Universitat Autònoma de Barcelona

USE OF SOYBEAN LECITHIN IN BROILER CHICKEN DIETS

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TESIS DOCTORAL PRESENTADA POR:

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BAJO LA DIRECCIÓN DE LOS DOCTORES:

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PARA ACCEDER AL GRADO DE DOCTOR DENTRO DEL PROGRAMA DE DOCTORADO DE PRODUCCIÓN ANIMAL DEL DEPARTAMENTO DE CIENCIA ANIMAL Y DE LOS ALIMENTOS

Bellaterra, 2019

FACULTAT DE VETERINÀRIA

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Certifican:

Que la memoria titulada "**Use of soybean lecithin in broiler chicken diets**", presentada por Alberto Viñado Martínez con la finalidad de optar al Grado de Doctor en Veterinaria, ha sido realizada bajo su dirección y, considerándola finalizada, autorizan su presentación para que sea juzgada por la comisión correspondiente.

Y para que conste a efectos oportunos, firman la presente en Bellaterra a 6 de junio de 2019.

Dra. Ana Cristina Barroeta Lajusticia Dra. Lorena Castillejos Velázquez

“We learned more from a three-minute record

than we ever learned in school”

No Surrender, by Bruce Springsteen and the E Street Band, 1984.

Agradecimientos

"*Una tesis doctoral, es una carrera de fondo.*"

Justo enfilábamos los primeros meses del año 2016, cuando cierta persona me soltó esta frase. Esa sencilla oración voló por el despacho, donde hacía más bien poco que me había instalado, y se diluyó sin pena ni gloría. Curiosamente aquí estoy, acabando de escribir los agradecimientos y cerrar una memoria escrita de casi cuatro años de trabajo, que aún la recuerdo. La autora de dicho comentario fue **Ana Cris**, que además de haber dirigido junto con **Lorena** mis primeros pasos como investigador (con bastante acierto, todo sea dicho), ha sido mi compañera de "oficina" durante esta etapa de mi vida. Cuanta razón en tan pocas palabras. Aunque, como aficionado a correr populares y medias maratones, debo matizarla. Cuando corres y quieres mejorar tus marcas, dependes exclusivamente de ti. En cambio, una tesis no sólo desgasta física y mentalmente, sino que, afrontarla de manera individual supone un fracaso con toda seguridad.

Es por tanto que los "Agradecimientos" no son una parte baladí de esta tesis. Porque, aunque suene a típico tópico (y más en estas épocas de "postureo" llevado al extremo), no hubiera llegado a este punto sin ayuda.

A mis directoras, **Ana Cris** y **Lorena**, gracias por esa infinita paciencia, por haberme dado la oportunidad, y por todas las cosas que he aprendido de vosotras, tanto del método científico como de otros aspectos de la vida. A esa maravillosa compañera de despacho, **Raquel**, que no sólo le echo un cable al novato de manera desinteresada, sino que además se ha convertido en una persona a la que puedo considerar amiga. Y me tengo que considerar afortunado, porque no es la única. A **Héctor**, **Sergi**, **Lourdes**, **Montse** y **Bea**, gracias, no sólo para ayudar en la granja o tomarnos una cerveza en el bar, sino por estar ahí cuando necesitaba hablar y desahogarme. Por animarme en los momentos personales, no diré difíciles, pero si complicados.

A todas esas magníficas personas con las que he tenido el placer de compartir trabajo, despacho, cafés y cervezas: **David S.**, **Nedra**, **Ismael**, **Emili**, **Laia**, **Carmen**, **Paola**, **Meritxell**, **Anwar**, **Lluis**, **Manel**, **Nabil**, **Gerard**, **Elena**, **Francesc**, **Inma**, **Elisa** y **Mónica**. Y sería tremendamente injusto olvidarme de todas aquellas personas, que sin pertenecer al Departamento de Ciencia Animal y de los Alimentos, de alguna manera u otra, han participado durante esta etapa de mi vida: **Antón**, **Blasco**, **Álvaro**, **Calaf**, **Berta**, **Sara T.**, **Oriol**, **Anais**, **Etna** y **Sara M.**

Por último, a mi familia, que aun haciendo el esfuerzo de entender que hacía exactamente en la "universidad" y no atinar demasiado, me han animado y motivado para seguir dando guerra. A **Montse**, **Plácido**, **Andrea**, **Javier** y **Ester**.

"I ja no importen tant i semblen lluny tots els desastres que hagis fet,
I passa un enemic i feu les paus amb un brindis de cubates."

Teresa Rampell, per Manel, 2013.

Summary

Fat addition is a common practice in feed manufacturing in order to increase the energetic density of diets and provide essential nutrients to livestock animals. The availability of conventional energetic ingredients for broiler chicken diets may be compromised by a constant growing world-wide population and the current tendency to use vegetable oils for biodiesel production. In this context, soybean lecithin (**L**), as a co-product obtained during soybean oil (**S**) refining process, may represent an economical and alternative energy source due to its high content in phospholipids, triacylglycerols, free fatty acids (**FFA**), phosphorus, choline and antioxidant compounds. Therefore, the global aim of the present thesis was to investigate the potential use of L, as energy source for broiler chicken diets.

Several trials were performed with the aim to evaluate the inclusion of L as energy source in broiler feeding and study its influence on performance, energy utilization, fatty acid (**FA**) digestibility and the FA profile of the abdominal fat pad. A basal diet was supplemented at 3% with either S or acid oil (**A**) and increasing amounts of L (crude and high in FFA for Chapter Three and Four, respectively) were included in replacement (1%, 2% and 3%). In relation to S replacement, despite no effects were observed on performance parameters, results from the digestibility balances indicated that S replacement by L, in starter broiler chickens, lowered FA digestibility and the apparent metabolizable energy content of the diets. However, in grower-finisher broiler chickens, partial replacements up to a 2%, did not modify performance, and the utilization of energy and total FA. Regarding the replacement of A by L, in starter and grower-finisher broiler chicken diets, it was observed that blending both co-products have resulted in improvements on energy and nutrient utilizations. Finally, the FA profile of the adipose tissue was a clear reflect of the FA composition of the added fats, and S replacement by L produced slight changes on the FA profile of the abdominal fat pad.

The last experiment (Chapter Five) consisted in a field trial under experimental conditions with the main objective to study, in grower and finisher broiler chicken diets, different levels of L inclusion in replacement of S and its effects on growth performance. In addition, ileal absorption of FA, FA profile of the abdominal fat pad and gut health markers were assessed. Soybean oil total replacement by L (2% of inclusion), in diets that also contained palm and A (3.25% and 4.5% of total added fats for grower and finisher diets, respectively), did not modify performance parameters, total FA ileal digestibility

and jejunal morphology. On the other hand, a reduction on the digestibility of the polyunsaturated FA and an increase on *Lactobacillus* spp. counts at the jejunum were linked to total replacement; however, with no significant consequences on growth efficiency. Slight modifications were observed on the saturation degree of the abdominal fat pad, associated to the FA profile of the different added fats.

Taking all the results into account, it was evidenced that L is a suitable energetic ingredient for grower and finisher broiler chicken diets due to it was observed that S partial replacements up to a 2% by L do not alter growth performance and the utilization of energy and FA. Besides, the blending of L and A results an interesting option, for adult broiler diets, due the existence of positive interactions on energy and FA utilization.

Resumen

La adición de grasas es una práctica habitual en la fabricación de piensos para avicultura, ya que son fuente de energía y ácidos grasos esenciales. La disponibilidad de ingredientes con alto contenido energético se puede ver reducida a causa del aumento de población mundial y la utilización de aceites vegetales para la producción de biodiesel. Por ello, la lecitina de soja (**L**), un coproducto del refinado del aceite de soja (**S**), puede ser una fuente de energía alternativa, ya que presenta un alto contenido en fosfolípidos, triacilgliceroles, ácidos grasos libres (**AGL**), fósforo, colina y compuestos antioxidantes. Por todo ello, el objetivo de la tesis fue estudiar el uso potencial de L como fuente de energía para piensos de pollos de carne.

Para ello, se realizaron varios ensayos con el objetivo de estudiar el uso de L como fuente de energía en la alimentación de pollos y evaluar su influencia sobre el rendimiento productivo, digestibilidad de los ácidos grasos (**AG**), utilización de la energía y perfil de AG de la grasa abdominal. Una dieta base fue suplementada al 3% con S o aceite ácido (**A**), que fueron sustituidos por niveles crecientes (1%, 2% y 3%) de L (cruda o con alto contenido en AGL en el Capítulo Tres y Cuatro, respectivamente). En relación con la sustitución de S por L, aunque durante la fase de iniciación no se observaron efectos sobre el rendimiento productivo, los balances de digestibilidad demostraron que la incorporación de L disminuyó la digestibilidad de los AG y el contenido en energía metabolizable aparente del pienso. Sin embargo, en la fase de crecimiento-acabado, la sustitución parcial de L (hasta un 2%), no dio lugar a modificaciones en el rendimiento productivo ni la utilización de la energía y de AG. Respecto a la sustitución de A por L, tanto en fase de iniciación como en la de crecimiento-acabado, se observó que la combinación de ambos coproductos dio lugar a una mayor utilización de la energía y los nutrientes. Finalmente, el perfil de AG de la grasa abdominal estaba directamente relacionado con el perfil de AG del pienso, y no se observaron modificaciones importantes al sustituir S por L.

En el último ensayo (Capítulo Cinco) se desarrolló una prueba de campo bajo condiciones experimentales con el objetivo de estudiar, diferentes niveles de inclusión de L en sustitución de S en dietas de pollos de crecimiento y de acabado, y su efecto sobre el rendimiento productivo. Además, se estudió el efecto sobre la digestibilidad ileal de los AG, el perfil de AG de la grasa abdominal y la salud intestinal. La sustitución total del S (2% de inclusión) por L, en dietas que también contenían aceite de palma y A

(3,25% y 4,5% de grasas añadidas en crecimiento y acabado, respectivamente) no modificó el rendimiento productivo, la digestibilidad ileal de los AG totales y la morfología yeyunal. Por otro lado, se observó una reducción de la digestibilidad ileal de los AG poliinsaturados y un incremento en los recuentos de *Lactobacillus* spp. en yeyuno; aunque, sin consecuencias significativas sobre los parámetros productivos. El perfil de AG de la grasa abdominal reflejó el perfil de AG de las grasas, sin observarse importantes modificaciones.

Como conclusiones podemos decir que L es una fuente de energía alternativa adecuada para pollos de carne en crecimiento y acabado, pudiendo sustituir S hasta un 2% sin alterar el rendimiento productivo, la utilización de energía y los AG. Además, la combinación L y A es una alternativa interesante para pollos adultos gracias a la existencia de interacciones positivas sobre la utilización de la energía y los AG.

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Abbreviations

FA: fatty acids

SFA: saturated fatty acids

MUFA: monounsaturated fatty acids

PUFA: polyunsaturated fatty acids

FFA: free fatty acids

TAG: triacylglycerols

DAG: diacylglycerols

MAG monoacylglycerols

PL: phospholipids

AME: apparent metabolizable energy

LPL: lysophospholipids

NSP: non-starch polysaccharides

AMEn: nitrogen corrected AME

AI: acetone-insoluble matter

PC: phosphatidylcholine

PE: phosphatidylethanolamine

PI: phosphatidylinositol

VH:CD: VH to CD ratio

UFA:SFA: unsaturated-FA to SFA ratio

PUFA:SFA: PUFA to SFA ratio

PS: phosphatidylserine

PA: phosphatidic acid

O/W: oil-in-water

GE: gross energy

FCR: feed conversion ratio

ADG: average daily gain

DM: dry matter

CP: crude protein

EE: ether extract

OM: organic matter

BW: body weight

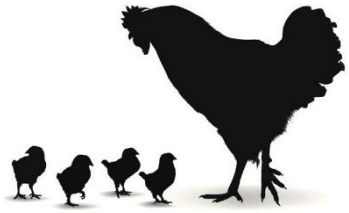
ADFI: average daily feed intake

AFP: abdominal fat pad

TFA: total FA. **VH**: villous height

CD: crypt depth

TiO_2: titanium dioxide



Chapter One

Literature review

"Perhaps it does us good to have a fall every now and then.

As long as we don't break".

J. M. Coetzee, in *Disgrace*, 1999.

1.1. Introduction

A literature review regarding the importance of fat addition in poultry feeding will be presented. The following chapter highlights a basic overview of lipids biochemistry, classification (from a nutritional point of view) and animal physiology. Afterwards, in the last section, the review is focused about lecithins and their inclusion in poultry and swine nutrition.

1.2. Lipid sources in broiler feeding

1.2.1. What is a lipid?

"**Fats**" and "**oils**" are terms used habitually as **synonyms of lipids** (Ravindran et al., 2016). Fats refers to those lipids in solid state at room temperature whereas the oil refers to those lipids that are liquid at room temperature (Baião and Lara, 2005). Lipids include a wide range of different compounds, thus, defining them is complex. In general, mostly authors agreed that lipids should meet the following conditions described by Kates (1986):

a) Mostly composed by long-chain hydrocarbon groups.
b) Insoluble in water.
c) Soluble in organic solvents such as benzene, ether and chloroform.
d) Present in living organism.

However, it is important to highlight that not all lipids satisfy and meet these requirements, existing many exceptions. For example, steroids are structured as core formations; phospholipids present variable degrees of water solubility; and *trans*-fatty acids are not derived directly from living organisms (Watson, 2006).

In order to standardize the nomenclature in the present dissertation, lipids (or fats and oils) are going to be considered by the definition stated by the lipid library of the American Oil Society Chemistry (AOCS, 2018a). Lipids are composed by fatty acids (**FA**), their combinations (e.g. triglycerides and phospholipids), and substances related to these compounds by biochemistry pathways (e.g. lipoproteins) or by functionality (e.g. sterols). Thus, **FA are the defining components of lipids**. Fatty acids (Figure 1.1) are organic acids with hydrocarbon chains composed of 4 to 36 carbons long (C4 to C36) connecting with a carboxyl group. An overview of the nomenclature, number of carbons

and double bonds of FA present in most commons fats and oils used in animal feeding are shown in Table 1.1.

Table 1.1. Common fatty acids present in fats and oils. Adapted from AOCS (2019a).

CA:DB	Fatty acid (trivial nomenclature)	Carbon atoms	Double bonds
C4:0	Butyric acid	4	0
C6:0	Caproic acid	6	0
C8:0	Caprylic acid	8	0
C10:0	Capric acid	10	0
C12:0	Lauric acid	12	0
C14:0	Myristic acid	14	0
C16:0	Palmitic acid	16	0
C16:1	Palmitoleic acid	16	1 (ω-7)
C18:0	Stearic acid	18	0
C18:1	Oleic acid	18	1 (ω-9)
C18:2	Linoleic acid	18	2 (ω-6)
C18:3	α-Linolenic acid	18	3 (ω-3)
C20:0	Arachidic acid	20	0
C20:4	Arachidonic acid	20	4 (ω-6)
C20:5	Eicosapentaenoic	20	5 (ω-3)
C22:1	Erucic acid	22	1 (ω-9)
C22:6	Docosahexaenoic acid	22	6 (ω-3)

CA:DB = Number of carbon atoms (CA) in the FA and the number of double bonds (DB) in the FA. The place of the first double bond when counting starting from methyl group (end of the FA) is commonly indicated with ω-DB (ω: Greek alphabet last letter).

Because their chemistry heterogenicity and complexity, a variety of **classification systems** for lipids exist. A detailed classification based on different criteria is reviewed by O'Keefe (2002). The most commonly used systems are:

a) Presence or absence of double bonds (saturated/unsaturated).
b) Chemical structure (derived/simple/complex).
c) Physical properties at room temperature (fats/oils).
d) Water solubility behavior (neutral/polar).
e) Essentiality for animals and humans (essential/not essential).

The **physical and chemical properties of lipids are determined by the FA structure**, more specifically, by the **length** and **saturation degree** of the hydrocarbon chain (Nelson and Cox, 2014). It is possible to classify the FA depending on the number of double bonds

present in the molecule: saturated FA (**SFA**: absence of double bonds), monounsaturated FA (**MUFA**: one double bond), and polyunsaturated FA (**PUFA**: more than one double bond).

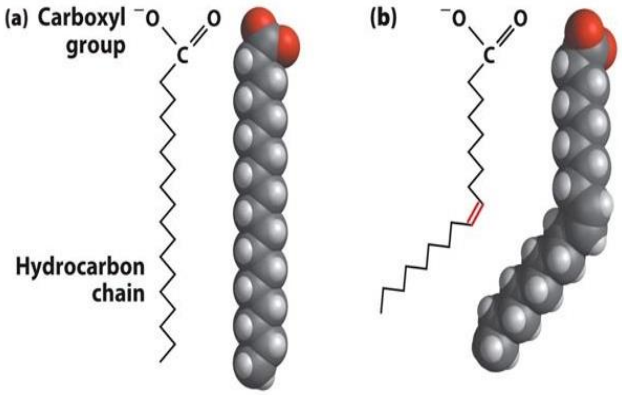


Figure 1.1. Saturated (a) and unsaturated (b) fatty acid representation. Each zigzag is a single bond between carbons and the double bond is represented by a red line. Figure extracted from Nelson and Cox (2014).

Classify lipids by its chemical structure is also habitually used. **Derived lipids** are basically free fatty acids (**FFA**) and alcohols (e.g. glycerol), which are the base for building simple and complex lipids. **Simple lipids** are derived from the combination of FFA and an alcohol, being triacylglycerols (**TAG**; commonly known as triglycerides), diacylglycerols (**DAG**), monoacylglycerols (**MAG**) and sterols the most representative. Finally, **complex lipids** yield three or more structures that can be separated after a hydrolyzation, such as glycerophospholipids (**PL**; commonly known as phospholipids), sphingolipids and glycolipids.

1.2.2. Why lipids are included in broiler feeding?

Biological functions of lipids are diverse as their chemistry. For example, TAG are the principal storage forms of energy in many organisms whereas PL and sterols are structural elements of biological membranes (Nelson and Cox, 2014). Other lipids present in fat sources in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, anchors for proteins, emulsifying agents, hormones, and intracellular messengers (Nelson and Cox, 2014).

One of the most important features of broiler production is the high energy requirements of the animals for an efficient productive performance. Cereals are the main energy source for broilers thanks to they represent the main ingredient in commercial

diets. However, the **inclusion of added fats and oils in broiler diets is a widespread activity** in order to **increase the dietary energetic density** with the aim to satisfy the energetic requirements of broiler chickens. Carbon atoms located in FA chains are chemically more reduced than carbon atoms within other nutrients, such as proteins and carbohydrates. More concretely, the oxidation of one gram of TAG releases at least twice energy than the oxidation of one gram of protein or carbohydrate: 9 kcal/g *vs.* 4 kcal/g (NRC, 1994). From a **nutritional point of view**, lipids of importance are TAG, PL, sterols and fat-soluble vitamins and their dietary addition have very important implications:

a) Dietary lipids **slow feed passage** at gastrointestinal tract, improving the absorption of all nutrients (lipidic and non-lipidic) presents in the diet. Thus, lipid addition leads to obtain a higher dietary apparent metabolizable energy (**AME**) value than can be accounted from the summation of ingredients (Mateos and Sell, 1980; 1981).
b) **Essential FA supply**. Avian species are not capable to synthetize all FA, such as linoleic, linolenic and arachidonic acid, which are recognized as metabolically essential (Baião and Lara, 2005). A deficient essential FA supply is translated in a reduced productive performance and impairment in immunity system functions (Watkins, 1991).
c) **Fat-soluble vitamins supply**, such as vitamin A, D, E and K. These vitamins are essential for a correct animal development, playing crucial roles in the organism as: bone formation, immune system modulation, antihemorrhagic factors production, and many more functions (Aslam et al., 1998; Fan et al., 2015). Furthermore, lipids supply also enhance fat-soluble vitamins digestion and absorption (Villaverde et al., 2004).
d) **Increases feed efficiency** by lowering metabolic heat production. As reviewed by Hausman and Grossman (2017), high lipid diets reduce heat increment compared to diets with high content in carbohydrates.
e) Lipids can **regulate intestinal microbiota**. For example, it has been observed that monocaprin is particularly effective in controlling pathogen flora, especially in the case of *Campylobacter jejuni* (Thormar et al., 2006).

Besides, lipid dietary addition also presents several added values, especially related with **technological** and **organoleptic** aspect. Lipid inclusion enhances the production rate in pellet meals due to their lubricating effect, and in addition, lipid inclusion **decreases dustiness of mash feed** and **improve** the **durability** and **quality** of **pelleted feed** (Thomas et al., 1998). On the other hand, **high amounts of added lipids cause negative effect** on **pellet hardness** and **durability**. Mostly feeding particles of a pellet are soluble

in water, whereas mostly lipids are hydrophobic. This situation interferes in binding properties of the pellet, causing lower pressures in pelleting and lower quality and durability of the pellet. This is the main reason in aviculture why there is a top limit of lipid inclusion (Mateos et al., 1996). On the other hand, lipids play a crucial role in the quality of poultry products, as broiler meat. Diet directly influences on the FA profile of meat or regulates fat deposition (Crespo and Esteve-Garcia, 2001; Cortinas et al., 2004). This fact is important because it is possible to modulate meat quality parameters such as meat oxidation susceptibility and the shelf-life of end-products.

1.2.3. Conventional *vs.* alternative lipid sources

In the present dissertation, fats and oils included in broiler feeding will be classified according to their frequency of use as an ingredient (conventional or alternative lipids).

Conventional lipid sources (also known as **native oils** and **fats**) used in feed manufacturing are mainly composed by TAG (> 95%) and, in a lesser extent, contain a variable proportion of other compounds such as DAG, MAG, FFA and PL. Furthermore, their inclusion in commercial diets is widespread. Triacylglycerols consist in three FA chains attached with an ester bond (esterified) to a glycerol molecule (Figure 1.2). The three hydroxyl groups are numbered by the stereospecific numbering system, designating the FA to the position *sn*-1, *sn*-2, and *sn*-3 (AOCS, 2018b).

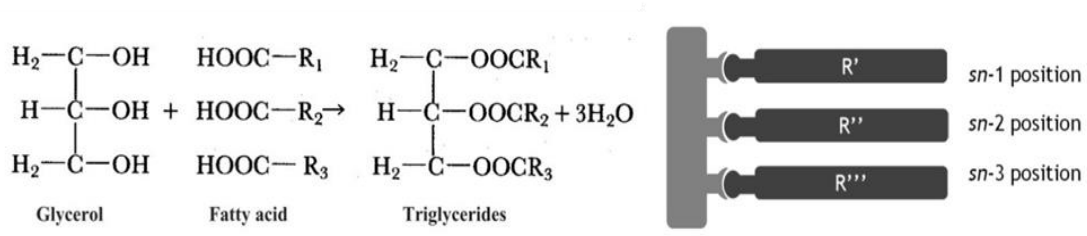


Figure 1.2. Stereochemical configuration and representation of triacylglycerol, adapted from AOCS (2018b) and Vilarrasa (2014).

This kind of lipids are habitually derived from a few vegetable and animal sources. On one hand, **soybean oil**, **sunflower oil** and **poultry fat** represents the most commonly unsaturated lipid sources used in monogastric feeding; on the other hand, **palm oil**, **beef tallow** and **pig lard** are the most common saturated lipid sources (Baião and Lara, 2005). Despite their common feature of a high content in TAG, they differ in their chemical

composition (Table 1.2), stability to oxidation, nutritive value and economic cost, thus, their use as energy sources for broiler feeding depends of many factors.

Table 1.2. Fatty acid profile and gross energy values of conventional lipid sources used in broiler feeding.

	Animal fats			Vegetable oils			
Item	PF[a]	L[b]	T[c]	PO[d]	nd-S[c]	d-S[b]	SF[e]
Fatty acid profile (%)							
C16:0	22	25	24	43	10	12	6.8
C16:1	4.8	2.3	6.1	0.0	0.0	0.1	0.1
C18:0	7.2	13	21	4.4	3.7	4.3	4.5
C18:1	42	42	41	38	24	27	26
C18:2	23	13	5.0	11	55	50	62
C18:3	0.0	0.5	0.6	0.3	7.2	5.7	0.0
Minor fatty acids	1.1	3.8	2.3	3.4	0.0	1.2	0.8
UFA:SFA	2.43	1.54	1.16	1.00	6.41	5.18	7.78
Gross energy (kcal/kg)	9,336	9,264	9,513	9,407	9,458	9,443	N.D.

PF = poultry fat; L = pig lard; T = beef tallow; PO = palm oil; nd-S =non-degummed soybean oil; d-S = degummed soybean oil; SF = sunflower oil; N.D. = not determined; Unsaturated-to-saturated fatty acids ratio..

Data extracted from: [a] Lessire et al., 1982; [b] Vieira et al., 2015; [c] Huyghebaert et al., 1988; [d] Roll et al., 2018; [e] Crespo and Esteve-Garcia, 2002.

Alternative lipid sources, such as coproducts derived from vegetable oil refining process (**lecithins**, **FA distillates from physical refining and acid oils**), **re-esterified oils** and **recycled lipids** may represent an alternative to conventional lipids. The price of some native oils, such as soybean oil, have been constantly increasing regardless to biodiesel production (Statista, 2018). Therefore, there is a growing interest of including alternative energy sources in replacement of conventional lipids with the aim to reduce production cost associated to feed production. Lecithins will be explained extensively in Chapter 1.5 of this dissertation, and the rest of alternatives will be summarized as follow.

1.2.3.1. Fatty acid distillates from physical refining and acid oils

Fatty acid distillates from physical refining and acid oils are coproducts generated during vegetable oil refining during physical and chemical refining, respectively (Figure 1.3). The refining method of choice is determined by the characteristics of the individual crude oils. The objective of oil refining process is to **remove crude oil FFA** content and

reduce gross impurities, such as oxidized materials, to ensure the quality, stability and nutritional value of the end-product (Fediol, 2018).

The Catalogue of Feed Materials (Commission Regulation No 68/2013; European Commission) define fatty acids distillates from physical refining as products obtained during the deacidification of vegetable or animal oils by means of distillation. Fatty acids distillates from physical refining, as the name implies, are obtained after a **physical refining** consisting in a steam distillation (180-270 ºC) due to FFA present a lower boiling point than TAG (Nuchi et al., 2009).

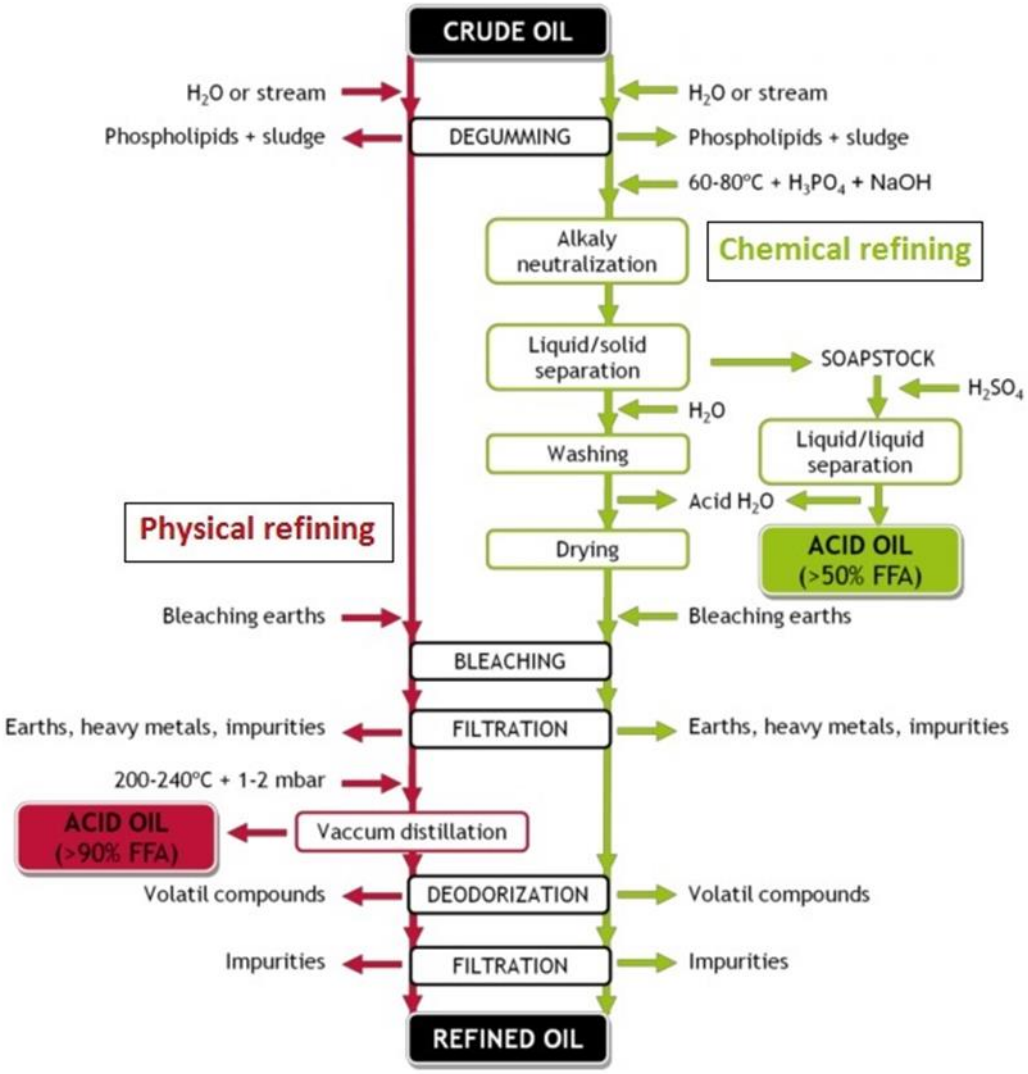


Figure 1.3. Physical and chemical refining of vegetable crude oils. Extracted from Vilarrasa (2014). *FFA = free fatty acids.*

Crude oils with a low content in PL need to be treated by this way, the most common is **palm oil**, and these coproducts are characterized by containing high levels of FFA (> 90%; Nuchi et al., 2009; Tres et al., 2012).

On the other hand, oils with a high content in PL, such as **soybean** and **sunflower oil**, are chemically refined by an **alkali neutralization**, generating acid oils. This chemical refining process provokes a large reduction of FFA through their conversion into water-soluble soaps. Then, these soaps are treated with sulfuric acid in hot aqueous solution giving an acidulated oil as a coproduct derived from the crude oil refining process (Baião and Lara, 2005). These acid oils present a similar FA profile than the original native oil, but present a similar proportion of FFA and TAG, containing around the 40-60% of their composition in FFA (Nuchi et al., 2009; Barroeta, 2017).

Wiseman and Salvador (1991) used in broiler feeding three different native oils and their respective acid oils as energy sources, evaluating their combination at different levels. The experiment showed that no important differences on FA profile were observed between the native oil and their respective coproduct obtained after refining process. Basically, the main difference was located on their FFA content (Table 1.3).

Table 1.3. Fatty acid profile of fats used in Wiseman and Salvador (1991). The main difference between native oils and acids oils is in their free fatty acid content.

	Tallow		Palm oil		Soybean oil	
Item	T	T-AO	P	P-AO	S	S-AO
Fatty acid profile (%)						
C16:0	25	28	49	47	16	7.0
C16:1	3.9	3.5	0.1	0.5	0.6	0.4
C18:0	21	24	5.5	5.5	6.1	2.4
C18:1	41	37	36	35	25	39
C18:2	6.6	3.3	7.3	9.4	45	44
C18:3	1.0	0.3	0.2	0.8	6.6	7.9
Minor fatty acids	2.5	3.7	1.7	1.8	0.3	0.1
Ratio U:S	1.14	0.86	0.81	0.88	3.49	9.63
Free fatty acids (%)	14	95	5.8	92	1.4	68

T = native tallow; T-AO = tallow acid oil; P = native palm oil; P-AO = palm acid oil; S = native soybean oil; S-AO = soybean acid oil.

It is important to remark that these differences (between native oils and coproducts) regarding how their FA are structured in different **lipid molecular structures** are important in order to evaluate its **AME value**. It is well documented that lipid with a high content in FFA are worse absorbed by poultry than those lipids mainly composed by TAG

(Sklan, 1979). However, several authors have stated that the inclusion of these coproducts maintains poultry performance efficiency. For example, in Perez-Bonilla et al. (2011), was observed that the use of three different fats (non-degummed soybean oil, vegetable acid oil and pig lard) supplemented to three different cereals-based diets (corn, wheat and barley), in laying hens from 22 weeks old to 54 weeks, did not modify performance results and dietary AMEn value. In the same way, in Pardio et al. (2005), the full replacement of soybean oil by acidulated soybean soapstocks, in laying hen diets, did not modify quality parameters and the FA composition of eggs. Similar results in broiler chickens were reported by Pekel et al. (2013), concluding that sunflower acid oil may be included in diet formulation replacing soybean oil as energy source without modifying performance results.

1.2.3.2. Re-esterified oils

Table 1.4: Fatty acid profile, acylglycerol fraction and gross energy content of re-esterified oils selected from different studies.

	S[a]	P[b]	ReSL[a]	ReSH[a]	ReP[c]	RePL[b]	RePH[b]
Fatty acid profile (%)							
C16:0	10.7	44.9	11.4	10.9	47.2	47.8	47.9
C18:0	4.4	4.5	4.1	3.9	5.1	4.74	4.8
C18:1	21.2	38.1	21.3	22.1	36.8	35.3	35.5
C18:2	54.2	9.8	53.6	54.5	4.8	8.0	8.2
C18:3	7.7	0.27	7.8	6.8	0.0	0.2	0.2
Minor fatty acids	1.8	2.4	1.8	1.8	6.1	3.9	3.5
Ratio U:S	5.5	0.98	5.32	5.62	0.82	0.82	0.83
Acylglycerol fraction (%)							
TAG	98.2	76.0	78.6	34.6	12.9	48.0	26.3
DAG	0.8	15.5	11.5	36.0	45.7	40.1	50.8
MAG	0.3	0.5	8.9	28.1	41.4	10.9	22.9
FFA	0.8	8.0	1.0	1.3	0.0	1.1	0
Gross energy (kcal/kg)	9,465	9,305	9,280	8,955	8,479	9,171	8,991

TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids; S = soybean oil; P = palm oil; ReSL = re-esterified soybean oil with low content in MAG and DAG; ReSH = re-esterified soybean oil with high content in MAG and DAG; ReP = re-esterified palm oil; RePL = re-esterified palm oil with low content in MAG and DAG; RePH = re-esterified palm oil with high content in MAG and DAG.

Data extracted from: [a] Vilarrasa et al, 2015a; [b] Vilarrasa et al, 2015b; [c] Roll et al, 2018.

Another alternative energy source to keep in mind are **technical lipids**, **especially re-esterified FA**. Technical lipids are the result of chemical modifications of lipids (fractionation, interesterification or hydrogenation) by a technological process with the aim to change special features of the molecule for specific nutritional purposes.

Re-esterified oils are obtained by **interesterification** (Vilarrasa et al., 2015a)). Interesterification is a chemical reaction based in the esterification of FFA with free hydroxyl groups derived from the crude oil or from added glycerol molecules, mostly derived from biodiesel industry (Bhosle and Subramanian, 2005; Mandalawi et al., 2017). After this process, a blend of molecules such as FFA, MAG, DAG and TAG are obtained in different proportions depending on the temperature, the catalyst used and the amount of glycerol added (Kombe et al., 2013). Re-esterified lipids are usually obtained **using acid oils** derived from oil refining industry as a raw material, whereas **glycerol** is usually obtained from biodiesel production (Vilarrasa et al., 2014). This process gives to acid oils and glycerol an added value and the chance to reintroduce these coproducts in animal feeding chain. The main difference between a re-esterified oil with their native oil is that the molecular structure is modified due to changes in acylglycerol fraction. In addition, re-esterified oils present an increased proportion of *sn-2* SFA compared to their respective native oil (Table 1.4). Results reported in Vilarrasa et al. (2014) demonstrated that the use of a re-esterified palm oil instead of a native palm oil, in broiler chicken diets, did not modify performance, fat absorption and postprandial lipemia.

1.2.3.3. Recycled lipids

Finally, another alternative option are recycled lipids and their blends. Recycled lipid is a generic term that englobes all kind of **fats** and **oils used for cooking and recycled for animal feeding** purposes (Blas et al., 2010). Their nutritive value depends of many factors, especially influenced by the raw material and the frying process that they submit. These products result highly variable in terms of final quality and safety. If these products are not processed correctly it might include undesirable substances. However, some studies reported that these kind of energy sources may be included in poultry feeding without modify performance results (Zumbado, 1999; Pesti et al., 2002; Irandoust et al., 2012). However, it is important to highlight that there is no much data about their inclusion in animal feeding, in general.

1.3. Lipid physiology in broiler chickens

1.3.1. Lipid digestion: basic concepts

Dietary components present in animal feed need to go through the digestive tract in order to be digested and absorbed. The final objective of lipid digestion and absorption is to release the lipids from the matrix (animal feed) and break complex molecules (mostly TAG) to smaller molecules (FFA and MAG), making possible lipid absorption by enterocytes. The main obstacle of this process is due to fat is not soluble in the medium where it must be digested and absorbed (Tancharoenrat, 2012).

Fat digestion is a complex process that involves several physicochemical and enzymatic steps throughout the digestive tract. In the case of avian species, the process is almost equal to monogastric mammals, however, some peculiarities and features should be highlighted.

First, the **digestive tract** of **avian species** is **shorter** than mammals (Turk, 1982). This fact is important due to this leads to shorter times of retention for feedstuffs in the gut and **less efficiency** in **nutrient digestion** and **absorption** compared to monogastric mammals. The existence of **refluxes** (or **reverse peristalsis**) in concrete parts of the gastrointestinal tract compensates the shorter times of feed retention in order to improve nutrients digestion and absorption (Turk, 1982). Three gastrointestinal refluxes are documented in the bibliography in different anatomic points: between proventriculus and gizzard (gastric reflux), between duodenum and gizzard (small intestinal reflux) and the cloacal-caecal reflux (Duke, 1982; Ravindran et al., 2016).

Second, the existence of **specials organs** that birds do not share with other livestock animals and influence on digestive physiology. The most obvious is the presence of a **beak** in the buccal cavity (and the absence of teeth). Following the gastrointestinal tract, we find the **crop**, which its basic function is to act as a storage organ for birds under situations of discontinuous feeding. Thus, the crop is not thought to have an important nutritional role, however, it has been observed that crop play a role in moisturization the feed ingested (Svihus, 2014). Regarding the stomach, is divided in two chambers: **proventriculus** (glandular stomach) and **gizzard** (muscular stomach). In this place nutrient digestion starts due to feed will be grounded by the gizzard and digestive enzymes will be secreted by the proventriculus. After the small intestine (duodenum, jejunum and ileum) we can find the **caecum**. The main function of avian caecum is related

with water and mineral absorption, fermentation of undigested nutrients and nitrogen recycling from urine (Svihus et al., 2013). Finally, gastrointestinal, urinary and reproductive tract converge and exit at the same organ, the **cloaca**.

Third, the **absence of a developed lymphatic system**, with important implications for lipid metabolism and deposition (Hermier, 1997).

A brief review of lipid digestion, absorption and deposition is described below.

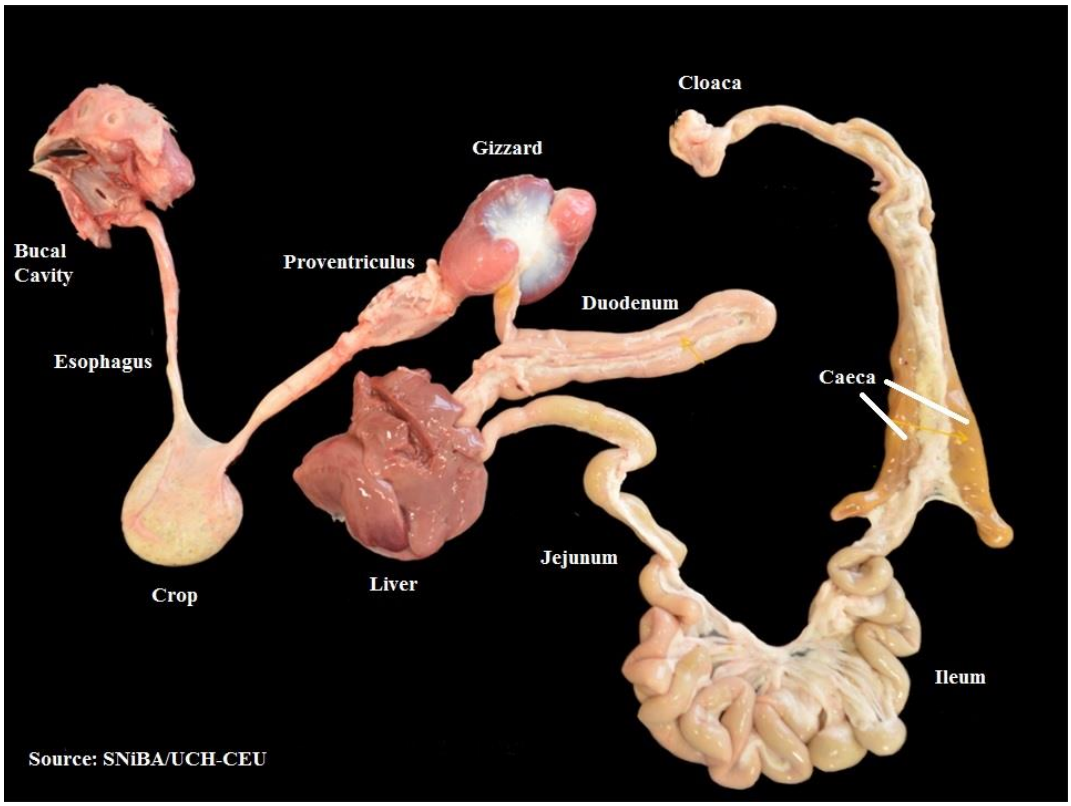


Figure 1.4. Gastrointestinal tract of a broiler chicken (38 days of live). Picture originally taken by SNiBA/UCH-CEU (2017).

1.3.2. Buccal cavity to crop: first step before lipid digestion

It could be said that digestion process begins with feed consumption by the animal, but, in contrast to mammalians, in avian species the feed enters in buccal cavity without any grounding process and is swallowed directly to the esophagus (Svihus, 2014). In mammals, fat digestion starts in buccal cavity due to the presence of lingual lipases that starts hydrolyzing lipidic molecules (Hamosh and Scow, 1973), but in the case of avian species, saliva does not have lingual lipases (Krogdahl, 1985). In avian species there are numerous ducts of salivary glands in mouth cavity, but their only function is lubricating the feed. Once is swallowed, the next step is the esophagus. The esophagus only has the

function of connecting mouth cavity with the crop (a dilatated extension of the esophagus). As mentioned before, the crop is an anatomical feature from avian species which play a role as a storage organ, especially for those animals under situations of discontinues feeding, as wildlife birds. In the case of broiler chickens, that feed is usually offered *ad libitum*, so the crop does not play any important role in nutrients digestion (Svihus et al., 2010). In this first step no considerable actions are taken place for fat digestion before the feed arrives to the proventriculus and gizzard.

1.3.3. Proventriculus, gizzard and enzymatic secretion

Proventriculus and gizzard are commonly known as the glandular and the muscular stomach of avian species, respectively. These avian gastrointestinal organs are often seen as one entity due to exist a reflux process between them (gastric flux) that improves the mixing process of feed digesta with endogenous secretions of proventriculus as hydrochloric acid (HCl) and pepsinogen (Duke, 1982; Bauer et al., 2005). Once the feed arrives to proventriculus, it secretes HCl with a low pH, and at the same time, the particles from feed are reduced mechanically by the grinding action of gizzard (Lentle et al., 2013). During the grinding cycle of the gizzard it is possible to find duodenal content thanks to the intestinal reflux (content from duodenum and jejunum that is pushed back to gizzard). The presence of **bile acids** and **pancreatic lipase** from duodenum and jejunum in the gizzard initiates **fat emulsion** at this level and facilitate the proteolytic activity of pepsinogen (Sklan, et al., 1978).

Bile acids are formed at the hepatocytes of the liver, synthesized from cholesterol, transported and stored at the gallbladder and delivered into distal duodenum (Marin et al., 2016). Bile acids are composed by bile salts and PL, but also pigments, cholesterol and electrolytes (Lai et al., 2018). Bile salts needs to be **conjugated** with glycine or taurine before its secretion, due to this process increases their hydrophilic properties and make them less cytotoxic (King, 2018). Bile acids causes that dietary **fat globules** break to fine stable **droplets**, thus increasing the surface area for pancreatic lipase activity (Bauer et al., 2005). The lipid droplets consist in a hydrophobic core of TAG, fat soluble vitamins and esterified cholesterol surrounded by an amphipathic surface monolayer (Bauer et al., 2005). This step, together the colipase presence, is essential to allow pancreatic lipase action. Pancreatic lipase is secreted by pancreas and act as a catalyst after bile emulsification, breaking TAG specifically at *sn-1* and *sn-3* position, releasing more water-soluble molecules, concretely two FFA and a *sn-2* MAG (Bauer et al., 2005).

1.3.4. Small intestine: where lipid absorption occurs

The small intestine is the place where absorption process of nutrients mostly occurs (Svihus, 2014). The small intestine segment is divided in three anatomical differentiated parts: **duodenum**, **jejunum** and **ileum**.

Lipids enter the duodenum as emulsified droplets due to bile acids action at gizzard level with the rest of the digesta. Once the bolus arrives to duodenum, the acidic feed bolus is neutralized by sodium bicarbonate secretion, raising a pH of 2.0-3.0 to a valor of between 5.0 to 7.5. This sudden change in the pH causes a modification on the behavior of the FFA inside the droplets. These FFA become partly ionized and migrate to the interface doing a spontaneous emulsion (Salentinig et al., 2011). At the same time, the presence of digesta content in duodenum stimulates the secretion of **cholecystokinin**, which will induce bile and pancreatic juices secretion at distal duodenum by gallbladder and pancreas, respectively (Degolier et al., 2013). Pancreatic juices are composed by bicarbonate sodium and a large variety of hydrolytic enzymes such as amylase, trypsin, lipase and **phospholipase A_2** that will play a role in nutrient digestion and absorption, including lipids (Svihus, 2014). Phospholipase A_2 is responsible to cleave dietary phospholipids at *sn-2* position, releasing a lysophospholipid (**LPL**) and one FFA (Joshi et al., 2006).

The aqueous solubility of *sn-2* MAG and FFA is relatively low, especially influenced by their chemical composition and structure, and need to be incorporated to a **mixed micelle** (also known as mixed-bile-salt-micelles) for being absorbed (Wilde and Chu, 2011). Released FA with short and medium chains and unsaturated FFA are solubilized and spontaneously incorporated to the surface of the mixed micelles with polar lipids and bile salts (Wilde and Chu, 2011). On the other hand, low water-soluble molecules, such as long chain FA and saturated FFA are incorporated at the core of the mixed micelle (Wilde and Chu, 2011). Thus, mixed micelles result an assembly of a **hydrophilic layer** whereas the **hydrophobic** molecules constitute the **core**. These assemblies will be transported to apical part of plasma membrane of the epithelial lining of small intestine and finally absorbed. This process is **mostly located** anatomically at **jejunum**, but also a small portion of the dietary fat is absorbed at ileum (Rodriguez-Sanchez, et al., 2019). It is important to highlight that most of the secreted bile salts are reabsorbed at distal ileum (95%) by an active transport process and are returned to liver by **enterohepatic**

circulation (Hofmann and Hagey, 2008). The aim of this process is to reincorporate and recycle this bile salts for further digestions.

Lipids that are not absorbed at distal ileum will arrive to **colon** and **caecum**. There are theories about lipid utilization by colon and caecum microbiota as a carbon source (Fujita et al., 2007), however actually there is a lack of data regarding undigested fat utilization by microbiota. At this point, all undigested fat will travel as insoluble fat droplets until arrive to the cloaca and finally will be excreted. Some authors have concluded that little or inexistent lipid absorption occurs after ileum passage due to crude fat digestibility calculations from ileal samples are close similar to crude fat digestibility results from excreta (Renner, 1965; Ajuyah et al., 1996).

1.3.5. Inside the enterocytes to the liver

Mixed micelles contact with the apical part of epithelial cells of small intestine, also knowns as **enterocytes**. This apical portion consist in a microvillus membrane with absorption capacities (Karcher and Applegate, 2008).

The absorption process of *sn-2* MAG, short and medium FA chains is produced by **passive diffusion** across the enterocyte membrane, driven by a concentration gradient and thank to their higher water solubility (Lo and Tso, 2009). On the other hand, low water-soluble molecules integrated in the core of mixed micelles as long FA chains or saturated FFA requires **active** and **protein-mediated transport** mechanisms. There are a few families of proteins involved in the active absorption process, but the most important are the fatty acid transfer proteins, and within this family, the most common is **FATP4** (Lo and Tso, 2009). Other lipidic compounds, such as cholesterol or PL are absorbed by other specific protein mediated transports, however the information regarding these proteins is scarce (Gajda and Storch, 2015; Glatz, 2015).

Once inside the enterocyte cytosol, *sn-2* MAG and long FA chains need to be **re-esterified** with another molecules, such as cholesterol or PL. As mentioned before, birds present a poorly developed lymphatic system and due to that, FA must be re-esterified as **lipoproteins** and released directly to portal venous blood system, and for that reason are known as **portomicrons** (Hermier, 1997). On the other hand, short and medium FA chains can be released directly to portal blood bounded with albumin without a re-esterification process (Hermier, 1997). In mammals, these lipoproteins, are known as chylomicrons and are transported to the general circulation via lymph (Sethi et al., 1993).

In the case of avian species, the great part of absorbed lipids will travel to liver due to is the tissue where mostly fat metabolic processes happen (Ravindran et al., 2016).

1.3.6. Fat deposition

Once a lipidic molecule is digested and absorbed, can be used by the organism in different ways. Depending on the physiological state of the animal (energetic balance), dietary FA will be **β-oxidized** to ATP releasing energy or will be deposited as energy storage in different tissues in the **adipocytes** (also known as fat cells). Adipocytes will receive lipoproteins from liver and will re-esterify the FA and *2-sn* MAG in TAG and store them. In addition, adipocytes regulate the energy balance and glucose homeostasis (Rosen and Spiegelman, 2006). In broiler production, animals usually dispose *ad libitum* their feed and are always in a positive energetic balance, so there will be always a trend on fat deposition.

1.4. Factors affecting lipid digestion, absorption and deposition in broiler chickens

Among all the lipids described, exist a huge variety of chemical structures with different physicochemical behaviors with important consequences on fat digestion, absorption and deposition. The AME value of a fat is highly dependent of numerous factors classified in two groups: diet-related and animal-related factors. A brief summary of the most important factors is described down below.

1.4.1. Diet-related factors

1.4.1.1. Fatty acid saturation degree and chain length

A lot of authors have demonstrated and well documented **differences in fat AME** value **depending on** their **FA saturation** degree and **chain length** (Wiseman et al., 1991; Blanch et al., 1996; Sanz et al., 2000a; Smink et al., 2010, Tancharoenrat et al., 2014).

Monogastric animals digest and absorb less those fats with a high content in SFA than those fats with a high content in unsaturated FA. Saturated FA present a higher hydrophobic activity and superior melting point than unsaturated FA (Table 1.5). Concretely, SFA present melting points above body temperature, making even harder their solubility in gut aqueous medium. It has been demonstrated, in broiler chickens, that animals feed with a saturated FA source showed lower fat utilizations than those fed with

unsaturated FA sources (Tancharoenrat el al., 2014). Moreover, SFA present a higher tendency to form insoluble soaps with divalent cations, such as calcium, reducing their intestinal absorption (Denke et al., 1993; Bendsen et al., 2008). Tancharoenrat and Ravindran (2014) observed that increasing dietary calcium concentration decreased fat digestibility (and calcium digestibility) by increasing the formation of insoluble calcium-soaps in the small intestine.

It is important to highlight the existence of **synergistic interactions** between the unsaturated and saturated FA. There are evidences suggesting that blending different saturated and unsaturated lipid sources improves fat digestibility in broiler chickens (Wiseman and Lessire, 1987; Wiseman et al., 1990; Baião and Lara, 2005; Tancharoenrat et al., 2013). Saturated FA utilization is enhanced by the presence of high concentrations of unsaturated FA because they act as emulsifiers improving the mixed micelles formation and supporting pancreatic lipase hydrolysis (Garret and Young, 1975).

Regarding the effect of the FA chain length, the absorption rate of a FA is negatively related its chain length. Shorter FA chains are easier to absorb, while long FA chains are less soluble in water (Tancharoenrat et al., 2014). In a similar way, lipolytic enzymes seems to catalyze more efficiently those TAG with short and medium FA chains (Papamandjaris et al., 1998). Deschod-Lanckman et al. (1971) observed, in rats, that pancreatic lipase activity was twice superior in unsaturated TAG than saturated TAG.

Table 1.5. Melting point of different fatty acids depending on their carbon chain length. Adapted from Knothe and Dunn (2009).

Fatty acid	Abbreviation (c:d)	Carbon atoms	Melting point (ºC)
Caprylic acid	C8:0	8	16.5
Capric acid	C10:0	10	31.5
Lauric acid	C12:0	12	44.0
Myristic acid	C14:0	14	58.0
Palmitic acid	C16:0	16	63.0
Stearic acid	C18:0	18	71.2
Arachidic acid	C20:0	20	77.0

c:d = carbon (c) to double bounds (d) ratio.

Saturation degree also plays an important role on lipid metabolism and deposition. It has been reported that unsaturated FA are easier and faster catabolized than SFA (Leyton et al., 1987). At the same time, fat deposition depends directly from energetic balance (energy intake/energy waste), and many studies have demonstrated that heat loss may be

greater when unsaturated FA are abundant in the diet (Clarke, 2000; Newman et al., 2002). Thus, it has been observed that broiler that consume diets rich in unsaturated FA present a reduced abdominal and total body fat compared to chickens fed saturated diets (Sanz et al., 1999; Crespo and Esteve-Garcia, 2001; Ferrini et al., 2008; González-Ortiz et al., 2013).

1.4.1.2. Free fatty acid content

Quality of fats and oils is one of the most important factors influencing its bioavailability. As mentioned above, FA profile may be the most important factor to consider, but there are more. Moisture, FFA content, unsaponifiable fraction, oxidation products and impurity content are important quality aspect to take account because they decrease fat digestibility and its AME content (Codony et al., 2017; Barroeta, 2017).

In conventional lipids, most of their FA are esterified as TAG molecules. However, some alternative fat sources, such as **acid oils**, present FFA levels between 50 to 90% of their total composition. Many studies, in monogastric species, have reported that lipid sources with **a high FFA content** show **lower utilizations** compared to animals fed diets with conventional added fats mainly composed in TAG (Powles et al., 1993;1994; Vilà and Esteve-Garcia, 1996a, b). This lower utilization values are especially pronounced in those fat sources with a high saturated FFA content (Wiseman and Salvador, 1991). This issue is due to the absence of MAG molecules, because they are capable to facilitate the incorporation of hydrophobic FFA into the mixed micelle core (Lo and Tso, 2009). Besides, TAG and MAG presence at duodenum stimulates endogenous secretions of bile acids, and as a consequence, animals fed rich FFA sources have a reduced bile secretion (Sklan, 1979). In addition, FFA tend to form insoluble soaps with divalent cations as calcium, especially in the case of saturated FFA (Garret and Young, 1975).

1.4.1.3. Fatty acid position

Pancreatic lipase hydrolyze TAG specifically at *sn*-1,3 positions, releasing two FFA and one intact *sn*-2 MAG. These MAG are well absorbed regardless of the saturation degree of its FA, due to glycerol hydrophilic character. On the other hand, FFA absorption is highly dependent of their saturation degree, as mentioned before. Unsaturated FFA are incorporated into the mixed micelle and then, are easily absorbed by the enterocytes, while on the other hand, saturated FFA not (Leeson and Summer, 2001). For this reason, the molecular structure and FA distribution within the TAG (and, probably, other lipid

molecular structures as PL) play an important role on mixed micelle formation and the posterior lipid absorption and metabolism (Innis, 2011; Michalski et al., 2013).

This issue is important to understand the differences between vegetable and animal fat sources and their respective digestibility. Animal fat sources usually present SFA at *sn*-1,3 positions, whereas vegetable fat sources present unsaturated FA at *sn*-1,3 position (Meng et al., 2004). Experiments performed in laboratory rats (Renaud et al., 1995; Lien et al., 1997) have observed a better fat digestibility in animals fed diets with a high content in unsaturated *sn*-1,3 TAG and saturated 2-MAG than animals fed diets containing a high amount of saturated *sn*-1,3 TAG with unsaturated 2-MAG. This fact has been also confirmed in broiler chickens (Lin and Chiang, 2010; Vilarrasa et al., 2015b, c).

1.4.1.4. Level of fat inclusion

The level of fat inclusion also has an important role in fat digestibility. Many studies have reported that **fat** is **better digested and absorbed at lower inclusion levels** than in higher inclusion rates (Wiseman et al., 1991; Villaverde et al., 2006; Smink et al., 2010).

This fact is explained by an insufficient secretion of pancreatic lipase and bile acids for the increasing amounts of dietary fats, especially in young birds (Krogdahl, 1985). For example, Tancharoenrat and Ravindran (2014) studied three inclusion levels of tallow (0, 4 and 8%) in corn-soy-based diets and observed that animals fed 4% of tallow obtained better fat digestibility than those fed 0 and 8%. They theorized about a certain minimal level ofinclusion to optimize fat digestion and in addition, the existence of a possible threshold for effective emulsification of fat by bile salts. However, the certain reasons about this issue are still unclearly and further studies are required.

1.4.1.5. Non-starch polysaccharides

Non-starch polysaccharides (**NSP**), such as arabinoxylans and β-glucans, are known for their anti-nutritional effects on poultry diets and also influence on fat digestion and absorption (Lee et al., 2004). Non-starch polysaccharides are known basically for increasing digesta viscosity and due to that, slow the gut motility and impair the diffusion and transport of the nutrients within the intestinal enterocytes (Meng et al., 2005).

In addition, NSP can stimulate the overgrowth of intestinal bacteria capable to deconjugate primary bile salts to secondary bile salts, by a **bile salt hydrolase**, reducing its emulsifying capacity (Knarreborg et al., 2002a, 2004). Besides, this deconjugated bile acids cannot be absorbed and recycled by enterohepatic circulation and would be lost with

the excreta, causing a low concentration of bile secretion of the animals fed diets high in NPS content, thus, producing a fat digestibility reduction (Smits and Annison, 1996).

1.4.2. Animal-related factors

1.4.2.1. Animal age

It has been extensively documented that **young birds** have a **limited capacity** of **fat digestion and absorption** (Krogdahl, 1985; Noy and Sklan, 1995; Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019).

In general, fat digestion and absorption are low at post-hatch and develops rapidly after the first week of life, improving with age (Carew et al., 1972). A **lower bile secretion** capacity and an inefficient enterohepatic bile recycling process seems to be the principal reasons of the lower fat digestion and absorption capacity of young poultry (Krogdahl, 1985; Noy and Sklan, 1995). Some authors have tested bile acid inclusion in broilers feeding (Polin et al., 1980; Maisonnier et al., 2003; Lai et al., 2018) demonstrating an improvement on fat digestibility and performance parameters, especially in young chicks. Furthermore, during first week of life, digestive enzymes such as amylase, trypsin and pancreatic lipase are secreted in low quantities to gastrointestinal tract but increases 20- to 100-fold between 4 to 21 days of life (Noy and Sklan, 1995).

Table 1.6. Influence of broilers age on the apparent metabolizable energy value (kcal/kg) and total tract apparent digestibility coefficient of the following fats (in parenthesis). Adapted from Tancharoenrat et al. (2013).

Lipid source	Weeks of life			
	1	2	3	5
Tallow	2,925 (0.368)	5,305 (0.653)	6,413 (0.736)	6,363 (0.726)
Soybean oil	4,003 (0.591)	8,465 (0.898)	9,043 (0.965)	8,902 (0.948)
50:50 T/S	4,228 (0.500)	7,476 (0.831)	7,896 (0.830)	8,436 (0.856)
Poultry fat	4,311 (0.600)	8,065 (0.845)	8,379 (0.928)	8,159 (0.911)
Refined Palm Oil	3,798 (0.603)	7,832 (0.806)	8,276 (0.836)	8,412 (0.843)

50:50 T/S = blend of tallow and soybean oil (proportion 1:1).

Tancharoenrat et al. (2013) also confirmed the limited capacity of lipid digestion of newly hatched chickens. Different fat sources were included in a maize-soy based basal diet to quantify the fat AME value and the total tract apparent digestibility of fat

depending on the animal age (Table 1.6). It was concluded that fat AME value and its digestibility were improved with age, so it is necessary to assign a lower AME values for fat sources in the formulation of starter diets.

1.4.2.2. Intestinal pathology and microbiota

Some intestinal pathogens are capable of damaging enterocytes and consequently, reduce the absorption of lipids (and other nutrients). **Diseases** such as necrotic enteritis, malabsorption syndrome, coccidiosis and dysbiosis can decrease energy utilization by broiler chickens. **Coccidia** are capable of damaging enterocytes of intestinal epithelium (Perez-Carbajal., 2010), influence on intestinal morphology by shortening villi (Kettunen et al., 2001) and reduce the activity of digestive enzymes (Williams, 2005). The sum of all these pathogenia is translated in nutrients absorption impairment. Amerah and Ravindran (2015) challenged broiler chickens with a mix of *Eimeria* species to evaluate its effects on nutrient utilization, concluding that the negative effects were especially pronounced in fat absorption. Whereas protein absorption was reduced in a 25% and starch absorption was reduced in a 19%; fat digestibility was reduced in a 96%.

Not only pathogens can modify nutrient absorption, microbiota located along gastrointestinal tract also have an influence. **Bacteria community** is capable to **affect fat digestion** as observed by some authors (Knarreborg et al., 2002a; van der Hoeven-Hangoor et al., 2013). This modulation is bidirectional, due to broiler microbiota is also influenced by dietary components, such as fat (Knarreborg et al., 2002b; Rodríguez et al., 2012). For example, in Knarreborg et al. (2004) was studied the influence of bile salt hydrolase bacteria as *Clostridium perfringens* and it was demonstrated that the use of antibiotics to reduce *C. perfringens* populations enhanced FA digestibility.

1.4.2.3. Gender

There are a few studies about gender influence on fat digestibility, however the conclusions are scarce and controversial. Guirguis (1975; 1976) reported significant gender effects on energy utilization, concluding that feed AME value was higher for female broiler chickens than male broiler chickens. On the contrary, Zelenka (1997) studied the effect of gender on nitrogen corrected AME (**AMEn**) and no differences were observed.

However, there are **gender differences in fat deposition**, due to female broiler chicken shown a higher fat deposition than male broilers (Rondelli et al., 2003).

1.5. Lecithins

1.5.1. Definition and chemical composition

Lecithin is defined as a **lipid mixture** with a minimum of **60% of acetone-insoluble** (**AI**) **matter** (van Nieuwenhuyzen and Tomás, 2008; EFSA, 2016).

The AI (%) matter is a commercial and legal specification that indicates the number of polar lipids of a substance, because neutral lipids as FFA and TAG dissolve in acetone (Nguyen et al., 2014; Bueschelberguer et al., 2015). As a reminder, lipids can be classified depending on their behavior on an aqueous phase, differentiating two main groups: neutral or polar lipids (Figure 1.5.). Neutral lipids are non-polar, with a hydrophobic behavior and habitually have an energetic function. On the other hand, **polar lipids** are **amphipathic** (hydrophobic and hydrophilic behavior) and usually play structural functions.

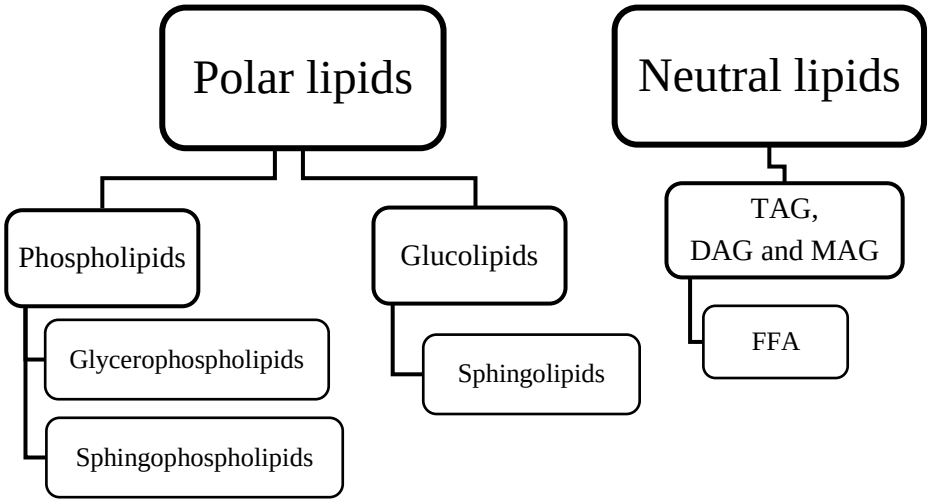


Figure 1.5. Lipid classification depending on their behavior with water. Adapted from Nelson and Cox (2014). *TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids.*

Lecithins consist in a mixture of polar lipids, mainly PL, and a variety of other extracted substances during degumming, such as TAG and FFA (van Nieuwenhuyzen and Tomás, 2008). **This definition is going to be used throughout this dissertation** in order to avoid term misleading due to the existence of various definitions. For example, in chemistry and medicine, lecithin term is used to refer exclusively to the molecule of phosphatidylcholine (**PC**; one of the most common PL in vegetable lecithins), also defined as "pure" or "chemical" lecithin to distinguish it from the lipid mixture. However,

lecithin term is habitually used to refer about the lipid mixture. There are a great variety of **lecithin sources** (vegetable, animal and microbiological) but those obtained from **vegetable oils**, in particular, **soybean oil** and sunflower oil with the **92%** and 5% of global lecithin production, respectively, and eggs (1% of lecithin global production), are the most important (Bueschelberguer et al., 2015).

Lecithins are composed mostly by the following PL: PC, phosphatidylethanolamine (**PE**; also known as cephalin), phosphatidylinositol (**PI**), phosphatidyl serine (**PS**) and phosphatidic acid (**PA**), but also other minor phospholipids could be present (van Nieuwenhyzen, 2010). The PL composition of a lecithin depends on the raw materials they are derived from as we can see in Table 1.7. Even when discussing soy lecithin exclusively, the soy variety, the geographic region, weather, storage and processing conditions all have a significant influence on the various quality aspects of lecithin (Jansen, 2015). This is appreciable in Table 1.8., where is summarized different PL compositions of soybean lecithin reported by different authors.

Table 1.7. Composition in polar lipids in different de-oiled lecithins (oil-free basis, %). Adapted from Wendell (2000).

Item (%)	Soy lecithin	Sunflower lecithin	Rapeseed lecithin	Corn lecithin	Egg lecithin	Bovine brain lecithin
PC	21	14	37	31	69	18
PE	22	24	29	3	24	36
PI	19	13	14	16	N.D.	2
PA	10	7	N.D.	9	N.D.	2
PS	1	N.D.	N.D.	1	3	18
Sphingomyelin	N.D.	N.D.	N.D.	N.D.	1	15
Glucolipid	12	N.D.	20	30	N.D.	N.D.

PC= phosphatidylcholine; PE= phosphatidylethanolamine; PI= phosphatidylinositol; PI= phosphatidylinositol; PA = phosphatidic acid; PS= phosphatidylserine; N.D. = not detected/determined.

Fats and oils commonly used in animal feeding consist predominantly of glyceryl esters of FA with some non-glyceridic materials present in small quantities (Baião and Lara, 2005). It is important to highlight that TAG have an energetic function and consist in three FA chains attached to a glycerol. Otherwise, in the case of lecithin, PL consist in a glycerol backbone, a phosphate head group typically found at *sn*-3 position and two FA chains (Bueschelberguer et al., 2015; Cui and Decker, 2016). Phospholipids are present in cell membranes of all living organism, disposed as lipid bilayers playing multiple roles.

The most important function is forming the permeable barrier of the cell membrane and intracellular organelles, but also provide precursors for signaling processes and are the matrix for many catalytic processes (Nelson and Cox, 2014).

The coexistence of lipophilic (FA chains) and hydrophilic (glycerol and phosphate) compounds within a PL molecule makes it surface-active (**emulsifying capacity**). Phospholipids act as emulsifiers because they are able to form a bridge between water-soluble and fat-soluble materials, in other words, they stabilize mixtures of immiscible products. Oils and fats are insoluble in water and do not solubilize in the aqueous phase of the gastrointestinal tract, thus need to be emulsified first by bile secretions. For that reason, some authors have suggested that dietary lecithin can improve fat digestion and absorption (Jones et al., 1992; Ravindran et al., 2016; Siyal et al., 2017b).

Table 1.8. Composition in phospholipids of different soybean lecithins.

	Phospholipids (%):				
References	PC	PE	PI	PA	PS
Scholfield (1981)	19-21	8-20	20-21	N.D.	N.D.
Szuhaj (1989)	12-21	8-9.5	1.7-7	0.2-1.5	0.2
Wendell (2000)	21	22	19	10	1
Helmerich et al. (2003)	23	12	11	7	2
Nieuwenhuyzen et al. (2008)	15	11	10	4	N.D.
Bueschelberger et al. (2015)	9-17	8-15	8-11	3-10	5-10

PC= phosphatidylcholine; PE= phosphatidylethanolamine; PI= phosphatidylinositol; PI= phosphatidylinositol; PS= phosphatidylserine; N.D. = not detected/ determined.

1.5.2. Crude soybean lecithin production

Soybean lecithin is a coproduct extracted during soybean oil refining process, specifically, during the **degumming** step. Vegetable oil refining process is large and complex with a lot of steps where many coproducts and byproducts are produced. Degumming is necessary due to oil refiners need crude oil with low phosphorus content with the aim to perform an efficient refining and to avoid the sedimentation of refined oils (van Nieuwenhuyzen, 2010; Bueschelberger et al., 2015). Crude soybean lecithin is

obtained after several chemical and physical treatments applied to soybean seeds in the crusher plants.

Degumming process (Figure 1.6) is well described in van Nieuwenhuyzen and Tomás (2008) and van Nieuwenhuyzen (2010), a brief summary of their work is described below:

1) Soybeans are first cleaned, dehulled and dried. Foreign material (like stones, glass and metal) is taken out from the process. Then, beans need to be preheated and then **cracked** and **flacked** with the aim to break the vegetable cell membranes and facilitate the oil extraction. This process varies depending on the vegetable source.
2) Next step is based in a **solvent extraction** with hot hexane for obtaining a non-degummed oil rich in PL mixed with the hexane (this mixture is also known as **miscella**). During this solvent extraction, the miscella is separated from the solid phase or "wet cake", which further processed will generate the **protein meals**.

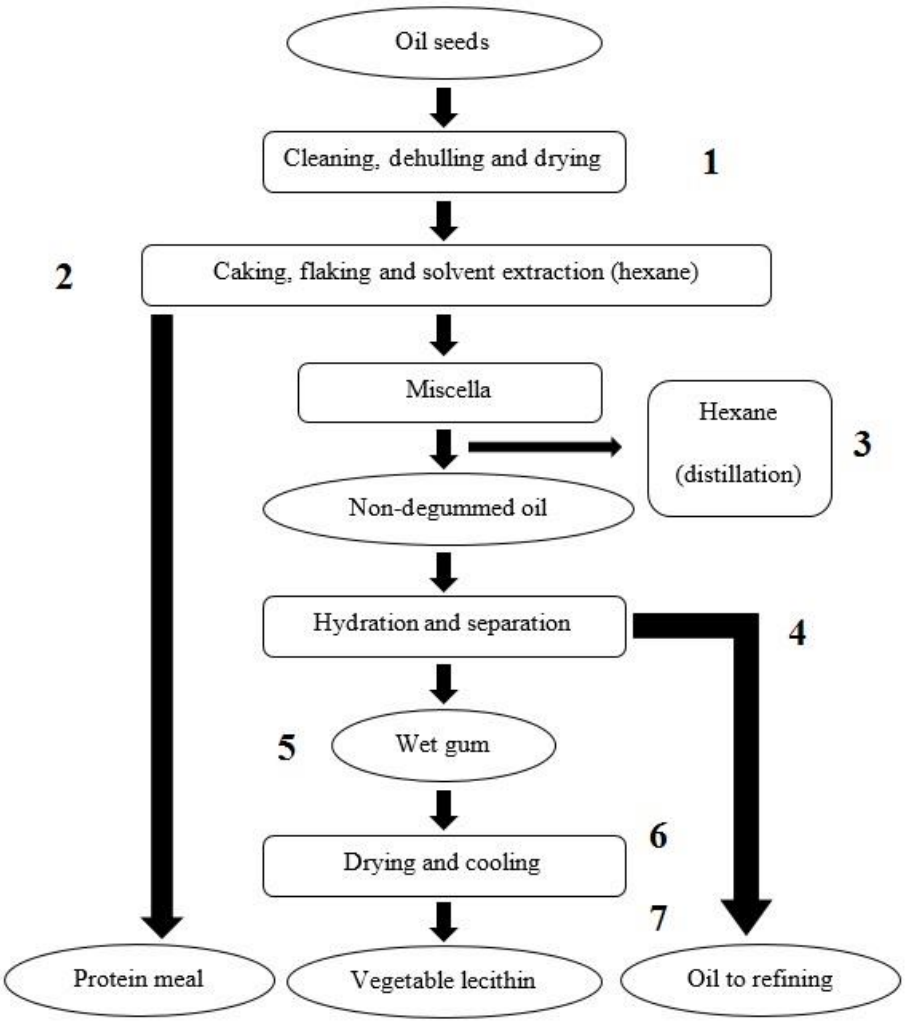


Figure 1.6. Schematic overview of protein meals, refined oil and vegetable lecithin production process.

3) The next step is removing the hexane from the **non-degummed soybean oil** by a sequence of several distillation processes due to its high toxicity.
4) The non-degummed oil must be subjected to a **hydration** of the PL: a 1% to 3% of water is mixed with non-degummed soybean oil at 50-70 ºC. This addition of water influence on the hydratable PL and other polar lipids making them insoluble in the oil. Temperature, water amount added, and mixing time may vary between different vegetable sources. After this step, **oil** is separated from the gums and **will be further refined** in order to reduce its content in FFA and non-desirable material.
5) These PL will form a **wet gum** with a higher density than oil and will be separated by centrifuges within 1 hour. This gum is usually composed by a maximum of 50% of water and a minimum 33% of AI matter. It is important to remark that the wet gums also contain crude oil (< 17%).
6) The next step is **drying** the wet gum using film evaporators. Lecithin is dried until arrive to a < 1% of moisture content, achieving a long-time shelf life and fluidity.
7) The last step is **cooling** the gum for obtaining a **dried lecithin**.

Phospholipids constitute a total of the 0.3%-0.6% of soybean seeds and the 1.5%-3.0% of the crude soybean oil. In other words, one kilogram of crude soybean oil generates approximately 20 to 30 grams of commercial soybean lecithin (Jansen, 2015). The commercial lecithin product with an approximate content of PL between 60 to 70% and the rest composed by crude oil (30 to 40%) is known as unrefined lecithin or **crude lecithin**. The crude oil contained in the crude lecithin can be removed by extraction with acetone (PL are insoluble in acetone) to obtain a product classified as **refined lecithin** (this process will be described in detail further in this chapter).

1.5.3. Refined lecithins

The objective of modifying lecithins consists in the separation of individual compounds, with a wide range of different compositions and functionalities, from the crude lecithin (Bueschelberger, et al., 2015). The process implicates **modifications** in the **lecithin chemical structure**, particularly on PL polar head. Refined lecithins present an improved emulsifying capacity, but, in counterpart, present a higher market price than crude lecithins. These modified products are generally termed as lecithins too, despite of their composition and its applications present several differences regarding to crude

lecithins (Bueschelberger, et al., 2015). As a consequence, it is important to know and understand the particular features of these kind of products.

1.5.3.1. Lysolecithins

Lysolecithins are derived from an **enzymatic hydrolyzation** of crude lecithins by a phospholipase (Joshi et al., 2006). Several phospholipases can catalyze this reaction, but the most commonly used is **phospholipase A_2** (Cabezas et al., 2011). Phospholipase A_2 cleave off a PL at *sn*-2 position, releasing one FFA and a LPL, which is composed by a MAG with a phosphate head in *sn*-3 position and a FA chain at *sn*-1 position (Hasenhuettl, 2008). Like lecithins, lysolecithins are defined as a blend of polar lipids, with LPL as their main component (Jansen, 2015). Lysolecithins have an important commercial interest because are excellent oil-in-water (**O/W**) emulsifiers (Vikbjerb et al., 2006).

1.5.3.2. De-oiled lecithins

De-oiled lecithins are obtained after a **physical fractionation**. Fractionate a lecithin is based on an isolation of one or various components of a crude lecithin, obtaining a uniformed and pure product (Wu and Wang, 2003). Two kinds of physical fractionation are habitually used with two different resultant products: acetone fractionation and alcohol fractionation (van Nieuwenhuyzen, 2010). De-oiling a crude lecithin with acetone (acetone fractionation) is based on PL, in contrast to TAG and FFA, are almost insoluble in acetone. Thus, the process consists in removing the TAG and FFA, obtaining lecithins with an AI matter of 95% (but maintaining the ratios of the PL types).

De-oiled lecithins can be further fractioned with alcohol. Each PL present their own solubility for different solvents, for example, PC is soluble in ethanol while PI not. The differences between PL in the above solvents provide possibilities for isolation, purification and quantification of PL. This is interesting in order to obtain different products with different applications. Phosphatidylcholine, for example, present O/W emulsification properties, but on the contrary, PI present better water-in-oil (W/O) emulsification properties (Joshi et al., 2006).

1.5.3.3. Chemically modified lecithins

Exist a great variety of chemical processes capable to modify lecithins, however, only the most important three are going to be commented in the present dissertation:

a) **Hydroxilation**: This process is based in the addition of hydroxyl groups, using H_2O_2 combined with acetic or lactic acid as a reactive, at the FA unsaturations of the PL. The lecithin resulting from this reaction has an improved water dispersibility with an increased O/W emulsifier ability (List, 2015).
b) **Acetylation**: This process is exclusive of PE. The PE is able to react with acetic-anhydre resulting in acetyl-PE, obtaining an improved O/W emulsifier ability (Nasir et al., 2007).
c) **Hydrogenation**: This process involves the addition of hydrogen to double bonds inside unsaturated FA chains. Hydrogenated lecithins are more stable to FA oxidation and present better emulsifying properties than crude lecithins (Joshi et al., 2006).

1.6. Use of lecithin and its derivatives in animal feeding

The use of lecithins and its derived products is extended in multiple areas, especially for technological, food, feed and pharmaceutical applications.

The chapter section is focused on reviewing the use of lecithin (from multiple raw sources, not only soybean lecithin), and their derivatives (refined lecithins), exclusively in poultry and swine feeding. It is important to highlight, that **not always dietary lecithin inclusion is pursuing the same objectives**, and due to this, the review is organized depending on the main goal attributable to dietary lecithin inclusion.

1.6.1. Lecithin as an alternative energy source

The use of vegetable lecithin, as an **ingredient**, may result interesting as a **replacer** for **conventional fats** due to its high content in energy. Data obtained from **FEDNA (2015)** estimated that crude soybean lecithin (> 60% of AI matter; EFSA, 2016) contains 7,860 kcal/kg of gross energy (**GE**). Furthermore, **FEDNA (2015)** also estimated an AMEn value for crude soybean lecithin in 6,850 and 7,620 kcal/kg for < 20 days-old and 20-days old broiler chickens, respectively. Similarly, **Peña et al. (2014)** concluded that crude soybean lecithin AMEn value for 25-days old broiler was 7,502 kcal/kg (averages values by regression analysis) whereas **Borsatti et al. (2018)** estimated an AMEn value of 7,051 kcal/kg (averages values by regression analysis). On the other hand, soybean oil (mainly composed in TAG) contain higher levels of GE and AMEn (9,350 and 9,000 kcal/kg, respectively; Pérez-Bonilla et al., 2011). However, in **Mateos et al. (2012)**, was suggested that soybean lecithin is capable to improve nutrient absorption and due to this,

can be considered as a possible replacer for conventional fats without impairing animal growth.

Following this line, some **researchers have tried to assess the energetic value of lecithin**, as an alternative dietary ingredient for monogastric livestock animals, and its **effects on performance and energy and nutrients digestibility**.

Regarding the effects of **soybean lecithin** inclusion, as **alternative energy source**, on **poultry performance**, results reported by **Cantor et al. (1997)** indicated that the 50% replacement of the added fats of a broiler diet by soybean lecithin did not modify any performance parameter. On the other hand, other authors have indicated that partial **(Cox et al., 2000)** and total replacement **(Cox et al., 2000**; **Huang et al., 2007)** of the added fats by soybean lecithin reduced the feed intake. In a similar way, **Azman and Ciftci (2004)** reported that partial replacement (25 and 50%) of soybean oil also reduced the feed intake. Moreover, the bibliography seems to indicate an improving effect on the feed conversion ratio (**FCR**) with the partial replacement of the added fats by soybean lecithin **(Cox et al., 2000**; **Huang et al., 2007)**. Following this line, **Mandalawi et al. (2015)** observed in **laying hens**, that the partial and total replacement of pork fat by soybean lecithin also reduced the FCR and improved egg production performance parameters. As a counterpart, **Azman and Ciftci (2004)** indicated a negative effect, associated to soybean lecithin inclusion as a partial replacer for soybean oil in starter diets, on broilers average daily gain (**ADG**).

The use of soybean lecithin in **swine diets**, as **alternative energy source**, and its **effect on performance** has been also assessed. **Jones et al. (1992)** conducted a performance trial, in weaning piglets, using tallow as added fat, partially replacing it by a lecithin and concluding with no significant effects on any performance parameter during the overall period. These findings were also confirmed by **Reis de Souza et al. (1995)**, in weanling piglet diets, with a similar experimental design. Results reported by **Jin et al. (1998)** clearly indicated a positive effect associated to the partial replacement of tallow (10%), as added fat for weaned pigs, by lecithin, due to an enhancement was observed on ADG and FCR. Furthermore, **Soares and Lopez-Bote (2002)** concluded that a 20% replacement of different fats by soybean lecithin (soybean oil, lard and a blend of both fats at 5:1), did not alter weaned pigs performance. Nevertheless, **Øverland et al. (1993a)** and **Øverland et al. (1993b)** observed no positive effects on performance with the addition of a 3% of lecithin in weaned and growing-finishing pigs regarding diets without added fats.

Table 1.9. Effect of replacing different added fats by lecithin, as energy source, on performance parameters and energy and nutrient utilization.

Reference	Specie (Age)	Fat Type (% Inclusion)	Lecithin Type (% Inclusion)	Rpl (%)	Total added fats (%)	Lecithin effect on	
						Performance	Utilization
Jones et al. (1992) *Experiment I*	Weanling piglets (21-28 d)	Tallow, Lard (10)	Lecithin* (1)	10	10	Not studied	DM, GE **(=)** TFD: Tallow **(↑)** and Lard **(↓)**
Jones et al. (1992) *Experiment II*	Weanling piglets (Phase I and II)	Tallow (PI: 10; PII: 5)	Lecithin* (PI: 0.5, 1, 3) (PII: 0.25, 0.5, 1.5)	5, 10, 30	(PI: 10) (PII: 5)	N.S.	PI: TFD **(↑)** DM and GE **(=)**
Jones et al. (1992) *Experiment III*	Weanling piglets (21-56 d)	Tallow (10)	Lecithin* (10)	10	20	ADFI **(↑)**	SFA **(↑)** DM and GE **(=)**
Øverland et al. (1993a)	Weanling piglets (27-62 d)	No (0)	Lecithin* (0, 1, 2, 3)	No (0)	0, 1, 2, 3	N.S.	Not studied
Øverland et al. (1993b)	Growing-finishing pigs (23.2 kg-105 kg)	No (0)	Lecithin* (0, 1, 2, 3)	No (0)	0, 1, 2, 3	N.S.	Not studied
Reis de Souza et al. (1995)	Weanling piglets (21-42 d)	Tallow (6.5 and 8)	Soybean (1.5)	18.75	8	N.S.	TFD **(↑)** DM, OM, GE **(=)**
Blanch et al. (1996)	Adult roosters (1 year)	Tallow (3.8 and 4)	Soybean (0 and 0.2)	5	4	Not studied	C16:0 **(↓)**, Rest of FA **(=)** Energy **(=)**
Cantor et al. (1997)	Broiler chickens (*)	Blend of fats* (2.5 to 5)	Soybean (2.5 to 5)	(*)	5	N.S.	Not studied

*Rpl = level of replacement; * = non-specified; N. S. = no significative (P > 0.05); DM = dry matter; GE = gross energy utilization; TFD =total fat digestibility; ADFI = average daily feed intake; SFA = saturated fatty acids; OM = organic matter.*

Table 1.9. Effect of replacing different added fats by lecithin, as energy source, on performance parameters and utilization. *Continued*.

Reference	Specie (Age)	Fat Type (% Inclusion)	Lecithin Type (% Inclusion)	Rpl (%)	Total added fats (%)	Lecithin effect on	
						Performance	Utilization
Jin et al. (1998)	Weanling piglets	Tallow (10)	Lecithin* (1)	10	10	ADFI, ADG **(↑)** FCR **(↓)**	EE, DM, N **(↑)**
Cox et al. (2000)	Broiler chickens (0-39 d)	No specified (0 to 3.5)	Soybean (0 to 3.5)	0, 25, 50, 75 and 100	2.5 to 3.5	ADFI, FCR **(↓)**	Not studied.
Soares and Lopez-Bote (2002)	Weanling piglets (21-49 d)	Soybean oil, lard, S/L (5)	Soybean (0 and 1)	20	5	N.S.	N.S.
Azman and Ciftci (2004)	Broiler chickens (5-35 d)	Soybean oil (St 2 to 4; Gf 3 to 6)	Soybean (St 0 to 2; Gf 0 to 3)	25 and 50	St 4; Gf: 6	St: ADG **(↓)** St & Gf: ADFI **(↓)**	Not studied
Huang et al. (2007)	Broiler chickens (0-42 d)	Soybean oil (0 to 2)	Soybean (0 to 2)	0, 25, 50 and 100.	2	25% rpl **(↑)** 100% rpl **(↓)**	St DM, CP EE, AME **(↓)**
Cho et al. (2008)	Nursery pigs (9 kg)	Soybean oil (5)	Lecithin*(0.5)	10	5	Not studied	N **(↓)**
Mandalawi et al. (2015)	Laying hens (23-51 wk)	Pork fat (0 to 4)	Soybean (0 to 4)	0, 50, and 100	4	100% rpl EW, EM **(↑)** FCR **(↓)**	50-100% rpl OM, EE, GE **(↑)**

S/L = soybean oil blended with lard; St = starter period; Gf = grower-finisher period; ADG =average daily gain; FCR = feed conversion ratio; N = nitrogen retention; AME = apparent metabolizable energy; CP = crude protein; EW = egg weight; EM = egg mass.

Several researchers have been focused in study the use of lecithin, as a replacer of added fats in poultry and swine diets, and its **effects on energy and nutrient utilization** with the aim to evaluate its **use an alternative energy source** from a nutritional point of view.Available literature in poultry feeding is scarce, only **Huang et al. (2007)** reported that soybean oil partial and total replacements (50% and 100%) by soybean lecithin reduced, in starter diets, dietary AME and the digestibility of dry matter (**DM**) and crude protein (**CP**). However, this negative influence was not observed in grower-finisher chickens, due to total replacement of soybean oil by soybean lecithin did not reduce feed AME value, nor ether extract (**EE**), DM and CP digestibility (**Huang et al., 2007**). Similarly, **Blanch et al. (1996)** concluded that partial replacement of tallow, in **adult rooster** diets, did not reduce energy and fat utilization, however no improvements were observed, as they expected. **Mandalawi et al. (2015)** reported, in **laying hens**, that partial and total pork fat replacement by soybean lecithin improved EE and organic matter (**OM**) digestibility. Regarding lecithin use in **swine diets**, **Jones et al. (1992)** performed two experiments in weanling piglets. In the first trial it was observed that the partial replacement of different added fats by lecithin modified fat digestibility. Total fat digestibility was improved when tallow was partially replaced by lecithin; however, soybean oil and coconut oil showed no effects and the lard replacement reduced fat digestibility. In the second experiment, partial replacements (5, 10 and 30%) of tallow, improved total fat digestibility. Again, these findings were also confirmed by **Reis de Souza et al. (1995)**. Results reported by **Jin et al. (1998)** indicated an improvement on nitrogen retention, DM utilization and EE digestibility due to a partial replacement of tallow by lecithin in weaned pig diets. **Soares and Lopez-Bote (2002)** partially replaced soybean oil, as added fat in weanling pig diets, and confirmed its use as an alternative to soybean oil due to no modifications were observed on nutrient utilization. Finally, **Cho et al. (2008)** observed, in cannulated barrows, that the partial replacement (10%) of soybean oil by a lecithin, reduced nitrogen retention in fecal samples, however, that effect was not observed at the ileum, concluding that lecithin can partially replace soybean oil without modifying energy and nutrient utilization.

Despite the absence of an extensive bibliography, and the existence of some controversial results in poultry and swine, the literature seems to indicate that lecithin, particularly **soybean lecithin**, **may represent an added fat as an alternative for conventional lipid sources**. Furthermore, in general terms, **soybean lecithin inclusion in replacement of other fats**, **does not affect negatively on animal performance**, and

some authors have indicated an **enhancement on nutrient digestibility when its used as a replacer of saturated fat sources**.

1.6.2. Lecithin and lysolecithin as dietary emulsifiers

Phospholipids and LPL are widely used as emulsifier in many applications, especially in food industry (van Nieuwenhuyzen and Tomás, 2008). Thus, several authors have tried to link their **addition** into poultry and swine diets, as an **ingredient** (lecithin) or **additive** (lecithin and lysolecithin), **to evaluate its emulsifying effect** on performance, nutrient absorption and animal health parameters.

The use of **lecithin**, as an **exogenous emulsifier** in **broiler chicken** diets, has been tested by a few authors. **Polin (1980)** demonstrated that a 2% addition of soybean lecithin to broiler diets containing tallow improved total fat digestibility, thus the author suggested a possible emulsifying effect on tallow absorption. These findings were supported by **Abbas et al. (2016)**, who observed that the addition of 0.035% of soybean lecithin in broiler diets containing vegetable oil, improved adult body weight (**BW**), ADG and FCR. Furthermore, also improved DM and EE digestibility. In a similar way, **Siyal et al. (2017a)** reported that the addition of soybean lecithin in three different levels (0%, 0.05%, and 0.1%) to diets containing palm oil allowed improvements on the overall ADG, ADFI and FCR. Moreover, the addition of 0.1% of soybean lecithin improved, in starter diets, the utilization of DM, EE, GE and CP, whereas in adult broilers, an improvement on GE and EE was observed (**Siyal et al., 2017a**). On the contrary, **Jansen (2015)**, in Chapter five, concluded that the addition of a rapeseed lecithin at 0.025% to basal diets containing lard did not improved the final BW and the overall ADFI, ADG and FCR. Furthermore, lecithin addition reduced ileal DM digestibility. Again, **Jansen (2015)**, in Chapter six, observed that the addition of a lecithin at 0.025% as a potential improver of the digestibility of palm oil, in starter broilers, was useless, due to the feed AMEn value and the digestibility of the rest of FA were unaffected.

Regarding the use of **lecithin as an exogenous emulsifier in swine feeding** is controversial. It was concluded in **Øverland et al. (1993a)**, that the addition of a lecithin to soybean oil, in weaned pig diets, did not improved fat and DM digestibility and the performance parameters were unaffected; however, an increase in dietary AME was reported. Contrasting with these findings, **Øverland and Sundstøl (1995)** reported that the addition of soybean lecithin to weaned pig diets containing a rendered fat increased the overall ADG, FCR. **Øverland et al. (1993b)** conducted a performance trial in growing

finishing pigs and observed that the addition of lecithin to diets containing soybean oil allowed an improvement on FCR and the gain/metabolic energy intake. Contrasting, the results observed in adult pigs by **Øverland et al. (1994)** indicated that lecithin addition to diets containing a rendered fat did not produce any interaction on nutrient absorption, thus they concluded that soybean lecithin did not modify fat digestibility.

On the other hand, **lysolecithins** (Table 1.11) have generated a lot of interest during the recent years. The **literature** regarding lysolecithin inclusion on poultry and swine feeding is **huge**, and only a few studies are going to be reviewed here, due to in general, **are consistent** between them. Several authors have demonstrated that the addition of this kind of additives allow improvements on numerous animal production aspects. For example, **Zhao et al. (2015)** reported, in weanling piglets, that the addition of soybean lysolecithin on reduced energy diets containing a tallow improved ADG, FCR, DM and fat digestibility and the AME value of the diets to similar levels of the positive control diets. **Jansen et al. (2015)** performed a 2 x 2 experiment, using soybean oil or lard, as energy source, at 5.30 and 5.81% of total addition, respectively. Then, soybean and rapeseed lysolecithin were added at 0.025% to evaluate its effects on nutrient digestibility. Both lysolecithins were demonstrated useful as emulsifiers due to an improvement on DM digestibility and dietary AMEn of lard diets was observed. In such study, it was concluded that the emulsifying capacity of a lysolecithins is highly dependent of the fat source used, due to saturated fat sources are more enhanced that unsaturated fat sources. **Zampiga et al. (2016)** observed that the addition of a 0.001% of soybean lysolecithin to broiler diets containing soybean oil improved the global FCR, but no effects were observed on nutrient utilization. **Polycarpo et al. (2016)** reported that the addition (0.1%) of a lysolecithin to broiler diets containing tallow as added fat, improved the fat digestibility and FCR in starter and grower diets. It was also observed a lysolecithin effect that improved overall ADG on diets containing either tallow or soybean oil as added fat. Finally, **Boontiam et al. (2017)** added soybean lysolecithin to energy reduced diets (-150 kcal/kg than the positive control) at 0.05, 0.10 and 0.15%. The addition of lysolecithin improved final BW and the overall ADG and ADFI to similar results obtained in the positive control. The global FCR was only improved with 0.15% lysolecithin addition. Regarding nutrient digestibility, lysolecithin addition improved CP and fat digestibility, showing similar levels to positive control.

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Table 1.10. Effect of adding lecithin, as dietary emulsifier, on performance, energy and nutrient utilization and health parameters.

Reference	Specie (Age)	Fat Type (% Inclusion)	Lecithin Type (% Inclusion)	Total added fats (%)	Lecithin effect on		
					Performance	Utilization	Health
Polin et al. (1980)	Broiler chickens (0-21 d)	Tallow (4)	Soybean (0.02, 0.2 and 2)	4.02, 4.2 and 6	Not studied	2% add: TFD **(↑)**	Not studied
Øverland et al. (1993a)	Weanling piglets (21-56 d)	Soybean oil (6)	Lecithin* (0 and 2)	6 and 8	N.S.	AME **(↑)** TFD, DM, N **(=)**	Not studied
Øverland et al. (1993b)	Growing-finishing pigs (23.2 kg-105 kg)	Soybean oil (6)	Lecithin* (0 and 2)	6 and 8	FCR **(↑)** gain/ME intake **(↑)**	Not studied	Not studied
Øverland et al. (1994)	Cannulated barrows (39.8-73 kg)	Rendered fat (6)	Soybean lecithin (0 and 0.24)	0, 0.24, 6 and 6.24	Not studied	N.S.	Not studied
Øverland and Sundstøl (1995)	Weanling piglets (21-56 d)	Rendered fat (6)	Soybean lecithin (0 and 2)	0, 2, 6 and 8	ADG, FCR **(↑)**	AME **(↑)**	Not studied
Jansen (2015) *Chapter Five*	Broiler chicken (0-39 d)	Lard (St:4.9; G:5.71; F:6.22)	Rapeseed (0.025)	St:4.925; G:5.735 F:6.245	N.S.	DM **(↓)** Fat **(=)**	AFP **(=)** Serum **(=)**
Jansen (2015) *Chapter Six*	Broiler chickens (0-7 d)	Palm oil (4)	Lecithin*(0.025)	4.025	Not studied	AMEn, TFD **(=)**	Not studied
Abbas et al. (2016)	Broiler chickens (0-35 d)	Vegetable oil* (1, 2, 3)	Soybean (0.035)	1.035, 2.035 3.035	BW, ADG **(↑)** FCR **(↓)**	DM, EE **(↑)**	Heart Weight **(↑)**

** = non-specified; N. S. = no significative (P > 0.05); DM = dry matter; ADG =average daily gain; FCR = feed conversion ratio; N = nitrogen retention; AME = apparent metabolizable energy; TFD = total fat digestibility; St =starter period; G = grower period; F = finisher period; AFP =abdominal fat pad; EE = ether extract.*

Table 1.10. Effect of adding lecithin, as dietary emulsifier, on performance, energy and nutrient utilization and health parameters. *Continued*.

Reference	Specie (Age)	Fat Type (% Inclusion)	Lecithin Type (% Inclusion)	Total added fats (%)	Lecithin effect on		
					Performance	Utilization	Health
Siyal et al. (2017a)	Broiler chickens (Start: 0-42 d)	Palm oil (St: 5.51; Gf: 4.16)	Soybean (0.0, 0.05, 0.1)	St: 5.51, 5.56, 5.61 Gf: 4.16, 4.21, 4.26	ADG **(↑)**, ADFI, FCR **(↓)**	St 0.1% add: GE, DM, EE, CP **(↑)** Gf 0.1 add: GE, EE **(↑)**	Liver rel. weight **(↑)** Chol, TAG, LDL **(↓)** Antioxidant effect

GE = gross energy; Chol = cholesterol; TAG = triacylglycerols; Gf =grower-finisher.

Table 1.11. Effect of adding lysolecithin, as dietary emulsifier, on performance, energy and nutrient utilization and health parameters. *Continued*.

Reference	Specie (Age)	Fat Type (% Inclusion)	Lysolecithin Type (% Inclusion)	Total added fats (%)	Lysolecithin effect on		
					Performance	Utilization	Health
Zhao et al. (2015)	Weanling piglets	Tallow (3)	Soybean (0.05, 0.1)	3.05, 3.1	0.1% add: ADG **(↑)** 0.1% add: FCR **(↓)**	DM, TFD, AME **(↑)**	Serum TAG **(↓)**
Jansen et al. (2015)	Broiler chickens (0-28 d)	Soybean oil/lard (5.3/5.81)	Soybean, rapeseed (0.025)	5.325/5.835	Not studied	Lard DM, AMEn **(↑)**	Not studied
Zampiga et. al. (2016)	Broiler chickens (0-42 d)	Soybean oil (St: 2.53; GI:2.51; GII:3.43; F:3.61)	Soybean (0.001)	St: 2.531; GI: 2.511; GII: 3.431 F: 3.611	Global period: FCR **(↓)**	N.S.	N.S. (food pad lesions, skin colour)
Polycarpo et al. (2016)	Broiler chickens (0-42 d)	Soybean/Tallow (St & GI:2.7; GII & F:3.15)	Lysolecithin* (0 or 0.1)	St & GI: 2.7 or 2.8 GII & F: 3.15 or 3.25	St & GI T d: FCR **(↓)** Overall: ADG **(↑)**	St & GI T d: TFD **(↑)**	Jejunal microbiota: T d Cocci + **(↓)**
Boontiam et al. (2017)	Broiler chickens (0-21 d)	Soybean oil (St:1; G:2.5; F:2.8)	Soybean (0.05, 0.10, 0.15)	St:1, 1.1, 1.15; G:2.5, 2.6, 2.65; F:2.8, 2.9, 2.95	0.15% add: FCR **(↓)** Global: BW, ADG, ADFI **(↑)**	CP, EE **(↑)**	Morphology **(=)** Inflammation **(↓)** Breast, Leg weight **(↑)**

*DM = dry matter; ADG =average daily gain; FCR = feed conversion ratio; AME = apparent metabolizable energy; TAG = triacylglycerols. N. S. = no significative (P > 0.05); * = non-specified; TFD = total fat digestibility; St: starter period; GI = grower I period; GI = grower II period; GII = grower II period; F = finisher period; G = grower period; T d = tallow diets; BW = body weight; CP =crude protein; EE = ether extract.*

The use of **lecithin as dietary emulsifier** in poultry and swine **is controverted**, due the lack of an extensive bibliography and the existence of some contradictory results. However, the use of **lysolecithins as additive** seem to clearly **improve several aspects related to animal production**

1.6.3. Lecithin as essential nutrient supplier

Lecithins are particularly rich in essential nutrients as **linoleic acid**, **phosphor**, **choline**, **vitamin E** and **inositol** (Mateos et al., 2012).

Choline chloride is habitually used as the main source for choline supply in conventional animal feeding. However, choline chloride is an acidic substance and it can cause corrosion damage on feeding technology and destroy dietary nutrients and vitamins by oxidation. Some authors have suggested the possibility of using **soybean lecithin** as **alternative choline source** in order to avoid this problematic. It has been demonstrated, in poultry and swine, that crude soybean lecithin is well utilized as synthetic choline chloride do **(Lipstein et al., 1976; Kuhn et al., 1998)**. Similarly, **Emmert et al. (1996)** demonstrated that a de-oiled soybean lecithin, in broiler chicken diets, was an efficient replacer for choline chloride.

Finally, crude soybean lecithin high content in vitamin E could play a role as antioxidant in the animal feed (**FEDNA, 2015; Judde et al, 2003**). In addition, PL can act as antioxidants through chelating prooxidative metals and forming antioxidative Maillard reaction products (**Cui and Decker, 2016**).

Chapter Two

Background, hypothesis and objectives

"Las tres frases cortas que sacaran tu vida adelante:

la primera, no digas que he sido yo;

la segunda, ¡oh, buena idea jefe!;

y la tercera, ¡estaba así cuando llegué!"

Homer Simpson, en *Los Simpsons*, 1991

As seen in the literature review, oils and fats have a vital importance as energetic ingredients in broiler chicken diets. However, the energetic value of a lipid source is highly variable and depends on many factors. Furthermore, the price and availability of most common commodities used as dietary ingredients is extremely volatile, thus, feed producers are interested in increasing the range of possible ingredients. Based on this context, coproducts derived from vegetable oil chemical refining may represent an interesting alternative.

The present PhD dissertation is part of a project with a public grant from the *Ministerio de Agricultura, Alimentación y Medio Ambiente* of the Spanish Government given to livestock producer groups (*Orden AAA/1229/2015*). The mentioned project was focused on the inclusion of soybean lecithin in broiler chicken diets with the objective to reduce costs production.

Soybean lecithin is an important source of energy and essential nutrients, such as linoleic acid, phosphor, choline, inositol and vitamin E. In addition, several authors have suggested the possibility that its elevated phospholipid content is capable to improve lipid absorption in young chicks. However, previous published studies using soybean lecithin as energy source in monogastric feeding are scarce and inconsistent, thus it is necessary to perform an extensive evaluation about its inclusion. Therefore, it was **hypothesized** that soybean lecithin is an alternative energy source to soybean oil for broiler feeding, capable to maintain energy and nutrient utilization, performance efficiency, and carcass lipid composition.

For all the reasons above, the **general aim** of the present thesis is to investigate the **potential use of soybean lecithin as energy source for broiler chicken diets**. The specific objectives are:

1. To evaluate the replacement and combination of soybean oil by soybean lecithin, in starter and grower-finisher broiler chicken diets, and its effects on performance parameters, energy utilization, fatty acid (**FA**) digestibility and the FA profile of the abdominal fat pad (**AFP**).

2. To evaluate the replacement and combination of acid oil by soybean lecithin, in starter and grower-finisher broiler chicken diets, and its effects on performance parameters, energy utilization, FA digestibility, and the FA profile of the AFP.

3. To study the best strategy to combine soybean lecithin with soybean oil as well as acid oil, in grower and finisher broiler chicken diets, in terms of growth efficiency, FA digestibility, gut health and the FA profile of the AFP.

In order to accomplish these objectives, several experiments were performed using increasing inclusion levels of soybean lecithin in starter and grower-finisher broiler chicken diets.

Two trials were performed in parallel, using soybean oil (Experiment 1) or acid oil (Experiment 2) at 3% and both were gradually replaced by soybean lecithin (1, 2 and 3%). Crude soybean lecithin was used in the two experiments of Chapter Three, whereas soybean lecithin high in free fatty acids was used in the two experiments of Chapter Four. During all the experiments two digestibility balances were performed (between day 9 to 11 and day 36 to 37) in order to determine the feed apparent metabolizable value and the FA digestibility. Moreover, performance parameters were measured, and the FA profile of the AFP was analyzed.

Finally, a field trial was performed under experimental conditions (Chapter Five), taken in account the previous results obtained, in order to study different levels of soybean lecithin inclusion in grower and finisher broiler chicken diets. The main objective of the experiment was based on evaluating the effects on productive performance; however, ileal FA digestibility, gut health and the FA profile of the AFP were assessed too.

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Chapter Three

Crude soybean lecithin as alternative energy source for broiler chicken diets

"You have power over your mind - not outside events.

Realize this, and you will find strength."

Marcus Aurelius, in *Meditations*, 170-180.

Crude soybean lecithin as alternative energy source for broiler chicken diets

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Poultry Science (Accepted May 18, 2019)

DOI: 10.3382/ps/pez318

3.1. Abstract

Two experiments were conducted to evaluate the use of crude soybean lecithin (L) as an alternative energy source in broiler feeding and to study its influence on performance, fatty acid (FA) digestibility between 9 to 11 d and 36 to 37 d, feed AME content and the FA profile of the abdominal fat pad (AFP). A basal diet was supplemented at 3% with soybean oil (S; Experiment 1) or a monounsaturated vegetable acid oil (A; Experiment 2) and increasing amounts of L (1%, 2% and 3%) were included in replacement. The inclusion of L did not modify performance results ($P > 0.05$). In starter diets, the replacement of S by L reduced feed AME content ($P < 0.001$) and lowered PUFA digestibility ($P = 0.028$), whereas in the grower-finisher phase, a blend of 2% of S and 1% of L did not modify feed AME content or FA digestibility. When L was included instead of A, no effects on feed AME value and total FA digestibility ($P > 0.05$) were shown in the starter phase, whereas in grower-finisher diets, a blending of 2% of A and 1% of L enhanced feed AME content ($P < 0.001$) and total FA digestibility ($P = 0.001$). The FA profile of the AFP reflected the FA composition of the diets. Crude soybean lecithin represents an alternative energy source for broiler chickens, and it can be used in growing-finishing diets in replacement of 1% S. The best option to include both alternative fats (L and A) was 2% of L with 1% of A in starter diets and 1% of L with 2% of A in grower-finisher diets because showed positive synergic effects. The results suggest that dietary FA profile have a bigger impact on the AFP saturation degree than the different dietary lipid molecular structures.

3.2. Introduction

Fat inclusion in broiler feeding is a widespread activity in aviculture which allows the reaching of the high energetic requirements of fast-growing birds. Fat inclusion also presents other positive features, such as essential fatty acids and vitamins supply, slowing the passage rate and lubricating the feed milling equipment, among others (Ravindran et al., 2016). The price of conventional added fat sources in broiler feeding has been increasing in the last few years, in part by the rising demand of vegetable fats for biodiesel production and, according to soybean oil current forecasts, this trend is going to be maintained in the following years (Statista, 2018). This context explains why there is an increasing interest in the search and the use of alternative energy sources in broiler feeding in order to reduce production costs. Co-products derived from the vegetable oil

refining process represent an attractive alternative to conventional energetic sources due to their competitive price and the possibility to recycle products in order to avoid environmental contamination.

A great variety of lecithin sources (vegetable and animal) exists, but those obtained from soybean seeds are the most relevant in terms of applications and worldwide production (Cui and Decker, 2016). Crude soybean lecithin is obtained prior to the refining process during the degumming step and consists of a lipid mixture mainly composed of polar lipids (> 60%), in particular phospholipids (**PL**), and, to a lesser extent, of neutral lipids such as triacylglycerols (**TAG**; Van Nieuwenhuyzen and Tomás, 2008). The chemical structure of PL consists of a sn-1,2-diacylglycerol backbone with two fatty acid (**FA**) chains and a phosphate head group bound to a functional moiety (choline, ethanolamine and inositol, among others) at the sn-3 position (Bueschelberguer et al., 2015). The presence of both hydrophilic (FA chains) and lipophilic (glycerol, phosphorus and the functional moiety) components confers to lecithin emulsifying properties, giving many applications to these kinds of co-products. Although crude soybean lecithin represents an economic alternative and an important source of gross energy (**GE**), phosphorus, choline, linoleic and linolenic acid, there is not enough information available to recommend its use in broiler chickens.

On the other hand, vegetable acid oils are co-products derived from the crude vegetable oil refining process. These co-products are normally obtained by treating crude oil through an alkali reaction (chemical refining) with the aim to reduce free fatty acid (**FFA**) content and other impurities (Baião and Lara, 2005). In other words, acid oils are characterized by a high content in FFA (40%-60%), a variable proportion of TAG, diacylglycerols and monoacylglycerols, and a similar FA profile to their corresponding crude oils (Roll et al., 2018). In addition, their high content in GE gives an interesting and economic relevance to acid oils for all kinds of poultry species (Mateos et al., 2012).

The present study has been carried out in order to determine the potential use of crude soybean lecithin as an energy source in broiler feeding. The aim of the current work is to evaluate the impact of crude soybean lecithin dietary supplementation and its combination with other fats (soybean oil as a conventional source or a monounsaturated vegetable acid oil as an alternative source) on performance parameters, feed AME content, FA digestibility, and the FA profile of the abdominal fat pad (**AFP**).

3.3. Material and methods

The experiments were performed at *Servei de Granges i Camps Experimentals* (Universitat Autònoma de Barcelona, Bellaterra, Spain). These experimental procedures were approved by the Animal Ethics Committee of the Universitat Autònoma de Barcelona (CEEAH) and were in accordance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010).

3.3.1. Experimental design and diets

Two trials of 38 days (**d**) were performed with a feeding programme in two phases: starter (from 0 d to 21 d) and grower-finisher (from 22 d to 38 d). Diets were presented in mash form, and the wheat- and soybean-meal-based diets were formulated to meet or exceed FEDNA (2008) requirements, as is seen in **Table 3.1**. In addition, titanium dioxide (**TiO_2**) was added as an indigestible marker at 0.5%.

Experiment 1. A total of 96 Ross 308 newly hatched female broiler chickens were randomly allocated in metabolic cages (4 birds per cage) and assigned to one of 4 experimental treatments (6 replicates per treatment). The experimental diets were the result of a basal diet supplemented at 3% with different proportions of soybean oil (**S**) and crude soybean lecithin (**L**). The S3 treatment included S at 3% and was gradually replaced by L: S2-L1 (2% of S and 1% of L), S1-L2 (1% of S and 2% of L) and L3 (L at 3%).

Experiment 2. A total of 120 Ross 308 newly hatched female broiler chickens were allocated in metabolic cages (4 birds per cage) and assigned to one of 5 experimental treatments (6 replicates per treatment). The experimental diets consisted of a basal diet supplemented with 3% of different fat sources. A monounsaturated vegetable acid oil (**A**; a blending 50:50 of olive pomace acid oil and sunflower acid oil) was included at 3% in treatment A3 and increasing amounts of L were added in replacement of A: A2-L1 (2% of A and 1% of L), A1-L2 (1% of A and 2% of L) and L3 (L at 3%). The S3 diet was included as a reference treatment.

Table 3.1. Ingredient composition of the starter and grower-finisher broiler chicken diets, as-fed basis (Experiments 1 and 2).

	Experiment 1		Experiment 2	
Ingredients (%)	Starter diet (0 to 21 days)	Grower-finisher diet (22 to 38 days)	Starter diet (0 to 21 days)	Grower-finisher diet (22 to 38 days)
Wheat	36.55	46.84	36.64	45.92
Soybean meal 47%	29.43	21.09	30.46	24.25
Corn	9.71	-	9.71	-
Barley	9.71	15.58	8.33	15.76
Extruded full-fat soybean	4.76	-	4.73	-
Added fats[1]	3.00	3.00	3.00	3.00
Rapeseed meal 00	-	3.42	-	3.41
Sunflower meal 28%	-	2.44	-	-
Sepiolite	1.93	1.90	2.03	2.03
Palm oil	-	1.50	-	1.51
Calcium carbonate	1.19	1.08	1.16	1.00
Monocalcium phosphate	0.97	0.57	0.93	0.48
Trace mineral-vitamin premix[2]	1.15	1.01	1.44	1.17
Titanium dioxide	0.50	0.50	0.50	0.50
Salt	0.30	0.23	0.30	0.23
L-lysine	0.30	0.35	0.28	0.28
DL-methionine	0.28	0.21	0.28	0.22
L- threonine	0.08	0.09	0.07	0.07
Sodic bicarbonate	0.07	0.12	0.07	0.11
Clorure choline 75%	0.07	0.07	0.07	0.06

[1] *Soybean oil, crude soybean lecithin and acid oil in different blending proportions.*

[2] *Provides per kg feed: vitamin A (from retinol), 13,500 IU; vitamin D3 (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 4.5 mg; vitamin B12, 16.5 µg; vitamin K3, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from FeSO4·7H2O), 54 mg; I [from Ca(I2O3)2], 1.2 mg; Co (from 2CoCO3·3Co(OH)2·H2O), 0.6 mg; Cu (from CuSO4·5H2O), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na2SeO3), 0.18 mg; Mo [from (NH4)6Mo7O24], 1.2 mg; Organic acids (starter diets at 4 g/kg; grower-finisher diets at 3 g/kg); β-glucanase 350 IU; xylanase 1,125 IU.*

3.3.2. Animal husbandry and controls

The animals were obtained from a local hatchery (Pondex S.A.U.; Juneda, Spain), weighed, wing-tagged and randomly allocated in metabolic cages with a grid floor and a tray for excreta collection. Birds were allowed to consume both feed and water *ad libitum* in an environmentally controlled room. The temperature and light programme used was consistent with the specifications in the Ross 308 lineage management handbook (Aviagen, 2014). Twice daily, animals and housing facilities were inspected for the general health status, constant feed and water supply, as well as temperature and ventilation.

Broiler bodyweight (**BW**) was recorded individually at the d of hatching and d 21 and 38 post-hatch, whereas feed intake was measured by replicate (cage) on d 21 and 38 post-hatch. The data were used to measure BW and calculate the average daily gain (**ADG**), average daily feed intake (**ADFI**), the feed conversion ratio (**FCR**) of each period and the global results of the experiments. Mortality was recorded daily to adjust ADFI and ADG. Two nutritional balances were performed for each experiment between d 9 to 11 (starter period) and d 36 to 37 (grower-finisher period), and excreta samples (free of contaminants such as feed, scales and feathers) were taken each day of the digestibility balance. The excreta samples were homogenized, freeze-dried, ground and kept at 4ºC until further analysis. On d 21, one bird per replicate was euthanized by cervical dislocation due to stocking density reasons. At the end of the experiment, broiler chickens were fasted for 3h, stunned, slaughtered, bled, plucked, and chilled at 4ºC for 12h in a commercial slaughterhouse (GIMAVE S.A.; Ripollet, Spain), and carcasses were recovered for further study. Carcasses (total BW excluding blood and feathers) were weighed, and AFP (from the proventriculus surrounding the gizzard down to the cloaca) of each bird was removed and weighed in order to calculate carcass yield and the AFP carcass percentage. Finally, a representative sample of AFP of each bird was taken, pooled by replicate, frozen at -20ºC and analysed to determine the FA profile.

3.3.3. Laboratory analyses

Experimental feed samples were taken at the beginning and end of each experimental period and were ground and kept at 4ºC until further analysis.

Table 3.2. Chemical analysis of the fat sources[1] included in the experimental diets of Experiments 1 and 2.

	Experimental fats				
	Experiment 1		**Experiment 2**		
Item	S	L	S	A	L
Gross energy (kcal/kg)	9,396	7,952	9,621	9,429	8,105
Fatty acid composition (%)					
SFA	16.56	18.00	16.04	15.05	21.90
C16:0	11.65	14.21	10.63	9.97	17.84
C18:0	3.55	3.79	4.26	3.84	4.06
MUFA	24.25	29.70	23.51	54.22	22.69
C18:1 ω-9	22.29	29.70	21.81	51.25	22.69
PUFA	59.19	52.30	60.45	30.73	55.41
C18:2 ω-6	53.43	46.52	52.78	29.18	50.95
C18:3 ω-3	5.76	5.78	7.67	1.55	4.46
Minor FA	3.32	N.A.	2.85	4.21	N.A.
UFA:SFA	5.04	4.55	5.23	5.60	3.56
PUFA:SFA	3.57	2.91	3.77	2.04	2.53
Acidity (%)[2]					
FFA	2.41	13.22	1.49	52.92	14.48
Phospholipids (%)[2]					
Acetone insoluble	N.A.	62.70	N.A.	N.A.	60.10
PC	N.A.	15.88	N.A.	N.A.	12.54
PI	N.A.	10.57	N.A.	N.A.	10.28
PE	N.A.	7.79	N.A.	N.A.	6.14
AP	N.A.	3.52	N.A.	N.A.	4.83
LPC	N.A.	1.23	N.A.	N.A.	1.08

[1] S = soybean oil; L = crude soybean lecithin; A = acid oil.

[2] Percentage in total product.

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio; PC = phosphatidylcholine; PI = phosphatidylinositol; PE = phosphatidylethanolamine; AP = phosphatidic acid; LPC = lysophosphatidylcholine; FFA = free fatty acids; N.A.= not analysed.

Diet proximate analyses were performed according to the methods of AOAC International (2005): ether extract (**EE)** by the Soxhlet analysis (method 920.39), crude protein (Method 968.06) and crude fiber (Method 962.09). In addition, feed and excreta samples analyses included ash determination (Method 942.05), dry matter (Method

934.01), and GE content by adiabatic bomb calorimeter (IKA-Kalorimeter system C4000; Staufen, Germany. The inert marker was determined in feed and excreta by different ways in each experiment: in Experiment 1, TiO_2 was determined by spectrophotometry ICP-OES (Optima 3200 RL, Perkin Elmer; Waltham, MA, USA), while in Experiment 2, TiO_2 was determined by using the method described by Short et al. (1996).

Oil samples (soybean oil, monounsaturated acid oil and crude soybean lecithin) were analysed in duplicate for FA composition by gas chromatography according to the method described by Guardiola et al. (1994). In addition, the acid value was determined according to ISO 660 (2009) method and the acidity was inidcated as the FFA percentage by expressed in oleic acid. Regarding PL content of the two batches of crude soybean lecithin used, acetone insoluble determination was performed following the Ja 4-46 analytical method from AOCS (2017), and the PL composition was determined by HPLC (D450 MT1, Kontron, Eching, Germany) following the method described by Helmerich and Koehler (2003). In the case of feed and excreta, FA content was analysed adding nonadecanoic acid (Sigma–Aldrich Chemical Co.; St. Louis, MO) as an internal standard and following the method described by Sukhija and Palmquist (1988), whereas in the case of AFP, the method described by Carrapiso et al. (2000) was used. The final extract obtained was injected in a gas chromatograph (HP6890, Agilent Technologies, Waldbronn, Germany) following the method conditions previously described by Cortinas et al. (2004). The FA were identified by matching their retention times with those of their relative standards (Supelco 37 component FAME Mix; Sigma-Aldrich Co., St. Louis, MO) and quantified by internal normalization. Nonadecanoic acid was used for the calibration curves and quantification of FA.

3.3.4. Calculations and statistical analysis

Apparent digestibility of FA (%) in excreta was calculated by the index method using the following equation:

$$Apparent\ digestibility\ of\ nutrient = 1 - \{(TiO_2/N)_d / (TiO_2/N)_e\}$$

Where $(TiO_2/N)_d$ is the concentration of the inert marker and the nutrient in the diet, and $(TiO_2/N)_e$ is the concentration of the inert marker and the nutrient in the excreta. The AME of the diets was calculated by the following equation:

$$AME\ (kcal/kg) = Apparent\ digestibility\ of\ GE\ (\%) * GE\ of\ the\ diet.$$

Cage means were used as the experimental unit (6 replicates/treatment) in performance parameters (except BW), FA digestibility, FA profile of the AFP and AME values of the

diets. Data were analysed by one-way ANOVA using R Statistics (version 3.3.1), with treatment as the main factor. Tukey's multiple range test was performed to determine whether means were significantly different ($P \leq 0.05$). In Experiment 2, soybean oil treatment (S3) was compared against A3 treatment separately with one-way ANOVA.

3.4. Results and discussion

3.4.1. Characterization of experimental fats and diets

The chemical analyses of the fat sources included in the diets of both experiments are presented in **Table 3.2**. Regarding average FA profile, S and L were characterized by a high content in linoleic (S = 53.11%; L = 48.74%) and oleic acid (S = 22.05%; L = 26.20%). In general, L showed a higher palmitic acid concentration (16.02%) in comparison with S (11.14%). The composition results agreed with data reported in the literature for both fat sources (Soares and Lopez-Bote, 2002; FEDNA, 2015). On the other hand, A (Experiment 2) was mainly composed of MUFA, in particular oleic acid (51.25%), but also contained linoleic (29.18%) and palmitic acid (9.97%). The average unsaturated-to-saturated FA ratio (**UFA:SFA**) was lower for L (4.06) than for S and A (S = 5.14; A = 5.60), whereas the average polyunsaturated-to-saturated FA ratio (**PUFA:SFA**) was higher for S (3.67) than for L and A (L = 2.72; A = 2.04).

Regarding added fats acidity (**Table 3.2**), S presented a lower content average of FFA (1.95%) than L (13.85%), whereas A was mainly composed of FFA (52.92%). In addition, L presented higher levels of PL (> 38%), where phoshatidylcholine, phosphatidylinositol and phosphatidylethanolamine were the most abundant. The L composition is according to results provided by Van Nieuwenhuyzen and Tomás, 2008. However, it is important to highlight that the lecithin FA profile and PL content is highly dependent on the raw materials they are derived from (vegetable or animal source) and, even within crude soybean lecithin, the soy variety, geographic region, weather, storage and processing conditions have an important influence on the various composition aspects of lecithin (Nguyen et al., 2014). In addition, the GE content was markedly different among the three fats sources included in the diets, S and A presented higher GE values (S: 9,396-9,621 kcal/kg; A: 9,429 kcal/kg) than L (7,952-8,105 kcal/kg), justified by the lower heat combustion provided by PL in comparison with TAG and FFA.

Table 3.3. Analysed[1]gross energy, macronutrient content and fatty acid composition for starter and grower-finisher diets[2] (Experiment 1).

	Starter diet (from 0 to 21 d)				Grower-finisher (from 22 to 38 d)			
Item	S3	S2-L1	S1-L2	L3	S3	S2-L1	S1-L2	L3
Gross energy (kcal/kg)	4,159	4,121	4,111	4,059	4,165	4,135	4,121	4,125
Macronutrient content (%)								
Dry matter	91.79	91.24	91.22	91.14	90.85	90.62	90.62	91.15
Crude protein	23.66	22.99	22.95	23.54	21.77	20.62	22.25	21.21
Ash	8.43	8.98	8.86	9.47	8.89	8.25	9.43	8.83
Crude Fat	5.42	5.49	5.45	5.18	6.33	5.95	5.98	6.08
Crude Fiber	3.83	4.43	4.28	3.91	4.90	5.14	4.84	4.26
Fatty acid composition (%)								
SFA	18.18	18.83	18.98	19.25	25.02	24.44	26.85	28.60
C16:0	13.91	14.24	14.75	15.10	20.67	20.17	22.49	24.18
C18:0	3.53	3.85	3.85	3.77	3.43	3.35	3.44	3.46
MUFA	21.08	20.98	21.12	21.11	26.37	25.25	26.88	27.50
C18:1 ω-9	19.43	19.35	19.47	19.47	24.49	23.47	25.05	25.68
PUFA	60.74	60.19	59.90	59.64	48.61	50.31	46.27	43.90
C18:2 ω-6	54.65	54.08	53.42	53.00	44.14	45.49	41.90	39.74
C18:3 ω-3	6.09	6.11	6.14	6.31	4.72	4.98	4.61	4.41
Minor fatty acids	2.39	2.37	2.37	2.35	2.55	2.54	2.51	2.53
UFA:SFA	4.50	4.31	4.27	4.20	3.00	3.09	2.72	2.50
PUFA:SFA	3.34	3.20	3.16	3.10	1.94	2.06	1.72	1.53

[1] All samples were analysed twice.

[2] S3 = soybean oil (S) at 3.00%; S2-L1 = S at 2.00% and crude soybean lecithin (L) at 1.00%; S1-L2 = S at 1.00% and L at 2.00%; L3 = L at 3.00%.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio.

Table 3.4. Analysed[1] gross energy, macronutrient content, and fatty acid composition for starter and the grower-finisher diets[2] (Experiment 2).

	Starter diet (from 0 to 21 d)					Grower-finisher (from 22 to 38 d)				
Item	S3	A3	A2-L1	A1-L2	L3	S3	A3	A2-L1	A1-L2	L3
Gross energy (kcal/kg)	4,124	4,122	4,083	4,063	3,996	4,198	4,194	4,195	4,172	4,119
Macronutrient content (%)										
Dry matter	91.78	91.78	91.24	91.44	91.14	90.86	90.89	91.26	91.18	90.75
Crude protein	22.32	22.23	21.98	22.33	22.87	22.09	21.25	20.67	21.17	20.38
Ash	8.50	8.36	8.27	8.60	9.24	9.86	9.21	10.31	10.48	10.13
Crude Fat	5.46	5.33	5.03	5.30	5.10	6.60	6.60	6.33	6.66	5.87
Crude Fibre	4.38	3.84	3.83	4.67	3.48	4.62	4.17	4.09	3.34	4.57
Fatty acid composition (%)										
SFA	17.21	17.94	18.65	19.15	19.81	23.66	24.71	25.44	26.44	27.80
C16:0	13.51	14.28	14.97	15.68	16.47	19.44	20.48	21.31	22.33	23.75
C18:0	3.70	3.66	3.68	3.47	3.35	3.96	3.95	3.85	3.82	3.74
MUFA	22.11	34.96	30.58	26.18	21.11	27.06	40.10	35.92	31.66	27.32
C18:1 ω-9	20.72	33.51	29.14	24.74	19.68	25.55	38.27	34.22	30.13	25.81
PUFA	60.68	47.10	50.77	54.67	59.08	49.28	35.19	38.64	41.90	44.88
C18:2 ω-6	54.13	43.36	46.57	49.98	53.77	43.72	32.46	35.50	38.28	40.84
C18:3 ω-3	6.55	3.74	4.20	4.69	5.31	5.56	2.73	3.14	3.61	4.04
Minor fatty acids	1.39	1.45	1.44	1.44	1.42	1.77	2.11	1.98	1.83	1.82
UFA:SFA	4.81	4.58	4.36	4.22	4.05	3.23	3.05	2.93	2.78	2.60
PUFA:SFA	3.53	2.63	2.72	2.85	2.98	2.08	1.42	1.52	1.58	1.61

[1] All samples were analysed twice.

[2] S3= soybean oil at 3.00%; A3= acid oil (A) at 3.00%; A2-L1= A at 2.00% and crude soybean lecithin (L) at 1.00%; A1-L2= A at 1.00% and L at 2.00%; L3= L at 3.00%.

SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio.

Chemical analysis of diets from Experiment 1 (**Table 3.3**) showed that as S was replaced by L, dietary SFA increased, in particular palmitic acid, whereas PUFA concentration decreased and, consequently, the UFA:SFA ratio decreased. Similar modifications on the FA profile were observed in piglet diets by Soares and Lopez-Bote (2002). They reported that a partial replacement of soybean oil by crude soybean lecithin (5% of S inclusion versus 4% of S plus 1% of L) decreased the UFA:SFA ratio from 4.93 to 4.31. On the other hand, in Experiment 2 (**Table 3.4**), an increasing incorporation of L in replacement of A led to a reduction in dietary MUFA concentration and increased dietary SFA and PUFA content, and, consequently, the dietary UFA:SFA ratio decreased and the PUFA:SFA ratio increased slightly. The addition of L at the expense of A modified the FA diet profile to similar contents of MUFA and PUFA present in diet S3. Regarding S3 treatment, results obtained showed a similar FA profile in both experiments.

3.4.2. Growth performance and abdominal fat deposition

The trial was successfully carried out and animals showed good health throughout the entire study. The effect of the different dietary fat sources on growth performance in the starter (from d 0 to 21), the grower-finisher (from d 22 to 38) and the global (from d 0 to 38) periods, and abdominal fat deposition are presented in **Table 3.5** (Experiments 1 and 2).

The incorporation of L in replacement of S, in Experiment 1, did not modify any performance parameter in any phase nor in the global period ($P > 0.05$). In the case of Experiment 2, no differences were observed on growth performance among those experimental treatments with co-products as energy sources (A and L) in any feeding phase or the global period of the experiment ($P > 0.05$). Moreover, animals fed S3 obtained better FCR in the grower-finisher phase and the global period of the experiment when compared to those fed A3 ($P \leq 0.05$). Regarding the effect of L supplementation on growth performance, as an alternative to S, our findings agree with the results of Azman and Ciftci (2004). They observed that the partial replacement (50:50) of soybean oil by soybean lecithin (total added fat inclusion, 4% for starter and 6% for grower-finisher diets) did not modify BW (35 d) and global FCR.

Table 3.5. Growth performance and abdominal fat deposition of broiler chickens according to different dietary added fats[1] (Experiments 1 and 2).

	Experiment 1						Experiment 2						
Item	S3	S2-L1	S1-L2	L3	RSE	P-value	S3[2]	A3	A2-L1	A1-L2	L3	RSE	P-value
From 0 to 21 days													
BW at 0 days (g)	43.9	42.9	43.0	43.0	2.67	0.996	45.1	45.2	45.1	45.1	45.10	2.47	0.999
BW at 21 days (g)	834	827	850	818	81.6	0.580	876	878	871	863	834	104.0	0.602
ADFI (g/d/bird)	54.5	55.1	54.6	53.0	2.74	0.585	56.2	57.1	54.8	57.0	56.9	3.79	0.677
ADG (g/d/bird)	37.8	37.6	37.5	37.2	2.02	0.956	39.6	39.7	39.3	39.0	37.4	3.29	0.649
FCR (g/g)	1.42	1.46	1.42	1.43	0.045	0.286	1.40	1.44	1.40	1.47	1.53	0.084	0.127
From 22 to 38 days													
BW at 38 days (g)	2,432	2,442	2,420	2,342	178.2	0.333	2,469	2,395	2,487	2,405	2,367	253.6	0.576
ADFI (g/d/bird)	165.4	169.1	166.2	159.3	10.77	0.473	163.5	160.9	164.4	163.4	164.0	8.49	0.896
ADG (g/d/bird)	92.7	93.4	90.8	88.6	5.47	0.448	91.7	87.8	93.4	89.3	88.8	7.26	0.563
FCR (g/g)	1.78	1.81	1.80	1.80	0.078	0.940	1.78^{x}	1.86^{y}	1.77	1.83	1.81	0.099	0.600
From 0 to 38 days													
ADFI (g/d/bird)	103.6	106.1	104.5	100.6	5.84	0.436	104.2	103.5	103.8	104.6	104.8	5.12	0.968
ADG (g/d/bird)	62.4	63.8	60.6	60.0	3.16	0.185	62.7	61.2	63.5	61.3	61.5	4.52	0.788
FCR (g/g)	1.66	1.66	1.69	1.68	0.045	0.666	1.66^{x}	1.71^{y}	1.64	1.71	1.68	0.078	0.401
Carcass weight (g)	2,169	2,184	2,175	2,100	81.7	0.309	2,229	2,183	2,247	2,194	2,133	151.8	0.641
Abdominal fat depot													
g	45.4	37.3	38.7	37.4	6.46	0.143	43.9	40.6	44.2	41.6	40.1	4.00	0.379
(%)	2.07	1.65	1.79	1.77	0.274	0.083	1.97	1.88	1.99	2.04	1.89	0.214	0.554

^{x,y} ANOVA A3 *vs* S3: Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[1] S3 = soybean oil (S) at 3.00%; S2-L1 = S at 2.00% and crude soybean lecithin (L) at 1.00%; S1-L2 = S at 1.00% and L at 2.00%; L3 = L at 3.00%; A3= acid oil (A) at 3.00%; A2-L1= AO at 2.00% and L at 1.00%; A1-L2= A at 1.00% and L at 2.00%.

[2] S3 was not included in the statistical analysis against diets containing co-products.

BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; RSE = residual standard error.

In contrast with our results, Huang et al. (2007) reported that a 75:25 soybean oil-soybean lecithin blending proportion (2% of total added fat in both starter and grower-finisher diets) improved the global ADG and FCR, whereas the total replacement of soybean oil by soybean lecithin negatively affected final BW, global ADG, ADFI and FCR, justified by a suppression of food intake and a delay in gastric emptying (Nishimukai et al., 2003). However, in the present experiment, no effect of S total replacement by L on broiler feed intake was observed ($P > 0.05$).

Previous data regarding A replacement by L and its influence on performance parameters are scarce. Results of Experiment 2 (**Table 3.5**) show that the replacement of A by L of up to 3% of inclusion does not negatively affect broiler chicken performance ($P > 0.05$). Nevertheless, several studies showed controversial results concerning the inclusion of acid oils instead of conventional added fats in broiler feeding and their influence on performance parameters. Some authors reported a negative influence on growth efficiency due to its high FFA content and, thus, a lower FA digestibility (Sklan, 1979; Blanch et al., 1996). This is consistent with the differences observed in the FCR results between S3 and A3 treatments. On the contrary, Vieira et al. (2006) concluded that the inclusion of soybean acid oil as an added fat source allowed the animals to obtain similar BW and FCR to broiler chickens fed soybean oil as energy source.

Regarding the effect of the dietary fat source on fat deposition, it is widely recognized that the dietary FA profile modifies abdominal fat deposition. Many authors have reported that animals fed diets with a lower UFA:SFA ratio presented higher levels of abdominal fat deposition, as compared to animals fed diets with a higher UFA:SFA ratio (Ferrini et al., 2008; González-Ortiz et al., 2013). It has been demonstrated that dietary PUFA inhibits lipid synthesis and increases FA oxidation, causing a reduction in abdominal and total body fat deposition (Sanz et al., 2000b; Crespo and Esteve-García, 2001). However, no differences were observed between diets in either experiment ($P > 0.05$). This situation may be explained by the average narrow range of the UFA:SFA ratio presented in the grower-finisher experimental diets (Experiment 1: S3 = 3.00 and L3 = 2.50; Experiment 2: S3 = 3.23, A3 = 3.05 and L3 = 2.60).

3.4.3. Digestibility balances

The feed AME value and the apparent digestibility of individual FA of the experimental diets in both periods (starter and grower-finisher periods) are given in **Tables 3.6** (Experiment 1) and **3.7** (Experiment 2).

Table 3.6. Apparent fatty acid digestibility (%) and feed AME value (kcal/kg) of starter diet and grower-finisher diet according to the fat included in the diet (Experiment 1).

	Dietary Treatments[1]					
Item	S3	S2-L1	S1-L2	L3	RSE	P-value
From 9 to 11 d						
AME	3,050[a]	2,709[bc]	2,848[b]	2,621[c]	109.7	<0.001
Total FA	79.59	74.48	71.82	70.76	6.298	0.088
SFA	59.46	52.58	51.53	51.57	9.577	0.372
C16:0	66.59	61.53	60.40	61.42	8.246	0.488
C18:0	50.99	45.13	41.65	46.32	12.680	0.531
MUFA	76.71	72.37	69.10	71.57	7.382	0.295
C18:1 ω-9	77.55	73.69	70.30	72.54	7.208	0.311
PUFA	86.61[a]	81.73[ab]	78.48[b]	77.41[b]	5.372	0.028
C18:2 ω-6	86.29[a]	81.63[ab]	78.58[b]	75.85[b]	5.412	0.019
C18:3 ω-3	89.53[a]	86.26[ab]	83.60[b]	82.19[b]	4.039	0.023
From 36 to 37 d						
AME	3,092[ab]	3,141[a]	2,944[b]	2,966[b]	96.7	0.007
Total FA	83.56	83.72	82.04	82.42	2.620	0.222
SFA	78.71	82.56	80.75	81.63	2.644	0.447
C16:0	81.12	84.04	83.01	84.07	2.167	0.583
C18:0	78.48	83.87	80.43	81.14	3.423	0.210
MUFA	86.05	86.08	85.22	85.81	1.798	0.295
C18:1 ω-9	87.59	87.13	86.76	87.48	1.751	0.315
PUFA	84.71[a]	83.88[ab]	80.93[b]	80.80[b]	3.323	0.037
C18:2 ω-6	84.57[a]	83.83[ab]	80.87[ab]	80.59[b]	3.357	0.038
C18:3 ω-3	86.04[a]	85.35[ab]	82.67[b]	82.64[b]	2.982	0.037

[a-c] Values within the same row with no common superscripts are different, $P \leq 0.05$.

[1] S3= soybean oil (S) at 3.00%; S2-L1= S at 2.00% and crude soybean lecithin (L) at 1.00%; S1-L2= S at 1.00% and L at 2.00%; L3= L at 3.00%.

[2] Values are pooled means of 6 replicates.

AME = apparent metabolizable energy; FA = fatty acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; RSE = residual standard error.

In both experiments, FA digestibility increased, numerically, from the starter to the grower-finisher period. It has been largely demonstrated that FA digestibility is lower in young broilers, as compared to adult broilers, and especially in the case of SFA (Baião and Lara, 2005; Tancharoenrat et al., 2013; Vilarrasa et al., 2015b; Ravindran et al., 2016). Furthermore, in the present study, the apparent digestibility of unsaturated FA was higher, when compared to SFA, especially in young chicks. Young birds present a limited capacity to digest and absorb fat; nevertheless, this capacity improves with age. This fact is due to many reasons, but especially by a limited bile secretion, an inefficient enterohepatic bile recycling process and the difficulty to digest and absorb long-chain FA and SFA due to their physicochemical behavior (Krogdahl, 1985; Tancharoenrat et al., 2013).

The starter digestibility balance in Experiment 1 (**Table 3.6**) showed that L added diets presented a lower AME content, as compared to the S3 diet ($P < 0.001$). This fact may be explained because, as S is replaced by L, animals showed lower apparent PUFA absorption ($P = 0.028$) and, in addition, total FA absorption tended to be worse ($P = 0.088$). These results are in line with data reported by Huang et al. (2007), who observed a lowering effect on the dietary AME value as soybean lecithin was added in substitution of soybean oil (replacement of 50% and 100% of a 2% dietary fat supplementation) in starter diets (excreta collected from broilers of 19 d to 21 d of life). In contrast with our results, they also reported that the EE utilization was not modified by total replacement of soybean oil by soybean lecithin, and its utilization was enhanced with a 75:25 blending of soybean oil-soybean lecithin. It is important to highlight that our results did not show any emulsifying effect from lecithin inclusion, as was expected, especially in the case of young chicks and SFA digestibility. Phosholipids, as the main components of L, are amphiphilic substances with surface-active activity, which is the key origin of its application as commercial emulsifiers (Bueschelberguer et al., 2015). Some researchers have suggested that dietary exogenous emulsifiers may enhance the endogenous bile emulsification process during animal fat digestion and absorption, especially in the case of SFA (Jansen et al., 2015; Siyal et al., 2017b). However, the crude soybean lecithin emulsifying effect on animal digestion and absorption have been controversial in the literature, due to some authors having proven it (Polin, 1980; Jones et al., 1992), but other authors not (Øverland et al., 1994; Blanch et al., 1996). The effectiveness in emulsifying activity is highly dependent on the saturation degree of the added fat source incorporated in the feed (Jones et al., 1992). The lack of an emulsifier effect on FA digestibility and

AME value in Experiment 1 could be attributable to using a highly digestible lipid source such as S in the starter diets, instead of a saturated lipid source such as tallow or palm oil.

In the grower-finisher digestibility balance (**Table 3.6**), no differences were found in AME between S3 and any of the L supplemented treatments, moreover S2-L1 treatment showed a higher AME value than S1-L2 and L3 ($P < 0.007$). Total replacement of S by L (L3) caused a decrease in PUFA digestibility ($P = 0.037$), in particular linoleic and linolenic acid, but no differences were observed in total FA digestibility ($P = 0.222$). The results on the grower-finisher dietary AME value were according to those obtained by Huang et al. (2007), who did not observe, in adult broilers (excreta collected from 40 d to 42 d of life), any modification in dietary AME content and EE utilization induced by the total replacement of soybean oil by soybean lecithin as the energy source (2% of added fats). The inclusion of L as a substitute of S in starter diets caused a reduction in AME content and FA digestibility, however, a 1% replacement in grower-finisher diets caused no adverse effects on feed AME content and FA digestibility.

Results extracted from the starter balance of Experiment 2 (**Table 3.7**) showed that the inclusion of A3 at the expense of S3 presented a lower feed AME value ($P \leq 0.05$) and the animals showed lower FA digestibility ($P \leq 0.05$). Similar effects were observed on the grower-finisher balance, where S3 showed a higher AME content ($P \leq 0.05$) and animals absorbed total FA and PUFA better in comparison with animals fed A3 ($P \leq 0.05$). The depressing effect of acid oils on AME content and FA digestibility has been reported by many authors, especially in the case of fat sources with a high saturated FFA content (Sklan, 1979; Wiseman and Salvador, 1991; Roll et al., 2018). Wiseman and Salvador (1991) reported that the feed AME value linearly decreased with increasing FFA content when the combination or replacement of soybean oil (FFA content: 1.44%) by a soybean acid oil (FFA content: 68.34%) was performed. This effect was more pronounced in the case of young broilers and using saturated added fat sources (experiments comparing tallow and palm acid oil against their respective native oils). It has been demonstrated that FFA are more poorly absorbed than TAG because the presence of monoacylglycerol molecules are essential to the mixed micelle formation and, in addition, FFA tend to form insoluble soaps with cations, such as magnesium or calcium (Small, 1991; Ravindran et al., 2016). This fact explains why a high content in FFA (> 50%) is directly related to a reduction in FA digestibility and feed AME value, especially in the starter period, as we have seen in Experiment 2 (S3 vs A3; **Table 3.7**).

Table 3.7. Apparent fatty acid digestibility (%) and feed AME (kcal/kg) value of starter diet and grower-finisher diet according to the fat included in the diet[1](Experiment 2).

	Dietary treatments[2]						
Item	S3[3]	A3	A2-L1	A1-L2	L3	RSE	P-value
From 9 to 11 d							
AME	2,977^{x}	2,873^{y}	2,877	2,876	2,786	90.02	0.254
Total FA	79.59^{x}	65.90^{y}	71.65	74.07	69.10	5.011	0.062
SFA	68.10^{x}	51.74^{y,b}	59.55^{ab}	63.83^{a}	51.97^{b}	6.819	0.018
C16:0	73.10^{x}	61.47^{y}	67.68	70.37	65.28	5.786	0.088
C18:0	70.26^{x}	47.57^{y}	59.71	63.86	53.28	10.130	0.058
MUFA	80.33^{x}	70.53^{y}	76.05	77.48	71.12	5.223	0.081
C18:1 ω-9	80.75	71.89	76.80	79.02	71.12	5.248	0.094
PUFA	82.60^{x}	67.85^{y,b}	73.45^{ab}	76.02^{a}	73.35^{ab}	4.298	0.027
C18:2 ω-6	82.08^{x}	67.60^{y,b}	73.22^{ab}	75.81^{a}	72.99^{ab}	4.395	0.030
C18:3 ω-3	86.70^{x}	70.76^{y,b}	75.96^{ab}	78.25^{a}	76.94^{a}	3.287	0.005
From 36 to 37 d							
AME	3,026^{x}	2,940^{y,b}	3,098^{a}	2,851^{b}	2,916^{b}	66.86	<0.001
Total FA	87.01^{x}	84.25^{y,ab}	85.79^{a}	82.83^{bc}	81.39^{c}	1.658	0.001
SFA	83.30	81.05^{b}	84.30^{a}	82.11^{ab}	81.51^{ab}	1,744	0.021
C16:0	86.13	84.08^{b}	86.80^{a}	84.73^{ab}	84.22^{b}	1,414	0.036
C18:0	88.14^{x}	84.43^{y,b}	87.33^{a}	85.53^{ab}	84.05^{b}	1,640	0.011
MUFA	88.23	88.63^{ab}	89.40^{a}	86.75^{b}	84.57^{c}	1,170	<0.001
C18:1 ω-9	90.05	90.01^{ab}	90.88^{a}	88.54^{b}	86.64^{c}	1,075	<0.001
PUFA	87.85^{x}	81.52^{y,ab}	83.40^{a}	80.31^{ab}	79.38^{b}	2,413	0.050
C18:2 ω-6	87.51^{x}	81.64^{y,ab}	83.47^{a}	80.30^{ab}	79.30^{b}	2,403	0.039
C18:3 ω-3	90.45^{x}	80.05^{y}	82.61	80.42	81.06	0,176	0.287

$^{x-y}$ ANOVA S3 vs A3: values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

$^{a-c}$ ANOVA diets with coproducts: values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[1] S3= soybean oil at 3.00%; A3= acid oil (A) at 3.00%; A2-L1= A at 2.00% and crude soybean lecithin (L) at 1.00%; A1-L2= A at 1.00% and L at 2.00%; L3= L at 3.00%.

[2] Values are pooled means of 6 replicates.

[3] S3 was not included in the statistical analysis against diets containing co-products.

AME = apparent metabolizable energy; FA = fatty acid; SFA= saturated fatty acid; MUFA = mono-unsaturated fatty acid; PUFA = polyunsaturated fatty acid; RSE= residual standard error.

Regarding the substitution of A by L (Table 9), the feed AME value resulted unaffected ($P = 0.254$) during the starter period. Nevertheless, SFA and PUFA absorption were influenced by the added fat source ($P \leq 0.05$), and a tendency in total FA and MUFA apparent absorption was shown ($P < 0.10$). Young broiler chickens fed A1-L2 digested and absorbed dietary SFA and PUFA better, when compared to those animals fed A3. On the other hand, in the grower-finisher period, treatment A2-L1 obtained the highest feed AME content value ($P < 0.001$), a higher TFA, MUFA and PUFA digestibility, when compared to L3 treatment ($P \leq 0.05$), and a higher SFA digestibility, as compared to A3 treatment ($P = 0.021$). It has been widely demonstrated that blending fats with variated physicochemical properties enhance, by an interaction, the energetic value of fats in comparison with the sum of the energetic values of each individual fat (Peña et al., 2014; Borsatti et al., 2018). The establishment of a synergism between different fat sources could be related to the combination of complementary FA profiles or lipid molecular structures (TAG, FFA, PL, among others). For example, this effect is particularly marked in mixtures of saturated fat sources with unsaturated fat sources, due to the digestibility of SFA and non-polar molecules being improved by the emulsifying properties of unsaturated FA (Sibbald, 1978; Borsatti et al., 2018). The emulsifying effect of crude soybean lecithin with a saturated native oil is another example of synergism. Results from a digestibility balance in laying hens conducted by Mandalawi et al. (2015) confirmed that the combination of a saturated animal fat with soybean lecithin in a blend of 50:50 (4% of added fat inclusion) improved total tract apparent retention of EE and GE. Another experiment in broiler chickens (Polin, 1980) combined tallow at 4% with different inclusion rates of soybean lecithin (0.02%, 0.2% and 2%) and stated that 2% of soybean lecithin supplementation significantly improved tallow absorption in comparison with those diets containing 0.02% and 0.2% of soybean lecithin. Similar results were obtained in piglet diets (Jones et al., 1992). In this study, the combination of L and A, with a similar UFA:SFA ratio, but with a different (and complementary) FA profile and lipid molecular structures (FFA from A combined with surface-active PL from L; **Table 3.2**), obtained the best results. Dietary and endogenous PL play an important role in mixed micelle formation displacing monoacylglycerol and FFA molecules from the interface to the hydrophobic core of the mixed micelle and, due to this, they are capable of improving the absorption of lipids (Krogdahl, 1985). In Experiment 2, the addition of L at 2% in starter diets and at 1% in grower-finisher diets in replacement of A resulted the best option in terms of FA digestibility and feed AME content.

3.4.4. Fatty acid composition of abdominal fat pad

The effect of dietary fat sources on the FA composition of AFP is presented in **Table 3.8**. In Experiment 1, S replacement by L produced changes between treatments for palmitic acid concentration (P = 0.019), and also a tendency (P = 0.087) for SFA was observed, however, in general, the use of one added fat source instead of the other did not modify the FA profile of the AFP. In the case of Experiment 2, the AFP of animals fed S3 presented a higher percentage of PUFA ($P \leq 0.05$) and a lower percentage of MUFA ($P \leq 0.05$) than animals fed A3. Regarding the replacement of A by L, the total replacement of A by L in the diet (L3) caused a significant increase in linolenic acid concentration (P = 0.004) and also tended to increase linoleic acid (P = 0.066), whereas a decrease ($P < 0.001$) in oleic acid concentration was observed.

As other authors reported before, the FA composition found in abdominal fat tissue reflected the FA profile of the experimental diets (Ferrini et al., 2008; Smink et al., 2010; Vilarrasa et al., 2015b). In Experiment 1, both fat sources presented a similar FA profile, UFA:SFA and PUFA:SFA ratios (**Table 3.2**); for this reason few differences were observed between treatments in most FA. On the other hand, in Experiment 2, both fat sources influenced and changed FA composition of AFP, and these changes were according to the main differences shown between A and L diets (**Table 3.2**), especially in the case of MUFA and PUFA concentration. Results show that dietary FA composition has a greater impact on the saturation degree of AFP than the lipid molecular structures have.

3.5. Conclusions

In conclusion, crude soybean lecithin is a suitable energy source for broiler chickens in the grower-finisher period. The inclusion of 1% of L in replacement of S in the grower-finisher phase did not affect feed AME content, FA digestibility and, in turn, preserved productive performance and the FA profile of the AFP. On the other hand, the combination of L and A (2% of L and 1% of A in the starter and 1% of L and 2% of A in the grower-finisher) is the best strategy to include both alternative fats as energy source in broiler chicken diets, probably related to the positive synergism between FFA and PL. It was observed that the dietary FA profile has a greater impact on AFP saturation degree in comparison to the lipid molecular structures (TAG, PL and FFA). Further studies might bring a better understanding of the mechanisms underlying these effects

Table 3.8. Fatty acid composition of abdominal fat pad of broiler chickens according to different fat sources[1] in diet (Experiments 1 and 2).

	Experiment 1						Experiment 2						
Item	S3	S2-L1	S1-L2	L3	RSE	P-value	S3[2]	AO3	AO2-L1	AO1-L2	L3	RSE	P-value
Fatty acid profile (%)													
SFA	30.88	29.94	31.50	31.56	1.104	0.087	29.19	29.81	30.16	30.95	31.62	1.423	0.154
C16:0	24.57^{ab}	23.62^{b}	25.10^{a}	25.10^{a}	0.802	0.019	23.13	23.61	24.38	25.06	25.18	1.170	0.112
C18:0	5.44	5.44	5.51	5.52	0.523	0.961	5.37	5.34	5.21	5.03	5.56	0.429	0.217
MUFA	45.41	46.20	46.88	46.54	1.797	0.645	46.79^{y}	53.64^{x,a}	51.72^{a}	50.25^{ab}	46.69^{b}	2.422	0.001
C18:1 ω-9	37.54	38.30	39.48	38.97	1.295	0.182	38.96^{y}	5.41^{x,a}	43.27^{b}	41.40^{b}	38.78^{c}	1.278	<0.001
PUFA	23.71	23.86	21.84	21.90	1.826	0.226	24.45^{x}	16.55^{y}	18.46	18.80	21.69	3.103	0.068
C18:2 ω-6	21.09	21.06	19.25	19.39	1.621	0.206	21.27^{x}	14.94^{y}	16.33	16.82	19.37	2.711	0.066
C18:3 ω-3	2.03	2.10	1.93	1.93	0.164	0.375	2.47^{x}	1.06^{y,b}	1.32^{ab}	1.44^{ab}	1.71^{a}	0.253	0.004
UFA:SFA	2.24	2.34	2.20	2.17	0.118	0.157	2.43	2.36	2.32	2.24	2.17	0.151	0.168

[x-y] ANOVA S3 *vs* AO3: values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[a-c] Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[1] S3 = soybean oil (S) at 3.00%; S2-L1 = S at 2.00% and soybean lecithin high in free fatty acids (L) at 1.00%; S1-L2 = S at 1.00% and L at 2.00%; AO3 = acid oil (AO) at 3.00%; AO2-L1 = AO at 2.00% and L at 1.00%; AO1-L2 = AO at 1.00% and L at 2.00%; L3 = L at 3.00%.

[2] S3 was not included in the statistical analysis against diets containing co-products.

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio; RSE = residual standard error.

Chapter Four

Soybean lecithin high in free fatty acids for broiler chickens: impact on performance, fatty acid digestibility and saturation degree of adipose tissue

"Queridos amigos, lo que han cambiado las cosas,

y lo rápido que se mueve todo aquí fuera.

Cuando era niño vi una vez un coche, pero ahora están por todas partes.

Este maldito mundo va demasiado deprisa."

Brooks Hatlen, en *Cadena Perpetua*, 1994.

Soybean lecithin high in free fatty acids for broiler chicken diets: impact on performance, fatty acid digestibility and saturation degree of adipose tissue

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Submitted to Animal: The International Journal of Animal Biosciences.

4.1. Abstract

Soybean lecithin is a co-product extracted during the process of oil refining that may represent an energy source for broiler feeding. Two experiments were conducted to evaluate the use of a soybean lecithin (L) with a high free fatty acid content (23-26%) as an energy source in starter (0-21 days) and grower-finisher (22-38 days) broiler diets. The influence of the added fat source was assessed on performance, fatty acid (FA) digestibility, feed apparent metabolizable energy (AME) content and the FA profile of the abdominal fat pad (AFP). A basal diet was supplemented with soybean oil at 3% (S; Experiment 1) or vegetable acid oil at 3% (AO; Experiment 2), and increasing amounts of L (1%, 2% and 3%) were included in replacement. The inclusion of L did not modify performance parameters ($P > 0.05$). In starter diets of Experiment 1, the inclusion of L in the replacement of S reduced feed AME and total FA digestibility ($P \leq 0.05$). However, in grower-finisher diets, a partial replacement of S by L (up to 2%) did not modify feed AME value or FA digestibility. In Experiment 2, AO replacement by L, in starter diets, caused no changes in feed AME or total FA digestibility ($P > 0.05$). Nevertheless, in the grower-finisher phase, animals fed diets with a blend of 1% of AO and 2% of L obtained the best results, showing no differences in monounsaturated FA and a higher linolenic acid digestibility than with 3% of AO. In both experiments, the FA profile of the AFP reflected the FA composition of the experimental diets. On the contrary, the influence of dietary lipid molecular structures (triacylglycerols, phospholipids and free FA) on the AFP saturation degree was limited. The present study suggests that soybean lecithin high in free fatty acids could replace soybean oil, up to 2%, or be blended with a vegetable acid oil (2% of L and 1% of AO) in broiler grower-finisher diets, without negative effects on performance and FA digestibility.

4.2. Introduction

Co-products derived from the vegetable oil refining process may represent an interesting and economic alternative to conventional fat sources used in broiler feeding. During degumming, most phospholipids (**PL**) present in crude soybean oil are extracted, generating a co-product known as crude soybean lecithin. Lecithins are defined as a lipid mixture highly composed of PL, but also rich in glycolipids, carbohydrates and neutral lipids, such as triacylglycerols (EFSA, 2016). Soybean lecithin is an available low-cost energetic source (Borsatti et al., 2017) with a similar fatty acid (**FA**) profile to soybean

oil (Soares and Lopez-Bote, 2002; Huang et al., 2007). In addition, its elevated surface-active PL content represents an added value as emulsifiers, hence its dietary inclusion may improve fat absorption (Jones et al., 1992; Ravindran et al., 2016). However, soybean lecithin presents high viscosity that hampers its inclusion during feed manufacturing. For this reason, in order to facilitate its homogeneous blend in feed, mixing lecithin at different ratios with acid or crude oils is a common practice (van Nieuwenhuyzen and Tomás, 2008). On the other hand, vegetable acid oils derived from the chemical refining process of native oils are normally composed of a large quantity of free fatty acids (**FFA**; 40%-60%) and represent an important source of energy.

We hypothesized that soybean lecithin can be considered as an alternative energy source for broiler chicken diets, in replacement or combined with other fats, with no negative effects on performance, nutrient digestibility and FA composition of adipose tissue. Therefore, a total of two experiments were conducted to assess the potential use of a soybean lecithin high in FFA (**L**), as an alternative energy source in broiler feeding, combined with soybean oil (**S**; Experiment 1) or a vegetable acid oil (**AO**; Experiment 2). The evaluation was based on studying the influence of L inclusion on performance, feed energetic content, FA digestibility, thus the effect on the FA profile of the abdominal fat pad (**AFP**) of the broiler carcass.

4.3. Material and methods

4.3.1. Experimental design and diets

The experiments were performed at *Servei de Granges i Camps Experimentals* (Universitat Autònoma de Barcelona, Bellaterra, Spain). These experimental procedures were in accordance with the European Union Guidelines (2010/63/EU) and approved by the Animal Ethics Committee of the same institution. Two trials of 38 days were performed with a feeding programme in two phases: starter (0 to 21 days) and grower-finisher (22 to 38 days). Experimental diets (**Table 4.1**) were based on wheat and soybean meal, presented in mash form, and were formulated to meet or exceed FEDNA (2008) requirements. Titanium dioxide was added at 0.5%.

Experiment 1. A total of 96 Ross 308 newly hatched female broiler chickens were randomly assigned to one of four experimental treatments (six replicates/treatment) and allocated in cages (four birds/cage). A control basal diet was supplemented with S at 3% (S3) and increasing amounts of L (soybean lecithin blended with soybean acid oil in a 5:1

proportion) were included in replacement of S as added fat: 1% (S2-L1), 2% (S1-L2) and 3% (L3).

Experiment 2. A total of 120 Ross 308 newly hatched female broiler chickens were randomly assigned to one of five experimental treatments (six replicates/treatment) and allocated in cages (four birds/cage). A control basal diet was supplemented with AO (a 1:1 blend of olive pomace acid oil and sunflower acid oil) at 3% (AO3) and increasing amounts of L were included in replacement of A: 1% (AO2-L1), 2% (AO1-L2) and 3% (L3). The S3 diet was included as a reference treatment.

4.3.2. Animal husbandry and controls

The animals were obtained from a local hatchery (Pondex S.A.U.; Juneda, Spain), weighed, wing-tagged and randomly allocated in cages with a grid floor and a tray for excreta collection. Birds were allowed to consume feed and water *ad libitum*. The temperature and light programme used was consistent with the specifications in the Ross 308 lineage management handbook (Aviagen, 2014). Broiler bodyweight (**BW**) was recorded individually at days 21 and 38 post-hatch, whereas feed intake was measured by cage at days 21 and 38 post-hatch. The data were used to measure BW and calculate the average daily gain, average daily feed intake and feed conversion ratio of each period and the global results. Mortality was daily recorded to adjust average feed intake and average daily gain. Two nutritional balances were performed for each experiment between days 9-11 (starter period) and days 36-37 (grower-finisher period), where excreta samples (free of contaminants) were taken each day of the digestibility balance. Samples of excreta were homogenized, freeze-dried, ground and kept at 4ºC until further analysis. At the end of the experiment, broiler chickens were slaughtered in a commercial abattoir and carcasses were recovered for further study. Carcasses (total BW excluding blood and feathers) were weighed, and AFP (from the proventriculus surrounding the gizzard down to the cloaca) of each bird were removed and weighed in order to calculate carcass yield and the AFP carcass percentage. A representative sample of AFP of each bird was taken, pooled by replicate and frozen at -20ºC for further analysis.

Table 4.1. Ingredient composition of the starter and grower-finisher broiler chicken diets, as-fed basis (Experiments 1 and 2).

	Experiment 1		Experiment 2	
Ingredients (%)	Starter diet (0 to 21 days)	Grower-finisher diet (22 to 38 days)	Starter diet (0 to 21 days)	Grower-finisher diet (22 to 38 days)
Wheat	36.55	46.84	36.64	45.92
Soybean meal 47%	29.43	21.09	30.46	24.25
Corn	9.71	-	9.71	-
Barley	9.71	15.58	8.33	15.76
Extruded full-fat soybean	4.76	-	4.73	-
Added fats[1]	3.00	3.00	3.00	3.00
Rapeseed meal 00	-	3.42	-	3.41
Sunflower meal 28%	-	2.44	-	-
Sepiolite	1.93	1.90	2.03	2.03
Palm oil	-	1.50	-	1.51
Calcium carbonate	1.19	1.08	1.16	1.00
Monocalcium phosphate	0.97	0.57	0.93	0.48
Trace mineral-vitamin premix[2]	1.15	1.01	1.44	1.17
Titanium dioxide	0.50	0.50	0.50	0.50
Salt	0.30	0.23	0.30	0.23
L-lysine	0.30	0.35	0.28	0.28
DL-methionine	0.28	0.21	0.28	0.22
L- threonine	0.08	0.09	0.07	0.07
Sodic bicarbonate	0.07	0.12	0.07	0.11
Clorure choline 75%	0.07	0.07	0.07	0.06

[2] *Soybean oil, soybean lecithin high in free fatty acids and acid oil in different blending proportions.*

[2] *Provides per kg feed: vitamin A (from retinol), 13500 IU; vitamin D3 (from cholecalciferol), 4800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; vitamin B1, 3mg; vitamin B2, 9mg; vitamin B6, 4.5 mg; vitamin B12, 16.5 µg; vitamin K3, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from FeSO4·7H2O), 54 mg; I [from Ca(I2O3)2], 1.2 mg; Co (from 2CoCO3·3Co(OH)2·H2O), 0.6 mg; Cu (from CuSO4·5H2O), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na2SeO3), 0.18 mg; Mo [from (NH4)6Mo7O24], 1.2 mg; Organic acids (starter diets at 4 g/kg; grower-finisher diets at 3 g/kg); β-glucanase 350 IU; xylanase 1,125 IU.*

4.3.3. Laboratory analyses

Diet proximate analyses were performed according to AOAC (2005): ether extract (Method 920.39), crude protein (Method 968.06) and crude fibre (Method 962.09). Feed and excreta samples analysis included ash determination (Method 942.05), dry matter (Method 934.01), and gross energy content by adiabatic bomb calorimeter (IKA-Kalorimeter system C4000; Staufen, Germany). Titanium dioxide was determined by spectrophotometry ICP-OES (Optima 3200 RL, Perkin Elmer; Waltham, MA, USA) in Experiment 1, while in Experiment 2 the method described by Short et al. (1996) was used. Oil samples (S, AO and L) were analysed for FA composition by gas chromatography according to the method described by Guardiola et al. (1994). The acid value was determined according to ISO (2009), and acidity was indicated as FFA percentage expressed in oleic acid. Acetone insoluble determination was performed following the Ja 4-46 method (AOCS, 2017), and PL composition was determined by HPLC (D450 MT1, Kontron; Eching, Germany) following the method described by Helmerich and Koehler (2003).

The FA content of feed and excreta was analysed adding nonadecanoic acid (C19:0, Sigma–Aldrich Chemical Co.; St. Louis, MO) as an internal standard and following the method described by Sukhija and Palmquist (1988), whereas in the case of AFP, the method described by Carrapiso *et al*. (2000) was used. The final extract obtained was injected in a gas chromatograph (HP6890, Agilent Technologies; Waldbronn, Germany) following the method conditions described by Cortinas et al. (2004).

4.3.4. Calculations and statistical analysis

Apparent digestibility of FA (%) was calculated using the following equation: *Apparent digestibility of nutrient = 1- {(Titanium dioxide concentration in feed/Nutrient concentration in feed)/(Titanium dioxide concentration in excreta/Nutrient concentration in excreta)}*.

The apparent metabolizable energy (**AME**) of the diets was calculated multiplying the apparent absorption of the gross energy by its corresponding diet gross energy. Cage means were used as the experimental unit (six replicates/treatment) in performance (except BW), FA digestibility, FA profile of the AFP and AME values of the diets. Data were analysed by one-way ANOVA using R Statistics (version 3.3.1), with treatment as the main factor. In Experiment 2, soybean oil treatment (S3) was compared against AO3

treatment separately with one-way ANOVA. Tukey's multiple-range test was performed to determine whether means were significantly different ($P \leq 0.05$). The linear model used was: $Y_{ij} = \mu + \alpha_i + \varepsilon_j$, where μ is the global mean, α is the treatment effect and ε is the residual error.

Table 4.2. Chemical analysis of the added fat sources.

	Experimental fats[1]				
	Experiment 1		**Experiment 2**		
Item	S	L	S	AO	L
Fatty acid profile (%)[2]					
SFA	16.5	20.4	16.0	15.1	21.9
C16:0	11.7	15.7	10.6	9.97	16.1
C18:0	3.55	4.68	4.26	3.84	5.86
MUFA	24.3	19.4	23.5	54.2	19.6
C18:1 ω-9	22.3	19.4	21.8	51.3	19.6
PUFA	59.2	60.2	60.5	30.7	58.5
C18:2 ω-6	53.4	54.2	52.8	29.2	52.6
C18:3 ω-3	5.76	6.09	7.67	1.55	5.90
Minor FA	3.32	N.D.	2.85	4.21	N.D.
UFA:SFA	5.06	3.90	5.25	5.62	3.57
PUFA:SFA	3.59	2.95	3.78	2.03	2.67
Acidity (%)[2]					
FFA	2.41	22.6	1.49	52.9	25.5
Phospholipids (%)[2]					
AI	N.D.	48.7	N.D.	N.D.	46.8
Total PL	N.D.	24.6	N.D.	N.D.	27.8
PC	N.D.	9.42	N.D.	N.D.	9.96
PI	N.D.	5.80	N.D.	N.D.	7.38
PE	N.D.	4.62	N.D.	N.D.	5.56
AP	N.D.	2.11	N.D.	N.D.	3.58
LPC	N.D.	2.68	N.D.	N.D.	1.31
Gross energy (kcal/kg)					
GE	9,387	8,121	9,625	9,434	8,288

[1] S = soybean oil; L = soybean lecithin high in free fatty acids; AO = acid oil.

[2] Percentage of total product.

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio; AI = acetone insoluble matter; Total PL = total phospholipids; PC = phosphatidylcholine; PI = phosphatidylinositol; PE = phosphatidylethanolamine; AP = phosphatidic acid; LPC = lysophosphatidylcholine; FFA = free fatty acid; GE = gross energy; N.D. = not determined.

Table 4.3. Analysed gross energy, macronutrient content and fatty acid composition of starter and grower-finisher broiler diets[1] (Experiment 1).

	Starter (0 to 21 days)				Grower-finisher (22 to 38 days)			
Item[2]	S3	S2-L1	S1-L2	L3	S3	S2-L1	S1-L2	L3
Gross energy (kcal/kg)	4,156	4,132	4,084	4,080	4,180	4,156	4,150	4,108
Macronutrient content (%)								
Dry Matter	91.7	91.4	91.2	91.3	90.6	90.2	90.7	90.3
Crude Protein	23.7	23.1	22.7	23.2	21.5	20.8	21.4	20.7
Crude Fat	5.37	5.33	4.91	5.38	6.24	6.03	5.82	5.75
Crude Fibre	3.94	4.20	3.50	3.95	4.88	3.75	4.86	3.80
Ash	8.54	8.86	8.51	8.97	8.71	8.53	8.22	8.87
Fatty acid profile (%)								
SFA	18.2	18.7	19.1	19.9	25.0	25.9	27.0	28.0
C16:0	13.9	14.4	15.0	15.6	20.6	21.5	22.5	23.2
C18:0	3.55	3.58	3.70	3.88	3.44	3.51	3.55	3.80
MUFA	21.1	20.4	19.6	18.7	26.3	25.8	25.4	24.4
C18:1 ω-9	19.4	18.8	18.1	17.2	24.4	24.0	23.7	22.9
PUFA	60.7	60.9	61.3	61.4	48.7	48.3	47.6	47.6
C18:2 ω-6	54.6	54.7	54.7	54.7	44.2	43.8	43.1	43.0
C18:3 ω-3	6.16	6.18	6.23	6.39	4.80	4.68	4.64	4.75
Minor fatty acids	2.39	2.34	2.27	2.23	2.56	2.51	2.51	2.35
UFA:SFA	4.49	4.35	4.24	4.03	3.00	2.86	2.70	2.55
PUFA:SFA	3.34	3.26	3.21	3.09	1.95	1.86	1.76	1.70

[1] S3 = soybean oil (S) at 3.00%; S2-L1 = S at 2.00% and soybean lecithin high in free fatty acids (L) at 1.00%; S1-L2 = S at 1.00% and L at 2.00%; L3 = L at 3.00%.

[2] Samples were analysed twice.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio; GE = gross energy

Table 4.4. Analysed gross energy, macronutrient content, and fatty acid composition of starter and grower-finisher chicken diets[1] (Experiment 2).

	Starter (0 to 21 days)					Grower-finisher (22 to 38 days)				
Item[2]	S3	AO3	AO2-L1	AO1-L2	L3	S3	AO3	AO2-L1	AO1-L2	L3
Gross energy (kcal/kg)	4,132	4,125	4,083	4,079	4,036	4,203	4,201	4,179	4,176	4,155
Macronutrient content (%)										
Dry Matter	91.8	91.8	91.4	92.1	91.0	90.9	90.9	90.9	90.7	91.0
Crude Protein	22.3	22.2	23.1	22.5	22.5	22.1	21.3	20.6	20.6	20.8
Crude Fat	5.46	5.33	5.58	5.30	5.10	6.60	6.60	6.37	6.15	6.23
Crude Fibre	4.38	3.84	4.04	3.98	4.13	4.62	4.17	3.81	4.02	4.20
Ash	8.50	8.36	8.73	8.53	8.65	9.86	9.21	10.2	10.5	9.53
Fatty acid profile (%)										
SFA	17.2	17.9	18.2	18.8	19.6	23.6	24.7	25.4	25.9	26.5
C16:0	13.5	14.3	14.6	15.1	15.7	19.4	20.5	21.1	21.6	22.2
C18:0	3.70	3.66	3.63	3.68	3.87	3.96	3.95	3.98	3.99	3.99
MUFA	22.1	35.0	30.5	25.5	19.9	27.1	40.1	35.1	30.5	25.4
C18:1 ω-9	20.7	33.5	29.1	24.1	18.7	25.6	38.3	33.4	29.0	24.0
PUFA	60.7	47.1	51.3	55.7	60.5	49.3	35.2	39.5	43.6	48.1
C18:2 ω-6	54.1	43.4	46.8	50.6	54.6	43.7	32.5	36.0	39.6	43.3
C18:3 ω-3	6.61	3.74	4.45	5.14	5.90	5.56	2.73	3.46	4.07	4.81
Minor fatty acids	1.39	1.40	1.42	1.38	1.23	1.78	2.02	2.06	1.74	1.70
UFA:SFA	4.81	4.59	4.49	4.32	4.10	3.24	3.05	2.94	2.86	2.77
PUFA:SFA	3.53	2.63	2.82	2.96	3.09	2.09	1.43	1.56	1.68	1.82

[1] S3= soybean (S) oil at 3.00%; AO3= acid oil (AO) at 3.00%; AO2-L1= AO at 2.00% and soybean lecithin high in free fatty acids (L) at 1.00%; AO1-L2= AO at 1.00% and L at 2.00%; L3= L at 3.00%

[2] Samples were analysed twice.

SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio; GE = gross energy.

4.4. Results

4.4.1. Experimental fats and diets composition

The chemical composition of the added fat sources is presented in **Table 4.2**. Both S and AO presented higher average values of gross energy (9,506 and 9,434 kcal/kg, respectively) than did L (8,216 kcal/kg). The FA profile of S and L was similar regarding polyunsaturated FA (**PUFA**) content; nevertheless, L presented higher content in saturated FA (**SFA**) and lower content in monounsaturated FA (**MUFA**) than did S. In the case of AO, oleic acid was the most abundant FA, followed by linoleic acid. The three added fats differed in their average unsaturated-to-saturated FA ratio (**UFA:SFA**), where S and AO presented higher average values (5.14 and 5.60, respectively) than did L (3.74), and on their average polyunsaturated-to-saturated FA ratio (**PUFA:SFA**), where AO presented the lowest value (2.04), followed by L (2.82) and S (3.67). Concerning FFA content, AO presented the highest value, representing its main lipid molecular structure (52.9%) whereas L showed a medium average content (24.1%) and S the average lowest value (1.95%). The chemical composition of the experimental diets is shown in **Table 4.3** (Experiment 1) and **Table 4.4** (Experiment 2). In Experiment 1, the replacement of S by L increased dietary SFA in starter (9.4%) and grower-finisher diets (11.9%), whereas a decrease in MUFA was observed (11.3% and 7.1% for starter and grower-finisher diets, respectively), causing a reduction in dietary UFA:SFA. In Experiment 2, the replacement of AO by L increased dietary SFA (9.0% and 7.2%, for starter and grower-finisher diets, respectively) and dietary PUFA (28.4% and 36.8% for starter and grower-finisher diets, respectively). On the contrary, it reduced MUFA content (42.8% and 36.7% for starter and grower-finisher diets, respectively). The replacement of AO by L reduced UFA:SFA, whereas it increased PUFA:SFA.

4.4.2. Growth performance and fat deposition

Growth performance and abdominal fat deposition parameters figure in Table 5. In both experiments, performance parameters were not affected by the replacement of the added fats (S and AO) by L in any phase, nor the overall ones of the experiments ($P > 0.05$). Nevertheless, in Experiment 2, S replacement by AO impaired feed conversion ratio in grower-finisher phase and the global of the experiment ($P \leq 0.05$) and AO replacement by L tended to improve the feed conversion ratio ($P = 0.055$).

Table 4.5. Growth performance and abdominal fat deposition of broiler chickens according to dietary added fats[1] (Experiments 1 and 2).

	Experiment 1						Experiment 2						
Item	S3	S2-L1	S1-L2	L3	RSE	P-value	S3[2]	AO3	AO2-L1	AO1-L2	L3	RSE	P-value
From 0 to 21 days													
BW at 0 days (g)	43.0	42.9	42.9	43.1	2.64	0.996	45.1	45.2	45.1	45.1	45.1	2.44	0.999
BW at 21 days (g)	825	816	836	825	85.6	0.891	876	878	870	864	876	89.6	0.943
ADFI (g/bird/d)	54.9	55.7	52.5	54.3	3.03	0.338	56.2	57.1	57.7	55.6	57.6	2.35	0.400
ADG (g/bird/d)	37.1	37.7	38.3	36.6	2.19	0.618	39.6	39.7	39.3	39.0	40.7	2.06	0.561
FCR (g/g)	1.45	1.41	1.40	1.44	0.039	0.170	1.40	1.44	1.47	1.41	1.45	0.052	0.943
From 22 to 38 days													
BW at 38 days (g)	2,408	2,461	2,500	2,428	186.8	0.509	2,469	2,395	2,430	2,418	2,469	186.1	0.927
ADFI (g/bird/d)	167.1	172.0	170.6	171.1	10.14	0.855	163.5	160.9	165.4	159.8	164.7	9.90	0.706
ADG (g/bird/d)	93.8	94.5	95.6	91.9	5.75	0.724	91.7	87.8	90.3	89.8	90.0	6.36	0.897
FCR (g)	1.78	1.79	1.82	1.86	0.075	0.287	1.78[x]	1.86[y]	1.83	1.80	1.81	0.033	0.171
From 0 to 38 days													
ADFI (g/bird/d)	105.1	107.8	105.3	105.9	6.00	0.885	104.2	103.5	105.9	102.2	104.6	5.41	0.679
ADG (g/bird/d)	62.3	61.8	63.9	62.2	3.70	0.755	62.7	61.2	62.1	62.5	61.8	4.09	0.954
FCR (g/g)	1.69	1.71	1.68	1.70	0.054	0.780	1.66[x]	1.71[y]	1.71	1.66	1.67	0.032	0.055
Carcass weight (g)	2,147	2,224	2,241	2,173	109.1	0.463	2,229	2,183	2,193	2,172	2,193	141.3	0.999
Abdominal fat depot													
g	40.01	35.86	33.78	39.45	3.835	0.062	43.86	40.61	45.04	39.34	45.31	5.528	0.185
(%)	1.93	1.61	1.64	1.82	0.251	0.134	1.97	1.88	2.05	1.79	2.06	0.175	0.064

[x,y] ANOVA AO3 *vs* S3: Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[1] S3 = soybean oil (S) at 3.00%; S2-L1 = S at 2.00% and soybean lecithin high in free fatty acids (L) at 1.00%; S1-L2 = S at 1.00% and L at 2.00%; L3 = L at 3.00%; AO3= acid oil (AO) at 3.00%; AO2-L1= AO at 2.00% and L at 1.00%; AO1-L2= AO at 1.00% and L at 2.00%.

[2] S3 was not included in the statistical analysis against diets containing co-products.

BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; RSE = residual standard error

Concerning the effect of dietary added fats on abdominal fat deposition, no significant differences were observed between experimental treatments ($P > 0.05$).

4.4.3. Digestibility balances

The influence of the added fats on dietary feed AME and FA digestibility in both feeding periods can be seen in **Table 4.6** (Experiment 1) and **Table 4.7** (Experiment 2). Digestibility balance of Experiment 1 indicated, in starter diets, that S partial and total replacement by L (S2-L1, S1-L2 and L3), negatively affected feed AME value ($P < 0.001$) and FA digestibility. Animals fed diets with 2% and 3% of L (S1-L2 and L3) showed lower total fatty acid (**TFA**; $P = 0.017$), MUFA ($P = 0.026$) and PUFA ($P = 0.004$) digestibility and tended to absorb SFA worse ($P = 0.055$) than did animals fed S3. In the case of grower-finisher diets, animals fed L3 presented lower feed AME ($P < 0.001$), and lower TFA ($P = 0.020$), oleic acid ($P < 0.001$) and PUFA ($P = 0.003$) digestibility as compared to animals fed S3. No differences were observed between S3 and treatments with partial replacement by L (S2-L1 and S1-L2).

Results from Experiment 2 showed that S3 treatment presented a higher dietary AME and FA digestibility than did AO3 in both periods ($P \leq 0.05$). Regarding the use of co-products (AO and L) as added fats, in the starter period, no differences were observed by replacing AO by L on feed AME and the digestibility of TFA, SFA and MUFA ($P > 0.05$). Nevertheless, L3 presented higher digestibility of linolenic acid ($P = 0.011$), in contrast to AO3. On the other hand, grower-finisher diets showed differences between treatments in SFA, MUFA and PUFA digestibility. The total replacement of AO by L (L3) did not modify dietary AME or the digestibility of TFA and SFA, while it caused lower MUFA ($P < 0.001$) and higher linolenic acid ($P = 0.006$) digestibility. The lowest feed AME value was observed in AO2-L1 ($P < 0.001$), which was consistent with FA digestibility. Treatment AO2-L1 presented lower TFA and MUFA digestibility than did AO3 ($P \leq 0.05$), and lower SFA digestibility than did AO1-L2 and L3 ($P < 0.01$). Nonetheless, animals fed AO1-L2 did not show differences with AO3 treatment and presented higher MUFA digestibility in comparison to L3 ($P < 0.001$).

4.4.4. Fatty acid composition of abdominal fat adipose tissue

The effect of dietary added fat on the FA composition of AFP is seen in **Table 4.8**. Total replacement of S by L increased SFA, in particular palmitic acid concentration ($P < 0.01$), whereas it reduced UFA:SFA and PUFA:SFA ($P < 0.01$). Furthermore, a tendency in a reduction of linoleic acid concentration ($P = 0.069$) was observed. In contrast to S3, animals feed AO3 presented AFP with higher MUFA concentration, concretely oleic acid ($P \leq 0.05$), and lower PUFA content, concretely linoleic and linolenic acid ($P \leq 0.05$), thus reducing PUFA:SFA ($P \leq 0.05$). Finally, the use of L as a substitute for AO caused an increase in PUFA, specifically linoleic and linolenic acid ($P < 0.01$), and a reduction in MUFA content ($P < 0.01$). In this case, PUFA:SFA increased as long as L replaced AO ($P = 0.014$).

4.5. Discussion

4.5.1. Chemical composition of the experimental fats and diets

The gross energy content of the added fats indicated that L resulted in being less energetic than did S and AO. This fact is a direct consequence of PL release less energy than triacylglycerol and FFA. Furthermore, L included in both experiments contained high levels of FFA (24.1%) because it was blended with soybean acid oil. The standard FFA content of crude soybean lecithin products are established at between 1.0 to 3.0% (Bueschelberger et al., 2015; EFSA, 2016). It is important to mention that available literature using a soybean lecithin high in FFA in monogastric nutrition is scarce and the literature review is based on studies that used a regular soybean lecithin with a lower FFA content. The chemical composition of the experimental diets reflected the FA profile of the added fats. Dietary UFA:SFA was reduced as L was included in replacement of S, as also reported Soares and Lopez-Bote (2002) previously.

Table 4.6. Feed apparent metabolizable energy value and fatty acid digestibility of starter and grower-finisher broiler chicken diets according to added fat sources (Experiment 1).

	Dietary treatments[1]					
Item	S3	S2-L1	S1-L2	L3	RSE	P-value
From 9 to 11 days						
AME (Kcal/kg)	3,089[a]	2,771[b]	2,763[b]	2,723[b]	0.36	<0.001
Fatty acid digestibility (%)						
TFA	81.5[a]	77.5[ab]	71.1[b]	70.9[b]	5.91	0.017
SFA	62.3	56.6	48.9	49.7	7.70	0.055
C16:0	69.5	65.0	58.4	60.4	6.83	0.098
C18:0	50.2	50.1	38.7	37.9	11.1	0.153
MUFA	79.2[a]	75.1[ab]	67.9[b]	68.8[b]	6.50	0.026
C18:1 ω-9	80.3[a]	78.0[a]	69.8[b]	69.8[b]	3.98	<0.001
PUFA	88.0[a]	83.6[ab]	75.6[b]	78.0[b]	5.45	0.004
C18:2 ω-6	87.7[a]	83.2[ab]	74.9[b]	77.3[b]	5.60	0.003
C18:3 ω-3	90.6[a]	87.4[ab]	80.6[b]	83.0[b]	4.31	0.003
From 36 to 37 days						
AME (Kcal/kg)	3,105[a]	3,057[a]	3,081[a]	2,818[b]	0.39	<0.001
Fatty acid digestibility (%)						
TFA	85.0[a]	83.5[ab]	83.0[ab]	79.0[b]	2.96	0.020
SFA	80.7	81.6	81.4	79.0	2.96	0.446
C16:0	82.3	83.3	83.3	81.0	2.84	0.480
C18:0	80.9	81.1	81.7	79.9	3.21	0.807
MUFA	84.8	83.8	81.8	78.7	4.25	0.141
C18:1 ω-9	88.5[a]	86.6[a]	87.9[a]	83.9[b]	1.46	<0.001
PUFA	85.3[a]	82.2[ab]	84.7[a]	79.9[b]	2.29	0.003
C18:2 ω-6	85.1[a]	82.1[ab]	84.5[a]	79.7[b]	2.33	0.003
C18:3 ω-3	86.4[a]	83.4[ab]	85.7[a]	81.7[b]	2.00	0.003

[a,c] Values within the same row with no common superscripts are different, $P \leq 0.05$.

1 S3 = soybean oil (S) at 3.00%; S2-L1 = S at 2.00% and soybean lecithin high in free fatty acids (L) at 1.00%; S1-L2= S at 1.00% and L at 2.00%; L3= L at 3.00%.

AME = apparent metabolizable energy; TFA = total fatty acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; RSE = residual standard error.

4.5.2. Growth performance and abdominal fat deposition

The inclusion of L as a substitute for S did not lead any negative effect on growth efficiency. Results agree with Azman and Cifti (2004), who observed that a partial replacement (50%) of soybean oil by a soybean lecithin (4 and 6% of total added fats for starter and grower-finisher diets, respectively) did not modify final BW or global feed conversion ratio. However, the use of AO instead S reduced feed conversion efficiency in grower-finisher phase and the global of Experiment 2. Some authors have stated that acid

oils present a lower nutritive value than do native oils due to their main lipid molecular structure being FFA, negatively affecting FA absorption (Sklan, 1979; Ravindran et al., 2016). Regarding abdominal fat deposition, results indicated that the different added fats included had no influence. It has been demonstrated that animals fed a diet high in SFA content (PUFA:SFA = 0.25) presented a higher AFP deposition than did animals fed diets rich in PUFA (PUFA:SFA = 6.72; Ferrini et al., 2008). The lack of differences observed in fat deposition in the present studies could be related to the slight changes in saturation degree between treatments (grower-finisher S3 and L3 PUFA:SFA = 1.95 and 1.70, respectively).

4.5.3. Digestibility balances

Results extracted from Experiment 1 shown that the substitution of S by L at any level in starter diets is not recommended, in terms of FA and energy utilization. However, the results in adult broilers suggest that L can partially replace S up to 2%. According to our results, Huang et al. (2007) observed, in young broiler chickens, that the partial (1%) and total replacement (2%) of soybean oil by soybean lecithin reduced feed AME content. In the case of adult broilers, they reported that the partial (0.5 and 1%), but also the total (2%), replacement of soybean oil by soybean lecithin did not affect feed AME value or ether extract utilisation.

In Experiment 2, the comparison between S3 and AO3 demonstrated the lowering effect of high FFA content on FA digestibility and feed AME, as other authors stated before (Blanch et al., 1996; Roll et al., 2018). The blending of AO and L, in starter diets, did not modify the FA digestibility except for linolenic acid, which was enhanced with L inclusion. However, in grower-finisher diets, the blending of 1% of AO plus 2% of L resulted in being the best option in terms of energy and FA utilization. Some authors have suggested that soybean lecithin, as an emulsifier, may enhance lipid absorption, in particular SFA and long-chain FA, by facilitating FA incorporation inside the micelles (Bauer et al., 2005; Ravindran et al., 2016). However, in accordance with Soares and Lopez-Bote results (2002), no improvement of SFA digestibility related to L inclusion has been demonstrated in the present experiments. This lack of effect could be related to the highly unsaturated degree of the experimental diets used in the present study.

Table 4.7. Feed apparent metabolizable energy value and fatty acid digestibility of starter and grower-finisher broiler chicken diets according to added fat sources (Experiment 2).

	Dietary treatments[1]						
Item	S3[2]	AO3	AO2-L1	AO1-L2	L3	RSE	P-value
From 9 to 11 days							
AME (kcal/kg)	2,986^{x}	2,866^{y}	2,794	2,890	2,842	0.41	0.252
Fatty acid digestibility (%)							
TFA	79.6^{x}	65.9^{y}	66.1	71.4	70.1	7.06	0.478
SFA	68.1^{x}	51.7^{y}	53.4	59.9	60.6	9.62	0.344
C16:0	73.1^{x}	61.5^{y}	61.6	68.1	66.2	7.94	0.428
C18:0	70.3^{x}	47.6^{y}	58.8	62.6	62.3	12.69	0.186
MUFA	80.3^{x}	70.5^{y}	70.6	75.1	70.5	7.60	0.678
C18:1 ω-9	80.8	71.9	71.5	76.3	71.9	7.26	0.649
PUFA	82.6^{x}	67.9^{y}	68.0	75.4	73.8	5.67	0.097
C18:2 ω-6	82.1^{x}	67.6^{y}	67.7	73.3	73.3	6.13	0.234
C18:3 ω-3	86.7^{x}	70.8^{y,b}	71.3^{b}	77.3^{ab}	78.2^{a}	4.12	0.011
From 36 to 37 days							
AME (kcal/kg)	3,033^{x}	2,940^{y,a}	2,801^{b}	2,992^{a}	2,962^{a}	0.25	<0.001
Fatty acid digestibility (%)							
TFA	87.0^{x}	84.3^{y,a}	81.4^{b}	84.6^{a}	83.3^{ab}	1.73	0.022
SFA	83.3	81.1^{ab}	78.6^{b}	82.8^{a}	82.6^{a}	1.80	0.002
C16:0	86.1	84.1^{a}	81.4^{b}	85.4^{a}	85.0^{a}	1.58	0.001
C18:0	88.1^{x}	84.4^{y,ab}	84.0^{b}	86.2^{a}	86.2^{a}	1.51	0.041
MUFA	88.2	88.6^{a}	85.5^{bc}	87.5^{ab}	84.7^{c}	1.24	<0.001
C18:1 ω-9	90.1	90.0^{a}	87.5^{bc}	89.1^{ab}	87.1^{c}	1.18	0.001
PUFA	87.9^{x}	81.5^{y}	79.3	82.9	82.9	2.52	0.071
C18:2 ω-6	87.5^{x}	81.6^{y}	79.2	82.8	82.6	2.52	0.080
C18:3 ω-3	90.5^{x}	80.1^{y,b}	80.1^{b}	83.9^{ab}	84.9^{a}	2.63	0.006

^{a,c} Values within the same row with no common superscripts are different, $P \leq 0.05$.

^{x,y} Values within the same row with no common superscripts are different, $P \leq 0.05$.

[1] S3= soybean oil at 3.00%; AO3= acid oil (AO) at 3.00%; AO-L1= AO at 2.00% and soybean lecithin high in free fatty acids (L) at 1.00%; AO-L2= AO at 1.00% and L at 2.00%; L3= L at 3.00%.

[2] S3 was not included in the statistical analysis against diets containing co-products.

AME = apparent metabolizable energy; FA = fatty acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; RSE = residual standard error.

On the other hand, in the grower-finisher phase of Experiment 2, AO1-L2 treatment resulted in the best option, thanks to an improvement in linolenic acid, along with a tendency in PUFA digestibility ($P = 0.071$), which suggests an emulsifying effect. It is well known that blending fats with a complementary FA profile and different lipid molecular structures (triacylglycerols, FFA and PL) produces positive interactions in terms of AME content and FA digestibility (Blanch et al. 1996; Borsatti et al., 2017; Roll et al., 2018). The synergic effect observed between 1% of AO and 2% of L can be explained because it might be an adequate proportion of PL capable of solubilising FFA in the mixed micelle better, facilitating its absorption.

4.5.4. Fatty acid composition of abdominal fat tissue

The FA profile of AFP reflected the FA profile of the diets, and that was according to most of the published data (Ferrini et al., 2008; Vilarrasa et al., 2015b). Although some authors have reported that the presence of different dietary lipid molecular structures, such as randomised FA, influence the FA profile of AFP (Smink et al., 2008; Vilarrasa et al., 2015b), our results demonstrate that the saturation degree of AFP is more influenced by the dietary saturation degree rather than by the lipid molecular structures (triacylglycerols, FFA and PL) present in the feed.

4.6. Conclusions

In summary, the inclusion of soybean lecithin high in FFA is suitable in grower-finisher diets, as a partial replacer of soybean oil up to 2%, without impairing performance, FA and energy utilisation. In relation to the use of a combination of co-products as an energy source, the best strategy, in grower-finisher diets, is the blend of 2% of high FFA soybean lecithin plus 1% of monounsaturated vegetable acid oil, due to synergistic interactions on FA and energy utilisation. Finally, the FA profile of the diets has a major impact on the FA profile of AFP rather than the different lipid molecular structures.

Table 4.8. Fatty acid composition of abdominal fat pad of broiler chickens according to different fat sources[1] in diet (Experiments 1 and 2).

	Experiment 1						Experiment 2						
Item	S3	S2-L1	S1-L2	L3	RSE	P-value	S3[2]	AO3	AO2-L1	AO1-L2	L3	RSE	P-value
Fatty acid profile (%)													
SFA	29.8^{b}	30.0^{b}	30.3^{b}	32.1^{a}	1.06	0.005	29.2	29.8	30.8	30.3	31.1	1.14	0.287
C16:0	23.6^{b}	24.0^{b}	24.3^{b}	25.7^{a}	0.76	<0.001	23.1	23.6	24.2	23.9	24.5	0.98	0.463
C18:0	5.31	5.46	5.17	5.55	0.64	0.779	5.37	5.34	5.73	5.39	5.44	0.40	0.364
MUFA	44.9	46.4	46.4	46.9	2.81	0.661	46.8^{y}	53.6^{x,a}	50.8^{ab}	48.5^{b}	46.7^{b}	2.76	0.002
C18:1 ω-9	37.4	38.5	37.3	38.3	1.45	0.468	39.0^{y}	45.4^{x,a}	42.8^{ab}	40.6^{bc}	38.6^{c}	2.06	<0.001
PUFA	25.3	23.7	23.3	21.0	2.85	0.107	24.5^{x}	16.6^{y,b}	17.8^{ab}	21.4^{a}	22.5^{a}	2.75	0.004
C18:2 ω-6	22.4	20.9	19.7	18.5	2.40	0.069	21.3^{x}	14.9^{y,b}	16.0^{ab}	19.0^{a}	19.9^{a}	2.41	0.006
C18:3 ω-3	2.19	2.06	2.03	1.88	0.27	0.293	2.47^{x}	1.06^{y,c}	1.42^{bc}	1.81^{ab}	2.02^{a}	0.26	<0.001
UFA:SFA	2.37^{a}	2.34^{a}	2.30^{ab}	2.11^{b}	0.12	0.007	2.43	2.36	2.25	2.32	2.22	0.12	0.223
PUFA:SFA	0.85^{a}	0.79^{ab}	0.79^{ab}	0.65^{b}	0.09	0.009	0.84^{x}	0.55^{y,b}	0.60^{ab}	0.74^{a}	0.73^{a}	0.10	0.014

[a-c] Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[x-y] ANOVA S3 *vs* AO3: values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[1] S3 = soybean oil (S) at 3.00%; S2-L1 = S at 2.00% and soybean lecithin high in free fatty acids (L) at 1.00%; S1-L2 = S at 1.00% and L at 2.00%; AO3 = acid oil (AO) at 3.00%; AO2-L1 = AO at 2.00% and L at 1.00%; AO1-L2 = AO at 1.00% and L at 2.00%; L3 = L at 3.00%.

[2] S3 was not included in the statistical analysis against diets containing co-products.

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio; RSE = residual standard error.

Chapter Five

Soybean lecithin as energy source for adult broilers: effects on performance, fatty acid absorption, gastrointestinal health and adipose saturation degree

"Hagas lo que hagas ámalo,

como amabas la cabina del Paradiso cuando eras niño."

Alfredo, en *Cinema Paradiso*, 1988.

5.1. Abstract

Soybean lecithin, a co-product of soybean oil refining process rich in phospholipids, could be a suitable energy source for grower and finisher broiler chickens. An experiment was performed to assess the inclusion of soybean lecithin (L) in replacement of soybean oil (S) and its effects on performance, fatty acid (FA) absorption, gut health and saturation degree of the abdominal fat pad (AFP). A total of 1440 newly hatched female Ross-308 were distributed in 60 pens of an environmentally controlled farm and were fed with five experimental diets. The control diet (T1) was supplemented with S (grower and finisher diets at 2%) and four levels of L were included in replacement: T2 (0.25% in grower and 0.5% in finisher diet), T3 (0.5% in grower and 1% in finisher diet), T4 (0.75% in grower and 1.5% in finisher diet) and T5 (1% in grower and 2% in finisher diet). At day 39, titanium dioxide was added to finisher diets at 0.5% in order to perform a digestibility balance (six replicates per treatment). At day 46, AFP, tissue and gut digesta samples were collected to characterize adipose saturation degree, microbial groups and histomorphometry. No effects were associated to S replacement by L on performance parameters ($P > 0.05$), ileal apparent digestibility of total, saturated and monounsaturated FA ($P > 0.05$) nor jejunal morphology ($P > 0.05$). Total replacement of S by L reduced ileal apparent absorption of polyunsaturated FA ($P < 0.02$) and increased jejunal *Lactobacillus* spp. counts ($P = 0.049$). Higher levels of L inclusion (T4 and T5) lowered PUFA concentration of the AFP ($P = 0.002$), and thus, slightly reduced unsaturated-to-saturated FA ratio of carcass fat (T1: 2.45 vs. T5 = 2.25; $P = 0.005$). The present experiment demonstrates that L represents an alternative for S, as energy source for grower and finisher broiler diets, without modifying performance, total FA apparent ileal absorption nor jejunal morphology and causing minor changes on the FA profile of the AFP. Soybean oil total replacement by L increased jejunal *Lactobacillus* spp. counts and reduced the apparent ileal digestibility of polyunsaturated FA; however, these modifications had no impact on broiler chicken growth.

5.2. Introduction

Fats and oils are commonly included in broiler chicken feed in order to increase the energetic density of the diets, however, its inclusion is highly dependent on their chemical composition and economic cost. It has been demonstrated that the saturation degree and the composition in different lipid molecular structures have an important influence on

performance parameters (Vieira et al., 2006; Ferrini et al., 2008), fatty acid (**FA**) absorption (Tancharoenrat et al., 2013; Roll et al., 2018), gastrointestinal health (Thormar et al., 2006; Khatun et al., 2017) and carcass quality parameters (Smink et al., 2008; Gonzalez-Ortiz et al., 2013).

Currently, there is a growing interest in the search and use of alternative energy sources in broiler feeding. In this context, co-products derived from soybean oil (**S**) refinement process represents an economic alternative and permit to give an added value to residual products. Soybean lecithin (**L**), which is extracted from soybean oil degumming process, is mainly composed by polar lipids (> 60%), especially by phospholipids (**PL**), but also contains an important amount of neutral lipids (30%-40%), as triacylglycerols (**TAG**) and free fatty acids (**FFA**; van Nieuwenhuyzen and Tomas, 2008; EFSA, 2016). Furthermore, soybean lecithin represents a good source of phosphorus, choline and energy for broiler chickens (Mateos et al., 2012; Borsatti et al., 2017) and its combination with other fats and oils could be interesting in order to exploit synergies on lipid utilization (Ravindran et al., 2016).

Available literature indicates that soybean oil replacement by soybean lecithin, in starter broiler diets, impairs the growth performance (Azman and Cifti, 2004), reduces the feed apparent metabolizable energy content of the feed (Huang et al., 2007; Viñado et al., 2019[1]) and also lowers total tract digestibility of the polyunsaturated FA (**PUFA**; Viñado et al., 2019). On the contrary, in grower-finisher diets, a few authors have indicated that soybean lecithin can partially replace soybean oil without modifying energy utilization (Huang et al., 2007; Viñado et al., 2019) and total tract FA digestibility (Viñado et al., 2019).

Therefore, it has been hypothesized that soybean lecithin can replace soybean oil in grower and finisher broiler diets, as an energetic ingredient, without modifying performance parameters, nutrient absorption, gut health and carcass FA profile. Taking this into account, the present study was designed with the aim to evaluate increasing rates of soybean oil replacement by soybean lecithin in grower and finisher diets, assessing its

[1] Viñado, A., L. Castillejos, R. Rodriguez-Sanchez, and A.C. Barroeta. 2019. Crude soybean lecithin as alternative energy source for broiler chicken diets. Poultry Science, accepted May 18th. DOI: 10.3382/ps/pez318

effects on growth performance, ileal FA absorption, jejunal morphology and microbiota counts, and the FA profile of the abdominal fat pad (**AFP**).

5.3. Material and methods

5.3.1. Experimental design, animal husbandry and sampling

The experiment was in accordance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010) and was approved by the Animal Ethics Committee of the Universitat Autònoma de Barcelona (CEEAH).

The experiment was performed in a commercial farm under controlled conditions (Granja Sole, Vila-Rodona, Spain). A total of 1440 newly hatched female broiler chickens Ross 308 were obtained from a local hatchery (Pondex S.A.U, Juneda, Spain). On arrival, the animals were weighted (initial body weight: 42.1 ± 0.02 g) and randomly allocated to 60 pens with 24 birds. Replicates were allocated for a homogeneous distribution of treatments and started with an equal mean bodyweight (**Table 5.5**). Each pen consisted in 1.5 square meters and were equipped with a pan feeder and a bell waterer, and both, experimental feed and water, were provided *ad libitum* throughout the trial. Temperature, humidity level and light program used was according with the specifications in the Ross 308 lineage management handbook (Aviagen, 2014). Twice daily, animals and housing facilities were inspected for the general health status, constant feed, dead birds, water supply, as well as temperature and ventilation.

The feeding program was divided in three phases: starter (from 0 to 14 days), grower (from 15 to 28 days) and finisher (from 29 days to end of the experiment). All experimental diets were based in wheat and soybean meal and were formulated to meet or exceed FEDNA (2008) requirements, as it figures in **Table 5.1**. Animals were fed with five experimental treatments (12 replicates per treatment) during grower and finisher phase, as it is detailed in **Table 5.2.** The control diet (T1) was supplemented with S at 2% (grower and finisher) and four levels of L were included replacing S: T2 (0.25% in grower and 0.5% in finisher diet), T3 (0.5% in grower and 1% in finisher diet), T4 (0.75% in grower and 1.5% in finisher diet) and T5 (1% in grower and 2% in finisher diet).

Table 5.1. Ingredient composition of experimental basal diets (%, as fed basis).

Ingredient (%)	Starter diet (0 to 14 days)	Grower diet (15 to 28 days)	Finisher diet (from 29 days)
Wheat	29.75	49.37	46.06
Soybean meal 47%	27.40	23.65	22.82
Corn	15.10	0.00	0.00
Barley	9.78	11.74	17.62
Extruded full-fat soybean	9.17	4.58	0.00
Added fats[1]	2.00	3.25	4.50
Rapeseed meal 00	0.00	1.52	3.43
Sepiolite	1.99	1.99	1.99
Trace mineral-vitamin premix[2]	1.43	1.13	1.15
Calcium carbonate	1.23	1.05	0.93
Monocalcium phosphate	0.96	0.64	0.49
Salt	0.35	0.21	0.23
L-lysine	0.30	0.31	0.29
DL-methionine	0.30	0.25	0.22
Sodic bicarbonate	0.09	0.16	0.12
L-threonine	0.08	0.08	0.08
Clorure choline 75%	0.07	0.07	0.07

[1] *Soybean oil, soybean lecithin, palm oil and vegetable acid oil, in different blending proportions (see Table 5.2).*

[2] *Provides per kg feed: vitamin A (from retinol), 13,500 IU; vitamin D3 (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 4.5 mg; vitamin B12, 16.5 µg; vitamin K3, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from FeSO4·7H2O), 54 mg; I [from Ca(I2O3)2], 1.2 mg; Co (from 2CoCO3·3Co(OH)2·H2O), 0.6 mg; Cu (from CuSO4·5H2O), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na2SeO3), 0.18 mg; Mo [from (NH4)6Mo7O24], 1.2 mg; Organic acids (starter diets at 4 g/kg; grower and finisher diets at 3 g/kg); β-glucanase 350 IU; xylanase 1,125 IU.*

Table 5.2. Total added fat inclusion in the experimental diets (%, as fed basis).

	Starter diet (0 to 14 days)	Grower diet (15 to 28 days)					Finisher diet (from 29 days)				
Ingredient (%)		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Total added fats	2.00	3.25	3.25	3.25	3.25	3.25	4.50	4.50	4.50	4.50	4.50
Palm oil	0.00	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00
Acid oil[a]	0.00	0.75	0.75	0.75	0.75	0.75	1.50	1.50	1.50	1.50	1.50
Soybean oil	2.00	2.00	1.75	1.50	1.25	1.00	2.00	1.50	1.00	0.50	0.00
Soybean lecithin	0.00	0.00	0.25	0.50	0.75	1.00	0.00	0.50	1.00	1.50	2.00

[1] Vegetable monounsaturated acid oil (blending 50:50 of olive pomace acid oil and sunflower acid oil).

Broiler body weight (**BW**) and feed intake was recorded on pen (24 birds) at day of hatch, 14, 28 and 39. The data were used to measure BW and calculate the average daily gain (**ADG**), the average daily feed intake (**ADFI**) and the feed conversion ratio (**FCR**) of each period and the overall results of the trial. Mortality was daily recorded to adjust ADFI and ADG (1.8% of total mortality rate). At day 39, three animals of each pen (six replicates per treatment) were chosen based on pen mean BW (a total of 90 animals) and received the finisher diet with titanium dioxide (**TiO_2**) at 0.5% from day 40 to 46. The rest of animals were slaughtered in a commercial abattoir. At day 46, broiler chickens were euthanized with the aim to collect digesta samples and AFP. The ileal content of all birds within a pen was pooled, homogenized, freeze-dried, ground and kept at 4ºC until FA profile analysis. A representative sample of the AFP (from the proventriculus surrounding the gizzard down to the cloaca) of each bird was taken, pooled by replicate, frozen at -20 ºC and analyzed to determine the FA profile. Finally, jejunum tissue and digesta content from T1 and T5 animals were collected in order to evaluate the effect of using either S or L as added fats on jejunal morphology and microbiota.

5.3.2. Laboratory analyses

Soybean oil and L samples were analyzed in duplicate for FA composition, by gas chromatography, according to methylation method described by Guardiola et al. (1994). In addition, the acid value was determined according to ISO 660 (2009) and the acidity was indicated as FFA percentage by expressed in oleic acid. Regarding PL content of crude soybean lecithin used, acetone insoluble determination was performed following the Ja-4-46 analytical method from AOCS (2017), and the PL composition was determined by HPLC following the method described by Helmerich and Koehler (2003). Experimental feed samples were taken at the beginning and end of each experimental periods, were grounded and kept at 4ºC until further analysis. Diet proximate analysis were performed according to the methods of AOAC International (2005): ether extract (method 920.39), crude protein (Method 968.06) and crude fiber (Method 962.09). In addition, feed and excreta samples analysis included ash determination (Method 942.05), dry matter (Method 934.01), and gross energy content by adiabatic bomb calorimeter (IKA-Kalorimeter system C4000; Staufen, Germany).

Fatty acid content was analyzed adding nonadecanoic acid (Sigma–Aldrich; St. Louis, MO) as an internal standard and following the method described by Sukhija and Palmquist (1988) in feed and ileal content, whereas in the case of AFP, the method described by Carrapiso et al. (2000) was used. The final extract obtained was injected in a gas chromatograph following the method conditions previously described by Cortinas et al. (2004). Titanium dioxide was analyzed on finisher diets and ileal content following the methodology described in Short et al. (1996).

5.3.3. Jejunal histomorphometry and microbial counts of digesta

A distal jejunum segment of 5 cm (close from Meckel's diverticulum) was excised, flushed with sterile PBS and fixed by immersion in a 3.7-4.0% neutral buffered formaldehyde solution (PanReac AppliChem, Castellar del Vallès, Spain). Then, the tissue was embedded in paraffin, prepared and stained with hematoxylin/eosin for further observations in a light microscope (BHS-Olympus, Tokyo, Japan). The histomorphometric indexes evaluated were villus height (**VH**: from the villous tip to the crypt junction), crypt depth (**CD**: from the villous bottom to the crypt) and the ratio between VH and CD (**VH:CD**). The analysis was performed on 10 well-oriented and intact villi and 10 crypts according to Schiavone et al. (2018) methodology. Measures were taken using ImageJ (1.8.0) as software analysis.

Jejunal digesta samples were collected in sterile conditions and were stored at 4 ºC until further analysis. Samples were 10-fold serial diluted in Lactated Ringer's Solution (Sigma–Aldrich Química, Madrid, Spain) in order to perform microbiota counts. Diluted digesta samples were inoculated on azide glucose, MacConkey and MRS agar (Oxoid Limited, Hampshire, United Kingdom) for microbial counts of *Enterococcus faecium*, *Enterobacteria* and *Lactobacillus* spp., respectively. Counts were manually read after 24 h incubation at 37 ºC.

5.3.4. Calculations and statistical analysis

The FA apparent ileal digestibility coefficient (**AIDC**) was calculated as follows: $AIDC = 1-\{(TiO_2/N)_d / (TiO_2/N)_i\}$. Where $(TiO_2/N)_d$ is the concentration of the inert marker and the nutrient in the diet, and $(TiO_2/N)_i$ is the concentration of the inert marker and the nutrient in the ileal digesta.

Pen means (24 birds) were used as experimental unit (12 replicates per treatment) for performance parameters. In the case of AIDC calculations and the FA profile of the AFP,

pen means (3 birds) were used as experimental unit (six replicates per treatment). Microbial counts (log transformation for statistical analysis) and histomorphometric analysis were performed on T1 and T5, being the pen the experimental unit (six replicates per treatment). Data were analyzed by one-way ANOVA using R Statistics (version 3.3.1) with treatment as the main factor. Tukey's multiple range test was performed to determine whether means were significantly different ($P \leq 0.05$).

Table 5.3. Chemical analysis of soybean oil and soybean lecithin included in the experimental diets.

	Experimental fats[1]	
Item	S	L
Energy content (kcal/kg)	9,602	7,937
Fatty acid composition (%)[2]		
SFA	16.0	23.4
C16:0	10.6	20.4
C18:0	4.24	2.97
MUFA	23.4	18.4
C18:1 ω-9	21.8	18.4
PUFA	60.6	58.2
C18:2 ω-6	52.9	53.3
C18:3 ω-3	7.67	4.81
Minor fatty acids	2.8	N.D.
UFA:SFA	5.25	3.27
PUFA:SFA	3.79	2.49
Acidity (%)[2]		
FFA	1.29	14.6
Phospholipids (g/kg)[2]		
AI	N.D.	68.3
Total PL	N.D.	44.0
Phosphatidylcholine	N.D.	13.5
Phosphatidylinositol	N.D.	13.7
Phosphatidylethanolamine	N.D.	9.15
Phosphatidic acid	N.D.	7.02
Lysophosphatidylcholine	N.D.	0.58

[1] S = soybean oil; L = soybean lecithin.

[2] Expressed on total product.

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio; AI = acetone insoluble matter; Total PL = total phospholipids; FFA = free fatty acid; N.D. = not determined.

5.4. Results

5.4.1. Chemical composition of the experimental fats and diets

The chemical composition of S and L, and the chemical composition of experimental diets figure in **Table 5.3** and **Table 5.4**, respectively. Soybean oil and L were mainly composed by PUFA and differed on their respective saturated FA (**SFA**) and monounsaturated FA (**MUFA**) content. Soybean lecithin, in contrast to S, presented a higher SFA content (23.4% *vs.* 16%, respectively) and a lower MUFA content (18.4% *vs.* 23.5%, respectively), hence lower unsaturated-to-saturated fatty acid ratio (**UFA:SFA**) and polyunsaturated-to-saturated fatty acid ratio (**PUFA:SFA**). Furthermore, L presented higher acidity and lower gross energy content than S. The experimental diets were close similar between treatments regarding protein and ether extract content; however, differed on their respective FA composition.

5.4.2. Performance parameters

The effects of S substitution by L on productive performance figure in **Table 5.5**. Soybean oil partial and total replacement by L did not modify any performance parameter during the grower and the finisher phase ($P > 0.05$), and neither during the overall period ($P > 0.05$).

5.4.3. Apparent ileal digestibility coefficients of fatty acids

The effects of S replacement by L on the AIDC of FA are shown in **Table 5.6**. Soybean oil partial and total replacement by L in the finisher diets did not modify total FA (**TFA**), SFA and MUFA digestibility ($P > 0.05$). However, PUFA apparent ileal absorption, concretely linoleic and linolenic acid, were lower in T3, T4 and T5, in contrast to T1 ($P < 0.02$).

5.4.4. Jejunal histomorphometry and microbiological counts

The effects of either including S (T1) or L (T5) at 2%, in finisher diets, on jejunal morphology and microbiological counts of digesta are shown in **Table 5.7**. The different dietary added fats did not influence on any morphologic measurement ($P > 0.05$). Concerning jejunal microbiological counts, the use of L instead S increased *Lactobacillus* spp. population ($P = 0.049$); however, no differences were observed regarding *Enterobacteria* and *Enterococcus faecium* ($P > 0.05$).

Table 5.4. Analyzed gross energy, macronutrient content and fatty acid composition for starter, grower and finisher diets.

Item	Starter diet (0 to 14 days)	Grower diet[1] (15 to 28 days)					Finisher diet[2] (from 29 days)				
		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Energy content (kcal/kg)	4,182	4,249	4,259	4,235	4,156	4,180	4,237	4,182	4,197	4,196	4,180
Macronutrient content (%)											
Dry matter	89.73	90.88	91.26	90.45	90.36	90.35	90.31	89.91	90.15	90.64	90.61
Crude protein	21.62	19.16	19.42	19.26	19.49	19.16	18.45	18.32	18.35	18.26	18.44
Crude Fat	5.53	5.34	5.28	5.15	5.02	5.23	5.68	5.37	5.34	5.38	5.24
Crude Fiber	3.31	4.04	4.12	4.38	4.19	3.90	4.07	3.59	3.68	3.95	3.79
Ash	7.67	6.51	7.21	6.86	6.74	6.78	6.02	6.29	6.33	6.37	6.47
Fatty acid composition (%)											
SFA	17.6	21.0	21.1	21.6	21.8	22.0	24.3	24.9	25.3	25.8	26.5
C16:0	13.4	16.1	16.3	16.7	16.9	17.1	18.7	19.3	19.7	20.1	20.8
C18:0	3.02	3.63	3.57	3.57	3.66	3.65	4.16	4.16	4.18	4.21	4.27
MUFA	22.4	26.0	25.8	25.1	25.2	25.0	27.8	27.3	27.1	26.9	26.8
C18:1 ω-9	20.1	23.8	23.4	22.8	22.6	22.4	25.4	24.9	24.7	24.4	24.4
PUFA	60.0	53.0	53.1	53.3	53.0	53.0	47.9	47.8	47.6	47.3	46.7
C18:2 ω-6	54.1	47.9	47.9	48.1	47.9	47.9	43.6	43.7	43.5	43.4	42.9
C18:3 ω-3	5.96	5.42	5.20	5.14	5.13	5.12	4.29	4.19	4.09	3.98	3.82
Minor fatty acids	3.49	3.48	3.59	3.67	3.93	3.92	3.82	3.78	3.82	3.88	3.91
UFA:SFA	4.68	3.76	3.74	3.63	3.59	3.55	3.12	3.02	2.95	2.88	2.77
PUFA:SFA	3.41	2.52	2.52	2.47	2.43	2.41	1.97	1.92	1.88	1.83	1.76

[1] T1 = soybean oil (S) at 2%; T2 = S at 1.75% and crude soybean lecithin (L) at 0.25%; T3 = S at 1.5% and L at 0.5%; T4 = S at 1.25% and L at 0.75 %; T5 = S at 1% and L at 1%.

[2] T1 = S at 2%; T2 = S at 1.5% and L at 0.5%; T3 = S at 1 % and L at 1%; T4 = S at 0.5% and L at 1.5%; T5 = L at 2%.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio.

5.4.5. Fatty acid composition of abdominal fat adipose tissue

The effect of L inclusion on the FA composition of AFP is presented in **Table 5.8**. Animals fed T1 showed a higher PUFA (P = 0.002), concretely linoleic (P = 0.003) and linolenic acid (P < 0.001) content, and tended to show a lower palmitic acid content than animals fed diets with a high level of L supplementation (T4 and T5; P = 0.068). Thus, L inclusion, as a replacer for SO, reduced the UFA:SFA (T1: 2,42 *vs.* T5: 2,25; P = 0.005) and the PUFA:SFA (0.88 *vs.* 0.75; P = 0.004) of the AFP.

Table 5.5. Growth performance of broiler chickens according to soybean lecithin inclusion level in the grower and finisher diets.

	Dietary Treatments						
Item	T1	T2	T3	T4	T5	RSE	P-value
Starter period (0 to 14 days)[1]							
BW 0 days (g)	42.09	41.98	42.03	42.09	42.10	0.176	0.337
BW 14 days (g)	453	459	454	456	459	13.8	0.792
ADFI (g/d/bird)	40.5	40.4	40.4	39.8	40.4	1.07	0.571
ADG (g/d/bird)	29.4	29.8	29.4	29.6	29.8	0.99	0.812
FCR (g/g)	1.37	1.37	1.36	1.35	1.35	0.030	0.305
Grower period (15 to 28 days)[2]							
BW 28 days (g)	1,552	1,544	1,531	1,541	1,551	36.74	0.647
ADFI (g/d/bird)	121.6	120.3	120.1	119.5	121.8	2.65	0.182
ADG (g/d/bird)	77.7	77.6	76.9	77.8	78.4	0.07	0.436
FCR (g/g)	1.56	1.57	1.56	1.54	1.56	0.024	0.195
Finisher period (29 to 39 days)[3]							
BW at 39 days (g)	2,439	2,422	2,443	2,437	2,442	73.52	0.961
ADFI (g/d/bird)	173.1	173.5	174.7	174.5	175.5	6.59	0.909
ADG (g/d/bird)	81.2	78.5	83.3	81.2	80.7	5.27	0.324
FCR (g/g)	2.14	2.21	2.13	2.16	2.18	0.100	0.293
Global period (0 to 39 days)							
ADFI (g/d/bird)	106.0	106.8	106.5	106.4	107.7	2.57	0.612
ADG (g/d/bird)	61.2	60.6	61.4	61.2	61.5	1.99	0.842
FCR (g/g)	1.74	1.76	1.74	1.73	1.75	0.030	0.154

[1] All replicates consumed the same starter diet.

[2] T1 = soybean oil (S) at 2%; T2 = S at 1.75% and crude soybean lecithin (L) at 0.25%; T3 = S at 1.5% and L at 0.5%; T4 = S at 1.25% and L at 0.75 %; T5 = S at 1% and L at 1%.

[3] T1 = S at 2%; T2 = S at 1.5% and L at 0.5%; T3 = S at 1 % and L at 1%; T4 = S at 0.5% and L at 1.5%; T5 = L at 2%.

BW= body weight; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio.

5.5. Discussion

5.5.1. Chemical composition of the experimental fats and diets

In general terms, the chemical composition of S and L was in accordance with existing literature (Øverland et al., 1993; Soares and Lopez-Bote, 2002; Viñado et al., 2019). Furthermore, the FA profile of the experimental diets were a clear reflect of the FA profile of the different added fats, showing an increment of dietary SFA content and a reduction on UFA:SFA and PUFA:SFA as L was included in replacement of S. Similar effects due to soybean oil substitution by soybean lecithin on dietary FA profile were reported previously by Soares and Lopez-Bote (2002) and Viñado et al. (2019).

5.5.2. Performance parameters

The results obtained in the present experiment have indicated that S replacement by L, in grower and finisher diets, did not modify the performance parameters in any phase and the overall of the trial. This finding was consistent with the data reported in Viñado et al. (2019), where soybean oil partial and a total replacement by a soybean lecithin (33%, 66% and 100% of a 3% of total dietary added fat) did not alter broiler chicken growth performance. Furthermore, our results also partially agreed with Azman and Cifti (2004), who observed that a soybean oil partial replacement by soybean lecithin (25% and 50% replacement of a 6% of total added fat) did not modify grower-finisher broiler chickens BW and FCR. Other authors have stated that soybean lecithin inclusion, as alternative to saturated added fats, such as tallow, is acceptable without a negative impact on the productive performance of broiler chickens (Cantor et al., 1997; Cox et al., 2000), laying hens (Mandalawi et al., 2015) and weaned pigs (Jones et al., 1992; Øverland and Sundstøl, 1995; Reis de Souza et al., 1995). On the contrary, Huang et al. (2007) observed that soybean oil partial and total replacement (2% of total addition) by soybean lecithin improved broiler ADG during grower-finisher phase. They also indicated that a partial replacement (25%) enhanced global ADG and FCR, whereas a total replacement impaired global ADG and FCR.

Concerning its effects on the feed intake, no effects were observed despite of L contained less gross energy than SO (7,937 *vs.* 9,602 kcal/kg; **Table 5.3**). This finding is in accordance with Mandalawi et al. (2015), who observed in laying hens, that pork fat replacement by soybean lecithin did not modify the feed intake, even though that lecithin was a 16% less energetic (32.6 *vs.* 38.9 MJ/kg). It is important to remark the existence of

controversial results on feed intake in the literature, due to other authors have reported that soybean lecithin inclusion in replacement of soybean oil had a lowering effect on the ADFI (Azman and Cifti, 2004; Huang et al., 2007). Despite L contained more SFA a total than S (23.4% *vs.* 16%), it was also mainly composed by unsaturated FA (> 84%; **Table 5.3**) and its most abundant lipid molecular structures were the PL (44%; **Table 5.3**). Both facts are important, due to it is well known that the chemical composition of a fat (FA chain length, saturation degree, lipid molecular structures) has a major importance on fat utilization, and consequently on growth performance (Dänicke et al., 1997; Bãiao and Lara, 2005). Several authors have suggested that PL present in lecithin are responsible of enhancements in nutrient absorption due to positive interactions with other lipid molecules, indicating the suitability of soybean lecithin as an energetic ingredient for poultry feeding (Mandalawi et al., 2015; Ravindran et al., 2016; Borsatti et al., 2017). Therefore, the performance results confirmed the hypothesis that it is possible to use L in replacement of S, for adult broiler diets, without altering growth performance.

5.5.3. Fatty acid ileal digestibility

The use of L instead of SO did not modify the ileal absorption of TFA, SFA and MUFA. It is well established that FA absorption is a process highly influenced by the saturation degree of the FA (Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019), and as it was commented previously, the FA profile of both, L and S, was close similar. This fact might justify the absence of differences regarding the absorption of FA using either S or L. The present results were consistent with data reported by Huang et al. (2007), who observed that partial and total replacement of soybean oil by a soybean lecithin (2 % of total added fats) did not alter ether extract apparent total tract digestibility in finisher broiler chickens. More concretely, Viñado et al. (2019) studied the effect of replacing soybean oil by a soybean lecithin on total tract apparent digestibility of FA, and confirmed that soybean oil partial and total replacement by soybean lecithin (3% of total added fats) did not modify, in finisher broiler chickens, the total tract digestibility of TFA, SFA and MUFA. It is important to remark the importance of the chemical composition of the replaced fat on the results obtained. Several researchers have tested the inclusion of soybean lecithin as a replacer for saturated fat sources and its effects on lipid digestion and absorption. For example, Jones et al., (1992) concluded that tallow partial replacement by lecithin (10%), in weaned pig diets, improved total tract apparent digestibility of fat, whereas in the case of soybean oil replacement, no effects were

described. This finding was confirmed by Mandalawi et al. (2015), who reported, in laying hens, that partial and total replacement of pork fat by soybean lecithin improved the total tract apparent utilization of ether extract, organic matter and gross energy.

Table 5.6. Broiler apparent ileal digestibility coefficients (AIDC) of fatty acids according to soybean lecithin inclusion level in the digestibility balance performed from day 40 to 46 of live.

	Dietary treatments[1]						
AIDC	T1	T2	T3	T4	T5	RSE	P-value
TFA	0.84	0.81	0.79	0.79	0.80	0.036	0.229
SFA	0.77	0.77	0.78	0.76	0.78	0.046	0.945
C16:0	0.77	0.77	0.78	0.76	0.78	0.046	0.915
C18:0	0.78	0.79	0.80	0.79	0.79	0.045	0.960
MUFA	0.85	0.84	0.83	0.81	0.82	0.031	0.331
C18:1 ω-9	0.85	0.84	0.83	0.81	0.83	0.031	0.364
PUFA	0.85^{a}	0.82^{ab}	0.78^{b}	0.78^{b}	0.80^{b}	0.035	0.017
C18:2 ω-6	0.85^{a}	0.82^{ab}	0.78^{b}	0.78^{b}	0.80^{b}	0.035	0.019
C18:3 ω-3	0.85^{a}	0.83^{ab}	0.78^{c}	0.78^{c}	0.79^{bc}	0.039	0.016

[a-c] Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[1] T1 = Soybean oil (S) at 2%; T2 = S at 1.5% and soybean lecithin (L) at 0.5%; T3 = S at 1 % and L at 1%; T4 = S at 0.5% and L at 1.5%; T5 = L at 2%.

TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; RSE = residual standard error.

Nevertheless, S replacement rates equal or higher than a 1% by L affected negatively the absorption of PUFA, specifically to linoleic and linolenic acid. Same effect was also reported previously (Viñado et al., 2019), where PUFA digestibility was negatively affected with a 66% and 100% soybean oil replacement by soybean lecithin (3% of total added fats). Common fats and oils included in broiler feeding, as S, contain most of their FA esterified with glycerol forming TAG (Bãiao and Lara, 2005). In the present experiment, L contained ten times more FFA than S (14.6% *vs.* 1.3%). It is well studied that FA absorption is more reduced in their free form than if they are esterified to glycerol (Blanch et al., 1995; Roll et al., 2018). This fact may partially explain the differences observed in PUFA, linoleic and linolenic acid apparent digestibility at ileum. However, the reason of this finding is still unclear.

5.5.4. Jejunal histomorphometry and microbiological counts

Morphology of intestinal mucosa and microbial population represents two of the most important biomarkers for evaluating the gastrointestinal health in poultry species (Yegani and Korver, 2008; Ducatelle et al., 2018). Several studies have demonstrated that dietary fat is capable to modify intestinal morphology (Khatun et al., 2017; Sabino et al., 2018) and microbiota (Dänicke et al., 1999; Józefiak et al., 2014).

Enterocytes present in the jejunal villus tips play an important role in fat absorption, due to it has been demonstrated that jejunum is the major site of lipid absorption (Rodriguez-Sanchez et al., 2019). An increase of the villus height is related to enhancements in nutrient absorption whereas deeper crypts are associated to higher tissue turnover rates (Choct, 2009; de Verdal et al., 2010). In the current experiment, the replacement of S by L did not produce modifications on jejunal morphology, supporting the performance and the FA digestibility results. Several authors have demonstrated that poultry feed addition of lysolecithins, products mainly composed in lysophospholipids and derived from the hydrolyzation of lecithins, promotes an increase in the villous heights and the VH:CD ratio of the gut (Boontiam et al., 2017; Brautigan et al., 2017; Chen et al., 2019). Lysophospholipids are capable to alter the lipid bilayer of cell membranes (Arouri and Mouritsen, 2013) and reduce the production of inflammatory mediators (Hartmann et al., 2009). Our hypothesis was based on L high content in PL is hydrolyzed at duodenum by pancreatic phospholipase A2 (Jansen, 2015), releasing lysophospholipids capable to enhance jejunal morphology. However, the hypothesis cannot be confirmed with the results obtained, thus, further experiments should be performed in order to assess a possible beneficial effect of soybean lecithin on broilers gut morphology.

Concerning gut microbiota, results have suggested that using L instead of S caused *Lactobacillus* spp. overgrowth at the jejunum, whereas no modifications were observed on *Enterobacteria* and *E. faecium* counts. An *Enterobacteria* overgrowth is linked to gastrointestinal inflammation and nutrient malabsorption (Ducatelle, et al., 2018). On the other hand, certain inhabitants of poultry gut, such as *Lactobacillus* spp. and *E. faecium*, are widely used as dietary probiotics by controlling overgrowth of pathogenic bacteria (Gaggia et al., 2010). Despite of the beneficial effect of *Lactobacillus* spp. overgrowth in broiler gut, these bacteria present bile salt hydrolase capacity, an enzyme that catalyzes primary bile salts to secondary bile salts, which are less efficient emulsifiers (Dierick and

Decuypere, 2004). It has been suggested the possibility that an overgrowth of these kind of bacteria may decrease lipid digestion and absorption (Pan and Yu, 2014), and this fact, may justify the effects described regarding PUFA absorption. However, there is a lack of information about a possible relationship between a jejunal overgrowth of *Lactobacillus* spp. with a reduction on the apparent digestibility of FA. On the other hand, Knarreborg et al. (2002) linked the high presence of *E. faecium* in the small intestine with a lower FA digestibility in broiler chickens. Moreover, further experiments should be performed in order to understand why PUFA seem to be more affected than SFA by this kind of bacteria.

Table 5.7. Effects of either including 2% of soybean oil (T1) or soybean lecithin (T5) on jejunum morphology and microbiological plate counts of digesta in broiler chickens of 46 days of age.

	Dietary treatments			
Item	T1	T5	RSE	P-value
Morphology measurements				
Villous height (µm)	1,347	1,254	167.0	0.358
Crypt depth (µm)	210	212	26.2	0.912
VH:CD ratio	6.21	6.11	0.235	0.585
Microbiology (log UFC/g)[d]				
Enterococcus faecium	6.47	6.33	0.760	0.761
Enterobacteria	4.17	5.65	1.523	0.143
Lactobacillus spp.	8.31	8.54	0.158	0.049

RSE = residual standard error; VH:CD = villus height-to-crypt depth ratio.

5.5.5. Fatty acid composition of abdominal fat adipose tissue

The FA profile of the AFP was influenced by the dietary FA profile, in accordance with most of the published data (Ferrini et al., 2008; Gonzalez-Ortiz et al., 2013; Vilarrasa et al., 2015). However, in general terms, the use of L instead of S caused minor changes on the FA profile of the AFP and the presence of different lipid molecular structures (TAG, FFA and PL) have not played an important influence, as it was described in Viñado et al. (2019). In general terms, the dietary inclusion of L reduced the PUFA content of the AFP, thus a reduction on UFA:SFA and PUFA:SFA was observed. A reduction on the carcass unsaturated content may result interesting, in terms of carcass quality, in order to reduce fat melting point (Sanz et al., 1999) and the meat oxidation susceptibility (Gonzalez-Ortiz et al., 2013). However, the reduction observed on the unsaturated content

of the carcass due to O replacement by L, numerically, is slightly and probably with a limited effect from a nutritional point of view.

Table 5.8. Fatty acid profile of the abdominal fat pad according soybean lecithin inclusion level at 46 days of age.

	Dietary treatments[1]						
Fatty acid content (%)	T1	T2	T3	T4	T5	RSE	P-value
SFA	29.7	31.3	31.2	31.3	30.8	1.03	0.064
C16:0	23.0	24.2	23.9	24.4	24.4	0.77	0.068
C18:0	5.80	6.24	6.45	6.05	5.84	0.468	0.120
MUFA	44.3	44.4	43.1	45.6	44.7	1.66	0.191
C18:1 ω-9	36.3	37.0	36.3	37.9	36.8	1.11	0.145
PUFA	26.7^{a}	25.7^{ab}	25.7^{ab}	23.1^{c}	24.2^{bc}	1.35	0.002
C18:2 ω-6	23.8^{a}	23.1^{ab}	23.1^{ab}	20.7^{c}	21.8^{bc}	1.22	0.003
C18:3 ω-3	2.27^{a}	2.12^{b}	2.04^{b}	1.90^{c}	1.91^{c}	0.101	<0.001
UFA:SFA	2.42^{a}	2.19^{b}	2.20^{b}	2.20^{b}	2.25^{b}	0.095	0.005
PUFA:SFA	0.88^{a}	0.83^{ab}	0.82^{ab}	0.74^{b}	0.75^{b}	0.059	0.004

[a-c] Values within the same row with no common superscripts are different, $P \leq 0.05$.

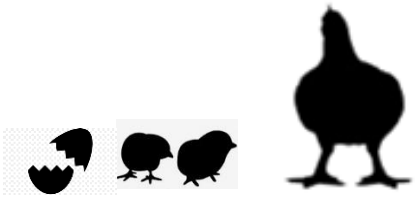
[1] T1 = Soybean oil (S) at 2%; T2 = S at 1.5% and soybean lecithin (L) at 0.5%; T3 = S at 1% and L at 1%; T4 = S at 0.5% and L at 1.5%; T5 = L at 2%.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA:SFA = unsaturated-to-saturated fatty acid ratio; PUFA:SFA = polyunsaturated-to-saturated fatty acid ratio

5.6. Conclusions

In conclusion, results have shown that soybean lecithin can be included in grower and finisher broiler diets as added fat, in replacement of soybean oil, without modifications on performance, total fatty acid ileal absorption nor jejunal morphology of broiler chickens. Higher levels of soybean oil replacement by soybean lecithin slightly reduced the polyunsaturated fatty acid content of the abdominal fat pat, causing minor changes on carcass lipid composition. On the other hand, soybean oil total replacement by soybean lecithin reduced ileal digestibility of polyunsaturated fatty acids and increased jejunal *Lactobacillus* spp. counts; however, these modifications presented a limited influence on broiler chicken performance.

Chapter Six



General discussion

"He disfrutado mucho con esta obra de teatro.

Especialmente en el descanso."

Groucho Marx.

6.1. Introduction

The general aim of the present thesis was to evaluate the inclusion of soybean lecithin as an energetic ingredient for broiler diets, replacing or mixed with other dietary added fats. Knowing in detail how the different added fats are digested and absorbed can give us valuable information in order to increase the range of different ingredients that can be included in feed manufacturing. Fat digestion and absorption process are highly dependent of several animal- and diet-related factors and have a major influence on performance and carcass composition.

The objective of this general discussion is to report and integrate all the results obtained during the experiments (Chapter Three, Chapter Four and Chapter Five). The present work in this dissertation tried to reveal the effect of including different soybean lecithin products on **(a)** performance parameters, **(b)** energy and fatty acid utilization **(c)** jejunal morphology and microbiota, and finally, **(d)** fat tissue saturation degree. In addition, some insights and suggestions for further investigations will be presented in order to increase and improve relevant data regarding the use of soybean lecithin as an alternative energy source for broiler chickens.

6.2. Performance parameters

Previous results about the inclusion of soybean lecithin in monogastric feeds and its influence on performance are scarce and inconsistent (Jones et al., 1992; Øverland et al., 1993a, b; Azman and Ciftci, 2004; Mandalawi et al., 2015). Some authors have suggested that soybean lecithin may represent a good energy source for broiler chickens capable to reduce production costs and maintain a normal growth rate (Mateos et al., 2012; Ravindran et al., 2016). Results reported in the digestibility balances performed in metabolic cages (Chapter Three and Four), indicated that **soybean lecithin can be used as energy source**, reaching up to a 3% of inclusion, without impairing body weight, feed intake, daily gain and feed conversion ratio.

In order to obtain more information about soybean lecithin (**L**) inclusion and its effects on performance, an experiment was designed and performed in a commercial farm under controlled conditions. The main objective of the field trial (Chapter Five) consisted in evaluate the effect of including L in replacement of soybean oil (**S**), as energy source, in diets with other added fats, such as palm oil and acid oil. Based on digestibility results (explained in detail in the next section), S was the added fat of choice until the end of the

starter phase (14 days of life), then L was gradually included during the grower and finisher phase. Furthermore, the level of replacement increased along with the age of the animals due to it was observed that adult broilers utilized L better than young ones. Again, **partial** and **total substitution** of S by L **maintained the bodyweight, daily gain, feed intake and feed conversion ratio**, supporting the results showed in the digestibility balances.

Our results were, at the same time, in agreement and contrasting with available literature. On one hand, Azman and Ciftci (2004) stated that soybean oil can be replaced at 50% by soybean lecithin in grower-finisher diets (6% of added fats) without modifying bodyweight, daily gain and feed conversion. On the other hand, several authors have reported that soybean oil partial and total replacement by soybean lecithin in grower-finisher diets caused a decrease on feed intake (Azman and Ciftci, 2004; Huang et al., 2007) and improved the feed conversion ratio (Huang et al. 2007). The consisting results with the digestibility balances and the existence of published data in other monogastric species with similar conclusions (Soares and Lopez-Bote, 2002) demonstrates the use of soybean lecithin as an efficient energy source for adult broiler chickens.

6.3. Energy and fatty acid utilization

Fatty acid (**FA**) absorption has a major importance on feed apparent metabolizable energy (**AME**) content due to FA contains twice energy than carbohydrates and proteins (NRC, 1994). Fatty acid digestibility can be evaluated by different methodologies, however, *in vivo* digestibility balances with the addition of dietary inert markers are extensively standardized (Ravindran et al., 2016). In the present thesis, titanium dioxide was used as inert marker during the experiments and was analyzed on feed, excreta (Chapter Three and Chapter Four) and ileal digesta samples (Chapter Five).

As it figures in **Table 6.1**, L and soybean lecithin high in FFA (**AL**) contained less gross energy than S and monounsaturated acid oil (**A**; 7,998 and 8,193 kcal/kg for L and LA, respectively *vs.* 9,542 kcal/kg and 9,429 kcal/kg for S and A, respectively), because phospholipids (**PL**) release less energy than triacylglycerols (**TAG**) and free fatty acids (**FFA**). Regarding FFA content, A, as a coproduct derived from olive and sunflower oil chemical refining, contained most of their FA in free form (52.9%), whereas S, as a native oil, presented the average lowest content (1.73%). Soybean lecithin and AL, as a blend of extracted components during degumming, contained average medium levels of FFA (14.1 and 24.1%, respectively). Finally, both L and LA showed a higher average content

of saturated FA (**SFA**) than S (21% *vs.* 16%), whereas S contained higher average content of monounsaturated FA (**MUFA**) than AL (24% *vs.* 20%) and higher average PUFA content than L (60% *vs* 55%). In the case of A, MUFA were the most abundant (54%) followed by PUFA (31%). As a consequence, the unsaturated-to-saturated fatty acid ratio (**UFA:SFA**) of L and AL ranged between 3.28 to 4.55, whereas the UFA:SFA of S and A ranged between 5.04 and 5.67.

Table 6.1. Average fatty acid profile, acidity and gross energy content of soybean oil, monounsaturated acid oil and soybean lecithin products.

	Chemical composition of added fats			
Item	A	S	L	AL
Fatty acid profile (%)				
Saturated fatty acids	15	16 ± 0.3	21 ± 2.8	21 ± 1.1
C16:0	10	11 ± 0.6	18 ± 3.1	16 ± 0.3
C18:0	5	5 ± 0.4	3 ± 0.6	5 ± 0.8
Monounsaturated fatty acids	54	24 ± 0.4	24 ± 5.7	20 ± 0.1
C18:1 ω-9	51	24 ± 0.3	24 ± 5.7	20 ± 0.1
Polyunsaturated fatty acids	31	60 ± 0.8	55 ± 2.9	59 ± 1.2
C18:2 ω-6	29	53 ± 0.4	50 ± 3.5	53 ± 1.1
C18:3 ω-3	2	7 ± 1.1	5 ± 0.7	6 ± 0.1
UFA:SFA	5.67	5.25 ± 0.1	3.76 ± 0.7	3.76
Acidity (%)				
Free fatty acids	52.9	1.73 ± 0.6	14.1 ± 0.8	24.1 ± 0.2
Energy content (kcal/kg)				
Gross energy	9,429	9,542 ± 127	7,998 ± 93	8,193 ± 118

UFA:SFA = unsaturated-to-saturated fatty acid ratio; A = monounsaturated acid oil; S = soybean oil; L = soybean lecithin; AL = soybean lecithin high in free fatty acids.

First of all, the effects of replacing S by L in starter, and then, in grower-finisher broiler diets is going to be discussed. After that, the discussion will be focused regarding A replacement by L in starter and grower-finisher broiler diets.

The digestibility balances performed during the **starter phase** in Chapter Three and Chapter Four (Figure 6.1), indicated that the **partial** and **total replacement of S by both kind of soybean lecithins reduced dietary AME**. In addition, the **digestibility of total FA** and **polyunsaturated FA** (**PUFA**) **were reduced** with soybean lecithin inclusion equal or higher than a 2% (Figure 6.2).

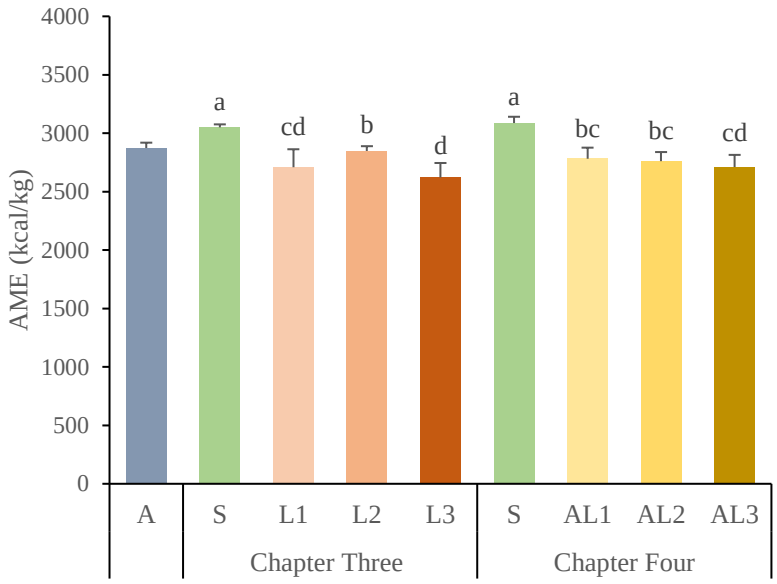


Figure 6.1. Effect of soybean oil replacement by soybean lecithin on feed apparent metabolizable energy in young broilers diets (from 9 to 11 days of life). *A =acid oil at 3%; S = soybean oil (S) at 3%; L1 = S at 2% and soybean lecithin (L) at 1%; L2 = S at 1% and L at 2%; L3 = L at 3%; AL1 = S at 2% and soybean lecithin blended with acid oil (AL) at 1%; AL2 = S at 1% and AL at 2%; AL3 = AL at 3%.*

In the present thesis, it was observed a direct relationship between the addition of dietary lecithin in replacement of S and the rising of the saturation degree of the experimental diets. It is well known that **as dietary saturation degree is increased, a reduction of energy and FA utilization is observed, particularly in young broiler chickens** (Wiseman et al., 1991; Sanz et al., 2000a; Tancharoenrat et al., 2014; Rodriguez-Sanchez et al., 2019). Besides, it has been demonstrated that the **FFA content of a fat source is inversely related to its FA digestibility** (Sklan, 1979; Roll et al., 2018; Rodriguez-Sanchez et al., 2019). The presence of monoacylglycerol (**MAG**) molecules is essential for the mixed micelle formation, so their low presence or absence is responsible of reductions on FA digestibility (Lo and Tso, 2009). This effect is clearly shown in the comparisons of S treatments against A. Despite of A presented a similar UFA:SFA ratio to S, starter diets presented, numerically, lower AME value and FA digestibility. Therefore, the elevated content in FFA of lecithins also have negatively influenced on its nutritive value. For that reason, **highly unsaturated fat sources with a low content in FFA**, such as S, are **preferred** as energetic ingredients **for starter diets**

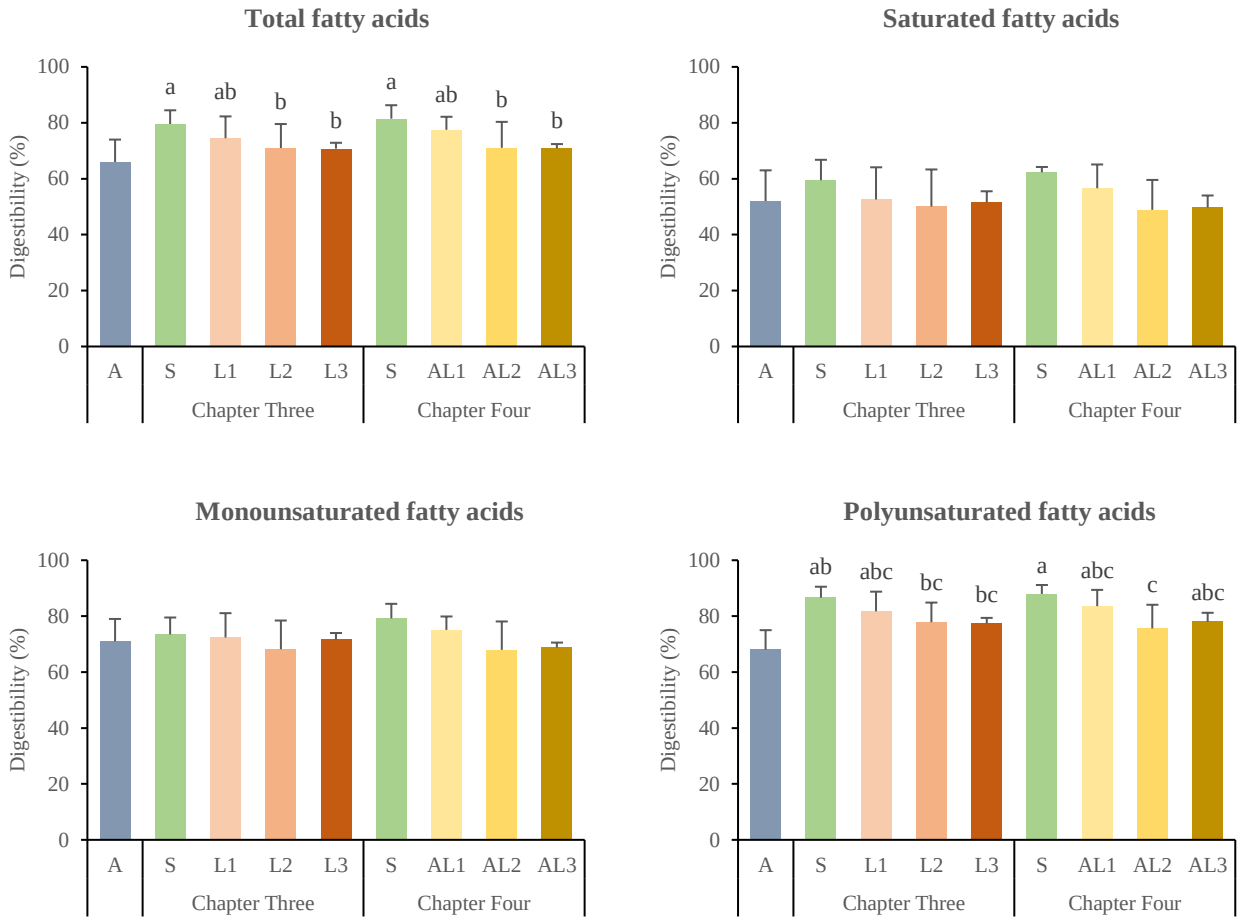


Figure 6.2. Effect of soybean oil replacement by soybean lecithin on total, saturated, monounsaturated and polyunsaturated fatty acids digestibility in young broiler chickens (from 9 to 11 days of life). *A =acid oil at 3%; S = soybean oil (S) at 3%; L1 = S at 2% and soybean lecithin (L) at 1%; L2 = S at 1% and L at 2%; L3 = L at 3%; AL1 = S at 2% and soybean lecithin blended with acid oil (AL) at 1%; AL2 = S at 1% and AL at 2%; AL3 = AL at 3%.*

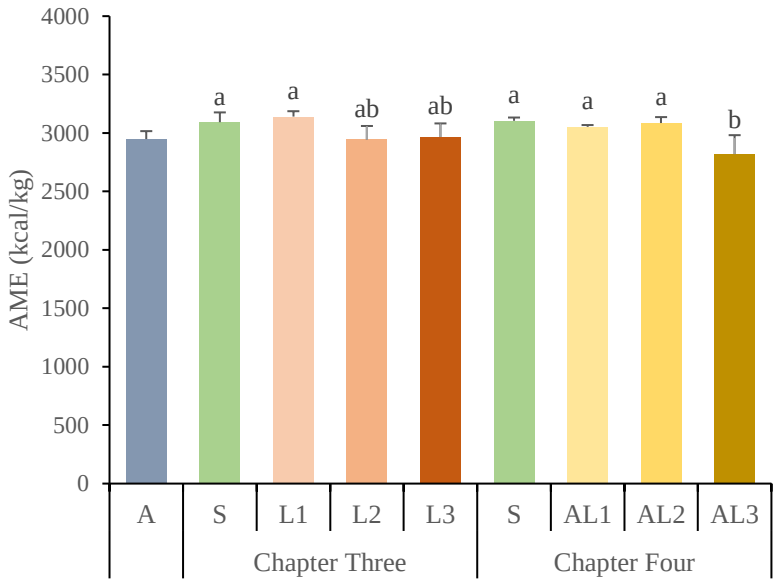


Figure 6.3. Effect of soybean oil replacement by soybean lecithin on feed apparent metabolizable energy in adult broilers (from 36 to 37 days of life). *A =acid oil at 3%; S = soybean oil (S) at 3%; L1 = S at 2% and soybean lecithin (L) at 1%; L2 = S at 1% and L at 2%; L3 = L at 3%; AL1 = S at 2% and soybean lecithin blended with acid oil (AL) at 1%; AL2 = S at 1% and AL at 2%; AL3 = AL at 3%.*

On the other hand, results observed during the **grower-finisher** digestibility balances indicated that **soybean lecithin was better digested by adult broilers than young ones** (Figure 6.3 and 6.4). This fact confirmed that the **age of the animals played an important role on the fat and energy utilization**, as other authors reported previously (Kroghdahl, 1985; Bãiao and Lara, 2005; Tancharoenrat et al., 2013; Rodriguez-Sanchez et al., 2019). It is well demonstrated that bile salt synthesis is improved during the first weeks of life of newly hatched chicks, and, as a consequence, fat absorption is improved with age (Carew et al., 1972; Krogdahl, 1985).

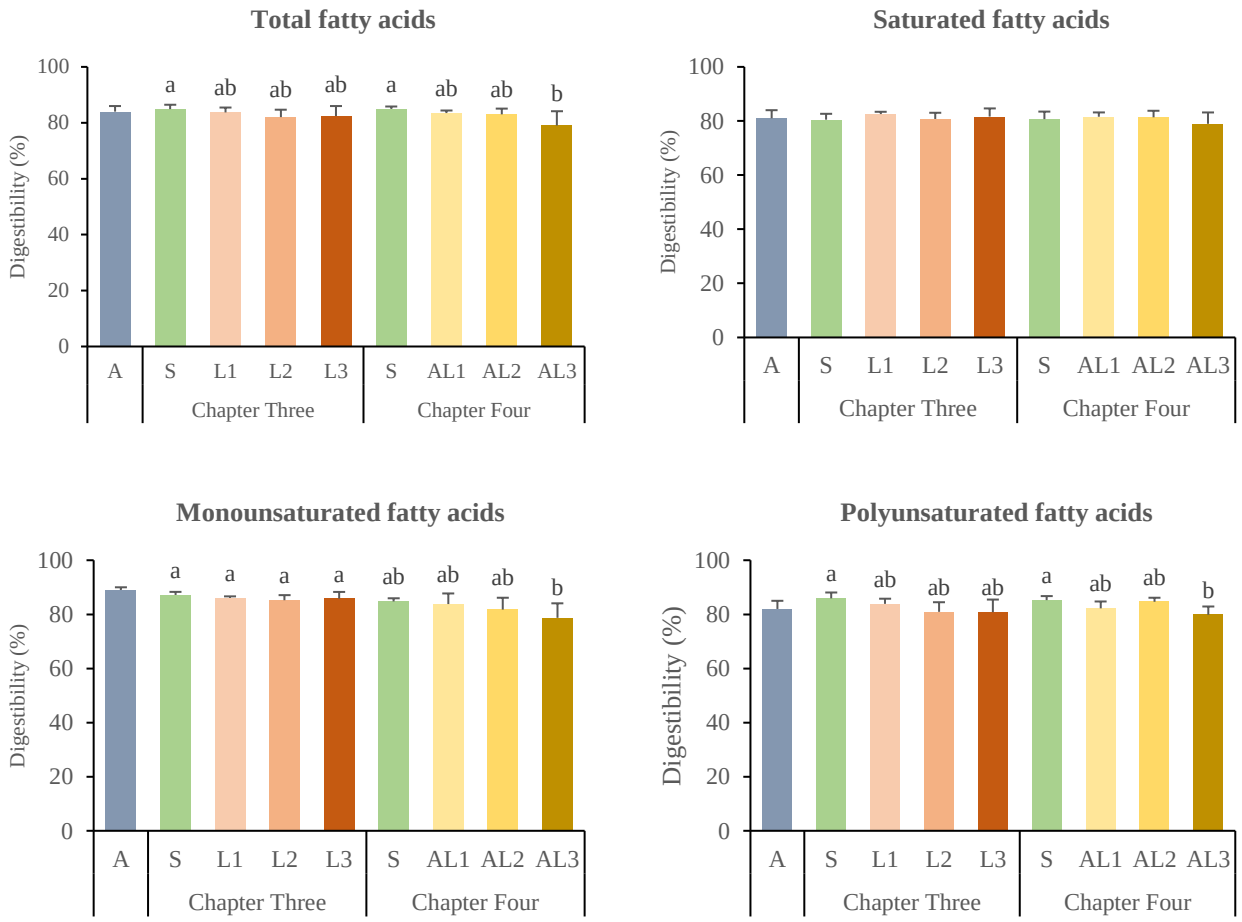


Figure 6.4. Effect of soybean oil replacement by soybean lecithin on total, saturated, monounsaturated and polyunsaturated fatty acids digestibility in adult broiler chickens (from 36 to 37 days of life). *A =acid oil at 3%; S = soybean oil (S) at 3%; L1 = S at 2% and soybean lecithin (L) at 1%; L2 = S at 1% and L at 2%; L3 = L at 3%; AL1 = S at 2% and soybean lecithin blended with acid oil (AL) at 1%; AL2 = S at 1% and AL at 2%; AL3 = AL at 3%.*

In addition, the secretion of digestive enzymes such as amylase and trypsin are also undeveloped in young animals (Noy and Sklan, 1995). It was observed that partial and total replacement of S by L did not reduce feed AME content neither total FA (**TFA**), SFA, MUFA and PUFA digestibility. However, in the case of AL, the total replacement of S (3%) reduced FA and energy utilization, probably due to its low ratio PL:FFA. Results suggest that **PL are better absorbed than FFA** by adult broilers. Again, the use of an acid oil instead S, as energetic ingredient, impaired energy and FA utilization due to its lower content in MAG.

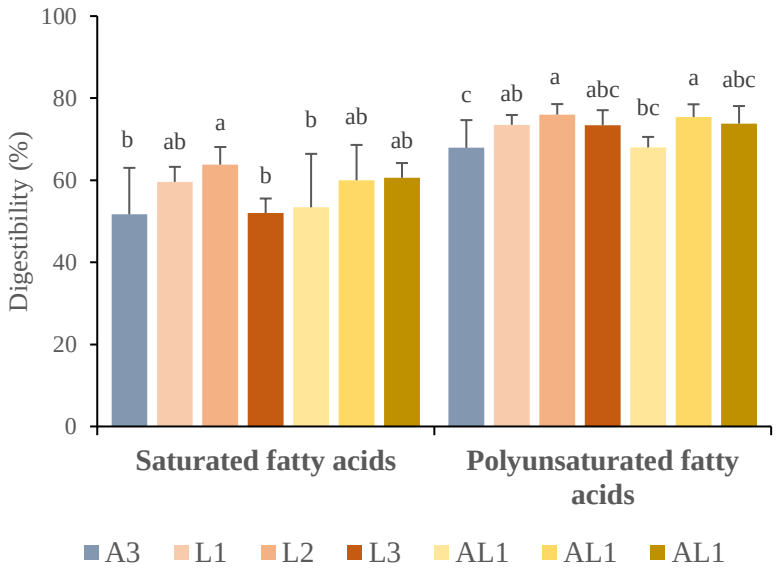


Figure 6.5. Effect of acid oil replacement by soybean lecithin on saturated and polyunsaturated fatty acid digestibility in young broiler diets (from 9 to 11 days of life). *A = monounsaturated acid oil (A) at 3%; L1 = A at 2% and soybean lecithin (L) at 1%; L2 = A at 1% and L at 2%; L3 = L at 3%; AL1 = A at 2% and soybean lecithin blended with acid oil (AL) at 1%; AL2 = A at 1% and AL at 2%; AL3= AL at 3%.*

In Chapter Three and Chapter Four was also described the effect of replacing A by L and AL. The **starter digestibility balance** clearly indicated that this kind of coproducts should be avoided in order to maintain an efficient nutrient retention. However, it is important to remark that an **emulsifying effect**, due to the combination of either L or AL with A on SFA and PUFA digestibility (Figure 6.5).

On the other hand, the adult digestibility balance (Figure 6.5 and 6.6) showed that **blending both coproducts in concrete proportions may result an interesting option as alternative energy source**. It was observed that blending 1% of L plus a 2% of A improved feed AME and SFA digestibility, probably as a result of a **positive interaction** or **emulsion**.

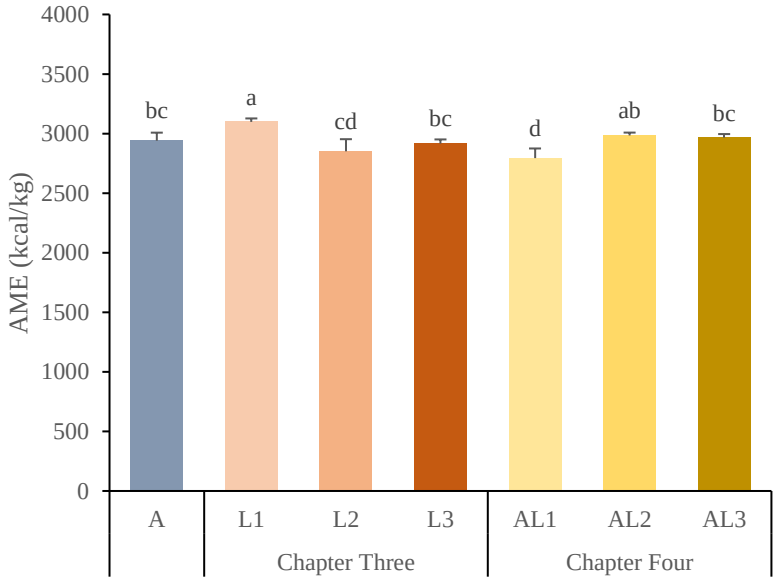


Figure 6.6. Effect of acid oil replacement by soybean lecithin on feed apparent metabolizable energy in adult broilers diets (from 36 to 37 days of life). *A = monounsaturated acid oil (A) at 3%; L1 = A at 2% and soybean lecithin (L) at 1%; L2 = A at 1% and L at 2%; L3 = L at 3%; AL1 = A at 2% and soybean lecithin blended with acid oil (AL) at 1%; AL2 = A at 1% and AL at 2%; AL3= AL at 3%.*

On the contrary, a 1% inclusion of AL plus a 2% of A reduced dietary AME and MUFA digestibility. Despite of LA1 was a blending treatment, the bad results observed were probably caused by a low ratio of PL:FFA. Therefore, **the chemical composition of the coproducts** and the **ratios between the different lipid molecular structures** seem to play an **important** role.

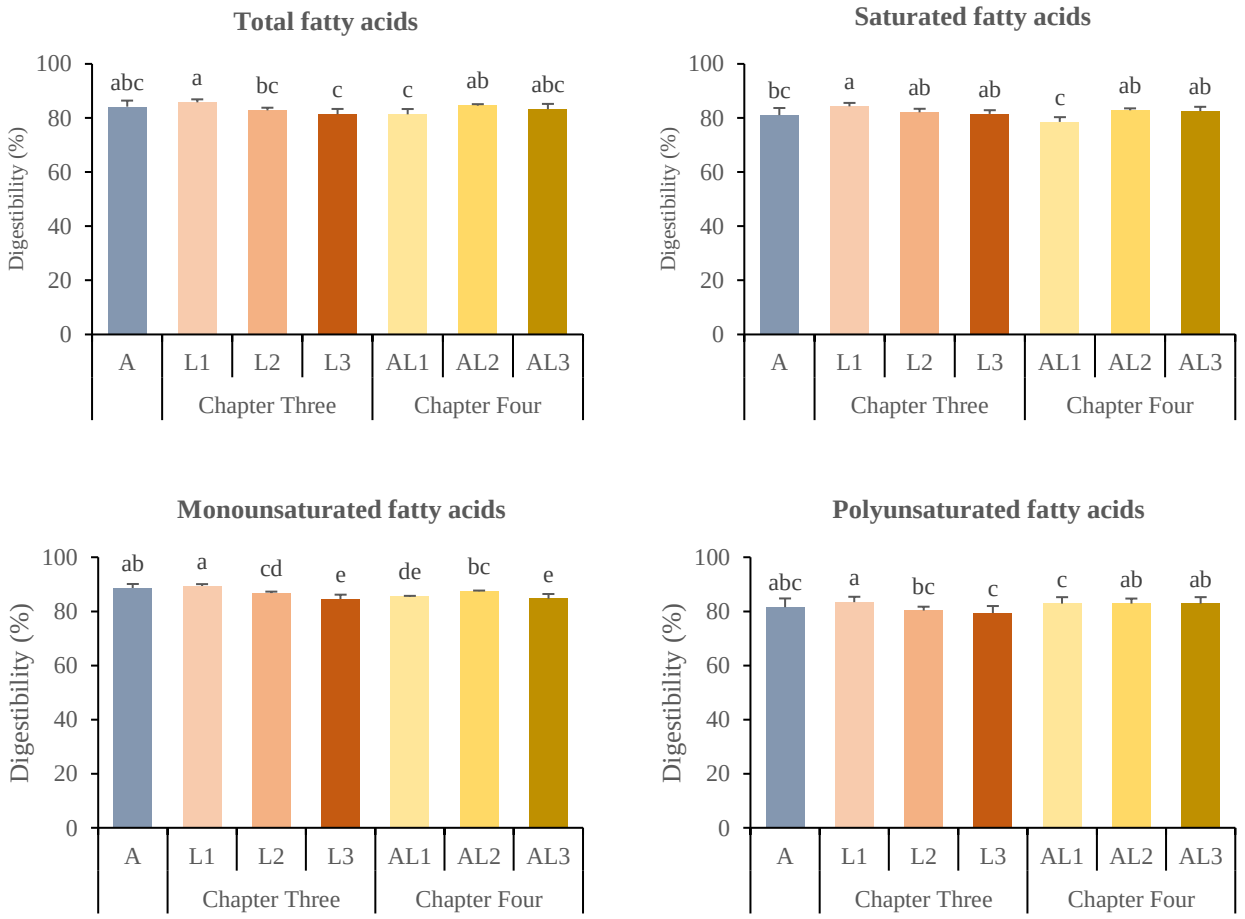


Figure 6.7. Effect of acid oil replacement by soybean lecithin on total, saturated, monounsaturated and polyunsaturated fatty acids digestibility in adult broiler chickens (from 36 to 37 days of life; Chapter three and Chapter four). *A3 = acid oil (A) at 3%; L1 = A at 2% and soybean lecithin (L) at 1%; L2 = A at 1% and L at 2%; L3 = A at 3%; LA1 = A at 2% and soybean lecithin blended with acid oil (LA) at 1%; LA2 = A at 1% and LA at 2%; LA3= LA at 3%.*

It is well known that blending fats with different lipid molecular structures result in an improvement of dietary AME and FA digestibility (Blanch et al., 1996; Borsatti et al., 2018; Roll et al., 2018). For that reason, several authors have tried to demonstrate that the inclusion of **dietary emulsifiers**, such as bile salts, lecithins and lysolecithins, are capable to enhance nutrients digestibility (Jones et al., 1992; Khonyoung et al., 2015; Zampiga et al., 2016; Boontiam et al., 2017; Lai et al., 2018). In our case, **mixing soybean lecithin with soybean oil**, did not modify FA digestibility during the starter and grower-finisher phase, thus **no synergism was observed**, as Soares and Lopez-Bote (2002) reported previously in weanling piglets. However, it is important to remark that several authors reported, in monogastric species, an emulsifying effect of soybean lecithin on fat digestibility when it was blended saturated fat sources (Polin, 1980; Jones et al., 1992). Unfortunately, regarding the combination of soybean lecithin with an acid oil, there are no available literature to compare and confirm (or not) the possible emulsifying effect observed.

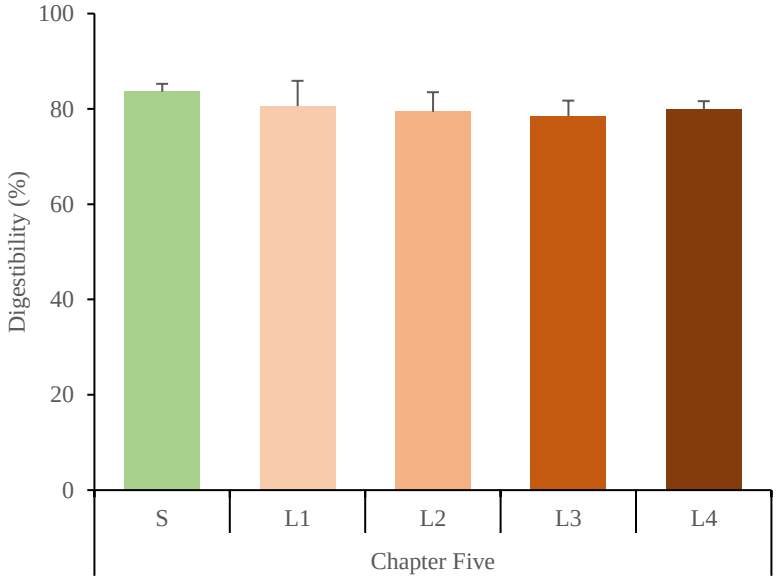


Figure 6.8. Effect of soybean oil replacement by soybean lecithin on total fatty acid ileal digestibility (P > 0.10) in adult broiler chickens (from 40 to 46 days of life). *S = soybean oil (S) at 2%; L1 = S at 1.5% and soybean lecithin (L) at 0.5%; L2 = S at 1% and L at 1%; L3 = S at 0.5% and L at 1.5%; L4 = L at 2%.*

Results obtained in Chapter Five were consistent with the results obtained in Chapter Three and Four. It was observed that the total replacement of S by L, as added fat, in finisher broiler chicken diets that also contained palm oil and acid oil (1% and 1.5%,

respectively) maintained the ileal TFA digestibility. Again, the suitability of L as energy source for adult broilers was confirmed.

6.4 Jejunal morphology and microbiota

Several studies have demonstrated that dietary fat is capable to modify gut morphology (Zeitz et al., 2015; Khatun et al., 2017; Sabino et al., 2018), and intestinal microbiota (Dänicke et al., 1999; Thormar et al., 2006). In addition, it also has been demonstrated that microbial community itself is also able to affect nutrient absorption (Knarreborg et al., 2002; Józefiak et al., 2014; Ducatelle et al., 2018).

At the end of the experiment described at Chapter Five, jejunal samples were collected in order to assess the effect of the added fat sources on jejunal morphology and microbiota. **The use of L instead S did not modify jejunal morphology and increased the *Lactobacillus* spp. presence at the jejunum**. It is well established that reductions on the villous heights are linked to nutrient absorption impairments and the increase of the crypt depth indicate tissue turn-over (Choct, 2009). Based on that, the absence of effects on jejunal morphology support the performance and digestibility results obtained during the grower-finisher phase in all the experiments.

Regarding the increment of *Lactobacillus* spp. in broilers gut, results published by Knarreborg et al. (2002) associated the presence of concrete bacteria with bile salt hydrolase capacity, such as *Lactobacillus* spp. and *Enterococcus faecium*, with a reduction on the apparent digestibility of the FA. Thus, despite microbiota results are obtained in adult broilers, the effect observed on FA utilization during the starter phase may be also justified by an increase on bile salt hydrolase bacteria populations. However, the results observed in the present dissertation are based on the observations between two treatments and only in adult broilers, so it is necessary to research in more detail all the effects of using soybean lecithin as energy source on gastrointestinal health markers.

6.5 Fatty acid profile of the abdominal fat pad

Abdominal fat pad (**AFP**) is the most representative fat depot in broiler chickens and its FA profile correlates well with the rest of fat tissues present in the broiler carcass (Crespo and Esteve-Garcia, 2001; Ferrini et al., 2008). **The FA profile of the AFP was a clear reflect of the FA profile of the dietary fats** and that was in agreement with the available literature (Ferrini et al., 2008; Gonzalez-Ortiz, 2013; Vilarrasa et al., 2015). It

is well known that dietary fats have an important influence on the carcass quality, especially on the FA profile of the carcass.

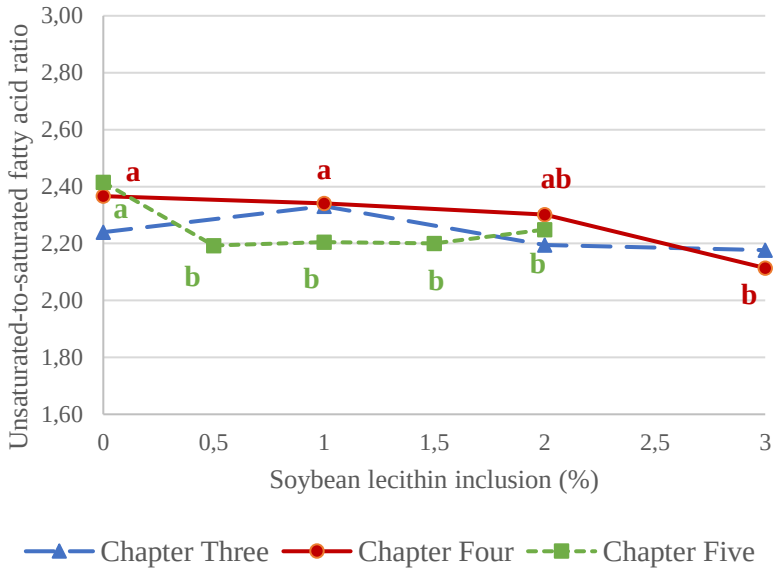


Figure 6.9. Unsaturated-to-saturated fatty acid ratio in the abdominal fat pad of adult broilers depending the of level of soybean oil replacement by soybean lecithin (Chapter Three and Five) and soybean lecithin high in free fatty acids (Chapter Four).

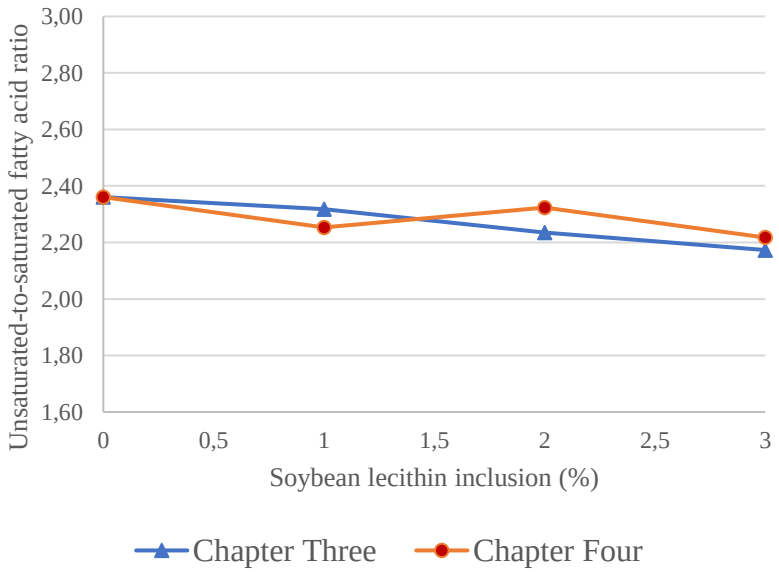


Figure 6.10. Unsaturated-to-saturated fatty acid ratio in the abdominal fat pad of adult broilers depending the of level of monounsaturated acid oil replacement by soybean lecithin (Chapter Three) and soybean lecithin high in free fatty acids (Chapter Four).

In Chapter Three, Four and Five was studied the effect of including different soybean lecithin sources on the FA profile of the AFP. **The replacement of S by soybean lecithin (L and AL) reduced, numerically or statistically, the UFA:SFA of the AFP (Figure 6.8)**, and these results were supported by the chemical composition of the soybean lecithins (Table 6.1). On the other hand, the replacement of an acid oil by soybean lecithins did not modify the UFA:SFA of the AFP (Figure 6.9). Finally, it was also observed that **the saturation degree of the AFP was more influenced by the dietary saturation degree rather than the different lipid molecular structures** present in the feed (TAG, PL and FFA).

6.6. Future perspectives

Despite of the results clearly indicate that soybean lecithin can be used as an energetic ingredient for adult broiler chickens, several doubts may be elucidated with new experiments.

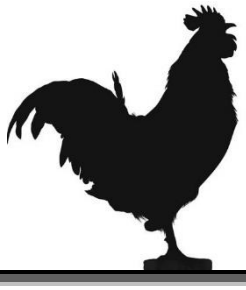
An interesting point would be to study the soybean lecithin **digestibility** and **absorption by *in vitro* methodologies**, as it is described at Soler-Rivas et al. (2010) and Jimenez-Moya et al. (2018). An *in vitro* digestion model may give us valuable information to justify the differences observed on FA digestibility between soybean oil and soybean lecithin. It has been observed that the percentage of MAG and FFA released as lipid products during the hydrolysis partially indicates its respective bioavailability. Furthermore, the isolation and characterization of the mixed micelle fraction and the precipitated phase (TAG, diacylglycerol, MAG, lysophospholipids and FFA) obtained during the hydrolysis is essential to understand the results observed in the current dissertation. The absorption model may be performed applying the micellar phase obtained during the digestion model to Caco-2 cells. Again, the detailed study of the MAG and FFA content at the apical portion of Caco-2 cells (non-absorbed) is an interesting tool to characterize the absorbability of a fat.

The emulsifying effect of soybean lecithin on animal feeding remain unclear due to no improvements were observed, in young animals, when it was blended with S, and slight enhancements were observed when it was blended with an acid oil. In order to **demonstrate (or not) the emulsifying effect of soybean lecithin**, in **young broilers** fat digestion and absorption, it would be interesting to use it as energy source in **combination** with saturated fat sources as **palm oil**. Another interesting point to study its emulsifying

activity is the combination of a soybean lecithin with **fatty acid distillates from physical refining**, due to their elevated content in saturated FFA.

Furthermore, in order to allow the possibility of using soybean lecithin as an energetic ingredient in starter diets, it would be interesting to evaluate the addition of lysolecithins (as additives) to diets containing soybean lecithin as added fat, with the aim to demonstrate an improvement on FA and energy utilization by young chicks. The use of the mentioned ***in vitro*** methods can result an interesting tool in order to demonstrate possible emulsifying effects of lysolecithins on lecithin digestibility. In addition, study the ileal digestibility and the comparison against the fecal digestibility may indicate possible differences due to cecal fermentations.

Finally, assessing the use of soybean lecithin as energy source and study in detail its effects on the **gut morphology** and **microbiota** is also important. Despite of it is well established that energetic ingredients influence on gut health (Khatun et al., 2017; Sabino et al., 2018) there is no data regarding soybean lecithin inclusion in broiler diets and its effects on gastrointestinal health. Recent published studies have linked the addition of lecithin derivatives (mainly lysophospholipids) with positive effects on gut morphology (Boontiam et al., 2018; Chen et al., 2019). Thus, the study of soybean lecithin effect on morphology (villous height, crypt depth, mitosis, goblet cells, lymphocytes) and microbiota (microbioma) of duodenum, jejunum and ileum would be engaging. In addition, further studies should be performed in order to investigate the effects of the overgrowth of **bile salt hydrolase bacteria** and its consequences on fat utilization.



Chapter Seven

Conclusions

"Venceréis, pero no convenceréis. Venceréis porque tenéis sobrada fuerza bruta; pero no convenceréis, porque convencer significa persuadir.
Y para persuadir, necesitáis algo que os falta: razón y derecho en la lucha".
Miguel de Unamuno, en 1936.

From the results shown under the experimental conditions mentioned in the present dissertation, the following conclusions can be drawn:

1. Soybean oil combination or replacement by soybean lecithin is not recommended in starter broiler chicken diets due to reduces feed apparent metabolizable energy value and the fatty acid digestibility.
2. Soybean lecithin represents a suitable energy source for grower-finisher broiler chicken diets, as a partial replacer for soybean oil (up to 2% of total inclusion), without modifying energy utilization, total fatty acids digestibility and performance parameters.
3. Mixing a vegetable acid oil with a soybean lecithin low in free fatty acid content (1% and 2%, respectively) allows enhancements in saturated and polyunsaturated fatty acids digestibility in starter diets.
4. The combination of coproducts derived from vegetable oil chemical refining (lecithin and acid oils) represent an alternative energy source for grower-finisher broiler chicken diets (3% of total fat addition) due to improves total fatty acid digestibility and energy utilization.
5. The total replacement of soybean oil by soybean lecithin at 2% (4.5% of total added fats, including palm oil and acid oil) in grower and finisher broiler diets does not modify performance parameters, total fatty acids digestibility and jejunal morphology.
6. The total replacement of soybean oil by soybean lecithin in grower-finisher diets produces slight modifications on the fatty acid profile of the abdominal fat pat.

Chapter Eight

References

"Por nuestra codicia, lo mucho es poco;

por nuestra necesidad, lo poco es mucho."

Francisco de Quevedo.

Ajuyah, A.O., D. Balnave, and E. F. Annison. 1996. Determination of apparent and true dietary fatty acid digestibilities and metabolisable energy using ileal digesta and excreta from broiler chickens. Animal Feed Science and Technology, 62:131–139.

Amerah, A. M., and V. Ravindran. 2015. Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. Poultry Science, 94:673–680.

AOAC International. 2005. Official Methods of Analysis of AOAC International. 18th Edition. AOAC International, Gaithersburg, MD.

AOCS. 2017. Official Methods and Recommended Practices of the AOCS: Official Method Ja-4-46. 7th Edition. AOCS Press, Boulder, Urbana, IL.

AOCS. 2018a. A lipid essential: Structures, Occurrence, Basic Biochemistry and Function, ed by William W. Christy. American Oil Chemist's Society, Illinois. http://www.lipidhome.co.uk/lipids/lipids.html. Accessed in January 2018.

AOCS. 2018b. A lipid essential: Simple Lipids, Sterols, Lipoproteins and Other Miscellaneous Lipids, ed by William W. Christy. American Oil Chemist's Society, Illinois. http://www.lipidhome.co.uk/lipids/simple.html. Accessed in January 2018.

Arouri, A., and O. G. Mouritsen. 2013. Membrane-perturbing effect of fatty acids and lysolipids. Progress in Lipid Research, 52:130–140.

Aslam, S. M., J. D. Garlich, and M. A. Qureshi. 1998. Vitamin D Deficiency Alters the Immune Responses of Broiler Chicks. Poultry Science, 77:842–849.

Aviagen. 2014. Management handbook. Aviagen, Newbridge, Scotland, UK.

Azman, M. A., and M. Ciftci. 2004. Effects of replacing dietary fat with lecithin on broiler chicken zootechnical performance. Revue de Médecine Vétérinaire, 155:445–448.

Baião, N. C., and L. J. C. Lara. 2005. Oil and Fat in Broiler Nutrition. Brazilian Journal of Poultry Science, 7:129-141.

Bauer, E., S. Jakob, and R. Mosenthin. 2005. Principles of Physiology of Lipid Digestion. Asian-Australasian Journal of Animal Science, 18:282–295.

Bendsen, N. T., A-L. Hother, S. K. Jensen, J. K. Lorenzen, and A. Astrup. 2008. Effect of dairy calcium on fecal fat excretion: A randomized crossover trial. International Journal of Obesity, 32:1816-1824.

Bhosle, B. M., and R. Subramanian. 2005. New approaches in deacidification of edible oils-a review. Journal of Food Engineering, 69:481–494.

Blanch, A., A. C. Barroeta, M. D. Baucells, and F. Puchal. 1995. The nutritive value of dietary fats in relation to their chemical composition. Apparent fat availability and metabolizable energy in two-week-old chicks. Poultry Science, 74:1335-1340.

Blanch, A., A. C. Barroeta, M. D. Baucells, X. Serrano, and F. Puchal. 1996. Utilization of different fats and oils by adult chickens as a source of energy, lipid and fatty acids. Animal Feed Science and Technology, 61:335–342.

Blas, E., C. Cervera, L. Rodenas, E. Martínez, and J. J. Pascual. 2010. The use of recycled oils from the food industry in growing rabbit feeds in substitution of fresh oil does not affect performance. Animal Feed Science and Technology, 161:67–74.

Boontiam, W., B. Jung, and Y. Y. Kim. 2017. Effects of lysophospholipid supplementation to lower nutrient diets on growth performance, intestinal morphology, and blood metabolites in broiler chickens. Poultry Science, 96:593–601.

Borsatti, L., S. L. Vieira, C. Stefanello, L. Kindlein, E. O. Oviedo-Rondón, and C. R. Angel. 2018. Apparent metabolizable energy of by-products from the soybean oil industry for broilers: acidulated soapstock, glycerin, lecithin, and their mixture. Poultry Science, 97: 124-130.

Brautigan, D. L., R. Li, E. Kubicka, S. D. Turner, J. S. Garcia, M. L. Weintraut, and E. A. Wong. 2017. Lysolecithin as feed additive enhances collagen expression and villus length in the jejunum of broiler chickens. Poultry Science, 96:2889–2898.

Bueschelberger H., S. Tirok, I. Stoffels, and A. Schoeppe. 2015. Lecithins. Pages 21-60 In Emulsifiers in food technology. V. Norn ed. 1st Edition. John Wiley & Sons, West Sussex, UK.

Cabezas, D. M., R. Madoery, B. W. K. Diehl, and M.C. Tomás. 2011. Application of Enzymatic Hydrolysis on Sunflower Lecithin Using a Pancreatic PLA_2. Journal of the American Oil Chemists' Society, 88:443–446.

Cantor A. H., R. Vargas, A. J. Pescatore, M. L. Straw, and M. J. Ford. 1997. Influence of crude soybean lecithin as a dietary energy source on growth performance and carcass yield of broilers. Poultry Science, 76 (Suppl. 1):109.

Carew, L. B., R. H. Machemer, Jr., R. W. Sharp, and D. C. Foss. 1972. Fat Absorption by the Very Young Chick. Poultry Science, 51:738–742.

Carrapiso, A. I., M. L. Timón, M. J. Petrón, J. F. Tejeda, and C. García. 2000. In situ transesterification of fatty acids from Iberian pig subcutaneous adipose tissue. Meat Science, 56:159–164.

Chen, C., B. Jung, and W. K. Kim. 2019. Effects of lysophospholipid on growth performance, carcass yield, intestinal development, and bone quality in broilers. Poultry Science. DOI: https://doi.org/10.3382/ps/pez111

Cho, J. H., Y. J. Chen, J. S. Yoo, W. T. Kim, I. B. Chung, and I. H. Kim. 2008. Evaluation of fat sources (lecithin, mono-glyceride and mono-diglyceride) in weaned pigs: Apparent total tract and ileal nutrient digestibilities. Nutrition Research and Practice, 2:130-133.

Choct, M. 2009. Managing gut health through nutrition. British Poultry Science, 50:9–15.

Clarke, S. D. 2000. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. British Journal of Nutrition, 83, Suppl, 1:S59–S66.

Codony R, F. Guardiola, A. Tres, and A. C. Barroeta. 2017. Quality control and nutritional value of fats. In Proceedings of the 21st European Symposium on Poultry Nutrition, 8-11 May 2017, Salou/Vila-Seca, Spain, pp. 118-123.

Cortinas, L., C. Villaverde, J. Galobart, M. D. Baucells, R. Codony, and A. C. Barroeta. 2004. Fatty Acid Content in Chicken Thigh and Breast as Affected by Dietary Polyunsaturation Level. Poultry Science, 83:1155–1164.

Cox, W. R., S. J. Richie, M. Sifri, B. Bennett, and D. D. Kitts. 2000. The impact of replacing dietary fat with lecithin on broiler chicken performance. Poultry Science, 79 (Suppl. 1):67.

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

Crespo, N., and E. Esteve-Garcia. 2002. Nutrient and Fatty Acid Deposition in Broilers Fed Different Dietary Fatty Acid Profiles. Poultry Science, 81:1533–1542.

Cui, L. and E. A. Decker. 2016. Phospholipids in foods: Prooxidants or antioxidants? The Journal of the Science and Food Agriculture, 96:18–31.

Dänicke, S., O. Simon, H. Jeroch, and M. Bedford. 1997. Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens 2. Performance, nutrient digestibility and the fat-soluble vitamin status of livers. British Poultry Science, 38:546–556.

Dänicke S., W. Vahjen, O. Simon, and H. Jeroch. 1999. Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups

adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. Poultry Science, 78:1292-1299.

DeGolier, T. F., D. R. Brown, G. E. Duke, M. M. Palmer, J. R. Swenson, and R. E. Carraway. 2013. Neurotensin and cholecystokinin contract gallbladder circular muscle in chickens. Poultry Science, 92:2156–2162.

Denke M. A., M. M. Fox, and M. C. Schulte. 1993. Short-Term Dietary Calcium Fortification Increases Fecal Saturated Fat Content and Reduces Serum Lipids in Men. Journal of Nutrition, 123:1047–1053.

Deschodt-Lanckman, M., P. Robberecht, J. Camus, and J. Christophe. 1971. Short-term adaptation of pancreatic hydrolases to nutritional and physiological stimuli in adult rats. Biochimie, 53:789–796.

Dierick, N. A, and J. A. Decuypere. 2004. Influence of lipase and/or emulsifier addition on the ileal and faecal nutrient digestibility in growing pigs fed diets containing 4% animal fat. Journal of the Science of Food and Agriculture, 84:1443-1450.

Ducatelle, R., E. Goossens, F. De Meyer, V. Eeckhaut, G. Antonissen, F. Haesebrouck, and F. Van Immerseel. 2018. Biomarkers for monitoring intestinal health in poultry: Present status and future perspectives. Veterinary Research, 49:43.

Duke, G. E. 1982. Gastrointestinal Motility and Its Regulation. Poultry Science, 61:1245–1256.

EFSA FEEDAP Panel. 2016. Safety and efficacy of lecithins for all animal species. EFSA Journal 14, 4561.

Emmert, J. L., T. A. Garrow, and D. H. Baker. 1996. Development of an Experimental Diet for Determining Bioavailable Choline Concentration and its Application in Studies with Soybean Lecithin. Journal of Animal Science, 74:2738–2744.

European Parliament. 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Fan, X., S. Liu, G. Liu, J. Zhao, H. Jiao, X. Wang, Z. Song, and H. Lin. 2015. Vitamin A Deficiency Impairs Mucin Expression and Suppresses the Mucosal Immune Function of the Respiratory Tract in chicks. PLoS One, 10: e0139131.

Fediol. 2008. https://www.fediol.eu/web/refining/1011306087/list1187970096/f1.html. Accessed in January 2018.

FEDNA. 2008. Necesidades Nutricionales para Avicultura: Pollos de Carne y Aves de Puesta. FEDNA, Madrid, Spain.

FEDNA. 2015. Lecitinas comerciales (actualizado Feb. 2015). http://www.fundacionfedna.org/ingredientes_para_piensos/lecitinas-comerciales-actualizado-feb-2015. Accessed on January 2018.

Ferrini, G., M. D. Baucells, E. Esteve-Garcia, and A. C. Barroeta. 2008. Dietary Polyunsaturated Fat Reduces Skin Fat as Well as Abdominal Fat in Broiler Chickens. Poultry Science, 87:528–535.

Fujita, Y., H. Matsuoka, and K. Hirooka. 2007. Regulation of fatty acid metabolism in bacteria. Molecular Microbiology, 66:829–839.

Gaggìa, F., P. Mattarelli, and B. Biavati. 2010. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology, 141:S15–S28.

Gajda, A. M., and J. Storch. 2015. Enterocyte fatty acid-binding proteins (FABPs): Different functions of liver and intestinal FABPs in the intestine. Prostaglandins, Leukotrienes and Essential Fatty Acids, 93:9–16.

Garret R. L., and R. J. Young. 1975. Effect of micelle formation on the absorption of natural fat and fatty acids by the chicken. Journal of Nutrition, 105:827–838.

Glatz, J. F. C. 2015. Lipids and lipid binding proteins: A perfect match. Prostaglandins, Leukotrienes and Essential Fatty Acids, 93:45–49.

González-Ortiz, G., R. Sala, E. Cánovas, N. Abed, and A. C. Barroeta. 2013. Consumption of Dietary n-3 Fatty Acids Decreases Fat Deposition and Adipocyte Size, but Increases Oxidative Susceptibility in Broiler Chickens. Lipids, 48:705–717.

Guardiola, F., R. Codony, M. Rafecas, J. Boatella, and A. López. 1994. Fatty Acid Composition and Nutritional Value of Fresh Eggs, from Large- and Small-Scale Farms. Journal of Food Composition and Analysis, 7:171– 188.

Guirguis, N. 1975. Evaluating poultry feedstuffs in terms of their metabolizable energy content and chemical composition. Australian Journal of Experimental Agriculture and Animal Husbandry, 15:773–779.

Guirguis, N. 1976. Metabolizable energy values of fats and protein concentrates for poultry: effect of sex and inclusion level of feedstuffs. Australian Journal of Experimental Agriculture and Animal Husbandry, 16:691–695.

Hamosh, M., and R. O. Scow. 1973. Lingual Lipase and Its Role in the Digestion of Dietary Lipid. The Journal of Clinical Investigation, 52:88–95.

Hartmann, P., A. Szabó, G. Eros, D. Gurabi, G. Horváth, I. Németh, M. Ghyczy, and M. Boros. 2009. Anti-inflammatory effects of phosphatidylcholine in neutrophil leukocyte-dependent acute arthritis in rats. European Journal of Pharmacology, 622:58–64.

Hasenhuettl, G. L. 2008. Synthesis and Commercial Preparation of Food Emulsifiers. Pages 11-39 In Food Emulsifiers and Their Applications. Hasenhuettl and Harder, eds. 2nd Edition. Springer, New York, NY.

Hausman D. B, and B. M. Grossman. 2017. Dietary Fats and Obesity. In Food Lipids: Chemistry, Nutrition, and Biotechnology. Akoh and Min, eds. 2nd Edition. Marcel Dekker, New York, NY.

Helmerich, G., and P. Koehler. 2003. Comparison of Methods for the Quantitative Determination of Phospholipids in Lecithins and Flour Improvers. Journal of Agriculture and Food Chemistry, 51:6645-6651.

Hermier, D. 1997. Lipoprotein Metabolism and Fattening in Poultry. The Journal of Nutrition, 127:805S–808S.

van der Hoeven-Hangoor, E., J. M. B. M. van der Vossen, F. H. J. Schuren, M. W. A. Verstegen, J. E. de Oliveira, R. C. Montijn, and W. H. Hendriks. 2013. Ileal microbiota composition of broilers fed various commercial diet compositions. Poultry Science, 92:2713–2723.

Hofmann, A. F., and L. R. Hagey. 2008. Bile Acids: Chemistry, Pathochemistry, Biology, Pathobiology, and Therapeutics. Cellular and Molecular Life Sciences, 65:2461–2483.

Huang, J., D. Yang, and T. Wang. 2007. Effects of Replacing Soy-oil with Soy-lecithin on Growth Performance, Nutrient Utilization and Serum Parameters of Broilers Fed Corn-based Diets. Asian-Australasian Journal of Animal Science, 20:1880–1886.

Huyghebaert, G., G. De Munter, and G. De Groote. 1988. The Metabolisable Energy (AMEn) of Fats for Broilers in Relation to their Chemical Composition. Animal Feed Science and Technology, 20:45–58.

Innis, S. M. 2011. Dietary Triacylglycerol Structure and Its Role in Infant Nutrition. Advances in Nutrition, 2:275–283.

Irandoust, H., A. H. Samie, H. R. Rahmani, M. A. Edriss, and G. G. Mateos. 2012. Influence of source of fat and supplementation of the diet with vitamin E and C on performance and egg quality of laying hens from forty-four to fifty-six weeks of age. Animal Feed Science and Technology, 177:75–85.

ISO 660. 2009. Animal and vegetable fats and oils-Determination of acid value and acidity. International Organization for Standardization, Geneva, Switzerland.

Jansen, M. 2015. Modes of action of lysophospholipids as feed additives on fat digestion in broilers. PhD Thesis. Katholieke Universiteit Leuven, Leuven, Belgium.

Jansen, M., F. Nuyens, J. Buyse, S. Leleu, and Van Campenhout, L. 2015. Interaction between fat type and lysolecithin supplementation in broiler feeds. Poultry Science, 94: 2506–2515.

Jimenez-Moya, B., D. Martin, C. Soler-Rivas, A. Tres, F. Guardiola, A.C. Barroeta, and R. Sala. 2018. Evaluación de la digestibilidad in-vitro de aceite de soja y palma para su uso en avicultura. Proceedings of 55th Symposium Científico de Avicultura, Madrid, Spain.

Jin, C. F., J. H. Kim, In K. Han, H. J. Jung, and C. H. Kwon. 1998. Effects of Various Fat Sources and Lecithin on the Growth Performance and Nutrient Utilization in Pigs Weaned at 21 Days of Age. Asian-Australasian Journal of Animal Sciences, 11:176-184

Jones, D. B., J. D. Hancock, D. L. Harmon, and C. E. Walker. 1992. Effects of Exogenous Emulsifiers and Fat Sources on Nutrient Digestibility, Serum Lipids, and Growth-Performance in Weanling Pigs. Journal of Animal Science, 70:3473–3482.

Joshi, A., S. G. Paratkar, and B. N. Thorat. 2006. Modification of lecithin by physical, chemical and enzymatic methods. European Journal of Lipid Science and Technology, 108:363–373.

Józefiak, D., B. Kierończyk, M. Rawski, M. Hejdysz, A. Rutkowski, R. M. Engberg, and O. Højberg. 2014. Clostridium perfringens challenge and dietary fat type affect broiler chicken performance and fermentation in the gastrointestinal tract. Animal, 8:912–922.

Judde, A., P. Villeneuve, A. Rossignol-Castera, A., and A. L. Guillou. 2003. Antioxidant effect of soy lecithins on vegetable oil stability and their synergism with tocopherols. Journal of the American Oil Chemists' Society, 80:1209–1215.

Karcher, D. M., and T. Applegate. 2008. Survey of Enterocyte Morphology and Tight Junction Formation in the Small Intestine of Avian Embryos. Poultry Science, 87:339–350.

Kates, M. 1986. Definition and Classification of Lipids. In Techniques of Lipidology: Isolation, Analysis and Identification of Lipids. Kates, M. ed. 1st Edition. Elsevier, New York, NY.

Kettunen, H., K. Tiihonen, S. Peuranen, M. T. Saarinen, and J. C. Remus. 2001. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. Comparative Biochemistry and Physiology Part A, 130:759–769.

Khatun, J., T. C. Loh, H. Akit, H. L Foo, and R. Mohamad. 2018. Influence of different sources of oil on performance, meat quality, gut morphology, ileal digestibility and serum lipid profile in broilers. Journal of Applied Animal Research, 46:479–485.

Khonyoung, D., Yamauchi, K., Suzuki, K., 2015. Influence of dietary fat sources and lysolecithin on growth performance, visceral organ size, and histological intestinal alteration in broiler chickens. Livestock Science, 176:111–120.

King, M. W. 2018. Bile acids Synthesis and Utilizations. http://themedicalbiochemistrypage.org/bileacids.php. Accessed on September 2018.

Knarreborg, A., R. M. Engberg, S. K. Jensen, and B. B. Jensen. 2002a. Quantitative Determination of Bile Salt Hydrolase Activity in Bacteria Isolated from the Small Intestine of Chickens. Applied and Environmental Microbiology, 68:6425–6428.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002b. Effects of Dietary Fat Source and Subtherapeutic Levels of Antibiotic on the Bacterial Community in the Ileum of Broiler Chickens at Various Ages. Applied Environmental Microbiology, 68:5918-5924.

Knarreborg, A., C. Lauridsen, R. M. Engberg, and S. K. Jensen. 2004. Dietary Antibiotic Growth Promoters Enhance the Bioavailability of α-Tocopheryl Acetate in Broilers by Altering Lipid Absorption. The Journal of Nutrition, 134:1487–1492.

Knothe, G., and R. O. Dunn. 2009. A Comprehensive Evaluation of the Melting Points of Fatty Acids and Esters Determined by Differential Scanning Calorimetry. Journal of the American Oil Chemists' Society, 86:843–856.

Kombe, G. G., A. K. Temu, H. M. Rajabu, G. D. Mrema, J. Kansedo, and K. T. Lee. 2013. Pre-Treatment of High Free Fatty Acids Oils by Chemical Re-Esterification for Biodiesel Production-A Review. Advances in Chemical Engineering and Science, 3:242–247.

Krogdahl, Å. 1985. Digestion and Absorption of Lipids in Poultry. The Journal of Nutrition, 115:675–685.

Kuhn, M., B. Frohmann, A. Petersen, K. Rübesam, and C. Jatsch. 1998. Utilization of crude soybean lecithin as a native choline source in feed rations of fattening pigs. European Journal of Lipid Science and Technology, 100:78-84.

Lai, W., W. Huang, B. Dong, A. Cao, W. Zhang, J. Li, H. Wu, and L. Zhang. 2018. Effects of dietary supplemental bile acids on performance, carcass characteristics, serum lipid metabolites and intestinal enzyme activities of broiler chickens. Poultry Science, 97:196–202.

Lee, K.-W., H. Everts, H. J. Kappert, J. Van Der Kuilen, A. G. Lemmens, M. Frehner, and A. C. Beynen. 2004. Growth Performance, Intestinal Viscosity, Fat Digestibility and Plasma Cholesterol in Broiler Chickens Fed a Rye-containing Diet Without or with Essential Oil Components. International Journal of Poultry Science, 3:613–618.

Leeson, S., and J. D. Summers. 2001. Scott's Nutrition of the Chicken. Leeson and Summers, ed. 4th Edition. University of Guelph, Ontario, Canada.

Lentle, R. G., G. Reynolds, C. de Loubens, C. Hulls, P. W. M. Janssen, and V. Ravindran. 2013. Spatiotemporal mapping of the muscular activity of the gizzard of the chicken (*Gallus domesticus*). Poultry Science, 92:483–491.

Lessire, M., B. Leclercq, and L. Conan. 1982. Metabolisable energy value of fats in chicks and adult cockerels. Animal Feed Science and Technology, 7:365–374.

Leyton, J., P. J. Drury, and M. A. Crawford. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. British Journal of Nutrition, 57:383–393.

Lien, E. L., F. G. Boyle, R. Yuhas, R. M. Tomarelli, and P. Quinlan. 1997. The Effect of Triglyceride Positional Distribution on Fatty Acid Absorption in Rats. Journal of Pediatric Gastroenterology & Nutrition, 25:167–174.

Lin, C-S., and S-H. Chiang. 2010. Effect of sn-2 Saturated Fatty Acids in Dietary Triglycerides on Fatty Acid and Calcium Digestibility and Leg Abnormalities in Broiler Chickens. Japan Poultry Science Association, 47:156–162.

Lipstein, B., S. Bornstein, and P. Budowski. 1977. Utilization of choline from crude soybean lecithin by chicks. Poultry Science, 56:331-336.

List, G. R. 2015. Soybean Lecithin: Food, Industrial Uses, and Other Applications. Pages 1-33 In Polar Lipids: Biology, Chemistry and Technology. Ahmad and Xu, eds. 1st Edition. AOCS Press, Urbana, IL.

Lo, C. M, and P. Tso. 2009. Physicochemical basis of the digestion and absorption of triacylglycerol. Pages 94-125 In Designing Functional Food. McClements and Decker, eds. 1st Edition. Woodhead Publishing Limited, Oxford, UK.

Maisonnier, S., J. Gomez, A. Bree, C. Berri, E. Baéza, and B. Carré. 2003. Effects of Microflora Status, Dietary Bile Salts and Guar Gum on Lipid Digestibility, Intestinal Bile Salts, and Histomorphology in Broiler Chickens. Poultry Science, 82:805–814.

Mandalawi, H. A., R. Lázaro, M. Redón, J. Herrera, D. Menoyo, and G. G. Mateos. 2015. Glycerin and lecithin inclusion in diets for brown egg-laying hens: Effects on egg production and nutrient digestibility. Animal Feed Science and Technology, 209:145–156.

Mandalawi, H. A., J. J. Mallo, D. Menoyo, R. Lázaro, and G. G. Mateos. 2017. Metabolizable energy content of traditional and re-esterified lipid sources: Effects of inclusion in the diet on nutrient retention and growth performance of broilers from 7 to 21 days of age. Animal Feed Science and Technology, 224:124–135.

Marin, J. J. G., R. I. R. Macias, O. Briz, J. M. Banales, and M. J. Monte. 2016. Bile Acids in Physiology, Pathology and Pharmacology. Current Drug Metabolism, 17:4–29.

Mateos, G. G., and J. L. Sell. 1980. Influence of Carbohydrate and Supplemental Fat Source on the Metabolizable Energy of the Diet. Poultry Science, 59:2129–2135.

Mateos, G. G., and J. L. Sell. 1981. Nature of the Extrametabolic Effect of Supplemental Fat Used in Semipurified Diets for Laying Hens. Poultry Science, 60:1925–1930.

Mateos, G. G., P. G. Rebollar, and P. Medel. 1996. Utilización de grasas y productos lipídicos en alimentación animal. XII Curso de Especialización FEDNA, Madrid, Spain.

Mateos, G. G., M. P. Serrano, J. Berrocoso, A. Pérez-Bonilla and R. Lázaro. 2012. Improving the utilization of raw materials in poultry feeding: new technologies and inclusion levels. Proceedings of 24th World's Poultry Congress, Bahia-Salvador, Brazil, 5–9 August 2012, pp. 1–13.

Meng, X., B. A. Slominski, and W. Guenter. 2004. The Effect of Fat Type, Carbohydrase, and Lipase Addition on Growth Performance and Nutrient Utilization of Young Broilers Fed Wheat-Based Diets. Poultry Science, 83:1718–1727.

Michalski, M. C., C. Genot, C. Gayet, C. Lopez, F. Fine, F. Joffre, J. L. Vendeuvre, J. Bouvier, J. M. Chardigny, and K. Raynal-Ljutovac. 2013. Multiscale structures of

lipids in foods as parameters affecting fatty acid bioavailability and lipid metabolism. Progress in Lipid Research, 52:354–373.

Nasir, M. I., M. A. Bernards, and P. A. Charpentier. 2007. Acetylation of Soybean Lecithin and Identification of Components for Solubility in Supercritical Carbon Dioxide. Journal of Agriculture and Food Chemistry, 55:1961–1969.

Nelson, D. L., and M. M. Cox. 2014. Lipids. Pages 363-389 in Lehninger: Principles of Biochemistry. Nelson and Cox, eds. 6th Edition. Ediciones Omega, Madrid, Spain.

Newman, R. E., W. L. Bryden, E. Fleck, J. R. Ashes, W. A. Buttemer, L. H. Storlien, and J. A. Downing. 2002. Dietary *n*-3 and *n*-6 fatty acids alter avian metabolism: metabolism and abdominal fat deposition. British Journal of Nutrition, 88:11-18.

Nguyen, M. T., D. Van De Walle, C. Petit, B. Beheydt, F. Depypere, and K. Dewettinck. 2014. Mapping the chemical variability of vegetable lecithins. Journal of the American Oil Chemists' Society, 91:1093–1101.

van Nieuwenhuyzen, W., and M. C. Tomás. 2008. Update on vegetable lecithin and phospholipid technologies. European Journal of Lipid Science and Technology 110, 472–486.

van Nieuwenhuyzen, W. 2010. Lecithin and Other Phospholipids. Pages 191–212 In Surfactants from Renewable Resources. Kjellin and Johansson, eds. 1st Edition. John Wiley & Sons, West Sussex, UK.

Nishimukai, M., H. Hara, and Y. Aoyama. 2003. The addition of soybean phosphatidylcholine to triglyceride increases suppressive effects on food intake and gastric emptying in rats. The Journal of Nutrition, 133:1255–1258.

Noy, Y., and D. Sklan. 1995. Digestion and Absorption in the Young Chick. Poultry Science, 74:366–373.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Nuchi, C., F. Guardiola, R. Bou, P. Bondioli, L. Della Bella, and R. Codony. 2009. Assessment of the Levels of Degradation in Fat Co- and Byproducts for Feed Uses and Their Relationship with Some Lipid Composition Parameters. Journal of Agricultural and Food Chemistry, 57:1952-1959.

O'Keefe S.F. 2002. Nomenclature and classification of lipids. In Food Lipids: Chemistry, Nutrition, and Biotechnology. Akoh and Min, eds. 2nd Edition. Marcel Dekker, New York, NY.

Øverland M., M. D. Tokach, S. G. Cornelius, J. E. Pettigrew, and J. W. Rust. 1993a. Lecithin in Swine Diets: I. Weanling pigs. Journal of Animal Science, 71:1187–1193.

Øverland M., M. D. Tokach, S. G. Cornelius, J. E. Pettigrew, and M. E. Wilson. 1993b. Lecithin in Swine Diets: II. Growing-Finishing Pigs. Journal of Animal Science, 71:1194–1197.

Øverland M., Z. Mroz, and F. Sundstøl. 1994. Effect of Lecithin on the Apparent Ileal and Overall Digestibility of Crude Fat and Fatty Acids in Pigs. Journal of Animal Science, 72:2022-2028.

Øverland M., and F. Sundstøl. 1995. Effects of lecithin on fat utilization by weanling pigs. Livestock Production Science, 41:217-224.

Pan, D., and Z. Yu. 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes, 5:108–119.

Papamandjaris, A. A., D. E. MacDougall, and P. J. H. Jones. 1998. Medium chain fatty acid metabolism and energy expenditure: Obesity treatment implications. Life Sciences, 62:1203–1215.

Pardío, V. T., L. A. Landín, K. N. Waliszewski, F. Pérez-Gil, L. Díaz, and B. Hernández. 2005. The Effect of Soybean Soapstock on the Quality Parameters and Fatty Acid Composition of the Hen Egg Yolk. Poultry Science, 84:148–157.

Pekel, A. Y., G. Demirel, M. Midilli, T. Öğretmen, N. Kocabağli, and M. Alp. 2013. Comparison of broiler live performance, carcass characteristics, and fatty acid composition of thigh meat when fed diets supplemented with neutralized sunflower soapstock or soybean oil. Journal of Applied Poultry Research, 22:118-131.

Peña J. E. M., S. L. Vieira, L. Borsatti, C. Pontin, and H. V. Rios. 2014. Energy Utilization of By-Products from the Soybean Oil Industry by Broiler Chickens: Acidulated Soapstock, Lecithin, Glycerol and Their Mixture. Brazilian Journal of Poultry Science, 16:437–442.

Pérez-Bonilla, A., M. Frikha, S. Mirzaie, J. García, and G. G. Mateos. 2011. Effects of the main cereal and type of fat of the diet on productive performance and egg quality of brown-egg laying hens from 22 to 54 weeks of age. Poultry Science, 90:2801–2810.

Perez-Carbajal, C., D. Caldwell, M. Farnell, K. Stringfellow, S. Pohl, G. Casco, A. Pro-Martinez, and C. A. Ruiz-Feria. 2010. Immune response of broiler chickens fed

different levels of arginine and vitamin E to a coccidiosis vaccine and *Eimeria* challenge. Poultry Science, 89:1870–1877.

Pesti, G. M., R. I. Bakalli, M. Qiao, and K. G. Sterling. 2002. A Comparison of Eight Grades of Fat as Broiler Feed Ingredients. Poultry Science, 81:382–390.

Polin, D. 1980. Increased absorption of tallow with lecithin. Poultry Science, 59:1652.

Polin, D., T. L. Wing, P. Ki, and K. E. Pell. 1980. The Effect of Bile Acids and Lipase on Absorption of Tallow in Young Chicks. Poultry Science, 59:2738–2743.

Polycarpo, G. V., M. F. C. Burbarelli, A. C. P. CarÃo, C. E. B. Merseguel, J. C. Dadalt, S. R. L. Maganha, R. L. M. Sousa, V. C. Cruz-Polycarpo, and R. Albuquerque. 2016. Effects of lipid sources, lysophospholipids and organic acids in maize-based broiler diets on nutrient balance, liver concentration of fat-soluble vitamins, jejunal microbiota and performance. British Poultry Science, 57:788-798.

Powles, J., J. Wiseman, D. J. A. Cole, and B. Hardy. 1993: Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Animal Production, 57:137–146.

Powles, J.; J. Wiseman, D. J. A. Cole, and B. Hardy. 1994: Effect of chemical structure of fats upon their apparent digestible energy value when given to young pigs. Animal Production, 58:411–417.

Ravindran, V., P. Tancharoenrat, F. Zaefarian, and G. Ravindran. 2016. Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. Animal Feed Science and Technology, 213:1–21.

Reis, P. M., T. W. Raab, J. Y. Chuat, M. E. Leser, R. Miller, H. J. Watzke, and K. Holmberg. 2008. Influence of Surfactants on Lipase Fat Digestion in a Model Gastro-intestinal System. Food Biophysics, 3:370–381.

Reis de Souza, T., J. Peiniau, A. Mounier, and A. Aumaitre. 1995. Effect of addition of tallow and lecithin in the diet of weanling piglets on the apparent total tract and ileal digestibility of fat and fatty acids. Animal Feed Science and Technology, 52:77–91.

Renaud, S. C., J. C. Ruf, and D. Petithory. 1995. The Positional Distribution of Fatty Acids in Palm Oil and Lard Influences their Biologic Effects in Rats. The Journal of Nutrition, 125:229–237.

Renner, R. 1965. Site of Fat Absorption in the Chick. Poultry Science, 44:861–864.

Rodríguez, M. L., A. Rebolé, S. Velasco, L. T. Ortiz, J. Treviño, and C. Alzueta. 2012. Wheat- and barley-based diets with or without additives influence broiler chicken

performance, nutrient digestibility and intestinal microflora. Journal of the Science of Food and Agriculture, 92:184–190.

Rodriguez-Sanchez, R., A. Tres, R. Sala, F. Guardiola, and A. C. Barroeta. 2019. Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages. Poultry Science, 98:1341–1353

Roll, A. P., E. Vilarrasa, A. Tres, and A. C. Barroeta. 2018. The different molecular structure and glycerol-to-fatty acid ratio of palm oils affect their nutritive value in broiler chicken diets. Animal, 12: 2040–2048.

Rondelli, S., O. Martinez, and P. García. 2003. Sex Effect on Productive Parameters, Carcass and Body Fat Composition of Two Commercial Broilers Lines. Brazilian Journal of Poultry Science, 5:169–173.

Rosen, E. D., and B. M. Spiegelman. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. Nature, 444:847–853.

Sabino, M., K. Cappelli, S. Capomaccio, L. Pascucci, I. Biasato, A. Verini-Supplizi, A. Valiani, and M. Trabalza-Marinucci. 2018. Dietary supplementation with olive mill wastewaters induces modifications on chicken jejunum epithelial cell transcriptome and modulates jejunum morphology. BMC Genomics 19, 576.

Salentinig, S., L. Sagalowicz, M. E. Leser, C. Tedeschi, and O. Glatter. 2011. Transitions in the internal structure of lipid droplets during fat digestion. Soft Matter, 7:650–661.

Sanz, M., A. Flores, P. Perez de Ayala, and C. J. Lopez-Bote. 1999. Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. British Poultry Science, 40:95–101.

Sanz, M., A. Flores, and C. J. Lopez-Bote. 2000a. The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. British Poultry Science, 41:61–68.

Sanz, M., C. J. Lopez-Bote, D. Menoyo, and J. M. Bautista. 2000b. Abdominal Fat Deposition and Fatty Acid Synthesis Are Lower and β-Oxidation Is Higher in Broiler Chickens Fed Diets Containing Unsaturated Rather than Saturated Fat. The Journal of Nutrition, 130:3034–3037.

Schiavone, A., S. Dabbou, M. De Marco, M. Cullere, I. Biasato, E. Biasibetti, M. T. Capucchio, S. Bergagna, D. Dezzutto, M. Meneguz, F. Gai, A. Dalle Zotte, and L.

Gasco. 2018. Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animal 12, 2032-2039.

Sethi, S., M. J. Gibney, and C. M. Williams. 1993. Postprandial Lipoprotein Metabolism. Nutrition Research Reviews, 6:161–183.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology, 59:215–221.

Sibbald, I. R. 1978. The True Metabolizable Energy Values of Mixtures of Tallow with Either Soybean Oil or Lard. Poultry Science, 57:473–477.

Siyal, F. A., M. E. A. El-Hack, M. Alagawany, C. Wang, X. Wan, J. He, M. Wang, L. Zhang, X. Zhong, T. Wang, and K. Dhama. 2017a. Effect of Soy Lecithin on Growth Performance, Nutrient Digestibility and Hepatic Antioxidant Parameters of Broiler Chickens. International Journal of Pharmacology, 13:396–402.

Siyal, F. A., D. Babazadeh, C. Wang, M. A. Arain, M. Saeed, T. Ayasan, L. Zhang, and T. Wang. 2017b. Emulsifiers in the poultry industry. Worlds Poultry Science Journal, 73:611–620.

Sklan, D., B. Shachaf, J. Baron, and S. Hurwitz. 1978. Retrograde Movement of Digesta in the Duodenum of the Chick: Extent, Frequency, and Nutritional Implications. The Journal of Nutrition, 108:1485–1490.

Sklan, D. 1979. Digestion and Absorption of Lipids in Chicks Fed Triglycerides or Free Fatty Acids: Synthesis of Monoglycerides in the Intestine. Poultry Science, 58:885–889.

Small, D. M. 1991. The effects of glyceride structure on absorption and metabolism. Annual Review of Nutrition, 11:413–434.

Smink, W., W. J. J. Gerrits, R. Hovenier, M. J. H. Geelen, M. W. A. Verstegen, and A. C. Beynen. 2010. Effect of dietary fat sources on fatty acid deposition and lipid metabolism in broiler chickens. Poultry Science, 89:2432–2440.

Smits, C. H. M., and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition–towards a physiologically valid approach to their determination. Worlds Poultry Science Journal, 52:203–221.

Soares, M., and C. J. Lopez-Bote. 2002. Effects of dietary lecithin and fat unsaturation on nutrient utilisation in weaned piglets. Animal Feed Science and Technology, 95:169–177.

Soler-Rivas, C., F. R. Marin, S. Santoyo, M. R. García-Risco, F. J. Señoráns, and G. Reglero. 2010. Testing and enhancing the in vitro bioaccessibility and bioavailability of rosmarinus officinalis extracts with a high level of antioxidant abietanes. Journal of Agricultural and Food Chemistry, 58:1144-1152.

Statista 2018. https://www.statista.com/statistics/675815/average-prices-soybean-oil-worldwide/. Accessed Sept 2018.

Sukhija, P. S., and D. L. Palmquist. 1988. Rapid Method for Determination of Total Fatty Acid Content and Composition of Feedstuffs and Feces. Journal of Agriculture and Food Chemistry, 36:1202–1206.

Svihus, B., A. Sacranie, V. Denstadli, and M. Choct. 2010. Nutrient utilization and functionality of the anterior digestive tract caused by intermittent feeding and inclusion of whole wheat in diets for broiler chickens. Poultry Science, 89:2617–2625.

Svihus, B., M. Choct, and H. L. Classen. 2013. Function and nutritional roles of the avian caeca: a review. Worlds. Poultry Science Journal, 69:249–264.

Svihus, B. 2014. Function of the digestive system. Journal of Applied. Poultry Research, 23:306–314.

Tancharoenrat, P. 2012. Factors influencing fat digestion in Poultry. PhD Thesis. Massey University, Palmerston North, New Zealand.

Tancharoenrat, P., V. Ravindran, F. Zaefarian, and G. Ravindran. 2013. Influence of age on the apparent metabolisable energy and total tract apparent fat digestibility of different fat sources for broiler chickens. Animal Feed Science and Technology, 186, 186–192.

Tancharoenrat, P., V. Ravindran, F. Zaefarian, and G. Ravindran. 2014. Digestion of fat and fatty acids along the gastrointestinal tract of broiler chickens. Poultry Science, 93:371–379.

Tancharoenrat, P., and V. Ravindran. 2014. Influence of tallow and calcium concentrations on the performance and energy and nutrient utilization in broiler starters. Poultry Science, 93:1453–1462.

Thomas, M., T. van Vliet, and A. F. B. van der Poel. 1998. Physical quality of pelleted animal feed 3. Contribution of feedstuff components. Animal Feed Science and Technology, 70:59–78.

Thormar, H., H. Hilmarsson, and G. Bergsson. 2006. Stable Concentrated Emulsions of the 1-Monoglyceride of Capric Acid (Monocaprin) with Microbicidal Activities

against the Food-Borne Bacteria *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*. Applied and Environmental Microbiology, 72:522–526.

Tres, A., C. D. Nuchi, N. Magrinyà, F. Guardiola, R. Bou, and R. Codony. 2012. Use of palm-oil by-products in chicken and rabbit feeds: effect on the fatty acid and tocol composition of meat, liver and plasma. Animal, 6:1005–1017.

Turk, D. E. 1982. The Anatomy of the Avian Digestive Tract as Related to Feed Utilization. Poultry Science, 61:1225-1244.

de Verdal, H., S. Mignon-Grasteau, C. Jeulin, E. le Bihan-Duval, M. Leconte, S. Mallet, C. Martin, and A. Narcy. 2010. Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. Poultry Science, 89:1955–1961.

Vieira, S. L., E. S. Viola, J. Berres, A. R. Olmos, O. R. A. Conde, and J. G. Almeida. 2006. Performance of Broilers Fed Increased Levels Energy in the Pre-Starter Diet and on Subsequent Feeding Programs Having with Acidulated Soybean Soapstock Supplementation. Brazilian Journal of Poultry Science, 8:55–61.

Vieira, S. L., L. Kindlein, C. Stefanello, C. T. Simoes, G. O. Santiago, and L. P. Machado. 2015. Energy Utilization from Various Fat Sources by Broiler Chickens at Different Ages. International Journal of Poultry Science, 14:257–261.

Vilà, B., and E. Esteve-Garcia. 1996a. Studies on acid oils and fatty acids for chickens. II. Effect of free fatty acid content and degree of saturation of free fatty acids and neutral fat on fatty acid digestibility. British Poultry Science, 37:119–130.

Vilà, B., and E. Esteve-Garcia. 1996b. Studies on acid oils and fatty acids for chickens. III. Effect of chemical composition on metabolisable energy of by-products of vegetable oil refining. British Poultry Science, 37:131–144.

Vilarrasa, E. 2014. Use of re-esterified oils in pigs and broiler chicken diets. PhD Thesis. Universitat Autònoma de Barcelona, Barcelona, Spain.

Vilarrasa, E., 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 Chicks. Lipids 49:795–805.

Vilarrasa, E., A. C. Barroeta, A. Tres, and E. Esteve-Garcia. 2015a. Use of re-esterified palm oils, differing in their acylglycerol structure, in weaning-piglet diets. Animal, 9:1304-1311.

Vilarrasa, E., R. Codony, F. Guardiola, E. Esteve-Garcia, and A. C. Barroeta. 2015b. Use of re-esterified oils, differing in their degree of saturation and molecular structure, in broiler chicken diets. Poult. Sci. 94:1527–1538.

Vilarrasa, E., F. Guardiola, R. Codony, E. Esteve-Garcia, and A. C. Barroeta. 2015c. Use of combinations of re-esterified oils, differing in their degree of saturation, in broiler chicken diets. Poultry Science, 94:1539–1548.

Villaverde, C., L. Cortinas, A. C. Barroeta, S. M. Martin-Orue, and M. D. Baucells. 2004. Relationship between dietary unsaturation and vitamin E in poultry. Journal of Animal Physiology and Animal Nutrition, 88:143–149.

Villaverde, C., M. D. Baucells, L. Cortinas, and A. C. Barroeta. 2006. Effects of dietary concentration and degree of polyunsaturation of dietary fat on endogenous synthesis and deposition of fatty acids in chickens. British Poultry Science, 47:173–179.

Viñado, A., L. Castillejos, R. Rodriguez-Sanchez, and A.C. Barroeta. 2019. Crude soybean lecithin as alternative energy source for broiler chicken diets. Poultry Science, DOI: https://doi.org/10.3382/ps/pez318.

Watkins, B. A. 1991. Importance of Essential Fatty Acids and Their Derivatives in Poultry. The Journal of Nutrition, 121:1475–1485.

Watson, A. D. 2006. Lipidomics: a global approach to lipid analysis in biological systems. The Journal of Lipid Research, 47:2101-2111.

Wendel, A. 2000. Lecithin. Pages 1–19 in Kirk-Othmer Encyclopedia of Chemical Technology. Kirk-Othmer ed. 5th Edition. John Wiley & Sons, West Sussex, UK.

Vikbjerg, A. F., J. Y. Rusig, G. Jonsson, H. Mu, and X. Xu. 2006. Comparative Evaluation of the Emulsifying Properties of Phosphatidylcholine after Enzymatic Acyl Modification. Journal of Agriculture and Food Chemistry, 54:3310–3316.

Wilde, P. J. and B. S. Chu. 2011. Interfacial & colloidal aspects of lipid digestion. Advances in Colloid and Interface Science, 165:14–22.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34:159–180.

Wiseman, J., D. J. A. Cole, F. G. Perry, B. G. Vernon, and B. C. Cooke. 1986. Apparent Metabolisable Energy Values of Fats for Broiler Chicks. British Poultry Science, 27:561–576.

Wiseman, J., and M. Lessire. 1987. Interactions Between Fats of Differing Chemical Content: Apparent Metabolisable Energy Values and Apparent Fat Availability. British Poultry Science, 28:663–676.

Wiseman, J., D. J. A. Cole, and B. Hardy. 1990. The dietary energy values of soya-bean oil, tallow and their blends for growing/finishing pigs. Animal Production, 50:513–518.

Wiseman, J., and F. Salvador. 1991. The Influence of Free Fatty Acid Content and Degree of Saturation on the Apparent Metabolizable Energy Value of Fats Fed to Broilers. Poultry Science, 70:573–582.

Wiseman, J., F. Salvador, and J. Craigon. 1991. Prediction of the apparent metabolizable energy content of fats fed to broiler chickens. Poultry Science, 70:1527–1533.

Wu, Y., and T. Wang. 2003. Soybean lecithin fractionation and functionality. Journal of the American Oil Chemists' Society, 80:319–326.

Yegani, M., and D. R. Korver. 2008. Factors affecting intestinal health in poultry. Poultry Science, 87:2052–2063.

Zampiga, M., A. Meluzzi, and F. Sirri. 2016. Effect of dietary supplementation of lysophospholipids on productive performance, nutrient digestibility and carcass quality traits of broiler chickens. Italian Journal of Animal Science, 15:521–528.

Zeitz, J.O., Fennhoff, J., Kluge, H., Stangl, G.I., Eder, K., 2015. Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality in broilers. Poultry Science, 94:2404–2413.

Zelenka, J. 1997. Effects of sex, age and food intake upon metabolisable energy values in broiler chickens, 38:281-284.

Zhao, P. Y., H. L. Li, M. M. Hossain, and I. H. Kim. 2015. Effect of emulsifier (lysophospholipids) on growth performance, nutrient digestibility and blood profile in weanling pigs. Animal Feed Science and Technology, 207:190–195.

Zumbado, M. E. 1999. Chemical Composition, and Metabolizable Energy Content of Different Fat and Oil By-Products. Journal of Applied Poultry Research, 8:263–271.

Chapter Nine

Curriculum of the author

Personal information:

Surnames, Name: Viñado Martínez, Alberto **Date of birth:** 04/12/1992

Location: Barcelona (Spain) **E-mail:** avm1992@gmail.com

Education:

2018-2020	M.Sc. in Laboratory Animal Science and Welfare
	Universitat Autònoma de Barcelona
2016-Present	Ph.D Student in Animal Science (Thesis defense: July 2019)
	Universitat Autònoma de Barcelona
2010-2015	Degree in Veterinary Medicine
	Universitat Autònoma de Barcelona

Post-graduate courses:

2017 Training course for Researchers on Laboratory Animal management (FELASA).

Universitat Autònoma de Barcelona

2017 Statistical techniques in R

Universitat Autònoma de Barcelona

Professional experience:

2016-Present Member of the Animal Nutrition and Welfare Service (SNiBA)

- Collaboration in several research projects of the animal science field (experimental design, farm controls and samplings, laboratory and statistical analysis, reports writing and scientific divulgation).
- Write technical summaries (monthly) related to poultry production for AECA website.

2015-2016 Trader and consultant at Etalon SA.

- Selling and distribution of commodities to animal feed producers. Forecasts production of demands for raw materials.

2014-2015 Veterinarian in practice at Hospital Veterinari Glories.

- Veterinary assistant during consultations and surgeries. Responsible of the anaesthesia and analgesia of the patient, and realization of minor surgeries.
- Responsible of hospitalized animals. Administration of drug treatment.

Publications:

Viñado, A., L. Castillejos, R. Rodriguez-Sanchez, and A.C. Barroeta. 2019. Crude soybean lecithin as alternative energy source for broiler chicken diets. Poultry Science, DOI: https://doi.org/10.3382/ps/pez318.

Viñado, A., L. Castillejos, and A.C. Barroeta. 2019. Soybean lecithin high in free fatty acids for broiler chicken diets: impact on performance, fatty acid digestibility and saturation degree of adipose tissue. Animal (*in Revision*).

Scientific divulgation:

Viñado, A., L. Castillejos, and A. C. Barroeta (2019). Effect of dietary soybean lecithin on broiler chickens performance, ileal fatty acid digestibility and adipose saturation degree. 22nd European Symposium On Poultry Nutrition (ESPN), Gdansk, Poland.

Viñado, A., L. Castillejos, and A. C. Barroeta (2018). Crude soybean lecithin and vegetable acid oil as energy sources for broiler chickens. 6th Mediterranean Poultry Summit. Torino, Italy. Oral communication.

Viñado A., L. Castillejos, and A. C. Barroeta (2017). Combinación de oleína insaturada y lecitina vegetal en piensos para pollos de carne. 54 Simposio AECA, Leon, Spain. Poster presentation.

Viñado, A., L. Castillejos, and A. C. Barroeta (2017). Lecitina de soja como fuente de energía en la alimentación de pollos de carne. XVII Jornadas sobre producción animal, Zaragoza, Spain. Oral communication.

Viñado, A., D. Sola-Oriol, M. Jansen, A. Karwacinska, and A. C. Barroeta (2017). Dietary lysolecithin supplementation improves nutrient utilization in broiler chickens. 21st European Symposium On Poultry Nutrition (ESPN), Vila-Seca, Tarragona. Poster presentation.

A. Viñado, L. Castillejos y A. C. Barroeta. (2017). Crude soybean lecithin as alternative energy source in broiler chickens. 21st European Symposium On Poultry Nutrition (ESPN), Vila-Seca, Tarragona. Poster presentation.

Skills and competence:

Languages:	Native Spanish and Catalan
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Software:	High level in Microsoft Office.