Universitat Autònoma de Barcelona

# USE OF ACID OILS IN BROILER CHICKEN DIETS

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TESIS DOCTORAL PRESENTADA POR:
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PARA ACCEDER AL GRADO DE DOCTOR DENTRO DEL PROGRAMA EN PRODUCCIÓN ANIMAL DEL DEPARTAMENTO DE CIENCIA ANIMAL Y DE LOS ALIMENTOS

Bellaterra, 2018

FACULTAT DE VETERINÀRIA

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Que la memoria titulada “**Use of acid oils in broiler chicken diets**”, presentada por Raquel Rodríguez Sánchez con la finalidad de optar al grado de Doctor en Veterinaria, ha sido realizada bajo su dirección y, considerándola por finalizada, autorizan a su presentación para que sea juzgada por la comisión correspondiente.

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La presente memoria de tesis ha sido realizada gracias a una beca predoctoral *Ayudas para la Contratación de Personal Investigador Novel* (Ref. FI-DGR 2015– FI_B 00475) concedida por la Agencia de gestión de Ayudas Universitarias y de Investigación de la Generalitat de Cataluña y posteriormente por una beca predoctoral *Formación de Profesorado Universitario* (Ref. FPU1406063) concedida por el Ministerio de Educación y Formación Profesional del Gobierno de España y a la financiación proporcionada por el Ministerio de Economía y Competitividad del Gobierno de España (Proyecto AGL2015-64431-C2-1-R).

*To everyone who light up my life*
*A todos los que iluminan mi vida*

# Agradecimientos - Acknowledgments

Nunca me había planteado realizar un doctorado, pero me surgió la oportunidad, después de meditarlo decidí embarcarme en esta aventura y hoy puedo decir que no me arrepiento que este proyecto haya formado parte de mi vida. Durante estos cuatro años he vivido experiencias increíbles y aunque también ha habido momentos no tan buenos, todos, tanto los buenos como los malos, han sido enriquecedores y me han permitido crecer como persona. El doctorado ha sido un proceso de crecimiento constante, ya que como en la vida misma, aparecen retos constantemente. Retos que hay que solventar para poder seguir adelante, y aunque al principio todo parezca difícil, con esfuerzo, constancia, y también un poco de suerte, todo se consigue. Suerte, sí, porque a pesar de siempre haber hecho las cosas lo mejor que he sabido y podido, me considero muy afortunada por muchas personas que han aparecido en mi camino y lo han hecho todo más fácil, entretenido e interesante. Siempre agradezco a todas las personas que me ayudan o me han ayudado y aunque no pueda incluir a todas ellas en esta lista, no por ello dejan de ser menos importantes y por ello, quiero darles las gracias a todas ellas una vez más.

En especial quiero agradecer:

A Ana, por haber confiado en mí y haberme dado la oportunidad de iniciar este proyecto cuando prácticamente no me conocía. De ti he aprendido infinitas cosas. También darte las gracias por haberme apoyado en todas las experiencias relacionadas con el doctorado, nunca me hubiese imaginado en China exponiendo un trabajo, o en Nueva Zelanda haciendo una estancia, y todo ello ha sido en parte gracias a ti.

A Alba, por haberme dado la oportunidad de trabajar en un área de trabajo en la que no tenía nada de experiencia, de la que he aprendido muchas cosas y por haber aportado un enfoque distinto al trabajo de esta tesis. También por haberme enseñado que si algo tiene solución, no es un problema.

A todos los profesores y personas que forman o han formado parte tanto del grupo SNIBA como del grupo LiBiFOOD, también a Carlos y Mariola, por haberme dedicado su tiempo y ayudarme cuando he tenido dudas.

Al personal de la secretaría del departamento por ser de gran ayuda en la tramitación de documentación y al personal del *Servei de Granges i Camps Experimentals*, por ayudarme durante mis pruebas experimentales.

A Vittor, Gemma (mi Salernitana favorita) e Isamel por haberme ayudado incondicionalmente y porque de vosotros también aprendí muchas cosas. Mi trabajo sin vosotros hubiera sido mucho más difícil y aburrido. También a Julieta por haberme ayudado con parte de mis muestras y por su compañía en el laboratorio. Me alegro de teneros como amigos.

A todos mis compañeros, becarios del grupo SNIBA y del grupo LibiFOOD, así como a los que han realizado estancias temporales en estos grupos. Estoy segura de que de todos vosotros he aprendido algo. Especialmente me considero afortunada de haber conocido a Elisa, persona como la que quedan pocas en el mundo y a Alberto, por ser el mejor compañero de oficina y sacarme una sonrisa en los momentos de trabajo más duros. Estoy segura de que nuestra amistad seguirá muchos años más.

To James, the best English teacher I have ever had. Thanks for all the classes that made my English flourish. Four years ago, I could not imagine myself writing my thesis in English or presenting some of my work in a conferenece. I still have lots of things to improve and learn, but I would not be at this point without the help and support you gave me.

To Ravi, for giving me the opportunity to join his team for my internship. I learnt a lot from your way of working and also your way of being, which I personally admire. I’m very grateful for all the experiences I got to live in New Zealand because of you, and for the kindness of you and your family, Rana and Veena. To Reza and Fifi for their help and support, I also learnt a lot from you. To the team in the Poultry Unit, because you also taught me a lot, and you made the time I spent in the farm very nice. And to all the PhD students and office mates of the vet tower at Massey for spending time with me and make my life better. All of you made me feel like at home. I couldn't imagine a best way to spend part of my last year of PhD. I hope I can come back to New Zealand someday. Last but not least, to my family in New Zealand, to Ryan, Russell, Nipuna and Nick, because you have given me inspiration, light and strength. I'm really happy I got to know you.

A los no tan humanos, que también me han ayudado durante estos cuatro años, a mi pequeño Toby, que siempre está conmigo, a Cosmo, Holly y Alo.

A mis padres por ser el mejor equipo que tengo en la vida. Siempre estoy agradecida por todo lo que hacéis por mí.

*Develop an attitude of gratitude.*
*Say thank you to everyone you meet for everything they do for you.*

# Summary

The use of supplemental fats in feed formulation is a widespread practice for meeting both the energy and also essential fatty acid requirements. Food fat by-products, like those from the edible oil refining industry (acid oils), are an example of an economic alternative in comparison to conventional fats that can be revalorized as a feed fat ingredient.

Acid oils are rich in free fatty acids (**FFA**), and have similar fatty acid (**FA**) composition to their respective crude oils, but different molecular structures, which can affect their nutritional value. FFA have been negatively related to fat utilization, and it is well known that the saturation degree of dietary fat has a negative impact on fat utilization, as well as that the ability of chicks to digest and absorb dietary fat is poorly developed. Thus, the aim of the present thesis is to study in depth the digestion and absorption of fat in broiler chickens, and determine how the age of the chickens, the saturation degree and the FFA level of dietary fat affect these processes in order to raise recommendations on the use of oils with certain percentages of FFA in the diets with no negative repercussions on fat utilization. This is the first step to assess the use of acid oils in broiler chickens diets under up-to-date conditions.

The first trial (Chapter 3) was conducted in order to study the effect of the dietary fat saturation degree and age of the chickens (14 d and 35 d) on the fat digestion and absorption processes. For this purpose the dietary use of crude soybean oil and crude palm oil was studied, and the FA digestibility and lipid class composition were determined along the gastrointestinal tract (GIT; gizzard, duodenum, jejunum, and ileum), and excreta. The assessment of both the FA digestibility and lipid class content allowed for a better understanding of the fat digestion and absorption processes. It was seen that the FA absorption process was more affected than the hydrolysis process was by both the fat saturation degree and age of the chickens, being the jejunum the main place of FA absorption. However, the contribution of the ileum to FA absorption was also important, being the last GIT segment where FA absorption has been described. Furthermore, the utilization of unsaturated diets was higher than the utilization of saturated diets mainly due to a higher contribution of the ileum to saturated FA absorption, and the improvement on fat utilization in adult broiler chickens was due to an increase in the contribution of the jejunum to FA absorption.

The second trial (Chapters 4 and 5) was conducted to assess the effect of the dietary FFA level, fat saturation degree and age (14 d and 37 d) on fat digestion and absorption processes. Two crude oils and two fat by-products from the edible oil refining industry rich in FFA were used in order to produce eight dietary treatments with two different saturation degrees (soybean oil products as unsaturated fat source, and palm oil products as saturated fat source), and four levels of dietary FFA (5%, 15%, 35%, and 50%). FA digestibility and lipid class composition along the GIT, and excreta were determined. Concerning the dietary FFA level, the absorption process was more affected than the hydrolysis process was. The effect of dietary FFA level in the absorption process was found to be different depending on the saturation degree of dietary fat and age of the chicken. The results evinced that adult broiler chickens (37 d) were less affected by the dietary FFA level than young broiler chickens (14 d) were due to a more efficient FA absorption at the jejunum level. Thus, while at 14 d the diets with the highest dietary FFA level (50%) were related to lower fat utilization in comparison to the diets with the lowest dietary FFA level (5%), at 37 d this was less pronounced. Unsaturated diets were more affected by the dietary FFA level than saturated diets, and the suitable level of dietary FFA in grower-finisher diets was higher than in starter-broiler chicken diets. Regardless of the age, it was concluded that the FA profile of dietary fat has a bigger impact on fat utilization than the level of dietary FFA.

Taking all the results into account, it was evinced that the absorption process is more affected than the hydrolysis process is by the saturation degree and free fatty acid level of dietary fat, as well as by the age of the chicken. The greater utilization of unsaturated diets irrespective of the age was confirmed. Another interesting finding is that crude soybean oil could be partially replaced by acid soybean oil, being a good alternative fat source to be used in broiler chicken diets at least when the dietary FFA level does not exceed 15% and 35% in starter and grower-finisher diets, respectively. On the other hand, the saturated diet with 50% dietary FFA level did not differ from the saturated diet with the lowest dietary FFA level (5%), suggesting that acid palm oil (palm fatty acid distillate) could replace crude palm oil in grower-finisher diets, at least when the FFA level does not exceed 50% with no negative repercussions on fat utilization compared to the use of crude palm oil.

# Resumen

Las materias grasas se incorporan de forma habitual en los piensos para pollos de carne ya que son una fuente concentrada de energía y ácidos grasos esenciales. Los aceites ácidos (también conocidos como oleínas) son co-productos que derivan del proceso de refinación de los aceites para consumo humano y pueden reutilizarse en alimentación animal, siendo una fuente de grasa alternativa más económica que las grasas convencionales. Los aceites ácidos (aceites ácidos de refinación química y ácidos grasos destilados de refinación física) se caracterizan por tener un alto contenido en ácidos grasos libres (**AGL**) y aunque presentan un perfil en ácidos grasos (**AG**) similar a la de los aceites crudos de los que derivan, se diferencian de los últimos en su estructura molecular, lo que puede afectar a su valor nutricional. Se ha descrito que la utilización de la grasa de la dieta se ve afectada de forma negativa por el grado de saturación y se señala la presencia de AGL como un factor negativo. También es reconocido que la capacidad digestiva de los pollos mejora con la edad. Por todo ello, el objetivo de la presente tesis es profundizar en el estudio de los procesos de digestión y absorción de la grasa en pollos de carne y determinar cómo afectan a estos procesos, el nivel de saturación y nivel de AGL de la dieta, así como la edad de los pollos. Estos estudios son importantes para poder establecer recomendaciones sobre el uso de aceites ácidos (oleínas) en dietas para pollos de carne sin repercusiones negativas sobre la utilización de la grasa.

El primer ensayo (Capítulo 3) se llevó a cabo con el fin de evaluar el efecto del grado de saturación de la dieta y edad (14 d y 35 d) sobre los procesos de digestión y absorción de la grasa. Para ello se utilizaron dietas con un 6% de aceite crudo de soja y aceite crudo de palma y se determinó la digestibilidad de los AG y el contenido en fracciones lipídicas a lo largo del tracto gastrointestinal (**TGI**; molleja, duodeno, yeyuno e íleon) y en excreta. La determinación de la digestibilidad de los AG y contenido en fracciones lipídicas permitió entender mejor los procesos de digestión y absorción de la grasa. El proceso de absorción de los AG se vio más afectado que el proceso de hidrólisis por el grado de saturación de la grasa y edad de los pollos, siendo el yeyuno el principal lugar de absorción de los AG. Además, también destaca la importancia del íleon en la absorción final de los AG, ya que es el último tramo del TGI donde se ha descrito absorción de los AG. La mayor utilización de las dietas insaturadas se relaciona con una mayor capacidad de absorción de los AG saturados a nivel del

íleon y la mejor utilización de la grasa en pollos adultos se debe a una mayor absorción de los AG a nivel de yeyuno.

El segundo ensayo (Capítulos 4 y 5) se llevó a cabo para estudiar el efecto del nivel de AGL de la dieta, grado de saturación de la grasa de la dieta y edad (14 d y 37 d) sobre los procesos de digestión y absorción de la grasa. Se utilizaron ocho dietas experimentales con dos grados de saturación (derivados del aceite de soja como fuente insaturada y derivados del aceite de palma como fuente saturada) y cuatro niveles de acidez (5%, 15%, 35% y 50%). Para ello, se combinaron dos aceites crudos y dos co-productos ricos en AGL (oleínas). Se determinó la digestibilidad de los AG y el contenido en fracciones lipídicas en diferentes tramos del TGI y excreta. Los resultados demuestran que los pollos adultos (37 d) se vieron menos afectados por el nivel de AGL en la dieta que los pollos jóvenes (14 d) debido a que la absorción de los AG, en los primeros, fue más eficiente a nivel de yeyuno. A 14 d, las dietas con mayor nivel de acidez (50% AGL), en comparación con las dietas con menor nivel de acidez (5% AGL), se asociaron con una menor utilización de la grasa de la dieta, mientras que estas diferencias no se observaron a 37 d. En relación al nivel de AGL de las dietas el proceso de absorción se vio más afectado que el proceso de hidrólisis, dependiendo del grado de saturación de las dietas y edad del pollo. Las dietas insaturadas se vieron más afectadas por el nivel de acidez que las dietas saturadas y el nivel adecuado de AGL fue mayor para las dietas de crecimiento-acabado que para las de inicio. Independientemente de la edad, se observó que el perfil de AG de la grasa de la dieta tuvo un mayor impacto sobre la utilización de la grasa que el nivel de AGL de la dieta.

Teniendo en cuenta todos los resultados, se evidenció que las diferencias en la utilización de las grasas en relación al grado de saturación y nivel de AGL de la dieta, así como a la edad de los pollos se deben a cambios en el proceso de absorción de los AG más que a limitaciones en el proceso de hidrólisis. Se confirma la mejor utilización de las dietas insaturadas tanto en pollos jóvenes como adultos. Otro hallazgo interesante es que el aceite crudo de soja podría ser parcialmente reemplazado por el aceite ácido de soja, cómo grasa alternativa, siempre y cuando no se supere el 15% y 35% de AGL en dietas de inicio y crecimiento-acabado, respectivamente. Por otro lado, no se observaron diferencias destacables entre los resultados obtenidos con dietas saturadas con 50% y 5% de AGL, lo que lleva a sugerir que el aceite ácido de palma (ácidos grasos destilados de refinación física de palma) podría reemplazar el aceite crudo de palma en dietas de crecimiento-acabado, al menos cuando no se supere el 50% de AGL de la dieta, sin que se produzcan repercusiones negativas sobre la utilización de la grasa.

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# Abbreviations

| | |
|---|---|
| **A** | acid oil |
| **ADFI** | average daily feed intake |
| **ADG** | average daily gain |
| **AME** | apparent metabolizable energy |
| **DAG** | diacylglycerol |
| **DM** | dry matter |
| **FA** | fatty acid |
| **FCR** | feed conversion ratio |
| **FFA** | free fatty acid |
| **GIT** | gastrointestinal tract |
| **MAG** | monoacylglycerol |
| **ME** | metabolizable energy |
| **MUFA** | monounsaturated fatty acids |
| **O** | crude oil |
| **PA** | palm fatty acid distillate from physical refining |
| **PO** | crude palm oil |
| **PUFA** | polyunsaturated fatty acids |
| **RID** | refractive index detector |
| **SA** | acid soybean oil from chemical refining |
| **SO** | crude soybean oil |
| **SFA** | saturated fatty acids |
| **TAG** | triacylglycerol |
| **TFA** | total fatty acids |
| **UFA** | unsaturated fatty acids |

# CHAPTER 1

## Literature review

# 1. 1. General introduction

Fats and oils are the main sources of energy as they have the highest caloric value among all ingredients. For this reason, the use of supplemental fats in feed formulation is a widespread practice for meeting both the energy and also essential fatty acid requirements. Furthermore, fats and oils have other nutritional and technological beneficial effects when added to the diet such as dustiness reduction or palatability improvement. There are different available fat sources for poultry diets, and there is an increasing interest about searching new more affordable alternative fat sources, such as fat by-products, to meet the energy requirements and reduce the costs at the same time. In order to optimize the use of alternative fats is important to understand how fat is digested and absorbed. Ravindran et al. (2016) reviewed the digestive physiology and the most important factors that have an effect on fat utilization in poultry. However, there are still some controversial issues and the information about the utilization of fat by-products from the edible oil refining industry by broiler chickens is scarce. For this reason, the aim of the present work is to study in depth the digestion and absorption of fat in broiler chickens, and determine how the age of the chickens, the saturation degree and the free fatty acid (**FFA**) level of dietary fat affect these processes. This information can be of practical application, as it will allow for a better understanding of the limitations of the use of acid oils (a fat by-product from the refining process of edible oils) both in starter and grower-finisher diets for broiler chickens.

First, the basic terms and definitions related to the topic, as well as the digestion and absorption processes of fat, and the role of lipids in the body will be described. Then, the available fat sources and fat by-products from the oil refining industry, their main characteristics, and their use in broiler chicken diets will be explained.

# 1.2. Basic terms and definitions

The term **lipid** defines a group of organic compounds that are insoluble in water and soluble in organic solvents (Smith et al. 2008). As described by Fahy et al. (2011), these chemical features can be found in a wide range of molecules such as fatty acids, phospholipids, sterols, sphingolipids, and terpenes, among others.

There are different classifications for lipids. According to Christie (The Lipid Web, http://lipidhome.co.uk), lipids are fatty acids (**FA**) and their derivatives, and substances related biosynthetically or functionally to these compounds, and can be classified in simple or complex. **Simple lipids** are those that yield on hydrolysis at most two types of primary products per mole (e.g., acylglycerols: fatty acids and glycerol), and **complex lipids** are those that yield three or more primary hydrolysis products per mole (e.g., glycerophospholipids: fatty acids, glycerol, and head group). It is suggested that complex lipids are best considered in terms of glycerophospholipids, sphingolipids, or glycolipids. On the other hand, according to the LIPID MAPS consortium (http://lipidmaps.org), lipids can be divided into the following eight main categories: fatty acyls (or fatty acids), glycerolipids, glycerolphospholipids, sphingolipids, saccharolipids and polyketides, sterol and prenol lipids.

The terms "**fat**" and "**oil**" are generic names; the first term is usually applied to fats that are solid at room temperature, while the second term is applied to those that are liquid at room temperature. Acylglycerols (glycerides), esters of fatty acids and glycerol, are the primary constituents of natural fats and oils; this kind of lipids includes triacylglycerols (**TAG**), diacylglycerols (**DAG**), and monoacylglycerols (**MAG**) (Weete, 1974). TAG in which glycerol is esterified with three FA **(**Figure 1.1), are the main component of conventional fats and oils (> 90%); DAG, MAG, and FFA can also be present in different proportions in fats and oils.

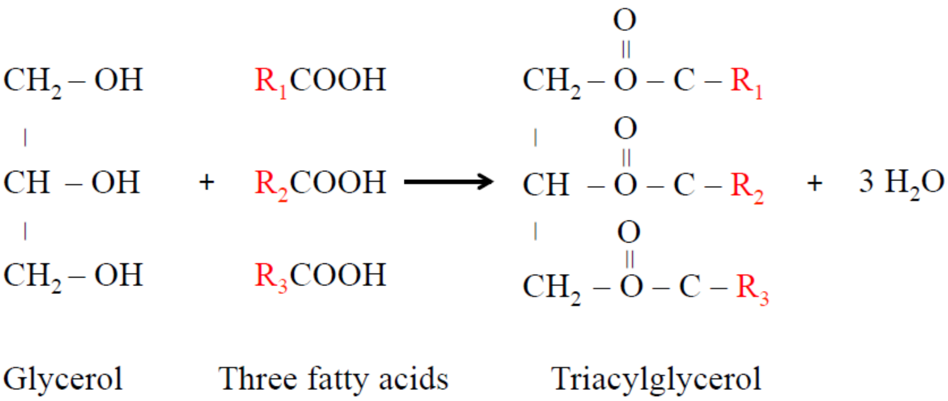


**Figure 1.1.** The structure of a triacylglycerol molecule. R1, R2, and R3 are the hydrocarbon chains of fatty acids.

Chemically, the differences between different fat and oil sources are due to their FA composition, which determines their physical and chemical properties. FA can be classified as unsaturated (**UFA**) or saturated (**SFA**). UFA have at least one double bond (usually in *cis* conformation, although some FA have *trans* configuration) between its carbon atoms, and they usually are liquid at room temperature; those FA with one double bond are called monounsaturated FA (**MUFA**) and those with two or more double bonds are called polyunsaturated FA (**PUFA**). SFA have no double bonds at the carbon chain and are usually solid at room temperature. The length of the carbon chain is another way to classify FA. According to Christie (The Lipid Web, http://lipidhome.co.uk), short FA are those with up to 6 carbons, medium FA those with 7-12 carbons, and long-chain FA those with more than 12 carbons in chain-length. The most common FA in plant and animal tissues contain from 12 to 22 carbon atoms. The five most common FA in animal feeds are presented in Table 1.1.

**Table 1.1.** The five most common fatty acids present in animal feeds

| Systematic name | Trivial name | Notation |
|---|---|---|
| Hexadacanoic | Palmitic | C16:0 |
| Octadecanoic | Stearic | C18:0 |
| Cis-9-octadecenoic | Oleic | C18:1n-9 |
| 9,12-octadecadienoic | Linoleic | C18:2n-6 |
| 9,12,15-octadecatrienoic | α-linolenic | C18:3n-3 |

# 1.3. Importance of the use of fat in poultry diets

## 1.3.1. Nutritional importance of fat

The main reason of the inclusion of supplemental fats in poultry feed formulation is their metabolizable energy (**ME**) content, which depends on their digestibility and absorption (Pesti et al., 2002). However, as it will be discussed below, fat is also a source of nutrients and has other non-nutritional roles.

It has to be taken into account that in terms of **energy-yielding potential**, fat is not essential and could be replaced by carbohydrates. However, the caloric value of fats and oils (9 kcal/g fat, approximately) is higher than the caloric value of carbohydrates, and proteins. According to Saleh et al. (2004) the determination of the level of energy of a diet is one of the most important things to do in the formulation of diets for poultry, as it represents a high proportion of the cost. Saleh et al. (2004) also suggested that higher energy levels could allow for a faster gain and higher quantity of meat produced leading to a reduction of the housing, equipment and labor costs. The relative advantage or disadvantage of using diets high in nutrient density should be determined by the price of the ingredients. Supplying energy as fat is usually a more economical alternative than supplying it with the use of carbohydrates, and it is for that reason that the interest about searching new more affordable fat sources to meet the energy requirements and reduce the costs has increased (Doreau and Chilliard, 1997). As there is no physiological limitation, the fat inclusion level in poultry diets can increase when the price of fat is competitive, in comparison to the price of cereal grains (Kellems and Church, 2010), as long as there are no technological limitations.

Fat is also used for nutritional purposes in order to provide linoleic acid, an essential FA for poultry. The lack of linoleic acid can result in abnormalities such as reduced growth rates, poor reproductive performance, skin lesions, increased water consumption, reduced resistance to disease, among others (Balnave, 1970). The linoleic acid requirement of broiler chickens has been estimated to be 1% of the diet (Balnave, 1970; NCR, 1994).

### 1.3.2. Other nutritional and non-nutritional effects of fat

Apart from the effects described above, the addition of fats in animal diets improves the absorption of liposoluble vitamins, such as vitamins A and E (Leeson and Atteh, 1995), as well as carotenoids, important for carcass pigmentation. It also reduces the dustiness and ingredient separation, increases the palatability and decreases the rate of food passage, which is related to a higher utilization of nutrients. The level of inclusion of fat can affect the presentation characteristics of the feed. For this reason, high levels of fat should be avoided in pelleted diets as it affects negatively the physical pellet quality (Rose, 2001; Abdollahi et al., 2013).

### 1.3.3. Effects of dietary fat on intestinal morphology and microbiota

According to Bischoff (2011) the gastrointestinal barrier is strongly associated to the maintenance of gut health. There is some evidence that dietary fat could influence both intestinal morphology and immune health.

On the one hand, intestinal mucosa is one of the first tissues to be in contact with dietary ingredients, and according to Jenkins et al. (1992), it is likely that dietary factors affect gut morphology, especially at their site of absorption. Regarding the effect of fat, in rats fed a high-fat diet, Goda and Takase (1994) reported a reduction on the length of microvilli, a slightly increase in their diameter, and an increase in the number of enterocytes per villus (hyperplasia) as a compensation. It was hypothesized that this change on villi structure was due to the presence of long chain FA. Li et al. (1990) reported that after feeding pigs with a diet supplemented with a mixture of 50% soybean oil (rich in long-chain FA) and 50% coconut oil (rich in short-chain FA), intestinal villi were higher and rounder. Zeitz et al. (2015) studied the effect of dietary fats rich in lauric and myristic acid on intestinal morphology and reported an effect on the villi length:crypt depth ratio, which was lower in those animals fed a diet with free lauric and myristic acids than in those fed a diet rich in esterified lauric and myristic acids.

On the other hand, there is also some available information about the role of FA as modulators of immune responses (Fritsche et al., 1991), and the beneficial effect of some FA (e.g., monocaprin) in the control of *Campylobacter jejuni* (Thormar et al., 2006). Baltić et al. (2017) associated medium chain FA with antibacterial, anticoccidial and antiviral effects, and related this with positive effects on health, production, feed digestibility, and lower body and muscle fat deposition both in broiler and pigs. However, Zeitz et al. (2015) did not see any effect of medium chain FA on gut bacteria. Bacterial activity can affect the nutrient utilization and improve or limit the available energy values of diets; bacteria can either benefit the host by producing energy in the form of short-chain FA (these FA come from the fermentation of non-hydrolysable oligo- and polysaccharides), or use part of the dietary energy especially when it is supplied as easily digestible substrates by the host (Lan et al., 2005).

Thus, considering both intestinal morphology and microbiota as indicators of gut health is important as it can influence the utilization of fat and other nutrients. However, the available information regarding the effect of dietary fat on intestinal morphology and intestinal microbiota in broiler chickens is scarce.

## 1.3.4. Effects of fat on meat quality

Another important aspect is the effect of dietary fat composition on meat quality. It is known that the dietary fat composition affects both the amount, and FA composition of the deposited fat in the carcass in broiler chickens (Esteve-Garcia, 2012), as well as, the stability, nutritional and sensory quality of meat (Wood et al., 2003; Bou et al., 2009).

On the one hand, the dietary fat source can modify the amount of deposited fat in the body. In particular, the use of vegetable oils rich in PUFA (mainly of n-3 series), like sunflower oil, soybean oil or linseed oil has been related to a decrease of abdominal fat deposition in broiler chickens (Crespo and Esteve-Garcia, 2001; Ferrini et al., 2008; Wongsuthavas et al., 2008; Smink et al., 2010). According to Ferrini et al. (2008) this reduction is about 30% in comparison to the use of dietary SFA.

On the other hand, the FA profile of dietary fat has a direct effect on lipid deposition, and it is reflected on the FA profile of the different body tissues (Crespo and Esteve-Garcia, 2001; Skrivan et al., 2018). Crespo and Esteve-Garcia (2001) reported that chickens fed tallow presented higher SFA (mainly myristic, palmitic, and stearic acids), chickens fed olive oil presented higher oleic acid, those fed sunflower oil had higher values of linoleic acid, and those fed linseed oil had the highest value of linolenic acid in abdominal fat, thigh and breast muscles. Similarly, Skrivan et al. (2018) reported that the breast meat of chickens fed rapeseed oil had the highest content of UFA, while the breast meat of those fed with palm oil had the highest content of SFA. As a consequence, it is expected that the profile of FA in the meat affect the fat tissue firmness (hardness), shelf life (lipid and pigment oxidation), flavor, and could also affect tenderness and juiciness. The FA profile affects meat firmness due to the different melting points of FA; as the unsaturation degree increases, the melting point decreases. For example, according to Bou et al. (2009), meat enriched with UFA is more prone to oxidize leading to the formation of off-flavors and off-odors, and also the loss of nutritional value. The structure of the molecule also has an effect on the melting point; *trans* FA melt at a higher temperature than their *cis*-isomers and branch chain FA have lower melting points than the straight chain FA with the same number of atoms (Enser, 1984; Wood and Enser, 1997).

For all of these reasons, dietary fat is important, not only for ensure a good utilization by the animal, but also because it will affect the consumer acceptability of the product and its stability (Enser, 1984; Bou et al., 2009).

## 1.4. Available vegetable fat sources for poultry diets

The use of conventional vegetable fat sources like crude palm oil, crude soybean oil or crude sunflower oil, among others, is a common practice in poultry diets. However, the use of alternative fat sources, like fat by-products, is becoming more important. In this section, a brief review of both the most used conventional vegetable oils and fat by-products from the oil refining industry will be presented, as well as a brief description about the obtaining process of the latter.

## 1.4.1. Conventional vegetable oils and fats

According the USDA report "Oilseeds: World Markets and Trade" (2017), the global vegetable oil production in 2016/2017 was 185.8 million tons, including coconut, cottonseed, olive, palm, palm kernel, peanut, rapeseed, soybean and sunflower seed oil. Over the last years, the global production of vegetable oils has experienced a constant growth. However, not all the fat sources mentioned above are destined to animal feeds, and just a small part is used for this purpose. According to the mentioned report, it is estimated that 90% of palm oil is used for human food consumption, being the remaining 10% used for other purposes such as industrial consumption (cosmetic products, fuel) and animal feed. Palm and soybean are the most produced and consumed vegetable oils worldwide (Figure 1.2).

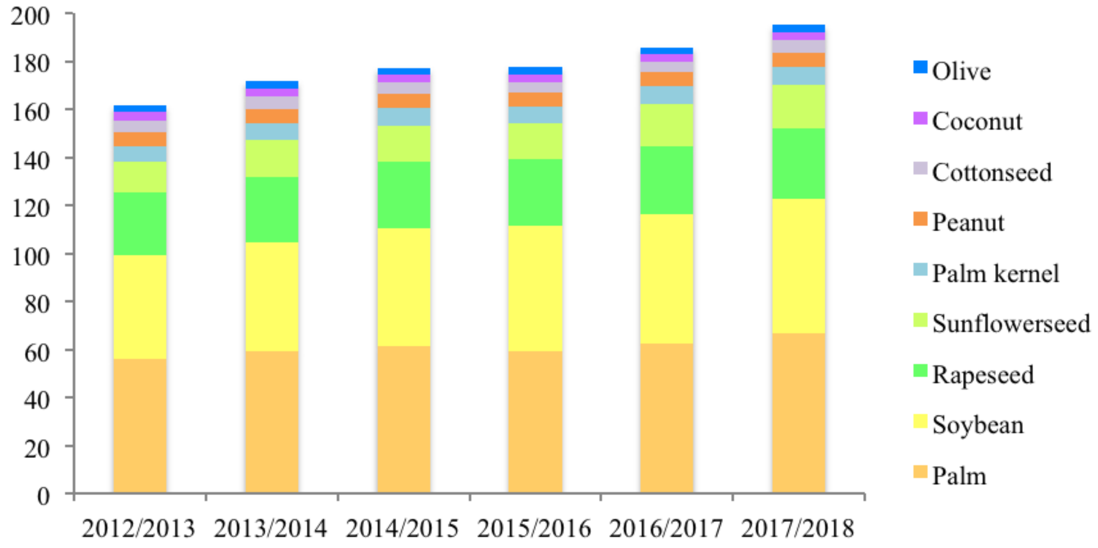


**Figure 1.2.** General production of major vegetable oils worldwide from 2012/13 to 2017/18, by type in million tons. Source: USDA 2017.

According to the forecast for the period 2017/2018 (USDA, 2017), the global production of palm and soybean oil is 66.9 and 56 million tons, respectively. The other two most produced and consumed oils are rapeseed and sunflower oils.

The two most commonly used vegetable oils for animal feeds are crude palm oil and crude soybean oil, and they will be explained below more in depth. The use of one or another will depend on different factors such as their chemical composition, which determines their nutritional value and stability to oxidation (Codony et al., 2010), as well as on their cost. The FA composition of the most common fats and oils in animal feeds is presented in Table 1.2.

**Table 1.2.** Fatty acid composition (%) of the most common vegetable oils used in animal feeds

| Fatty acid | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | C>20 |
|---|---|---|---|---|---|---|---|---|
| Palm oil | 0.99 | 42.57 | 0.30 | 4.75 | 39.60 | 9.90 | 0.00 | 0.00 |
| Soybean oil | 0.00 | 9.41 | 0.20 | 3.96 | 21.78 | 53.46 | 7.23 | 1.09 |
| Rapeseed oil | 0.00 | 4.95 | 0.30 | 2.18 | 56.93 | 20.30 | 8.91 | 4.36 |
| Sunflower oil | 0.00 | 6.34 | 0.00 | 4.95 | 22.37 | 62.37 | 0.50 | 1.09 |
| Olive oil | 0.00 | 9.90 | 0.20 | 3.47 | 77.22 | 6.44 | 0.30 | 0.00 |

Source: FEDNA (2010)

### 1.4.1.2. Crude palm oil

**Palm oil** is derived from the fleshy mesocarp of the fruit of the oil palm (*Elaeis guineensis*; Standard for Named Vegetable Oils, Codex Stan 210-1999). According to the USDA (2017) the two main producers of palm oil are Indonesia and Malaysia, and the main importers are India, EU-28, and China.

Palm oil is a vegetable oil rich in palmitic acid (C16:0), being the content of this FA about 45% of the TFA, and it is the only vegetable oil with almost 50%-50% composition of SFA and UFA. The high content of SFA makes palm oil semi-solid at room temperature (melting point: 30-35°C). Crude palm oil is a source of valuable minor components, particularly carotenes, especially β- (56%) and α –carotene (35%), which are responsible of the orange color of the oil (Nagendran et al., 2000), as well as tocols (150-1500 mg/kg), especially tocotrienols (Standard for Named Vegetable Oils, Codex Stan 210-1999). The main characteristics of crude palm oil are presented in Figure 1.3.

According to the Standard for Named Vegetable Oils (Codex Stan 210-1999), insoluble impurities (0.05% m/m), acid value (10.0 mg KOH/g oil), peroxide value (up to 15 milliequivalents of active oxygen/kg oil), and unsaponifiable matter (≤ 12 g/kg) are some values to ensure of the quality of palm oil for human consumption. It is important to consider the possible presence of pollutants and other non-desirable substances in crude palm oil.

The use of crude palm oil in broiler diets is interesting because it is a saturated fat source that can be associated with a positive influence on meat firmness. However, it is well known that saturated fats are less digestible than those fats high in medium-chain FA or UFA (Renner and Hill, 1961; Young, 1961; Garrett and Young, 1975; Vila and Esteve-Garcia, 1996a; Villaverde et al., 2005).

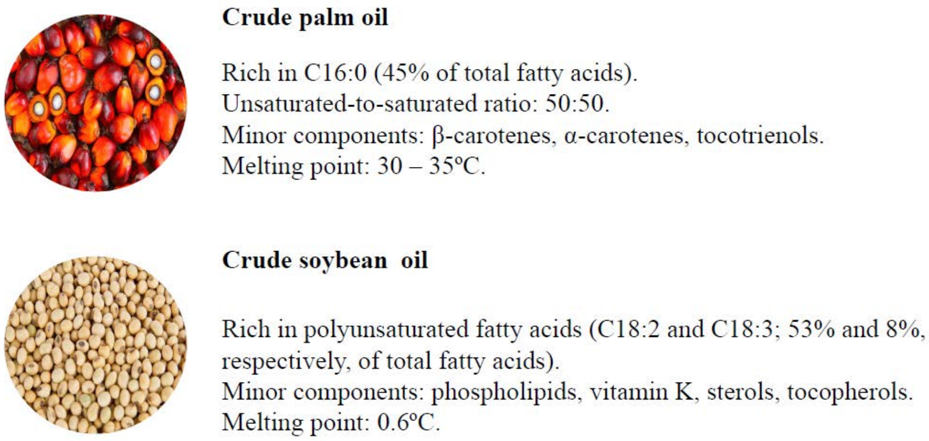
One important aspect that needs to be considered is the negative public perception of palm oil, being its use not always well accepted. This negative perception is related with the detrimental consequences for biodiversity, endangered species, and greenhouse gas emissions that have been associated with palm plantations especially as a result of the tremendous increase of palm oil production in the lasts decades. On the other hand, the health impact of palm oil (as SFA have been associated with cardiovascular disease) is also another sensitive issue (Disdier et al., 2013).

### 1.4.1.3. Crude soybean oil

**Soybean oil** is derived from soya beans (seeds of *Glycine max* (L.) Merr.; Standard for Named Vegetable Oils, Codex Stan 210-1999). The main producers are United States, Argentina and Brazil, and the main importers are China and EU-28 (USDA, 2017).

Soybean oil is an unsaturated vegetable oil rich in linoleic acid (C18:2) and linolenic acid (C18:3), being the content of these FA about 53% and 8% of TFA, respectively. Both, linoleic and linolenic acids have valuable nutritional properties. However, they also contribute to oxidative instability. The high energetic value of soybean oil is due to the high proportion of PUFA, which makes this oil liquid at room temperature (melting point: 0.6°C). According to the Standard for Named Vegetable Oils (Codex Stan 210-1999), crude soybean oil has some minor valuable components like phospholipids (3.7% approximately), vitamin K (about 1.9 ppm), sterols and tocopherols (600-3370 mg/kg), and the most important quality parameters are: insoluble impurities (0.05% m/m), acid value (4.0 mg KOH/g oil), peroxide value (up to 15 milliequivalents of active oxygen/kg oil), and unsaponifiable matter (≤ 15 g/kg). It is important to consider the possible presence of pollutants and other non-desirable substances in crude soybean oil. The main characteristics of crude soybean oil are presented in Figure 1.3.

Crude soybean oil is popularly used in poultry diets because of its high digestibility and ME content in comparison to other vegetable oils.



**Crude palm oil**

Rich in C16:0 (45% of total fatty acids).
Unsaturated-to-saturated ratio: 50:50.
Minor components: β-carotenes, α-carotenes, tocotrienols.
Melting point: 30 – 35ºC.

**Crude soybean oil**

Rich in polyunsaturated fatty acids (C18:2 and C18:3; 53% and 8%, respectively, of total fatty acids).
Minor components: phospholipids, vitamin K, sterols, tocopherols.
Melting point: 0.6ºC.

**Figure 1.3.** Main characteristics of crude palm oil and crude soybean oil

As mentioned before, the production of palm oil, and specially the production of soybean oil are expected to grow modestly during the period 2017/18. However, considering that the use of these fat sources is not exclusively for animals, the interest for finding new alternative and affordable fat sources for animal feeds has become more relevant during the last decades. In this context, fat by-products appear to try to cover this increasing necessity. The use of fat by-products would also be a way of re-valorizing them.

## 1.4.2. Alternative vegetable fat sources: fat by-products from the edible oil refining industry

Fat by-products from the edible oil refining industry are an example of alternative fat sources that can be used in broiler chicken diets. There are three main fat by-products that can be obtained from the oil refining industry and are suitable for animal feeds: acid oils from chemical refining, fatty acid distillates from physical refining, and crude lecithins (Catalogue of Feed Materials; Commission Regulation (EU) No 68/2013). The use of these fat by-products is interesting because they usually are an economic alternative to conventional fats, and they also are a way to revalorize the by-products of the refining process of edible oils, that otherwise would be wasted and could have a negative environmental effect.

Different fat by-products were identified as interesting fat sources for animal feeds In the European project "*Quality and safety of feeding fats obtained from co-products or by-products from the food chain*" (FOOD-CT-2004-007.020) (Nuchi et al., 2009; Tres et al., 2012). Furthermore, in the CICYT project (AGL2010-22008-C02) it was seen that acid oils are an interesting alternative to conventional fats, especially for certain species and growth periods (Tres et al., 2015; Vilarrasa et al., 2014; Trullàs et al., 2016). Indeed, different fat by-products such as acid oils from chemical refining, and fatty acid distillates from physical refining among others, have been assessed (Nuchi et al., 2009; Tres et al., 2012), and as described above, are included in the Catalogue of Feed Materials (Commission Regulation (EU) No 68/2013).

Crude oils might contain variable amounts of non-triglyceride components such as FA, DAG and MAG, phosphatides, sterols, tocopherols, hydrocarbons, pigments (gossypol, chlorophyll), vitamins carotene, sterol glucosides, glycolipids, protein fragments, traces of pesticides, and trace metals, as well as resinous and mucilaginous materials. The quantities of the non-triglycerides vary with the oil source, extraction process, season, and geographical source. Removal of the objectionable non-triglyceride constituents in the fat or oil with the least possible damage to the triglycerides and minimal loss of desirable constituents is the objective of the refining process (O’Brien, 2004), being the end product (refined oil) mainly intended for human consumption. However, in this section special attention will be given to those fat by-products obtained during the process, which are the subject of study of the present thesis.

The two major refining methods are chemical refining and physical refining, being the main difference between them the way by which FFA are removed: neutralization by the addition of an alkali (chemical refining) or by steam distillation (physical refining). The main steps from chemical and physical refining are summarized in Figure 1.4. Currently, the refining method of choice is determined by the characteristics of the individual crude fats and oils. Depending on the crude oil composition, the conditions of each step might be modified by the refining plant, and indeed some steps might not be applied.

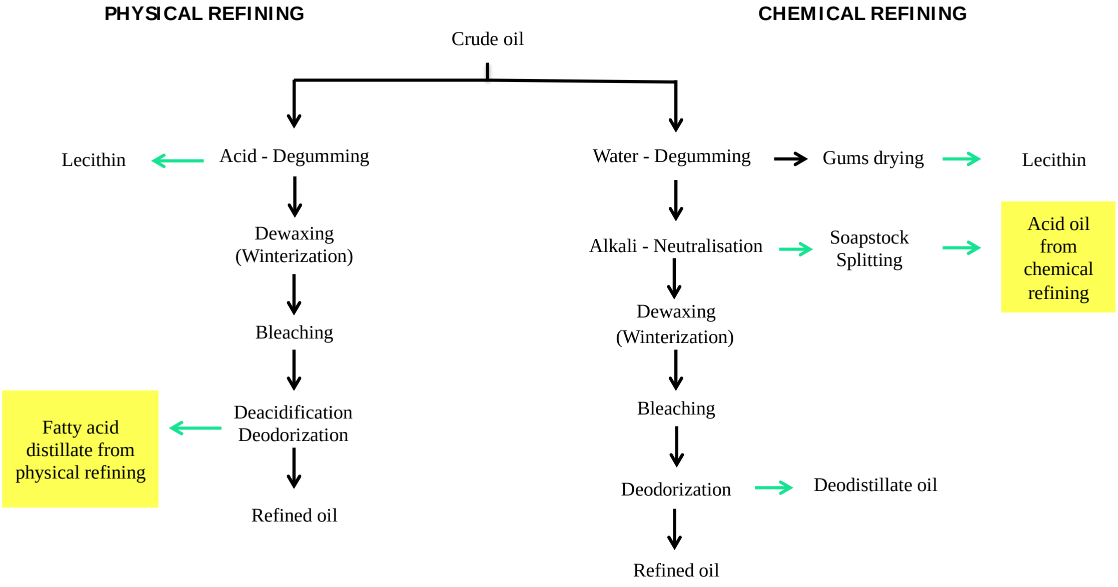


**Figure 1.4.** Physical and chemical refining of edible oils. The green arrows show the different by-products obtained in the different steps of each refining process. The two fat by-products studied in this thesis appear in yellow.
Adapted from FEDIOL (http://www.fediol.eu) and AOCS (http://www.lipidlibrary.aocs.org).

### 1.4.2.1. Chemical refining

The **chemical refining** (also known as alkali refining) is the most used technique for the purification of many seed vegetable oils, like soybean oil, as it successfully decreases the level of FFA, phospholipids (gums), waxes, aldehydes, and ketones among other components. It consists in the following steps: degumming, neutralization, dewaxing, bleaching, and deodorization. The end product is the refined oil, and some by-products are produced through all of these steps.

The main aim of the first step in chemical refining (degumming) is removing phospholipids and other compounds such as carbohydrates, proteins, and seed particles. In this first step oils are usually treated with water and/or acid, reaching temperatures of 100°C. In some cases, when the level of phospholipids is low, this step is not conducted. Lecithin is a fat by-product obtained from this step, and consists in a mixture of phospholipids (60-65%) and residual oil (35-40%; Mateos et al., 2012).

The second step of chemical refining is neutralization, which consists on the addition of an alkali solution, usually NaOH. Its main aim is to reduce the content of FFA, although other compounds, pigments and insoluble impurities can also be removed. The process leads to the formation of soap, and the resultant soapstock is a solid material mixed with some water. It may be sold to soap manufacturers or it may be treated with an acid treatment (sulfuric acid) to set FFA acids contained in it providing what is known as "**acid oil from chemical refining**" (Catalogue of Feed Materials; Commission Regulation (EU) No 68/2013). Thus, acid oils from chemical refining are rich in FFA (> 40 %), have lower proportions of DAG (0 - 13.1%), and MAG (0.4 - 4.8%), and moisture content of 0.34 - 4.53%, according to Nuchi et al. (2009). Their nutritional value depends on the conditions of the refining process and the cleaning of the final product in order to limit the water and FFA content. It is important to mention that this fat by-product can be named differently according to the country; thus, acid oils from chemical refining are commonly known in the feed sector as *acidulated soapstocks* in the USA, and as *oleínas* in Spain (FEDNA, 2010).

In the dewaxing step (which sometimes is an optional step) waxes are crystallized and removed in a filtering process to avoid clouding of the liquid fraction at cooler temperatures. In the bleaching step there is a reduction of the level of pigments (e.g., carotenoids and chlorophyll), and also residues of phosphatides, soaps, traces of metals, oxidation products and proteins by means of the addition of bleaching earths, with or without activated carbon to remove possible contaminants. Deodorization is the final step and is a distillation process that removes aldehydes, ketones and other volatile components that could give undesirable flavor, color and odor to the final product, refined oil, mainly intended for human consumption.

### 1.4.2.1. Physical refining

Regarding the **physical refining** (also known as dry or steam refining) is based on the higher volatility of FFA in comparison to TAG. It is also widely used and sometimes is preferred to the chemical refining as it reduces the loss of TAG, and also decreases the usage of water consumption and enables the recovery of high quality FFA, having a lower impact on the environment (Dumont and Narine, 2008; Piloto-Rodríguez et al., 2014). In consequence is usually less expensive than chemical refining, and the generation of by-products is lower. It requires high temperature and vacuum and can form side reaction products such as polymers and trans isomers among others, (Sengupta and Bhattacharyya, 1992; Dumont and Narine, 2008), and therefore it is not the first choice for refining some oils: while palm oil is usually refined by this method, physical refining it is not advisable to be applied to unsaturated oils, such as soybean oil, which is usually refined by chemical refining.

Physical refining consists in the following steps: degumming, dewaxing, bleaching, and deodorization. The steps are similar to the ones explained above for chemical refining. The biggest difference between both refining processes is the way by which FFA are removed. In chemical refining FFA are usually removed during the neutralization process while in the physical refining there is no neutralization process, and FFA are removed during the deodorization process. Thus, in the physical refining the aim of the deodorization is to reduce the levels of FFA and to remove odors, off-flavors and other volatile components such as pesticides and light polycyclic aromatic hydrocarbons (Čmolík and Pokorny, 2000). This process is carried out under vacuum and high temperatures (180-270°C) and generates a by-product called "**fatty acid distillates from physical refining**" (Catalogue of Feed Materials; Commission Regulation (EU) No 68/2013). It is important to mention that in Spain, fatty acid distillates from physical refining are commonly known as *oleínas* or *destilados* in the feed sector (FEDNA, 2010). Fatty acid distillates from physical refining are characterized for having high levels of FFA (> 90%), and 0.50 - 17.2% of DAG, 0 - 9.0% of MAG, and 0.17 - 6.10% of moisture (Nuchi et al., 2009).

Here, from now on, both acid oils from chemical refining and fatty acid distillates from physical refining will be generically named as acid oils unless otherwise stated.

Acid oils usually represent a challenge for nutritionists as their composition, and by extension their quality, is variable. Their variability, will determine the utilization by the animal. For instance, it is important to determine the content of various non-energetic substances such as moisture, impurities and pollutants, which can be very variable in acid oils (Doppenberg and van der Aar, 2010; Codony et al., 2017). The presence of these substances could in turn have an impact on the apparent metabolizable energy (**AME**) values of fats (Renner and Hill, 1961; Artman, 1964; Vilà and Esteve-Garcia, 1996). For the assessment of this non-energetic fraction, MIU (moisture, insoluble, unsaponifiable) has been described as an accurate determination (Codony et al., 2017). Other non-energetic substances are products of degradation processes, especially oxidative rancidity (Leeson, 1993), like peroxides, oxidized FA, secondary oxidation products, and polymers (Codony et al., 2017). These substances can affect the organoleptic characteristics of the product (flavor, aroma, color, texture). Moreover and more importantly, during the refining process the oxidative stability and nutritive value of fat by-products can be reduced due to the conditions of the process, which can destroy some fat-soluble vitamins, like vitamins A and E (Baião and Lara, 2005).

Regarding the energetic fraction of acid oils, FA are the main source of energy, providing 9.3 kcal/g. The FA composition and molecular structure of the lipids present in the diet will determine the utilization by the animal. The FA composition has been reported as a good estimation of the AME and digestibility of fats (Ketels and de Groote, 1989), and even though the FFA content is usually related negatively to AME, it is not always considered as the best AME predictor (Vilà and Esteve-Garcia, 1996b).

Thus, for all of these reasons it is important to have a good characterization of the most important quality parameters in order to know both the energetic and non-energetic content of acid oils.

## 1.4.3. Possible strategies to improve the final value of acid oils in poultry diets

Different processes have been described in order to improve the final value of acid oils. The **re-esterification** is an example, which involves the use of acid oils (rich in FFA) and glycerol (a by-product from biodiesel industries) that are esterified to form new acylglycerols by means of a chemical or enzymatic process. Depending on the process conditions, the position of the FA in the glycerol backbone might be at random or specific, thus differing from the position that they used to have in the acylglycerols in the crude oil. It is well known that long chain saturated FFA are less digestible than those SFA attached to a glycerol backbone, especially than those bound on the sn-2 position.

Some authors have studied the use of randomized or re-esterified fats in poultry diets as an economically interesting alternative to crude or acid oils (Smink et al., 2008; Vilarrasa et al., 2014; Roll et al., 2018). Smink et al. (2008) associated the chemical randomized palm oil with an increase on the fraction of C16:0 at sn-2 position of the glycerol molecule from 14% to 32%; however, no statistically significant differences were observed on total fat digestibility and the digestibility of individual FA. Vilarrasa et al. (2015a) reported that the re-esterification of palm fatty acid distillates from physical refining with glycerol had a positive effect on the SFA apparent absorption. In young broiler chickens this was explained by the increased sn-2 SFA content, and in adult broiler chickens, by the increased MAG and DAG content of re-esterified products. Thus, it was concluded that re-esterified oils, especially from palm sources, could be used in broiler chicken diets as alternative to crude and acid oils, as they showed similar FA digestibility than their corresponding crude and acid oils, and had a little repercussion on the FA composition of the abdominal adipose tissue. Roll et al. (2018) reported that increasing the proportion of re-esterified palm oil (rich in DAG and MAG) in a blend with acid palm oil (rich in FFA), improved the TFA apparent absorption. However, the price of the randomization or re-esterification cannot always compensate their use.

Mixing acid oils with **free glycerol** has also been described; however, increasing amounts of free glycerol to acid oils did not enhanced the TFA apparent absorption, and impaired the absorption of MAG and PUFA (Roll et al., 2018). This can be explained by the high absorption rate of glycerol because its small molecular weight can be absorbed passively without forming micelles (Min et al., 2010).

# 1.5. Digestion of fat in poultry

Several authors have studied the digestion and absorption of fat in poultry (Krogdahl, 1985; Doppenberg and van der Aar, 2010; Ravindran et al., 2016) However, due to the complexity of the whole process, there are some features that still need to be studied in depth. The digestion of fat consists in two different and consecutive steps: the hydrolysis and the absorption.

## 1.5.1. Hydrolysis

The emulsification of fat is essential prior to the hydrolysis, and starts in the gizzard, where there is also a reduction of the particle size of fat droplets due to the mechanic action that takes place in this gastrointestinal (**GIT**) segment. The reduction of the particle size of fat droplets is really important, as it increases the oil-water interface surface. Bile acids have a key role in the emulsification process (Figure 1.5) facilitating not only the hydrolysis, but also the absorption (Wang et al., 2013), as it will be discussed below.

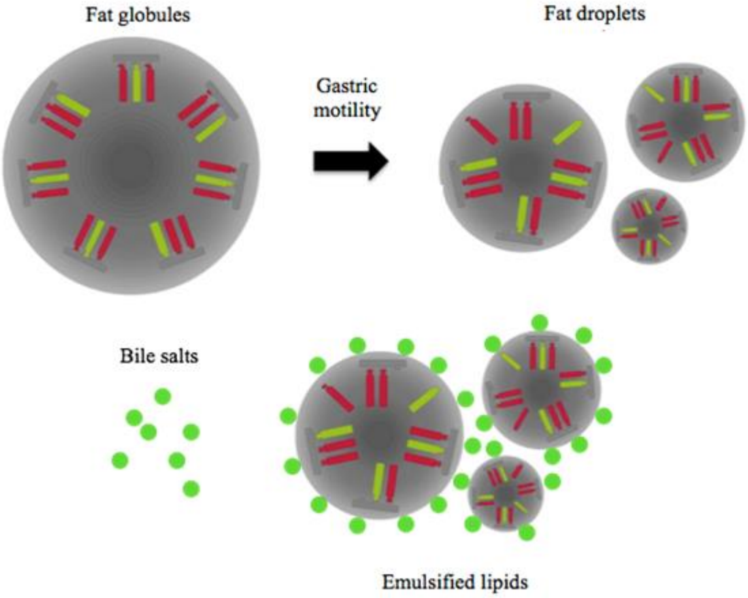


**Figure 1.5.** Schematic view of the first step of fat digestion, prior the hydrolysis: reduction of particle size and emulsification of lipids by the action of bile salts. Source: modified from Vilarrasa, 2014.

The hydrolysis implies the break of TAG molecules, the main lipids in dietary fats. No lipolytic action has been described in the proventriculus or gizzard in poultry. However, intact TAG cannot be absorbed and the action of pancreatic lipase is crucial. Pancreatic lipase is secreted into the duodenum and needs colipase and bile salts to act. Krogdahl et al. (1985) reported that the presence of fat in the duodenum stimulates the secretion of cholecystokinin, and in consequence regulates the secretion of pancreatic juice and bile. Pancreatic lipase has specificity for the FA in sn1- and sn3-positions of the glycerol backbone; which means that the enzyme has no preference to hydrolyze FA in one of these two positions, giving 1,2- or 2,3-DAG (intermediate lipolysis product) and then one 2-MAG and two FFA as end lipolysis products (Figure 1.6). Thus, for each TAG hydrolyzed, one 2-MAG and two FFA can be released into the intestinal lumen.

It has been reported that the activity of pancreatic lipase is influenced by the saturation degree of FA (Ravindran et al., 2016). Van Kuiken et al. (1994) reported that FA must have an angle of approximately 141º or less in order to maximally increase lipase activity. For example, oleic (C18:1) and linoleic (C18:2) acids have carbon backbones that form angles of 141º or less, and can stimulate the activity of the enzyme, while others like stearic acid (C18:0), with an angle of 180º, inhibit the activity of the enzyme. In rats has been described that the level and type of fat may regulate the pancreatic lipase gene expression (Ricketts and Brannon, 1994).

Thus, the digestion process of fat starts in the gizzard with the emulsification, but the hydrolysis of TAG starts in the duodenum (Ravindran et al., 2016).

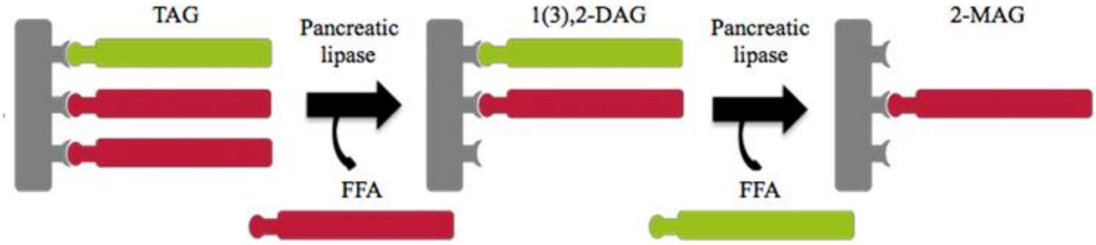


**Figure 1.6.** Schematic view of the hydrolysis of triacylglycerols by the action of pancreatic lipase. TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids. Source: modified from Vilarrasa, 2014

## 1.5.2. Absorption

On the other hand, the absorption of fat is the process that allows the resulting products of the hydrolysis (DAG, MAG and FFA) to pass the intestinal epithelial surface and reach different body tissues. However, the absorption of FA is not as simple, being a multistep process regulated by multiple genes at the enterocyte level (Wang et al., 2013).

The formation of mixed lipid-bile salt micelles is an essential process prior the absorption (Figure 1.7).

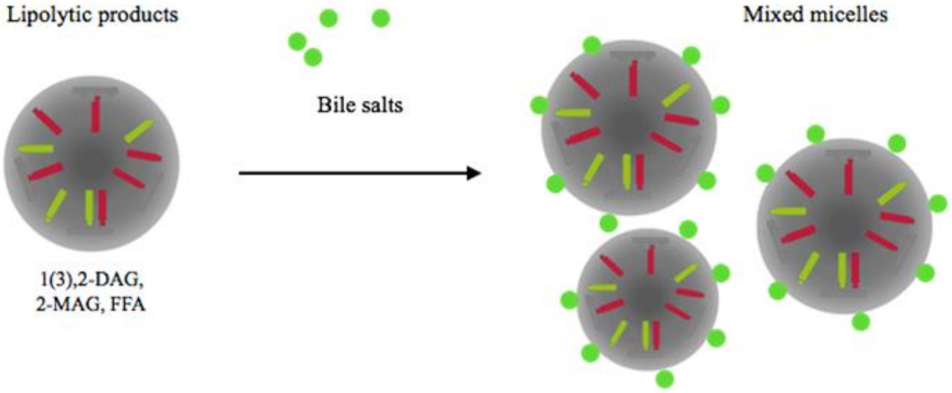


**Figure 1.7.** Schematic view of the solubilization of the lipolysis products through the formation of mixed micelles with bile salts. Source: modified from Vilarrasa, 2014.

Both FA and MAG are spontaneously incorporated into mixed micelles. However, not all the FA have the same ability to form mixed micelles (Freeman, 1969). Thus, while UFA form mixed micelles spontaneously as they are natural emulsifiers, SFA are non-polar and require the presence of the right amount of bile salts and UFA for their emulsification and incorporation into the mixed micelles (Krogdahl, 1985; Smulikowska and Mieczkowaka, 1996). Bile acids can form mixed micelles together predominantly with FFA, and to a lesser extent, with MAG, DAG and TAG, and are essential for this process due to their detergent properties (Wang et al., 2013). Short chain FA and MAG are highly soluble in simple bile acid micelles. However, medium- and long-chain SFA, DAG, fat-soluble vitamins and cholesterol esters need the prior solubilization in the hydrophobic cores of mixed micelles to be absorbed. Once long-chain SFA, DAG, fat-soluble vitamins and cholesterol esters are incorporated into mixed micelles they can reach the intestinal surface and be absorbed actively.

Mixed micelles promote the absorption of FA by facilitating their transport across the unstirred water layer adjacent to the surface of the apical membrane of enterocytes (Wang et al., 2013). According to Wang et al. (2013), the micelle particle itself does not penetrate the cell membrane, but facilitates the passage across a diffusion barrier that is located at the intestinal lumen-membrane interface for the uptake by the enterocytes. When micelles come into contact with microvilli, they are disrupted and lipid products can be passively absorbed into the cells (Ravindran et al., 2016).

The solubilization requires the right amount of bile salts, which are the main component of bile, and as explained before, play an important role both in the digestion and absorption of fat (Russell and Setchell, 1992). Apart from bile acids, MAG also have an important role on FA absorption; it has been described that non-ruminants are not able to absorb many FA without the presence of MAG (Ravindran et al., 2016). Despite all of this information there are still several things related to micelle formation that are not completely understood.

Regarding the absorption process of the lipolysis products at enterocyte level, Katongole and March (1979), demonstrated the presence of FA binding protein (FABP) in the mucosa of the intestine of the chicken. This intracellular protein participates in the translocation of FA from the microvilli to the endoplasmic reticulum through the cytosol (Ockner and Manning, 1974; Wang et al., 2013). According to Wang et al. (2013) this is based on the higher concentration of FABP in villi in comparison with crypts, in the jejunum in comparison with ileum, and in intestinal mucosa of animals fed high-fat diet compared with those fed a low-fat diet. Katongole and March (1980), also reported a higher concentration of this protein in the small intestine, as well as a relation between high-fat diets and higher concentrations of FABP in the intestine. According to Wang et al. (2013), the prevalent view is that FABP could act as acceptor for FA, and subsequently, FA make their way through the cell membrane possible by simple diffusion. Ockner and Manning (1974) reported that FABP has especially affinity for unsaturated long-chain FA, and to a lesser extent for SFA. They also reported a lower affinity for medium and short chain FA.

Once MAG and FFA are absorbed throughout the intestinal epithelial surface, they are re-esterified, combined with free and esterified cholesterol, lipoprotein and phospholipids (mainly via the consecutive actions of monoacylglycerol and diacylglycerol acyltransferases) to form triacylglycerols, which are then incorporated into portomicrons and secreted into the portal circulation. Portomicrons are transported to different tissues, where they can be used for the synthesis of different compounds (Scott et al., 1982). During the absorption and transport there are no changes in the composition of FA, it is for that reason that there is a big similarity between the ingested dietary fat and the fat deposited in the body (Baião and Lara, 2005).

Regarding the site where the absorption of fat takes place, it is accepted that the lipolysis products are absorbed in the small intestine. However, the results observed by different authors are contradictory. Hurwitz et al. (1973) described that both the jejunum and ileum were involved in the absorption of FA in laying hens. Contrary, Sklan et al. (1975) described that the absorption of FA mainly takes place in the duodenum and upper jejunum. Clément (1980) also described that FA were quickly absorbed in the proximal small intestine (duodenum). More recent studies have shown that almost 75% of the fat is absorbed in the jejunum and from 15% to 25% in the ileum, independently of the dietary saturation degree (Tancharoenrat et al., 2014). Tancharoenrat et al. (2014), used two corn-soybean meal diets with 5% of soybean oil or tallow, and reported some differences among FA regarding the site of fat digestion; thus, the digestion started in the duodenum for linoleic acid, in the upper jejunum for palmitic and oleic acids, and in the lower jejunum for stearic acid. According to Ravindran et al., (2013) part of this finding could be due to inefficiency in the emulsification of SFA, as in chickens the feed retention time in the duodenum is very short. However, the exact reasons of these differences are not clear and more studies are needed in order to understand why some fats and FA are better or easily digested and absorbed than others. The absorption of fat has been reported to be negligible in the hindgut of poultry (Renner, 1965). Thus, after the ileum no FA absorption is expected.

### 1.5.3. Especial features of fat digestion in birds

There are two different traits described in birds that need to be taken into account to understand better the whole digestion process. One of them is the **reverse peristalsis** or retrograde movement of digesta, which has been described in birds (Sklan et al., 1979; Duke, 1982, 1986). This process gives more time for the digestion and absorption of nutrients as increases the time the feed is exposed to digestive enzymes. Reverse peristalsis from the duodenum to the gizzard (gastro-duodenal reverse peristalsis) has been described as a continuous process and it has been evidenced for the presence of endogenous duodenal secretions in the gizzard (Sklan et al., 1979). Sklan et al. (1979) also described that about 30% of TFA in the gizzard were FFA, and that was due to the reflux of duodenal content, as lipolysis was not found to occur at the pH of the gizzard. Apart from the gastro duodenal peristalsis, which moves the digesta from the duodenum and jejunum back into the gizzard, gastric peristalsis and cloaca-caecal peristalsis have also been described in birds (Duke, 1986; Ravindran, et al., 2016). The first one moves the digesta between the gizzard and proventriculus, and the second one moves the content between the caecum and cloaca (Duke, 1986).

The other important trait that needs to be considered is the **secretion of endogenous fat** into the intestinal lumen. Significant losses have been described, not only lipids but also other nutrients, during digestion process (Sklan, 1979; Tancharoenrat et al., 2014). Endogenous fat losses take place into the lumen of the intestinal tract, especially into the duodenum. The main components are bile and desquamated epithelial cells (Clément, 1980). In order to determine the amount of endogenous fat lost, Tancharoenrat et al., (2014) used a fat-free purified diet to assess the endogenous fat losses in the lower half of the ileum and calculate the true digestibility of FA. The FA profile of ileal endogenous fat was found to be similar to that of the bile; only 48% of the endogenous fat was accounted by FA, the rest came from non-FA sources, being the endogenous FA profile mainly represented by palmitic, stearic, oleic and linoleic acids. The other 52% could be originated from bile acids, cholesterol, bile pigments, lipid-soluble intermediates and end products, and phospholipids in the bile (Tuchweber et al., 1996). Lindsay et al. (1969) and Tuchweber et al. (1996) reported that the type and level of dietary fat can modulate bile secretion; it has been observed that animals fed a fat-free diet exhibited a marked decrease of bile secretion, and in contrast animals fed a diet high in fat, specially those diets rich in polyunsaturated fat, resulted in a higher rate of bile secretion. According to Tancharoenrat et al. (2014) the measurement of these losses would be necessary for the estimation of the true ileal digestibility of lipids. However, its determination is not simple and in most of the studies is not determined, being the results an approximation of the true fat or FA digestibility.

### 1.5.4. Factors affecting the digestion and absorption process of fat

Lipid digestion is a complex process that not only depends on lipase, colipase and bile salts; there are different factors that can have an effect on it: on the one hand, those factors related to the animal, such as the age, the gender and the strain; on the other hand, those factors related to the diet, such as the degree of the saturation of dietary fat, the position of the FA in the glycerol backbone, the quality of the fat, the level of calcium in the diet, and some interactions between the basal diet and the dietary fat source. Briefly some of the most important factors will be explained.

Regarding the **age** of the birds, it is accepted that young chickens have some limitations in the digestion and absorption of lipids. The secretion of bile has been reported as limited in young birds, especially during the first week after hatching (Noy and Sklan, 1995). Noy and Sklan (1995) also reported that the secretion of pancreatic lipase into the duodenum increased 20 – 100 fold between days 4 and 21 post hatch. However, not all the studies agree with these findings. For instance, some authors have described a lack of response on fat digestibility after the dietary supplementation with lipase (Meng et al., 2004), and Sklan (2001) reported that the secretion of lipase in young chickens may not be as inadequate as expected when their feed intake is considered. Considering the intestinal FABP, its synthesis has been described as insufficient in young birds, but it has been seen than the concentration increases after the 4-week post hatch (Katongole and March, 1980). Wiseman and Salvador (1989) evidenced that the age of birds also affected the AME, being this feature related to a poor emulsification. Despite it is accepted adult chickens can better utilize dietary fat, there are still some things that need to be studied in depth in order to comprehend the main reason why that happens and thus, be able to optimize the utilization of fats according to the feeding period of the chickens.

Regarding the **gender**, it has been reported that females are related to higher AME values; however, no mechanisms have been identified in female broiler chickens for the better digestion of dietary fat (Guirguis, 1976). Katongole and March, (1980) also reported differences on fat utilization depending on the **breed** or **strain**. However, it was reported that these differences might not persist beyond the few first weeks of age.

Concerning the factors related to the **dietary fat**, it is well known and documented that the **saturation degree** and the **length of carbon chain** have an important effect on the utilization of fat by the animal. Thus, UFA are easier to digest and absorb than SFA (Renner and Hill, 1961; Sklan, 1979; Tancharoenrat et al., 2014; Vilarrasa et al., 2015b). Wiseman and Salvador (1991) reported the influence of the degree of saturation of FA on the AME of fats. The synergic effect between UFA and SFA is also well known; when the proportion of unsaturated to saturated FA increases, broilers can better use the saturated fat (Renner and Hill, 1960; Dvorin et al., 1998; Wiseman et al., 1998), resulting in an improvement of the nutritional value of the fat (Codony et al., 2010). This is because the presence of UFA assists in the absorption of SFA (Young, 1961; Young and Garret, 1963). For this reason, the formulation of fat or oil mixtures is a common practice as it allows mixing FA with different degree of saturation. However, the inclusion level of dietary fat has also an effect on the synergism and needs to be considered (Wiseman and Lessire, 1987). It has also been reported a synergic effect between different lipid molecular structures. For example, MAG have an important role in fat absorption; MAG have portions of their molecular structure that can interact both with aqueous systems and lipids, forming an interface between lipids and water (Ravindran et al., 2016).

The **position of the FA in the glycerol backbone** has also an important effect on fat digestion and absorption as pancreatic lipase has preference to hydrolyze those FA in the sn-1 and sn-3 positions of the glycerol backbone. This is important because long chain saturated FFA are less digested than those SFA attached to a glycerol backbone, and consequently those SFA bound at the sn-1 and sn-3 positions of the glycerol molecule are absorbed less efficiently than those bound on the sn-2 position (Smink et al., 2008; Ravindran et al., 2016).

The **level of dietary FFA** is also very important. As FFA are an end-product of the hydrolysis process, it would be possible to think that supplying them is beneficial in terms of fat utilization as they are ready to be absorbed. However, high levels of FFA have been related to low fat digestibility values. There are different hypotheses, which try to explain this feature. On the one hand, this can be due to the secretion of less bile salts; it has been suggested that while TAG and 2-MAG stimulate the secretion of bile salts, FFA do not have this effect (Sklan, 1979). Sklan (1979) reported that biliary secretion was negatively affected by the presence of FFA, and in addition the lack of MAG in the duodenum of FFA-fed chicks contributed to a depression in the amount of FA entering micellar solution and hence absorbed at this site. The negative effect of FFA is also related to the degree of saturation of FA (Wiseman and Salvador, 1991), and can be related to the capacity of FFA to react with ionized minerals (e.g., calcium) forming soaps (Atteh and Leeson, 1985). More studies are needed in order to better understand the mechanisms that affect the utilization of dietary FFA in poultry.

## 1.6. Use of acid oils in poultry diets

In this section a review of those studies that have assessed the use of fat by-products from the refining oil industry in broiler chicken diets will be presented, giving special importance to acid oils. As it has been previously stated, the term **acid oil** will be used without differentiating between acid oils from chemical refining and fatty acid distillates from physical refining, which is the nomenclature provided by the Catalogue of Feed Materials (Commission Regulation (EU) No 68/2013). Furthermore, given the complexity of the nomenclature of these fat by-products, as they can be named differently according to the country, the names given in the articles have been homogenized in order to facilitate the comprehension. Thus, instead of the term *acidulated soapstock*, the term acid oil will be used.

The available studies regarding the use of acid oils in broiler chicken diets will be presented in different subsections according to the assessed parameters: in the first subsection those studies that have assessed the **AME and/or digestibility of the added fat**, in the second subsection those studies that have studied the **feed AME and/or digestibility of dietary fat**, and in the third and fourth subsections the available results regarding the **performance parameters** and **meat quality**, respectively.

The main characteristic of acid oils is their high proportion of FFA, for this reason some studies which did not specifically study the use of acid oils but the effect of added fat FFA level on fat utilization will be also considered in this section.

### 1.6.1. Studies that assess the effect of the added fat FFA level on the AME value and digestibility of added fat

The available studies that have determined the AME value and/or digestibility of added fats (rich in FFA) are summarized in Table 1.3. It is important to mention that these studies evaluate the nutritional value exclusively attributable to the **added fat**. In other words, they evaluate the nutritional value of fat as an ingredient, without considering the fat provided by the basal diet.

On the one hand, some studies estimated the nutritive value of added fats using a basal diet and increasing the supplementation levels of the added fat. **Shannon (1971)** was one of the first authors who studied the effect of adding increasing tallow amounts with various FFA levels (20-97%) on the ME value and net absorption of added fat. The supplemental fats were prepared by hydrolyzing a portion of the tallow, removing the glycerol and mixing the FFA with the appropriate amounts of tallow TAG. This resulted in different tallows containing FFA levels of 20, 40, 60, 80, and 97% by weight. The net absorption of tallow decreased from 90 to 84% and the ME value decreased 12%, when the FFA level increased from 20 to 97%, being the reduction linear in both cases. It was concluded that increasing the level of inclusion of tallows with high FFA content decreased both net absorption and ME values of the added fat.

The research team headed by Wiseman carried out different studies to assess the influence of FFA level and degree of saturation of fat on the AME value and digestibility of different fat sources. Different fats or oils were selected for this purpose. Thus, **Wiseman and Salvador (1991)** selected three fats and their corresponding acid oils: tallow and acid tallow oil (13.8 and 95.2% FFA, respectively), palm oil and acid palm oil (5.8 and 91.7% FFA, respectively), soybean oil and acid soybean oil (1 and 68.3% FFA, respectively); **Wiseman et al. (1992)** chose sunflower oil (< 1% FFA) and acid sunflower oil (38.8% FFA); and **Wiseman and Blanch (1994)** chose a blend of coconut/palm kernel oil (< 1% FFA), and the respective acid oil (83.9%). In these three experiments the crude oil and the corresponding acid oil were blended in different proportions (100:0, 75:25, 50:50, 25:75, and 0:100), and were added into a basal diet at rates of inclusion of 4, 8, and 12%. In these studies, and as described by Wiseman et al.

(1991), the apparent digestibility of fat is obtained by extrapolation of a function obtained from the response of dietary fat digestibility to increasing rates of inclusion of fat. Thus, multiplying the estimated coefficient of apparent digestibility by the gross energy of the studied fat gives the AME value of the added fat, eliminating possible interactions between the added fat and basal diet. All of these studies reported that the AME value of a fat increases exponentially with increasing the U:S ratio, and declines linearly with increasing the FFA level, being the U:S ratio, FFA level and age of chicken the main factors influencing the AME value of fats. In Table 1.4 the AME values reported in the first experiment are summarized. It was concluded that the increase of the FFA level resulted on a progressive reduction in the AME values and apparent digestibility of added fats, being the decrease linear both in young and adult broiler chickens. Furthermore, they observed that this effect was more pronounced for palm than for soybean oil fat sources and for young than for adult broiler chickens. Based on the observations of these experiments a prediction equation was suggested for each one of the studied fats considering both the U:S ratio and FFA level, in order to predict the AME value of fats.

On the other hand, other authors have tried to assess the nutritive value of added fats (rich in FFA) in broiler chicken diets without increasing the levels of inclusion of the studied fat into the basal diet. According to **Wiseman et al. (1991)** this method is not appropriate when the aim is to assess the AME of added fat, as it is possible that some interactions occur between the added fat and the basal AME value. However, these studies will be explained as they also give valuable information about the use of fat by-products rich in FFA in broiler chicken diets.

**Young (1961)** used fat sources and FA mixtures with different level of FFA (from < 1 - 96%) added at 15% into a semi-purified basal diet and reported that the AME values of the studied fats were usually higher for conventional oils in comparison to their respective hydrolyzed products. Furthermore, it was observed that the mixture of unsaturated FFA with saturated FFA improved the utilization of the latter.

**Table 1.3.** Effect of added fat FFA level on AME value and digestibility of added fat. References are in chronological order.

| Reference | Fat sources | Inclusion level (%) | FFA of added fat (%) | Breed - Strain | Age | FFA effect on: | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Added fat AME | Added fat digestibility | Feed AME | Dietary fat digestibility |
| Young (1961) Experiment 1 and 2 | Soybean oil, corn oil, lard, beef tallow, soybean oil FA, corn oil FA, lard FA, beef tallow FA, yellow grease, hydrolyzed animal and vegetable fat | 15 | 0 - 99.3 | Peterson x White Rock cockerels | 3 - 4wk<br>7 - 8wk | Negative<br>* | Negative<br>* | ND | ND |
| Shannon (1971) | Tallows | 3, 5, 10, 20 | 20 - 97 | Laying hens | 1 - 2y | Negative<br>*** | Negative<br>*** | ND | ND |
| Wiseman and Salvador (1991)[a] | Blends of:<br>tallow and acid tallow oil, palm oil and acid palm oil, soybean oil and acid soybean oil | 4, 8, 12 | < 1 – 95 | Broiler chickens | 1.5wk<br>7.5wk | Negative<br>*** | Negative<br>*** | ND | ND |
| Wiseman et al. (1992) | Blends of sunflower oil and acid sunflower oil | 4, 8, 12 | 0 - 38.8 | Broiler chickens | 32d | Negative<br>*** | Negative<br>*** | ND | ND |
| Wiseman and Blanch (1994) | Blend of coconut and palm kernel oil with its hydrolyzed acid oil | 4, 8, 12 | < 1 - 83.9 | Ross I | 12d<br>52d | Negative<br>*** | ND | ND | ND |
| Blanch et al. (1995) | Palm oil, tallow, tallow + soybean oil (TSO), tallow + acid soybean oil (TSA), soybean oil (SO), linseed oil | 4 | < 1 - 34.2 | Broiler chickens | 14d | TSA vs. TSO,<br>TSA vs. SO<br>Negative<br>**<br>TSO = SO | TSA vs. TSO,<br>TSA vs. SO<br>Negative<br>**<br>TSO = SO | No effect<br>TSA = TSO = SO | TSA vs. SO<br>Negative<br>**<br>TSA = TSO<br>TSO = SO |

[a] See Table 1.4. for more details.

ND = not determined.

y = year; wk = week; d = day.

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

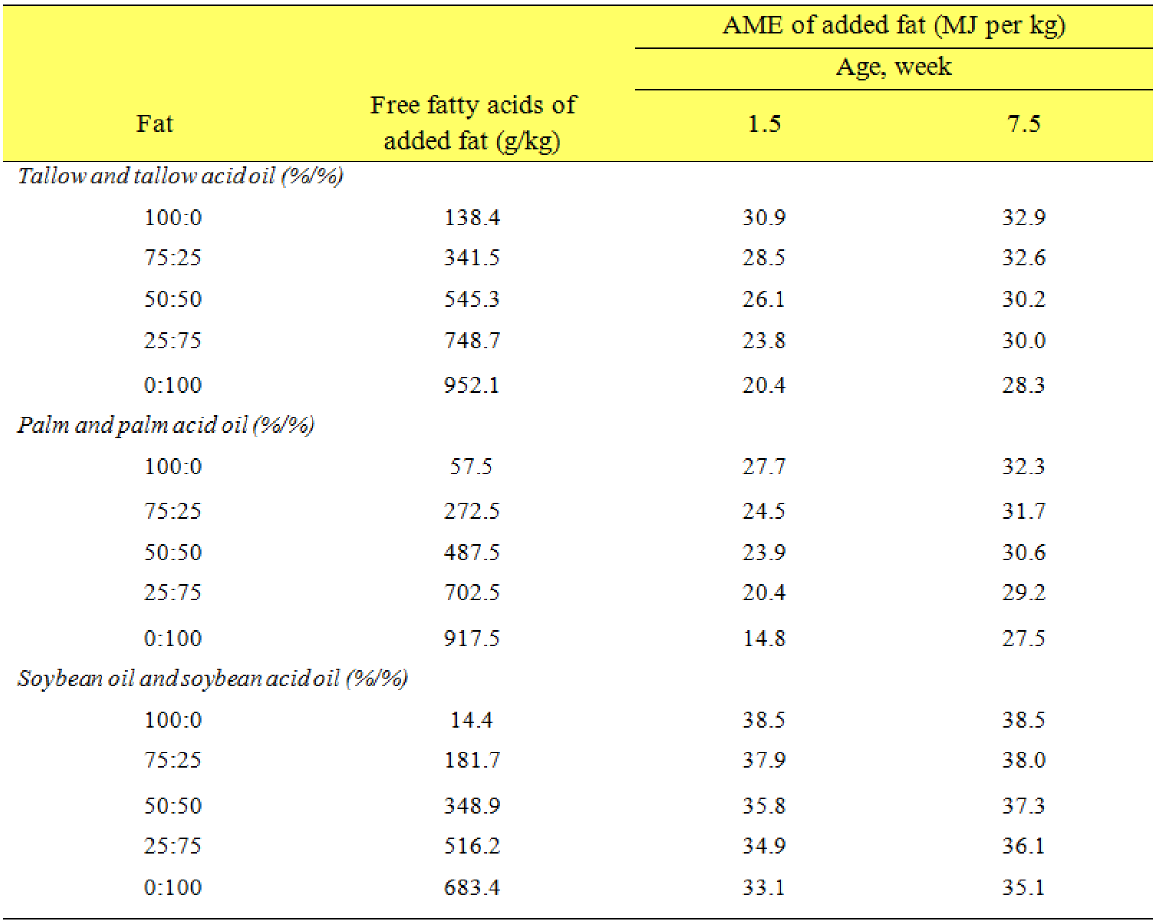
FFA = free fatty acids

**Table 1.3.** Effect of added fat FFA level on AME value and digestibility of added fat. References are in chronological order. Continued.

| Reference | Fat sources | Inclusion level (%) | FFA of added fat (%) | Breed - Strain | Age | FFA effect on: Added fat AME | Added fat digestibility | Feed AME | Dietary fat digestibility |
|---|---|---|---|---|---|---|---|---|---|
| Blanch et al. (1996) | Tallow, tallow + acid soybean oil (TSA), palm oil, palm oil + acid soybean oil (PSA), lard, acid soybean oil (SA), soybean oil (SO), linseed oil | 4 | < 1 - 64.7 | Warren roosters | 1y | SO vs. SA Negative **)<br>SO = PSA and TSA | SO vs. SA Negative (**)<br>SO = PSA and TSA | No effect | SO vs. SA Negative (**)<br>SO = PSA and TSA |
| Vilà and Esteve-Garcia (1996b) | Refined sunflower oil, different by-products of vegetable oil refining | 10 | < 1 - 80.8 | Broiler chickens | 14d | Negative (*) | Negative (*) | ND | ND |

ND = not determined.
y = year; wk = week; d = day.
*P < 0.05; ** P < 0.01; *** P < 0.001
FFA = free fatty acids

**Table 1.4**. Summary of the apparent metabolizable energy values of those fats evaluated by Wiseman and Salvador (1991).



| Fat | Free fatty acids of added fat (g/kg) | AME of added fat (MJ per kg) | |
|---|---|---|---|
| | | Age, week | |
| | | 1.5 | 7.5 |
| *Tallow and tallow acid oil (%/%)* | | | |
| 100:0 | 138.4 | 30.9 | 32.9 |
| 75:25 | 341.5 | 28.5 | 32.6 |
| 50:50 | 545.3 | 26.1 | 30.2 |
| 25:75 | 748.7 | 23.8 | 30.0 |
| 0:100 | 952.1 | 20.4 | 28.3 |
| *Palm and palm acid oil (%/%)* | | | |
| 100:0 | 57.5 | 27.7 | 32.3 |
| 75:25 | 272.5 | 24.5 | 31.7 |
| 50:50 | 487.5 | 23.9 | 30.6 |
| 25:75 | 702.5 | 20.4 | 29.2 |
| 0:100 | 917.5 | 14.8 | 27.5 |
| *Soybean oil and soybean acid oil (%/%)* | | | |
| 100:0 | 14.4 | 38.5 | 38.5 |
| 75:25 | 181.7 | 37.9 | 38.0 |
| 50:50 | 348.9 | 35.8 | 37.3 |
| 25:75 | 516.2 | 34.9 | 36.1 |
| 0:100 | 683.4 | 33.1 | 35.1 |

Source: Wiseman and Salvador, 1991

The studies carried out by Blanch assessed the AME value of the added fat and feed, as well as the digestibility of both the added fat and dietary fat. **Blanch et al. (1995)** used palm oil, tallow, tallow + soybean oil, tallow + acid soybean oil, soybean oil, and linseed oil in two-week-old broiler chickens, and **Blanch et al. (1996)** used tallow, tallow + acid soybean oil, palm oil, palm oil + acid soybean oil, acid soybean oil, lard, soybean oil, and linseed oil in one-year-old roosters. In both experiments fats/oils or mixtures were added at 4% of the basal diet by weight. In the first study (Blanch et al., 1995), when crude soybean oil (< 1% FFA) was compared with a tallow + acid soybean oil blend (34.2% FFA) it was reported that the apparent absorption for the blend tallow + acid soybean oil was lower when both the apparent absorption of dietary fat and apparent absorption of added fat were considered. The same was observed for the AME value of added fat. However, no differences were observed regarding the feed AME values between both diets. Furthermore, it was seen that both the apparent absorption and AME of added fat for the blend tallow + soybean oil (2.6% FFA) was higher than for the tallow + acid soybean oil blend; however, the AME value of the feed and apparent absorption of dietary fat between these two oil blends was similar. Similarly, in the second study (Blanch et al., 1996), the apparent digestibility of both the added fat and dietary fat, as well as the AME of added fat were lower for acid soybean oil (64.7% FFA) than for crude soybean oil (2.5% FFA), but the value observed for crude soybean oil was similar to the ones reported for the blend tallow + acid soybean oil (38.9% FFA), and the blend crude palm oil + acid soybean oil (39.1% FFA). Regarding the feed AME values no differences were reported.

**Vilà and Esteve-García (1996b)** studied different fat by-products from oil refining (a selection of different acid soybean oils, sunflower soapstocks, and deodorization distillates) with different FFA levels (from 23.1% to 80.9%) and compared them with refined sunflower oil as a reference fat (< 1% FFA). It was reported an effect of the added fat FFA level on AME and fat digestibility of added fat; however, those fats with high dietary FFA level had variable AME values, and they suggested that the FFA content was not a good predictor of the AME of fat by-products of oil refining.

## 1.6.2. Studies that assess the effect of the added fat FFA level on the feed AME value and digestibility of dietary fat

Some authors have assessed the feed AME value and fat digestibility of diets supplemented with fat by-products rich in FFA (Table 1.5). In this case, these studies evaluate the nutritional value **of dietary fat (added fat + fat present in the basal diet).** It is important to mention that some interactions between the added fat and the fat present in the basal diet, as well as with other dietary components, could occur and modify the total nutritional value of the diet.

**Artman et al. (1964)** used different fat sources differing in their saturation degree and FFA level (< 1% - 99%) in adult broiler chicken diets. Among the studied fats were refined soybean oil (< 1% FFA), soybean oil FA (98% FFA), prime beef tallow, tallow FA, acid soybean oil (38% FFA), and menhaden fish oil, which were added into a basal diet at 15%, alone or in mixtures. Soybean and tallow FA, were prepared by alkaline hydrolysis of the respective fats, followed by acidulation, water washing and stabilization with butylated hydroxyanisole. The results showed that the diet with refined soybean oil had higher digestibility of dietary fat and feed AME values than the diet with acid soybean oil. However, no differences were observed between the diet with refined soybean oil and soybean oil FA.

**Vilarrasa et al. (2015a)** studied the effect of different oils, added at 6% to a basal diet, on dietary FA apparent absorption and feed AME values. Among the studied fats were crude palm oil (5% FFA), acid palm oil (55.8% FFA), crude soybean oil (< 1% FFA), and acid soybean oil (55% FFA). The AME value and TFA digestibility of the diets supplemented with acid oils was lower than the diets supplemented with the respective crude oils in young broiler chickens. The negative FFA effect on TFA digestibility was also reported in adult broiler chickens. However, no effects were observed regarding the feed AME value in adult broiler chickens. Similarly, **Roll et al. (2018)** reported a negative FFA effect on TFA digestibility in both young and adult broiler chickens when compared a diet supplemented with crude palm oil (7% FFA) with a diet supplemented with acid palm oil (fatty acid distillate from physical refining; 89% FFA). Regarding the feed AME values, a negative FFA effect was reported in young broiler chickens, and no effects were reported in adult broiler chickens.

**Table 1.5.** Effect of added fat FFA level on feed AME value and digestibility of dietary fat. References are in chronologic order.

| Reference | Fat sources | Inclusion level (%) | FFA of added fat (%) | Breed - Strain | Age (days) | Free fatty acid effect on: Dietary AME | Free fatty acid effect on: Dietary fat digestibility |
|---|---|---|---|---|---|---|---|
| Artman et al. (1964) Experiment 4 | Soybean oil (SO), soybean oil FA, prime beef tallow, tallow FA, acid soybean oil (SA), menhaden fish oil and blends | 15 | < 1 - 99 | Arbor Acres White Rock cockerels | 56 | SO vs. SA<br>Negative<br>* | SO vs. SA<br>Negative<br>* |
| Vilarrasa et al. (2015) | Crude palm oil (PO), acid palm oil (PA), re-esterified palm oil low and high in mono- and diacylglicerides, crude soybean oil (SO), acid soybean oil (SA), re-esterified soybean oil low and high in mono- and diacylglicerides | 6 | < 1 - 55.8 | Ross 308 | 12 | PO vs. PA<br>SO vs. SA<br>Negative<br>* | PO vs. PA<br>SO vs. SA<br>Negative<br>*** |
| | | | | | 36 | PO vs. PA<br>SO vs. SA<br>No effect | PO vs. PA<br>SO vs. SA<br>Negative<br>* |
| Roll et al. (2018) | Crude palm oil (PO), acid palm oil (PA) | 6 | < 1 – 88.6 | Ross 308 | 10 | PO vs. PA<br>Negative<br>*** | PO vs. PA<br>Negative<br>*** |
| | | | | | 39 | PO vs. PA<br>No effect | PO vs. PA<br>Negative<br>*** |

ND = not determined.

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

FFA = free fatty acids

### 1.6.3. Studies that assess the effect of the added fat FFA level on growth performance parameters

The available studies regarding the effect of added fat FFA level on growth performance parameters in broiler chickens are presented in Table 1.6.

**Siedler et al. (1955)** studied the effect of the level of FFA (different grades of animal fats and tallows with 3 to 99% of FFA) added at levels of 0, 3 or 6% on the growth performance of broiler chickens. It was reported that when fats were properly stabilized with an antioxidant mixture they were utilized equally well regardless of their FFA level, which did not influence the productive parameters.

Similarly, **Young (1961)** did not report differences in growth performance when diets with fat sources and FA mixtures with levels of FFA from < 1% to 96% were compared. However, a numerically higher FCR was observed for those diets supplemented with the most saturated fats (beef tallow with < 1% FFA, and beef tallow FA with 96.4% FFA) both at 4 and 8 weeks of age.

**Bornstein and Lipstein (1961)** did not observe differences on the performance parameters of broiler chickens when compared prime tallow, methyl esters of vegetable fats, acid soybean oil, soybean lecithin, crude soybean oil, and non-extracted (but toasted) soybeans added at levels of 0, 3, or 6% to a basal diet; however it was seen a tendency for crude soybean oil to be superior. It was also reported that the FCR proportionally improved as the fat content of the diets increased. Soybean soapstock, an intermediate product of the chemical refining process, was also used in this experiment being the only fat source associated with negative results in comparison to the rest of fat sources, including acid soybean oil.

Later, **Bornstein and Lipstein (1963)** studied the use of some waste vegetable oils as fat supplements (acid sunflower oil, acid soybean oil, grape seed oil, and olive pomace oil) in broiler chicken diets, and no differences among the different diets were reported on growth performance and FCR when the studied fats were added at levels of 3 and 10%, both at 35 and 70 d, excluding the diet supplemented with grape seed oil, which was associated to a higher FCR. This exception was explained by the high content of impurities and oxidation products of grape seed oil.

**Artman et al. (1964)** also studied the effect of different diets differing in their saturation degree and FFA level on performance parameters in broiler chickens. The

BW of those chickens fed the diet with refined soybean oil (< 1% FFA) did not differ from the BW of the chickens fed the diet with acid soybean oil (38% FFA). However, a lower FCR was reported for the diet with refined soybean oil in comparison to the diet with acid soybean oil, and no differences were reported between the diet with refined soybean oil and the diet with soybean oil FA (soybean oil FA were prepared by alkaline hydrolysis, followed by acidulation, water washing and stabilization with butylated hydroxyanisole).

**Zumbado et al. (1994)** reported that up to 40% FFA in a crude palm oil – acid palm oil blend, did not have detrimental effects on broiler performance when the blend was included at a 6% rate in starter diets. Different blends of crude palm oil and acid palm oil were made to have diets with increasing levels of FFA, and crude soybean oil was used as reference. No differences were reported among the control diet (with crude soybean oil) and the palm diets with < 5%, 20%, and 40% FFA. However, the diet with 20% FFA had lower FCR in comparison to the diets with 60% and > 85% FFA. Regarding the BW, no significant effects were observed.

**Vilà and Esteve-García (1996b)** studied different fat by-products from oil refining (a selection of different acid soybean oils, sunflower soapstocks, and deodorization distillates) with different FFA levels (from 23.1% to 80.9%), and compared them with refined sunflower oil as a reference fat (< 1% FFA). The effect of the added fat FFA level did not seem to influence FCR, as the result reported for the chickens fed the diet supplemented with the fat with the highest FFA level (81%) was not significantly different than the one reported for those chickens fed the diet with refined sunflower oil.

**Zumbado et al. (1999)** reported a lower BW at 29 d for those chickens fed a diet with acid palm oil in comparison to a diet supplemented with crude palm oil. Furthermore, there were no differences between the diet supplemented with crude palm oil and the diet supplemented with a mixture of soybean soapstock, acid soybean oil and lecithin. A similar pattern was observed for FCR; the result reported for those animals fed the diet with acid palm oil was higher than for those fed the diet with crude palm oil which in turn was higher than that from those fed with the mixture of soybean oil by-products.

**Balevi et al. (2001)** investigated the use of fat by-products derived from the refining process of sunflower oil (sunflower soapstock, acid sunflower oil and deodistillate oil) supplemented at 5% to a basal diet for broiler chickens. No differences on BW at 49 d were observed among those chickens fed the diets with sunflower soapstock, acid sunflower oil and crude sunflower oil. It was concluded that none of the studied fat by-products had any harmful effect on the broiler performance and could be used in broiler chicken diets as an energy source instead of crude sunflower oil.

**Vilarrasa et al. (2015a)** reported no differences in any of the assessed performance parameters between those animals fed diets supplemented with crude oils and those fed diets supplemented with the respective acid oils. Similarly, **Roll et al. (2018)** reported no differences in the performance parameters between those chickens fed a diet with crude palm oil and those fed a diet with acid palm oil.

**Table 1.6.** Effect of added fat FFA level on performance parameters of broiler chickens. References are in chronologic order.

| Reference | Fat sources | Inclusion level (%) | FFA of added fat (%) | Breed - strain | Age (days) | FFA effect on: | |
|---|---|---|---|---|---|---|---|
| | | | | | | BW | FCR |
| Siedler et al. (1955) Experiment 1 and 2 | Several grades of animal greases and tallows stabilized with an antioxidant mixture: FA prepared from choice white grease, choice white grease, brown grease, yellow grease, prime tallow, tallow | 0, 3, 6 | 2.9 - 99.5 | White Rock cockerels | 63<br>70 | No effect | No effect |
| Young (1961) Experiment 1 and 2 | Soybean oil, corn oil, lard, beef tallow, soybean oil FA, corn oil FA, lard FA, beef tallow FA, yellow grease, hydrolyzed animal and vegetable fat | 15 | 0 - 99.3 | Peterson x White Rock cockerels | 28<br>56 | No effect | No effect |
| Bornstein and Lipstein (1961) Experiment 1 | Tallow<br>Feed oil (methyl esters of vegetable fat)<br>Crude soybean oil<br>Crude soybean lecithin Regular, untreated soybean soapstock<br>Acid soybean oil<br>Acid soybean oil / crude lecithin<br>Soybean oil meal / crude soybean oil, and processed unextracted soybeans | 0, 3, 6, 10 | No specified | Broiler chickens | 42<br>80 | No effect | No effect |
| Bornstein and Lipstein (1963) Experiment 3 | Acid soybean oil, tallow, acid sunflower oil, grape seed oil, olive pomace oil | 0, 3, 10 | No specified | Broiler chickens | 35<br>70 | No effect | No effect |
| Artman et al. (1964) Experiment 4 | Soybean oil (SO), soybean oil FA, prime beef tallow, tallow FA, acid soybean oil (SA), menhaden fish oil and blends | 15 | < 1 - 99 | Arbor Acre White Rock cockerels | 56 | SO vs. SA<br>No effect | SO vs.SA<br>Negative<br>* |

ND = not determined. FFA = free fatty acids

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

**Table 1.6.** Effect of added fat FFA level on performance parameters of broiler chickens. References are in chronologic order. Continued

| Reference | Fat sources | Inclusion level (%) | FFA of added fat (%) | Breed - strain | Age (days) | FFA effect on: | |
|---|---|---|---|---|---|---|---|
| | | | | | | BW | FCR |
| Zumbado et al. (1994) | Crude soybean oil, crude palm oil, blends of crude palm oil and acid palm oil | 6 | < 5 - > 85 | Broiler chickens | No specified | No effect | 20% < 60% and 85%<br>20% = 5%, 40%<br>Negative<br>* |
| Vilà and Esteve-Garcia (1996b) | Refined sunflower oil, different by-products of vegetable oil refining (acid soybean oils, sunflower soapstocks, and deodorization distillates) | 10 | < 1 - 80.8 | Broiler chickens | 14 | ND | No effect |
| Zumbado et al. (1999) | Acid palm oil (PA), soybean soapstock + acid soybean oil + lecithin, crude palm oil (PO), tallow, restaurant grease | 10 | 1 - 91.7 | Broiler chickens | 29 | PO vs. PA<br>Negative<br>* | PO vs. PA<br>Negative<br>* |
| Balevi et al. (2001) | Crude sunflower oil, sunflower soapstock, acid sunflower oil, deodistillate oil | 5 | No specified | Peterson x Avian commercial hybrid broilers | 49 | No effect | No effect |
| Vilarrasa et al. (2015a) | Crude palm oil (PO), acid palm oil (PA), re-esterified palm oil low and high in mono- and diacylglicerides, crude soybean oil (SO), acid soybean oil (SA), re-esterified soybean oil low and high in mono- and diacylglicerides | 6 | < 1 - 55.8 | Ross 308 | 40 | PO vs. PA<br>SO vs. SA<br>No effect | PO vs. PA<br>SO vs.SA<br>No effect |
| Roll et al. (2018) | Different palm oils (Crude palm oil (PO), acid palm oil (PA)) | 6 | 0 – 88.6 | Ross 308 | 42 | PO vs. PA<br>No effect | PO vs. PA<br>No effect |

ND = not determined. FFA = free fatty acids

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

### 1.6.4. Studies that assess the effect of the added fat FFA level on meat quality

The available studies regarding the effect of added fat FFA level on meat quality, as well as in some organoleptic characteristics, like meat pigmentation, are presented in Table 1.7.

**Bornstein and Lipstein (1963)** studied the effect of different fat by-products on meat pigmentation by visual inspection. The different treatments were classified in three degrees of pigmentation: strong, slight, and complete lack of pigmentation. The use of acid soybean oil led to strong pigmentation when added both at 3 and 10%. The use of acid sunflower oil was related to slight pigmentation when added at 3% and to a complete lack of pigmentation when added at 10%. In general, the other studied fat by-products were associated to a complete lack of pigmentation.

**Balevi et al. (2001)** did not report differences in carcass and abdominal fat weights when compared diets supplemented with crude sunflower oil, sunflower soapstock and acid sunflower oil. However, the supplementation with sunflower deodistillate oil was in all cases associated to worse results. It was also concluded that all the fat by-products studied (derived from the refining process of sunflower oil: soapstock, acid oil, and deodistillate oil), contained higher amounts of omega-3 FA in comparison to crude sunflower oil (crude sunflower oil: 0.82%; sunflower soapstock: 3.75%; acid sunflower oil: 2.28%; and deodistillate oil: 1.96%), being these FA found in higher proportion in the abdominal fat of the chickens fed the diets supplemented with the mentioned fat by-products.

**Vilarrasa et al. (2015a)** reported no differences in the weight of abdominal fat depot in those chickens fed diets with crude oils (crude palm oil or crude soybean oil) in comparison to those fed diets with acid oils (acid palm oil or acid soybean oil). Regarding the FA composition of abdominal adipose tissue lower MUFA content was observed in those chickens fed the diets with crude oils in comparison to those fed the diets with acid oils.

**Table 1.7.** Effect of added fat FFA level on meat quality. References are in chronologic order.

| Reference | Fat sources | Inclusion level (%) | FFA of added fat (%) | Breed - strain | Age (days) | Findings |
|---|---|---|---|---|---|---|
| Bornstein and Lipstein (1963) Experiment 2 and 3 | Acid soybean oil (SA), tallow, acid sunflower oil, grape seed oil, olive pomace oil | 0, 3, 10 | No specified | Broiler chickens | 70 | Stronger pigmentation in those chickens fed diets supplemented with SA |
| Balevi et al. (2001) | Crude sunflower oil, sunflower soapstock, acid sunflower oil, deodistillate oil | 5 | No specified | Broiler chickens | 49 | Similar carcass and abdominal fat weights for all fat supplements, except for deodistillate oil, which was associated to lower carcass weight and abdominal fat weight |
| Vilarrasa et al. (2015a) | Crude palm oil, acid palm oil, re-esterified palm oil low and high in mono- and diacylglicerides, crude soybean oil, acid soybean oil, re-esterified soybean oil low and high in mono- and diacylglicerides | 6 | < 1 - 55.8 | Ross 308 | 40 | Lower monounsaturated fatty acid content in abdominal adipose tissue of those chickens fed the diets with crude oils in comparison to those fed diets with acid oils ($P < 0.05$) |

FFA = free fatty acids

### 1.6.5. General summary

Most of the available information described in this section suggests that the level of FFA of a fat source has a negative effect on its utilization by the animal. Some reasons, such as the inefficient solubilization of fat in the intestinal lumen or the reaction of FFA with ionized minerals, have been suggested to explain the general low AME values and digestibility coefficients observed for fat by-products rich in FFA. However, there is a lack of information regarding this aspect, and none of the studies have assessed the effect of the level of FFA on both the hydrolysis and absorption processes. Knowing this would be the first step to determine the potential use of acid oils in broiler chickens diets.

Furthermore, none of the studies has taken into account the level of **dietary FFA** (including those ones that assessed the feed AME and dietary fat digestibility); instead they always give information regarding the **added fat FFA level**, and most of the studies just evaluated the use of acid oils as feed ingredients. In order to have more practical data regarding the use of these fat by-products in broiler chicken diets is essential to study their use on the whole diet, as it is likely that there are some synergistic effects with other dietary components.

Apart from the level of FFA, there are other factors that can influence fat utilization, such as the saturation degree of dietary fat. Thus, studying the use of diets supplemented with fats differing in both their FFA level, and saturation degree is needed in order to assess the inclusion of acid oils in diets for broiler chickens. In addition, this needs to be assessed both in young and adult broiler chickens, as it is well known that the age has a big influence on the whole digestion process.

Additionally, most of these studies, which have assessed the effect of the added fat FFA level on different parameters, were performed decades ago, using old broiler chicken strains. It is likely that the genetic improvement of modern broiler chicken strains (mainly Ross 308) has increased the potential of chickens to utilize dietary fat. Thus, it is also necessary to assess the use of acid oils under up-to-date conditions.

Another important factor is the quality of acid oils, as it can determine their energy value. In most of the studies there is no available information about this aspect, and it is likely that the quality control of fat by-products decades ago was not as important as it is nowadays.

All these reasons evince the necessity of studying in depth the use of acid oils in broiler chicken diets. Knowing the critical steps in their utilization by the animal as well as assessing their use in more practical conditions is necessary to study the potential use of acid oils as energy sources for broiler chicken diets. Moreover, this information will allow for an optimization of their use and also raising recommendations for its inclusion in the diets.

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# CHAPTER 2

## Background, hypotheses, objectives, and approach

As seen in the literature review, acid oils are an interesting alternative to conventional fats or oils due to their usually lower price and similar fatty acid (**FA**) composition to the crude fat or oil. However, their use in broiler chicken diets is controversial, mainly due to the lack of recent information regarding its use. Most of the available studies were carried out decades ago, being possible that the conditions tested are different than the ones present nowadays. Thus, it is necessary to study the use of acid oils under up-to-date conditions in broiler chickens, as well as to determine the critical steps on the utilization of these fat by-products to use them appropriately and efficiently in broiler chicken diets.

The present PhD dissertation is a part of a project (ref. AGL2015-64431-C2) conducted to improve the use of acid oils in monogastric animals. This project begun after previous projects (ref. FP6 FOOD-CT-2004-007020, and ref. AGL2010-22008-C02), where it was seen that currently acid oils could be an interesting alternative to conventional fats for monogastric animal diets. However, feed producers and farmers are still encountering variations in animal performance parameters when using acid oils, and this makes them to be reluctant of using them. Consequently, it is necessary to determine the critical points when using acid oils in animal feeding so that proper guidelines of their use can be set.

Similarly to conventional oils and fats, FA are the main constituents in acid oils. However, in the latter, FA are mainly found in free form, and it is for this reason that studying the effect of FFA should be one of the first points to address. It was hypothesized that both the degree of saturation of the dietary fat and age of the chicken can determine the effect of dietary free fatty acid (**FFA**) level on fat utilization.

For all the reasons described above, the general aim of the present thesis is to **investigate the potential use of acid oils in broiler chicken diets** and **to determine where the limitations of the use of acid oils are, both in young and adult broiler chickens**.

The specific objectives are:

- To better understand the fat digestion process through the study of the FA digestibility and lipid class composition along the gastrointestinal tract (**GIT**) in young and adult broiler chickens.
- To assess the effect of the saturation degree of dietary fat on the FA digestibility and lipid class composition along the GIT, and excreta in young and adult broiler chickens.
- To assess the effect of the dietary FFA level on FA digestibility and lipid class composition along the GIT, and excreta in young and adult broiler chickens.

In order to approach the mentioned objectives two *in vivo* experiments were performed in broiler chickens. In both experiments, the FA digestibility and the lipid class composition along the GIT (gizzard, duodenum, jejunum, ileum), and excreta were determined.

In the first experiment, two crude oils differing in their degree of saturation (crude soybean oil and crude palm oil) were used (Chapter 3). This experiment also helped setting the experimental and analytic methodologies for the second experiment.

In the second experiment, two crude oils differing in their degree of saturation (crude soybean oil and crude palm oil), and two fat by-products from the refining oil industry (acid soybean oil from chemical refining and palm fatty acid distillates from physical refining) were used alone or in blends with crude oils to have diets with different saturation degree and dietary FFA level (Chapter 4 and 5).

# CHAPTER 3

## Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages

# Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages

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Poultry Science (Accepted September 6, 2018)
DOI: 10.3382/ps/pey458
(Poultry Science, pey458, https://doi.org/10.3382/ps/pey458)

# 3.1. Abstract

The aim of the present study is to evaluate the effect of the dietary fat saturation degree and age on the lipid class (TAG, DAG, MAG and FFA) composition and fatty acid digestibility along the gastrointestinal tract (**GIT**) and excreta in broiler chickens. A total of 120 one-day-old female broiler chickens were randomly distributed in 2 dietary treatments (6 cages/treatment), which resulted from the supplementation of a basal diet with 6% of soybean oil or palm oil. Two digestibility balances were carried out at 14 and 35 days and fatty acid digestibility and lipid class composition were determined in the gizzard, duodenum, jejunum, ileum and excreta. Along de GIT, both fatty acid digestibility and lipid class composition were influenced by the dietary fat source and the age of the chickens. The absorption of the unsaturated fat was more efficient and faster than it was for the saturated fat. The ability of adult chickens to absorb fat was higher than for young chickens. The results show that the duodenum is the main place of fat digestion (hydrolysis), and the jejunum the main place of fat absorption. The role of the ileum on fat absorption is very important, as it is the last segment of the GIT where the absorption of fatty acids has been described. Thus, it was the contribution of the ileum that was responsible for the higher fat utilization observed for animals fed the unsaturated diet than for those fed the saturated diet at 14 days, and it was also responsible for the improvement on the utilization of the saturated diet between 14 and 35 days. All the results suggest that the absorption of fatty acids is more limiting than is hydrolysis, because the main differences were observed in the jejunum and ileum, where the absorption of fatty acids takes place.

# CHAPTER 4

## Effect of dietary free fatty acid level and saturation degree on lipid class composition and fatty acid digestibility along the gastrointestinal tract in young broiler chickens

# I. Effect of dietary free fatty acid level and saturation degree on lipid class composition and fatty acid digestibility along the gastrointestinal tract in young broiler chickens

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## 4.1. Abstract

The aim of the present study is to assess the effect of dietary free fatty acid (**FFA**) level with different saturation degrees, on the fatty acid (**FA**) digestibility and lipid class content along the gastrointestinal tract, and excreta in young broiler chickens. A wheat-and-soybean-meal-based diet was supplemented at 6% with crude oils, fat by-products from the edible oil refining industry rich in FFA or combinations of both. This resulted in eight dietary treatments in a 2 x 4 factorial arrangement: two different fat sources (soybean oil products as unsaturated fat source, **S**; and palm oil products as saturated fat source, **P**), and four levels of dietary FFA (5%, 15%, 35%, and 50%). Samples of digestive content (gizzard, duodenum, jejunum, and ileum) and excreta were collected at 14 d for the determination of the FA digestibility and lipid class content. The FA digestibility coefficients reported for the chickens fed S diets in the jejunum, ileum and excreta were higher than for those fed P diets. The higher utilization of the unsaturated fat was mainly explained by a higher contribution of the ileum in the absorption of SFA. The dietary FFA level mainly affected the FA absorption process, as the diets with 50% FFA were, in general, related to lower digestibility coefficients in the jejunum, and ileum, and to higher content of the lipolysis products in the ileum and excreta, in comparison to the diets with 5% FFA, and in some cases also with the diets with 15% FFA. It was concluded that unsaturated diets with moderate levels of dietary FFA (up to 15%) could be used in broiler-chicken starter diets, as they led to similar fat utilization and performance results than the diets with the lowest dietary FFA level. From the present study, it has also been concluded that the saturation degree has a greater impact on fat utilization than the dietary FFA level has.

## 4.2. Introduction

The cost of supplying energy in poultry diets is high, and supplemental fats are an interesting source to meet the energy requirements, due to their high-energy value. There are different available fat sources that can be used in poultry diets. Food fat by-products, like those from the edible oil refining industry, are an example of an economic alternative in comparison to conventional fats that can be revalorized as a feed fat ingredient. Fat by-products from the edible oil refining industry come from the chemical or physical refining processes of edible oils (named, respectively, acid oils from chemical refining and fatty acid distillates from physical refining, according to the Catalogue of Feed Materials; Commission Regulation (EU) No 68/2013), and are characterized by having high proportions of free fatty acids (**FFA**; 40%-90%; Nuchi et al., 2009). From now on both acid oils from chemical refining and fatty acid distillates from physical refining will be generically named as acid oils unless otherwise stated.

Acid oils have similar fatty acid (**FA**) composition to their respective crude oils, but different molecular structures, which, according to Roll et al. (2018), can affect their nutritional value. For this reason, evaluating the effect of both the FA and lipid class composition is essential in order to understand how acid oils affect the fat digestion and absorption processes, and determine their potential use in broiler chicken diets.

It is well known that the ability of chicks to digest and absorb dietary fat is poorly developed (Krogdahl, 1985). However, the information regarding the use of acid oils in young broiler chickens is scarce (Blanch et al., 1996; Vilarrasa et al., 2015; Roll et al., 2018), and the lack of consistent results and the high variability in their composition are the main reason why acid oils are still not widely utilized in poultry feeds.

Taking this into account, it has been hypothesized that both the degree of saturation of the dietary fat and the age of the chicken can determine the effect of dietary FFA on fat utilization. Thus, the objective of the present study is to assess the effect of the FFA level, and saturation degree of dietary fat on FA digestibility and lipid class composition along the gastrointestinal tract (**GIT**), and excreta in young broiler chickens (14-day-old). This information will be essential to determine where the limitation of the use of acid oils in starter broiler-chicken diets is found and, therefore, to raise recommendations on the use of oils with certain percentages of FFA in the diets with no negative repercussions on fat utilization and growth performance. For this aim, two acid oils and two crude oils of different origins were used in order to produce diets with different saturation degrees and FFA levels.

An accompanying paper (Part II; Rodriguez-Sanchez et al., Unpublished data) describes and discusses the results reported in adult (37 d) broiler chickens, as well as compares the results reported between the two studied ages.

# 4.3. Materials and methods

## 4.3.1. Animals and diets

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

A total of 528 one-day-old female broiler chickens of the Ross 308 strain were obtained from a commercial hatchery (Pondex SAU; Lleida, Spain). On arrival, chicks were wing-banded, weighed (initial BW, 36.88 g ± 2.30 g), and randomly assigned to one of the eight dietary treatments, with eleven chicks per cage and six cages per treatment. Birds were housed in wire-floor cages. Throughout the study, feed and water were supplied ad libitum, and animals were raised under controlled conditions of light and temperature, as recommended by the breeder.

Birds received a wheat- and soybean-meal-based starter diet (in mash form) that was formulated to meet or exceed FEDNA requirements (2008) and to minimize the basal level of fat. The ingredient composition of the basal diet is presented in Table 4.1. Titanium dioxide (**$TiO_2$**) was added (5 g/kg) as an inert marker for the determination of the digestibility of FA.

The basal diet was supplemented at 6% with different fats (crude oils, **O**; acid oils, **A**; or oil blends) in order to achieve eight dietary treatments. The eight dietary treatments resulted in a 2 x 4 factorial arrangement, with two different fat sources (soybean oil products as unsaturated fat source, and palm oil products as saturated fat source), and four levels of dietary FFA (5%, 15%, 35%, and 50%). Thus, there were four soybean diets (**S**) with four increasing levels of FFA, and four palm diets (**P**) with four increasing levels of FFA. The different levels of dietary FFA were achieved blending crude soybean oil (**SO**) or crude palm oil (**PO**), with low levels of FFA (< 5% FFA), with an acid soybean oil (**SA**, an acid soybean oil from chemical refining with 67% FFA) or an acid palm oil (**PA**, a palm fatty acid distillate from physical refining with 99% FFA) respectively, in the proportions show in Table 4.2.

**Table 4.1.** Ingredient composition of the experimental basal diet for the starter period

| Ingredients, % | Starter diet (from 0 d to 21 d) |
|---|---|
| Wheat | 54.46 |
| Soybean meal 48% | 35.4 |
| Experimental fats[1] | 6.00 |
| Calcium carbonate | 1.44 |
| Sodium chloride | 0.40 |
| Vitamin and mineral premix[2] | 0.40 |
| DL-Methionine | 0.23 |
| L-Lysine | 0.15 |
| Titanium oxide | 0.50 |
| Ethoxyquin 66% | 0.02 |

[1] Crude soybean oil, crude palm oil, acid soybean oil or acid palm oil in different proportions (See Table 2).

[2] Provides per kg of feed: vitamin A (from retinol), 13,500 IU; vitamin $D_3$ (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; vitamin $B_1$, 3 mg; vitamin $B_2$, 9 mg; vitamin $B_6$, 4.5 mg; vitamin $B_{12}$, 16.5 µg; vitamin $K_3$, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from $FeSO_4 \cdot 7H_2O$), 54 mg; I (from $Ca(I_2O_3)_2$), 1.2 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$), 0.6 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from $Na_2SeO_3$), 0.18 mg; Mo (from $(NH_4)_6Mo_7O_{24}$), 1.2 mg.

**Table 4.2**. Oil blends used in the experimental diets

| Saturation degree | Unsaturated - S diets | | | | Saturated - P diets | | | |
|---|---|---|---|---|---|---|---|---|
| FFA level % | 5 | 15 | 35 | 50 | 5 | 15 | 35 | 50 |
| Treatments | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 |
| *Proportion in oil blends, %* | | | | | | | | |
| Crude soybean oil (SO) | 100 | 70 | 30 | - | - | - | - | - |
| Acid soybean oil (SA)[1] | - | 30 | 70 | 100 | - | - | - | - |
| Crude palm oil (PO) | - | - | - | - | 100 | 80 | 53 | 33 |
| Acid palm oil (PA)[2] | - | - | - | - | - | 20 | 47 | 66 |

[1] SA, acid soybean oil from chemical refining (67% FFA)

[2] PA, palm fatty acid distillate from physical refining (99% FFA)

## 4.3.2. Controls and sampling

Feed consumption and BW were measured weekly to calculate the average daily feed intake (**ADFI**), the average daily gain (**ADG**), and the feed conversion ratio **(FCR)** throughout the experiment (0-21 d).

A digestibility balance was carried out from 11 to 14 d. At 14 d of age, eight birds per cage were killed by cervical dislocation and samples of content of the gizzard, duodenum (from the pyloric junction to the distal most point of insertion of the duodenal mesentery), jejunum (from the distal most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), ileum (from the junction with Meckel's diverticulum to a point 1 cm proximal to the ileocecal junction), and a representative sample of excreta of each cage were taken. The digestive content of each segment of the GIT from all birds within each cage was pooled, homogenized, frozen at -20ºC, and lyophilized. After lyophilization, samples were ground to pass through a 0.5-mm sieve, and they were kept at 5°C until further analyses.

## 4.3.3. Chemical analysis

Analytical determinations of the diets were performed according to the methods of the AOAC International (2005): dry matter (934.01), ash (942.05), crude protein (968.06), crude fat (2003.05) and crude fiber (962.09). Gross energy was determined by an adiabatic calorimeter (IKA C-4000, Janke-Kunkel; Staufen, Germany).

$TiO_2$ was analyzed following the procedures of Short et al. (1996), and determined by ICP-OES (Optima 3200 RL, Perkin Elmer; Waltham, MA, USA). The FA content of the feed, excreta and digestive content was determined according to the method of Sukhija and Palmquist (1988). This analytic procedure consists of a direct trans-esterification (the lipid extraction and FA methylation is achieved in only one step). Samples were incubated at 70°C with methanolic hydrochloric acid (a mixture of methanol and acetyl chloride) for the methylation. Nonadecanoic acid (C19:0; Sigma-Aldrich Chemical Co.; St. Louis, MO) was added as internal standard before the methylation. After extraction and methylation, potassium carbonate and toluene were added in order to separate the organic layer. The final extract was injected in a gas chromatograph (HP6890, Agilent Technologies; Waldbronn, Germany) following the conditions of the method previously described by Cortinas et al. (2004). FA methyl esters were identified by matching their retention times with those of their relative standards (Supelco 37 component FAME Mix, Sigma-Aldrich Co.; St. Louis, MO), and quantification was performed by means of their calibration curves. The macronutrient and FA composition of the experimental diets are presented in Table 4.3.

Lipid class composition (triacylglycerols, **TAG**; diacylglycerols, **DAG**, monoacylgycerols, **MAG** and FFA) of the extracted fat from the feed, excreta and digestive content was determined by size-exclusion chromatography on an Agilent 1100 series HPLC chromatograph equipped with a Refractive Index Detector (**RID**) set at

35°C. Previously, the lipids of the feed, excreta and digestive content were extracted as follows: 0.1 g of sample (lyophilized in the case of the digestive content and excreta) was weighed in a test tube, subsequently adding 3 mL of HCl 1N solution, 7.5 mL of diethyl ether, and 100 µL of a salicylic acid solution (0.0075 g/mL diethyl ether; purchased from Sigma-Aldrich Co.; St. Louis, MO, USA) as internal standard. The content of each tube was homogenized for 30 seconds at 20,000 rpm, using a high-speed homogenizer (Polytron® System PT 3100; Kinematica AG; Switzerland). After homogenization, the content of each tube was poured into a centrifuge test tube, the procedure of adding 7.5 mL of diethyl ether and homogenizing was repeated, and the mixtures were pooled. One mL of a saturated NaCl solution was added, and the tubes were centrifuged for 15 minutes at 618.5 *g*. After centrifugation, the upper layer was transferred into a round-bottom flask. Then, 15 mL of diethyl ether were added and the centrifugation process was repeated as described above. The upper layer was transferred into the round-bottom flask. The content in the round-bottom flask was evaporated completely in a rotatory evaporator set at 35ºC, the extracted fat was transferred by dissolving it in diethyl ether into a glass test tube and the content was evaporated under $N_2$ at 35ºC. After lipid extraction, lipids were dissolved in 2 mL of tetrahydrofuran and filtered through a Nylon filter (0.45 µm), and 100 µL were injected (20-µL loop) to an Agilent HPLC 1100 (Agilent Technologies) chromatograph equipped with an isocratic pump, oven and RID. Separation was conducted using two Styragel columns (Styragel HR 1 and Styragel HR 0.5) of 30 cm × 0.78 cm i.d., filled with a spherical styrenedivinylbenzene copolymer of 5-µm particle size and 100 Å and 50 Å, respectively (Water Associates; Milford, MA, USA), connected in series and placed in an oven set at 35°C. The mobile phase consisted of tetrahydrofuran (HPLC quality grade) at 1 mL/min. Lipid classes were identified by matching their retention times with those of standards (trioleoylglycerol for TAG, dioleoylglycerol for DAG, oleoylglycerol for MAG and oleic acid for FFA; Sigma-Aldrich Co.; St. Louis, MO, USA), and quantification was performed by means of their calibration curves.

### 4.3.4. Formulas used for the lipid class content and digestibility determination

In order to determine the lipid class content in the different GIT segments and excreta, the following formula was applied:

Lipid-class content = [LC]dig /[Ti]dig,

where [LC]dig is the concentration of the lipid class in the digesta of a GIT segment or excreta (mg/g DM) and [Ti]dig is the concentration of $TiO_2$ in the digesta of a GIT segment or excreta (mg/g DM). This ratio is an estimation of the content of each lipid class present in the digestive tract of the chickens at the moment of the sample collection.

The digestibility coefficients of FA in each segment of the GIT were determined using the $TiO_2$ ratio in the feed and digestive content or excreta as detailed in Rodriguez-Sanchez et al. (2018), and the apparent metabolizable energy (**AME**) was determined from the product of the energy utilization ratio and its corresponding gross energy of feed.

### 4.3.5. Statistical analysis

Productive parameters, lipid-class content and digestibility of FA were subjected to a univariate analysis using the GLM procedure of SPSS (SPSS statistics 25.0.0.0, IBM 2017) to study whether they depended on the added fat, and the dietary FFA level. In the case of the lipid-class content and digestibility of FA, this analysis was performed for each GIT segment.

A regression analysis was carried out for each GIT segment, and for S and P diets, in order to find the best-fit regression equations considering the saturated FA (**SFA**) digestibility as dependent variable and the level of dietary FFA as the independent variable.

The cage served as the experimental unit, so there were six replicates per treatment. The Tukey test was used to assess the differences among the eight treatments. Results in tables are reported as least square means, and differences were considered significant at $P < 0.05$.

# 4.4. Results

## 4.4.1. Characterization of experimental diets

The analyzed composition of the experimental diets is presented in Table 4.3. The FA profile reflected the composition of the added fat source (S or P). The main FA in S diets were linoleic (48-52%) and oleic (21-22%) acids, while the main FA in P diets were palmitic (36-38%), oleic (30-31%), and linoleic (21-22%) acids. In the S diets, as SO was replaced by SA, there was a decrease of linoleic acid and an increase of palmitic acid (as SA is richer in palmitic acid than SO is; data not shown), while in the P diets, as PO was replaced by PA, the change in the proportion of the different FA was not as evident (as the FA composition of PO and PA are similar; data not shown). This fact was reflected in the unsaturated-to-saturated FA ratios (**UFA:SFA**), which, in S diets, decreased progressively from S5 (4.43) to S50 (3.37), while in P diets they slightly decreased (P5: 1.31 - P50: 1.21). These UFA:SFA ratios, as well as the lipid class characterization of the experimental diets (Table 4.3), show the objective of the study of having four diets for each fat source (S and P) with four increasing levels of FFA. Thus, in both S and P diets, as A replaced the corresponding O, TAG% decreased (from more than 80% to 46% or less) and FFA% increased (from less than 6% to more than 47%). The % of DAG as A replaced O, increased in S diets (from 4.6% to 8.2%), and decreased in the P diets (from 8.7% to 4.8%). The MAG% remained more or less constant as the as A replaced the corresponding O.

A significant interaction between the fat source and the dietary FFA level was observed for the AME ($P < 0.001$; S5: 3212a, S15: 2996bc, S35: 2818de, S50: 2781e, P5: 2932cd, P15: 3068b, P35: 2997bc, P50: 2879de). The highest AME value was observed for S5, followed by P15, while the lowest values were observed for S35, S50, and P50 diets.

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

| | Starter diets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Unsaturated – S diets | | | | Saturated – P diets | | | |
| | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 |
| *Macronutrient content* | | | | | | | | |
| Dry matter % | 89.99 | 89.91 | 89.98 | 90.01 | 89.78 | 89.83 | 89.87 | 89.88 |
| Crude protein % | 20.66 | 21.67 | 22.55 | 21.30 | 20.76 | 20.81 | 19.44 | 20.83 |
| Crude fat % | 7.37 | 7.26 | 7.19 | 7.13 | 6.59 | 7.09 | 7.49 | 6.76 |
| Crude fiber % | 4.94 | 60.4 | 5.91 | 5.48 | 6.95 | 5.83 | 5.77 | 5.84 |
| Ash % | 5.75 | 6.89 | 7.44 | 6.45 | 5.81 | 6.01 | 5.32 | 6.35 |
| Gross energy, kcal/kg | 4147 | 4062 | 4056 | 4066 | 4038 | 4212 | 4091 | 4043 |
| AME[3] | 3212 | 2996 | 2818 | 2781 | 2932 | 3068 | 2997 | 2879 |
| *Fatty acid composition, %* | | | | | | | | |
| C14:0 | 0.20 | 0.20 | 0.21 | 0.21 | 1.06 | 1.06 | 1.03 | 0.96 |
| C16:0 | 13.02 | 14.04 | 15.52 | 16.28 | 36.87 | 37.92 | 38.37 | 36.84 |
| C18:0 | 4.19 | 4.67 | 4.77 | 5.12 | 4.13 | 4.05 | 4.04 | 4.26 |
| C18:1 n-9 | 21.08 | 21.26 | 21.94 | 22.30 | 31.59 | 31.84 | 31.35 | 30.02 |
| C18:1 n-7 | 1.43 | 1.41 | 1.43 | 1.43 | 0.91 | 0.90 | 0.88 | 0.88 |
| C18:2 n-6 | 52.61 | 51.24 | 49.34 | 48.18 | 22.29 | 21.64 | 21.77 | 21.61 |
| C18:3 n-3 | 6.17 | 5.81 | 5.13 | 4.66 | 1.63 | 1.58 | 1.63 | 1.74 |
| Minor fatty acids | 1.29 | 1.36 | 1.66 | 1.81 | 1.53 | 1.01 | 0.95 | 3.70 |
| SFA | 18.42 | 19.98 | 21.64 | 22.87 | 43.13 | 43.62 | 43.94 | 45.33 |
| MUFA | 22.80 | 22.97 | 23.90 | 24.28 | 32.95 | 33.16 | 32.66 | 31.33 |
| PUFA | 58.78 | 57.06 | 54.47 | 52.85 | 23.92 | 23.22 | 23.39 | 23.35 |
| UFA:SFA | 4.43 | 4.01 | 3.62 | 3.37 | 1.32 | 1.29 | 1.28 | 1.21 |
| *Lipid-class composition, %* | | | | | | | | |
| TAG | 90.20 | 79.67 | 56.49 | 34.69 | 83.02 | 70.11 | 56.32 | 46.02 |
| DAG | 4.65 | 5.11 | 6.64 | 8.23 | 8.74 | 7.86 | 6.70 | 4.80 |
| MAG | 1.52 | 1.27 | 1.57 | 1.60 | 2.30 | 1.86 | 1.76 | 1.85 |
| FFA | 3.63 | 13.95 | 35.30 | 55.49 | 5.95 | 20.17 | 35.22 | 47.33 |

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

[2] Dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P); S5: 100% crude soybean oil, S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50:oil blend with 33% crude palm oil and 66% acid palm oil.

[3] Values are pooled means of 6 replicates with 11 chickens/replicate.

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

## 4.4.2. Growth performance

The broiler chickens completed the trial successfully. The effect of the saturation degree and FFA level of dietary fat on growth performance from 0 to 21 d is presented in Table 4.4. Regarding the fat saturation degree, no significant differences were observed in any of the parameters. Regarding the dietary FFA level, an effect was observed for BW at 21 d, where the value reported for the chickens fed the 15% FFA diets was higher than for those fed the other diets.

**Table 4.4.** Growth performance of broiler chickens (0-21d) according to different fat sources in the diet[1]

| | Dietary treatments[2] | | | | | | | | Fat[3] | | FFA level (%)[4] | | | | | *P* - values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unsaturated – S diets | | | | Saturated – P diets | | | | | | | | | | | | | |
| Item | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 | S | P | 5 | 15 | 35 | 50 | SEM | Fat | FFA | Interaction |
| ADG, g/b/d | 37.36 | 39.20 | 36.02 | 37.32 | 35.61 | 37.97 | 36.48 | 36.82 | 37.47 | 36.72 | 36.48 | 38.59 | 36.25 | 37.07 | 0.355 | 0.30 | 0.10 | 0.71 |
| ADFI, g/b/d | 65.20 | 70.72 | 67.13 | 65.74 | 68.1 | 71.20 | 61.33 | 56.96 | 67.20 | 66.67 | 66.69 | 70.96 | 64.23 | 65.85 | 1.003 | 0.79 | 0.13 | 0.48 |
| FCR, g/g | 1.76 | 1.88 | 1.87 | 1.81 | 1.87 | 1.83 | 1.78 | 1.79 | 1.83 | 1.82 | 1.81 | 1.86 | 1.82 | 1.80 | 0.025 | 0.78 | 0.89 | 0.52 |
| BW at 21 d, g | 820.7 | 861.6 | 793.2 | 798.6 | 781.6 | 834.3 | 801.4 | 806.8 | 820.45 | 803.53 | 801.19b | 855.81a | 788.25b | 802.72b | 5.228 | 0.11 | <0.001 | 0.15 |

[1] Values are means of 6 replicates with 11 chickens / replicate (until day 14), and with 3 chickens / replicate (from day 15 to day 21) fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P).

[2] S5: 100% soybean crude oil, S15: oil blend with 70% soybean crude oil and 30% acid soybean oil, S35: oil blend with 30% soybean crude oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% palm crude oil, P15: oil blend with 80% palm crude oil and 20% acid palm oil, S35: oil blend with 53% palm crude oil and 47% acid palm oil, S50:oil blend with 33% palm crude oil and 66% acid palm oil.

[3] S is the average of S5, S15, S35 and S50 diets; P is the average of P5, P15, P35, and P50 diets.

[4] 5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets.

ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; BW = body weight; SEM = standard error of the grand mean.

*P*-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected growth performance values.

*P* < 0.05 was considered significant.

### 4.4.3. Lipid class content along the gastrointestinal tract

Regardless of the fat source and dietary FFA level, the general evolution of lipid class content throughout the GIT followed a similar pattern (Table 4.5). The lipid class content in the gizzard reflected the lipid class content of the feed. From the gizzard to the duodenum, TAG decreased and the lipolysis products (DAG, MAG and FFA) increased. The lipolysis products decreased from the duodenum on.

Despite the similar general evolution along the GIT, there were some differences in the lipid class content regarding the dietary fat source (S or P). In the gizzard and duodenum, the content of DAG, MAG, and FFA was higher for S than for P ($P \leq 0.014$). Continuing with the results observed in the jejunum, ileum and excreta, MAG content was higher and FFA content lower for S than for P ($P \leq 0.05$). In the ileum, DAG content was higher for S than for P ($P < 0.001$).

In relation to the effect of the dietary FFA level on the lipid class content, it was observed that in the gizzard, the higher the dietary FFA level, the lower the TAG and DAG content, this being especially evident when the lowest and the highest dietary FFA levels were compared (5% FFA vs. 50% FFA). While the dietary FFA level did not have an effect in any of the lipid classes in the duodenum and jejunum, an effect was observed in the ileum and excreta (Figure 4.1). In the ileum, the dietary FFA level had an effect on DAG (15% FFA diets < 50% FFA diets; $P = 0.022$), MAG (5% and 15% FFA diets < 50% FFA diets; $P = 0.015$) and FFA content (5% FFA diets < 50% FFA diets; $P = 0.014$). In the excreta, the dietary FFA level had an effect on FFA content, which was higher for the chickens fed the 35% and 50% FFA diets in comparison to the chickens fed the 5% and 15% FFA diets ($P \leq 0.001$).

A significant interaction between the fat source and the dietary FFA level was observed in the excreta for TAG; while TAG content was the same for the four different dietary FFA levels in the chickens fed P diets, in those chickens fed S diets the value observed for S15 was lower than was the value observed for S50, and there were no differences among the rest of dietary FFA levels.

**Table 4.5.** Lipid class content[1] along the gastrointestinal tract and excreta according to different fat sources in the diet[2] in 14-day-old broiler chickens

| | Dietary treatments[3] | | | | | | | | Saturation degree[4] | | FFA level (%)[5] | | | | | *P* - values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unsaturated – S diets | | | | Saturated – P diets | | | | | | | | | | | | | |
| Item | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 | S | P | 5 | 15 | 35 | 50 | SEM | Fat | FFA | Interaction |
| *Gizzard* | | | | | | | | | | | | | | | | | | |
| TAG | 21.35 | 19.16 | 15.28 | 11.89 | 17.88 | 14.33 | 12.36 | 11.74 | 16.92 | 14.10 | 19.61a | 16.74ab | 13.82bc | 11.82c | 0.486 | 0.006 | <0.001 | 0.392 |
| DAG | 3.03 | 3.14 | 2.90 | 2.56 | 2.87 | 2.35 | 1.86 | 1.88 | 2.91 | 2.24 | 2.95a | 2.75ab | 2.38ab | 2.22b | 0.081 | <0.001 | 0.011 | 0.286 |
| MAG | 1.03 | 1.26 | 1.07 | 1.02 | 0.97 | 0.78 | 0.74 | 0.80 | 1.10 | 0.83 | 1.00 | 1.02 | 0.91 | 0.91 | 0.042 | 0.003 | 0.705 | 0.372 |
| FFA | 7.61 | 9.37 | 13.32 | 13.76 | 6.33 | 7.44 | 9.91 | 10.79 | 11.02 | 8.62 | 6.97b | 8.41b | 11.61a | 12.27a | 0.194 | <0.001 | <0.001 | 0.209 |
| *Duodenum* | | | | | | | | | | | | | | | | | | |
| TAG | 6.61 | 8.22 | 5.43 | 7.88 | 8.40 | 4.59 | 4.83 | 5.77 | 7.03 | 5.90 | 7.50 | 6.41 | 5.13 | 6.82 | 0.426 | 0.190 | 0.264 | 0.156 |
| DAG | 30.87 | 21.07 | 23.90 | 24.40 | 30.87 | 21.07 | 23.90 | 24.40 | 33.38 | 25.06 | 31.48 | 26.66 | 28.77 | 29.99 | 1.164 | <0.001 | 0.520 | 0.378 |
| MAG | 12.21 | 10.77 | 11.31 | 12.43 | 9.96 | 7.05 | 7.35 | 9.70 | 11.68 | 8.52 | 11.09 | 8.91 | 9.33 | 11.07 | 0.612 | 0.014 | 0.463 | 0.954 |
| FFA | 179.01 | 168.07 | 167.76 | 182.96 | 155.05 | 97.75 | 108.26 | 120.17 | 174.45 | 119.81 | 167.03 | 131.91 | 138.01 | 151.56 | 6.982 | 0.001 | 0.305 | 0.635 |
| *Jejunum* | | | | | | | | | | | | | | | | | | |
| TAG | 0.81 | 0.99 | 1.02 | 1.15 | 0.62 | 0.61 | 0.54 | 0.45 | 0.99 | 0.56 | 0.72 | 0.80 | 0.78 | 0.80 | 0.046 | <0.001 | 0.906 | 0.283 |
| DAG | 1.37 | 1.27 | 1.48 | 1.76 | 1.49 | 1.19 | 1.16 | 1.38 | 1.46 | 1.31 | 1.43 | 1.21 | 1.32 | 1.57 | 0.076 | 0.324 | 0.353 | 0.614 |
| MAG | 0.75 | 0.75 | 0.74 | 1.08 | 0.57 | 0.50 | 0.59 | 0.50 | 0.83 | 0.54 | 0.66 | 0.63 | 0.66 | 0.79 | 0.052 | 0.008 | 0.686 | 0.458 |
| FFA | 4.53 | 3.64 | 3.70 | 4.85 | 8.12 | 7.26 | 8.44 | 8.73 | 4.18 | 8.14 | 6.33 | 5.45 | 6.07 | 6.79 | 0.301 | <0.001 | 0.480 | 0.895 |
| *Ileum* | | | | | | | | | | | | | | | | | | |
| TAG | 0.49 | 0.39 | 0.42 | 0.66 | 0.26 | 0.23 | 0.25 | 0.28 | 0.49 | 0.25 | 0.37 | 0.31 | 0.34 | 0.47 | 0.023 | <0.001 | 0.091 | 0.332 |
| DAG | 0.69 | 0.60 | 0.88 | 1.00 | 0.46 | 0.48 | 0.56 | 0.57 | 0.79 | 0.52 | 0.58ab | 0.54b | 0.72ab | 0.79a | 0.031 | <0.001 | 0.022 | 0.357 |
| MAG | 0.52 | 0.46 | 0.60 | 0.80 | 0.29 | 0.28 | 0.37 | 0.40 | 0.60 | 0.34 | 0.40b | 0.37b | 0.49ab | 0.60a | 0.026 | <0.001 | 0.015 | 0.496 |
| FFA | 2.27 | 1.88 | 2.65 | 3.30 | 4.42 | 4.87 | 5.79 | 5.55 | 2.53 | 5.16 | 3.35b | 3.38ab | 4.22ab | 4.43a | 0.141 | <0.001 | 0.014 | 0.499 |
| *Excreta* | | | | | | | | | | | | | | | | | | |
| TAG | 0.35ab | 0.28b | 0.46ab | 0.56a | 0.31b | 0.30b | 0.32ab | 0.27b | 0.41 | 0.30 | 0.33 | 0.29 | 0.39 | 0.41 | 0.019 | 0.005 | 0.082 | 0.038 |
| DAG | 0.77 | 0.71 | 0.98 | 0.99 | 0.69 | 0.71 | 0.81 | 0.69 | 0.86 | 0.73 | 0.73 | 0.71 | 0.90 | 0.84 | 0.037 | 0.075 | 0.229 | 0.539 |
| MAG | 0.39 | 0.47 | 0.52 | 0.57 | 0.41 | 0.36 | 0.40 | 0.45 | 0.49 | 0.41 | 0.40 | 0.42 | 0.46 | 0.51 | 0.021 | 0.049 | 0.265 | 0.591 |
| FFA | 2.38 | 2.38 | 3.91 | 3.33 | 5.30 | 5.61 | 6.49 | 6.73 | 3.00 | 6.03 | 3.84b | 4.00b | 5.20a | 5.03a | 0.127 | <0.001 | <0.001 | 0.681 |

[1] Lipid class concentration (mg/g) / Ti concentration (mg/g) in each gastrointestinal segment and excreta. [2] Values are pooled means of 6 replicates with 8 chickens / replicate fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P). [3] S5: 100% crude soybean oil, S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50: oil blend with 33% crude palm oil and 66% acid palm oil. [4] S is the average of S5, S15, S35 and S50 diets; P is the average of P5, P15, P35, and P50 diets. [5] 5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets. TAG = triacylglycerols, DAG = diacylglycerols, MAG = monoacylglycerols, FFA = free fatty acids, SEM = standard error of the grand mean. *P*-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected lipid-class results. $P<0.05$ was considered significant.[a–d]Means in a row not sharing a common superscript are significantly different ($P < 0.05$).

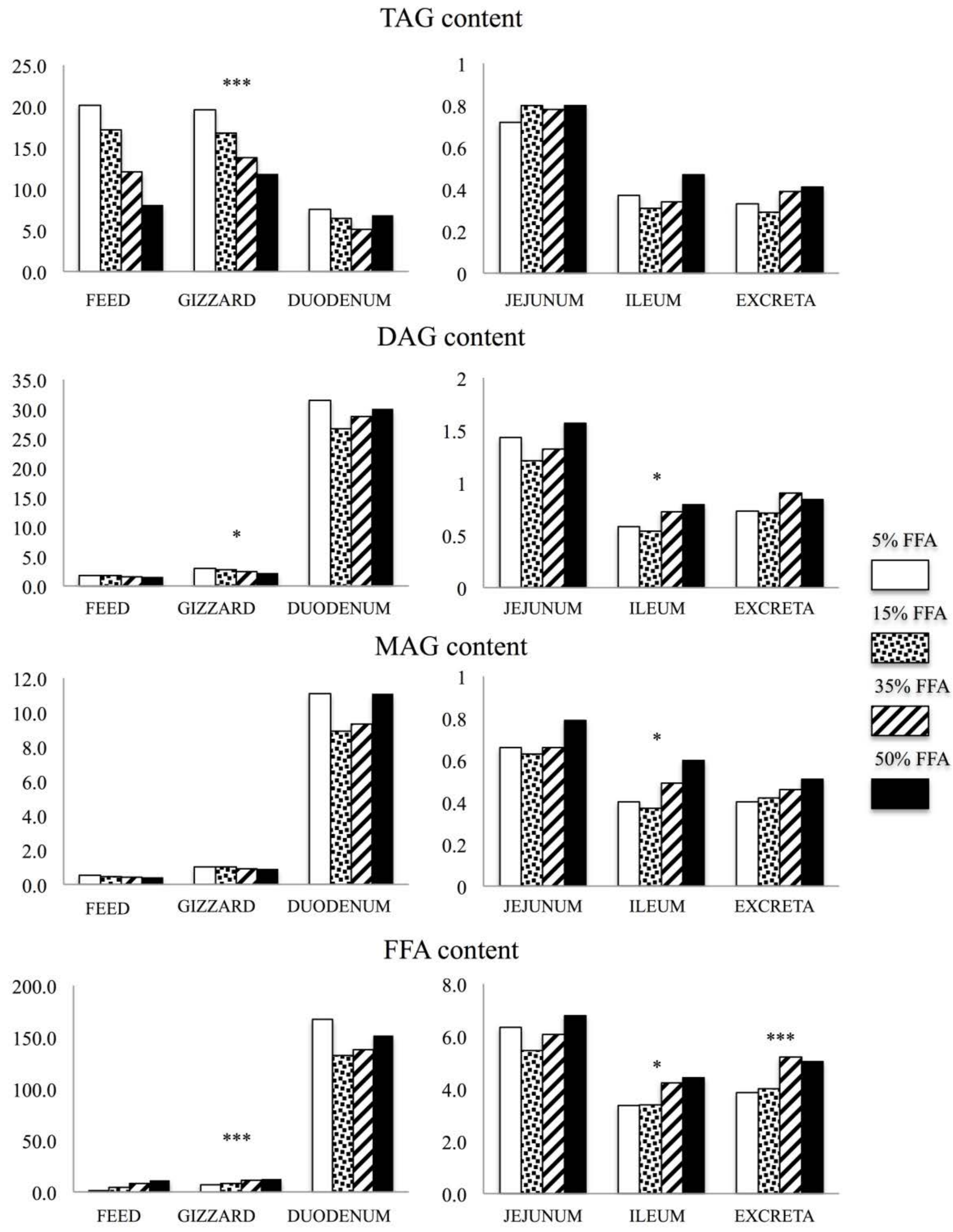

**Figure 4.1.** Lipid class content in the feed, along the gastrointestinal tract and excreta considering the average results for the four increasing levels of dietary free fatty acids[1] in 14-day-old broiler chickens.

[1] Values are means of 12 replicates per each dietary free fatty acid level with 8 chickens / replicate, and 11 chickens / replicate in the case of excreta. 5% free fatty acids; 15% free fatty acids; 35% free fatty acids: 50% free fatty acids.

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

P-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected the lipid-class content. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

### 4.4.4. Fatty acid digestibility along the gastrointestinal tract

Fatty acid digestibility coefficients were also studied in different segments of the GIT, and in the excreta for the eight dietary treatments (Table 4.6). SFA digestibility was mainly represented by palmitic (C16:0) and stearic (C18:0) acids, monounsaturated FA (**MUFA**) digestibility by oleic acid (C18:1n9), and polyunsaturated FA (**PUFA**) digestibility by linoleic (C18:2n6) acid.

Regarding the saturation degree of dietary fat, an effect was observed in the duodenum, where the digestibility coefficients of total FA (**TFA**) and SFA for those chickens fed the unsaturated diets (S) were lower (more negative) than for the chickens fed the saturated diets (P; $P \leq 0.001$). An effect was also observed in the jejunum and ileum. In the jejunum, the chickens fed the unsaturated diets had higher digestibility coefficients for both TFA ($P = 0.02$) and PUFA ($P \leq 0.001$), in comparison to those chickens fed the saturated diets, and the same feature was also observed in the ileum for TFA, SFA, MUFA, and PUFA ($P \leq 0.002$).

Regarding the FFA level of dietary fat, an effect was observed in the jejunum, and ileum (Figure 4.2). In the jejunum, TFA, SFA and MUFA digestibility coefficients were higher for the chickens fed the diets with the lowest level of dietary FFA (5%), in comparison to the ones fed the diets with the highest level of dietary FFA (50%; $P < 0.05$). The same was observed in the ileum for SFA digestibility coefficients. In the case of MUFA and SFA digestibility in the jejunum, the digestibility coefficient for the chickens fed the diets with 15% FFA was also higher, in comparison to the 50% FFA diets. A tendency was observed in the jejunum for PUFA, and in the ileum for MUFA, where the coefficient observed for the chickens fed the diets with the highest dietary FFA level (50%) was numerically lower in comparison to the rest of the diets.

A significant interaction between the fat source and the FFA level was observed in the gizzard and excreta for TFA, SFA, MUFA, and PUFA digestibility, which was explained by a different response in the digestibility coefficients between the chickens fed S diets and the chickens fed P diets as the FFA level increased. A tendency was also observed, both in the jejunum and ileum, for SFA, the pattern observed for SFA in the excreta being similar to these two GIT segments. The most relevant results regarding the regression analysis were found in the ileum (Figure 4.3), the last GIT segment where FA absorption has been described. The best-fit regression equation for the SFA ileal digestibility in S diets was quadratic ($P \leq 0.001$); the coefficients observed for the chickens fed S5 and S15 diets were numerically similar, while at higher dietary FFA levels the digestibility coefficients decreased progressively, reaching similar values to those obtained for the chickens fed P diets. On the other hand, the coefficients observed for the four increasing FFA levels in the chickens fed P diets were numerically similar, and did not fit any model.

It was seen that PUFA digestibility coefficients in the excreta were numerically lower than in the ileum. As the absorption of fat has been reported to be negligible in the hindgut of poultry (Renner, 1965), this decrease could be related, at least in part, to bacterial activity. In order to confirm this, the concentration of those FA determined in the excreta that could come from bacterial metabolism were added up (capric acid, C10:0; margaric acid, C17:0; and elaidic acid, C18:1 trans) and compared among the different diets (S5: 2.25; S15: 2.27; S35: 2.25; S50: 2.84; P5: 1.98; P15: 1.89; P35: 1.87; P50: 2.11 mg/g DM). An effect of the saturation degree ($P < 0.001$) and dietary FFA level ($P = 0.006$) was observed. The concentration of these three FA in the excreta was higher for the chickens fed S diets than for those fed P diets (S: 2.4 vs. P: 2.0), and their concentration was significantly higher for the chickens fed the diets with the highest FFA level (5% FFA: 2.1b, 15% FFA: 2.1b; 35% FFA: 2.1b; 50% FFA: 2.5a).

**Table 4.6.** Fatty acid digestibility coefficients[1] along the gastrointestinal tract and excreta according to different fat sources in the diet[1] in 14-day-old broiler chickens

| | Dietary treatments[2] | | | | | | | | Saturation degree[3] | | FFA level (%)[4] | | | | | *P* - values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unsaturated – S diets | | | | Saturated – P diets | | | | | | | | | | | | | |
| | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 | S | P | 5 | 15 | 35 | 50 | SEM | Fat | FFA | Interaction |
| *Gizzard* | | | | | | | | | | | | | | | | | | |
| TFA | -0.53ab | -0.85b | -0.76b | -0.51ab | -0.50ab | -0.39a | -0.16a | -0.23a | -0.66 | -0.32 | -0.52 | -0.62 | -0.46 | -0.37 | 0.029 | <0.001 | 0.03 | 0.01 |
| SFA | -0.57cd | -0.77d | -0.65cd | -0.45bc | -0.19ab | -0.08a | 0.07a | 0.06a | -0.61 | -0.03 | -0.38 | -0.43 | -0.29 | -0.20 | 0.023 | <0.001 | 0.01 | 0.04 |
| MUFA | -0.51bc | -0.83c | -0.74c | -0.49bc | -0.32ab | -0.24ab | -0.08a | -0.21ab | -0.65 | -0.21 | -0.42 | -0.54 | -0.41 | -0.35 | 0.027 | <0.001 | 0.12 | 0.01 |
| PUFA | -0.53a | -0.89ab | -0.81ab | -0.55a | -1.30b | -1.21b | -0.72a | -0.83ab | -0.69 | -1.02 | -0.92 | -1.05 | -0.76 | -0.69 | 0.042 | <0.001 | 0.02 | 0.01 |
| *Duodenum* | | | | | | | | | | | | | | | | | | |
| TFA | -8.36 | -10.23 | -9.45 | -10.82 | -8.95 | -4.89 | -4.76 | -6.35 | -9.72 | -6.24 | -8.66 | -7.56 | -7.10 | -8.58 | 0.439 | <0.001 | 0.51 | 0.08 |
| SFA | -15.79 | -17.99 | -15.63 | -17.16 | -8.23 | -4.48 | -4.29 | -5.47 | -16.64 | -5.62 | -12.01 | -11.23 | -9.96 | -11.32 | 0.652 | <0.001 | 0.73 | 0.45 |
| MUFA | -4.90 | -5.92 | -5.26 | -5.84 | -6.35 | -3.36 | -3.28 | -4.58 | -5.48 | -4.39 | -5.63 | -4.64 | -4.27 | -5.21 | 0.296 | 0.07 | 0.38 | 0.10 |
| PUFA | -7.38 | -9.25 | -8.83 | -10.37 | -13.84 | -7.84 | -7.72 | -10.42 | -8.96 | -9.95 | -10.61 | -8.55 | -8.28 | -10.39 | 0.516 | 0.34 | 0.26 | 0.03 |
| *Jejunum* | | | | | | | | | | | | | | | | | | |
| TFA | 0.64 | 0.60 | 0.57 | 0.46 | 0.50 | 0.53 | 0.50 | 0.46 | 0.57 | 0.50 | 0.57a | 0.56ab | 0.54ab | 0.46b | 0.014 | 0.02 | 0.03 | 0.45 |
| SFA | 0.46 | 0.46 | 0.34 | 0.23 | 0.41 | 0.44 | 0.41 | 0.41 | 0.38 | 0.42 | 0.44a | 0.45a | 0.38ab | 0.32b | 0.015 | 0.19 | 0.02 | 0.06 |
| MUFA | 0.69 | 0.64 | 0.57 | 0.51 | 0.65 | 0.66 | 0.62 | 0.58 | 0.60 | 0.63 | 0.67a | 0.65a | 0.59ab | 0.54b | 0.012 | 0.22 | 0.002 | 0.39 |
| PUFA | 0.67 | 0.64 | 0.66 | 0.53 | 0.47 | 0.51 | 0.51 | 0.40 | 0.63 | 0.47 | 0.57 | 0.57 | 0.59 | 0.46 | 0.017 | <0.001 | 0.05 | 0.91 |
| *Ileum* | | | | | | | | | | | | | | | | | | |
| TFA | 0.89 | 0.89 | 0.86 | 0.82 | 0.73 | 0.70 | 0.70 | 0.69 | 0.86 | 0.71 | 0.81 | 0.79 | 0.78 | 0.75 | 0.008 | <0.001 | 0.15 | 0.69 |
| SFA | 0.75 | 0.75 | 0.66 | 0.56 | 0.59 | 0.57 | 0.57 | 0.56 | 0.68 | 0.57 | 0.67a | 0.65ab | 0.61ab | 0.56b | 0.013 | <0.001 | 0.03 | 0.08 |
| MUFA | 0.87 | 0.87 | 0.85 | 0.81 | 0.83 | 0.81 | 0.80 | 0.79 | 0.85 | 0.81 | 0.85 | 0.84 | 0.82 | 0.80 | 0.007 | 0.002 | 0.06 | 0.75 |
| PUFA | 0.94 | 0.94 | 0.94 | 0.93 | 0.83 | 0.79 | 0.83 | 0.80 | 0.94 | 0.81 | 0.88 | 0.86 | 0.88 | 0.87 | 0.006 | <0.001 | 0.59 | 0.63 |
| *Excreta* | | | | | | | | | | | | | | | | | | |
| TFA | 0.89a | 0.86a | 0.78b | 0.77bc | 0.70cd | 0.70cd | 0.70cd | 0.67d | 0.83 | 0.69 | 0.80 | 0.78 | 0.74 | 0.72 | 0.006 | <0.001 | <0.001 | 0.001 |
| SFA | 0.81a | 0.73a | 0.60b | 0.55b | 0.57b | 0.57b | 0.58b | 0.54b | 0.67 | 0.57 | 0.68 | 0.65 | 0.59 | 0.54 | 0.009 | <0.001 | <0.001 | <0.001 |
| MUFA | 0.89a | 0.86ab | 0.78c | 0.77c | 0.82bc | 0.83abc | 0.83abc | 0.81bc | 0.83 | 0.82 | 0.86 | 0.85 | 0.81 | 0.79 | 0.005 | 0.71 | <0.001 | 0.001 |
| PUFA | 0.92a | 0.91ab | 0.85b | 0.86b | 0.75c | 0.73c | 0.76c | 0.76c | 0.88 | 0.75 | 0.83 | 0.81 | 0.80 | 0.81 | 0.005 | <0.001 | 0.14 | 0.002 |

[1] Values are pooled means of 6 replicates with 8 chickens / replicate fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P). [2] S5: 100% crude soybean oil, S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50: oil blend with 33% crude palm oil and 66% acid palm oil. [3] S is the average of S5, S15, S35 and S50 diets; P is the average of P5, P15, P35, and P50 diets. [4] 5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets. TFA = total fatty acids, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SEM = standard error of the grand mean. *P*-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected the FA digestibility results. P<0.05 was considered significant. [a–d] Means in a row not sharing a common superscript are significantly different ($P < 0.05$).

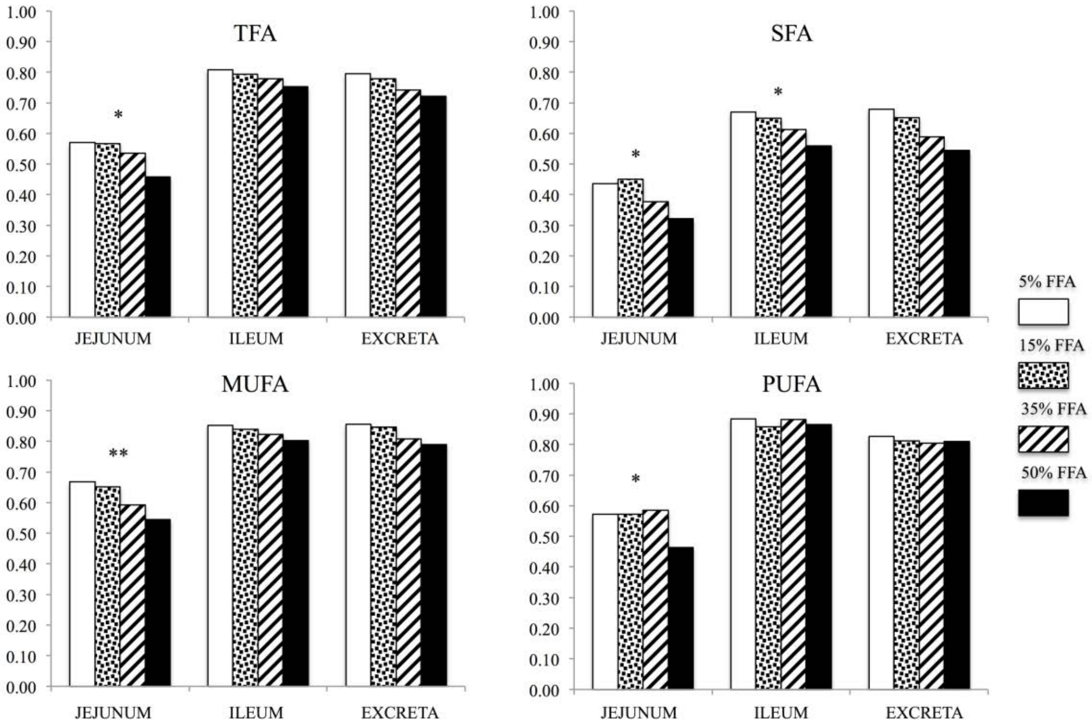

**Figure 4.2.** Fatty acid digestibility[1] in the jejunum, ileum and excreta considering the average results for the four increasing levels of dietary free fatty acids[2] in 14-day-old broiler chickens.

[1] Values are means of 12 replicates per each dietary free fatty acid level with 8 chickens / replicate, and 11 chickens / replicate in the case of excreta.

[2] 5% free fatty acids; 15% free fatty acids; 35% free fatty acids: 50% free fatty acids. TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; FFA = free fatty acids.

*P*-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected the fatty acid digestibility.

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

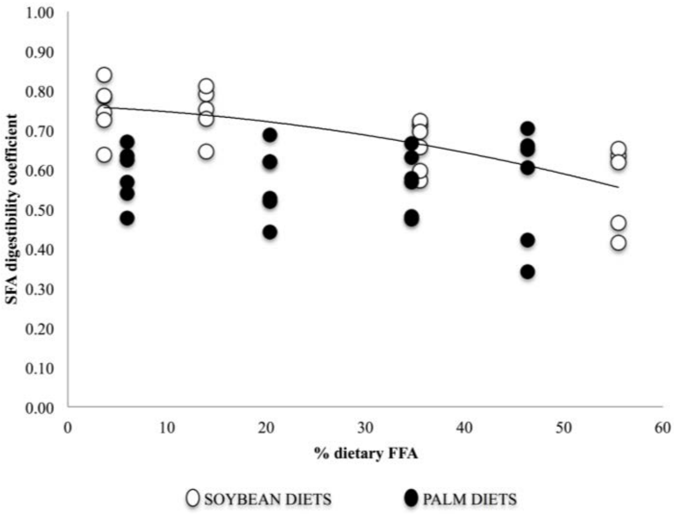

**Figure 4.3.** Relation between the saturated fatty acid digestibility calculated in the ileum and the four dietary free fatty acid levels[1] for two different fat sources in 14-day-old broiler chickens.
[1] Diets with an average of 5% FFA, 15% FFA, 35% FFA, 50% FFA.
Each point represents each replicate value (with 8 chickens / replicate).
SFA: saturated fatty acids; FFA: free fatty acids.
The black line illustrates the quadratic model observed for the unsaturated diets:
$y = -5^{-5}x^2 - 0.001x$, where $R^2 = 0.55$ and $P = 0.001$.
Interaction fat x dietary FFA: $P = 0.08$.

# 4.5. Discussion

## 4.5.1. Dietary fat saturation effect

As it has been previously documented (Renner and Hill, 1961; Young and Garrett, 1963), the saturation degree of dietary fat has an influence on fat utilization, and in the present study this fact was seen both in the fat hydrolysis and absorption processes. On the one hand, and in agreement with our previous study (Rodriguez-Sanchez et al., 2018), the lower digestibility coefficients (more negative) reported in the gizzard and duodenum for those chickens fed the unsaturated (S) diets, in comparison to the ones fed the saturated (P) diets, could be explained by a higher secretion of endogenous fat into the duodenum after consuming an unsaturated fat source. This was also supported by the lipid class content, which was higher for the chickens fed S diets than for those fed P diets, both in the gizzard and duodenum. The presence of fat in the duodenum has been reported as a stimulus for the secretion of cholecystokinin, and, consequently, the secretion of pancreatic enzymes and bile (Lindsay et al., 1969;

Krogdahl, 1985; Tuchweber et al., 1996). It is likely that unsaturated fat enhances the secretion of endogenous fat, like phospholipids, mucin-associated lipids or desquamated epithelial cells, into the duodenum lumen (Cotton, 1972; Hurwitz et al., 1973; Clément, 1980; Gong et al., 1990), which could explain the present results. It has also been described that unsaturated FA (**UFA**) have a greater ability to increase pancreatic lipase activity in comparison to SFA, due to their angle at the site of the double bond (Van Kuiken and Behnke, 1994). The gastro-duodenal reverse peristalsis could also be influenced by the dietary fat source; however, the authors have not found available information in the literature concerning the effect of the dietary fat source in this process. All of these findings agree with the results from a previous study (Rodriguez-Sanchez et al., 2018), and they suggest that the hydrolysis process in those animals fed the unsaturated diets (S) is faster and more efficient than in those fed the saturated diets (P).

On the other hand, and regarding the absorption process, the higher digestibility coefficients observed in the jejunum (for TFA and PUFA) and ileum (for TFA, SFA, MUFA and PUFA) for the chickens fed S diets, in comparison to the ones fed P diets, and the higher MAG content and the lower FFA content in those chickens fed S diets than in those fed P diets, suggest that the absorption of UFA is faster and more efficient, in comparison to the absorption of SFA.

The contribution of the jejunum and ileum on FA absorption was calculated considering the digestibility coefficient reported in the ileum as the maximum (100%) and expressing the digestibility coefficients observed in the jejunum and ileum towards that value. It was seen that the jejunum was the main site of fat absorption regardless of saturation degree of the added fat (68% and 63%, on average, of TFA and SFA, respectively, were absorbed in this GIT segment). However, differences were observed regarding the contribution of the ileum, which was higher for the chickens fed S diets, in comparison to those fed P diets, this being especially evident for SFA (S: 46% vs. P: 27%). In the present study, the greater utilization of the unsaturated diets in 14-day-old broiler chickens was supported by the higher ileum contribution to the SFA absorption in those animals fed S diets, in comparison to those fed P diets. This is in agreement with the results reported in our previous study in 14-day-old broiler chickens fed crude oils (Rodriguez-Sanchez et al., 2018).

### 4.5.2. Dietary free fatty acid effect

As explained before, one of the main characteristics of acid oils is their high content of FFA, which in general has been negatively associated with fat utilization. However, this negative association is not clear, and the information about the level of FFA that could be used in broiler chicken diets without having negative repercussions on fat utilization is scarce.

Regarding the performance parameters, the dietary FFA level did have an effect on BW at 21 d, however, the rest of the growth performance parameters were not affected, which is in agreement with different authors who did not report any effect of dietary FFA (at levels between 3% and 99%) on the productive parameters (Siedler et al., 1955; Bornstein and Lipstein, 1963). However, Bornstein and Lipstein (1963) also reported slight and inconsistent exceptions in their results, and Artman (1964) reported that broiler chickens fed diets with soybean oil had lower FCR in comparison to those fed acid soybean oil. Zumbado et al. (1999) reported that broiler chickens fed diets supplemented with soybean acid oil had the best FCR, and those supplemented with acid palm oil had the lowest weight gain and the worst FCR.

The present results do not allow to demonstrate whether the dietary FFA level influence the hydrolysis process. The differences observed in the gizzard reflected the lipid class composition of the different diets. The lack of differences in both the digestibility coefficients and lipid class content in the duodenum could be related to the presence of FFA in the diets, which are an end-product of the hydrolysis process and are not affected by pancreatic lipase activity. However, Larsson and Erlanson-Albertsson (1986) described that FFA could induce a high-affinity complex between lipase and colipase, and change the properties of the interface leading to an increased binding of lipase and colipase to the substrate; this effect has been especially attributed to lauric, oleic and linolenic acids.

On the other hand, a clear impact of dietary FFA level on fat absorption was reported, which was supported by the lower TFA, SFA, MUFA, and PUFA (tendency) digestibility coefficients in the jejunum, and the lower SFA, and MUFA (tendency) digestibility coefficients in the ileum observed for the chickens fed the diets with the highest dietary FFA level (50%), in comparison to those fed the diets with the 5% FFA level and, in some cases, with those fed the 15% FFA diets as well. Those chickens fed the diets with the highest dietary FFA level also presented higher content of all the lipolysis products in the ileum, especially of MAG and FFA, which were higher in the chickens fed the 50% FFA diets, in comparison to the chickens fed the 5% FFA ones (and also to the ones fed the 15% FFA diets in the case of MAG), and the highest content of FFA in the excreta, in this case both for the chickens fed the 35% FFA and 50% FFA diets, in comparison to the ones fed the 5% FFA and 15% FFA diets. Thus, according to these results, levels of dietary FFA above 15% were associated with a lower fat absorption rate. On the other hand, levels of FFA up to 15% did not have negative repercussions on fat utilization, suggesting that moderate levels of dietary FFA could be used in young broiler chicken diets with no detriments in both performance parameters and fat utilization. Blanch et al. (1995) also reported a lower fat apparent absorption in 2-week-old broiler chickens fed a diet supplemented with a blend tallow + acid soybean oil (34.2% FFA of added fat), in comparison to a diet supplemented with soybean oil (2.6% FFA of added fat). Contrary to the present findings, Artman (1964) did not negatively relate the dietary FFA level with fat utilization. Nevertheless, that study was performed in adult chickens, suggesting that the higher the age of the chickens, the greater the tolerance to FFA in the diets.

Some reasons have been hypothesized in the literature to explain the reduction of fat utilization due to the presence of dietary FFA. It has been described that TAG and 2-MAG stimulate the secretion of bile salts, in consequence being necessary for the emulsification of fat (Sklan, 1979). Sklan (1979) related diets with high levels of FFA to lower MAG content and bile secretion in the duodenum. This is also in accordance with Atteh and Leeson (1985), who reported an improvement on the ME of diets supplemented with FFA after supplementation with cholic acid, a primary bile acid. Blanch et al. (1995) also related the low fat utilization observed in the diets with high FFA levels with a low bile secretion, and consequently an incomplete micellar solubilization. In the present study, the lack of differences reported for both the FA digestibility and lipid class results in the duodenum, considering the dietary FFA level, suggest that the main reason for the different fat utilization observed after being fed a diet low or rich in FFA is more likely to be found in the absorption rather than in the hydrolysis process. This was also supported by the lower FA digestibility coefficients observed, in general, in the jejunum and ileum, and the higher content of lipolysis products in the ileum and excreta for the chickens fed the diets with the highest level of dietary FFA. It has been described that the key to absorption of lipolysis end-products is the formation of mixed lipid bile micelles (Ravindran et al., 2016), the presence of bile salts also being essential in the absorption process. Thus, this could be a reason for the lower fat utilization observed for those animals fed the diets with the highest dietary FFA level. On the other hand, dietary FFA have been related to some reactions in the intestine. Concretely, the acid group of FFA can react with ionized minerals (e.g., calcium) and lead to the formation of soaps. If these soaps are insoluble, then both, the FFA and the mineral, are unavailable to the chicken. Atteh and Leeson (1985) related the soap formation between FFA and calcium to the decrease in fat retention and dietary ME. Atteh and Leeson (1984) reported that those soaps formed by unsaturated FFA were absorbed more easily than those formed by saturated FFA. In the present study, the formation of soaps was not investigated, but could explain, at least in part, the worst fat utilization of the diets with the highest dietary FFA level.

Despite the general effect (irrespective of the fat source) of dietary FFA level on fat absorption, some differences were reported between the chickens fed the unsaturated diets and the chickens fed the saturated diets. This was supported by the tendency observed for SFA digestibility for the interaction between the fat source and the level of dietary FFA, both in the jejunum and ileum. In both cases the coefficients reported for the chickens fed S diets seemed to be more affected than the ones reported for the chickens fed P diets as the dietary FFA level increased. The regression analysis carried out for SFA digestibility in the ileum also supported the differences between dietary fat sources, suggesting that unsaturated diets with up to 15% FFA did not have negative repercussions on FA digestibility, and that unsaturated diets with 35% and 50% FFA had similar results to saturated diets. The contribution of the jejunum and ileum on fat utilization also supported the higher effect of dietary FFA level on the unsaturated diets. Thus, in those chickens fed S diets the contribution of the ileum on SFA absorption increased from S5 and S15 to S50 (S5: 39%, S15: 39%, S35: 49%, and S50: 59%); however, the higher contribution of the ileum did not compensate the lower digestibility coefficients reported for those chickens fed the diets with the highest FFA% level. On the other hand, in those chickens fed P diets there was a decrease from P5 to P15 on the contribution of the ileum on SFA absorption, the values for the other dietary FFA levels being similar among them (P5: 31%, P15: 23%, P35: 28%, and P50: 27%).

All of these findings suggest that the utilization of unsaturated diets was more affected by the dietary FFA level in comparison to the utilization of saturated diets, and that the saturation degree of dietary fat could affect fat utilization more than the level of dietary FFA, this being supported by the UFA:SFA ratios reported for the different diets. Thus, while in S diets there were two factors affecting fat utilization as the dietary FFA level increased: more saturated FA (UFA:SFA ratio: 4.43 and 3.37 for S5 and S50, respectively), and more FFA, in P diets, the dietary FFA level increased, but saturated FA remained more constant (UFA:SFA ratio: 1.32 and 1.21 for P5 and P50, respectively). Vilarrasa et al. (2015) also reported a greater effect of the fat saturation degree rather than of the molecular structure on FA apparent absorption. It also has to be taken into account that fat utilization in saturated diets was already low when crude oil was used, and so it probably had less chance to decrease.

The interactions observed in the excreta for TFA, SFA, MUFA and PUFA digestibility could support the different effect of dietary FFA level between fat sources. While the FA digestibility coefficients observed for the chickens fed P diets (in general lower than the ones observed for the chickens fed S diets) did not significantly changed as the dietary FFA level increased, there was a general decrease of the FA digestibility coefficients for those chickens fed S diets as the dietary FFA level increased, especially from S15 to S50. Furthermore, SFA and MUFA digestibility coefficients reported for the chickens fed S35 and S50 diets were not different than the ones reported for the chickens fed P diets. However, it is important to mention that the digestibility coefficients reported in the excreta were, in some cases, numerically lower in comparison to the ones reported in the ileum. The dietary fat saturation degree and FFA level effect observed for the concentration in the excreta of those FA that come from bacterial metabolism, suggest that bacterial activity could be a reason of this decrease. Nevertheless, it is likely that other FA endogenous losses like desquamated epithelial cells, also contributed to the decrease on digestibility coefficients from the ileum to the excreta. For this reason, it is likely that the results reported in the jejunum and ileum are better indicators of the FA absorption process rather than the ones reported in the excreta.

In conclusion, the results of the present study allow for a better understanding of the limitations of the incorporation of acid oils in starter broiler chicken diets. The better utilization of the unsaturated dietary fat, which was related to a higher contribution of the ileum in SFA absorption, was confirmed. It was seen that the absorption process is the most limiting part of fat utilization (in comparison to hydrolysis), and that the jejunum is the main place of FA absorption. The absorption process was also more affected than was hydrolysis by the dietary FFA level, the effect of the dietary FFA level on FA absorption being more evident in the unsaturated diets than in the saturated ones, which in general were related to low digestibility values. It was suggested that the saturation degree could have a greater impact on fat utilization, in comparison to the dietary FFA level. The results suggest that crude soybean oil is an adequate fat source for starter broiler chickens diets, and moderate levels of acid soybean oil could substitute soybean crude oil (as long as the dietary FFA level does not exceed 15%) without having negative repercussions on either fat utilization or growth performance. On the other hand, and irrespective of the dietary FFA level, palm oil sources are not suitable for starter broiler chickens diets as in general they were related to lower fat utilization than the diets supplemented with soybean oil sources.

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# CHAPTER 5

## Effect of dietary free fatty acid level and saturation degree on lipid class composition and fatty acid digestibility along the gastrointestinal tract in adult broiler chickens

# II. Effect of dietary free fatty acid level and saturation degree on lipid class composition and fatty acid digestibility along the gastrointestinal tract in adult broiler chickens

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Submitted to Poultry Science

# 5.1. Abstract

The aim of the present study is to investigate the effect of the dietary free fatty acid (**FFA)** level with different saturation degrees, on the fatty acid (**FA**) digestibility and lipid class content along the gastrointestinal tract in adult broiler chickens. Eight dietary treatments resulted from the supplementation at 6% of a wheat-and-soybean-meal-based diet with different fats (crude oils, fat by-products from the edible oil refining industry rich in FFA, or oil blends). This resulted in a 2 x 4 factorial arrangement: two different fat sources (soybean oil products as unsaturated fat source, and palm oil products as saturated fat source), and four levels of dietary FFA (5%, 15%, 35%, and 50%). The results suggest that the absorption process of FA was more limiting than the hydrolysis process, being the jejunum the main place of FA absorption. The contribution of the jejunum on FA absorption explained to a greater extent the higher FA digestibility coefficients reported in those chickens fed the unsaturated diets. However, the contribution of the ileum was also important, especially for the absorption of saturated FA. The dietary FFA level seemed to affect more the absorption than the hydrolysis process. However, the effect in the absorption process was found to be different depending on the saturation degree of dietary fat. It was concluded that unsaturated diets with up to 35% FFA did not negatively affect fat utilization, and saturated diets with 50% FFA led to similar results than diets with 5% FFA. Thus, the inclusion of acid soybean oil and palm fatty acid distillate in grower-finisher broiler chicken diets could replace the corresponding crude oils, at least when the FFA level does not exceed the mentioned values. Furthermore, the comparison of the results between 14- and 37-day-old broiler chickens showed that the latter were less affected by the level of dietary FFA, being the suitable level of dietary FFA in grower-finisher diets higher than in starter-broiler chicken diets.

# 5.2. Introduction

Fat by-products from the edible oil refining industry come from the chemical or physical refining process of edible oils, being the high proportion of free fatty acids (**FFA;** 40-99%; Nuchi et al., 2009) one of their main characteristics. According to the Catalogue of Feed Materials (Commission Regulation (EU) No 68/2013), acid oils are named according to their obtaining process: acid oils from chemical refining or fatty acid distillates from physical refining. From now on, they will be generically named as acid oils unless otherwise stated. Acid oils are an interesting economical alternative to conventional fats. Furthermore, they are a good source of energy and essential fatty acids.

It has been described that fat sources with high FFA levels, lead to low AME and digestibility values of added fat (Shannon, 1971; Wiseman and Salvador, 1991; Wiseman, 1992; Blanch et al., 1995, 1996; Vilà and Esteve-García, 1996). However, some authors have reported no differences on productive parameters (Young, 1961; Bornstein and Lipstein, 1961, 1963; Artman et al., 1964), as well as on the dietary AME when acid oils were included in adult broiler chicken diets (Vilarrasa et al., 2015; Roll et al., 2018).

It is accepted that the age has an influence on fat utilization, and several studies have reported an improvement on fat digestibility as the age increases (Renner and Hill, 1960; Batal and Parsons, 2002; Tancharoenrat et al., 2013, Rodriguez-Sanchez et al., 2018). Thus, it was hypothesized that adult broiler chickens are less affected by the dietary FFA level than young broiler chickens are, being able to use more efficiently not only saturated diets, but also diets with high levels of dietary FFA. For this reason, it is expected that the suitable inclusion level of acid oils in grower-finisher diets is higher than for starter diets.

This experiment was carried out in order to determine the potential use of acid oils in broiler chicken diets. The objective was to assess the effect of the FFA level, and saturation degree of dietary fat on fatty acid (**FA**) digestibility and lipid class composition along the gastrointestinal tract (**GIT**), and excreta in adult broiler chickens (37-day-old). Two acid oils and two crude oils from different origins were used in order to produce diets with different saturation degrees and FFA levels. This information will allow for a better understanding of the limitation of the use of acid oils in adult broiler chicken diets, as well as it will help to establish nutritional guidelines when using these fat by-products.

An accompanying paper (Part I) describes and discusses the results reported in young (14-day-old) broiler chickens.

# 5.3. Materials and methods

## 5.3.1. Animals and diets

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

A total of 528 one-day-old female broiler chickens of the Ross 308 strain were obtained from a commercial hatchery (Pondex SAU; Lleida, Spain), and randomly assigned to one of the eight dietary treatments, with eleven chicks per cage and six cages per treatment. Birds were housed in wire-floor cages. Throughout the study, feed and water were supplied for ad libitum consumption, and animals were raised under controlled conditions of light and temperature, as recommended by the breeder.

Birds received grower-finisher wheat- and soybean-meal-based diets (in mash form) that were formulated to meet or exceed FEDNA requirements (2008) and to minimize the basal level of fat. The ingredient composition of the basal diet is presented in Table 5.1. Titanium dioxide (**$TiO_2$**) was added (5 g/kg) as an inert marker for the determination of the digestibility of FA.

The basal diet was supplemented at 6% with different fats (crude oils, **O**; acid oils, **A**; or oil blends) in order to achieve eight dietary treatments. The eight dietary treatments resulted in a 2 x 4 factorial arrangement, with two different fat sources (soybean oil products as unsaturated fat source, and palm oil products as saturated fat source), and four levels of dietary FFA (5, 15, 35, and 50%). Thus, there were four soybean diets (**S**) with four increasing levels of FFA, and four palm diets (**P**) with four increasing levels of FFA. The different levels of dietary FFA were achieved blending crude soybean oil (**SO**) or crude palm oil (**PO**), with low levels of FFA (< 5% FFA), with an acid soybean oil (**SA**, a soybean acid oil from chemical refining with 67% FFA) or an acid palm oil (**PA**, a palm fatty acid distillate from physical refining with 99% FFA) respectively. Additional detail on the experimental design such as the proportions of each fat source in the blends is described in Part I (Rodriguez-Sanchez et al., Unpublished data).

**Table 5.1.** Ingredient composition of the experimental basal diet for the grower-finisher period

| Ingredients, % | Grower-finisher diet (from 22 to 37 d) |
|---|---|
| Wheat | 44.00 |
| Soybean meal 48% | 27.25 |
| Barley | 18.58 |
| Experimental fats[1] | 6.00 |
| Calcium carbonate | 1.39 |
| Sodium chloride | 0.35 |
| Vitamin and mineral premix[2] | 0.40 |
| DL-Methionine | 0.17 |
| L-Lysine | 0.12 |
| L-Threonine | 0.02 |
| Titanium oxide | 0.50 |
| Ethoxyquin 66% | 0.02 |

[1] Crude soybean oil, crude palm oil, acid soybean oil or acid palm oil in different proportions.

[2] Provides per kg of feed: vitamin A (from retinol), 13,500 IU; vitamin $D_3$ (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; vitamin $B_1$, 3 mg; vitamin $B_2$, 9 mg; vitamin $B_6$, 4.5 mg; vitamin $B_{12}$, 16.5 µg; vitamin $K_3$, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from $FeSO_4 \cdot 7H_2O$), 54 mg; I (from $Ca(I_2O_3)_2$), 1.2 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$), 0.6 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from $Na_2SeO_3$), 0.18 mg; Mo (from $(NH_4)_6Mo_7O_{24}$), 1.2 mg.

## 5.3.2. Controls and sampling

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

The results reported in young broiler chickens (14 d) are presented and discussed in Part I (Rodriguez-Sanchez et al., Unpublished data). The remaining birds (3 birds / cage) continued the experiment, and a second digestibility balance was carried out from 34 to 37 d. At 37 d of age, the 3 birds per cage were euthanized, and samples of content of the gizzard, duodenum (from the pyloric junction to the distal most point of insertion of the duodenal mesentery), jejunum (from the distal most point of insertion of the duodenal mesentery to the junction with Meckel’s diverticulum), ileum (from the junction with Meckel’s diverticulum to a point 1 cm proximal to the ileocecal junction), and a representative sample of excreta of each cage were taken. The digestive content of each segment of the GIT from all birds within each cage was pooled, homogenized, frozen at -20ºC, and lyophilized. After lyophilization, samples were ground to pass through a 0.5-mm sieve, and they were kept at 5°C until further analyses.

### 5.3.3. Chemical analysis and formulas used for the lipid class content and digestibility determination

All analytical determinations and formulas for the determination of the lipid class content and FA digestibility were performed as described in Part I (Rodriguez-Sanchez et al., Unpublished data). The macronutrient, FA and lipid class composition of the experimental diets are presented in Table 5.2.

### 5.3.4. Statistical analysis

Productive parameters, AME, lipid class content and digestibility of FA were subjected to a univariate analysis using GLM procedure of SPSS (SPSS statistics 25.0.0.0, IBM 2017) to study whether they depended on the added fat, and the dietary FFA level. In the case of the lipid class content and FA digestibility results, this analysis was performed for each GIT segment. A univariate analysis using the GLM procedure was also carried out for each one of the GIT segments in order to compare the results observed in young (Part I; Rodriguez-Sanchez et al., Unpublished data) and adult broiler chickens, both for lipid classes and FA digestibility coefficients.

For each GIT segment a regression analysis was carried out for S diets and for P diets in order to find the best-fit regression equations considering the saturated FA (**SFA**) digestibility as dependent variable and the level of dietary FFA as independent variable.

The cage served as the experimental unit, so there were six replicates per treatment. Tukey test was used to assess the differences among the eight dietary treatments. Results in tables are reported as least square means and differences were considered significant at $P < 0.05$.

**Table 5.2.** Analyzed[1] macronutrient content and fatty acid and lipid class composition of the experimental diets[2]

| | Grower-finisher diets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Unsaturated - S diets | | | | Saturated - P diets | | | |
| | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 |
| *Macronutrient content* | | | | | | | | |
| Dry matter % | 90.8 | 90.8 | 90.7 | 90.8 | 90.9 | 90.9 | 90.8 | 90.8 |
| Crude protein % | 19.3 | 19.9 | 20.2 | 19.9 | 19.8 | 20.1 | 19.5 | 19.8 |
| Crude fat % | 7.3 | 7.5 | 7.3 | 7.3 | 7.5 | 7.4 | 7.2 | 7.4 |
| Crude fiber % | 5.6 | 5.2 | 5.3 | 5.1 | 5.6 | 5.6 | 6.0 | 6.8 |
| Ash % | 6.1 | 6.2 | 6.0 | 6.3 | 5.8 | 6.0 | 6.0 | 6.1 |
| Gross energy, kcal/kg | 4134 | 4198 | 4175 | 4118 | 4301 | 4184 | 4172 | 4151 |
| AME[3] | 3019 | 3013 | 2987 | 2840 | 2985 | 2896 | 2758 | 2870 |
| *Fatty acid composition, %* | | | | | | | | |
| C14:0 | - | - | - | - | 1.1 | 1.1 | 1.0 | 1.0 |
| C16:0 | 14.39 | 14.73 | 16.21 | 17.21 | 37.80 | 37.64 | 38.38 | 39.94 |
| C18:0 | 4.02 | 4.18 | 4.62 | 4.71 | 3.85 | 3.83 | 3.78 | 3.88 |
| C18:1 n-9 | 20.50 | 20.56 | 20.96 | 20.92 | 31.26 | 30.67 | 30.19 | 30.32 |
| C18:1 n-7 | 1.35 | 1.36 | 1.34 | 1.34 | 0.85 | 0.85 | 0.82 | 0.80 |
| C18:2 n-6 | 52.37 | 51.94 | 50.20 | 49.52 | 22.38 | 23.13 | 23.09 | 21.45 |
| C18:3 n-3 | 6.12 | 5.90 | 5.26 | 4.79 | 1.71 | 1.78 | 1.79 | 1.71 |
| Minor fatty acids | 1.26 | 1.34 | 1.42 | 1.51 | 1.06 | 1.04 | 0.94 | 0.88 |
| SFA | 19.39 | 19.97 | 21.98 | 23.18 | 43.39 | 43.12 | 43.69 | 45.33 |
| MUFA | 22.12 | 22.19 | 22.56 | 22.51 | 32.51 | 31.97 | 31.43 | 31.52 |
| PUFA | 58.49 | 57.84 | 55.46 | 54.31 | 24.10 | 24.91 | 24.88 | 23.16 |
| UFA:SFA | 4.16 | 4.01 | 3.55 | 3.31 | 1.30 | 1.32 | 1.29 | 1.21 |
| *Lipid class composition, %* | | | | | | | | |
| TAG | 96.0 | 79.7 | 56.5 | 35.4 | 83.2 | 73.1 | 56.7 | 45.0 |
| DAG | 2.6 | 3.8 | 6.1 | 8.0 | 8.8 | 7.6 | 5.8 | 4.3 |
| MAG | 0.5 | 0.7 | 1.5 | 2.3 | 0.7 | 0.9 | 0.9 | 1.1 |
| FFA | 0.9 | 15.8 | 36.0 | 54.3 | 7.2 | 18.4 | 36.6 | 49.7 |

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

[2] Dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P); S5: crude soybean oil (S5), S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: acid soybean oil, P5: crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50:oil blend with 33% crude palm oil and 66% acid palm oil.

[3] Values are pooled means of 6 replicates with 3 chickens/replicate. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids; TAG = triacylglicerides; DAG = diacylglicerides; MAG = monoacylglicerides; FFA = free fatty acids.

# 5.4. Results

## 5.4.1. Characterization of experimental diets

The chemical analysis of the experimental diets for the grower-finisher period reflected the composition of the added oils and is presented in Table 5.2. The main FA in S diets were linoleic and oleic acids, while the main FA in P diets were palmitic, oleic, and linoleic acids. As it was observed in the S starter diets (Part I; Rodriguez-Sanchez et al., Unpublished data), in the S grower-finisher diets, linoleic acid decreased, and palmitic acid increased as the dietary FFA level increased because the FA composition of SA and SO differed. This feature was reflected in the unsaturated-to-saturated FA ratios (**UFA:SFA**); in S diets, this ratio decreased as the dietary FFA level increased (from 4.16 to 3.31), while in P diets this ratio remained constant regardless of the dietary FFA level (1.28 on average).

Regarding the lipid class composition of the experimental diets (Table 5.2), the triacylglycerols (**TAG**) and FFA% reflected the four increasing levels of dietary FFA; the higher the dietary FFA level, the lower TAG% and higher FFA%. Thus, TAG% decreased from S5 to S50, and from P5 to P50 (from more than 83% to 45% or less). Regarding diacylglycerols (**DAG**) %, the pattern was different for S and P diets, while in S diets there was an increase from S5 to S50 (from 2.6% to 8.0%), in P diets a DAG% decrease was observed from P5 to P50 (from 8.8% to 4.3%). The monoacylgycerols (**MAG**) %, both for S and P diets, increased as A replaced the corresponding O.

Considering the AME values, there was a significant interaction between the saturation degree of fat and the dietary FFA level ($P < 0.001$; S5: 3019a, S15: 3013a, S35: 2987a, S50: 2840cd, P5: 2985ab, P15: 2896bc, P35: 2758d, P50: 2870c). The AME values reported for S5, S15, S35, and P5 were equivalent and higher in comparison to the rest of the dietary treatments, and the AME value observed for S50 was similar to the values reported for P15, P35, and P50.

### 5.4.2. Growth performance

The broiler chickens completed the trial successfully. The effect of saturation degree and FFA level of dietary fat on growth performance from the grower-finisher period (22 to 37 d) is presented in Table 5.3.

Considering the saturation degree of fat, an effect was observed for the BW at 37 d ($P < 0.01$); the weight of the chickens fed the S diets was higher in comparison to those fed the P diets. An effect was also observed for FCR ($P < 0.01$), which was lower for the chickens fed the S diets than for those fed the P diets. The dietary FFA level had an effect on BW at 37 d ($P = 0.001$); the value reported for the chickens fed the 15% FFA diets was higher in comparison to the values reported for the chickens fed the 5% FFA and 35% FFA diets, and no differences were reported among the chickens fed the rest of the diets.

**Table 5.3.** Growth performance of broiler chickens (from 22 to 37 d) according to different fat sources in the diet[1]

| | Dietary treatments[2] | | | | | | | | Saturation degree[3] | | FFA level (%)[4] | | | | *P* - values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unsaturated – S diets | | | | Saturated – P diets | | | | | | | | | | | | | |
| Item | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 | S | P | 5 | 15 | 35 | 50 | SEM | Fat | FFA | Interaction |
| ADG, g/b/d | 85.3 | 90.9 | 87.4 | 90.4 | 86.1 | 90.4 | 88.5 | 85.5 | 88.5 | 87.6 | 85.7 | 90.7 | 88.0 | 87.9 | 0.622 | 0.49 | 0.06 | 0.30 |
| ADFI, g/b/d | 142.2 | 149.0 | 148.2 | 146.8 | 146.8 | 151.0 | 148.9 | 149.0 | 146.9 | 148.9 | 144.5 | 149.9 | 148.5 | 147.9 | 0.985 | 0.24 | 0.27 | 0.91 |
| FCR, g/g | 1.67 | 1.67 | 1.70 | 1.63 | 1.73 | 1.69 | 1.71 | 1.74 | 1.66 | 1.72 | 1.70 | 1.68 | 1.70 | 1.68 | 0.008 | <0.01 | 0.66 | 0.13 |
| BW at 37 d, g | 2180.6 | 2337.4 | 2190.2 | 2290.1 | 2156.2 | 2280.3 | 2194.3 | 2172.3 | 2256.0 | 2192.6 | 2152.0b | 2308.8a | 2192.3b | 2244.1ab | 11.994 | <0.01 | 0.001 | 0.196 |

[1] Values are means of 6 replicates with 3 chickens / replicate fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P).

[2] S5: crude soybean oil (S5), S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: acid soybean oil, P5: crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50:oil blend with 33% crude palm oil and 66% acid palm oil.

[3] S is the average of S5, S15, S35 and S50 diets; P is the average of P5, P15, P35, and P50 diets.

[4] 5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets.

ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; BW = body weight; SEM = standard error of the mean.

*P*-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected growth performance values. $P < 0.05$ was considered significant.

## 5.4.3. Lipid class composition along the gastrointestinal tract

The lipid class content in different GIT segments (gizzard, duodenum, jejunum, and ileum) and excreta for the eight dietary treatments is presented in Table 5.4.

The general evolution of lipid classes showed a decrease of TAG content from the gizzard to the duodenum, and a parallel increase of DAG, MAG and FFA content in the duodenum, which decreased progressively from the duodenum to the ileum.

Regarding the effect of the dietary fat source on the lipid class content in the gizzard, it was observed that the content of all lipid classes was higher in the chickens fed S diets than in those fed P diets ($P \leq 0.023$). In the duodenum, the content of the lipolysis products (DAG, MAG, and FFA) was also higher for the chickens fed S diets than for those fed  P diets ($P \leq 0.007$). A fat saturation degree effect was also observed for the FFA content in the jejunum and excreta, where the content was lower for the chickens fed S diets than for the chickens fed P diets ($P < 0.001$). In the jejunum, TAG content was higher for the chickens fed S diets than for those fed P diets ($P < 0.001$).

Regarding the dietary FFA level, an effect was observed in the gizzard for TAG and FFA. In the case of TAG, the value reported for the chickens fed the 50% FFA diets was lower in comparison to the values reported for the chickens fed the 5% and 15% FFA diets. Regarding the FFA, the content for the chickens fed the 5% FFA diets was the lowest, and the content reported for those fed the 35% and 50% FFA diets was higher in comparison to the rest. In the jejunum and excreta a dietary FFA effect was also observed. In the jejunum, the FFA content for the chickens fed the 5% FFA diets was lower in comparison to the ones fed the 15% FFA diets, but no differences were observed among the rest of the dietary FFA levels. In the excreta, the chickens fed the 5% FFA diets had lower FFA content than the ones fed the 15%, 35%, and 50% FFA diets, and no differences were observed among these three FFA levels.

**Table 5.4.** Lipid class content[1] along the gastrointestinal tract and excreta according to different fat sources in the diet[2] in 37-day-old broiler chicken

| | Dietary treatments[3] | | | | | | | | Saturation degree[4] | | FFA level (%)[5] | | | | *P* - values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unsaturated – S diets | | | | Saturated – P diets | | | | | | | | | | | | | |
| Item | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 | S | P | 5 | 15 | 35 | 50 | SEM | Fat | FFA | Interaction |
| *Gizzard* | | | | | | | | | | | | | | | | | | |
| TAG | 11.78 | 13.69 | 9.31 | 8.47 | 9.57 | 8.04 | 6.21 | 5.30 | 10.81 | 7.28 | 10.67a | 10.86a | 7.76ab | 6.89b | 0.419 | <0.001 | 0.003 | 0.465 |
| DAG | 2.78 | 3.02 | 2.96 | 3.33 | 2.26 | 2.30 | 2.09 | 2.22 | 3.02 | 2.22 | 2.52 | 2.66 | 2.52 | 2.78 | 0.067 | <0.001 | 0.492 | 0.462 |
| MAG | 1.16 | 1.21 | 1.16 | 1.47 | 0.93 | 0.99 | 0.99 | 1.01 | 1.25 | 0.98 | 1.05 | 1.1 | 1.07 | 1.24 | 0.030 | <0.001 | 0.119 | 0.373 |
| FFA | 7.67 | 9.19 | 12.79 | 15.41 | 6.67 | 9.81 | 11.26 | 12.13 | 11.27 | 9.97 | 7.17c | 9.51b | 12.03a | 13.77a | 0.273 | 0.023 | <0.001 | 0.106 |
| *Duodenum* | | | | | | | | | | | | | | | | | | |
| TAG | 4.23 | 4.16 | 4.29 | 3.53 | 4.31 | 6.04 | 4.31 | 4.55 | 4.05 | 4.80 | 4.27 | 5.10 | 4.30 | 4.04 | 0.336 | 0.273 | 0.684 | 0.728 |
| DAG | 27.02 | 23.42 | 23.77 | 18.34 | 17.34 | 19.30 | 16.00 | 18.99 | 23.14 | 17.91 | 22.18 | 21.36 | 19.89 | 18.66 | 0.914 | 0.007 | 0.508 | 0.192 |
| MAG | 9.47 | 8.57 | 10.14 | 8.52 | 5.38 | 5.84 | 5.03 | 5.34 | 9.17 | 5.40 | 7.42 | 7.21 | 7.58 | 6.93 | 0.434 | <0.001 | 0.953 | 0.793 |
| FFA | 157.77 | 133.05 | 129.74 | 96.81 | 82.45 | 104.02 | 76.90 | 98.60 | 129.34 | 90.49 | 120.11 | 118.54 | 103.32 | 97.70 | 5.723 | 0.002 | 0.386 | 0.082 |
| *Jejunum* | | | | | | | | | | | | | | | | | | |
| TAG | 0.74 | 0.89 | 0.91 | 1.00 | 0.40 | 0.39 | 0.40 | 0.37 | 0.88 | 0.39 | 0.57 | 0.64 | 0.65 | 0.68 | 0.027 | <0.001 | 0.479 | 0.298 |
| DAG | 1.02ab | 1.33ab | 0.94ab | 1.55a | 0.96ab | 1.14ab | 1.20ab | 0.88b | 1.21 | 1.05 | 0.99 | 1.24 | 1.07 | 1.21 | 0.048 | 0.093 | 0.222 | 0.013 |
| MAG | 0.60ab | 0.76ab | 0.71ab | 0.88a | 0.66ab | 0.56ab | 0.69ab | 0.45b | 0.74 | 0.58 | 0.62 | 0.66 | 0.70 | 0.66 | 0.027 | 0.007 | 0.761 | 0.016 |
| FFA | 3.33 | 4.42 | 3.78 | 4.31 | 5.18 | 6.52 | 6.49 | 4.88 | 3.96 | 5.77 | 4.26b | 5.47a | 5.13ab | 4.6ab | 0.153 | <0.001 | 0.033 | 0.103 |
| *Ileum* | | | | | | | | | | | | | | | | | | |
| TAG | 0.30b | 0.38ab | 0.53a | 0.53a | 0.30b | 0.33b | 0.28b | 0.30b | 0.44 | 0.30 | 0.30 | 0.36 | 0.41 | 0.41 | 0.013 | <0.001 | 0.022 | 0.002 |
| DAG | 0.45b | 0.58b | 0.71ab | 0.90a | 0.58b | 0.57b | 0.55b | 0.51b | 0.66 | 0.55 | 0.52 | 0.57 | 0.63 | 0.7 | 0.022 | 0.018 | 0.026 | 0.001 |
| MAG | 0.65 | 0.72 | 0.73 | 0.75 | 0.73 | 0.66 | 0.75 | 0.67 | 0.71 | 0.71 | 0.69 | 0.69 | 0.74 | 0.71 | 0.030 | 0.917 | 0.929 | 0.732 |
| FFA | 1.27d | 1.36d | 1.56d | 1.91d | 2.88c | 4.17a | 3.70ab | 3.27bc | 1.52 | 3.51 | 2.07 | 2.76 | 2.63 | 2.59 | 0.061 | <0.001 | 0.002 | 0.001 |
| *Excreta* | | | | | | | | | | | | | | | | | | |
| TAG | 0.31 | 0.44 | 0.48 | 0.49 | 0.43 | 0.40 | 0.36 | 0.40 | 0.43 | 0.40 | 0.37 | 0.42 | 0.42 | 0.44 | 0.019 | 0.42 | 0.528 | 0.118 |
| DAG | 0.61b | 0.88ab | 1.10a | 0.89ab | 0.66b | 0.87ab | 0.74ab | 0.67b | 0.87 | 0.74 | 0.64 | 0.89 | 0.93 | 0.78 | 0.028 | 0.02 | 0.004 | 0.045 |
| MAG | 0.40bc | 0.61ab | 0.49abc | 0.52abc | 0.40bc | 0.37c | 0.41bc | 0.63a | 0.51 | 0.45 | 0.40 | 0.49 | 0.45 | 0.58 | 0.016 | 0.118 | 0.005 | 0.007 |
| FFA | 3.13 | 4.19 | 4.18 | 4.30 | 4.48 | 5.40 | 6.40 | 7.20 | 3.95 | 5.87 | 3.8b | 4.80a | 5.29a | 5.75a | 0.129 | <0.001 | <0.001 | 0.097 |

[1] Calculated from the ratio lipid class concentration (mg/g) / Ti concentration (mg/g) in each segment of the gastrointestinal tract and excreta. [2] Values are pooled means of 6 replicates with 3 chickens / replicate fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P). [3] S5: crude soybean oil (S5), S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: acid soybean oil, P5: crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50:oil blend with 33% crude palm oil and 66% acid palm oil. [4] S is the average of S5, S15, S35 and S50 diets; P is the average of P5, P15, P35, and P50 diets. [5] 5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets. TAG = triacylglicerols, DAG = diacylglicerols, MAG = monoacylglicerols, FFA = free fatty acids, SEM = standard error of the mean. *P*-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected lipid classes results. $P < 0.05$ was considered significant. a,b,c,d allude to Tukey-test.

A significant interaction between the fat source and the dietary FFA level was observed for DAG and MAG in the jejunum. In both cases, the chickens fed the S50 diet had higher DAG and MAG content in comparison to the ones fed the P50 diet, while no differences were observed between those chickens fed S and P diets for the rest of the dietary FFA levels. An interaction was observed in the ileum for TAG, DAG, and FFA. Regarding TAG, while in the chickens fed S diets the content was higher for S35 and S50 than for S5, in those chickens fed P diets, the TAG content was similar among P5, P15, P35, and P50, as well as to S5 and S15. Regarding DAG, the chickens fed S50 diet had higher content than those fed S5, S15, and all P diets (P5, P15, P35, and P50), and no differences were observed among the chickens fed the rest of the diets. The FFA content in the ileum for the chickens fed S diets was lower than it was for those fed P diets; in P, the FFA content reported for P15 was higher than for P5 and P50. The interaction reported in the excreta for DAG could be explained by the numerically higher DAG content observed for the chickens fed S35 diet in comparison to the chickens fed S5, P5, and P50 diets. Regarding the interaction reported for MAG in the excreta, it was explained by the higher MAG content observed for the chickens fed S15 in comparison to the chickens fed the respective P diet (P15). Furthermore, MAG content in the chickens fed P diets was more affected by the dietary FFA level than it was for the chickens fed S diets, as the MAG content for P50 was higher than for P5, P15 and P35, and no differences were observed among S5, S15, S35, and S50.

## 5.4.4. Fatty acid digestibility along the gastrointestinal tract

The FA digestibility coefficients reported in different segments of the GIT, and excreta for the eight dietary treatments are presented in Table 5.5. The digestibility of SFA was mainly represented by palmitic (C16:0) and stearic (C18:0) acids, the digestibility of monounsaturated FA (**MUFA**) by oleic acid (C18:1n9), and the digestibility of polyunsaturated FA (**PUFA**) by linoleic (C18:2n6) acid.

**Table 5.5.** Fatty acid digestibility coefficients along the gastrointestinal tract and excreta according to different fat sources in the diet[1] in 37-day-old broiler chicken

| | Dietary treatments | | | | | | | | Saturation degree[3] | | FFA level (%)[4] | | | | *P* - values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unsaturated – S diets | | | | Saturated – P diets | | | | | | | | | | | | | |
| Item | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 | S | P | 5 | 15 | 35 | 50 | SEM | Fat | FFA | Interaction |
| *Gizzard* | | | | | | | | | | | | | | | | | | |
| TFA | -0.02 | -0.17 | -0.05 | -0.37 | 0.04 | 0.00 | 0.15 | -0.02 | -0.15 | 0.04 | 0.01a | -0.09ab | 0.05a | -0.19b | 0.024 | <0.001 | 0.006 | 0.219 |
| SFA | -0.13cd | -0.27de | -0.08bcd | -0.46e | 0.20ab | 0.09abc | 0.23a | 0.20ab | -0.24 | 0.18 | 0.04 | -0.09 | 0.07 | -0.13 | 0.023 | <0.001 | 0.009 | 0.035 |
| MUFA | 0.00 | -0.17 | -0.07 | -0.33 | 0.17 | 0.07 | 0.18 | 0.03 | -0.14 | 0.11 | 0.09a | -0.05ab | 0.06a | -0.15b | 0.020 | <0.001 | <0.001 | 0.328 |
| PUFA | 0.01 | -0.14 | -0.04 | -0.29 | -0.41 | -0.19 | -0.04 | -0.51 | -0.12 | -0.29 | -0.20ab | -0.17ab | -0.04a | -0.40b | 0.036 | 0.023 | 0.011 | 0.164 |
| *Duodenum* | | | | | | | | | | | | | | | | | | |
| TFA | -7.19 | -5.36 | -5.40 | -5.09 | -3.95 | -5.30 | -2.58 | -4.17 | -5.76 | -4.00 | -5.57 | -5.33 | -3.99 | -4.63 | 0.258 | 0.001 | 0.141 | 0.109 |
| SFA | -13.85 | -10.16 | -9.30 | -8.83 | -3.69 | -4.97 | -2.38 | -3.70 | -10.53 | -3.68 | -8.77 | -7.56 | -5.84 | -6.26 | 0.432 | <0.001 | 0.086 | 0.151 |
| MUFA | -4.10b | -2.87ab | -3.14ab | -2.91ab | -2.31ab | -3.59ab | -1.72a | -2.50ab | -3.26 | -2.53 | -3.21 | -3.23 | -2.43 | -2.71 | 0.165 | 0.033 | 0.259 | 0.045 |
| PUFA | -6.16 | -4.67 | -4.85 | -4.40 | -6.63 | -8.08 | -4.02 | -7.35 | -5.02 | -6.52 | -6.39 | -6.37 | -4.43 | -5.88 | 0.301 | 0.017 | 0.086 | 0.051 |
| *Jejunum* | | | | | | | | | | | | | | | | | | |
| TFA | 0.84a | 0.84a | 0.84a | 0.79a | 0.71b | 0.66b | 0.68b | 0.70b | 0.83 | 0.69 | 0.77 | 0.75 | 0.76 | 0.75 | 0.005 | <0.001 | 0.229 | 0.019 |
| SFA | 0.64 | 0.64 | 0.64 | 0.53 | 0.60 | 0.56 | 0.57 | 0.64 | 0.61 | 0.59 | 0.62 | 0.60 | 0.60 | 0.59 | 0.012 | 0.427 | 0.790 | 0.025 |
| MUFA | 0.83 | 0.83 | 0.82 | 0.78 | 0.81 | 0.78 | 0.78 | 0.80 | 0.81 | 0.79 | 0.82 | 0.81 | 0.80 | 0.79 | 0.005 | 0.028 | 0.185 | 0.036 |
| PUFA | 0.91 | 0.92 | 0.93 | 0.90 | 0.77 | 0.69 | 0.73 | 0.77 | 0.91 | 0.74 | 0.84 | 0.81 | 0.83 | 0.84 | 0.006 | <0.001 | 0.265 | 0.058 |
| *Ileum* | | | | | | | | | | | | | | | | | | |
| TFA | 0.92 | 0.90 | 0.90 | 0.86 | 0.79 | 0.75 | 0.77 | 0.79 | 0.89 | 0.78 | 0.85 | 0.82 | 0.83 | 0.83 | 0.006 | <0.001 | 0.259 | 0.055 |
| SFA | 0.81a | 0.76ab | 0.77ab | 0.69abc | 0.68bc | 0.60c | 0.66bc | 0.69abc | 0.76 | 0.66 | 0.74 | 0.68 | 0.72 | 0.69 | 0.010 | <0.001 | 0.086 | 0.025 |
| MUFA | 0.91a | 0.89ab | 0.90ab | 0.86b | 0.88ab | 0.86b | 0.88ab | 0.89ab | 0.89 | 0.88 | 0.90 | 0.88 | 0.89 | 0.87 | 0.004 | 0.086 | 0.078 | 0.035 |
| PUFA | 0.95 | 0.95 | 0.95 | 0.93 | 0.86 | 0.86 | 0.88 | 0.87 | 0.95 | 0.87 | 0.91 | 0.91 | 0.91 | 0.90 | 0.003 | <0.001 | 0.536 | 0.217 |
| *Excreta* | | | | | | | | | | | | | | | | | | |
| TFA | 0.86 | 0.83 | 0.82 | 0.79 | 0.74 | 0.69 | 0.69 | 0.70 | 0.82 | 0.71 | 0.80a | 0.76b | 0.75b | 0.75b | 0.005 | <0.001 | 0.002 | 0.287 |
| SFA | 0.78 | 0.72 | 0.72 | 0.67 | 0.65 | 0.55 | 0.54 | 0.57 | 0.72 | 0.58 | 0.71a | 0.63b | 0.63b | 0.62b | 0.008 | <0.001 | 0.001 | 0.313 |
| MUFA | 0.87a | 0.84abc | 0.82bc | 0.80c | 0.85ab | 0.84abc | 0.84abc | 0.85ab | 0.83 | 0.84 | 0.86 | 0.84 | 0.83 | 0.82 | 0.004 | 0.107 | 0.002 | 0.014 |
| PUFA | 0.88a | 0.86ab | 0.85ab | 0.82bc | 0.75d | 0.75d | 0.78cd | 0.77d | 0.85 | 0.76 | 0.82 | 0.80 | 0.82 | 0.79 | 0.004 | <0.001 | 0.122 | 0.006 |

[1] Values are pooled means of 6 replicates with 3 chickens / replicate fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P). [2] S5: crude soybean oil (S5), S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: acid soybean oil, P5: crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50:oil blend with 33% crude palm oil and 66% acid palm oil. [3] S is the average of S5, S15, S35 and S50 diets; P is the average of P5, P15, P35, and P50 diets. [4] 5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets. TFA = total fatty acids, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SEM = standard error of the mean. *P*-values were obtained from two-way ANOVA conducted to study whether the saturation degree and FFA level of dietary fat affected the FA digestibility results. $P < 0.05$ was considered significant. a,b,c,d allude to Tukey-test.

Regarding the saturation degree of dietary fat, an effect was observed in the gizzard for total FA (**TFA**) and MUFA ($P < 0.001$), being the digestibility coefficients lower (more negative) for the chickens fed S diets than for those fed P diets. The same was observed in the duodenum for TFA and SFA ($P \leq 0.001$). An effect was also observed for PUFA, both in the gizzard ($P = 0.023$) and duodenum ($P = 0.01$), in this case being the digestibility coefficient higher (less negative) for the chickens fed S diets than for the chickens fed P diets. In the jejunum, and ileum PUFA digestibility coefficient was higher for the chickens fed S diets than for those fed P diets ($P < 0.001$). The same effect was reported in the ileum and excreta for TFA ($P < 0.001$), and in the excreta for SFA ($P < 0.001$).

A FFA effect was observed in the gizzard for TFA, MUFA, and PUFA digestibility ($P \leq 0.01$). In the case of TFA and MUFA, the digestibility coefficient reported for the chickens fed the 50% FFA diets was lower (more negative) than it was for the chickens fed the 5% and 35% FFA diets, and no differences were reported among the chickens fed the other diets. In the case of PUFA, the digestibility coefficient observed for the chickens fed the 50% FFA diets was lower in comparison to the ones fed the 35% FFA diets, and no differences were observed among the chickens fed the rest of the diets. A FFA effect was also observed in the excreta for TFA and SFA digestibility ($P \leq 0.002$), where the coefficients reported for the chickens fed the 5% FFA diets were higher in comparison to the ones reported for the chickens fed the 15%, 35%, and 50% FFA diets. However, a significant interaction between the fat source and the dietary FFA level was observed for SFA in the gizzard ($P = 0.03$), jejunum ($P = 0.02$), and ileum ($P = 0.02$). These interactions showed that the SFA digestibility coefficients in those chickens fed S diets were more affected by the dietary FFA level than in those chickens fed P diets, as in general the coefficient reported for S5 was higher than the coefficient for S50, and the coefficients for P5 were not significantly different than for P50. In the duodenum, a significant interaction was observed for MUFA ($P = 0.04$); while in the chickens fed S diets the digestibility coefficients were numerically higher (less negative) as the FFA level increased (especially from the S5 to the S15 diet), in the chickens fed P diets, the digestibility coefficients numerically decreased from P5 to P15, and increased from P15 to P35. An interaction was also reported for MUFA in the jejunum, ileum, and excreta. In the ileum and excreta, MUFA digestibility coefficient for the chickens fed S5 diet was lower than for the chickens fed S50 diet (and also than for those fed S35 diet in the excreta); the coefficients reported for the chickens fed P5, P15, P35, and P50 diets were similar. The interaction reported for PUFA digestibility in the excreta was also explained by the different response of the chickens fed S diets and the chickens fed P diets to the increase of dietary FFA level; thus, while the coefficient for S5 was higher than for S50, no differences were reported among the chickens fed the four P diets.

Regarding the regression analysis, the most relevant results were found in the ileum. The best-fit regression equations between the SFA digestibility coefficients observed in the ileum and the four increasing levels of dietary FFA are presented in Figure 5.1. The best-fit regression equation for the SFA digestibility in S diets was quadratic (*P* = 0.007); the coefficients observed for S5, S15, and S35 were numerically similar, while the one reported for S50 was numerically lower, and similar to the values reported for P5, P15, P35, and P50. On the other hand, the coefficients observed for the four increasing dietary FFA levels in the chickens fed P diets were numerically similar, and did not fit any model.

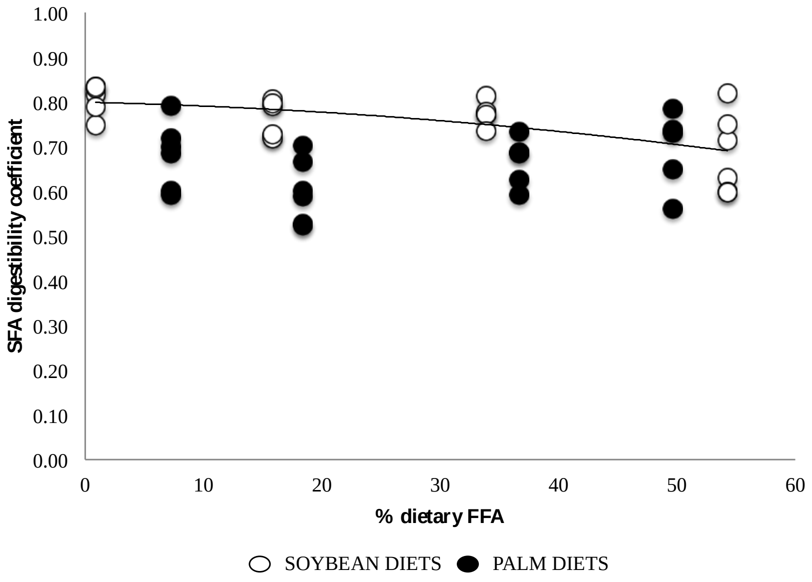

**Figure 5.1.** Relation between the saturated fatty acid digestibility calculated in the ileum and the four dietary free fatty acid levels[1] for two different fat sources in 37-day-old broiler chickens

[1] Diets with an average of 5% FFA, 15% FFA, 35% FFA, 50% FFA.

Each point represents each replicate value (with 3 chickens / replicate).

SFA: saturated fatty acids; FFA: free fatty acids.

The black line illustrates the quadratic model observed for the unsaturated diets:

$y = -3^{-5}x^2 - 0.006x$, where $R^2 = 0.39$, and $P = 0.007$

*P* Interaction fat x dietary FFA = 0.025.

The digestibility coefficients of TFA and all the FA families in the excreta were, in general, numerically lower than in the ileum. Part of this decrease could be related to bacterial activity in the hindgut as described at 14 d (Part I; Rodriguez-Sanchez et al., unpublished data). In order to confirm this, the FA detected in the excreta that could come from bacterial metabolism were added up (capric acid, C10:0; margaric acid, C17:0; elaidic acid, C18:1 9t; and vaccenic acid, C18:1 11t) and compared among the different diets (S5: 1.29; S15: 1.60; S35: 1.56; S50: 1.61; P5: 1.50; P15: 1.55; P35: 1.86; P50: 1.42 mg/g DM). A dietary FFA effect ($P$ = 0.05) was observed; the concentration of these four FA in the excreta was significantly higher for the chickens fed the 35% FFA diet in comparison to the chickens fed the 5% FFA diets, and no differences were observed among the chickens fed the other diets (5% FFA: 1.40b, 15% FFA: 1.58ab; 35% FFA: 1.71a; 50% FFA: 1.51ab).

### 5.4.5. Effect of the age on lipid class composition and fatty acid digestibility along the gastrointestinal tract

In order to determine the effect of the age of chickens on the studied parameters, the results reported at 14 d (Part I, Rodriguez et al. Unpublished data) were compared with the results reported at 37 d. The data of the gizzard and duodenum were compared to assess the effect of the age on the hydrolysis process, and the data of the jejunum and ileum were compared to assess the effect of the age on the absorption process.

The content of MAG in the gizzard was higher at 37 d than at 14 d ($P$ = 0.009), and the digestibility coefficients for TFA and all FA groups were higher (less negative) at 37 d than at 14 d ($P$ < 0.001). In the duodenum, the content of all the lipid classes was lower ($P$ < 0.001), and the digestibility coefficients of TFA and all FA groups were higher ($P$ < 0.001) in adult than in young broiler chickens.

In the jejunum and ileum, an effect of the age was observed for the lipid class content, this being especially important for FFA content. In fact, an interaction between the fat source and age was reported in these two GIT segments for FFA content ($P \leq$ 0.027); the FFA content at 37 d was lower than at 14 d, being this decrease more pronounced for the chickens fed P diets than for those fed S diets. The decrease of the FFA content at 37 d was also reflected on the digestibility coefficients: TFA digestibility in the jejunum was higher at 37 d than at 14 d, being this increase higher for the chickens fed S diets than for the chickens fed P diets. MUFA and PUFA digestibility were also higher in adult than in young broiler chickens, but in this case, the increase was higher for the chickens fed P diets than for those fed S diets. The digestibility of SFA was higher at 37 d both in the jejunum and ileum (*P* < 0.001).

In the jejunum, an interaction between the dietary FFA level and age was observed both for MUFA and PUFA digestibility coefficients (*P* ≤ 0.034); while at 14 d there was a tendency for MUFA digestibility coefficients to decrease as the dietary FFA level increased, at 37 d the coefficients among the four increasing levels of dietary FFA were similar (Figure 5.2).

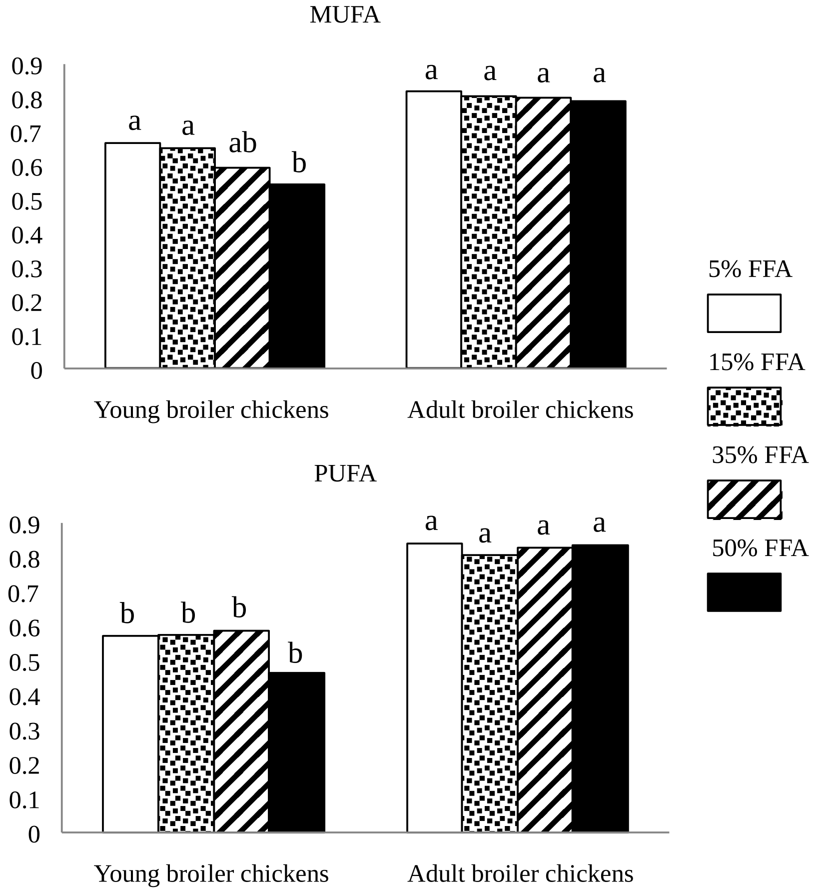

**Figure 5.2.** Digestibility coefficients for monounsaturated and polyunsaturated fatty acids in the jejunum for the four increasing levels of dietary free fatty acids[1] in young and adult broiler chickens.

[1] Diets with an average of 5% FFA, 15% FFA, 35% FFA, 50% FFA.

Values are means of 12 replicates per each dietary FFA level with 8 and 3 chickens / replicate in young (14 d), and adult (37 d) broiler chickens, respectively.

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; FFA = free fatty acids

*P* Interaction dietary FFA x age: 0.034 for MUFA, and 0.033 for PUFA. a, b allude to Tukey-test

# 5.5. Discussion

## 5.5.1. Dietary fat saturation effect

The data of this experiment show that adult broiler chickens can better utilize unsaturated diets rather than saturated diets. This better utilization was evident both in the hydrolysis and absorption processes. Considering the effect of fat saturation degree in the hydrolysis process, the higher content of all the lipid classes, and the more negative TFA and MUFA digestibility coefficients reported in the gizzard for those chickens fed S diets (which show that there were more TFA and MUFA in the gizzard than in the feed), could be explained by a higher secretion of endogenous fat into the duodenum lumen, and the gastro-duodenal reverse peristalsis, which allows for the movement of digesta from the duodenum to the gizzard. This was also supported by the higher content of all the lipolysis products (DAG, MAG and FFA) in the duodenum, as well as the lower (more negative) TFA and SFA digestibility coefficients reported for those animals fed S diets in comparison to those fed P diets. Furthermore, this could also be related to a higher hydrolysis rate of TAG. As explained in our previous study (Rodriguez-Sanchez et al., 2018), it is likely that the saturation degree of fat influences the secretion of endogenous fat, especially bile, which is the biggest source of lipids secreted into the duodenum (Tancharoenrat et al., 2014). There are also other components in the endogenous fat losses, like mucin-associated lipids, desquamated epithelial cells, lipids derived from blood circulating through the intestinal mucosa or newly synthesized by the enterocytes (Cotton, 1972; Hurwitz et al., 1973; Clément, 1980; Gong et al., 1990) that could explain the more negative TFA and SFA digestibility coefficients reported in the duodenum for the chickens fed S diets.

Regarding the effect of fat saturation degree in the absorption process, the lower FFA content observed in the jejunum, ileum, and excreta, and the higher digestibility coefficients observed for TFA in the jejunum, ileum and excreta, for PUFA in the jejunum and ileum, and for SFA in the excreta in those animals fed S diets, in comparison to those fed P diets, supported the more efficient absorption of the unsaturated fat. Considering the digestibility in the ileum as the maximum, as there is no effective utilization of fat by the animal after this GIT segment (Renner, 1965), and expressing the digestibility coefficients reported in the jejunum and ileum towards this value, it was seen that the jejunum was the main place of fat absorption irrespectively of the fat source (> 80%). The contribution of the ileum was especially important for SFA with differences depending on the dietary fat source: 19% of the absorption of SFA was reported in the ileum in those animals fed S diets, while in those fed P diets the contribution was 10%. This is in accordance with what it was reported in our previous study (Rodriguez-Sanchez et al., 2018), and it possibly explains the higher utilization of the unsaturated fat. The higher BW at 37d, and the lower FCR observed for those animals fed the S diets, in comparison to those fed the P diets also supported the higher utilization of the unsaturated diets.

## 5.5.2. Dietary free fatty acid effect

While some authors have reported no effects of the dietary FFA level on the performance parameters (Young, 1961; Bornstein and Lipstein, 1961, 1963; Vilarrasa et al., 2015), others have reported a negative effect (Artman et al., 1964; Zumbado et al., 1999). Regarding the results of the present study, the authors have not found an explanation for the higher BW reported at 37 d for the chickens fed the 15% FFA diets in comparison to the ones fed the 5% and 35% FFA diets.

According to the present results it is not possible to confirm an effect of the dietary FFA level on the hydrolysis process. The FFA effect reported in the gizzard for TAG and FFA, and the lower TFA, MUFA, and PUFA digestibility coefficients observed in general for the chickens fed the 50% FFA diets, reflected the composition of the different oils added in the diets. However, the interaction reported in the gizzard for SFA digestibility suggest that dietary FFA level could affect reverse peristalsis in those chickens fed S diets, being this supported by the highly negative digestibility coefficient reported for the chickens fed S50 diet, which could be due to an enhanced reverse peristalsis. The lack of differences in the duodenum (where the hydrolysis of TAG mainly takes place) among the four increasing levels of dietary FFA, both for the FA digestibility and lipid class content could suggest that the dietary FFA level do not affect the hydrolysis process. However, the results reported in this GIT segment are highly influenced by the secretion of endogenous fat into the duodenal lumen, as well as by the gastro-duodenal reverse peristalsis, which difficult their interpretation. On the other hand, it has been described that FFA can enhance the activity of pancreatic lipase (Larsson and Erlanson-Albertsson, 1986). However, this cannot be confirmed with the present results, as the enzymatic activity of pancreatic lipase was not determined.

Regarding the absorption process, the present results suggest that it was affected by the dietary FFA level. However, the interactions reported in the ileum for TAG, DAG, and FFA, as well as the interactions reported for SFA and MUFA digestibility, suggest that the effect was different for the chickens fed S diets than for those chickens fed P diets. Thus, in general, in the ileum while S50 was associated with a lower fat utilization in comparison to S5, the fat utilization among the chickens fed P5, P15, P35, and P50 was similar, and despite the fact P15 was associated with a high FFA content, P5 was not different than P50. This is also in accordance with the regression analysis. As explained before, the most relevant results were found in the ileum, which is a good indicator of the whole FA absorption process as no FA absorption has been described after this GIT segment (Renner, 1965). It is also well known that the absorption of SFA is more impaired than the absorption of UFA (Renner and Hill, 1961), for this reason, the regression analysis was performed considering the SFA digestibility coefficients. Thus, the regression analysis for ileal SFA digestibility coefficients supported the greater effect of dietary FFA level in those chickens fed the unsaturated diets, as the coefficient reported for the chickens fed S50 diet tended to be lower than for the chickens fed S5, S15, and S35 diets, and similar to P5, P15, P35, and P50 diets. Regarding P diets, the coefficients among the chickens fed P5, P15, P35, and P50 diets were similar, and in general, more variable in comparison to the coefficients reported for the chickens fed S diets.

All the mentioned above suggest that the chickens fed the unsaturated diets were more affected than the ones fed the saturated diets by increasing the dietary FFA level up to 50%. Also, fat utilization was more affected by the saturation degree of dietary fat rather than by the level of dietary FFA, which is in accordance with Vilarrasa et al. (2015), and it was also observed in young broiler chickens (Part I; Rodriguez-Sanchez et al., Unpublished data). The UFA:SFA ratios of the different diets evidenced this higher impact of the saturation degree on fat utilization; while in S diets SFA increased as the dietary FFA level increased (UFA:SFA ratios: 4.16 and 3.31, for S5 and S50, respectively), in P diets saturated FA remained more constant (UFA:SFA ratios: 1.30 and 1.21, for P5 and P50, respectively) as the dietary FFA level increased. The different trend in the UFA:SFA ratio was a consequence of the greater differences in the UFA profile between SO and SA in comparison to the differences between PO and PA, as well as to the higher proportion of inclusion of acid oil in the S blends.

Regarding the results reported in the excreta, the lower FFA content, and the higher TFA and SFA digestibility coefficients observed for the chickens fed the 5% FFA diets, in comparison to the chickens fed the rest of the diets, could suggest that diets with more than 5% of dietary FFA have a negative impact on FA absorption. However, as it has been mentioned above, no FA absorption is expected after the ileum, and the results reported in the excreta (numerically lower than the ones reported in the ileum), could have been influenced by the presence of lipids from bacterial activity, which was supported by the FFA effect observed on the concentration of those FA with possible bacterial origin. Apart from the possible contribution of bacterial activity, it is likely that other endogenous FA losses contributed to the decrease of the digestibility coefficients in the excreta. Cotton (1972) reported that the endogenous lipids in the rat can come from the exudation through the mucosa, and exfoliation of lipid-containing villus cells, and Croft and Cotton (1973) described the loss of cells in the normal human gut as a quantitatively important physiological process. However, the authors have not found information regarding broiler chickens. For all these reasons, jejunum and ileum results have been the focus of this discussion, as they are more representative of the FA absorption process.

## 5.5.3. Effect of the age

Considering the results reported in young broiler chickens (Part I; Rodriguez-Sanchez et al., Unpublished data) and the present results, it is possible to confirm that adult broiler chickens can better utilize fat. This agrees with other studies where fat digestibility between different ages was compared (Noy and Sklan, 1995; Batal and Parsons, 2002; Tancharoenrat et al., 2013).

The higher capacity of adult broiler chickens to utilize fat was reflected both in the hydrolysis and absorption processes. On the one hand, the higher content of MAG in the gizzard, and the lower content of all the lipolysis classes in the duodenum, as well as the higher (less negative) FA digestibility coefficients observed in both of these two GIT segments at 37 d suggest that gastro-duodenal reverse peristalsis could be enhanced in adult broiler chickens. However, it was in the jejunum and ileum were more differences were observed. The higher digestibility coefficients reported for TFA, and SFA in the jejunum, and the lower FFA content both in the jejunum an ileum support the improved capacity of adult broiler chickens in the absorption of FA. The interactions reported in the jejunum and ileum evidenced the higher utilization of the unsaturated diets at both ages, which agrees with our previous study (Rodriguez-Sanchez et al., 2018).

Regarding the contribution of the jejunum and ileum on the FA absorption process, it was seen that the contribution of the jejunum increased with the age of the chickens (14 d: 68% vs. 37 d: 91%, on average for TFA). The contribution of the ileum was especially important for the SFA absorption and was higher for the chickens fed S diets than for those fed P diets both at 14 d (S: 46% and P: 27%) and 37 d (S: 19% and P: 10%), probably explaining to a greater extent the higher utilization of the unsaturated fat. All of this suggests that the FA absorption process was more limiting than the hydrolysis process was, and that the higher contribution of the jejunum in the absorption of FA was the main responsible of the improvement on fat utilization from young to adult broiler chickens. This finding is in accordance with the findings of Sibbald and Kramer (1980), who reported that lipase activity was not a limiting factor in fat utilization, and with Katongole and March (1980), who reported that the absorption of fat could improve with the age of the chicken due to a higher concentration of FABP, a protein involved in the absorption of FA across the enterocytes. The gastrointestinal retention time could also influence this improvement, as it has been reported that reaches its maximum at 3 weeks of age approximately (Golian and Maurice, 1992). Thus, all of this could explain the higher capacity of adult broiler chickens to utilize both the unsaturated and saturated dietary fats.

Considering the dietary FFA level, the present results suggest that the absorption of FA in adult broiler chickens (37 d) was less affected by the presence of dietary FFA level than in young broiler chickens (14 d) was, where more differences were reported in the jejunum, the main place of fat absorption, and ileum (Part I; Rodriguez-Sanchez et al., Unpublished data); at 14 d a tendency for the digestibility coefficients to decrease as the dietary FFA level increased was observed, while at 37 d the digestibility coefficients for the four increasing levels of dietary FFA were more similar. Furthermore, the regression analysis carried out for the SFA ileal digestibility at 37 d showed that unsaturated diets with up to 35% of dietary FFA could be used in grower-finisher diets with no negative repercussions on fat utilization, while in young broiler chickens unsaturated diets with more than 15% FFA were negatively associated with fat utilization. Fewer differences among the diets with four increasing levels of FFA on fat utilization were observed in adult (37 d) than in young (14 d) broiler chickens, which agree with the higher capacity of adult broiler chickens to utilize fat.

In conclusion, the present results suggest that the hydrolysis process is likely to be improved in adult broiler chickens, which resulted in a higher hydrolysis rate of TAG. However, the absorption process was the one that differed the most between adult and young broiler chickens. Irrespective of the age, jejunum was the main place of FA absorption. However, the contribution of the jejunum increased with the age, being the main responsible of the improvement of fat utilization as the age increases. To a lesser extent, ileum contribution also explained the improvement on fat utilization in adult broiler chickens, especially regarding SFA. Thus, the utilization of both the unsaturated and saturated diets improved with the age, but the utilization of the unsaturated diets was always higher than the utilization of the saturated ones.

Considering the dietary FFA level, and regardless of the fat source, the present results do not allow demonstrating whether the dietary FFA level influence the hydrolysis process. On the other hand, the absorption process was clearly affected by the dietary FFA level, this effect being different for the unsaturated than for the saturated diets. This finding suggests that the saturation degree of dietary fat has a bigger impact on fat utilization than dietary FFA level has. According to the present results, acid soybean oil could replace crude soybean oil in grower-finisher diets if the dietary FFA level does not exceed 35%. On the other hand, the saturated diet with 50% dietary FFA level did not differ from the diet with the lowest dietary FFA level (5%), suggesting that acid palm oil (palm fatty acid distillate) could replace crude palm oil in grower-finisher broiler chicken diets, at least when the FFA level does not exceed 50%, with no negative repercussions on fat utilization compared to the use of crude palm oil. The comparison between the results reported at 14 d and 37 d suggest that adult broiler chickens are less affected by the dietary FFA level, being the suitable level of dietary FFA in grower-finisher diets higher than in starter-broiler chicken diets.

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# CHAPTER 6

## General discussion

## 6.1. Introduction

The general aim of this thesis was to study the use of acid oils in broiler chicken diets. It is well known that dietary fat saturation degree affects fat utilization, and the level of free fatty acids (**FFA**) of a fat source has been negatively related to fat utilization. Different theories have been suggested in order to explain this negative association. However, the reasons are not clear, and more information is needed in order to understand the reason why low fat digestibility coefficients and AME values have been associated with both the presence of high levels of FFA and saturated fatty acids (**SFA**). Knowing this is important, as it will allow for a more efficient utilization of supplemental fats in broiler chicken diets, being especially relevant when searching alternative fat sources such as fat by-products from the refining oil industry which are rich in FFA (e.g., acid oils).

There is a lack of information regarding the use of acid oils in the diets, as most of the studies have evaluated these fat by-products as ingredients without considering other aspects of the diet. Moreover, most of the available studies that have assessed the use of acid oils in broiler chicken diets are not up-to-date, being necessary to assess the use of fats rich in dietary FFA from the actual refining practices, and in the current farming conditions both in young and adult broiler chickens.

For this aim, in order to study the potential use of acid oils, and clarify the critic steps in their utilization by the animal, the impact of dietary FFA, saturation degree and age of the chicken on fat digestion and absorption processes was investigated.

In this general discussion the most relevant findings of this dissertation will be presented, as well as some comments and suggestions in order to improve future experiments in this area of study.

## 6.2. Advances in the methodological approach and critical considerations

In the present thesis fat digestion and absorption processes have been studied through the determination of both fatty acid (**FA**) digestibility, and lipid class composition in different segments of the gastrointestinal tract (**GIT;** gizzard, duodenum, jejunum and ileum) and excreta. On the one hand, FA digestibility coefficients are an estimation of the amount of FA that have been absorbed throughout the intestinal epithelial surface in comparison to the amount of FA that were present in the feed. FA digestibility has been assessed in several studies to evaluate fat utilization; however, most of these studies are confined to the determination of ileal or excreta digestibility, and do not offer information regarding the evolution of fat hydrolysis and absorption processes. On the other hand, lipid class composition gives extra information, as it is an estimation of the hydrolysis of triacylglycerols (**TAG**), and of the absorption of the lipolysis products (diacylglycerols, **DAG**; monoacylglycerols, **MAG**; and FFA). It is important to mention that in this case is more appropriate talking about "disappearance of the lipid classes" rather than "absorption of the lipid classes" because they are dynamic, and a decrease in the content of one is not necessarily related to their absorption (e.g., the decrease of DAG content could be related both to their absorption or their hydrolysis giving one MAG and one FFA). **Thus, combining the information provided by the assessment of the FA digestibility and lipid class content along the GIT allows for a better understanding of the fat digestion and absorption processes.** Furthermore, the assessment of both the FA digestibility and lipid class content using an inert marker in the diet was a step forward in our research group, as previously FA digestibility used to be assessed in excreta by the total collection method.

The inert marker in the diet used in the present experiments was $TiO_2$. According to Maynard et al. (1979) and Jagger et al. (1992), the ideal marker for the determination of digestibility coefficients should be indigestible and non-absorbed, physiologically inactive and non-toxic, maintain digestive transit at the same speed as other dietary nutrients in the tract, easily analyzed, as well as being homogenously mixed into a diet. It has been reported that $TiO_2$ is an appropriate inert marker for animal digestibility trials (Jagger et al., 1992), being easier to perform and giving similar trends than the total excreta collection method (Smeets et al., 2015). However, uncertainty exists to whether the flow of $TiO_2$ through the GIT is representative of the flow of feed through the digestive tract, probably due to the retrograde movement of digesta that takes place between different segments of the GIT, this being especially important in poultry (Svihus et al., 2002). Despite this fact, $TiO_2$ is the most used method for digestibility trials nowadays. Furthermore, the concentration of $TiO_2$ in digesta samples could be influenced by the method of collecting the samples. In both of the experiments presented in this thesis, samples of intestinal content (duodenum, jejunum, and ileum) were gently squeezed using digital pressure through the GIT segment. However, it is believed that certain amount of $TiO_2$ could remain attached to the intestinal walls. Thus, if this was true, the concentration of $TiO_2$ would be higher in a sample collected by flushing with distilled water than in one collected by the squeezing method. Moreover, it is also likely that the loss of some epithelial cells can occur when digesta samples are taken by squeezing. **For this reason, collecting digesta samples by flushing would be a consideration for future studies in order to improve the methodology.**

Regarding the <u>expression of the lipid class composition</u>, in the first experiment (Chapter 3) they were expressed in area normalization (%); this gives a general image of the evolution of lipid classes along the GIT. However, in the second experiment (Chapters 4 and 5) lipid class content was determined considering both the concentration of lipid class and the inert marker in each GIT segment (more information regarding this calculation can be found in Chapter 4). This ratio is an estimation of the content of each lipid class present in the digestive tract of the chickens at the moment of the sample collection. In Figure 6.1 these two different ways of expressing the lipid class composition (area normalization vs. lipid class: Ti ratio) are presented, considering the results of the second experiment reported at 14 d (Chapter 4) for those chickens fed the S5 diet (supplemented with crude soybean oil).

The global view between the two different representations is similar. However, the lipid class:Ti ratio is more appropriate in order to compare the lipid class content between different GIT segments, as the concentration of the inert marker ($TiO_2$) is supposed to be constant along the GIT. Contrary, area normalization makes difficult the comparison between different GIT segments, as it does not allow studying the evolution of lipid classes respect their value in the feed. Thus, few differences can be observed between these two ways of expressing the lipid classes; for example, DAG% was higher in the jejunum than in the duodenum, however when it was quantified and expressed using the mentioned ratio, DAG content was lower in the jejunum than in the duodenum.

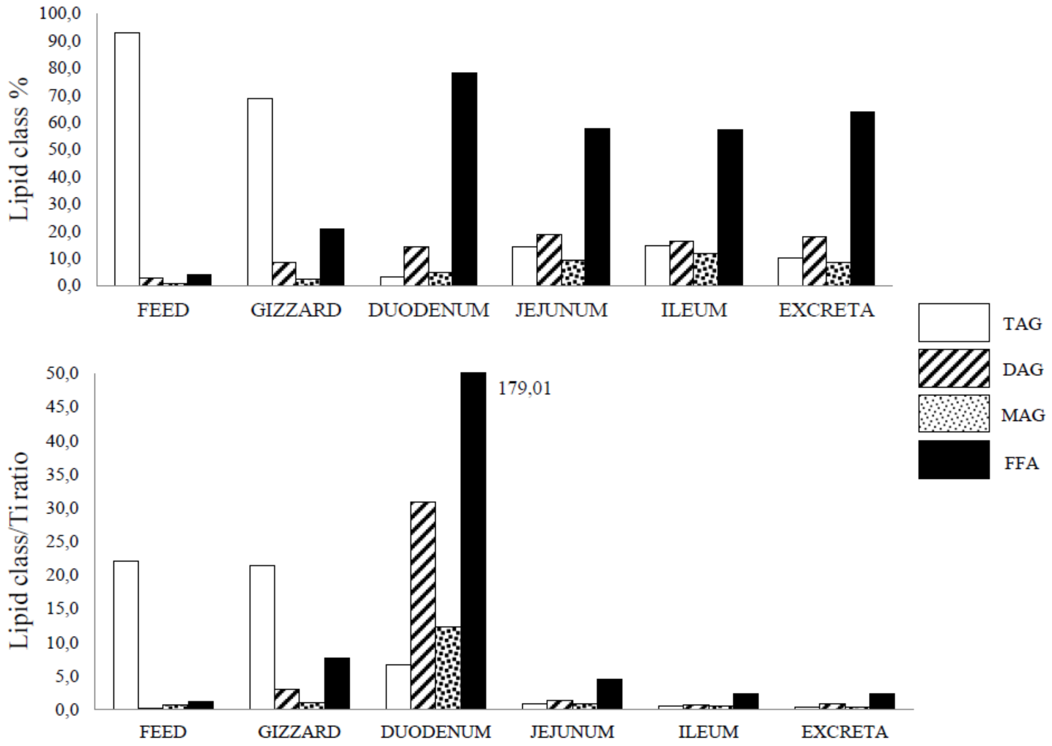

**Figure 6.1.** Lipid class composition expressed in area normalization (%) and lipid class/Ti ratio in the feed, gizzard, duodenum. jejunum, ileum, and excreta in chickens fed the diet supplemented with crude soybean oil (S5) at 14 d.

Regarding the fat hydrolysis, data reported in Chapters 3, 4, and 5 suggested that the **hydrolysis of fat starts in the gizzard and mainly takes place in the duodenum**. It has to be taken into account that the FA digestibility coefficients and lipid class composition reported in the gizzard and duodenum are also influenced by the presence of the gastro-duodenal reverse peristalsis and the secretion of endogenous fat into the duodenal lumen. For this reason it was not possible to differentiate between those lipids from the diet and those from endogenous origin. In some cases, no differences were reported regarding the lipid class content in the duodenum when chickens were fed different diets. However, it is possible that the different added fats affected in a different way the endogenous fat losses and reverse peristalsis, and that might have masked their differences over hydrolysis and absorption. It is possible that the dietary FFA level had an influence on fat solubilization as well on pancreatic lipase activity, which were not specifically studied in this thesis, and would be interesting to study in depth in future studies. In order to have more detailed information about the hydrolysis process, it would be interesting to determine and quantify the endogenous fat losses, and the intestinal retention time. This would help to confirm if the dietary fat saturation degree and level of dietary FFA have a real influence on the secretion of bile acids, and other endogenous fat losses, as well as if these factors enhance or inhibit the retention time of the digesta in the intestine, giving more or less time for the digestion and absorption of FA. Another possible way to determine this would be using radioisotope-labeled fatty acids as explained by Wang et al. (2013).

Regarding the absorption process, data reported in Chapters 3, 4, and 5 demonstrated that **the main place of fat absorption was the jejunum**, and **the absorption of FA continued in the ileum. The dietary FFA level, saturation degree of dietary fat, and age of the chicken influenced the efficiency and rate of FA absorption in these two segments.**

It has to be taken into account that the results presented for FA digestibility and lipid class content are an average of what happened in the whole segment because in both of the experiments presented in this thesis the content of the jejunum was taken from the distal most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum, and the content of the ileum was taken from the junction with Meckel's diverticulum to a point 1 cm proximal to the ileocecal junction. In order to have more accurate information about the absorption process of the individual FA it would be interesting to divide the content of the jejunum and ileum in two parts (upper and lower).

In Chapters 3, 4, and 5 the results reported in the excreta have also been presented. As it was explained, it is likely that bacterial activity had an influence on these results as no FA absorption has been described after the ileum. In the second experiment (Chapters 4 and 5), there was a decrease (of 3-9%) on the digestibility coefficients from the ileum to the excreta, and the FA detected in the excreta that could have bacterial origin (capric, margaric, elaidic, and vaccenic acids) were added up and compared among the eight different treatments to see if bacterial activity could explain the decrease. However, when the contribution of these FA on the digestibility coefficient was excluded, it was seen that the decrease observed from the ileum to the excreta was not exclusively due to bacterial activity. It is likely that the presence of endogenous FA as well the cloaca-caecal peristalsis affect the digestibility coefficients in the excreta. This was not observed in the first experiment (Chapter 3), where the digestibility coefficients in the excreta were higher (6-8% higher) than the ones reported in the ileum. Despite this difference, these results suggest that **ileal digesta samples**

**would be preferable than excreta samples to assess FA digestibility**. It has been described that protein digestion is affected by the caecal microbiota and it is generally accepted that ileal digesta is preferable rather than excreta for the determination of aminoacid digestibility in poultry (Ravindran et al. 1999). However, the information regarding the effect of bacteria on fat digestion in poultry is scarce. For this reason, the analysis of the microbiota would be useful in order to see the effect of bacteria on fat digestion, as well as to see if saturation degree and FFA level of dietary fat affect intestinal bacteria. Both in the first and second experiments, samples from caeca and jejunal tissue were taken to study the microbiota and intestinal morphology, respectively, as it is necessary to assess the effect of dietary FFA on these two parameters, as they are indicators of gut health. These results have not been included in the present dissertation.

## 6.3. Effect of dietary fat saturation degree on fat digestion and absorption

Data reported in the first (Chapter 3) and second experiments (Chapters 4 and 5) corroborated the **better utilization of unsaturated dietary fat both in young and adult broiler chickens**. Although it is likely that the saturation degree of dietary fat also affects the hydrolysis process, the present data confirmed that the **absorption process was more limiting than the hydrolysis process was**.

It is well known and accepted that unsaturated fat is better utilized than saturated fat, and data from first and second experiments showed: 1) **that the better utilization of unsaturated diets was related to a more efficient and faster absorption of FA,** and 2) **evinced that this difference was already found in the jejunum.**

Tancharoenrat et al. (2014) studied the digestibility of FA along the gastrointestinal tract of broiler chickens at 21 d, and reported that there was a difference among the different FA regarding the GIT segment where their digestion started. Data from the first and second experiments give valuable information regarding the absorption of the individual FA both in young (14 d) and adult broiler chickens (35-37 d), but it is not possible to confirm the exact point of GIT segment where the absorption of the individual FA starts, because as mentioned above, total jejunal and ileal digesta were collected. Dividing the content of the jejunum and ileum in an upper and lower part in future experiments, would give more detailed information, especially regarding the start point of absorption for the different FA.

Nevertheless, the digestibility coefficients reported for the individual FA along the GIT allow for a better understanding of the differences between unsaturated and saturated dietary fats, as the absorption of the different FA along the GIT, followed a different trend regarding the saturation degree of dietary fat. In Figure 6.2 the individual FA digestibility coefficients in the jejunum and ileum, both at 14 d and 37 d are presented; these data correspond to the second experiment (Chapters 4 and 5; results from the first experiment can be found in Table 3.4 of Chapter 3). Palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6), and linolenic (C18:3n3) acids showed different absorption rates along the jejunum and ileum. According to the present results (Figure 6.2) it is possible to see the effect of the carbon chain length on digestibility both in the jejunum and ileum. Thus, the absorption of C18:0 was less efficient and slower than C16:0. Regarding the effect of the number of double bonds on digestibility, the absorption of C18:1n9 was more efficient and faster than C16:0 and C18:0; this effect among C18:1n9, C18:2n6 and C18:3n3 was not as evident in the jejunum. This agreed with Tancharoenrat et al. (2014) in the sense that the absorption of C18:0 seemed to be delayed in comparison to the rest of the mentioned FA.

In Figure 6.2 it is also possible to see differences between unsaturated (soybean) and saturated diets (palm); thus, **at 14 d the absorption of C16:0 and C18:0 was higher for unsaturated diets, being this mainly due to the increased on the digestibility coefficients of these FA from the jejunum to the ileum.** This agrees with the fact that the <u>contribution of the ileum</u> was found to be different depending on the dietary fat source, being higher for SFA in those chickens fed the unsaturated diets than in the ones fed the saturated diets, which suggest that **the contribution of the ileum was the main responsible of the higher utilization of the unsaturated dietary fat at 14 d**. Regarding the digestibility of C18:1n9, C18:2n6 and C18:3n3, the digestibility coefficients in the jejunum were higher for unsaturated diets, but the digestibility coefficients between unsaturated and saturated diets at the ileum level were similar.

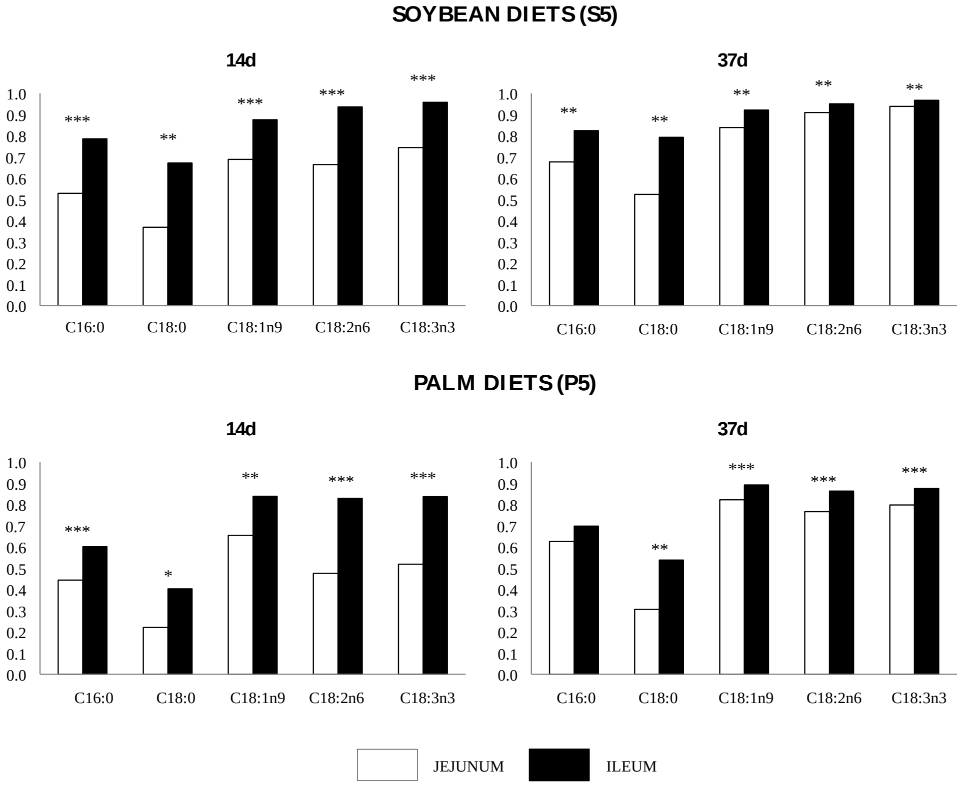

**Figure 6.2.** Digestibility coefficients in the jejunum and ileum for palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6), and linolenic (C18:3n3) acids in 14- and 37-day old broiler chickens. Soybean S5 – diet supplemented with crude soybean oil; Palm P5 – diet supplemented with crude palm oil.
Values are means of 6 replicates per each dietary fat source (soybean or palm diets) with 8 chickens / replicate at 14d, and 3 chickens / replicate at 37d. *P* values were obtained from Student's *t*-test conducted to see the effect of the gastrointestinal segment (jejunum and ileum) for each individual fatty acid. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

At 37 d, despite there was a general improvement of the digestibility coefficients, the absorption of SFA (C16:0 and C18:0) continued being higher for the chickens fed the unsaturated diets. The digestibility coefficients in the jejunum for C18:1n9, C18:2n6 and C18:3n3 were the ones that improved the most from 14 d to 37 d, in consequence being the increase on these digestibility coefficients from the jejunum to the ileum lower at 37 d than at 14 d. **The increase in the contribution of the jejunum from 14 d to 37 d mainly explained the improvement on fat utilization in adult broiler chickens**.

## 6.4. Effect of dietary fat free fatty acid level on fat digestion and absorption

As explained in the literature review (Chapter 1), FFA level has a negative association with fat utilization, which is mainly based on the studies that have assessed the nutritional value of added fats. The review of these studies evinced that there is lack of information and homogenization of the reported results, as most of the studies are quite old and some information is not clearly explained. Assessing the feed AME and dietary fat digestibility is important as it gives more practical information regarding the inclusion of acid oils in broiler chicken diets.

Data reported in the second experiment (Chapters 4 and 5) showed that **the fat absorption process was more affected than the hydrolysis process was by the level of dietary FFA.** In general, **the diets with the highest level of dietary FFA were related to lower fat utilization in comparison to the diets with the lowest level of dietary FFA at 14 d, this effect being less pronounced at 37 d**. However, **unsaturated diets with intermediate levels of dietary FFA, up to 15% and 35% at 14 d and 37 d respectively, seemed not to have a negative effect on fat utilization.**

It has been described that the more saturated the fat the greater the effect of FFA level on fat utilization, especially in young broiler chickens (Wiseman and Salvador, 1991). This was not observed in this study (Chapters 4 and Chapter 5), where **unsaturated diets were more affected by the dietary FFA level than saturated diets both at 14 d and 37 d**. This could be explained by different reasons: 1) as explained in Chapters 4 and 5, unsaturated diets had higher content of SFA as the dietary FFA level increased, while saturated diets had similar SFA content as the dietary FFA level increased (Table 6.1); 2) in the case of saturated diets, the diet with the highest dietary FFA level resulted from the supplementation with a blend (33:66) of crude palm oil – palm fatty acid distillate from physical refining (PFAD), and a diet with 100% PFAD was not included in the design; it is for this reason that was not possible to determine the effect of higher levels of dietary saturated FFA (85% approx.), which could negatively affect fat utilization; 3) in this study feed AME and dietary fat digestibility were considered in contrast to added fat AME and added fat digestibility, being likely that some interactions between the added fat and the fat present in the basal diet, or with other dietary components modify the utilization of acid oils. For example, the synergism between the FA present in the basal diet (mainly unsaturated FA) and the FA present in the added fat. Another example, would be the synergism between different fat molecular structures; it has been described that TAG and 2-MAG stimulate the secretion of bile salts (Sklan, 1979), facilitating the emulsification of FA, thus, their presence in the fat of the basal diet could facilitate the absorption of those FFA from the added fat.

**Table 6.1.** Unsaturated to saturated fatty acid ratios for the different dietary treatments at 14 and 37 d

| | Dietary treatments[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S5 | S15 | S35 | S50 | P5 | P15 | P35 | P50 |
| Dietary FFA level (%) | 5 | 15 | 35 | 50 | 5 | 15 | 35 | 50 |
| *At 14 d* | | | | | | | | |
| UFA:SFA Ratio | 4.43 | 4.01 | 3.62 | 3.37 | 1.32 | 1.29 | 1.28 | 1.21 |
| *At 37 d* | | | | | | | | |
| UFA:SFA Ratio | 4.16 | 4.01 | 3.55 | 3.31 | 1.30 | 1.32 | 1.29 | 1.21 |

[1] S5: 100% crude soybean oil, S15: oil blend with 70% crude soybean oil and 30% soybean acid oil, S35: oil blend with 30% crude soybean oil and 70% soybean acid oil, S50: 100% soybean acid oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% palm acid oil, S35: oil blend with 53% crude palm oil and 47% palm acid oil, S50:oil blend with 33% crude palm oil and 66% palm acid oil.
FFA = free fatty acids; UFA = unsaturated fatty acids; SFA = saturated fatty acids.

As it was in the absorption process where more differences were observed regarding the dietary FFA level, the digestibility coefficients for different individual FA in the jejunum and ileum and the four increasing levels of dietary FFA are presented in Figure 6.3. These data evinced that at the jejunum level there were already differences on the digestibility coefficients regarding the dietary FFA level. Thus, **at 14 d the digestibility coefficients for C16:0 and C18:0 were the most affected ones by the dietary FFA level**, as the coefficient value for these FA progressively decreased from the chickens fed the diets with the lowest dietary FFA level to the chickens fed the diets with the highest dietary FFA level, both in the jejunum and ileum. Similarly, the digestibility coefficients for the unsaturated FA in the jejunum at 14 d, especially for C18:1n9, progressively decreased as the dietary FFA level increased. However, the digestibility coefficients for this UFA in the ileum were higher than in the jejunum, being the coefficients in the ileum more similar for the different dietary FFA levels. This is in accordance with the increase in the contribution of the ileum to the absorption of TFA as the dietary FFA level increased, as it was higher in the chickens fed the diets with the highest FFA level (39%) than in those fed the diets with the lowest FFA level (30%).

**At 37 d, the digestibility coefficients of C16:0 and C18:0 were also the most affected ones numerically by the dietary FFA level**, but no significant differences were observed among the four levels of dietary FFA, both in the jejunum and ileum. No differences were observed for C18:1n9, and C18:2n6, both in the jejunum and ileum, and for C18:3n3 in the ileum. In this case the contribution of the jejunum and ileum to TFA absorption was similar for the different dietary FFA levels (on average 91%, and 9%, respectively).

**These results suggest that the FA profile of dietary fat has a bigger impact on the FA absorption process than the dietary FFA level does. Moreover, the dietary FFA level has a greater effect in 14- than in 37-day-old broiler chickens; the digestibility coefficients for adult chickens were less affected by the dietary FFA level due to a higher contribution of the jejunum to the absorption of TFA (14 d: 68% vs. 37 d: 91%)**

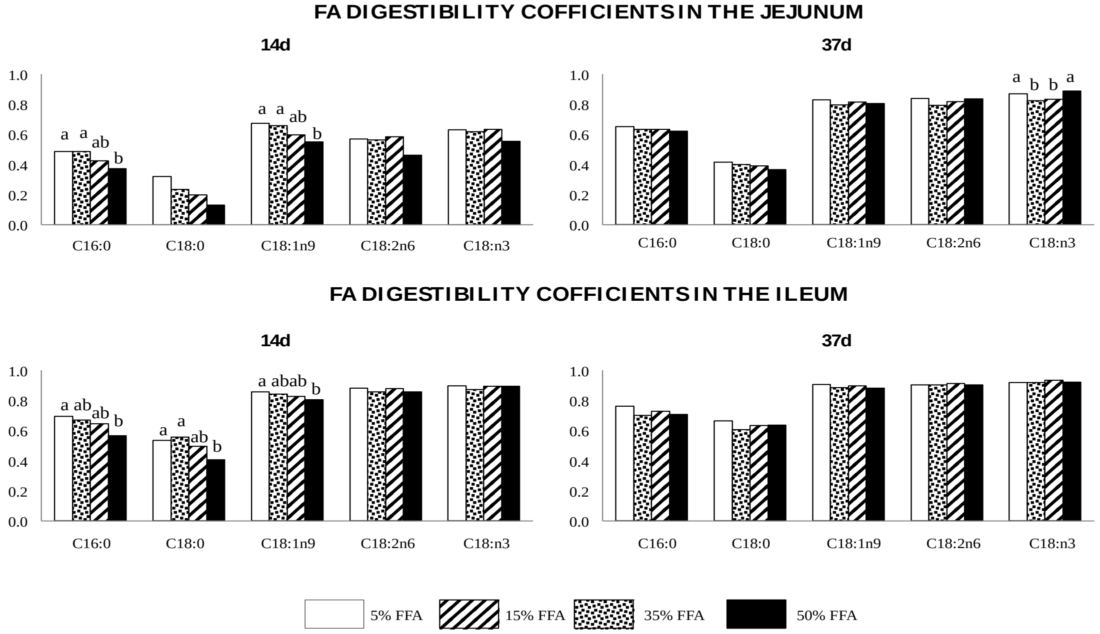

**Figure 6.3.** Digestibility coefficients in the jejunum and ileum for the four increasing levels of dietary free fatty acids (5%, 15%, 35%, and 50%) in 14- and 37-day old broiler chickens.
Palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6), and linolenic (C18:3n3) acids.
Values are means of 12 replicates per each dietary free fatty acid level with 8 chickens / replicate at 14 d, and 3 chickens / replicate at 37 d. FFA = free fatty acids. a, b, c allude to Tukey-test.

One of the main reasons usually given in the literature in order to explain the negative effect of FFA level on fat utilization is the capacity of FFA to react with ionized minerals forming soaps. This was not investigated in the present thesis, and cannot be confirmed. However, according to the present results it is likely that the greatest limitation on the utilization of FFA is their solubilization prior their absorption through the intestinal epithelial surface. The lack of MAG has also been associated with the low fat utilization of diets with high levels of FFA. Data from the second experiment did not evince a lack of MAG in duodenal, jejunal and ileal lumen as the dietary FFA level increased. However, dietary FFA could have a negative effect on the secretion of bile impairing the FA absorption process, as bile acids are necessary for the formation of mixed lipid bile micelles (Ravindran et al., 2016). Therefore, the study of different <u>strategies to improve the solubilization of FFA</u> and lipolysis products in general, would be interesting in order to confirm this. Some examples would be studying the inclusion of: 1) lecithin (or lysolecithin) + acid oil blends in broiler chickens diets due to the emulsifying properties of phospholipids, even greater for lysophospholipids (Joshi et al., 2006; Jansen et al., 2015); 2) bile acids in diets supplemented with acid oils; 3) blends of fats sources rich in MAG + acid oils in order to confirm if MAG content limit the absorption of FFA; or 4) blends of crude unsaturated oils + acid oils in order to take advantage of the natural emulsifying properties of UFA. Some of these strategies are already included in the project where the present thesis is included (ref. AGL2015-64431-C2), as well as in other research projects developed in our research group.

As it has been previously explained, bacterial activity and also other endogenous FA losses could influence the FA content in the excreta (Figure 6.5), and therefore affect FA digestibility coefficients. The level of dietary FFA had an effect on the concentration of those FA from bacterial origin present in the excreta both in young (14 d) and adult (37 d) broiler chickens (Chapters 4 and 5), suggesting that **ileal digestibility would be more accurate than excreta digestibility in the assessment of the use acid oils**, as it could lead to an undervaluation of acid oils.

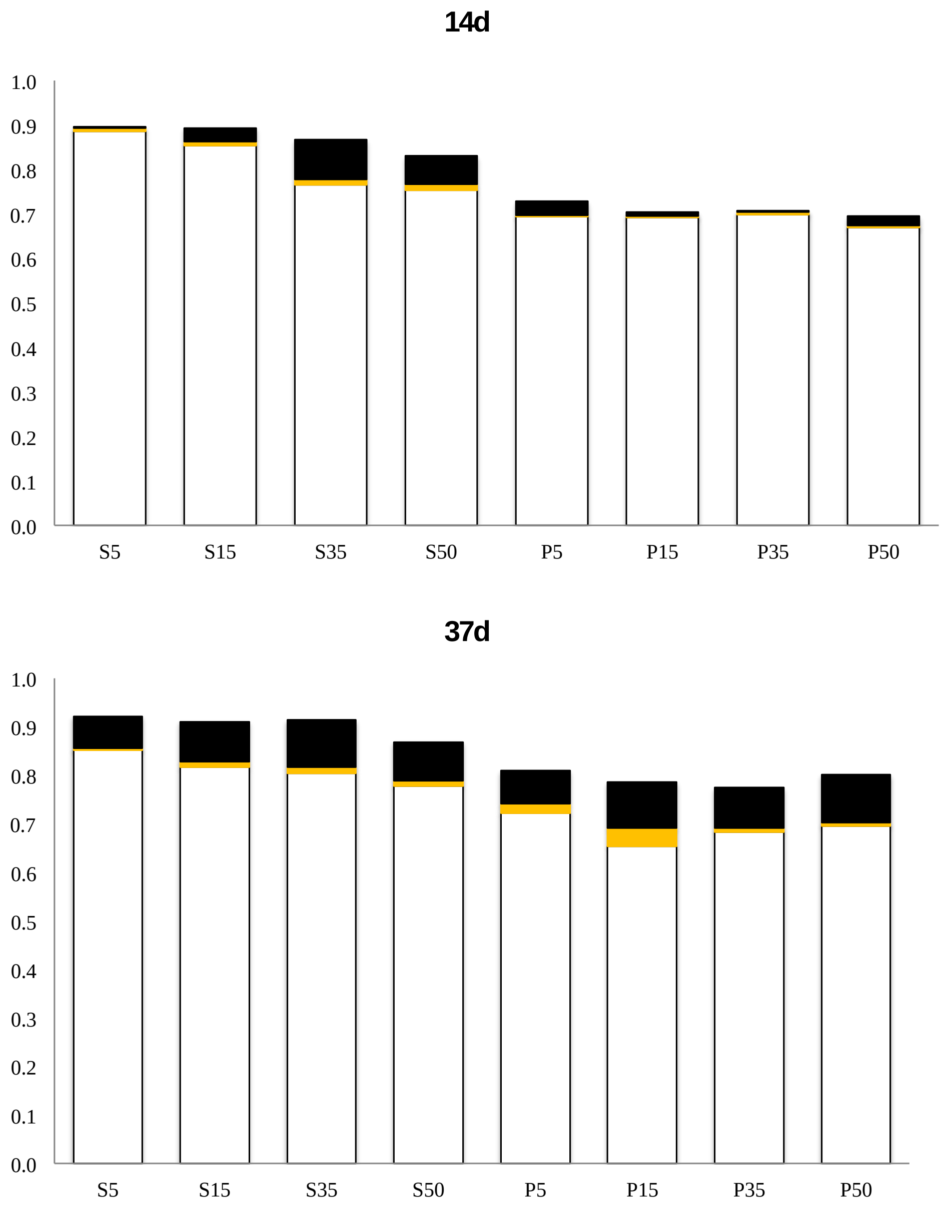

**Figure 6.4.** Representation of the contribution of bacterial activity on the decrease of total fatty acid digestibility coefficients from the ileum to excreta in 14- and 37-day-old broiler chickens.

The white part represents the TFA digestibility coefficient in the excreta, the yellow part represents the contribution of bacterial activity on the decrease of the coefficient from the ileum to the excreta, and the black part represents the rest of the difference between ileal and excreta TFA digestibility coefficient.

S5: 100% crude soybean oil, S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50: oil blend with 33% crude palm oil and 66% acid palm oil.

TFA = total fatty acids

Another important aspect to mention is the quality control of acid oils. Most of the studies that have assessed the use of acid oils comment about the importance of the presence of non-nutritive factors, such as moisture, polymerized and oxidized products, and it is accepted that knowing this is very important to ensure the quality of acid oils. However, the available information regarding the best parameters to determine, as well as the acceptable limits is scarce. Therefore, the characterization of these fat by-products is essential to study more in depth their composition and raise recommendations about the best parameters to analyze and determine their nutritional value. This part is also considered in the project where this present thesis is included (ref. AGL2015-64431-C2), and it is being studied. Regarding the fats used in the experiments included in this thesis, the MIU content for crude oils was lower than 3%, and lower than 5.5% for acid oils.

The present results suggest that acid oils could be used in broiler chicken diets, being this dependent on the dietary FFA level and feeding period. Thus, crude oils could be partially replaced by acid oils, when the price of the latter is competitive. It is important to consider the feeding period, as it has been observed that **up to 15% dietary FFA level in starter broiler chicken diets do not have negative repercussions on fat utilization, while in grower-finisher the dietary FFA level can be increased to 35% in the case of unsaturated diets. Regarding the saturated diets, in the grower-finisher period, the 50% dietary FFA level was not negatively related to fat utilization**, as no differences were reported between the chickens fed the saturated diet with the lowest dietary FFA level (5%) and the chickens fed he diet with the highest dietary FFA level (50%).

## 6.5. Future considerations

The information provided in the present dissertation has been essential in order to understand the physiological limitations of the chickens on the utilization of diets rich in FFA, which has been possible through the determination of both FA digestibility and lipid class content along the GIT. Furthermore, the results have set the **basis for the design of more experiments in the same line of investigation**.

As it has been explained along the general discussion, some of the findings presented in this thesis have raised new hypotheses and need further research. On the one hand, the **determination of endogenous fat losses**, as well as the **intestinal**

**retention time** after feeding fat sources with different saturation degree and dietary FFA level would be necessary to assess if these two factors affect the hydrolysis process. On the other hand, it would be interesting to study more in depth the absorption process, where most of the differences were found, regarding both the saturation degree and dietary FFA level. One of the first things to asses would be the **solubilization step of the lipolysis products** into the intestinal lumen. It is likely that improving the solubilization would lead to an improvement on the FA absorption by the animal. ***In vitro* digestibility trials** would help to study in depth this step on FA absorption, without the necessity of using animals. One possible way to improve this solubilization could be the supplementation of diets with acid oils and **products with emulsifying properties** (like lecithin), as well as the other strategies described in the previous subsection. In order to corroborate that FA profile of dietary fat is more important than the level of dietary FFA, it would be interesting to study the supplementation of diets with **blends of acid oils or blends of acid oil and crude oil** from fat sources with different saturation degree, in order to have diets with different levels of both saturation degree and FFA. Furthermore, it would be interesting to study if the inclusion of 100% palm fatty acid distillate has negative repercussions on fat utilization. In these studies, both the FA digestibility coefficients and lipid class content assessment would be appropriate as these two determinations give valuable information of the whole fat digestion process. Furthermore, it would be recommended to perform these determinations in the upper and lower jejunum, and in the upper and lower ileum, as well as collecting the samples of digestive content by flushing with distilled water instead of by squeezing the content by digital pressure.

Dividing the ileum in an upper and lower part, would allow for a better study of the differences between the digestibility coefficients observed in this GIT segment and the ones observed in the excreta. This, altogether with the study of the microbiota and intestinal histological measurements would give more information regarding the effect of acid oils on gut health.

Another important thing to assess is the effect of dietary FFA level on meat quality and stability, which is also part of the global project where this thesis is included.

In conclusion, the work presented in this thesis allows for a better understanding of the physiological limitations of young and adult broiler chickens regarding fat digestion and absorption processes, providing interesting information concerning the impact of both the saturation degree and FFA level of dietary fat on these processes. Given the fact the use of acid oils in broiler chicken diets is still controversial, the results presented in this thesis provide some recommendations on the use of oils with certain percentages of FFA in the diets with no negative repercussions on fat utilization and growth performance. Moreover, they give the basis to continue with the study of the use of these fat by-products in broiler chicken diets. The presented results also leave some doubts and questions, which would be interesting to determine in future experiments.

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# CHAPTER 7

## Conclusions

The following conclusions can be drawn from the present dissertation:

1) The assessment of the fatty acid digestibility and lipid class content along the gastrointestinal tract allows for a better understanding of the fat digestion and absorption processes.

2) The duodenum is the main place where hydrolysis of triacylglycerols takes place, and the fatty acid absorption mainly takes place in the jejunum, being the contribution of the ileum also important for the global fat utilization.

3) The fat absorption process is more affected than the hydrolysis process is by the dietary free fatty acid level and saturation degree as well as by the age of the chicken.

4) The higher utilization of unsaturated diets is related to a more efficient and faster absorption of fatty acids mainly due to:
   a) The higher contribution of the ileum, especially for saturated fatty acids, in young broiler chickens.
   b) The increase in the contribution of the jejunum in adult broiler chickens.

5) The saturation degree of dietary fat has a bigger impact on fat utilization than the dietary free fatty acid level does.

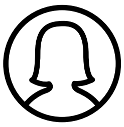
6) The utilization of fat in adult broiler chickens is less affected by the saturation degree and free fatty acid level of dietary fat due to a more efficient absorption at the jejunum level.

7) Acid palm oil (palm fatty acid distillate from physical refining) could replace crude palm oil in grower-finisher diets, at least when the dietary FFA level does not exceed 50% (maximum level of dietary free fatty acids used in this study).

8) Acid soybean oil from chemical refining could replace crude soybean oil in broiler chickens diets when the dietary free fatty acid level does not exceed:
    a) 15% in starter diets.
    b) 35% in grower-finisher diets.

# CHAPTER 8

## Resume of the author



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## Education

**2018-01 - 2018-08**

### IVABS, Massey University (New Zealand) - Internship

Internship at Massey University (Institute of Veterinary, Animal, and Biomedical Sciences) under the supervision of Professor Ravi Ravindran and his team.

- Involvement in the research topic "Fat digestion phisiology in Poultry".
- Involvement in different experimental trials and seminars of the research team.

**2015-03 - present**

### Universidad Autónoma de Barcelona (UAB) - PhD in Animal Science

I am conducting my PhD at the Department of Animal and Food Science from UAB at the Animal Nutrition and Welfare Group (SNIBA; http://sniba.es/?lang=en). I am also doing part of my research at the Department of Nutrition, Food Science and Gastronomy from the Universitat of Barcelona at the Lipids and Bioactive Compounds in the Food Chain Group (LiBiFOOD; https://libifooden.wordpress.com/8-2/libifood/).

My thesis is about the study of lipid digestion in order to see the effect of the dietary fat saturation degree and level of free fatty acids on both lipid digestion and absorption processes, and gut health (histology, microbiota) in broiler chickens.

Main equipment - techniques used in my thesis:

- HPLC-RID (lipid class composition)
- Gas chromatography (fatty acid composition)
- DNA extraction (DNA quantification - microbiota analysis)
- Histology measurements (gut)

During my thesis I have improved and developed several skills, and I have been awarded with grants in order to present my research at various international conferences. I have a pre-doctoral research grant from the Spanish Government (FPU/06063).

**2009-09 - 2014-09**

### Universidad Autónoma de Barcelona (UAB) - DVM

Degree in Veterinary Science

## Experience

**2016-11 - present**

### Teaching - Higher Education

*Universidad Autónoma de Barcelona (UAB) - Veterinary Faculty*

- Teaching 30h/year of practical classes in the 1st and 3rd course of the DVM.
- From 11/2016 to 02/2017 I attended to different teacher training courses in higher education in the institution (UAB).

**2015-03 - present**

### Involvement in different research projects

*Animal Nutrition and Welfare Service (SNIBA) http://sniba.es/?lang=en*

I have been involved in the following projects:

- *Use of acid oils in mono gastric animals: characterisation, comparative nutrition, and meat quality repercussions* (reference: AGL2015-64431-C2-1-R). Duration: 2016-2019.
- *Use of lecithins to reduce the cost of broiler diets* (reference: MAGRAMA 2 0150020003054). Duration: 2015-2017.
- *Reduction of the production costs in poultry farms through the optimisation of the use of fats and emulsifiers in feed, and thermographic analysis* (reference: MAGRAMA 20130020000782). Duration: 2013- 2015.

I have been involved in the following projects:

- *Use of acid oils in mono gastric animals: characterisation, comparative nutrition, and meat quality repercussions* (reference: AGL2015-64431-C2-1-R). Duration: 2016-2019.
- *Use of lecithins to reduce the cost of broiler diets* (reference: MAGRAMA 2 0150020003054). Duration: 2015-2017.
- *Reduction of the production costs in poultry farms through the optimisation of the use of fats and emulsifiers in feed, and thermographic analysis* (reference: MAGRAMA 20130020000782). Duration: 2013- 2015.

**2014-11 - 2017-11**

### Technical Summaries Writer

*Asociación Española de Ciencia Avícola (AECA). Spanish branch of World Poultry Science Association (WPSA) http://www.wpsa-aeca.es/index.php*

- Writing and translating technical summaries related to poultry (nutrition, health, welfare...) for AECA website (monthly).

**2014-12 - 2015-03**

### Research Technician

*Animal Nutrition and Welfare Service (SNIBA)* http://sniba.es/?lang=en

- Conducting trials with broiler chickens
- Writing reports
- Planning and decision making

## Publications

**2018-08** Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages. Accepted. Poultry Science Journal. doi: 10.3382/ps/pey458.

**2018-12** Dietary free fatty acid level and fat saturation degree affect lipid class composition and fatty acid digestibility along the gastrointestinal tract in young broiler chickens. Undergoing review.

**2018-12** Dietary free fatty acid level and fat saturation degree affect lipid class composition and fatty acid digestibility along the gastrointestinal tract in adult broiler chickens. Undergoing review.

## Conferences

**2018-10** XXXIV Curso de especialización FEDNA. *Use of acid oils in broiler chicken diets. Effect of dietary free fatty acid level and saturation degree on fat utilization at different ages* (Oral presentation for eligibility for the FEDNA award).

**2018-06** 6th Mediterranian Poultry Summit. *The effect of the dietary free fatty acids and its saturation degree on the morphometry of intestinal mucosa in 14-day-old broiler chickens* (Poster). Torino (Italy).

**2017-09** LIV Poultry Scientific Symposium (Symposium Cientifico de Avicultura; AECA: WPSAs Spanish branch). *Effect of free fatty acids on intestinal morphology in broiler chickens* (Oral presentation and poster). León (Spain).

**2017-05** 21st European Symposium on Poultry Nutrition (ESPN) - *Dietary free fatty acids and saturation degree modify lipid absorption dynamics in broiler chickens* (Oral presentation). Salou-Vila Seca (Spain).

**2016-10** 5th Mediterranian Poultry Summit. *Jejunal histomorphological changes according to dietary fat consumption in broiler chickens* (Poster). Italy-France.

**2016-09** XXV World's Poultry Science Congress. *Gastrointestinal lipid classes changes according to dietary fat consumption in broiler chickens at different ages* (Oral presentation). Beijing (China).

**2015-10** LII Poultry Scientific Symposium (Symposium Cientifico de Avicultura; AECA: WPSAs Spanish branch). *Relation between the fat saturation degree and excretion of free fatty acids in broiler chickens* (Oral presentation and poster). Málaga (Spain).

## Courses

**2016-11 - 2017-02** Teacher training courses in higher education

| | |
|---|---|
| **2016-11 - 2016-12** | Introduction to R software |
| **2015-07 - 2015-07** | Fundamentals of mass spectrometry: proteomics, small molecules, and metabolites |
| **2015-05 - 2015-05** | Introduction to QIIME software |

## Languages

| | |
|---|---|
| Spanish | ●●●●● |
| English | ●●●●● |
| Catalan | ●●●●● |

## Skills

| | |
|---|---|
| Organization - Planning | ●●●●● |
| Communication - Public Speaking - Ability to impart knowledge to both technical and non-technical audiences | ●●●●○ |
| Capacity of decision | ●●●●○ |
| Teamwork | ●●●●● |
| Creativity | ●●●●● |
| Goal oriented, self-motivated, hardworking | ●●●●● |
| Adaptability | ●●●●○ |
| Graphic design | ●●●●○ |
| Keen attention to detail | ●●●●● |

## Software

| | |
|---|---|
| Microsoft Office | ●●●●● |
| SPSS | ●●●●● |
| R | ●●○○○ |
| Lightroom | ●●●○○ |

## Licenses

| | |
|---|---|
| **2009-10** | Car driving licence |

## Interests

- Photography, music, travelling, nature, fashion, health, nutrition and fitness