.017x + 15.3 ± 0.47	0.945	***
C18:0	1.02	0.55	y = -0.41 ± 0.208x + 6.80 ± 0.820	0.092	NS
C18:1 n-9	0.50	3.91	y = -4.93 ± 1.077x + 193 ± 33.1	0.416	***
C18:2 n-6	23.8	13.7	y = 0.57 ± 0.021x – 1.05 ± 0.731	0.963	***
C18:3 n-3	3.54	1.95	y = 0.55 ± 0.013x – 0.12 ± 0.051	0.984	***
SFA	29.1	10.1	y = 0.35 ± 0.018x + 20.4 ± 0.59	0.932	***
MUFA	1.80	5.87	y = -2.87 ± 0.409x + 140 ± 13.1	0.634	***
PUFA	27.3	16.0	y = 0.58 ± 0.021x – 1.13 ± 0.797	0.965	***
UFA:SFA	3.76	1.09	y = 0.29 ± 0.012x + 1.46 ± 0.036	0.953	***

MUFA = monounsaturated fatty acids; NS = not significant; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; UFA = unsaturated fatty acids.

[1] The independent variable (x) corresponds to the dietary fatty acid content (%) and the dependent variable (y) corresponds to the deposited fatty acid content in abdominal adipose tissue (%).

*** $P < 0.001$.

To gain insight into the mechanism by which the dietary fat degree of saturation affects the FA composition of abdominal adipose tissue, we calculated the deposited-to-absorbed FA ratio, according to the dietary UFA:SFA (**Figure 6.2**). Whilst values above 1 indicate an increased abdominal adipose tissue content of a particular FA, with respect to the fat absorbed (i.e., net *de novo* synthesis, FA inter-conversions, or deposit preference), values below 1 indicate the opposite (i.e., net β-oxidation or FA inter-conversions). Regardless of the dietary UFA:SFA, the deposited-to-absorbed PUFA ratio remained almost constant and below 1, as has already been observed by other authors (Villaverde et al., 2006; Wongsuthavas et al., 2011) using native oils. This is consistent with the well-known preferential β-oxidation of PUFA (DeLany et al., 2000; Leyton et al., 2000; Sanz et al., 2000b; Ferrini et al., 2010; Wongsuthavas et al., 2011), and the fact that the parent PUFA, linoleic and linolenic acids, are essential FA and, by definition, are not synthesized by the birds (NRC, 1994).



Dependent variable	Equation	R^2	*P*-value
■ SFA	y = 0.21 ± 0.006x + 0.61 ± 0.017	0.980	***
▲ MUFA	y = -0.04 ± 0.006x + 1.56 ± 0.016	0.650	***
● PUFA	y = 0.01 ± 0.004x + 0.51 ± 0.011	0.142	*

Figure 6.2. Deposited-to-absorbed fatty acid ratio of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in response to increasing dietary unsaturated-to-saturated fatty acid ratio (UFA:SFA). ** P < 0.05; *** P < 0.001.*

The relationship between dietary and tissue concentration of SFA and MUFA is more complex because these FA in animals have a double origin: exogenous (dietary) and *de novo* FA synthesis (from carbohydrate and protein precursors). As the dietary UFA:SFA increased, the deposited-to-absorbed MUFA ratio slightly decreased and that of SFA markedly increased (**Figure 6.2**). The deposited-to-absorbed MUFA ratio was always above 1, indicating that, regardless of the dietary degree of saturation, there was always a net synthesis, as has also been observed by other authors (Villaverde et al., 2006; Wongsuthavas et al., 2011) using native oils. Actually, MUFA are the main FA synthesized from glucose in broiler chickens (Ferrini et al., 2010). In contrast, the deposited-to-absorbed SFA ratio was lower than 1 in birds receiving diets with a UFA:SFA below 1.89. Given that FA oxidation is more related to PUFA than to SFA (DeLany et al., 2000; Leyton et al., 2000; Sanz et al., 2000b; Ferrini et al., 2010; Wongsuthavas et al., 2011), the disappearance of SFA in broilers fed highly saturated

diets was probably, in part, due to the desaturation process leading to the formation of MUFA. However, in more unsaturated diets, the deposited-to-absorbed SFA ratio increased above 1, probably due to decreased conversion of SFA into MUFA. In fact, it has been observed that high dietary linoleic acid content inhibits the Δ9-desaturase enzyme in the liver (Kouba and Mourot, 1998), resulting in a lower oleic acid synthesis.

Feeding broiler chickens with increasing dietary UFA:SFA, by adding re-esterified soybean oil in replacement of re-esterified palm oil, resulted in an improved total FA apparent absorption, although no synergism was observed between re-esterified oils.

CHAPTER 7

General discussion

In monogastric animals, the biological role of fatty acid (**FA**) chain length, FA degree of saturation, and free fatty acid (**FFA**) content have been the main foci of lipid nutrition research for many years. However, less attention has been given to the intramolecular structure of acylglycerol molecules, including the FA positional distribution and the number of FA bound to glycerol molecules. Evidence is accumulating that the intramolecular structure of dietary fats is also of importance when considering the nutritional effects of a given fat, because it can affect its rates of digestion and absorption, and also its subsequent metabolism. Re-esterified oils, besides of being obtained from the reuse of two low-cost by-products (acid oils and glycerin), show variable amounts of saturated fatty acids (**SFA**) located at the *sn*-2 position, and mono- (**MAG**) and diacylglycerol (**DAG**) molecules. For this purpose, in this thesis we have investigated the potential use of re-esterified oils in pig and broiler chicken diets, in order to see if these technical fats provide a greater nutritive value than their corresponding acid oil and also than their analogous native oil. In general, we have observed positive effects regarding the use of these new fat sources, both in pigs and broiler chickens, but with some differences between species and the physiological state of the animals. Thus, the aim of this general discussion is to integrate all the results reported in this thesis and add some new insights that will complement the results.

7.1. Experimental fats

7.1.1. Molecular structure

In this thesis, a significant effort has been done to characterize in depth the molecular structure of experimental fats. We have conducted some analyses that are not commonly performed in routine fat characterization. The acylglycerol and FFA composition of experimental fats was analyzed by HPLC, in order to separate TAG, DAG, MAG, and FFA according to their molecular size. Moreover, given the potential importance of different positional isomers of MAG and DAG molecules in the digestion and absorption processes, we also analyzed the experimental fats by high-resolution ^{1}H NMR spectroscopy, in order to distinguish the proportion of 2-MAG vs. 1(3)-MAG and 1(3),2-DAG vs. 1,3-DAG. The FA composition at the *sn*-2 position of the acylglycerol molecules was analyzed by hydrolyzing the original fat with pancreatic lipase to selectively cleave the ester bonds at the *sn*-1,3 positions. Then, 2-MAG were isolated by

thin-layer chromatography and the FA composition of this spot was analyzed by gas chromatography.

The results of these analyses have provided detailed information about the molecular structure of experimental fats. In the next two sections, this issue is discussed in depth.

7.1.1.1. Acylglycerol and free fatty acid composition

As it has been observed in *Chapters 3, 4, 5, and 6*, re-esterified oils are always a blend of MAG, DAG and triacylglycerols (**TAG**) molecules, with a very low FFA content. When the glycerol-to-FA ratio added to the reactor increases (above 0.33), the formation of TAG decreases and that of MAG and DAG increases. However, the proportion of each acylglycerol molecule is very difficult to predetermine. Therefore, a limitation of our study has been the impossibility to discern the effect of MAG and DAG molecules separately. To discriminate the effects between them, we should have used pure MAG and DAG oils, which can only be obtained by distillation.

Furthermore, considering that chemical esterification, unlike enzymatic esterification, has no position specificity, one would have expected similar proportions of different MAG and DAG positional isomers. However, re-esterified palm oils were mainly composed of 1(3)-MAG (90.7 ± 2.59%) and 1,3-DAG (72.3 ± 4.07%) positional isomers, since primary esters are chemically more stable than the secondary ester (Crossley et al., 1959; Lo et al., 2008). Therefore, due to the specificity of pancreatic lipase for primary ester bonds (Mattson and Beck, 1956), 1(3)-MAG and 1,3-DAG molecules could be completely hydrolyzed to glycerol and FFA during the digestion process, as it has already been suggested by other authors (Murata et al., 1994; Watanabe et al., 1997a; Kondo et al., 2003). As a consequence, the expected emulsifying effect of MAG and DAG molecules, due to their amphiphilic properties, would disappear and the esterification process would have no sense. However, in this thesis, the use of re-esterified palm oils has achieved, in general, positive results when compared with their corresponding native and acid oils (*Chapter 3, 4, and 5*).

To verify the importance of ester bonds, we performed another experiment (Vilarrasa et al., 2014a), in which the importance of the esterification process to give added value to acid oils was confirmed. Keeping the glycerol-to-FA ratio constant (0.33 mol/mol), the treatment with a high MAG and DAG content achieved higher SFA apparent absorption values than did the treatment with a high TAG content (P = 0.028). In turn, the treatment with a high TAG content resulted in higher SFA apparent

absorption values than did the treatment with a high FFA and free glycerol content ($P < 0.001$). Furthermore, the nutritive value of oils with a certain glycerol-to-FA ratio differed depending on whether glycerol was in a free state or esterified as part of acylglycerol molecules. **Figure 7.1** shows how increasing amounts of glycerin to acid oil (from physical refining; 89% FFA) did not enhance the total FA apparent absorption ($P > 0.05$), whereas the addition of increasing amounts of MAG and DAG (re-esterified oil; 45% DAG and 41% MAG) to acid oil did enhance the total FA apparent absorption ($P < 0.001$), primarily due to the increased absorption of SFA ($P < 0.001$).

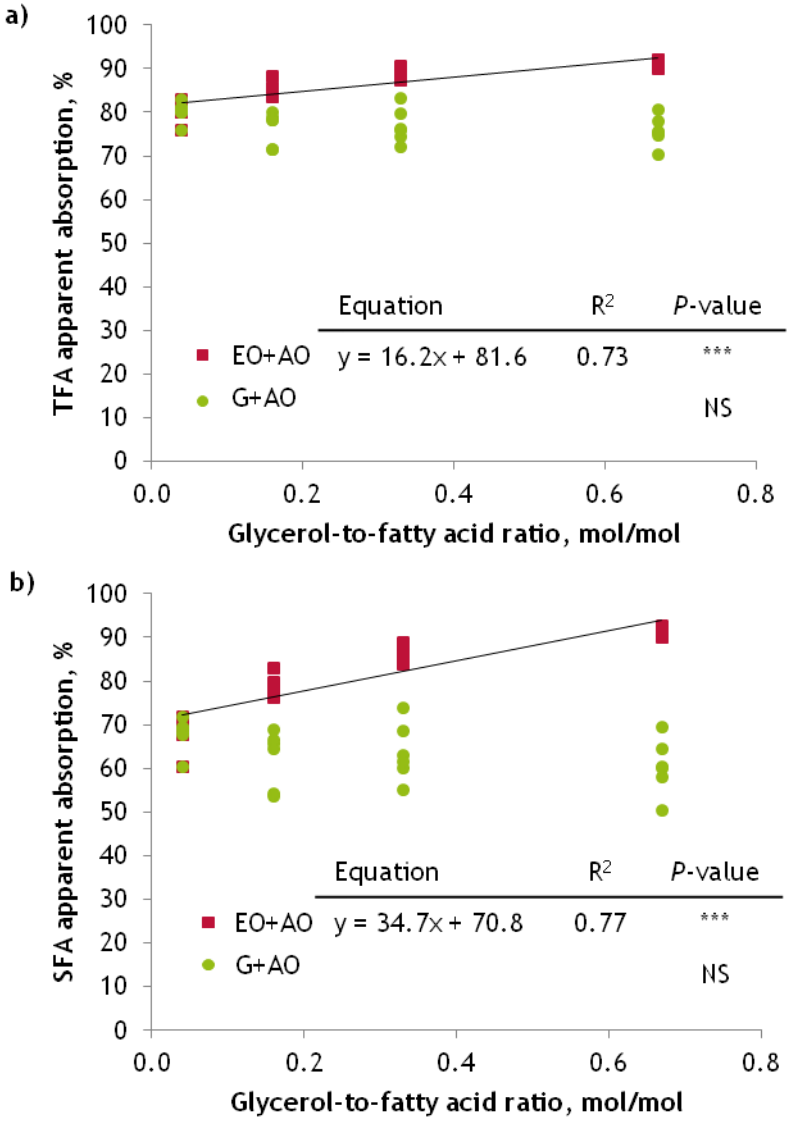


Figure 7.1. Apparent absorption of (a total fatty acids (TFA) and (b saturated fatty acids (SFA), according to increasing glycerol-to-fatty acid ratios achieved by blends of re-esterified oil with acid oil (EO + AO), or glycerol with acid oil (G + AO). **** P < 0.001.*

The results of this experiment refute the hypothesis that 1,3-DAG and 1(3)-MAG are completely hydrolyzed to glycerol and FFA during the digestion process. These findings are in agreement with the results observed by Mattson and Volpenhein (1964), who suggested that 1(3)-MAG escape from the hydrolytic activity of pancreatic lipase due to their rapid absorption. As a result, we can conclude that MAG and DAG molecules from re-esterified palm oils exert beneficial effects on the process of micelle formation, favoring, therefore, the absorption of fat digestion products.

7.1.1.2. Fatty acid positional distribution within acylglycerol molecules

Despite the different FA positional distribution observed among experimental fat sources, described in detail in *Chapters 3, 4, 5 and 6*, re-esterified oils did not show a random rearrangement of FA within acylglycerol molecules, as was initially expected. Several authors (Filer et al., 1969; Renaud et al., 1995; Innis and Dyer, 1997; Innis et al., 1997; Yli-Jokipii et al., 2001; Scheeder et al., 2003; Smink et al., 2008) have reported that randomization of native oils leads to an equal distribution of FA among the three positions of the glycerol backbone; i.e. one-third of each FA is on the *sn*-1 position, one-third on the *sn*-2 position, and one third on the *sn*-3 position of the TAG. However, the maximum percentage of SFA at the *sn*-2 position achieved in our re-esterified oils was 20.5% (PEL oil of *Chapter 5*). If the chemical esterification process had achieved re-esterified oils with a higher percentage of *sn*-2 SFA, we might have observed an even greater improvement in the SFA apparent absorption than the one obtained with our re-esterified oils. Under pancreatic lipase action, SFA in *sn*-2 position remain bound to the glycerol molecule forming a MAG of SFA, which is more easily incorporated into micelles and absorbed, than a free SFA. A reason explaining the lower percentage of *sn*-2 SFA of our re-esterified oils, when compared with those in the literature, could be their high proportion of 1,3-DAG and 1(3)-MAG and, therefore, a low amount of FA located at the *sn*-2 position. For this reason, in another experiment we tried to produce a re-esterified oil with a high TAG content (86.2%) but, contrary to what was expected, the amount of SFA located at the *sn*-2 position (18.3%) did not increase (Vilarrasa et al., 2014b). A possible explanation to this could be that the FA positional distribution of the preformed acylglycerol molecules (TAG, DAG, and MAG) remaining in acid oils might be conserved during the esterification process, preventing a random distribution of FA. This suggests that, if re-esterified oils had been produced from acid oils coming from physical refining (>90% FFA), instead of chemical refining

(50-60% FFA), FA might have been rearranged in acylglycerol molecules more randomly.

7.1.2. Physical properties

Changes in both FA positional distribution and acylglycerol and FFA composition of fats also exert important effects on their physical properties. In fact, transesterification is a process widely adopted by the food industry as a novel alternative to partial hydrogenation of fats, because it increases the melting point of fats without leading to the generation of *trans* FA (Sanders et al., 2003; Destaillats et al., 2007). However, melting points above body temperature might hinder the emulsification and hydrolysis processes, and thereby decrease fat absorption (Bonnaire et al., 2008; Michalski et al., 2013). For this reason, we determined the melting behavior of the experimental fats by differential scanning calorimetry (Vilarrasa et al., 2014b). **Figure 7.2** shows how melting occurs over a wide temperature range, since fats are complex blends of acylglycerol and FFA molecules.

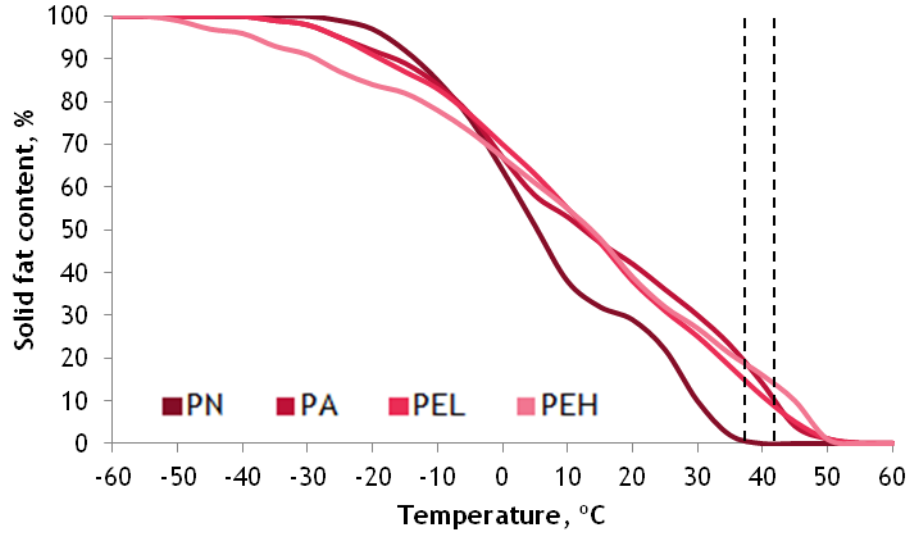


Figure 7.2. Melting profile of native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), and re-esterified palm oil high in mono- and diacylglycerols (PEH). The vertical, dashed lines show the solid fat content at the body temperature of pigs (38.5ºC) and broiler chickens (41.5ºC).

It can be observed that PA, PEL, and PEH oils started to melt earlier and finished melting later than PN oil, expanding their melting range (PN: -25 to 35ºC; PA: -35 to

50ºC; PEL: -35 to 50ºC; PEH: -50 to 50ºC). Nevertheless, the most important physical property of fats that will determine their nutritive value is their solid fat content at the animal's body temperature. While PN oil was totally or almost completely liquid at the body temperature of chickens (41.5ºC) and pigs (38.5ºC), PA, PEL and especially PEH oil still had an important proportion of solid fat content at these temperatures. However, re-esterified palm oils did not exert any negative effect on total FA apparent absorption in pigs and broiler chickens, despite their remarkably higher solid fat content at the animal's body temperature, in agreement with the results of Scheeder et al. (2003). Indeed, the beneficial effects of *sn*-2 SFA, and MAG and DAG molecules in re-esterified palm oils may have exceeded the detrimental effect of their higher solid fat content.

7.1.3. Occurrence of lipid hydrolysis during feed storage

If the basal diet has a low fat content, dietary fat should be a reflection of the fat source added to the diet, in terms of FA composition and molecular structure. However, a finding of this project has been the occurrence of lipid hydrolysis during feed storage, which could have important economical implications for feed and meat producers. The extent to which lipid hydrolysis might occur in feed might depend on the feed processing conditions, storage conditions and on the composition of other feed ingredients. Therefore, a study was carried out to follow the changes of feed lipid fractions during the storage of different feed types (Tres et al., 2013). Commercial feed samples (n = 86) were collected directly from seven feed producers. They included samples used for eight different animal species, and feeds produced according to different manufacturing processes (mash, pelleted, and extruded feeds). Feed were collected soon after they had been produced, and stored for different time periods (30, 60, and 120 days) at room temperature. Lipid hydrolysis in pelleted and mash-feed samples increased during storage time, while it remained less affected in extruded feeds. The manufacturing conditions applied during extrusion (high temperature) might have led to an inactivation of endogenous lipases coming from feed ingredients, reducing lipid hydrolysis during storage. Furthermore, differences were observed between feeds formulated for different animal species, which could indicate an influence of certain feed ingredients on lipid hydrolysis. A good control of feed manufacturing conditions would be useful in preventing lipid hydrolysis and thus in

maintaining the feed energetic value. The determination of the acylglycerol and FFA composition of feeds is not a routine analysis. Thus, while feed manufacturers may be adding high quality fat sources to feeds, degradation during storage can result in animals consuming hydrolyzed fat sources with a lower energy value, such as acid oils, leading to economic losses. These are only preliminary results, so more feed samples need to be analyzed to understand how to prevent the hydrolysis of acylglycerol molecules during feed storage.

7.2. Differences between species and physiological states

In this thesis, the comparison between species and physiological states can only be done qualitatively, because the experiments did not use the same experimental procedures (differences related to animals, percentages of fat inclusion in diets, sample collection methods, chemical analyses, environmental conditions, etc.).

7.2.1. Fat absorption

Figure 7.3 shows the total FA apparent absorption coefficients obtained between species (broiler chickens vs. pigs) and physiological states (young vs. older) in *Chapters 3, 4, and 5*. We have only selected the common treatments for both species.

Two ages for both pigs and broilers chickens were employed, because the age of the animals has a marked influence on the utilization of dietary fat. The physiological ability to absorb fat is poorly developed in very young chicks and piglets in the post-weaning period, but a marked improvement has been reported with age (Wiseman and Salvador, 1991; Powles et al., 1993, 1994). In agreement to this, a time effect was found for all absorption coefficients, reaching higher values during the second experimental period (older animals). Moreover, an interaction between physiological states and dietary fat sources was observed, since the behavior of the four different palm oil treatments was different depending on the species and the age of the animals.

According to what was expected, young animals fed SN achieved the best results in terms of total FA apparent absorption. However, PA oils, despite their high FFA content, only exerted negative effects in young broiler chickens, possibly because young broiler chickens have a more limited secretion of bile salts than young pigs.

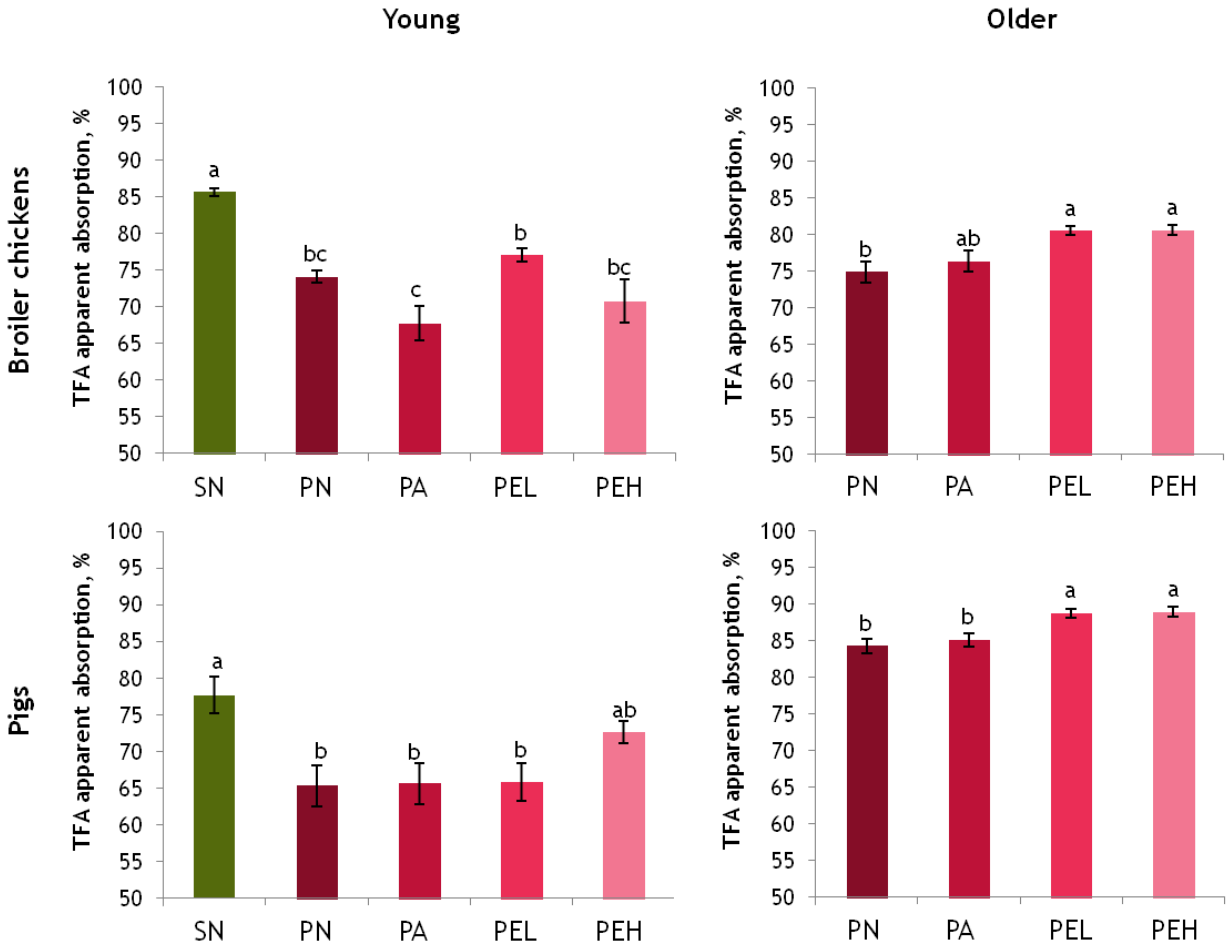


Figure7.3. Total fatty acid (TFA) apparent absorption coefficients obtained between species (broiler chickens vs. pigs) and physiological states of the animals (young vs. older), according to different dietary fat sources: native soybean oil (SN), native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), and re-esterified palm oil high in mono- and diacylglycerols (PEH).

Regarding re-esterified oils, whereas in young broiler chickens the treatment achieving the highest total FA apparent absorption results was PEL, in young pigs it was PEH, although no statistically significant differences were found between both re-esterified palm oils. This discrepancy may be attributed to differences in the activity of pancreatic lipase and micellar solubilization between the two species.

In older animals, the magnitude of the differences among treatments was not as pronounced as in young animals. PN and PA treatments achieved similar apparent absorption coefficients, whereas re-esterified palm oils (PEL and PEH) exerted favorable effects on the total FA apparent absorption, due to the increased SFA apparent absorption.

Taken together, it can be concluded that young animals are more sensitive to dietary fat sources and show a greater variability of response (within the same dietary treatment) than do older animals. This can be expected, considering that young animals have more unfavorable conditions to absorb dietary fat (insufficient secretion of bile salts and/or lipases) than have adult animals. On the other hand, it has been observed that re-esterified oils are interesting alternative fat sources to be considered for both young and older animals.

It is important to know the limitations in fat absorption, and how it is affected by dietary fat sources in order to select the best nutritional strategies throughout the rearing period. Therefore, in this thesis, we have conducted the analysis of the acylglycerol and FFA composition of feces, in order to determine the limiting factor of fat absorption in young and older pigs and broiler chickens. **Figure 7.4** shows the acylglycerol and FFA composition of feces obtained between species (broiler chickens vs. pigs) and physiological states of the animals (young vs. older) in *Chapters 3, 4, and 5*. As it has already been discussed in the corresponding chapters, FFA were the major lipid fraction in feces, for both species and for both periods, suggesting that the main limiting factor of fat absorption is the emulsifying effect of bile salts, rather than the hydrolytic activity of pancreatic lipase. Moreover, the amount of FFA excreted in feces paralleled, in most cases, the SFA apparent absorption coefficients, and this was the only lipid fraction that decreased with age. This suggests that the amount of TAG, DAG, and MAG excreted in feces may correspond to constant endogenous fat losses. Thus, we can suggest that the low fat absorption in young animals is not due to an insufficient secretion of pancreatic lipase, as other authors have suggested in weaned piglets (Lindemann et al., 1986; Cera et al., 1990). Another important aspect is that, in both periods, pigs excreted a greater proportion of DAG and a lower proportion of TAG than did broiler chickens, which suggests some differences between species.

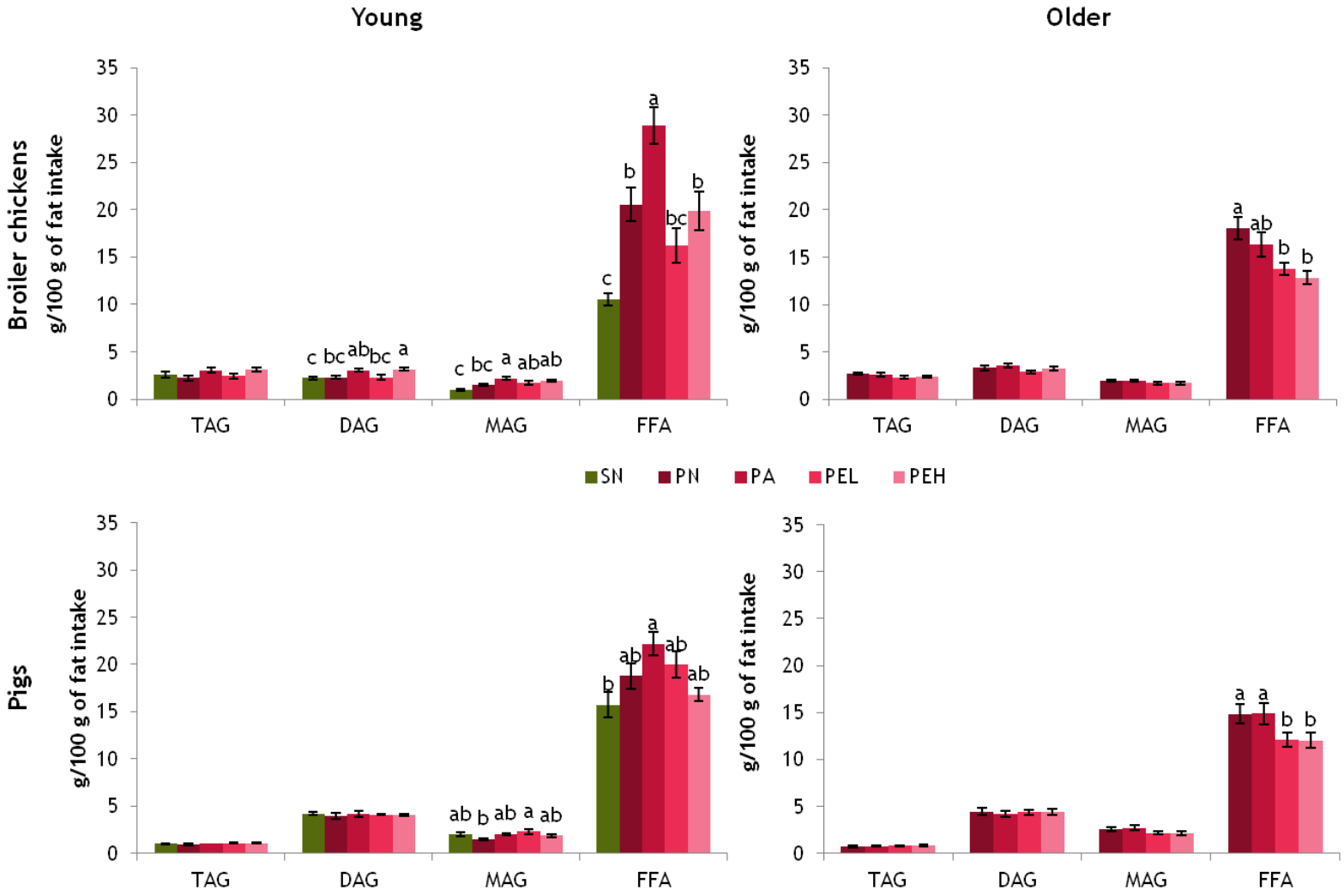


Figure 7.4. Acylglycerol and free fatty acid composition of excreta and feces (g/100 g of fat intake) obtained between species (broiler chickens vs. pigs) and physiological states of the animals (young vs. older), according to different dietary fat sources: native soybean oil (SN), native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), and re-esterified palm oil high in mono- and diacylglycerols (PEH). *DAG = diacylglycerols; FFA = free fatty acids; MAG = monoacylglycerols; TAG = triacylglycerols.*

7.2.2. Fat deposition

In monogastric animals it is well established that the FA composition of feed directly affects the FA composition of fat depots. This is why dietary fat sources in the finisher phase of the rearing period are selected with regard to product quality of the carcass fat. **Figure 7.5** shows the unsaturated-to-saturated FA ratio (**UFA:SFA**) of fat depots of broiler chickens and pigs fed different palm oil sources.

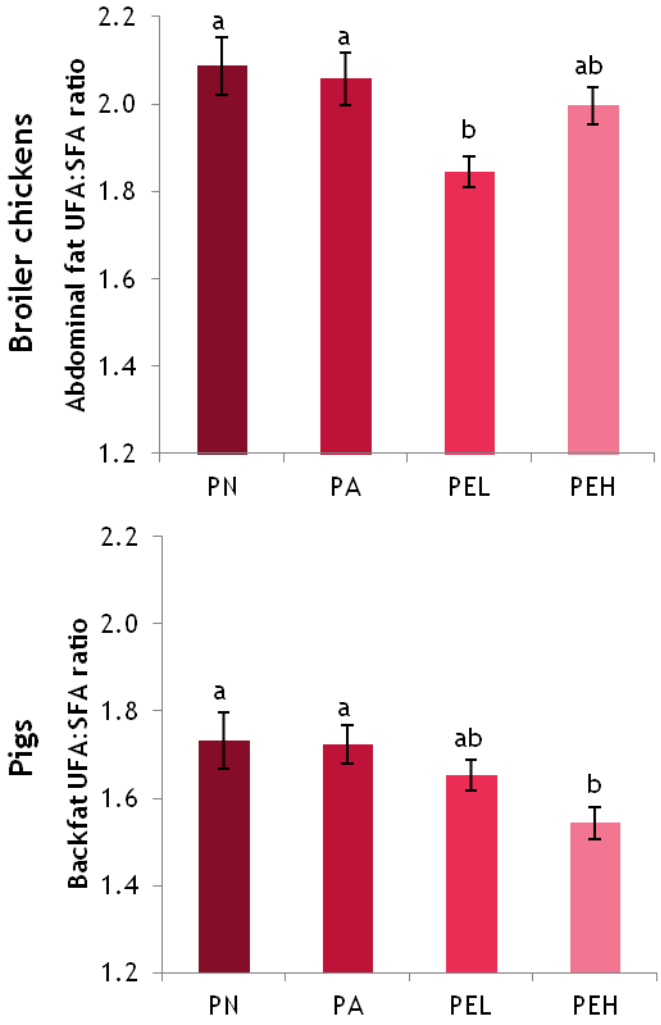


Figure 7.5. Unsaturated-to-saturated fatty acid ratio (UFA:SFA) of fat depots of broiler chickens (abdominal adipose tissue) and pigs (backfat) fed different palm oil sources: native palm oil (PN), acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), and re-esterified palm oil high in mono- and diacylglycerols (PEH).

The reason why we selected the abdominal fat pad in broiler chickens and the backfat in pigs is because they are the most representative fat depots, usually selected

to evaluate the FA composition and the degree of fat deposition, for each species. The comparison between species shows that the abdominal fat of broiler chickens contained a very similar proportion of PUFA, but less SFA and more MUFA than the pig backfat, resulting in an overall higher UFA:SFA for broiler chickens than for pigs, as also reported Scheeder et al. (2003). These differences may be related to differences between species, fat depots, percentages of fat inclusion in diets, and levels of *de novo* FA synthesis

Furthermore, despite the similar FA composition of the four dietary palm oil sources, significant differences were observed in fat depots for both species. Broiler chickens fed PEL showed a lower UFA:SFA ratio in the abdominal fat pad than those fed PN and PA. In contrast, in pigs, the treatment that achieved the lowest UFA:SFA ratio was the PEH. We cannot give an explanation to this phenomenon, given that from the SFA apparent absorption results of older animals, we would have expected that both animals fed PEL and PEH had deposited more SFA than those fed PN and PA.

An increased proportion of SFA has a positive effect upon organoleptic characteristics of pig and poultry meat, but this has also been identified as a negative feature with respect to human health. However, until recently, little attention has been given to the position of FA in TAG molecules. It is unknown if FA located at the *sn*-2 position in the glycerol molecule before the digestion process, remains unchanged in this *sn*-2 position through the absorption, transport and tissue storage processes. It is important to ensure that meat products from animals consuming re-esterified oils compared to native ones, do not present higher SFA in the *sn*-2 TAG position, which could produce a higher availability of SFA for the consumer and, subsequently, negative repercussion in human health.

7.3. Economic viability

Because of the positive results reported above, in terms of total FA apparent absorption coefficients, it seems that re-esterified oils could be interesting alternative fat sources to be considered in feed formulation. However, from a practical point of view, besides the energetic value, the availability and the price are what determine the end use of a particular raw material. Therefore, we calculated the price of our experimental fat sources in €/1,000 kcal of apparent metabolizable energy (**AME**; poultry) or digestible energy (**DE**; pigs). Only for this purpose, we estimated the AME (poultry) and DE (pigs) of our experimental fat sources by multiplying the coefficients

of fat apparent absorption by their corresponding gross energy values (Wiseman et al., 1998).

Concerning the price of fat sources, we took, as a reference, the price of PN oil on 2013/14 (612 €/t; USDA, 2014). Because no official data are available for PA oil prices, we considered a price range (from 612 to 112 €/t), depending on the price differential between PN and PA oils (from 0 to 500 €/t), which oscillates greatly throughout the year and among years, according to the law of supply and demand. This is the key point that will decide whether PA oil and, subsequently, P-EL and PEH oils (their price is approximately 100 €/t more expensive than their corresponding acid oil, due to production costs) are profitable.

Figure 7.6 shows how the price (in €/1,000 kcal of AME or DE) of PA, PEL, and PEH oils varies depending on the price differential between PN and PA oils in young and older broiler chickens and pigs. The vertical dashed lines set the price differential between PN and PA oils after which, the use of these alternative fat sources becomes economically advantageous to PN oil. Acid palm oil (< 60% of FFA and < 3% of moisture, impurities, and unsaponifiable matter) was, in all situations (except in young broiler chickens), the most economical fat source. Re-esterified palm oils, on the other hand, were also good alternative fat sources to PN oil (from a price differential of about 100 €/t between PN and PA oils, except for PEH oil in young broiler chickens), but they were always more expensive than their corresponding PA oil. Re-esterified palm oils, despite showing, in most cases, higher total FA apparent absorption coefficients than PA oils, they were always more expensive (due to the additional cost of the esterification process) and had a lower gross energy content (due to their increased glycerol-to-FA ratio) than PA oils. In case we had used acid oils with a higher FFA content, and therefore, with a supposed lower nutritive value, then re-esterified oils had probably surpass PA oils, from an economic standpoint. Thus, it can be concluded that the additional cost of re-esterified palm oils is not always justified by the benefit obtained in their utilization.

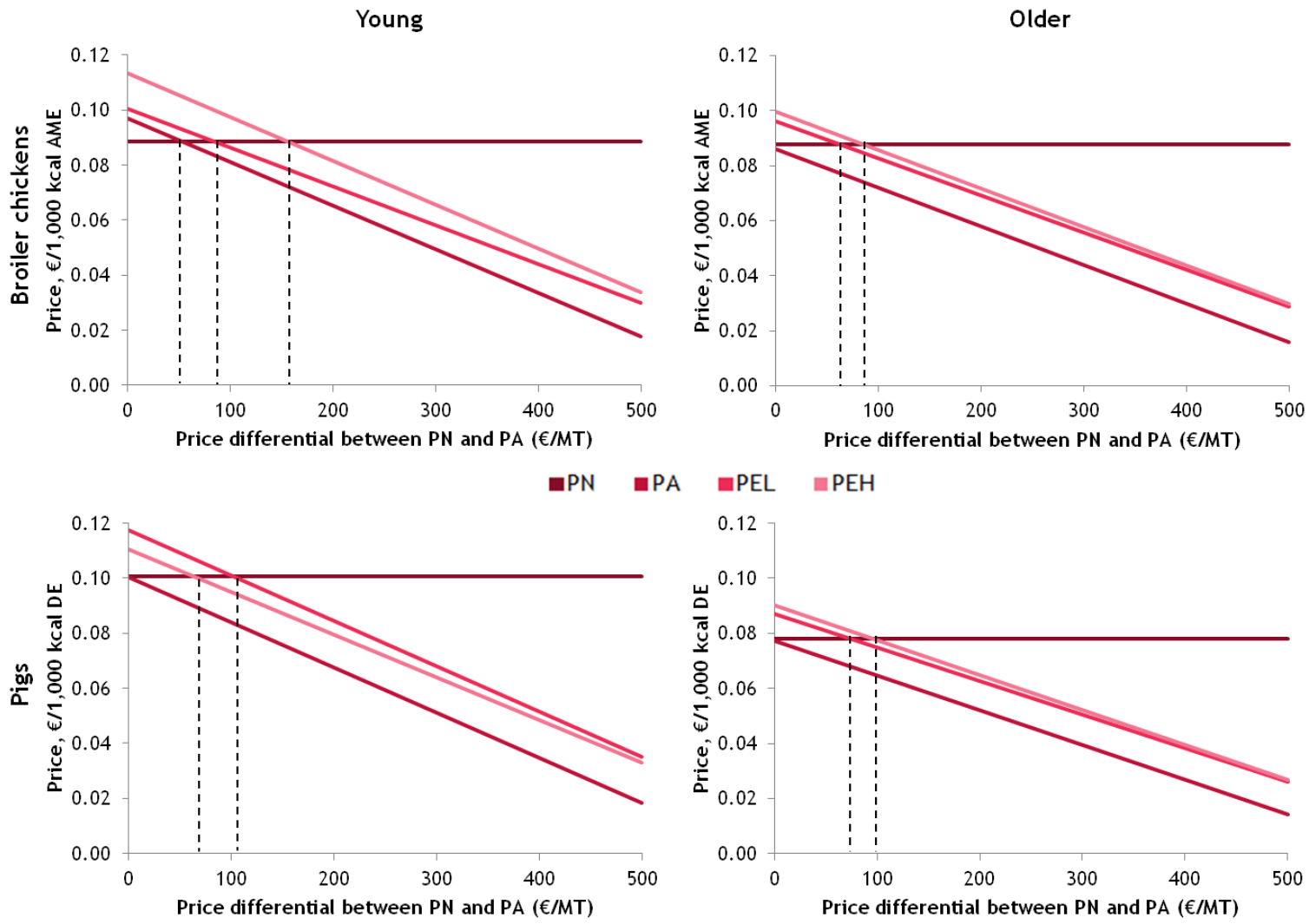


Figure 7.6. Price (in €/1,000 kcal of AME or DE) of acid palm oil (PA), re-esterified palm oil low in mono- and diacylglycerols (PEL), and re-esterified palm oil high in mono- and diacylglycerols (PEH) depending on the price differential between native palm oil (PN) and PA in young and older broiler chickens and pigs. The vertical, dashed lines set the price differential between PN and PA oils after which, the use of these alternative fat sources becomes economically advantageous to PN oil.

7.4. Future perspectives

As a conclusion of this project, acid oils seems to be an interesting subject for study, since, in contrast to what was expected, their use has led to positive results (except in young broiler chickens), comparable to those obtained with native oils. Therefore, their cost per unit of energy (€/1,000 kcal AME or DE) has proven to be substantially lower than that of their corresponding native oil. Moreover, these by-products constitute a residual product to be recycled in order to avoid environmental contamination. However, it must be taken into account that the acid oils used in this thesis have proved to be of good quality (moderate amount of FFA and low moisture, impurities and unsaponifiable matter content). Nevertheless, the origin and the quality of these by-products in the market place can be highly variable.

There have been numerous programs designed to estimate the dietary energy value of fats and it has been appreciated for some considerable time that the two chemical variables of most importance are the degree of saturation of FA and the FFA content. Wiseman's equations (1998), which have been worked out to calculate the AME or the DE values of lipids in pigs and broiler chickens at different ages, are currently being used as a tool in feed formulation programs. **Table 7.1** shows the relationship between the calculated and the predicted energy values of our PN and PA oils for young and older broiler chickens and pigs. The differences between the calculated and the predicted energy values show important discrepancies for both PN and PA fat sources. It is true that some of our experimental procedures do not exactly correspond with those used by Wiseman (1998), but considering the genetic improvement in pigs and broiler chickens during the last 20 years, it is possible that the prediction equations of Wiseman et al. (1998) need to be updated. Moreover, these equations do not consider other aspects such as the chain length of SFA, the FA positional distribution of SFA, and the presence of MAG and DAG molecules, which, as has been observed through this thesis, also exert important effects on the nutritive value of fat sources.

Finally, given that the main limiting factor of fat absorption has been found to be the emulsifying effect of bile salts, rather than the hydrolytic activity of pancreatic lipase, it opens new opportunities to study the application of some emulsifiers such as bile salts, lecithins, purified MAG, etc. which could enhance micelle formation and, therefore, FA apparent absorption, mainly at early ages.

Table 7.1. Comparison between calculated and predicted apparent metabolizable energy (broiler chickens) or digestible energy (pigs) values

Species	Age	Oil	Calculated AME or DE[1] (kcal/kg of fat)	Predicted AME or DE[2] (kcal/kg of fat)	Calculated-Predicted (kcal/kg of fat)
Broiler chickens	Young	PN	6,895	6,814	81
		PA	6,347	5,444	903
	Older	PN	6,969	7,863	-894
		PA	7,152	6,934	218
Pigs	Young	PN	6,076	7,694	-1618
		PA	6,141	6,902	-761
	Older	PN	7,890	7,987	-97
		PA	7,810	7,316	494

AME = apparent metabolizable energy; DE = digestible energy; PA = acid palm oil; PN = native palm oil.

[1] AME (broiler chickens) or DE (pigs) = (apparent fat absorption × fat gross energy)/100

[2] From Wiseman et al. (1998) prediction equations and corrected by a coefficient corresponding to MIU content:

AME broiler chickens 1.5 weeks of age = $239 \times [38.112 - 0.009 \times FFA - 15.337 \times e^{(-0.506 \times UFA:SFA)}] \times MIU$

AME broiler chickens 7.5 weeks of age = $239 \times [39.025 - 0.006 \times FFA - 8.505 \times e^{(-0.403 \times UFA:SFA)}] \times MIU$

DE pigs 15 kg live weight = $239 \times [37.890 - 0.005 \times FFA - 8.200 \times e^{(-0.515 \times UFA:SFA)}] \times MIU$

DE pigs 30-85 kg live weight = $239 \times [36.898 - 0.005 \times FFA - 7.330 \times e^{(-0.906 \times UFA:SFA)}] \times MIU$

CHAPTER 8

Conclusions

From the results presented in this dissertation, the following conclusions can be drawn:

1) Re-esterified palm and soybean oils (obtained by reacting acid oils with glycerin), when compared with their corresponding acid and native oils show:
    a. A very similar fatty acid composition.
    b. A greater proportion of saturated fatty acids located at the acylglycerol *sn*-2 position. However, the esterification process does not achieve a completely random distribution of fatty acids within acylglycerol molecules.
    c. A greater amount of mono- and diacylglycerols (with fatty acids mainly esterified at the *sn*-1,3 positions) and, therefore, a lower amount of triacylglycerols.
    d. An increased glycerol-to-fatty acid ratio, which results in a lower gross-energy content.

2) Re-esterified palm oils exert favorable effects on the apparent absorption of saturated fatty acids, resulting in a similar or even greater total fatty acid apparent absorption than that of their corresponding native oils in pigs and broiler chickens.

3) In general, the fat degree of saturation has a greater impact on fatty acid apparent absorption than does the fat molecular structure in broiler chickens.

4) The addition of re-esterified soybean oil in replacement of re-esterified palm oil in broiler chicken diets improves fat absorption and increases the unsaturated-to-saturated fatty acid ratio of abdominal adipose tissue.

5) The different molecular structure of re-esterified oils causes minor differences in the fatty acid composition of fat depots of pigs and broiler chickens, when compared with their corresponding native and acid oils.

6) From an economic standpoint, the additional cost of re-esterified palm oils is not always justified by the benefit obtained in their utilization.

7) Acid palm oils (< 60% of free fatty acids and < 3% of moisture, impurities, and unsaponifiable matter) are economically interesting alternative fat sources for pig and broiler-chicken diets, showing similar fatty acid apparent absorption coefficients as their corresponding native oils, except in young broiler chickens.

8) In both pigs and broiler chickens, and regardless of age, fat present in feces or excreta is mainly composed of free fatty acids, which suggests that micelle formation, and not fat hydrolysis, is the rate-limiting step of fat absorption.

CHAPTER 9

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CHAPTER 10

Curriculum vitae of the author

Personal information

Surname, Name: Vilarrasa Gustems, Ester Nationality: Spanish
E-mail: ester.vilarrasa@gmail.com Date of birth: 12/02/1987

Education

2011-present **Ph.D. Student in Animal Science**
Universitat Autònoma de Barcelona

2010-2011 **M.Sc. in Research in Animal and Food Science**
Universitat Autònoma de Barcelona

2005-2010 **B.Sc. in Veterinary Medicine**
Universitat Autònoma de Barcelona

Post-graduate courses

2014 **Writing and Presenting Scientific Papers**
EAAP/CUP Workshop – Cambridge University

2013 **Statistics One** - An introduction to R programming language
MOOC on Coursera - University of Princeton

2013 **Biological Agents: Risk and Preventive Measures**
Universitat Autònoma de Barcelona

2012 **Training Course for Researchers on Laboratory Animal Management**
Universitat Autònoma de Barcelona

Professional experience

2010-present **Member of the Animal Nutrition and Welfare Service** (SNiBA)
Universitat Autònoma de Barcelona (Bellaterra)

- Collaboration in several research projects (experimental design, farm controls, laboratory analyses, and statistical analyses)
- Collaboration in practical sessions of the subject Animal Production and Management (Veterinary Degree)

- Write technical summaries (monthly) related to:
  - Poultry production for AECA webside (AECA mail) http://www.wpsa-aeca.es/
  - Pig nutrition for 3tres3 webside (Nutrimail) http://www.3tres3.com/

2014 **Technical department assistant** (3 months)
Tecnología & Vitaminas (Alforja)

2011-2013 **Research trainee at the Monogastric Animal Nutrition Unit**
Institut de Recerca i Tecnologia Agroalimentàries (IRTA) – Centre Mas de Bover (Reus)

2009-2010 **Veterinarian in practice**
Albet, S.A. Clínica Veterinària (Vic)

2008 **Veterinary assistant** (2 months)
Vetxarxa, Centre Veterinari Manlleu (Manlleu)

2007 **Farmer and veterinary assistant** (2 months)
Granja Murucuc, Associació Frisona d'Osona (Vic)

Fellowships

2012-present **Pre-doctoral research grant (FI-DGR 2012)**
Agència de Gestió d'Ajuts Universitaris i de Recerca. Generalitat de Catalunya

2014 **Scholarship to attend to the 65th Annual Meeting of the EAAP**
European Federation of Animal Science

2013 **Scholarship to attend to an international conference**
Spanish branch of the World's Poultry Science Association

2012 **Student Program of the World's Poultry Congress 2012**
Brazilian branch of the World's Poultry Science Association

2011-2012 **Research assistant grant**
Universitat Autònoma de Barcelona

Scientific publications

E. Vilarrasa, R. Codony, E. Esteve-Garcia, and A.C. Barroeta. Use of re-esterified oils, differing in their degree of saturation and molecular structure, in broiler chicken diets. *Poultry Science* (in revision).

E. Vilarrasa, F. Guardiola, R. Codony, E. Esteve-Garcia, and A.C. Barroeta. Combination of re-esterified oils, differing in their degree of saturation, in broiler chicken diets. *Poultry Science* (submitted).

E. Vilarrasa, A.C. Barroeta, A. Tres, and E. Esteve-Garcia. Use of re-esterified palm oils, differing in their acylglycerol structure, in weaning-piglet diets. *Animal* (submitted).

E. Vilarrasa, A.C. Barroeta, A. Tres, and E. Esteve-Garcia. Use of re-esterified palm oils, differing in their acylglycerol structure, in fattening-pig diets. *Animal* (submitted).

E. Vilarrasa, A. Tres, L. Bayés-García, T. Parella, E. Esteve-Garcia, and A.C. Barroeta (2014). Re-esterified palm oils, compared to native palm oil, do not alter fat absorption, postprandial lipemia or growth performance in broiler chickens. *Lipids*, 49: 795-805.

V Fragua, **E Vilarrasa**, EG Manzanilla, C Villaverde, and AC Barroeta (2013). Comparison of postprandial lipaemia between native and palm random esterified acid oils in two different monogastric species (dogs and broiler chickens). *Journal of Animal Physiology and Animal Nutrition*, **97**: 74-79.

Divulgative publications

E. Vilarrasa and A.C. Barroeta (2014). Factores que afectan a los procesos de digestión y absorción de las grasas. *AviNews*, **9**: 101-107.

A.C. Barroeta and **E Vilarrasa** (2011). Millora nutricional dels ous i la carn de pollastre mitjançant l'alimentació. *Tecnologia i Ciència dels Aliments*, **13**: 28-35.

Conference proceedings

E. Vilarrasa, A.P. Roll, and A.C. Barroeta (2014). Uso de aceites de palma com distinta estructura molecular y distinta proporción glicerol:ácidos grasos en la alimentación de pollos de carne. *LI Symposium Científico de Avicultura*. Valencia, Spain. Type of presentation: Oral

E. Vilarrasa, A.C. Barroeta, and E. Esteve-Garcia (2014). Use of esterified palm acid oils with different acylglycerol structure in fattening pig diets. *65th Annual Meeting of the European Federation of Animal Science - EAAP 2014*. Copenhagen, Denmark. Type of presentation: Oral

E. Vilarrasa, A.C. Barroeta, and E. Esteve-Garcia (2014). Use of esterified palm acid oils with different acylglycerol structure in weaning piglet diets. *65th Annual Meeting of the European Federation of Animal Science - EAAP 2014*. Copenhagen, Denmark. Type of presentation: Poster

A.P. Roll, **E. Vilarrasa**, and A.C. Barroeta (2014). Proporções de mono- e digliceridos e ácidos graxos livres de óleo de palma na dieta de frangos de corte. *XIII Seminário Técnico Científico de Aves e Suínos - AveSui 2014*. Florianópolis, Brazil. Type of presentation: Poster

A.P. Roll, **E. Vilarrasa**, and A.C. Barroeta (2014). Combinação de glicerol com ácidos graxos livres de óleo de palma na dieta de frangos de corte. *XIII Seminário Técnico Científico de Aves e Suínos - AveSui 2014*. Florianópolis, Brazil. Type of presentation: Poster

A. Tres, R. Codony, **E. Vilarrasa**, R. Buonfiglio, J. Zoldan, N. Magrinya, T. Gallina, R. Bou, and F. Guardiola (2013). Occurrence of lipid hydrolysis during feed storage. *11th Euro Fed Lipid Congress*. Antalya, Turkey. Type of presentation: Poster

E. Vilarrasa and A.C. Barroeta (2013). Interacciones entre aceites ácidos esterificados saturados e insaturados en la alimentación de pollos de carne. 1. Rendimientos productivos, digestibilidad y depósito graso. *L Simposio Científico de Avicultura*. Lleida, Spain. Type of presentation: Poster

E. Vilarrasa and A.C. Barroeta (2013). Interacciones entre aceites ácidos esterificados saturados e insaturados en la alimentación de pollos de carne. 1. Rendimientos productivos, digestibilidad y depósito graso. *L Simposio Científico de Avicultura*. Lleida, Spain. Type of presentation: Poster

E. Vilarrasa, L. Bayés-García, and A.C. Barroeta (2013). Utilización de aceites ácidos esterificados de palma con distintas características físico-químicas en la ración de pollos de carne. *L Simposio Científico de Avicultura*. Lleida, Spain. Type of presentation: Oral

A. Tres, R. Codony, **E. Vilarrasa**, R. Buonfiglio, J. Zoldan, N. Magrinya, T. Gallina, R. Bou, and F. Guardiola (2013). Occurrence of lipid hydrolysis during feed storage. *11th Euro Fed Lipid Congress*. Antalya, Turkey. Type of presentation: Oral

E. Vilarrasa, C. Trullàs, V. Fragua, and A.C. Barroeta (2013). Use of palm esterified acid oils in monogastric animal nutrition. *17th European Society of Veterinary and Comparative Nutrition Congress*. Ghent, Belgium. Type of presentation: Poster

E. Vilarrasa, L. Bayés-García, and A.C. Barroeta (2013). Use of dietary palm random esterified acid oils with different physicochemical properties in broiler chicks. *19th European Symposium on Poultry Nutrition*. Potsdam, Germany. Type of presentation: Poster

A. Tres, R. Codony, **E. Vilarrasa**, R. Buonfiglio, J. Zoldan, N. Magrinyà, R. Bou, and F. Guardiola (2013). Lipid hydrolysis during processing and storage of commercial feed. *Feed for Health Final Conference "Healthy Food from Healthy Animals"*. Milán, Italy. Type of presentation: Oral

E. Vilarrasa, L. Bayés-García, M.T. Calvet, and A.C. Barroeta (2012). Factores que intervienen en la digestión de distintos tipos de aceites ácidos esterificados de palma en la ración de pollos de carne. *XLIX Symposium Científico de Avicultura*. Bellaterra, Spain. Type of presentation: Poster

E. Vilarrasa, V. Fragua, and A.C. Barroeta (2012). Effects of the use of esterified acid oils with different saturation degree and different monoglyceride content in broiler chicken diets. *XXIV World's Poultry Congress*. Salvador de Bahía, Brazil. Type of presentation: Poster

E. Vilarrasa, V. Fragua, and A.C. Barroeta (2012). Influence of dietary fat saturation degree on postprandial lipaemia in broiler chickens. *XXIV World's Poultry Congress*. Salvador de Bahía, Brazil. Type of presentation: Poster

E. Vilarrasa, V. Fragua, and A.C. Barroeta (2011). Efectos del uso de aceites ácidos esterificados con diferente contenido en monoglicéridos en la ración del pollo de carne. *XLVIII Symposium Científico de Avicultura*. Santiago de Compostela, Spain. Type of presentation: Poster

V. Fragua, **E. Vilarrasa**, C. Villaverde, E.G. Manzanilla, and A.C. Barroeta (2011). Comparision of postprandial lipaemia between native and randomized palm oils in two different monogastric species (dogs and broiler chickens). *15th Meeting of the European Society of Veterinary and Comparative Nutrition*. Zaragoza, Spain. Type of presentation: Poster

Awards

2011	**Extraordinary degree award in Veterinary Medicine** *Universitat Autònoma de Barcelona*
2006	***Gerbert Quadrivium* award for high school research projects** *Ajuntament de Ripoll*
2005	***Abraham Cresques* award for high school research projects** *Universitat de Vic*
2005	**High school degree award** *Centre Escorial de Vic*

Skills and competences

Languages		**Softwares**	
Catalan	● ● ● ● ●	Microsoft Office	● ● ● ● ●
Spanish	● ● ● ● ●	SAS	● ● ● ○ ○
English	● ● ● ● ○	R	● ● ○ ○ ○
		Allix	● ● ○ ○ ○