Universitat Autònoma de Barcelona

Use of soybean acid oil and palm fatty acid distillate in broiler chicken diets

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Doctoral Thesis

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Summary

The supplementation of fats and oils in broiler chicken feeds is a common practice to increase the energy content of diets and provide essential fatty acids. Soybean acid oil (**SA**) and palm fatty acid distillate (**PFAD**) are by-products derived from the soybean oil (**S**) and palm oil (**P**) refining industry, respectively. They represent a sustainable and economically interesting alternative to conventional oils to include as feed ingredients.

These by-products have similar fatty acid (**FA**) composition to their respective crude oil, but the different molecular structure, rich in free fatty acids (**FFA**), represents a limitation to upcycle these by-products in the animal feed industry. The strategy of including fat blends in poultry feed, combining unsaturated FA (**UFA**) with saturated FA (**SFA**), or varying the proportion of the different molecular structures (triacylglycerols, TAG; diacylglycerols, DAG; monoacylglycerols, MAG; FFA) has been positively related to fat utilization. In this context, it was hypothesized that these by-products blended with conventional oils of different saturation degrees, can be a good alternative source to feed broiler chickens. The global aim of this thesis was to investigate the potential use of soybean acid oil and palm fatty acid distillate in broiler chicken diets, focusing the study on the fat intestinal absorption in both starter and grower-finisher chickens.

In the first experiment (section 3.1), *in vitro* methodologies were performed with the aim to evaluate the hydrolysis and bioaccessibility of SA, PFAD, and their respective crude oils. The results supported the findings reported in *in vivo* studies, the bioaccessibility was the most limiting step in oil digestion, not the hydrolysis. Moreover, the assessment of the bioaccessibility corroborated that the FFA level of dietary fat influences fat utilization less than its saturation degree.

The second and the third trial (section 3.2 and 4.1) were conducted in parallel in order to study the effect of the replacement of S by PFAD and P by SA on lipid-class content and FA digestibility along the intestinal tract (upper and lower jejunum, upper and lower ileum) and in the excreta in broiler chickens at 11 and 35 days (d). In the second trial (section 3.2) a basal diet was supplemented at 6% with S and increasing amounts of PFAD were included in replacement (2%, 4%, 6%), and P at 6% was included as a control diet for PFAD. In the third trial (section 4.1) the same procedure was followed, P at 6% was replaced with increasing levels of SA (2%, 4%, 6%), and S was used as a positive control diet.

The assessment of the lipid-class content and the apparent FA digestibility in the different intestinal segments was crucial for a better understanding of the utilization of different fat sources in broiler chickens, being the lower ileum the most important segment to evaluate the final fat utilization. Concerning the absorption dynamics, the contribution of the upper and lower segments of the jejunum and ileum to FA absorption was influenced by the characteristics of the dietary fat (FA profile and FFA content), and the age of the chicken. In particular, as the SFA and FFA dietary content increased, the absorption of dietary fat was reduced and delayed in starter chickens. On the other hand, as the age of the chicken increased, was seen an improvement in the absorption of SFA and FFA in the jejunum and an increase in the contribution of the upper ileum to FA absorption. The results revealed that for 11-d-old broiler chickens the inclusion of PFAD at 6% or blended with S impaired fat utilization, whereas the replacement of P by SA improved FA absorption, although did not reach the fat absorption obtained with S. In broiler chickens at 35 d, the inclusion of PFAD blended with S (2% and 4%, respectively) represented a suitable fat alternative, when the blend has from 2.6 UFA:SFA ratio and the FFA% does not exceed 30%, which led to adequate fat utilization. Moreover, the use of SA at 6% or the blend between P and SA (2% and 4%, respectively) led to obtaining similar fat utilization than S at 6%. Furthermore, in 35-d-old broiler chickens, the synergism obtained in the apparent metabolizable energy (AME) of diets when both by-products were blended with the conventional oils suggested that the increase in both the UFA:SFA ratio and the proportion of TAG to FFA in the diet is a good way to introduce these by-products in broiler chicken diets. An interesting result was that the dietary FFA digestibility was higher when FFA were provided from unsaturated rather than saturated fat sources. This potential strategy to use SA and PFAD implies a reduction in economic costs of feed and a way to upcycle them decreasing their environmental impact.

Resumen

La incorporación de materias grasas en los piensos para pollos de carne tiene como principal objetivo incrementar su concentración energética. La refinación de los aceites destinados a consumo humano genera diversos subproductos, algunos de ellos considerados como ingredientes alternativos a los aceites convencionales ya que sus características nutricionales, precio competitivo e implicaciones medioambientales resultan interesantes para su utilización en alimentación animal. En concreto, el aceite ácido de soja (**SA**) y los destilados de ácidos grasos de palma (**PFAD**) son subproductos derivados de la refinación del aceite de soja (**S**) y del aceite de palma (**P**), respectivamente. Estos subproductos tienen una composición en ácidos grasos (**AG**) similar al aceite del que provienen, pero diferente estructura molecular. Su principal componente son los ácidos grasos libres (**AGL**), lo que puede limitar su utilización por parte del animal. En alimentación de aves, se ha demostrado que combinar diferentes grasas en el pienso, es una buena estrategia para mejorar su utilización, ya que potencia el sinergismo entre los AG saturados (**AGS**) e insaturados (**AGI**) y permite una adecuada proporción de diferentes estructuras moleculares (triacilgliceroles, TAG; diacilgliceroles, DAG; monoacilgliceroles, MAG; AGL).

En este contexto, la hipótesis de partida de este estudio es que la mezcla de materias grasas con diferente grado de saturación (saturadas con insaturadas) así como con diferente estructura molecular (subproductos ricos en AGL con aceites convencionales ricos en TAG), es una buena opción para incorporar estos subproductos como grasas alternativas en la alimentación de pollos de carne. El objetivo global de esta tesis fue investigar el uso potencial del aceite ácido de soja y el destilado de ácidos grasos de palma en la alimentación de pollos de carne, tanto en piensos de iniciación como en piensos de crecimiento-acabado. Para ello, se profundizó en los procesos de digestión y absorción de la fracción lipídica.

En el primer experimento (sección 3.1), se realizó un estudio *in vitro* con el objetivo de evaluar la hidrólisis y la bioaccessibilidad de SA, PFAD y sus respectivos aceites crudos. Los resultados obtenidos coincidieron con los derivados de los estudios *in vivo*, determinándose que la bioaccessibilidad es más limitante que la hidrólisis en la utilización de los aceites. Además, la evaluación de la bioaccessibilidad permitió corroborar que la utilización de los aceites se ve más influenciada por el grado de saturación que por el nivel de AGL de la dieta.

El segundo y el tercer ensayo (sección 3.2 y 4.1) se realizaron de forma simultánea, con el fin de estudiar el efecto de reemplazar S por PFAD y P por SA, sobre el contenido en fracciones lipídicas y la digestibilidad de los AG a lo largo del tracto intestinal (yeyuno anterior y posterior, íleon anterior y posterior) y en las heces, en pollos de carne de 11 y 35 días (d). En el segundo ensayo (sección 3.2) se utilizó una dieta base que fue suplementada al 6% con S y sustituida por niveles crecientes (2%, 4%, 6%) de PFAD. También se incluyó P al 6% a modo de dieta control para PFAD. En el tercer ensayo (sección 4.1) se siguió el mismo procedimiento, la substitución de P al 6% por niveles crecientes (2%, 4%, 6%) de SA y se utilizó S al 6% como control positivo. Por un lado, la determinación del contenido en fracciones lipídicas y de la digestibilidad aparente de los AG en los diferentes segmentos intestinales fue determinante para obtener una mejor compresión sobre la utilización de las diferentes fuentes de grasa de la dieta por parte de los pollos de carne. Cabe destacar, que el íleon posterior es el segmento más importante para evaluar la absorción final de la grasa por parte del pollo de carne. Respecto a la dinámica de la absorción de los AG, se observó que la contribución de los segmentos anteriores y posteriores del yeyuno e íleon se ve influenciada por las características de la grasa de la dieta (perfil de AG y contenido de AGL) y por la edad del animal. En concreto, se observó que a medida que aumentaba el contenido de AGS y AGL de la dieta, la absorción de grasa en pollos de iniciación disminuía y era más lenta. Por otro lado, conforme aumentaba la edad del pollo, mejoraba la absorción de AGS y AGL en el yeyuno y se incrementaba la contribución del íleon anterior en la absorción de los AG. Los resultados en pollos de 11d demuestran que la utilización de la grasa disminuía al incluir PFAD al 6% o mezclado con S; sin embargo, la utilización mejoró con la substitución de P por SA, aunque no alcanzó el nivel de absorción obtenido con la dieta S. En los pollos de carne de 35 d, el uso de PFAD mezclado con S (2% y 4%, respectivamente) dio lugar a una alta utilización de la grasa por parte del animal, por lo que se deduce que su incorporación en la dieta es una alternativa viable, siempre y cuando tenga al menos una relación de AGI:AGS de 2.6 y no supere el 30% de AGL de la dieta. Asimismo, se observó que la utilización de la grasa obtenida con SA al 6% o con la mezcla de P y SA (2% y 4%, respectivamente) era similar a la de S al 6%. Por otra parte, en las dietas de crecimiento-acabado, cuando ambos subproductos se mezclaron con los aceites convencionales (aumentando tanto la relación de AGI:AGS como la proporción de TAG respecto AGL en la dieta) se observó un sinergismo que repercutió de forma positiva en los valores de energía metabolizable aparente (EMA). Así pues, de los resultados obtenidos se desprende que una forma adecuada de introducir estos subproductos en las dietas de pollos de carne es a través de mezclas con aceites y grasas convencionales.

Otro de los resultados novedosos fue que, la digestibilidad de los AGL de la dieta fue mayor cuando los AGL provenían de una grasa insaturada en lugar de una saturada. Estas estrategias para reutilizar SA y PFAD en la alimentación de los pollos de carne, implica una mejora de la rentabilidad, y al mismo tiempo una forma de reciclar estos subproductos, disminuyendo así su impacto medioambiental.

Publications

During the development of this thesis, I have contributed as a co-author in the following publications. Articles 1 and 2 are an integral part of this thesis, corresponding to sections 3.1 and 3.2, respectively.

1. **Jimenez-Moya, B.**, Martin, D., Soler-Rivas, C., Barroeta, A.C., Tres, A., Sala, R. Acid versus crude oils for broiler chicken diets: in vitro lipid digestion and bioaccessibility. Animal Feed Science and Technology, 2021, 114926, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2021.114926.

2. **Jimenez-Moya, B.**, Barroeta, A.C., Tres, A., Soler, M.D., Sala, R. Soybean oil replacement by palm fatty acid distillate in broiler chicken diets: Fat digestibility and lipid-class content along the intestinal tract. Animals, 2021, 11, 1035. https://doi.org/10.3390/ani11041035.

Abbreviations

ADC	Apparent Digestibility Coefficients
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AME	Apparent Metabolizable Energy
AO	Acid Oils
BW	Body Weight
CMC	Critical Micellar Concentration
d	days
DAG	Diacylglycerols
DM	Dry Matter
DMM	Dietary Mixed Micelles
FA	Fatty Acid(s)
FAD	Fatty Acid Distillates
FCR	Feed Conversion Ratio
FFA	Free Fatty Acid(s)
GIT	Gastrointestinal Tract
LCFA	Long-Chain Fatty Acid(s)
MAG	Monoacylglycerols
MCFA	Medium-Chain Fatty Acid(s)
MIU	Moisture, Impurities, Unsaponifiable
MP	Micellar Phase
MUFA	Monounsaturated Fatty Acid(s)
NEM	Non Elutable Material
P	Palm Oil
PFAD	Palm Fatty Acid Distillate
PL	Phospholipids
PP	Precipitated Phase
PUFA	Polyunsaturated Fatty Acid
S	Soybean Oil
SA	Soybean Acid Oil
SCFA	Short-Chain Fatty Acid(s)
SFA	Saturated Fatty Acid(s)
TAG	Triacylglycerols
TFA	Total Fatty Acid(s)
TiO_2	Titanium dioxide
UFA	Unsaturated Fatty Acid(s)
UFA:SFA	Unsaturated Fatty Acid to Saturated Fatty Acid ratio

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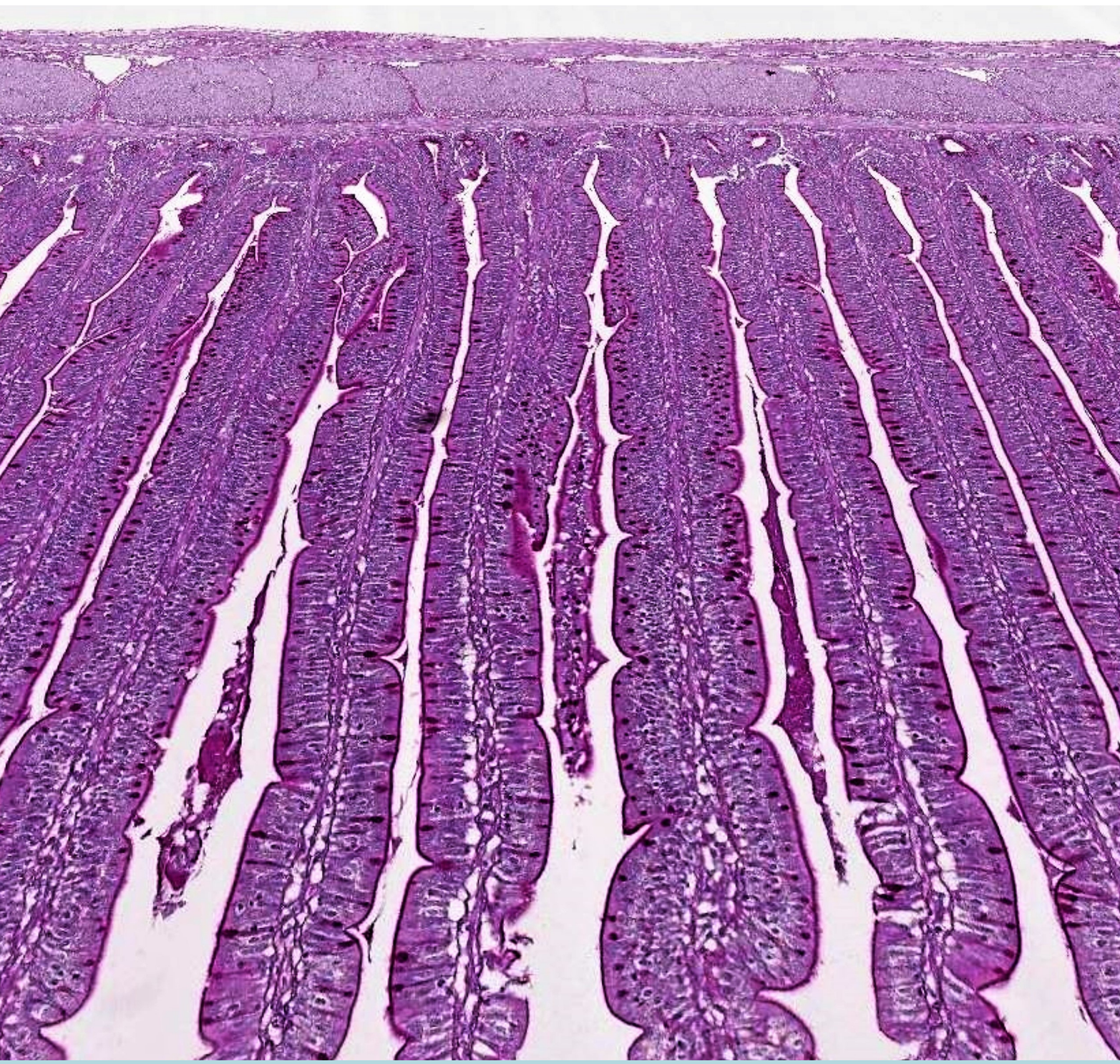
1.GENERAL INTRODUCTION



1.General Introduction

The present PhD dissertation is part of the project "Use of acid oils in monogastric animals: characterization, comparative nutrition, and meat quality repercussions" (AGL2015-64431-C2-1-R) and constitutes the next step after the results presented in the previous thesis "Use of acid oils in broiler chicken diets" (Rodríguez-Sánchez, 2018). The project emerged from intensive research to find alternatives to conventional fats for animal feed formulation and their impact on the quality of the food products. That includes European and national projects to advance in the upcycle of fat by-products in animal feed, contributing to the transition to a circular economy in animal food production. Results obtained and knowledge gaps detected in a previous European project "Feeding fats safety. Quality and safety of feeding fats obtained from waste or by-products from the food chain" (FOOD-CT-2004-07020), and in the national project "Use of random esterified acid oils in feeding monogastric animals. Comparative nutrition and lipid meat quality repercussions" (AGL2010-22008-C02) constitute the background of the present project.

The following general introduction aims to contextualize the importance of the research on acid oils or fatty acid distillates as alternative lipid sources for broiler chicken diets. First, the social relevance of poultry meat and the importance of lipids in broiler diets are described. Afterward, the physiology of fat digestion and absorption in poultry is explained, and finally, the literature review of the use of acid oils in chicken diets is presented, focused on soybean acid oil and palm fatty acid distillate.

1.1 The role of poultry meat in human nutrition

Nutrition of broiler chickens is an essential area to address since broiler chicken production has an important role to meet the future demand for food.

According to the current world population growth rate, around 1.05% per year, it is estimated that the world population will reach 9.7 billion people by 2050 (United Nations, 2019). This situation entails an important food demand for human consumption that implies that productivity will have to increase to satisfy this future demand (FAO, 2018). Poultry and pig meat are the most important meat sources produced globally (Figure 1.1), although egg production is also another important source of quality protein, with a total of 88 million Tn (FAOSTAT, 2019). In 2019, the production of poultry (131.6 million Tn) represented about 39% of meat produced worldwide (337.2 million Tn) and was expected to increase up to 137 million Tn for 2020 (FAO, 2020).

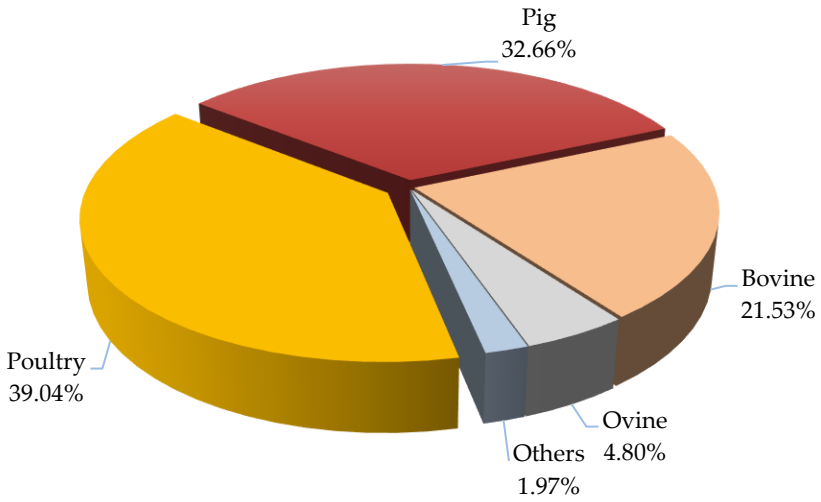


Figure 1.1 World meat production 2019. Graph by author. Adapted from FAOSTAT 2019.

Considering poultry meat production worldwide (Figure 1.2), chicken meat is the most produced (around 90%) meat product (FAOSTAT, 2019). In January of 2021, about 62% of its production took place in: United States of America (20.4 million Tn; 20.0%), China (14.9 million Tn; 14.6%), Brazil (14.1 million Tn; 13.9%), and the European Union (12.1 million

Tn; 11.8%). However, the global broiler meat trade is leading by Brazil, which is the most major exporter, reporting one-third of total exports (3.9 million Tn) (USDA, 2021a).

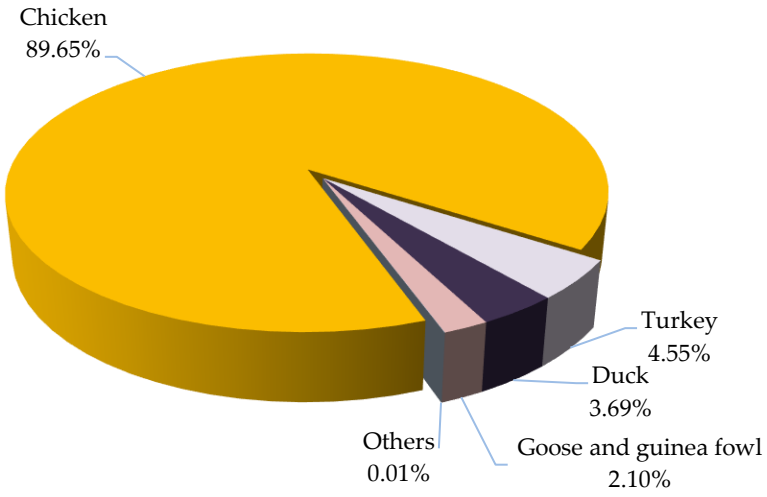


Figure 1.2. World poultry meat production 2019. Graph by author. Adapted from FAOSTAT 2019.

In the European Union, the largest producer in 2019 was Poland (19% of the total EU-27+UK poultry meat production) followed by Spain (13%) (USDA, 2020). Spain's chicken meat production was 1.4 million Tn, which represents the 80% of a total of 1.7 million Tn of poultry meat (MAPA, 2020).

The current highest and most efficient broiler chicken production is mainly related to a long-term genetic improvement of traits related to broiler productivity (85-90%), but also to nutrition (10-15%) (Havenstein et al., 2003). From 1957 to 2005, the growth of broiler increased about 464% together with an improvement of the feed conversion ratio (FCR) about 50% (Figure 1.3). In 2005, the production of 1g of chicken of 42 days of age required 1.208 less g of feed compared to 1957 (Zuidhof et al., 2014). From 2005 to 2019, FRC continued to improve from 1.674 to 1.611 (Aviagen, 2019).

In this context, the poultry sector, particularly broiler chicken production has an important role to play in answering the current and the future demand for food. Its meat provides a high-quality protein, it is relatively inexpensive to produce, widely available, and its consumption has no major taboos worldwide.

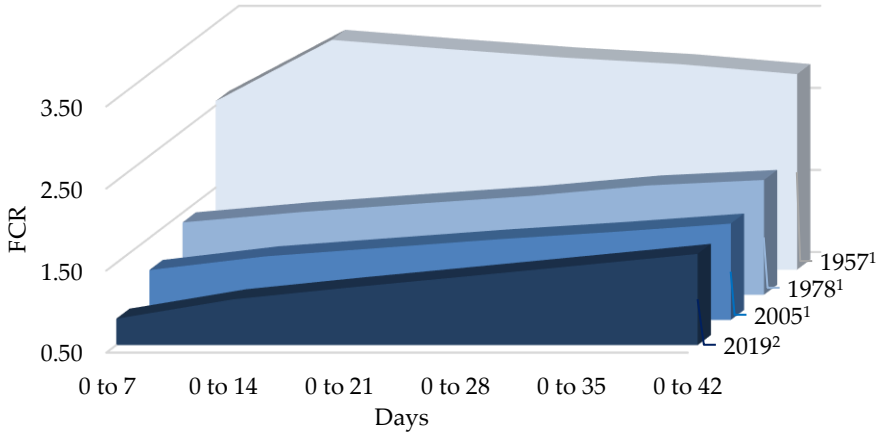


Figure 1.3. Cumulative feed conversion ratio of broiler chickens since 1957 to 2019. Graph by author. Data extracted from: [1]Zuidhof et al. (2014) and [2]Aviagen (2019).

In addition, poultry production has a less environmental impact than other livestock (Figure 1.4), with less greenhouse gas emissions across the supply chain per kilogram of food product (Poore and Nemecek, 2018; Ritchie, 2020). In poultry, around 30% of the total greenhouse emission is attributed to feed (on-farm emissions from crop production and its processing into feed for livestock). Consequently, poultry nutrition is an important area to address to meet future challenges.

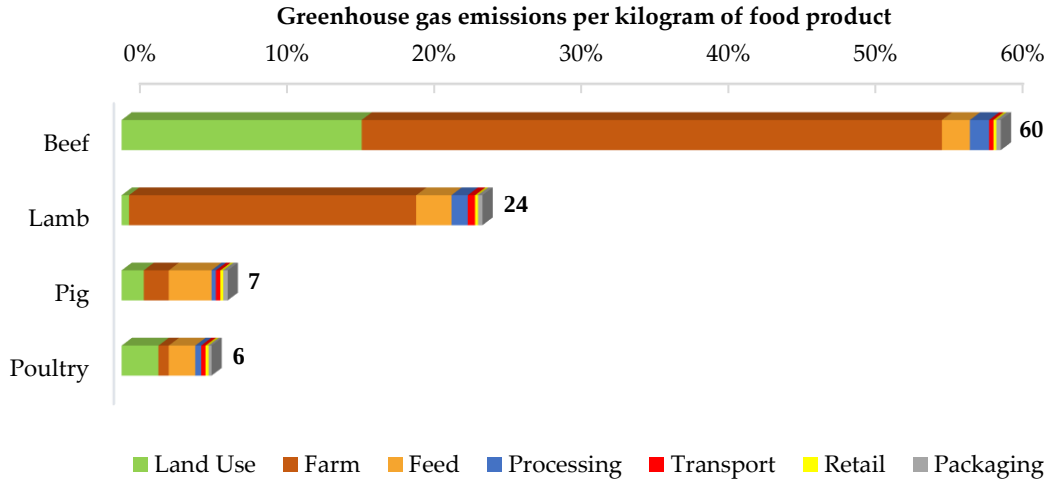


Figure 1.4. Greenhouse gas emissions across the supply chain. Data are kg CO_2 -equivalents per kg products. Graph by author. Data extracted from OurWorldinData.org. (Ritchie and Roser, 2020).

1.2 Lipids in broiler diets

Lipids are more than just an expensive energy source for broiler chicken feeds. The potential use of acid oils and fatty acid distillates in broiler feeding allows to valorize by-products with nutritional interest.

The feed is the highest cost of broiler production (60-70% of the total production cost) which major proportion is led by the cost of ingredients. In 2019, global broiler feed production was 307.3 million Tn, which represents about 29% of total feed production (1126 million Tn) (Alltech, 2020). In Spain, broiler feed represents 9% (3.4 million Tn) of total feed production (37.4 million Tn) (Subdirección General de Productos Ganaderos, 2019).

The rapid growth that characterizes the current broiler chicken production implies high energy demands by the animals. Broiler chicken adjusts its feed intake based on its energy requirements, and from the different feedstuffs present in their diets, lipids have the highest caloric value (approximately 9 kcal/g) (National Research Council, 1994). The use of lipids in broiler diets is a common and recommendable practice as besides providing energy, they supply essential fatty acids. Also, fat improves the absorption of fat-soluble vitamins, increases feed efficiency, and reduces the rate of food passage through the gastrointestinal tract (GIT), which allows greater absorption of all dietary nutrients. Moreover, its supplementation increases diet palatability, reduces feed dust, and due to lubricating effects, improves the yield and lifetime of feed milling equipment. The level of inclusion of lipid in commercial poultry diets is usually around 10 to 60 g/kg, however, the choice of lipid level incorporation in the diet formulation depends on the relative cost of lipids and cereal grains. In Spain, fats and oils represent 2.1% of the different feedstock groups used for animal feed, being cereals the most important (66.7%) followed by oilseeds (18%) (Dirección general de producciones y mercados agrarios, 2020).

The high and rising cost of lipids during the last decades is the main reason to search for alternative lipid sources at a lower price. Moreover, it is important to note that one of the

challenges in the 21st century related to sustainability is the use of ingredients not suitable for human consumption (by-products) in animal nutrition. The research to find and use available, cheaper, cost-effective, sustainable, and reliable alternative to conventional lipids for feed formulation allows to valorize by-products with nutritional interest and parallelly reduce environmental impact, enabling animal food producers to move toward circular agroindustry.

1.2.1 Fats and oils

Fats and oils are terms to designate lipids that are solid or liquid, respectively at room temperature. Chemically, fats and oils used in animal nutrition are generally triacylglycerols (TAG), which is a molecule constituted by three fatty acids (FA) bonded to a backbone structure of glycerol. The differences in FA composition determine the physical and chemical properties of lipids. Based on the saturation degree, the FA are classified into saturated FA (SFA; absence of double bonds in its structure) or unsaturated FA (UFA; the presence of one or more double bonds), the last one is divided into monounsaturated FA (MUFA; one double bond) or polyunsaturated FA (PUFA; more than one double bond). On the other hand, regardless of the saturation degree, FA can be classified as a function of its number of carbon atoms (chain length): short-chain FA (SCFA; contains less than 6 carbon atoms), medium-chain FA (MCFA; from 6 to 12 carbon atoms), long-chain FA (LCFA; from 14 to 20 carbon atoms), and very-long-chain FA (more than 20 carbon atoms). The increase in chain length and lower number of double bonds increases the melting point of the FA, so it will be less soluble. It is fundamental to know the FA profile of each lipid source since factors such as carbon chain length and the saturation degree influence the digestion process by the animal, its energy value, and the quality of the end-product.

Although the conventional lipid sources used in animal nutrition are mainly composed of TAG, other lipid classes also influence its energy value. Diacylglycerols (DAG), monoacylglycerols (MAG), free fatty acids (FFA), and phospholipids (PL) can be present at low levels, all of which supply lower energy content compared to TAG since their

energy is directly related to their FA content in the molecule structure. Moreover, there are other components that have negligible nutritive value, being energy dilution factors. Non elutable material (NEM) is the total amount of substances non-identified as a FA which includes moisture, impurities, and unsaponifiable (MIU) material, oxidized and polymerized FA, and glycerol. All these factors influence the quality of the lipid, therefore the analysis of these parameters, together with the determination of the FA profile, is a more precise quality control to characterize the different lipid sources before their use.

1.2.2 Common fats and oils used in broiler feeding

The lipid sources commonly used for broiler feeding are derived from vegetable and animal sources (Figure 1.5). Based on their FA composition, the most common saturated sources are palm oil, beef tallow, and pig lard, while soybean oil, sunflower oil, and rapeseed oil represent the unsaturated ones. Related to their FA profile, saturated sources have a higher melting point and lower apparent metabolizable energy (AME) than the unsaturated sources.

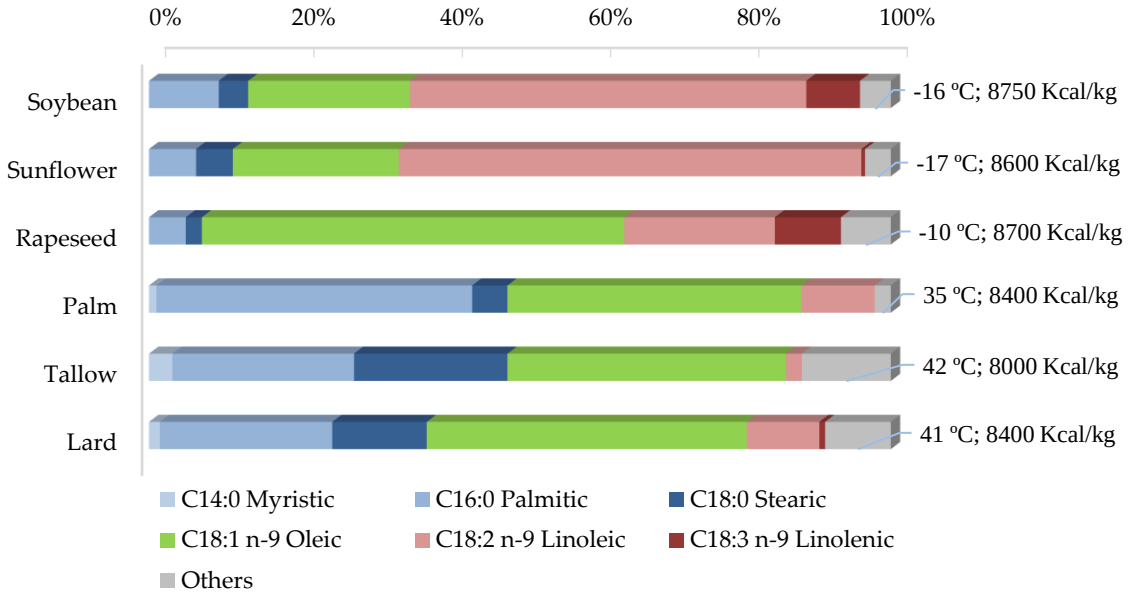


Figure 1.5. Fatty acid composition, melting point (ºC) and apparent metabolizable energy (Kcal/kg) of common lipid sources used in broiler chickens. Graph by author. Data extracted from Engineering ToolBox, 2008 and FEDNA (Fundación Española para el Desarrollo de la Nutrición Animal), 2019.

Palm oil is the most widely produced edible oil worldwide (35% of world production in 2020/21) (Figure 1.6), being Indonesia the largest producer, followed by soybean oil (29% of world production in 2020/21), mainly produced by China (USDA, 2021b). Their higher stock availability and cost/quality price with its lower price compared with the rest of the vegetable oils (palm: 955 US$/Tn; soybean: 1068 US$/Tn; corn: 1097 US$/Tn; rapeseed: 1148 US$/Tn; cottonseed: 1509 US$/Tn; coconut: 1427 US$/Tn; sunflower: 1433 US$/Tn, and peanut: 2141 US$/Tn) (USDA, 2021b) explain why are widely used as lipid sources in broiler feed.

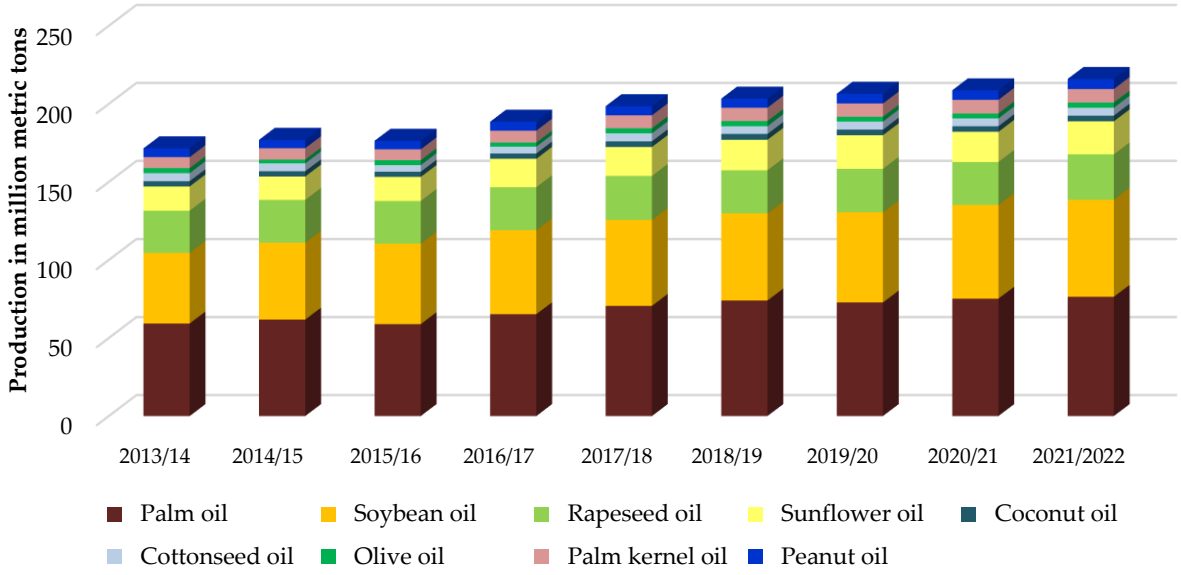


Figure 1.6. Global production (million metric tons) of the major vegetable oils from 2013/14 to 2021/22 (forecast). Graph by author. Data extracted from USDA Economic Research Service, 2020 and USDA, 2021b.

1.2.3 Acid oils and fatty acid distillates

In a previous European research project (FOOD-CT-2004-07020) it was concluded that acid oils (AO) and fatty acid distillates (FAD) were interesting alternative lipid sources for animal nutrition, and more economical in comparison with the conventional crude oils. Similarly, the national research project (AGL2010-22008-C02), focused on the possibility to give added value to AO through direct esterification of FFA with glycerol, also concluded that AO seemed to be an interesting subject for study because their use led to positive results comparable to those obtained with crude oils.

Both AO and FAD are by-products generated from the refining process of crude oils to obtain edible oils (Figure 1.7). The refining process is conducted to reduce the undesirable odor, flavor, color and to remove most of the undesirable components such as FFA, gums, waxes, metals, or other impurities (FEDIOL). The refining process is carried out by either a physical or chemical process. The choice of the refining method is based on the characteristics of the crude oil. Physical refining is more suitable for saturated oils as palm oil with high FFA content, but low PL, and compared to chemical refining, results in less by-products generated and reduces the amount of oil lost. In contrast, chemical refining is more suitable for unsaturated oils such as soybean oil with low FFA content and higher PL (Pawar and Marathe, 2015), this process is time-consuming, more expensive, and has a considerable oil loss (up to 50% of crude oil) but achieves the desired reduction of FFA content (Dumont and Narine, 2007). Both AO and FAD are by-products generated during the chemical and the physical refining process, respectively.

Palm crude oil is usually refined by a physical process, where FFA are removed by distillation obtaining as a by-products palm fatty acid distillate (**PFAD**). On the other hand, the chemical method is usually used to refine soybean crude oil, in which FFA are removed by alkali neutralization, obtaining as a by-product soybean acid oil (**SA**).

The refining process includes different process steps (Figure 1.7), degumming, bleaching, and deodorization. In chemical refining, a neutralization step is carried out before bleaching. The details of these steps are the following (Pawar and Marathe, 2015):

- **Degumming:** remove PL (gums) from the crude oil. This process represents 0.6-2.0% of crude oil loss, and gums are generated as a major by-product from the degumming process, which are the raw materials for lecithin processing. There are two main degumming processes:
    - Water degumming: addition of hot water (60-80ºC) to remove hydratable PL by centrifugation.
    - Acid degumming: addition of phosphoric acid or citric acid to remove non-hydratable PL.

- **Neutralization:** present only in chemical refining, consists of three steps: neutralization, washing, and drying. Almost all the FFA present in the crude oil are removed by the addition of an alkali solution (NaOH) that reacts with the FFA and converts them into soapstocks, which is the main by-product of this process (represents 1.6-2.0% of crude oil loss). Soapstocks are separated from the crude oil by centrifugation and the FFA contained in it can be extracted by adding sulfuric acid. These latter are the AO (represents approximately 1.7% of crude oil loss).
- **Bleaching:** remove coloring compounds and residual phosphatides, soaps, metals, and oxidation products by adding the adsorptive bleaching earth. The bleaching chemicals and impurities are subsequently removed by filtration (represent 0.5-2.0% of crude oil loss).
- **Deodorization:** it is the most important step to remove the FFA in the physical refining. It is a vacuum steam distillation process that also removes volatile components such as tocopherols, sterols, and sterols esters. Different degrees of pressure (2-10hPa) and temperature (180-270ºC) allow separating the different components (Dijkstra and van Duijn, 2016). In physical refining, the FFA distillates extracted from palm crude oil during deodorization represent about 4-5% of crude oil loss (Chang et al., 2016). This is the last step of the refining process, and the end-product obtained after that is the refined edible oil for human consumption.

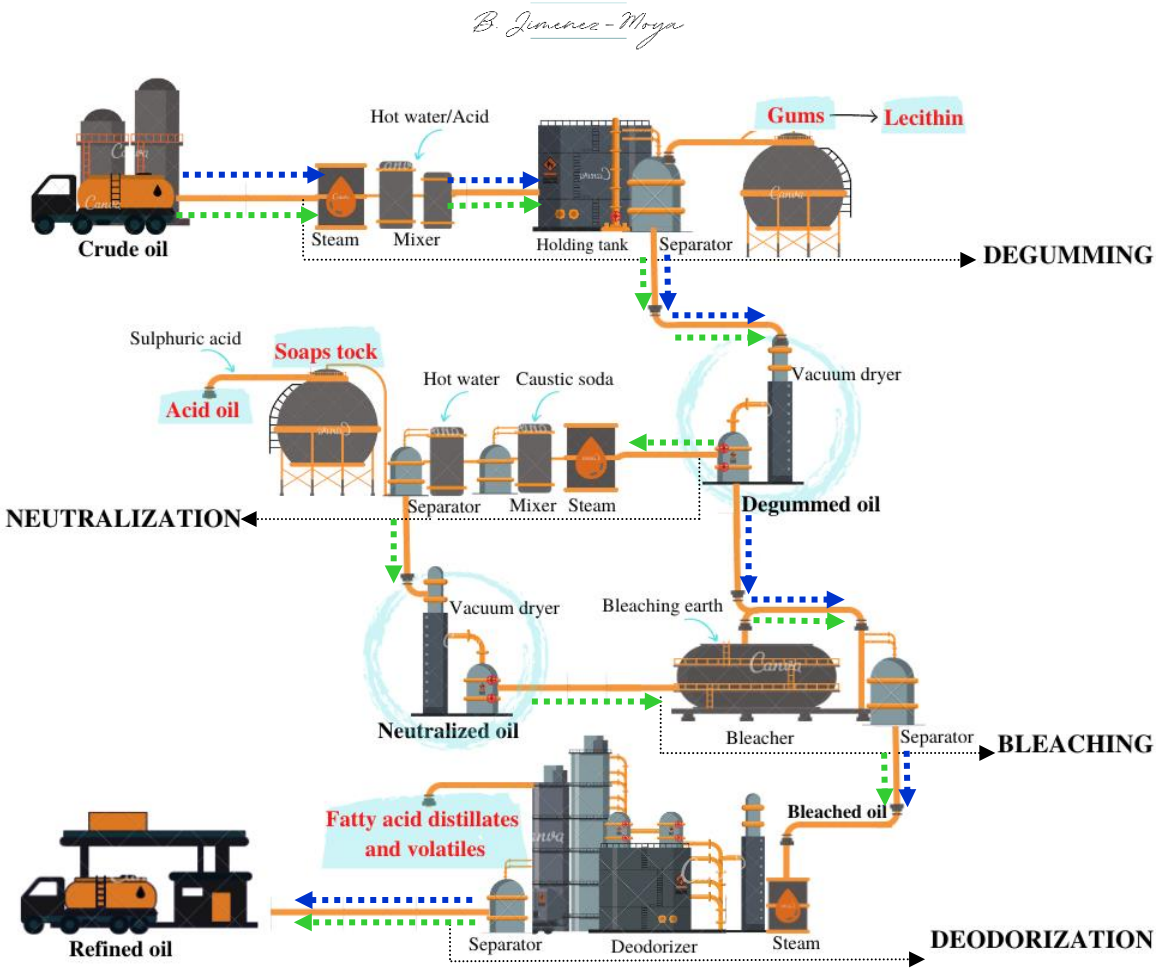


Figure 1.7. The process of crude oil refining. Blue arrows: physical refining process; Green arrows: chemical refining process. Graph by author created using Canva.

These by-products (SA and PFAD) are characterized by having a FA profile similar to the FA composition of their corresponding crude oil. However, they present high content of FFA (Table 1.1), which is higher for FAD (about 90%) than for AO (30-65%), and inversely for the rest of the lipid classes (TAG, DAG, and MAG). On the other hand, MIU content is lower in FAD than in AO (FAD: 5.37%; AO: 7.62%). In addition, AO and FAD, as by-products, can be quite variable in their composition, oxidation level, and presence of contaminants (Nuchi et al., 2009, Chang et al., 2016). According to Varona et al. (2021), the lipid-class composition and moisture of these fat by-products depend on the conditions of the refining process while their quantity of tocopherols, tocotrienols, unsaponifiable, and FA composition is related to the botanical origin.

Table 1.1. **Characterization of acid oils and fatty acid distillates.**

Parameter (%)	Acid oils (AO)	Fatty acid distillates (FAD)
Moisture and volatile matter	0.17 – 8.32 (1.31)	0.00 – 0.37 (0.12)
Insoluble impurities	0.33 – 10.24 (1.95)	0.05 – 8.74 (3.17)
Unsaponifiable matter	1.67 – 10.06 (4.36)	0.32 – 9.67 (2.07)
MIU	2.60 – 18.50 (7.62)	0.63 – 10.44 (5.37)
UFA:SFA ratio	1.0 – 6.2 (4.0)	0.8 – 6.8 (1.9)
TAG	9.3 – 54.2 (23.8)	2.1 – 8.6 (5.3)
DAG	6.5 – 28.2 (16.8)	2.6 – 6.3 (4.4)
MAG	ND – 6.2 (4.1)	ND – 1.6 (0.2)
FFA	31.7 – 65.3 (52.7)	87.2 – 93.6 (90.1)

Results expressed as min – max (mean). ND, not detected; MIU, sum of moisture, insoluble impurities, and unsaponifiable matter; UFA:SFA, unsaturated-to-saturated fatty acid ratio; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids.
Table by author. Data extracted from Varona et al. (2021).

According to the data of soybean oil production (60,49 thousand metric tons) and palm oil production (73,67 thousand metric tons) in 2020/21 (USDA, 2021b), the production of SA (estimated as 1.7% of soybean oil production) could be approximately 1,03 thousand metric tons. In the case of PFAD, which is considered about 4% of palm oil production, the estimated production could be 2,95 thousand metric tons. Regarding their price, these by-products are usually sold at 65-75% of the value of crude oils (Francesc Guardiola, personal communication), which could be estimated at 694-801 US$/Tn for SA oil, and 621-716 US$/Tn for PFAD.

1.3 Digestion and absorption of fats in poultry

The anatomical and functional knowledge of the gastrointestinal tract in poultry is of the outmost importance to study the fat utilization by the animal.
Fat digestion and absorption are complex processes, and the study of different lipid sources on fat utilization along the gastrointestinal tract provides a better comprehension of these processes.

1.3.1 The gastrointestinal tract

Fat utilization in poultry diets requires the digestion and absorption process during the passage through the GIT, processes that are similar in birds to those in other monogastric animals (Bauer et al., 2005). However, the GIT of poultry has some peculiarities (Figure 1.8) (Svihus, 2014, Denbow, 2015). It begins with the beak (without teeth or jaw muscles), then food particles pass through the esophagus and can either enter the crop, which is an extension of the esophagus and acts as a transient storage cavity, or pass directly to the proventriculus, where food particles are softened. After that, food moves into the gizzard, where food particles are broken down due to muscular movements. Once the food is ground, it passes into the small intestine, where fat digestion and absorption occur. The small intestine, where most of the released nutrients are absorbed, is composed of three distinct parts: the duodenum or duodenal loop, the jejunum (until the Meckel's diverticulum), and the ileum. The ileum ends at the ileo-cecal junction, where ceca are located, and the large intestine or colon starts. The ceca are two blind sacs, where part of the water and electrolytes remaining in the digested are reabsorbed, and fermentation of undigested nutrients takes place. The large intestine connects with the cloaca, a flexible cavity that represents the junction of the digestive, urinary, and reproductive tracts.

For the digestion of fats, is important the accessory organs: the pancreas and the liver. The pancreas lies in the center of the duodenal loop and the liver is located in the anterior end of the body cavity. In birds, in contrast to mammals, two bile ducts enter the duodenum,

the hepato-enteric duct (resulted from the combination of the right and left hepatic ducts) and the cysto-enteric duct (emerges from the connection between the right hepatic duct and the gallbladder). These two bile ducts and pancreatic ducts drain into the distal end of the duodenal loop (Denbow, 2015).

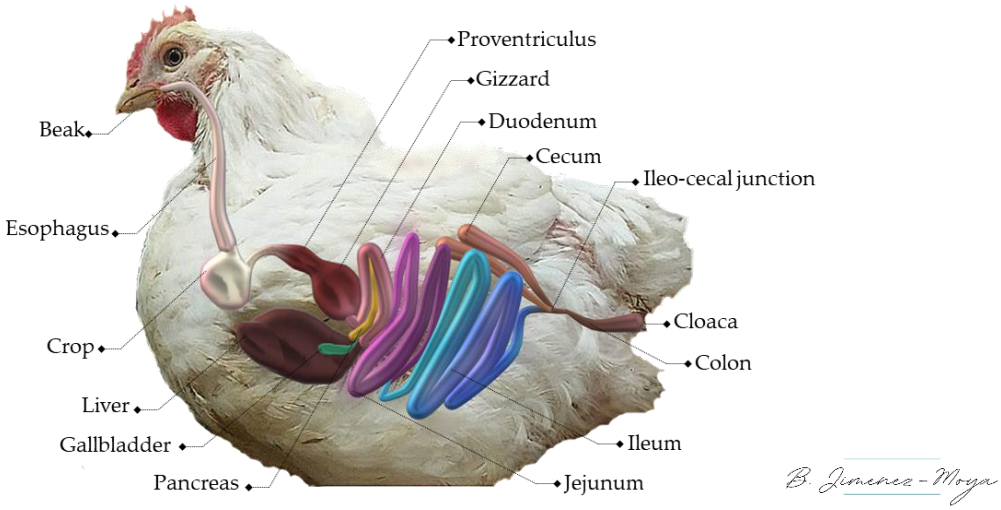


Figure 1.8. Digestive tract of a 35-day-old broiler chicken. Image by author.

Another difference of poultry digestive tract compared to mammals is that poultry has a shorter GIT and a faster passage rate of feed through the GIT, which in turn cause a short retention time of the ingested feed that can affect the optimal nutrient utilization (Angel et al., 2013). However, this fact can be compensated with another peculiarity present in poultry, the reverse peristalsis or refluxes (Duke, 1994), that occurs throughout the GIT (Sacranie et al., 2008). The first retrograde movement of digesta is from the gizzard to the proventriculus (gastric flux), the second one occurs among the upper ileum, jejunum, duodenum and into the gizzard (small intestinal flux). The third retrograde movement is in the large intestine, from the cloaca and colon to the cecum and lower ileum (cloacal-cecal flux). These refluxes increase the time the feed is re-exposed to digestive enzymes since these enzymes are not denatured although the variations in the pH conditions among the different compartments of the GIT (Sklan et al., 1978).

1.3.2 Broiler lipid digestion and absorption

The digestion and absorption process consists of several sequential steps. After feed ingestion, fats require the emulsification process since fats are insoluble in the aqueous medium of the GIT. Then, the TAG are hydrolyzed, releasing the absorbable lipid products (MAG and FFA). Finally, MAG and FFA are included in the dietary mixed micelles (**DMM**), which is the transport medium to attain the enterocyte for subsequent absorption.

1.3.2.1 Emulsification

Fat digestion starts in the gizzard with the grinding action (Figure 1.9). The contraction muscular movements of this organ cause a reduction in the feed particle size, which releases the TAG molecules from the feed matrix. Besides, due to the small intestinal flux, digesta from the duodenum refluxes to the gizzard and initiates fat emulsification. This process requires an emulsifier, mechanical movements, and a stabilizer (Holt, 1971). The molecules with potential emulsifying properties present in the gizzard, refluxed from the duodenum, include DAG, MAG, lysophospholipids, amphipathic peptides derived from protein hydrolysis, and especially bile salts. The gizzard provides sufficient motility for vigorous mixing of all substances and the stabilization is aided by lipolytic products from TAG hydrolysis (Bauer et al., 2005). Bile salts play an important role as an emulsifier because of their detergent-like properties, reducing the tension at the oil-water interphase. Their amphiphilic structure has a hydrophobic side that can dissolve at an oil/water interphase and a hydrophilic side that interacts with the aqueous medium. After the emulsification process, most lipids are present in emulsified droplets, including dietary TAG, DAG, and other fat-soluble nutrients (e.g. cholesteryl ester, vitamins) into the core, coated by a monolayer of PL, bile salts, denature proteins, oligosaccharides, and a small amount of cholesterol (Krogdahl, 1985, and Carey and Hernell, 1992).

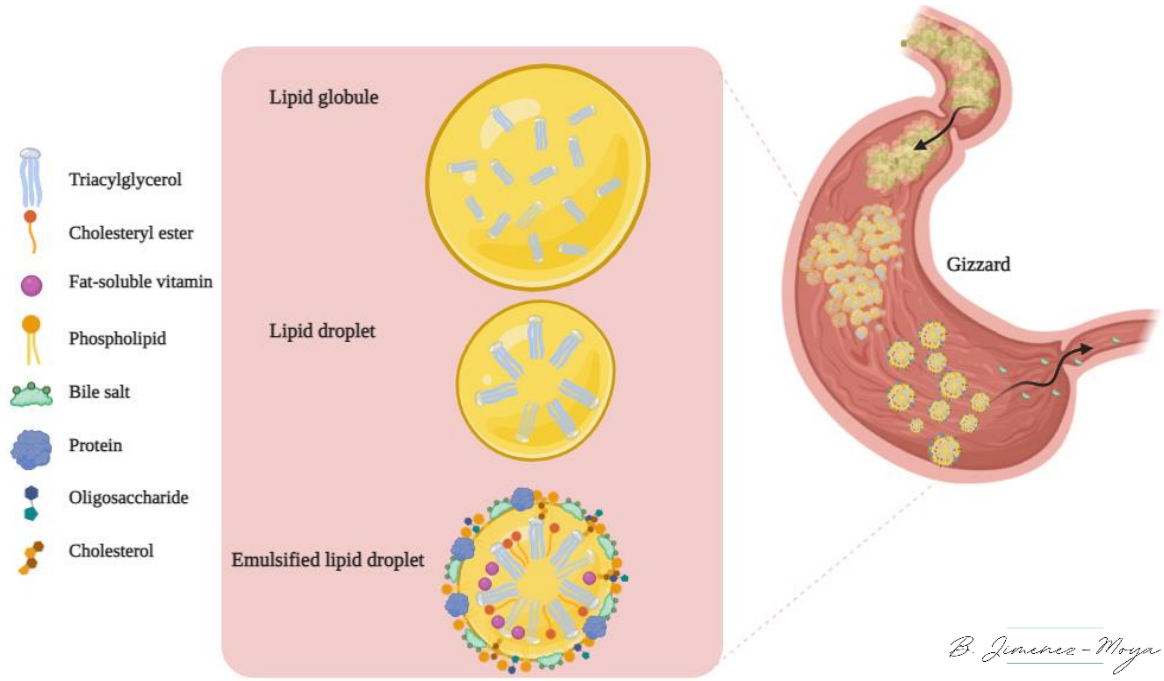


Figure 1.9. Simplified schematic view of the emulsification process in the gizzard. Image by author created using BioRender.com

1.3.2.2 Hydrolysis

The digesta, including the lipids as emulsion droplets, enters the duodenum (Figure 1.10), which stimulates the secretion of cholecystokinin. The presence of cholecystokinin causes the release of bile and pancreatic enzymes (Krogdahl, 1985). Bile produced in the liver enters the duodenum via the bile ducts. It consists of water, electrolytes, bile acids, bile salts, cholesterol, glycerides, PL, bile pigments, and some proteins (Zaefarian et al., 2019). The pancreas secretes water, bicarbonate ions, and different enzymes (Denbow, 2015). For the hydrolysis of TAG, the pancreatic secretion of lipase and colipase is essential. The presence of bile salts has great importance for the hydrolysis process, by their action lipid droplets break into very fine droplets, that are stable because bile salts prevent aggregation and coalescence of the droplets. This fact increases the surface area for the action on the lipase (Ravindran et al., 2016). Also, bile salts can stabilize lipase at low concentrations against surface denaturation, however, at higher concentrations inhibit the lipase activity. Lipase will not bind to a lipid droplet interface containing bile salts, phospholipids, proteins, or other surface-active constituents, preventing their hydrolysis

activity. However, the inhibitory effect is reversed by the colipase, which can form a one-to-one complex with lipase, binding to the surface of the lipid droplet, and acting as an anchor for lipase to reach its substrate (Bauer et al., 2005). Once lipase is binding to the lipid droplet interface, it hydrolyzes the TAG into DAG (intermediate lipolysis product) and FFA, and then DAG is hydrolyzed into MAG and FFA, which are the end lipolysis products. Lipase has specificity for the FA in sn1- and sn3- positions of the glycerol backbone, therefore the products resulted from the hydrolysis of each TAG are generally two FFA from sn1- and sn3-positions, and one MAG from sn2-position. It is reported that the saturation degree of the FA influences the lipase activity (Ravindran et al., 2016); the three-dimensional structure of unsaturated FA enables to bind with lipase more readily than saturated FA, which structure hinders the FA binding site (van Kuiken and Behnke, 1994).

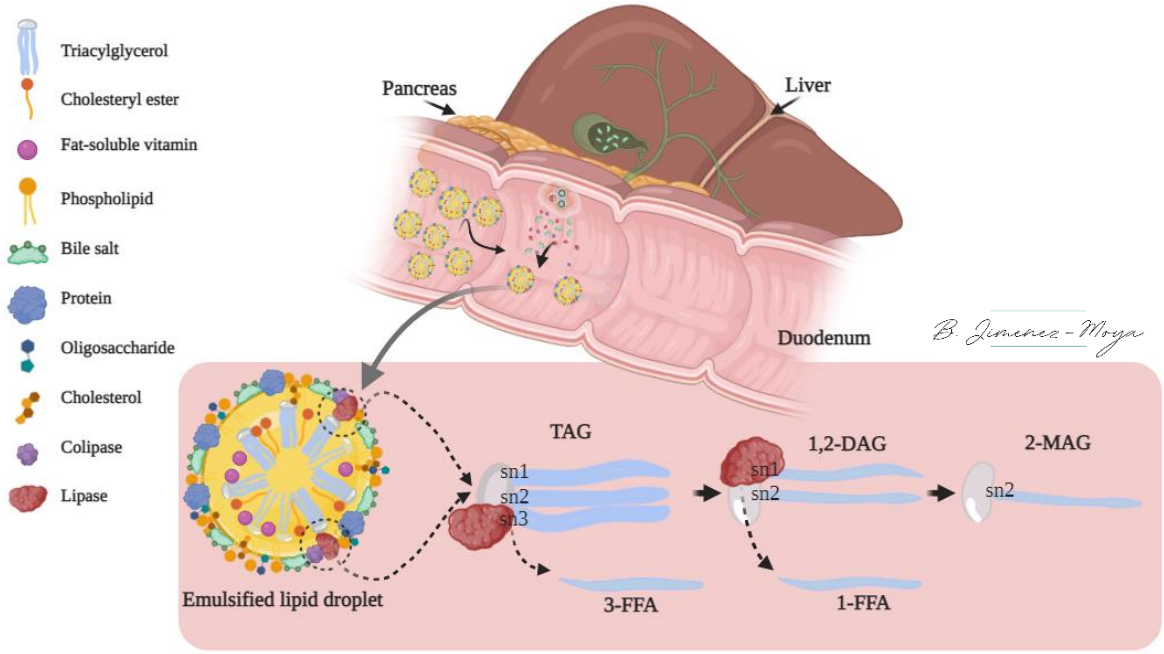


Figure 1.10. Simplified schematic view of the hydrolysis process in the duodenum. TAG = triacylglycerols; DAG = Diacylglycerols; MAG = Monoacylglycerols; FFA = Free fatty acids. Image by author created using BioRender.com.

1.3.2.3 Absorption

Bile salts have a key role to complete the digestion and absorption process. It solubilizes the end lipolysis products, removing them from the site of lipase action because the accumulation of MAG and FFA can inhibit its activity (Reis et al., 2009). Besides, bile salts inhibit the re-formation of TAG from MAG and FFA. Then, these end products, which are the potentially absorbable products, need to reach the absorptive site of the enterocytes. Although SCFA and MCFA are solubilized as individual components in the gut lumen, LCFA and MAG need to combine with bile salts and lysophospholipids to form DMM (Figure 1.11) (Sek et al., 2002; Pond et al., 2005). The ability to form DMM differs between unsaturated and saturated LCFA (Freeman, 1969); unsaturated LCFA are incorporated spontaneously in the DMM, while saturated LCFA need swelling amphiphiles such as MAG and unsaturated LCFA for its solubilization and its inclusion in the core of the DMM (Krogdahl, 1985). These structures are essential to transport the lipolytic products through the aqueous intestinal medium arriving at the intestinal microvillus membrane where the absorption takes place. The formation of DMM depends on bile salt concentration in the media, this fact is known as critical micellar concentration (CMC; Bauer et al., 2005). The DMM are composed of a hydrophobic core of LCFA, fat-soluble vitamins, and cholesteryl esters, coated by a monolayer of bile salts, lysophospholipids, and to a lesser degrees MAG, PL, and free cholesterol (Lairon, 2009; Reis et al., 2009; and Wang et al., 2013). Once the DMM, SCFA, and MCFA reach the brush border of the enterocytes, the absorption takes place. For this, it is necessary the disaggregation of DMM, facilitated by the low pH value of the unstirred water layer, to release the lipolytic products (Krogdahl, 1985), then MAG, SCFA, and MCFA are absorbed by a simple passive diffusion mechanism across the enterocyte membrane. On the other hand, LCFA are absorbed by an active protein-mediated process (Lo and Tso, 2009). From the different FA transporters identified there is the cluster determinant 36 (CD36), plasma membrane-associated fatty acid-binding protein (FABP), and a family of FA transport proteins 1–6 (FATP1–6) (Wang et al., 2013).

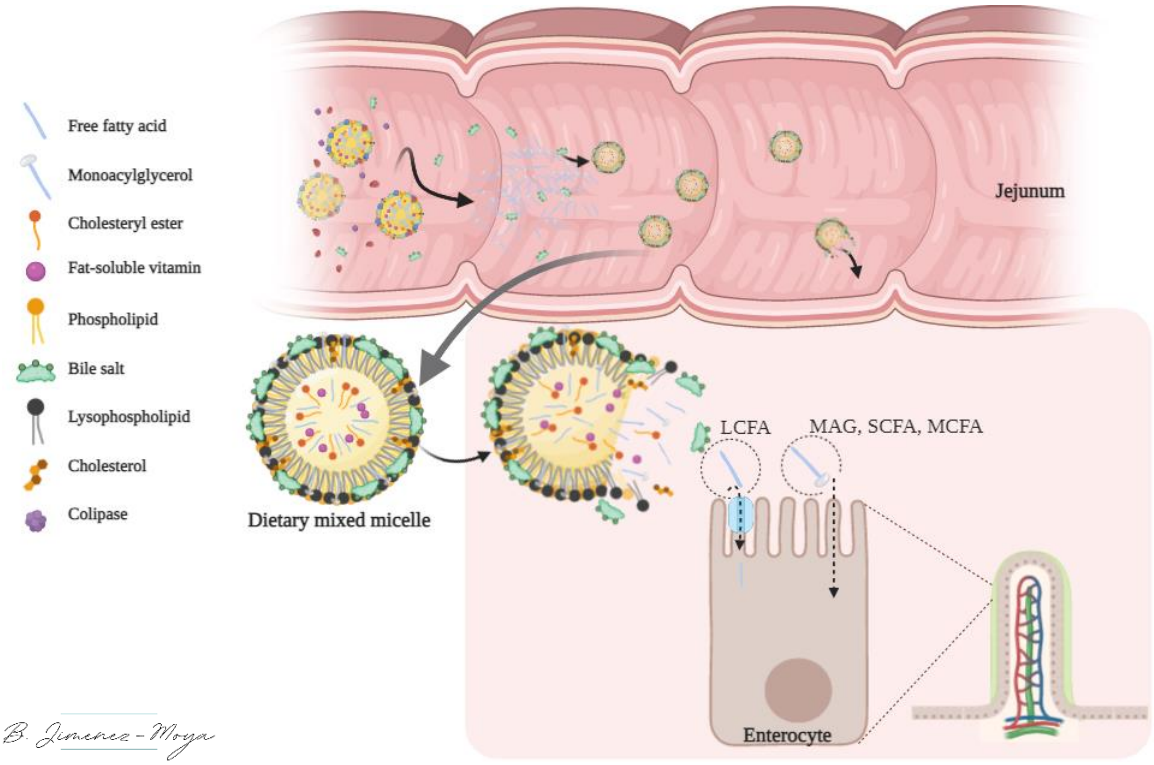


Figure 1.11. Simplified schematic view of the dietary mixed micelles formation and absorption of lipolytic products in the jejunum. LCFA = Long chain fatty acids; MAG = Monoacylglycerols; SCFA = Short chain fatty acids; MCFA = Medium chain fatty acids. Image by author created using BioRender.com.

Once inside the enterocyte, SCFA and MCFA pass directly to the portal blood bounded to albumin. On the other hand, the absorbed LCFA and MAG molecules reach the endoplasmic reticulum and are re-esterified to TAG. There are two possible ways for the re-esterification process, the first and predominant one is the MAG pathway in which MAG and LCFA form TAG by the action of MAG and DAG acyltransferases (enzymes located on the cytoplasmic surface of the endoplasmic reticulum). The second route, in the absence of MAG, is the α-glycerophosphate or glycerol-3-phosphate pathway. Then, TAG are included in lipoproteins, which include TAG and cholesteryl esters in the core coated by a monolayer of PL, cholesterol, and apolipoproteins (Wang et al., 2013). In birds, the intestinal lymphatic system is poorly developed, so these lipoproteins are drained directly into the portal blood system as very-low-density lipoproteins, which are termed portomicrons (Krogdahl, 1985). Portomicrons pass through the liver before they reach the rest of the circulation. The TAG from portomicrons can be stored in the liver hepatocytes,

can be used in the synthesis of different compounds, or at the vascular endothelium TAG can be hydrolyzed by the lipoprotein lipase; the FA released are β-oxidized to ATP as a source of energy (especially in muscle) or re-esterified to TAG and stored in tissues as fat deposits (Zaefarian et al., 2019).

1.4 Acid oils and fatty acid distillates in poultry diets: Literature review

The use of acid oils in poultry is controversial and the available studies conducted under up-to-date conditions in broiler chickens are scarce.
The last contributions point out the potential use of soybean acid oil and palm fatty acid distillate for broiler chicken feeding if these by-products are previously standardized.

1.4.1 Effect of dietary FFA level on fat utilization

The effect of the FFA level of added fat has been studied in poultry diets, however, there is a lack of agreement on their use and its inclusion level recommended in the diet. More information regarding their use in poultry diets is available in Rodríguez-Sánchez (2018).

Different authors have been studied the effect of the FFA level on the AME and digestibility of the added fat, but the results are controversial. In general, an increment in the FFA level was associated with a decrease in AME and digestibility values of the added fat; in cockerels (Young, 1961; Blanch et al., 1996), in laying hens (Shannon, 1971), and in broiler chickens (Wiseman and Salvador, 1991; Wiseman et al., 1992; Wiseman and Blanch, 1994; Blanch et al., 1995; Zumbado et al., 1999; Vieira et al., 2015). Nonetheless, Vilà and Esteve-Garcia (1996) reported that FFA content of the fats was a poor predictor of their AME values, and Mateos et al. (2012) considered the AO as good ingredients for poultry diets and recommended their use especially for finisher broilers and laying hens, when these by-products are of quality and cost-controlled. More recently, Borsatti et al. (2018) and Viñado et al. (2019) concluded that SA can be an adequate energy source for

broiler chickens, especially when it is blended with other by-products from the soybean oil industry, such as lecithin or glycerin, due to a positive synergism.

As mention above, lower AME values of the dietary fat have been associated with the FFA content. In fact, Wiseman and Salvador (1991) reported a linear decrease in the AME values of fats as FFA content increase (Figure 1.12) being the decrease more pronounced for saturated sources and more remarkable in young birds. According to this, Wiseman et al. (1998) developed the most known prediction equation for determining the AME values of fat sources used in the diets based on its UFA:SFA ratio (exponential response; from 1 to 3.5) and FFA content (linear response; 10% vs 50%) for young and adult chickens (1.5 vs 7.5 weeks, respectively). As it is present in Figure 1.12 and Figure 1.13, it is important to highlight that high FFA% and low UFA:SFA had a great detriment in young birds.

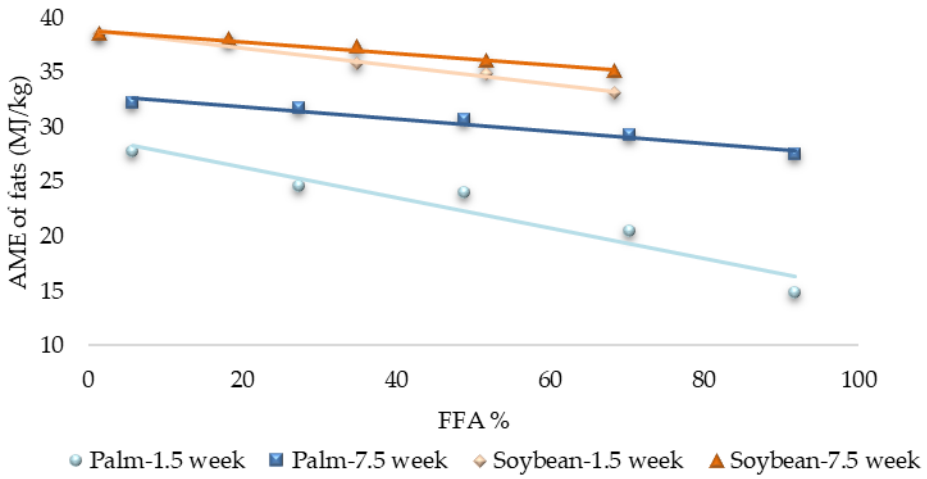


Figure 1.12. Summary of the apparent metabolizable energy (AME) values of fat sources (palm and soybean oil) with different free fatty acid (FFA) content. Responses are shown for young and old broiler chickens evaluated by Wiseman and Salvador, 1991. Graph by author.

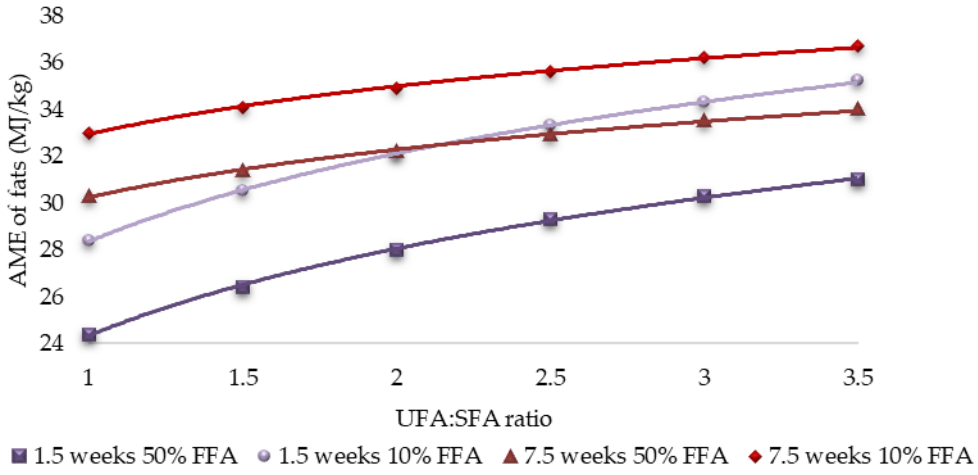


Figure 1.13. Apparent metabolizable energy (AME) of fat sources determined in low or high FFA content fats with different UFA:SFA ratio. Responses are shown for young and old broiler chickens evaluated by Wiseman et al., 1998. Data represent the solution of prediction equations for specific values of UFA:SFA and FFA (%). Graph by author.

Another factor that has been negatively related to the fat utilization by the animal is the content of non-energetic compounds measured as MIU or NEM analyses which have been negative correlated with AME values of fats (Wiseman et al., 1992; Vilà and Esteve-Garcia, 1996). According to this, a modification of Wiseman's equation has recently been suggested considering MIU content (Bierinckx, 2020).

The AO and FAD present in the market had a non-standardized quality and variable composition (Nuchi et al., 2009). In fact, results obtained from the characterization of AO and FAD available in the Spanish market showed high variability in their composition mainly influenced by both the botanical origin and the refining process (Varona et al., 2021). Also, most of the samples analyzed had MIU values above those recommended by FEDNA's (Fundación Española para el Desarrollo de la Nutrición Animal) guidelines (< 5%) (FEDNA, 2002), so when the MIU correction factor was applied to Wiseman's equation, the AME values of AO and FAD decreased (Varona et al., 2021). Thus, the authors point out the importance of the analytical control and standardization of these by-products to revalorized them as feed ingredients.

In general, results regarding the effect of the FFA in broiler nutrition are controversial. Most of the available studies are from decades ago, being far from modern improved and

efficient broiler chickens used nowadays. In addition, in most of them, the utilization of the added fat was determined without considering the quality of fats used. Furthermore, it is important to point out that the studies assessing the AME of the added fat as an ingredient did not evaluated the AME of the whole diet, so the synergism between the fat and other dietary components was ignored, underestimating the fat energy value of the diet (Mateos et al., 2019).

1.4.2 Use of soybean acid oil and palm fatty acid distillate in broiler chicken diets

In Table 1.2, a summary of different studies performed in chickens to assess the effect of the FFA level of SA or PFAD in comparison to their corresponding crude oil (S and P, respectively) is presented.

Young (1961) studied the utilization of various fats and FA supplemented at 15% by chickens at 4 and 8 weeks of age. Among them, there was S (0% FFA; 5.3 UFA:SFA) and soybean FA (S-FA; 76% FFA at 4 weeks, 93% FFA at 8 weeks; 4.9 UFA:SFA). No differences were reported for body weight (BW), nor for FCR among the different dietary fats or FA in any feed period. Similarly, Bornstein and Lipstein (1961) aimed to study the effect of different fat supplements in the diets added at 6% to a basal diet on the performance parameters. Among the different fats studied, no differences were observed in the performance parameters; chickens fed SA diets and birds fed S diets did not differed in BW, nor in FCR.

Artman (1964) studied different mixtures of fats and FA differing in their degree of saturation and FFA level. They included 15% of unsaturated sources (S, SA or soybean FA; S-FA), saturated sources (tallow or tallow FA) or blends between them. Animals fed SA (38% FFA) showed worst FCR, lower feed AME values and dietary fat digestibility than those fed S (<1% FFA), but no differences were reported for BW. However, S and S-FA (98% FFA) did not differ in fat digestibility, feed AME values, and BW, but worst FCR was reported in chickens fed S-FA. Moreover, a synergism effect between unsaturated

and saturated sources was reported for blend diets, being the feed efficiency, fat digestibility, and feed AME values obtained better than those calculated for these blends.

Zumbado et al. (1992, 1994) studied the impact of the FFA on fat utilization in broiler chickens, using blends of P + PFAD at different proportions obtaining diets (with 6% of fat or fat blend) with graded FFA levels (<5, 20, 40, 60, and >85% FFA). No differences in performance parameters (BW and FCR) and lipid digestibility were obtained among diets. In contrast, feed AME values were lower for chickens fed the blend diet with 40% FFA compared to chickens fed P diet (<5% FFA). No differences were observed between P and diets with 20%, 60%, and >85% FFA. However, considering the comparison with the positive control (chickens fed S), the authors concluded that up to 45% FFA in a blend of P + PFAD had not a negative effect on performance when that blend was added at 6% in starter diets.

Blanch et al. (1995, 1996) evaluated the effect of the dietary FFA level, the dietary saturation degree, and the use of blends of saturated and unsaturated sources on the feed AME and digestibility of the dietary fat when the experimental fats, oils or blends were added at 4% into a basal diet. When blends of tallow + S (50:50; 2.6% FFA) and tallow + SA (50:50; 34.2% FFA) were compared (Blanch et al., 1995), no differences on AME values of the feed and digestibility of dietary fat were obtained, regardless of the differences in the dietary FFA%. Similarly, no differences for feed AME and digestibility of dietary fat were reported between birds fed S (<1% FFA; 6.8 UFA:SFA) and those fed the blend of tallow + S (2.6% FFA; 2.5 UFA:SFA). The lack of differences supported that unsaturated sources enhanced the absorption of the saturated ones, and chickens fed blends with equal proportions of tallow and S had similar dietary fat utilization than chickens fed S alone. In another study, Blanch et al. (1996) used unsaturated (linseed oil and S) and saturated (tallow and P) sources. Although no differences were obtained for feed AME values, a negative FFA effect was reported for the apparent absorption of dietary fat; chickens fed SA diets (64.7% FFA) had lower values than birds fed S diets (2.5% FFA). The authors related this fact not only with the dietary FFA, but also with the high unsaponifiable content of SA (4.5% in SA vs 0.2% in S). Also, the results of those birds fed blends of SA +

tallow (38.9% FFA; 1.9 UFA:SFA) and SA + P (39.1% FFA; 1.8 UFA:SFA) reported the synergism between SA and the saturated sources (tallow or palm); the fat digestibility and AME values were higher than the calculated ones from the corresponding proportion of each source included into the blend. This was explained in part due to the higher content of TAG in tallow and palm oil, which could be hydrolyzed, providing enough MAG for improving the absorption of FFA from SA by their incorporation into DMM.

Zumbado et al. (1999) evaluated broiler performance from 19 to 29 days, fed a basal diet supplemented at 10% with different experimental fat sources. The authors reported lower BW and higher FCR for broiler fed palm FFA diet (91.7% FFA; 0.8 UFA:SFA) compared to those birds fed P diet (4.9% FFA; 1.1 UFA:SFA). Similarly, worst performance was obtained for broilers fed P + palm FFA blend in comparison to chickens fed P diet. In contrast, birds fed soybean FFA diet (50.6% FFA: 4.9 UFA:SFA) had the lowest FCR, and those that received palm FFA + soybean FFA blend did not differ in BW, nor FCR in comparison to animals fed P diet; this fact was related to a synergism effect between unsaturated and saturated FA, regardless of the FFA content of the lipid source.

Vieira et al. (2002) evaluated the incorporation of SA in broiler chicken diets, supplemented at 4% or 8% to a basal diet. The comparison between SA and degummed S showed no differences in BW. In contrast, higher FCR and lower feed AME values were reported for SA than for degummed S.

Vilarrasa et al. (2015) aimed to study the use of re-esterified oils, with different degree of saturation and molecular structure, to feed starter and grower broiler chickens. For this, crude oils (S: 0.8% FFA, and P: 4.8% FFA) and their corresponding acid oils (SA: 55% FFA, and PFAD: 55.8% FFA) were added at 6% in the basal diet, as a positive and negative control, respectively. It was reported a better utilization of unsaturated sources in comparison to saturated sources; the dietary saturation degree had a greater impact on FA digestibility than did the fat molecular structure. A negative FFA effect was reported on feed AME values and total FA (TFA) digestibility in starter chickens; chickens fed the acid oils had lower values than those chicks fed the corresponding crude oils. In grower broiler chickens, the negative FFA effect was also observed on TFA digestibility between

chickens fed S and those fed SA, but not between birds fed P or PFAD. However, no differences were reported for feed AME values in chickens at 36 days. Moreover, no effect was observed regarding any performance parameter, nor any feeding period.

Roll et al. (2018) assessed the effect of the fat molecule structure and its glycerol-to-fatty acid ratio on dietary fat apparent absorption using palm oils in starter and grower broiler chickens. In this study, the experimental fats were added at 6% to a basal diet; being P (7.5% FFA) the positive control, and PFAD (88.6% FFA) the negative control. In starter chicks, feed AME values and SFA digestibility were lower for birds fed PFAD compared to those fed P, but no effect was reported for TFA apparent absorption. Contrary, in grower broiler chickens, the negative FFA effect was reported in TFA digestibility, but not in feed AME values. Moreover, the authors concluded that the utilization of PFAD could be improved by adding glycerol esterified as a part of acylglycerol molecules (DAG, MAG).

Rodriguez-Sanchez et al. (2019) studied the effect of the dietary FFA content and dietary saturation degree on FA digestibility along the GIT in starter broiler chickens. For this purpose, S and P were replaced by graded levels of their corresponding acid oil (SA and PFAD, respectively), which were added into a basal diet at 6%. In relation to the saturation degree, unsaturated sources had higher FA digestibility coefficients than saturated sources, regardless of the FFA level. The authors concluded that the saturation degree influenced more on the FA digestibility compared to the FFA level. This study reported an interaction between the fat source and the dietary FFA% regarding the feed AME values; S diet (3.6% FFA; 4.4 UFA:SFA) had the highest value and SA diet (55.5% FFA; 3.4 UFA:SFA) the lowest one, while no significant difference was reported between P diet (5.9% FFA; 1.3 UFA:SFA) and PFAD diet (47.3% FFA; 1.2 UFA:SFA). Regarding the FFA effect on the maximum FA digestibility coefficients reached at ileum level, no differences were reported for TFA, MUFA and PUFA digestibility coefficients, regardless the FFA level (5, 15, 35, and 50% FFA), but a negative effect was observed for SFA apparent absorption, and a regression analysis described the quadratic model observed for soybean diets, where lower FFA% (<15%) did not cause a negative effect, but higher

FFA% (≥35%) caused lower SFA digestibility coefficients. In contrast, palm diets did not fit any model because the SFA digestibility was similar, regardless the dietary FFA%. Performance parameters (BW and FCR) were not affected by the FFA% of the diet when S and P were compared with their respective acid oil (SA and PFAD). It is important to highlight that among soybean diets the UFA:SFA changed from 4.43 to 3.37 as FFA level increased, whereas among palm diets the UFA:SFA had less variations (1.32 to 1.21). These authors suggested that SA could be a good alternative fat source for starter chicks at least when the dietary FFA content does not exceed 15% with UFA:SFA around 4. In addition Rodríguez-Sánchez, (2018) concluded that SA could replace S in grower-finisher chickens when the FFA% does not surpass 35%.

1.5 Justification of the Topic

Considering the studies described above, it is clear that there is a lack of consensus regarding the effect of dietary FFA level on performance parameters, feed AME values, and dietary fat digestibility. Two factors undoubtedly affect dietary fat utilization, the saturation degree of the fat and the age of the bird, and both seem to have a greater impact on fat utilization than the FFA content of the fat has. Regardless of the molecular structure, the unsaturated sources, such as those derived from soybean oil, are better digested than saturated ones, such as palm oil, and this is more evident in starter chickens. The fat utilization improves with the age of the bird, mainly from saturated sources, but also improves the capacity of FFA utilization. When the FFA level is assessed, it is important to consider the refining process conducted in each case since this factor determines the percentage of FFA of the AO or FAD. For instance, PFAD can reach a high FFA level (90%), which could lead to worst fat utilization than its corresponding crude oil, regardless of the age of the chicken. However, when PFAD is combined with P, decreasing the FFA content until a moderate percentage (around 50%), its utilization does not differ from the utilization of its corresponding crude oil, mainly in grower-finisher chickens. Regarding the use of SA, grower-finisher chickens are more capable to use it than starter chicks; it is described that this by-product blended with its corresponding

crude oil, with 15% FFA in 11-day-old broiler chickens, and 35% FFA in 37-day-old broiler chickens it is a suitable energy source to include in the broiler feeding. Moreover, it has been demonstrated the synergistic effect between saturated and unsaturated sources, and the beneficial effect of increasing the ratio of TAG and/or DAG to FFA. Thus, the study of replacement of a conventional fat source by an acid oil modifying the UFA:SFA, and the lipid-class composition in both starter and grower-finisher broiler chickens is needed in order to know if these blends could be a good option to use these by-products to feed broiler chickens, under up-to-date conditions.

From our knowledge, only Blanch et al. (1996) studied the effect of SA+P blends in 1-year-roosters feed, but no studies are available on broiler chickens, nor using PFAD+S.

For this, it is important to understand the dynamics of fat hydrolysis and absorption process when using these by-products, as is described by Rodriguez-Sanchez et al. (2019). However, no previous research has assessed the hydrolysis and absorption process when using SA blended with a conventional saturated source or PFAD blended with a conventional unsaturated source.

Table 1.2. **Effect of the FFA content of fat sources on performance parameters, feed AME values, and dietary fat digestibility in chickens.**

Reference	Fat sources	Inclusion (%)	FFA (%)	UFA:SFA ratio	MIU (%)	Breed - Strain	Age (days)	FFA effect on:			
								BW	FCR	Feed AME	Dietary fat digestibility
Young (1961) Experiment 2	S, corn oil, lard, beef tallow, S-FA, corn oil FA, lard FA, beef tallow FA, yellow grease, hydrolyzed animal and vegetable fat	15	0-96.4	0.9-5.8	IU 0.2-2.3	Peterson x White Rock cockerels	28 56	No effect	No effect	ND	ND
Bornstein and Lipstein (1961) Experiment 1	Tallow, feed oil (methyl esters of vegetable fat), S, soybean lecithin, soybean soapstock, SA, SA+ lecithin, S meal + S processed unextracted soybeans	6	NS	NS	NS	White Rock x Leghorn chickens	42 80	No effect	No effect	ND	ND
Artman (1964) Experiment 4	S, SA, S-FA, tallow, tallow FA, blends	15	<1-99	NS	NS	Arbor Acre White Rock cockerels	56	S = SA	S < SA*	S > SA*	S > SA*
Zumbado et al. (1992) Experiment 1	S, P, blends of P + PFAD	6	<5->85	NS	NS	Broiler chickens	NS	ND	ND	P = PFAD (20%, 60%, >85% FFA) P > PFAD (40% FFA)*	No effect
Zumbado et al. (1994)	S, P, blends of P + PFAD	6	<5->85	NS	NS	Broiler chickens	NS	No effect	No effect	ND	ND

FA = Fatty acids; FFA = Free fatty acids; NS = non-specified; ND = non determined; S = soybean oil; SA = Soybean acid oil; S-FA = Soybean fatty acids; P = palm oil; PFAD: palm fatty acid distillate.
* = P-value < 0.05. ‡ = P-value < 0.01

Table 1.2. **Effect of the FFA content of fat sources on performance parameters, feed AME values, and dietary fat digestibility in chickens. Continued.**

Reference	Fat sources	Inclusion (%)	FFA (%)	UFA:SFA ratio	MIU (%)	Breed - Strain	Age (days)	FFA effect on:			
								BW	FCR	Feed AME	Dietary fat digestibility
Blanch et al. (1995)	P, tallow, tallow + S (50:50, TS), tallow + SA (50: 50, TSA), S, linseed oil	4	<1-34.2	1.0-7.5	0.8-1.5	Broiler chickens	14	ND	ND	No effect	S = TS S > TSA‡ TS = TSA
Blanch et al. (1996)	Tallow, tallow + SA (50:50, TSA), P, P + SA (50:50, PSA), SA, lard, S, linseed oil	4	2.5-64.7	0.9-9.1	3.2-58.8	Warren roosters	365	ND	ND	No effect	S > SA‡ S = PSA or TSA
Zumbado et al. (1999)	Palm free fatty acids (PFFA), soybean free fatty acids (SFFA), P, tallow, restaurant greases	10	1.1-91.7	0.7-4.9	0.3-1.3	Broiler chickens	29	P > PFFA P > P+PFFA* P = SFFA = PFFA+SFFA	P < PFFA P < P+PFFA* SFFA < P = PFFA+SFFA	ND	ND
Vieira et al. (2002)	Degummed S (DS), SA, blends	4, 8	NS	3.8-4.8	<1	Ross x Ross 308	42/30†	DS = SA	DS < SA*	DS >SA*	ND

FA = Fatty acids; FFA = Free fatty acids; NS = non-specified; ND = non determined; S = soybean oil; SA = Soybean acid oil; P = palm oil; PFAD: palm fatty acid distillate. * = P-value < 0.05. ‡ = P-value < 0.01.

† first number for performance parameters (BW, FCR), second number for feed AME and dietary fat digestibility.

Table 1.2. **Effect of the FFA content of fat sources on performance parameters, feed AME values, and dietary fat digestibility in chickens. Continued.**

Reference	Fat sources	Inclusion (%)	FFA (%)	UFA:SFA ratio	MIU (%)	Breed - Strain	Age (days)	FFA effect on:			
								BW	FCR	Feed AME	Dietary fat digestibility
Vilarrasa et al. (2015)	P, PFAD, re-esterified P low in MAG and DAG, re-esterified P high in MAG and DAG, S, SA, re-esterified S low in MAG and DAG, re-esterified S high in MAG and DAG	6	<1-55.8	0.9-5.7	0.3-2.4	Ross 308	20/12†	S = SA P = PFAD	S = SA P = PFAD	S > SA P > PFAD⁑	(TFA) S > SA P > PFAD⁂
							40/36†	S = SA P = PFAD	S = SA P = PFAD	S = SA P = PFAD	(TFA) S > SA⁑ P = PFAD
Roll et al. (2018)	P, PFAD, re-esterified P, blends	6	0-88.6	0.8-1.0	NS	Ross 308	21/10†	No effect	No effect	P > PFAD⁂	(TFA) P = PFAD (SFA) P > PFAD⁂
							42/39†	No effect	No effect	No effect	(TFA) P > PFAD⁂
Rodriguez-Sanchez et al. (2019)	S, SA, P, PFAD blends (5, 15, 35, 50% FFA)	6	3.6-55.5‡	1.21-4.43‡	NS	Ross 308	21/14†	S = SA P = PFAD	S = SA P = PFAD	S > SA⁂ P = PFAD	Ileum: TFA, MUFA, PUFA No effect SFA: S > SA* P = PFAD

FA = Fatty acids; FFA = Free fatty acids; NS = non-specified; S = soybean oil; SA = Soybean acid oil; P = palm oil; PFAD: palm fatty acid distillate. TFA = Total fatty acids. SFA = saturated fatty acids. MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids.
* = P-value < 0.05. ⁑ = P-value < 0.01. ⁂ = P-value <0.001.
† first number for performance parameters (BW, FCR), second number for feed AME and dietary fat digestibility.
‡ values determined in the experimental diets.

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2.OBJECTIVES

2.Objectives

This PhD dissertation is part of a project (AGL2015-64431-C2-1-R) conducted to characterize the acid oils coming from the industrial refining applied to edible oils and to generate practical information on their use in feeding monogastric animals (poultry, swine, and fish) and their impact on the lipid quality of the meat produced. This project is included in the priority line of our research group in the search for new alternative fat sources for monogastric animal feeding, as it is evident in the various projects that have been carried out in last years focused on different by-products coming from refining processes (ref. FP6 FOOD-CT-2004-007020; ref. AGL2010-22008-C02) to up-cycled and by reintroducing them into the food chain.

As seen in the literature review, the use of vegetable acid oils as lipid sources in animal diets seems to be quite controversial. Although their high FFA content has been considered as responsible for a lower nutritive value, recent advances in the use of soybean acid oil and palm fatty acid distillate in broiler diets put into relevance how the inclusion in the diet of oils with moderate levels of FFA, achieved by blending each by-product with its corresponding crude oil, could be a suitable energy source. However, the potential strategy to use these by-products - rich in FFA -, blended with conventional oils of different saturation degrees - rich in TAG -, remained largely unexplored. To the best knowledge of the authors and to date, there are no studies in the literature reporting its effect.

In this context, it was hypothesized that these by-products blended with conventional oils of different saturation degrees can be a good alternative source to feed broiler chickens. The main objective of this thesis is to provide information about the potential use of soybean acid oil and palm fatty acid distillate in broiler chicken diets, paying special attention to the effect on intestinal absorption in both starter broiler chickens and grower-finisher chickens. This study contributes to expanding knowledge about the relation

between the UFA:SFA and the FFA content of the diet. To reach this aim, we established the following specific objectives:

- To determine the hydrolysis and bioaccessibility of soybean acid oil and palm fatty acid distillate and their corresponding crude oils by simulation of their *in vitro* intestinal digestion. Addressed in section 3.1.
- To assess the effect of the replacement of soybean oil by palm fatty acid distillate on lipid-class content and FA digestibility along the intestinal tract and in the excreta in 11 and 35-day-old broiler chickens. Addressed in section 3.2.
- To study the effect of the replacement of palm oil by soybean acid oil in the starter and grower-finisher broiler chicken diets on fat digestion and absorption process in broiler chickens at 11 d and at 35 d. Addressed in section 4.1.

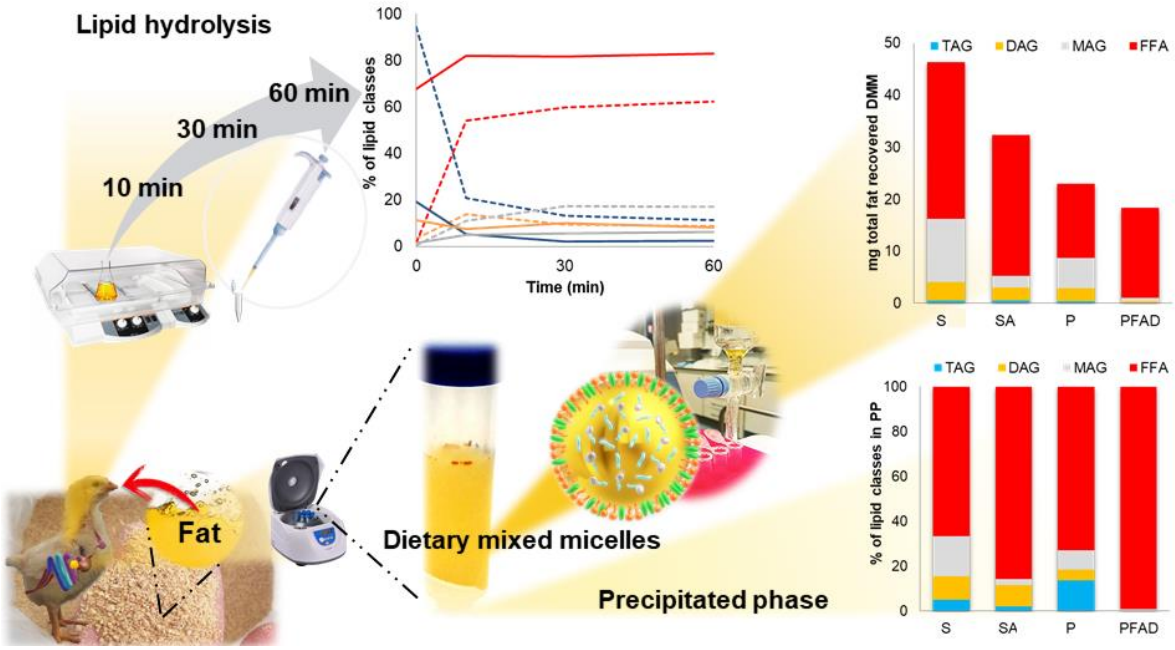
The two *in vivo* trials were conducted in parallel (sections 3.2 and 4.1) evaluating the dynamics of the lipid-class content and the FA digestibility along the intestinal tract and in the excreta.

3.PUBLISHED ARTICLES

USE OF SOYBEAN ACID OIL AND PALM FATTY ACID DISTILLATE IN BROILER CHICKEN DIETS

3.1 Acid versus crude oils for broiler chicken diets: in vitro lipid digestion and bioaccessibility

Jimenez-Moya, B.; Martin, D.; Soler-Rivas, C.; Barroeta, A.C.; Tres, A.; Sala, R. Acid versus crude oils for broiler chicken diets: in vitro lipid digestion and bioaccessibility. Animal Feed Science and Technology, 276 (2021) 114926.



https://doi.org/10.1016/j.anifeedsci.2021.114926

3.2 Soybean oil replacement by palm fatty acid distillate in broiler chicken diets: fat digestibility and lipid-class content along the intestinal tract

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Article

Soybean Oil Replacement by Palm Fatty Acid Distillate in Broiler Chicken Diets: Fat Digestibility and Lipid-Class Content along the Intestinal Tract

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Simple Summary: Palm fatty acid distillate is a by-product of palm oil refining. It is of both environmental and economic interest to include it in the diets of broiler chickens. However, its high saturation degree and acidity level limit its use. This study aimed to assess the effect of replacing soybean oil with increasing levels of palm fatty acid distillate on the utilization of fat by broilers. Dietary fat hydrolysis was mostly affected by the age of the bird and including palm fatty acid distillate mainly affected the absorption process. The replacement of soybean oil by palm fatty acid distillate reduced the total fat utilization, and in starter chicks delayed the site of fatty acid absorption. As the age increased, the digestibility of saturated fatty acids improved, and, above all, it improved the free fatty acid utilization. Therefore, the potential inclusion of palm fatty acid distillate for broiler feeds depends on the age of the bird. It would not be recommended to include this by-product in starter feeds. However, for the grower-finisher phase, blending palm fatty acid distillate with soybean oil (1:3, w/w) could be a suitable alternative, that does not have negative repercussions for either fatty acid absorption or growth performance.

Abstract: Palm fatty acid distillate (PFAD) is a by-product of palm oil (P) refining. Its use in chicken diets is a way to reduce the cost of feed and the environmental impact. Its low unsaturated:saturated fatty acid ratio (UFA:SFA) and its high free fatty acid (FFA) level could be partially counteracted by its blending with soybean oil (S). The objective was to assess the effect of replacing S with different levels of PFAD on lipid-class content and fatty acid (FA) digestibility along the intestinal tract and in the excreta of 11 and 35-day-old broiler chickens. Five experimental diets were prepared by supplementing a basal diet with S (S6), PFAD (PA6), two blends of them (S4-PA2 and S2-PA4), or P (P6) at 6%. Replacing S with PFAD did not affect performance parameters ($p > 0.05$) but negatively affected feed AME, FA digestibility, and FFA intestinal content ($p < 0.05$), especially in starter chicks. Including PFAD delayed total FA (TFA) absorption ($p < 0.05$) at 11 days, but at 35 days it did not affect the TFA absorption rate. The use of PFAD blended with S, when FFA $\leq$ 30% and UFA:SFA $\geq$ 2.6, led to adequate energy utilization in broiler grower-finisher diets.

Keywords: fat digestibility; lipid classes; free fatty acid; fat by-product; fatty acid distillate; alternative energy source; broiler chickens; poultry; intestinal tract

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1. Introduction

Fats are usually used in poultry diets as they satisfy a large fraction of the energy requirements. Palm fatty acid distillate (PFAD) is a fat by-product from the production of refined palm oil (P) which is one of the most produced and consumed vegetable oils worldwide [1]. Usually, P is obtained by a physical refining process that includes different steps, namely degumming, winterization (optional), bleaching, and deodorization [2]. The latter step is a vacuum steam distillation process that removes the FFA that are accumulated in the fatty acid distillate [3]. PFAD is characterized by having a high proportion of free fatty acids (FFA: 87–94%, being rich in saturated FA (SFA) and including other compounds such as tocopherols [4]). Based on a circular economy and taking into account the rising cost of conventional fats, there is increased interest in upcycling by-products from the fat industry for animal feeding to reduce the cost of feed formulation and also the environmental impact [5,6].

Assessing the digestibility of a fat ingredient is one of the clearest ways of defining its nutritional value for an animal. Conventional fat and oil sources used in poultry feed mainly consist of triacylglycerol (TAG) molecules. During digestion, TAGs, and diacylglycerols (DAGs) are hydrolyzed into monoacylglycerol (MAG) and FFA, which are incorporated into dietary mixed micelles (DMM) to attain the enterocytes for their absorption. Therefore, studying the evolution of the lipid classes (TAG, DAG, MAG, and FFA) and FA digestibility along the different segments of the gastrointestinal tract (GIT) may be of great interest for understanding the dynamics of fat digestion [7,8], mainly in new alternative fat sources rich in FFA and also in fat blends.

The PFAD is rich in FFA and also in SFA. It is well known that the degree of FA saturation plays and important role in fat absorption. In broiler chickens, although SFAs are not digested as well as unsaturated FA (UFA), several authors have found a synergistic effect when saturated sources are blended with unsaturated ones [9–11]. In fact, recent studies have found that the saturation degree of the dietary fat has more influence on fat digestibility than its FFA content [5,12]. Moreover, it has been suggested that there is a positive effect on FFA digestibility when there are increasing amounts of DAG or MAG, because their emulsifying effect enhances the inclusion of FFA in DMM [13]. However, there are few studies on FA absorption using blends of crude (rich in TAG) and acid (rich in FFA) oils.

Furthermore, it is accepted that the absorption of FA is also affected by the age of the chickens. Better results in the hydrolysis–absorption process along with the GIT of conventional and alternative fats have been obtained in grower-finisher chickens compared to starter broiler chickens [8].

Therefore, our hypothesis is that PFAD in combination with soybean oil (S) could be considered as an alternative energy source for broiler chicken diets, but the use of PFAD might be influenced by the age of the chicken. Thus, the aim of the present study was to research the effect of replacing S with graded levels of PFAD on lipid-class content and FA digestibility along the intestinal segments of the GIT (upper and lower jejunum, upper and lower ileum) and in the excreta in starter and grower-finisher broiler chickens.

2. Materials and Methods

2.1. Housing and Animals

The study was carried out at the animal experimental facilities of the Servei de Granges i Camps Experimentals (Universitat Autònoma de Barcelona; Bellaterra, Barcelona, Spain). All management practices and procedures were approved by the Animal Ethics Committee (CEEAH) of the same institution (number code: 3938), in accordance with the European Union guidelines for the care and use of animals in research (2010/63/EU).

A total of 480 newly hatched female broiler chickens (Ross 308) were obtained from a commercial hatchery (Pondex SAU; Lleida, Spain). On arrival, birds were wing-banded, individually weighed and randomly allocated to cages (16 birds per cage) and assigned to one of the five dietary treatments (six replicas per treatment). Birds were housed in

metabolic cages, with a grid floor and excreta collection tray, located in an environmentally controlled room. Throughout the study, feed and water were offered ad libitum. The temperature, humidity, ventilation, and illumination were automatically controlled, as recommended by the specifications in the Ross 308 lineage management handbook [14]. The animals and housing facilities were inspected, at least twice a day (d).

2.2. Experimental Design and Diets

All birds were raised with a starter feed until d 22 and a grower-finisher feed from d 23 to d 35, both in mash form. The wheat- and soybean meal-based diet was formulated to meet or exceed FEDNA's (Fundación Española para el Desarrollo de la Nutrición Animal) requirements [15] and to minimize basal fat levels, as shown in Table 1. Titanium dioxide (TiO_2) was included (5 g/kg) as an inert marker for determining the digestibility of FA.

Table 1. Ingredient composition of the experimental basal diet.

Ingredients, %	Starter Diet (from 0 d to 22 d)	Grower-Finisher Diet (from 23 d to 35 d)
Wheat	54.49	44.02
Soybean meal 47%	35.40	27.25
Barley	-	18.58
Experimental fats [1]	6.00	6.00
Calcium carbonate	1.44	1.39
Monocalcium phosphate	0.99	1.20
Titanium dioxide	0.50	0.50
Vitamin and mineral premix [2]	0.40	0.40
Sodium chloride	0.40	0.35
DL-Methionine	0.23	0.17
L-Lysine	0.15	0.12
L-Threonine	-	0.02

[1] Soybean oil, palm oil and palm fatty acid distillate in different proportions. [2] Provides per kg of feed: vitamin A (from retinol), 10,000 IU; vitamin D3 (from cholecalciferol), 4800 IU; vitamin E (from alfa tocopherol), 45 mg; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 4.5 mg; vitamin B12, 40 µg; vitamin K3, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 150 µg; Fe (from $FeSO_4 \cdot 7H_2O$), 54 mg; I (from $Ca(I_2O_3)_2$), 1.2 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; β-glucanase 150 U; xylanase 270 U.

The experimental diets consisted in a basal diet supplemented with 6% of the different fat sources (Table 2). The S was included at 6% (S6) and increasing amounts of PFAD were added in replacement of S: S4-PA2 (4% of S and 2% of PFAD), S2-PA4 (2% of S and 4% of PFAD) and PA6 (PFAD at 6%). The P was included at 6% (P6) as a control treatment for PFAD. Thus, 5 different diets were compared that were replicated 6 times. The composition of the experimental diets is shown in Table 3. The basal diet was manufactured at Pinsos Molinet S.A., (Prats de Lluçanès, Barcelona, Spain) and the addition of the experimental fat sources or fat blends to manufacture the experimental diets was performed at Lindo Pet Global S.A. (Castellar del Vallès, Barcelona, Spain).

Table 2. Chemical analyses of the experimental fats [1].

Item	S	PFAD	P
Moisture (g/100 g)	ND	0.01	ND
Insoluble impurities (g/100 g)	1.27	3.76	0.59
Unsaponifiable matter (g/100 g)	0.99	1.34	0.21
Fatty acid composition (%) [2]			
C16:0	10.98	46.59	43.94
C18:0	3.47	6.62	4.64
C18:1 n-9	25.11	34.96	38.43
C18:2 n-6	51.70	8.49	9.70
C18:3 n-3	5.34	0.29	0.13
Minor fatty acids	3.40	3.05	3.15
SFA	15.86	55.13	50.64
cis-MUFA	27.06	35.87	39.44
trans-C18:1	0.04	0.22	0.08
PUFA	57.04	8.78	9.83
UFA:SFA	5.29	0.82	0.98
Lipid class composition (%) [3]			
TAG	96.27	4.01	92.46
DAG	3.23	3.04	7.54
MAG	ND	ND	ND
FFA	0.50	92.94	ND
T (mg/kg)	1007.31	42.79	199.40
T3 (mg/kg)	ND	52.59	431.87

Abbreviations: S, soybean oil; P, palm oil; PFAD, palm fatty acid distillate; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA:SFA, unsaturated to saturated fatty acid ratio, calculated as described by Varona et al. [4]; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids; T, sum of α-, β-, γ- and δ-tocopherols; T3, sum of α-, β-, γ- and δ-tocotrienols; ND, not detected. [1] Chemical composition analyzed as described by Varona et al. [4]. [2] Percentage of total fatty acids (normalized data, area %); [3] Percentage of total lipid classes (normalized data, area %).

Table 3. Analyzed [1] macronutrient content and fatty-acid and lipid-class composition of the experimental diets [2].

Item	Starter Diets (from 0 to 22 d)					Grower-Finisher Diets (from 23 to 35 d)				
	S6	S4-PA2	S2-PA4	PA6	P6	S6	S4-PA2	S2-PA4	PA6	P6
Macronutrient content										
Dry matter (g/100 g)	91.00	91.03	91.14	90.89	90.93	90.14	90.27	90.37	90.43	90.02
Crude protein (g/100 g)	23.61	23.87	23.47	23.60	23.15	21.04	22.03	21.45	20.59	20.84
Crude fat (g/100 g)	7.51	7.39	8.16	7.78	7.70	8.18	8.08	8.36	8.10	7.49
Crude fiber (g/100 g)	3.29	3.14	3.14	2.86	3.20	3.08	3.10	3.32	3.13	3.41
Ash (g/100 g)	5.54	5.58	6.92	7.13	7.09	6.21	6.69	6.51	6.46	5.75
Gross energy, kcal/kg	4367	4402	4368	4332	4332	4339	4355	4320	4308	4324
Fatty acid composition (%)										
C14:0	-	0.40	0.66	0.91	0.87	0.06	0.39	0.65	0.90	0.85
C16:0	14.43	21.99	30.79	39.07	37.38	13.24	21.93	30.24	39.18	36.85
C18:0	3.48	4.19	5.07	5.79	4.29	3.35	4.19	4.97	5.69	4.17
C18:1 n-9	22.83	25.07	27.57	30.31	32.34	22.61	25.31	27.85	29.96	32.49
C18:1 n-7	1.46	1.28	1.05	0.78	0.83	1.50	1.25	1.00	0.74	0.80
C18:2 n-6	50.78	41.48	30.59	20.30	21.37	52.04	41.29	30.99	20.72	22.02
C18:3 n-3	5.27	4.13	2.83	1.55	1.46	5.50	4.20	2.93	1.61	1.60
Minor fatty acids	1.75	1.46	1.45	1.29	1.46	1.69	1.44	1.38	1.19	1.22
SFA	18.72	27.26	37.14	46.14	43.13	17.70	27.20	36.38	46.07	42.47
MUFA	25.24	27.13	29.44	32.01	34.04	24.76	27.31	29.70	31.60	33.92
PUFA	56.04	45.61	33.42	21.85	22.83	57.54	45.49	33.92	22.33	23.62
UFA:SFA	4.16	2.60	1.66	1.14	1.30	4.54	2.61	1.70	1.15	1.34

Table 3. *Cont.*

Item	Starter Diets (from 0 to 22 d)					Grower-Finisher Diets (from 23 to 35 d)				
	S6	**S4-PA2**	**S2-PA4**	**PA6**	**P6**	**S6**	**S4-PA2**	**S2-PA4**	**PA6**	**P6**
Lipid class composition (%)										
TAG	71.88	54.76	37.54	14.98	78.67	76.67	58.78	37.77	14.20	78.53
DAG	11.73	10.05	7.47	4.67	10.54	10.23	9.03	7.28	5.58	10.44
MAG	2.19	2.10	1.66	1.19	1.84	2.49	2.20	1.93	1.77	2.31
FFA	14.20	33.08	53.33	79.17	8.96	10.61	29.99	53.01	78.44	8.72

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA:SFA, unsaturated to saturated fatty acid ratio, calculated as described by Varona et al. [4]; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids. [1] All samples were analyzed at least in duplicate. [2] Dietary treatments supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). In all cases, fatty acids and lipid classes are expressed as internal area normalization (in %).

2.3. Controls and Sampling

Individual body weight (BW) and feed consumption by cage were measured at 11, 22 and 35 d of age to calculate the average daily gain (ADG), average daily feed intake (ADFI) and the feed conversion ratio (FCR) throughout the experiment. Mortality was recorded and weighed to correct these parameters.

Two digestibility balances were performed in young animals from 9 to 11 d and in older animals from 33 to 35 d. At 11 d of age, 12 birds per cage were killed by cervical dislocation, and the jejunum (from the distal-most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), and ileum (from the junction with Meckel's diverticulum to a point 1 cm proximal to the ileocecal junction) were carefully excised. Then, both segments (jejunum and ileum) were divided into 2 equal portions, named as upper and lower. Thus, for each cage, samples were taken of the digestive content from the upper and lower jejunum and the upper and lower ileum. The samples from the 12 birds from each cage were then homogenized and pooled (n = 6 per type of sample and dietary treatment). A representative sample of excreta (free of contaminants, such as feed or feathers) was also taken from each cage. Samples were frozen at −20 °C, and lyophilized. Thus, 5 different digesta samples were taken: 4 intestinal segments and excreta. Samples of diets, digesta and excreta were ground to pass through a 0.5-mm sieve and kept at 4 °C until further analyses. At 35 d of age, 2 birds per cage were euthanized, and the same procedure described above was carried out for sampling. In addition, at 35 d the abdominal fat pad (from the proventriculus surrounding the gizzard down to the cloaca) of each bird was removed and weighed. Abdominal fat pad weights were expressed in absolute values and as a percentage of BW.

2.4. Chemical Analysis

Oil samples were analyzed in triplicate for moisture and volatile matter according to the AOCS official method Ca 2d-25 [16], insoluble impurities [17], unsaponifiable matter according to the AOCS official method Ca 6b-53 [18], lipid-class composition according to IUPAC (2508 method) [19], and total FA composition [20], that were adapted to acid oils by Varona et al. [4]. The chemical analyses of the experimental fats are shown in Table 2.

Analytical determinations of the diets were performed according to the methods of AOAC International [21]: dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fiber (Method 962.09), and ether extract (EE) by Soxhlet analysis (Method 920.39). Gross energy was determined by an adiabatic bomb calorimeter (IKA C-4000, Janke-Kunkel; Staufen, Germany).

TiO_2 in feed, digestive content and excreta was analyzed following the procedures of Short et al. [22] and determined by spectrophotometry ICP-OES (Optima 3200 RL, Perkin Elmer, Waltham, MA, USA).

The FA content of the feed, digestive content, and excreta was determined according to the method of Sukhija and Palmquist [23]. A direct extraction-transesterification procedure using nonadecanoic acid (C19:0; Sigma Aldrich Chemical Co.; St. Louis, MO, USA) as internal standard was performed. Then, the lipid extract was injected in a gas chromatograph (HP6890, Agilent Technologies; Waldbronn, Germany) under the conditions of the method previously described by Cortinas et al. [24]. FAs were identified based on the retention times of commercial standards of major FA (Supelco 37 component FAME Mix; Sigma-Aldrich Co.). Quantification was carried out by internal normalization. The macronutrient and FA compositions of the experimental diets are presented in Table 3.

The lipid-class composition (TAG, DAG, MAG, and FFA) of the feed, digestive content, and excreta was determined according to the IUPAC, 2508 method [19] by size-exclusion chromatography on an Agilent 1100 series HPLC chromatograph equipped with an isocratic pump, with the oven and a Refractive Index Detector (RID) both set at 35 °C (Agilent Technologies, Santa Clara, USA). Lipid extraction was previously performed following the methodology described by Rodriguez-Sanchez et al. [8] with slight modifications. Briefly, 0.1 g of lyophilized sample was weighed to extract the fat content with diethyl ether after acidification with HCl 1N. After lipid extraction, lipids were dissolved in 2 mL of tetrahydrofuran and filtered through a Nylon filter (13 mm, 0.45 µm), then 100 µL were injected (20 µL loop) into the HPLC. Separation was conducted using 2 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 pore sizes of 100 Å and 50 Å, respectively (Water Associates; Milford, MA, USA), connected in series. The mobile phase consisted of tetrahydrofuran (HPLC quality grade) at 1 mL/min. Lipid classes were identified by using standards for each lipid-class (trioleoylglycerol for TAG, dioleoylglycerol for DAG, oleoylglycerol for MAG and oleic acid for FFA; Sigma-Aldrich GmbH; Steinheim, Germany) and they were quantified according to their calibration curves.

2.5. Calculations

The apparent digestibility coefficients (ADC) of FA in each intestinal segment and the excreta were calculated according to the following formula using the TiO_2 marker ratio in the diet and digesta or excreta.

$$\text{ADC of FA} = 1 - \{(TiO_2/FA)_d/(TiO_{2/}FA)_e\}, \quad (1)$$

where $(TiO_2/FA)_d$ is the concentration of the inert marker and the FA in the diet (g/g DM), and $(TiO_2/FA)_e$ is the concentration of the inert marker and the FA in the digestive content or excreta (g/g DM).

The apparent metabolizable energy (AME) was calculated with the following equation:

$$\text{AME (kcal/kg)} = \text{Apparent digestibility coefficient of gross energy (\%)} * \text{gross energy of the diet} \quad (2)$$

To determine the lipid-class content in the different GIT segments and excreta, the content of each lipid class present in the digestive tract of the chickens was estimated according to the following formula [12]:

$$\text{Lipid-class content} = [LC]/[TiO_2], \quad (3)$$

where [LC] is the concentration of the lipid-class in the digesta of the GIT segment or excreta (mg/g DM) and $[TiO_2]$ is the concentration of TiO_2 in the digesta of the GIT segment or excreta (mg/g DM).

2.6. Statistical Analysis

The study design included 2 main factors: diet (5 treatments) × intestinal segment (5 types, being 4 intestinal segments and the excreta). The effect of the age was also compared as described below (11 vs. 35 d). The normality of the data and homogeneity of variance were verified. For each age, the effect of the diet on productive parameters

(including abdominal fat depot at 35 d) and AME were statistically analyzed by one-way ANOVA using the GLM procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) (n = 30; 5 diets × 6 replicas). For each age, and for each intestinal segment and excreta, the effect of the diet on the lipid-class content, FA digestibility, and its contribution on FA absorption was also evaluated by one-way ANOVA (n = 30; 5 diets × 6 replicas).

For each age, the effect of the intestinal tract on the lipid-class content was also analyzed by one-way ANOVA with the intestinal segments and the excreta as the main factor (n = 150; 30 samples × 5 types of digesta samples).

On each intestinal segment, the effect of the age (11 or 35 d) on FA absorption was statistically analyzed by one-way ANOVA using the age as the main factor (n = 60; 5 dietary treatments replicated 6 times × 2 ages). Additionally, for each dietary treatment, one-way ANOVA was used to test the effect of the age on feed AME, and at lower ileum level on lipid-class content and FA digestibility, (n = 12; 6 replicas of lower ileum × 2 ages).

The differences between treatments means were tested using Tukey's correction for multiple comparisons. The cage served as the experimental unit, so there were six units per diet.

The results shown in tables are reported as least-square means, and in all statistical analyses, differences were considered significant at $p < 0.05$.

3. Results

3.1. Characterization of Experimental Oils and Diets

The characterization of the experimental oils included in the diets is shown in Table 2. The main FAs in S were linoleic and oleic acids, whereas in P and PFAD they were palmitic and oleic acids. The unsaturated-to-saturated FA ratio (UFA:SFA) was higher for S (5.29) than for PFAD and P (0.82 and 0.98, respectively). Regarding lipid-class composition, S and P were mainly composed of TAG (>92%), whereas PFAD was mainly composed of FFA (92.94%). The rest of parameters observed for PFAD were in line with those usually found in PFAD [4], being insoluble impurities and unsaponifiable matter higher in PFAD than in P.

The chemical analysis of the experimental diets is shown in Table 3. Replacing S with PFAD led to an increment in both saturation degree and FFA content. Therefore, a progressive decrease in the UFA:SFA from 4.16 to 1.14 in starter diets, and from 4.54 to 1.15 in grower-finisher diets was obtained. In parallel, a large increase was achieved in the FFA content from 14.20% to 79.17% in starter diets and from 10.61% to 78.44% in grower-finisher diets. Although the FA profile and saturation degree of P6 and PA6 were similar, their FFA content was different (P6: 8–9% FFA; PA6: 78–79% FFA).

3.2. Growth Performance and Abdominal Fat Deposition

The effect of dietary fat source on growth-performance traits in starter (from 0 to 22 d), grower-finisher (from 23 to 35 d) and the global (from 0 to 35 d) periods, and on abdominal fat deposition is reported in Table 4. No significant differences in any of the performance parameters, nor any feeding period ($p > 0.05$), were observed due to the saturation degree or FFA content of the diet. Concerning the effect of the diet on abdominal fat deposition, a tendency for a reduction of fat weight (%) was observed as S was replaced by PFAD ($p = 0.08$).

Table 4. Growth performance and abdominal fat pad deposition of broiler chickens according to different fat sources in diet [1].

Item	Dietary Treatments [2]					SEM [3]	*p*-Value
	S6	S4-PA2	S2-PA4	PA6	P6		
			From 0 to 22 d				
ADFI, g/d/bird	48.7	53.2	50.7	54.0	54.5	2.25	0.335
ADG, g/d/bird	37.2	39.1	38.6	39.7	40.6	1.24	0.373
FCR, g/g	1.31	1.36	1.31	1.36	1.34	0.036	0.733
BW at 22 d, g	856	899	888	913	933	27.1	0.361
			From 23 to 35 d				
ADFI, g/d/bird	134	141	141	144	143	2.92	0.148
ADG, g/d/bird	87.8	90.2	89.8	90.3	89.9	2.18	0.929
FCR, g/g	1.53	1.57	1.57	1.59	1.60	0.022	0.175
BW at 35 d, g	1997	2072	2055	2086	2101	44.4	0.526
			From 0 to 35 d				
ADFI, g/d/bird	80.3	85.8	84.3	87.3	87.5	2.01	0.130
ADG, g/d/bird	56.0	58.1	57.6	58.5	58.9	1.27	0.535
FCR, g/g	1.43	1.48	1.46	1.49	1.49	0.017	0.154
Abdominal fat, g	29.62	30.35	29.52	25.66	32.36	1.938	0.136
Abdominal fat, %	1.46	1.47	1.42	1.23	1.53	0.082	0.080

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; BW, body weight. [1] Diets supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). [2] Values are pooled means of 6 replicates with 16 chickens/replicate from 0 to 11 d and 4 chickens/replicate from 11 to 35 d. In the case of BW, values are means of 24 chickens each treatment from 22 to 35 d. For abdominal fat, values are means of 2 chickens/replicate: 12 for each treatment at 35 d. [3] SEM, standard error of means of 6 observations per treatment (the experimental unit is the cage).

3.3. Lipid-Class Content along the Intestinal Tract

The lipid-class content (TAG, DAG, MAG, and FFA) in the upper and lower jejunum, upper and lower ileum and excreta determined in 11 and 35-d-old broiler chickens fed the different experimental diets is shown in Tables 5 and 6, respectively. In general, a significant decrease in TAG, DAG, and FFA content was observed from the upper jejunum to lower ileum ($p < 0.001$) (Supplementary Table S1).

Significant differences in TAG, DAG and MAG content in the different diets were obtained at the jejunum level in starter broiler chickens (Table 5; $p \leq 0.027$). In contrast, grower broiler chickens showed no differences among the different dietary treatments for TAG, DAG, and MAG content in any intestinal segment (Table 6). For each experimental diet, TAG and DAG content at the lower ileum level was significantly lower in grower chickens than in starter chicks ($p \leq 0.02$) (Supplementary Table S2).

Table 5. Lipid-class content [1] along the intestinal tract and excreta according to different fat sources in the diet [2] in 11-d-old broiler chickens.

Item	Dietary Treatments					SEM [3]	*p*-Value
	S6	S4-PA2	S2-PA4	PA6	P6		
				Upper Jejunum			
TAG	0.53 ^{ab}	0.57 ^{a}	0.44 ^{ab}	0.19 ^{b}	0.49 ^{ab}	0.082	0.027
DAG	1.30	1.60	1.91	2.37	1.99	0.413	0.440
MAG	0.18 ^{ab}	0.28 ^{a}	0.16 ^{ab}	0.12 ^{b}	0.15 ^{ab}	0.033	0.018
FFA	6.58 ^{c}	10.04 ^{bc}	16.69 ^{ab}	24.82 ^{a}	18.20 ^{ab}	2.215	<0.001
				Lower Jejunum			
TAG	0.34 ^{ab}	0.34 ^{ab}	0.47 ^{a}	0.32 ^{b}	0.30 ^{b}	0.031	0.008
DAG	0.82 ^{ab}	0.69 ^{b}	1.16 ^{a}	0.98 ^{ab}	0.76 ^{b}	0.091	0.008
MAG	0.18	0.18	0.21	0.17	0.17	0.025	0.729
FFA	3.65 ^{c}	5.50 ^{c}	10.45 ^{b}	14.21 ^{a}	9.70 ^{b}	0.471	<0.001
				Upper Ileum			
TAG	0.25	0.24	0.25	0.32	0.24	0.053	0.775
DAG	0.64	0.55	0.72	0.87	0.62	0.121	0.426
MAG	0.16	0.15	0.15	0.16	0.13	0.030	0.929
FFA	3.05 ^{c}	5.02 ^{c}	8.82 ^{b}	12.76 ^{a}	8.69 ^{b}	0.530	<0.001
				Lower Ileum			
TAG	0.32	0.29	0.27	0.33	0.19	0.045	0.239
DAG	0.64	0.60	0.76	0.71	0.45	0.084	0.114
MAG	0.23	0.25	0.25	0.23	0.15	0.029	0.137
FFA	3.02 ^{d}	5.23 ^{c}	8.78 ^{b}	12.70 ^{a}	8.47 ^{b}	0.471	<0.001
				Excreta			
TAG	0.38	0.39	0.28	0.27	0.29	0.044	0.219
DAG	0.98	0.95	1.33	1.20	0.80	0.167	0.205
MAG	0.19	0.13	0.15	0.13	0.21	0.031	0.282
FFA	4.16 ^{c}	5.98 ^{c}	10.11 ^{b}	14.18 ^{a}	10.24 ^{b}	0.794	<0.001

Abbreviations: TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids. [1] Lipid-class concentration (mg/g)/Ti concentration (mg/g) in each intestinal segment and excreta. [2] Values are pooled means of 6 replicates with 12 chickens/replicate fed diets supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). [3] SEM = standard error of the mean. a–d: means in a row not sharing a common letter are significantly different ($p < 0.05$).

Regardless of the diet, FFA was the major lipid-class present in the digesta from the upper jejunum to the excreta, both in starter and grower chickens. In starter broiler chickens, FFA content decreased notably from the upper to lower jejunum ($p < 0.001$), while in grower broiler chickens, the significant decrease reached the upper ileum ($p < 0.001$) (Supplementary Table S1).

Differences in FFA content were also observed among the dietary treatments in the digesta in all intestinal segments and in the excreta, at 11 d ($p < 0.001$) and 35 d ($p \leq 0.011$) (Table 5; Table 6, Figure 1). It was found that the higher the replacement of S by PFAD in the diet, the higher the FFA content in the digesta, which was more evident from the lower jejunum on.

In both starter and grower broiler chickens, birds fed the most unsaturated diets (S6 and S4-PA2) showed the lowest FFA values in the digesta of most of the GIT segments studied and the excreta ($p > 0.001$). No differences were observed in the FFA content of the digesta between chickens fed the S2-PA4 and P6 diets along the intestinal tract and excreta. Chickens fed PA6 had the highest FFA content. For each experimental diet, it was observed that starter chicks had a higher FFA content at the lower ileum level than grower chickens ($p < 0.001$) (Supplementary Table S2).

Table 6. Lipid-class content [1] along the intestinal tract and excreta according to different fat sources in the diet [2] in 35-d-old broiler chickens.

Item	Dietary Treatments					SEM [3]	*p*-Value
	S6	S4-PA2	S2-PA4	PA6	P6		
				Upper Jejunum			
TAG	0.21	0.20	0.20	0.20	0.26	0.048	0.871
DAG	1.32	1.69	1.47	2.22	1.20	0.256	0.073
MAG	0.24	0.27	0.30	0.27	0.17	0.048	0.436
FFA	8.28 ^{b}	10.05 ^{ab}	10.40 ^{ab}	14.18 ^{a}	7.40 ^{b}	1.296	0.011
				Lower Jejunum			
TAG	0.10	0.26	0.27	0.23	0.22	0.045	0.108
DAG	0.43	0.61	0.61	0.72	0.58	0.115	0.496
MAG	0.18	0.26	0.19	0.23	0.14	0.041	0.342
FFA	3.10 ^{c}	4.29 ^{bc}	5.72 ^{b}	7.89 ^{a}	5.08 ^{b}	0.412	<0.001
				Upper Ileum			
TAG	0.14	0.16	0.13	0.13	0.09	0.021	0.254
DAG	0.16	0.21	0.18	0.17	0.17	0.021	0.482
MAG	0.11	0.12	0.16	0.14	0.11	0.015	0.090
FFA	1.20 ^{c}	1.65 ^{c}	3.32 ^{b}	4.47 ^{a}	2.67 ^{b}	0.189	<0.001
				Lower Ileum			
TAG	0.09	0.11	0.09	0.07	0.07	0.022	0.676
DAG	0.14	0.17	0.11	0.18	0.17	0.020	0.141
MAG	0.13	0.14	0.21	0.21	0.15	0.022	0.051
FFA	0.87 ^{c}	1.32 ^{c}	2.77 ^{b}	4.82 ^{a}	2.92 ^{b}	0.257	<0.001
				Excreta			
TAG	0.12	0.21	0.18	0.15	0.15	0.021	0.080
DAG	0.13	0.20	0.14	0.24	0.15	0.027	0.055
MAG	0.09 ^{b}	0.12 ^{ab}	0.12 ^{ab}	0.16 ^{a}	0.13 ^{ab}	0.011	0.005
FFA	0.96 ^{c}	1.63 ^{bc}	2.62 ^{b}	6.04 ^{a}	2.66 ^{b}	0.265	<0.001

Abbreviations: TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids. [1] Lipid-class concentration (mg/g)/Ti concentration (mg/g) in each intestinal segment and excreta. [2] Values are pooled means of 6 replicates with 2 chickens/replicate fed diets supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). [3] SEM = standard error of the mean. a–c: means in a row not sharing a common letter are significantly different ($p < 0.05$).

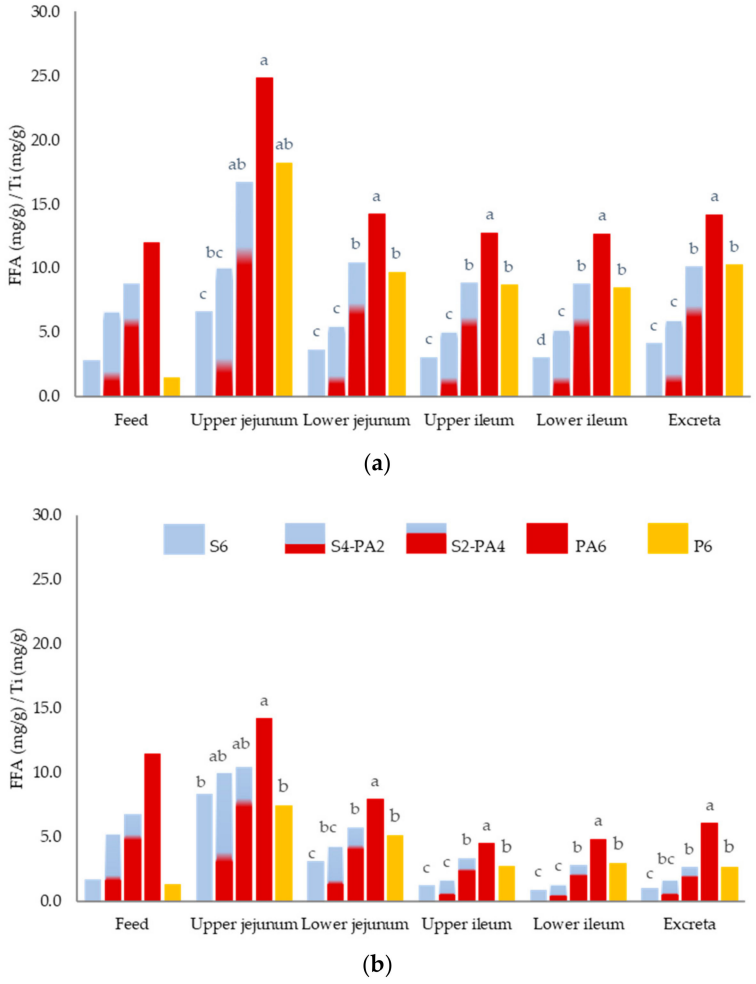

Figure 1. FFA content [1] in the feed, upper jejunum, lower jejunum, upper ileum, lower ileum, and excreta for the five different diets; with 6% of soybean oil (S6), blend with 4% soybean oil and 2% palm fatty acid distillate (S4-2PA), blend with 2% soybean oil and 4% palm fatty acid distillate (S2-2PA), with 6% of palm fatty acid distillate (PA6) and with 6% palm oil (P6) in (**a**) 11-d-old broiler chickens and (**b**) 35-d-old broiler chickens. [1] FFA concentration (mg/g)/Ti concentration (mg/g) in each intestinal segment and excreta. Values are pooled means of 6 replicates per each diet with 12 chickens/replicate at 11 d, and 2 chickens/replicate at 35 d. a–d: columns not sharing a common letter within each intestinal segment are significantly different ($p \leq 0.01$).

3.4. Apparent Fatty-Acid Digestibility along the Intestinal Tract

Tables 7 and 8 show the feed apparent metabolizable energy and apparent FA digestibility coefficients in the different intestinal segments and excreta determined in 11 and 35-d-old broiler chickens fed the different dietary treatments, respectively.

Differences were observed in the feed AME values among the different diets ($p < 0.001$) both in 11-d-old broiler chickens (Table 7) and in 35-d-old broiler chickens (Table 8). In general, at both ages, the lowest values were obtained in the PA6 diet and the highest in the most unsaturated diets (S6 and S4-PA2). An increase in AME values was observed in grower chickens compared to starter chicks fed diets with higher SFA and FFA contents (PA6, S2-PA4, and P6; $p \leq 0.002$) (Supplementary Table S3).

Starter broiler chicks fed the diets containing PA6 showed the lowest digestibility coefficients, mainly for TFA and SFA from the lower jejunum on ($p < 0.001$) (Table 7). For S6 and S4-PA2, no differences were obtained in TFA, MUFA or PUFA along the GIT or in the excreta, and these diets showed the highest TFA digestibility coefficient values. In contrast, birds fed the S2-PA4 diet had lower SFA digestibility coefficients than those fed the S6 diet from the lower jejunum on ($p < 0.001$). Comparing S2-PA4 and P6, no differences were

obtained in either the TFA or all FA group digestibility coefficients in the GIT segments and excreta examined (except for PUFA at the lower jejunum).

In grower chickens (35 d), birds fed the PA6 diet showed the lowest digestibility coefficients for TFA and SFA only in the lower ileum and excreta ($p < 0.001$) and the lowest MUFA digestibility coefficient in the excreta ($p < 0.001$) (Table 8). No differences were observed between S6 and S4-PA2 in TFA and all FA group digestibility coefficients. The highest TFA, SFA, and PUFA digestibility coefficients were shown from the lower jejunum on. Comparing S2-PA4 and P6, no significant differences were observed in TFA or SFA digestibility (except for SFA in the lower ileum). In contrast, birds fed the S2-PA4 diet had lower MUFA digestibility coefficients than those fed P6 diet from the lower jejunum on ($p < 0.001$), and higher PUFA digestibility coefficients in the upper ileum and excreta ($p < 0.001$).

For each experimental diet, chickens at d 35 had higher FA digestibility coefficients at the lower ileum level than chicks at d 11 ($p \leq 0.05$) (Supplementary Table S3).

Table 7. Feed apparent metabolizable energy values and apparent fatty-acid digestibility coefficients along the intestinal tract and excreta according to different fat sources in the diet in 11-d-old broiler chickens.

Item	Dietary Treatments [1]					SEM [4]	*p*-Value
	S6	S4-PA2	S2-PA4	PA6	P6		
AME, kcal/kg [2]	3348 [a]	3340 [a]	3074 [b]	2760 [c]	3014 [b]	26.08	<0.001
Upper Jejunum [3]							
TFA	0.61 [a]	0.61 [a]	0.29 [b]	0.05[b]	0.20 [b]	0.071	<0.001
SFA	0.20 [ab]	0.36 [a]	0.19 [ab]	-0.01[b]	0.19 [ab]	0.076	0.044
MUFA	0.51 [a]	0.51 [a]	0.35 [ab]	0.16 [b]	0.31 [ab]	0.059	<0.001
PUFA	0.78[a]	0.71 [a]	0.34 [b]	0.02 [b]	0.04 [b]	0.078	<0.001
Lower Jejunum [3]							
TFA	0.72 [a]	0.67 [a]	0.51 [b]	0.30 [c]	0.48 [b]	0.020	<0.001
SFA	0.60 [a]	0.47 [b]	0.32 [c]	0.12 [d]	0.37 [c]	0.026	<0.001
MUFA	0.69 [a]	0.67 [ab]	0.58 [b]	0.44 [c]	0.58 [b]	0.025	<0.001
PUFA	0.77 [ab]	0.78 [a]	0.67 [b]	0.48 [c]	0.56 [c]	0.026	<0.001
Upper Ileum [3]							
TFA	0.74 [a]	0.68 [a]	0.53 [b]	0.35 [c]	0.51 [b]	0.027	<0.001
SFA	0.65 [a]	0.49 [b]	0.32 [c]	0.12 [d]	0.36 [bc]	0.033	<0.001
MUFA	0.73 [a]	0.69 [a]	0.61 [ab]	0.52 [b]	0.62 [ab]	0.031	<0.001
PUFA	0.78 [a]	0.78 [a]	0.69 [ab]	0.59 [b]	0.63 [b]	0.028	<0.001
Lower Ileum [3]							
TFA	0.79 [a]	0.73 [a]	0.65 [b]	0.41 [c]	0.62 [b]	0.020	<0.001
SFA	0.69 [a]	0.55 [b]	0.47 [b]	0.18 [c]	0.49 [b]	0.022	<0.001
MUFA	0.76 [a]	0.74 [a]	0.71 [a]	0.56 [b]	0.74 [a]	0.025	<0.001
PUFA	0.83 [a]	0.83 [a]	0.79 [a]	0.68 [b]	0.75 [ab]	0.028	0.002
Excreta [3]							
TFA	0.80 [a]	0.73 [ab]	0.63 [bc]	0.47 [d]	0.60 [c]	0.023	<0.001
SFA	0.64 [a]	0.53 [b]	0.43 [bc]	0.23 [d]	0.39 [c]	0.025	<0.001
MUFA	0.79 [a]	0.77 [ab]	0.74 [ab]	0.68 [b]	0.73 [ab]	0.021	0.018
PUFA	0.85 [a]	0.81 [a]	0.75 [ab]	0.67 [b]	0.80 [a]	0.031	0.004

Abbreviations: AME, apparent metabolizable energy; TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [1] Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). [2] Values are pooled means of 6 replicates with 16 chickens/replicate. [3] Values are pooled means of 6 replicates with 12 chickens/replicate. [4] SEM = standard error of the mean. a–d: means in a row not sharing a common letter are significantly different ($p < 0.05$).

Table 8. Feed apparent metabolizable energy values and apparent fatty-acid digestibility coefficients along the intestinal tract and excreta according to different fat sources in the diet in 35-d-old broiler chickens.

Item	Dietary Treatments [1]					SEM [4]	*p*-Value
	S6	S4-PA2	S2-PA4	PA6	P6		
AME, kcal/kg [2]	3364 [a]	3379 [a]	3212 [bc]	3121 [c]	3279 [ab]	32.48	<0.001
Upper Jejunum [3]							
TFA	0.48 [ab]	0.53 [a]	0.32 [ab]	0.29 [b]	0.51 [a]	0.052	0.005
SFA	0.21 [b]	0.46 [ab]	0.26 [ab]	0.25 [ab]	0.48 [a]	0.062	0.009
MUFA	0.60 [ab]	0.66 [ab]	0.52 [ab]	0.50 [b]	0.67 [a]	0.042	0.015
PUFA	0.51[a]	0.49[a]	0.20[bc]	0.06[c]	0.34[ab]	0.063	<0.001
Lower Jejunum [3]							
TFA	0.81 [a]	0.78 [a]	0.65 [b]	0.62 [b]	0.69 [b]	0.021	<0.001
SFA	0.73 [ab]	0.76 [a]	0.59 [c]	0.55 [c]	0.64 [bc]	0.031	<0.001
MUFA	0.85 [a]	0.84 [a]	0.76 [b]	0.77 [b]	0.83 [a]	0.016	<0.001
PUFA	0.81 [a]	0.76 [a]	0.62 [b]	0.54 [b]	0.56 [b]	0.031	<0.001
Upper Ileum [3]							
TFA	0.89 [a]	0.89 [a]	0.77 [bc]	0.72 [c]	0.82 [b]	0.015	<0.001
SFA	0.85 [a]	0.86 [a]	0.67 [bc]	0.61 [c]	0.77 [ab]	0.030	<0.001
MUFA	0.91 [a]	0.91 [a]	0.85 [b]	0.84 [b]	0.91 [a]	0.008	<0.001
PUFA	0.90 [a]	0.90 [a]	0.82 [b]	0.77 [c]	0.78 [c]	0.008	<0.001
Lower Ileum [3]							
TFA	0.92 [a]	0.92 [a]	0.82 [b]	0.76 [c]	0.84 [b]	0.010	<0.001
SFA	0.90 [a]	0.90 [a]	0.71 [c]	0.64 [d]	0.78 [b]	0.017	<0.001
MUFA	0.93 [a]	0.93 [a]	0.88 [b]	0.88 [b]	0.93 [a]	0.006	<0.001
PUFA	0.93 [a]	0.93 [a]	0.87 [b]	0.85 [b]	0.83 [b]	0.013	<0.001
Excreta [3]							
TFA	0.93 [a]	0.92 [a]	0.84 [b]	0.72 [c]	0.84 [b]	0.009	<0.001
SFA	0.87 [a]	0.87 [a]	0.76 [b]	0.59 [c]	0.77 [b]	0.016	<0.001
MUFA	0.93 [a]	0.93 [a]	0.89 [b]	0.85 [c]	0.92 [a]	0.005	<0.001
PUFA	0.94 [a]	0.94 [a]	0.90 [b]	0.82 [c]	0.84 [c]	0.009	<0.001

Abbreviations: AME, apparent metabolizable energy; TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [1] Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). [2] Values are pooled means of 6 replicates with 4 chickens/replicate. [3] Values are pooled means of 6 replicates with 2 chickens/replicate. [4] SEM = standard error of the mean. a–d: means in a row not sharing a common letter are significantly different ($p < 0.05$).

3.5. Contribution of Each Intestinal Segment to FA Absorption

To better understand the importance of the different intestinal segments in the FA absorption, the contribution of each intestinal segment was calculated as a proportion of the total digestibility coefficient obtained in the lower ileum, since it is well known that this is the last segment where absorption can take place [25]. The contributions of the different intestinal segments to the digestibility of TFA and the four major FAs (palmitic and stearic, representing SFA; oleic, MUFA; and linoleic, PUFA) are shown in Figure 2.

The results show that jejunum was the main site of TFA absorption (Jejunum: 84%; Ileum: 16%; these results indicate the percentage of FA disappearance), when all diets at 11 d and 35 d are considered. It was also the most important place for the absorption of palmitic (11 d: 80%; 35 d: 87%), oleic (11 d: 85%; 35 d: 89%), and linoleic acids (11 d: 85%; 35 d: 75%).

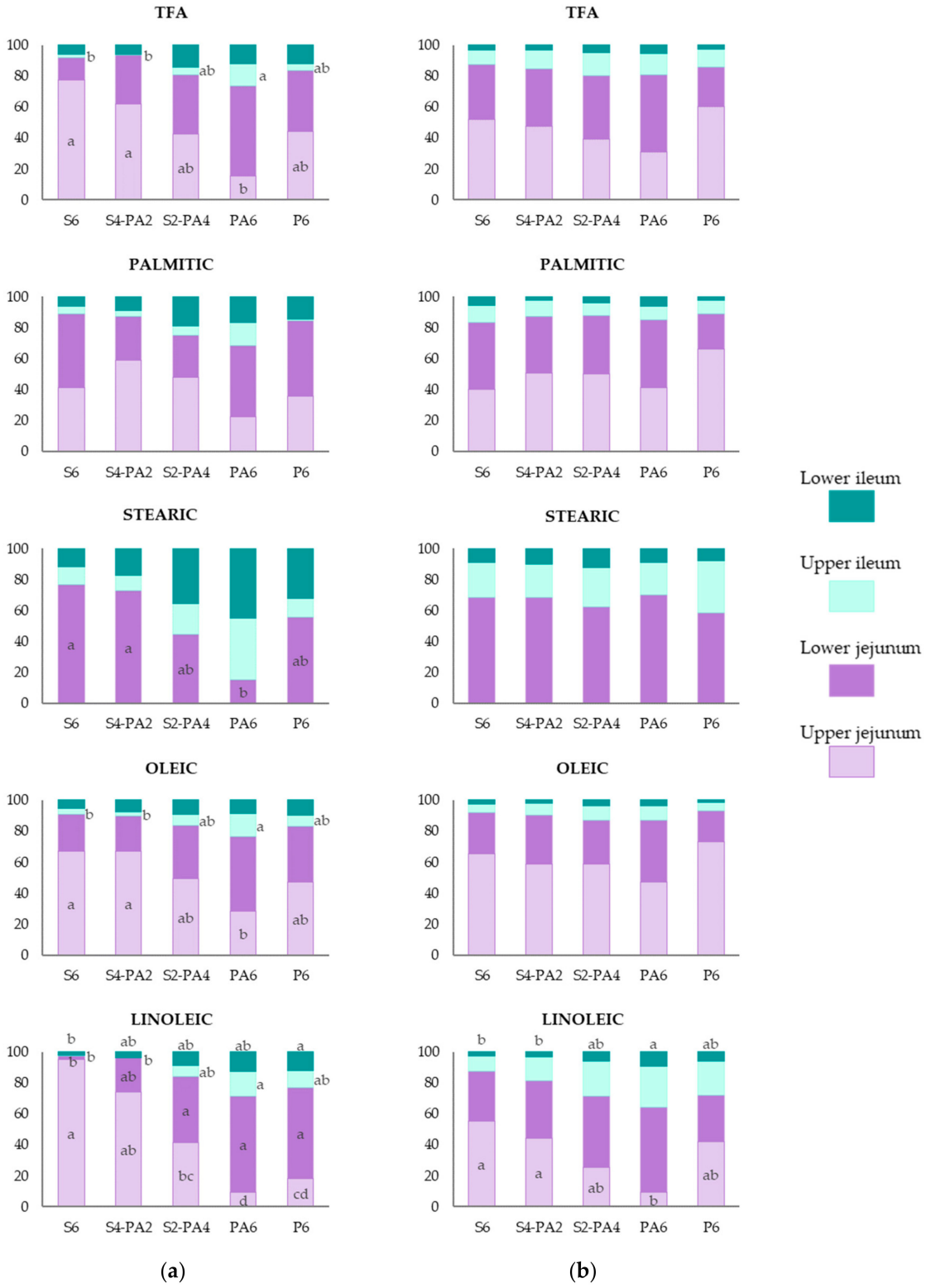

Figure 2. Contribution of each intestinal segment to the apparent fatty acid digestibility, calculated as a proportion of total digestibility reached at the lower ileum, along the intestinal tract for the five different diets; with 6% of soybean oil (S6), blend with 4% soybean oil and 2% palm fatty acid distillate (S4-2PA), blend with 2% soybean oil and 4% palm fatty acid distillate (S2-2PA), with 6% of palm fatty acid distillate (PA6) and with 6% palm oil (P6) in (**a**) 11-d-old broiler chickens and (**b**) 35-d-old broiler chickens. TFA (Total Fatty Acids), Palmitic (C16:0), stearic (C18:0), oleic (C18:1n-9) and linoleic (C18:2n-6) acids. Values are means of 6 replicates per each diet with 12 chickens/replicate at 11 d, and 2 chickens/replicate at 35 d. a–d: columns with the same intestinal segment not sharing a common letter are significantly different ($p < 0.01$).

In starter broiler chickens (Figure 2a), the contribution of the upper jejunum to the absorption of TFA decreased as S was replaced by PFAD (S6: 77%a, S4-PA2: 62%a, S2-PA4: 42%ab, PA6: 15%b, P6: 44%ab; $p = 0.002$) inversely to the increased contribution of the following segments, mainly the lower jejunum. A similar pattern was observed for the absorption of oleic and linoleic acids. In relation to SFA, no differences among diets in the contribution of the jejunum (both upper and lower) were observed for palmitic acid. For stearic acid the absorption started in the lower jejunum and was higher for chicks fed S6 (77%) and S4-PA2 (72%) than for those fed the PA6 (15%) diet ($p = 0.004$). In parallel, the contribution of the ileum (upper and lower segments) to the absorption of stearic acid increased as more S was replaced by PFAD (higher saturation and higher FFA content), reaching 85% in the PA6 diet.

In grower broiler chickens, no differences among diets in the contribution of the different intestinal segments to FA absorption were observed, except for linoleic acid. The absorption of linoleic acid in the upper jejunum was 55% and 44% for S6 and S4-PA2, respectively, and 9% for PA6 (Figure 2b; $p = 0.003$). Conversely, in the lower ileum, 9% of linoleic acid was absorbed for chickens fed PA6, compared to 3% for chickens fed the S6 and S4-PA2 diets ($p = 0.010$). As observed in starter chicks, the absorption of stearic acid is delayed, starting at the lower jejunum level, but no effect of the degree of saturation and FFA content of the diet was obtained. The upper and lower ileum make a large contribution to the absorption of stearic acid (25% and 10% on average, respectively).

The TFA absorption was higher in the upper ileum for grower chickens ($p < 0.001$) and in the lower ileum for starter chicks ($p < 0.001$) (Supplementary Table S4).

4. Discussion

Studying the lipid classes and FA digestibility along the intestinal tract leads to a better understanding of the dynamics of the hydrolysis-absorption process of PFAD alone and in blends in broiler chickens. Our results show that lipolysis, based on the disappearance of TAG and DAG in the digesta, is extended until the ileum. In addition, the results obtained support that hydrolysis is not the most limiting step for fat utilization when compared with the absorption process, which is in accordance with our previous studies in vitro [26] and in vivo [12]. Furthermore, our results suggest that hydrolysis efficiency is mainly affected by the age of the bird, whereas the lipid composition of the diet (saturation and FFA level) has less influence on this process. An improvement in the hydrolysis capacity with chicken age was demonstrated by the higher disappearance of TAG and DAG at 35 d compared to 11 d. In starter broiler chickens, some limitations in the hydrolysis process due to low bile and lipase secretion have been described [27]. However, Noy and Sklan [28] reported an increase of 80% in the duodenal bile acid secretion between 10 d and 21 d, and a 20-fold increase in lipase secretion between 4 d and 21 d.

The absorption process takes place as a dynamic process parallel to the hydrolysis of fat. The content of the end lipolysis products, mainly FFA, decreased from the upper jejunum to ileum and the maximum digestibility coefficients of FA were reached in the lower ileum. These results show that the lower ileum is the last segment where FA absorption occurs. Moreover, the evolution of the FFA content and the digestibility values throughout the gut confirmed that the jejunum was the main site of FA absorption, in line with previous studies on broiler chickens [7,12]. However, the absorption dynamics along the GIT are different according to the FA, and the stearic acid is the one that is absorbed later with no absorption observed until the lower jejunum at both ages. This is related to the lower solubilization into DMM for long-chain saturated FA compared to unsaturated long-chain FA [29]. This is also reflected in the lower digestibility coefficients for SFA along the GIT compared to MUFA or PUFA, regardless of the age of the chicken or the diet.

The results on the effect of the diet provide evidence of the detriment to the dietary AME values, FA digestibility coefficients, especially SFA, and FFA absorption associated with both the higher SFA% and FFA% of the broiler chicken diet. The lower FA absorption together with the higher residual FFA content in the digesta at the lower ileum obtained

for chickens fed the higher level of PFAD (6%; PA6), could be explained by two factors. First, the association of FFA, mainly SFA, with minerals to form insoluble soaps has been described, so that both the FFA and the mineral become unavailable for the absorption [30]. This has a greater impact on young birds than on older ones [31]. In our last in vitro study [26], we found that fat content from PFAD compared to other fat sources (P, S, or soybean acid oil) was less available for micellar solubilization. Second, and related to the lipid-class content, the lowest MAG content in PA6 diets (Table 3; 1.5% on average for both ages) may hinder the absorption of many FFAs [9] since the emulsifying properties of MAG improve the rate of FA incorporation into DMM [32]. This in turn could explain that birds fed PA6 tended to show the lowest abdominal fat weight (%).

The potential inclusion of PFAD in feed for broiler chickens is influenced by the age of the bird. In 11-d-old broiler chicks, the supplementation of PFAD at any level studied had a negative effect on fat utilization compared to S. Consistent with our results, several authors (Wiseman and Salvador, [33]; Vilarrasa et al. [5]; Rodriguez-Sanchez et al. [12]) have found a negative impact of dietary saturation and FFA level on fat utilization in broiler chickens. At 35 d the PA6 showed the worst fat utilization, however, adding PFAD in substitution of S with a feed FFA content up to 30% and a UFA:SFA ratio higher than 2.6 made it possible to achieve a high level of fat digestibility, similar to that obtained using S. This could be partially related to the higher FA digestibility coefficients and higher dietary AME values obtained for the S4-PA2 diet compared to those calculated from the proportions of the components. This suggests a positive synergic effect by the presence of UFA together with the presence of different lipid-class structures provided by S, since UFA and MAG obtained from the lipolysis of TAG are natural emulsifiers, which might enhance the incorporation of SFA, mainly FFA of PFAD, in the DMM and increase its absorption [34]. This synergistic effect is in agreement with the reported positive results of blending saturated and unsaturated conventional lipid sources [11] and acid oils [10]. However, the similarities obtained in feed AME values, lipid-class content in digesta, and apparent FA digestibility coefficients for S2-PA4 (UFA: SFA ratio: 1.7; FFA%: 53) and P6 (UFA: SFA ratio: 1.3; FFA%: 9) suggest that changes in the saturation degree might have a greater impact on FA utilization than the changes in the FFA level of the diet, as reported by Vilarrasa et al. [5] and Rodriguez-Sanchez et al. [12].

The present results also demonstrated that replacing S with PFAD led to a delay in the FA absorption along the GIT, which was more evident in starter animals (11-d-old chicks), and for the absorption of linoleic acid in 35-d-old chickens. Thus, even though the jejunum is the main site of fat absorption, the differentiation between the upper and the lower segments should be considered for future studies, at least in starter broiler chickens.

The comparison of the results between starter (11 d) and grower (35 d) broiler chickens confirms that the age has a positive effect on the FFA lipid-class absorption, FA absorption, and AME values of all the diets, which is consistent with the findings of Batal and Parsons [35], Tancharoenrat et al. [11], Roll et al. [13], Rodriguez-Sanchez et al. [8], and Viñado et al. [6]. However, it is important to highlight that the observed improvement with age in FA digestibility (especially for SFA), FFA absorption, and dietary AME values, was higher for those chickens fed the most saturated diets (especially with higher FFA%) than for those fed the most unsaturated diets. That there were no differences among diets in grower chickens in the contribution of intestinal segments to FA absorption suggests that the absorption of diets with higher SFA% and FFA% is advanced to the upper intestinal segments at 35 d. This was especially evident for the absorption of stearic acid, and the contribution of the lower jejunum increased due to the absorption of this FA acid in grower broiler chickens fed the PA6 diet. In addition, the higher contribution of the upper ileum in TFA and linoleic acid absorption at 35 d suggests that this segment plays a key role in improving FA absorption with age. In starter chickens, the limited capacity of fat absorption [27] together with the shorter feed retention time in the GIT (3.15 h in 11-d-old chicks and 5.10 h in 42-d-old chickens) [36] could explain the lower efficiency in the absorption process. This in turn could explain the higher implication of the lower ileum in young

chicks as it is the last part of the GIT for the remaining FA to be absorbed. Therefore, it may be recommendable to separate the ileum into upper and lower segments for further studies. Determining the maximum fat utilization at the lower ileum level instead of from the pool of digesta of the whole ileum may give more accurate results.

The maximum digestibility coefficients of SFA reached at the lower ileum, show that at 11 d both the dietary FFA content (PA6: 0.18 vs. P6: 0.48) and the SFA level (P6: 0.48 vs. S6: 0.69) had a great impact. At 35 d the magnitude of the negative effect was lower than at 11 d, for the dietary FFA content (PA6: 0.64 vs. P6: 0.78) and for the SFA level (P6: 0.78 vs. S6: 0.90). This in turn suggests that as age increases, the digestibility of SFA improves and, most notably, the utilization of FFA improves.

5. Conclusions

The present study confirms that determining the lipid classes together with the FA digestibility along the GIT provides valuable information for better understanding the dynamics of FA utilization in diets with different FA profiles and FFA contents. The results demonstrate that the effect of dietary saturation degree (UFA:SFA) on dietary fat utilization is higher than the effect of dietary FFA level. A clear improvement in the efficiency of both the lipolysis and the absorption process was observed with age. Fat hydrolysis is more affected by the age of the chicken than by the saturation degree and/or FFA content of the diet. The absorption results demonstrated that most of the FA absorption occurs in the jejunum (from 73% to 92%), but the ileum also plays a key role (from 8% to 27%). The contribution of the upper and lower segments of the jejunum and ileum to FA absorption is influenced by the characteristics of the dietary FA (degree of saturation, chain length, and FFA%), and the age of the chicken. There is a notably higher implication of the upper ileum for grower broiler chickens.

Replacing soybean oil with palm fatty acid distillate affected the extent and the site of FA absorption. The results show that the increase in SFA% and FFA% in the diet reduced and delayed the absorption of the dietary FA, especially the SFA in starter broiler chicks. As age increased, the FA absorption increased, and advanced to the upper intestinal segments, especially in the most saturated and rich FFA diets. Age has a positive effect on the digestibility of SFA and, above all, on the FFA utilization. For 11-day-old starter broiler chickens, it is not recommended to use this by-product alone or in blends. For grower broiler chickens, it is possible to include palm fatty acid distillate blended with a conventional unsaturated oil, such as soybean oil, in feed formulation, when the blend has from 2.6 UFA:SFA and the FFA% does not exceed 30%, without impairing FA utilization or growth performance. This potential strategy for using palm fatty acid distillate without negatively impacting fat utilization by the animal implies a reduction in costs and a way to upcycle and valorize this by-product.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani11041035/s1, Table S1: Lipid-class content according to different intestinal segments and excreta in 11- and 35-day-old broiler chickens, Table S2. Lipid-class content in the lower ileum according to different fat sources in the diet in 11- and 35-day-old broiler chickens, Table S3. Feed apparent metabolizable energy value and apparent fatty-acid digestibility coefficients in the lower ileum according to different fat sources in the diet in 11- and 35-day-old broiler chickens, Table S4. Contribution of each intestinal segment to FA absorption according to the age of the chicken.

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Article

Soybean Oil Replacement by Palm Fatty Acid Distillate in Broiler Chicken Diets: Fat Digestibility and Lipid-Class Content along the Intestinal Tract

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Simple Summary: Palm fatty acid distillate is a by-product of palm oil refining. It is of both environmental and economic interest to include it in the diets of broiler chickens. However, its high saturation degree and acidity level limit its use. This study aimed to assess the effect of replacing soybean oil with increasing levels of palm fatty acid distillate on the utilization of fat by broilers. Dietary fat hydrolysis was mostly affected by the age of the bird and including palm fatty acid distillate mainly affected the absorption process. The replacement of soybean oil by palm fatty acid distillate reduced the total fat utilization, and in starter chicks delayed the site of fatty acid absorption. As the age increased, the digestibility of saturated fatty acids improved, and, above all, it improved the free fatty acid utilization. Therefore, the potential inclusion of palm fatty acid distillate for broiler feeds depends on the age of the bird. It would not be recommended to include this by-product in starter feeds. However, for the grower-finisher phase, blending palm fatty acid distillate with soybean oil (1:3, w/w) could be a suitable alternative, that does not have negative repercussions for either fatty acid absorption or growth performance.

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Table S1. Lipid-class content according to different intestinal segments and excreta in 11- and 35-day-old broiler chickens.

	Intestinal Segment and Excreta [1]					*p*-Values	
Item	**Upper Jejunum**	**Lower Jejunum**	**Upper Ileum**	**Lower Ileum**	**Excreta**	**SEM**	**P**
11-day-old broiler chickens							
TAG	0.44 ^{a}	0.35 ^{ab}	0.26 ^{b}	0.28 ^{b}	0.32 ^{b}	0.022	<0.001
DAG	1.83 ^{a}	0.88 ^{bc}	0.68 ^{bc}	0.63 ^{c}	1.05 ^{b}	0.097	<0.001
MAG	0.18 ^{ab}	0.18 ^{ab}	0.15 ^{b}	0.22 ^{a}	0.16 ^{b}	0.014	0.004
FFA	15.22 ^{a}	8.70 ^{b}	7.67 ^{b}	7.64 ^{b}	8.93 ^{b}	0.913	<0.001
35-day-old broiler chickens							
TAG	0.21 ^{a}	0.22 ^{a}	0.13 ^{bc}	0.09 ^{c}	0.16 ^{ab}	0.015	<0.001
DAG	1.58 ^{a}	0.60 ^{b}	0.18 ^{c}	0.15 ^{c}	0.17 ^{c}	0.062	<0.001
MAG	0.25 ^{a}	0.20 ^{ab}	0.13 ^{c}	0.17 ^{bc}	0.12 ^{c}	0.015	<0.001
FFA	10.06 ^{a}	5.29 ^{b}	2.60 ^{c}	2.54 ^{c}	2.78 ^{c}	0.417	<0.001

[1] Values are pooled means of 30 replicates from chickens fed diets at 11-day-old (n = 150) or 35-day-old (n = 150). TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids. SEM = standard error of the mean. p-Values were obtained from univariate ANOVA conducted for each age to study whether the intestinal segment (or excreta) affected the lipid-class content. a–c: means in a row not sharing a common letter are significantly different (p < 0.05).

Table S2. Lipid-class content in the lower ileum according to different fat sources in the diet in 11- and 35-day-old broiler chickens.

Item	Dietary Treatments [1]										*p*-Values									
	11-day-old broiler chickens					35-day-old broiler chickens					S6		S4-PA2		S2-PA4		PA6		P6	
	S6	S4-PA2	S2-PA4	PA6	P6	S6	S4-PA2	S2-PA4	PA6	P6	SEM	P	SEM	P	SEM	P	SEM	P	SEM	P
TAG	0.32	0.29	0.27	0.33	0.19	0.09	0.11	0.09	0.07	0.07	0.020	<0.001	0.044	0.015	0.029	0.001	0.051	0.006	0.021	0.002
DAG	0.64	0.60	0.76	0.71	0.45	0.14	0.17	0.11	0.18	0.17	0.069	<0.001	0.052	<0.001	0.072	<0.001	0.032	<0.001	0.071	0.021
MAG	0.23	0.25	0.25	0.23	0.15	0.13	0.14	0.21	0.21	0.15	0.014	<0.001	0.036	0.051	0.027	0.384	0.027	0.483	0.019	1.000
FFA	3.02	5.23	8.78	12.70	8.47	0.87	1.32	2.77	4.82	2.92	0.252	<0.001	0.290	<0.001	0.443	<0.001	0.212	<0.001	0.574	<0.001

[1]Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids. SEM = standard error of the mean. *p*-Values were obtained from univariate ANOVA conducted for each dietary treatment to study whether the age affected the lipid-class content ($n = 12$). $p < 0.05$ was considered significant.

Table S3. Feed apparent metabolizable energy value and apparent fatty-acid digestibility coefficients in the lower ileum according to different fat sources in the diet in 11- and 35-day-old broiler chickens.

Item	Dietary Treatments [1]										*p*-Values									
	11-day-old broiler chickens					35-day-old broiler chickens					S6		S4-PA2		S2-PA4		PA6		P6	
	S6	S4-PA2	S2-PA4	PA6	P6	S6	S4-PA2	S2-PA4	PA6	P6	SEM	P	SEM	P	SEM	P	SEM	P	SEM	P
AME, kcal/kg [2]	3348	3340	3074	2760	3014	3364	3379	3212	3121	3279	24.64	0.656	30.21	0.381	23.86	0.002	35.64	<0.001	31.27	<0.001
FA digestibility																				
TFA	0.79	0.73	0.65	0.41	0.62	0.92	0.92	0.82	0.76	0.84	0.011	<0.001	0.011	<0.001	0.019	<0.001	0.012	<0.001	0.022	<0.001
SFA	0.69	0.55	0.47	0.18	0.49	0.90	0.90	0.71	0.64	0.78	0.011	<0.001	0.023	<0.001	0.018	<0.001	0.016	<0.001	0.026	<0.001
MUFA	0.76	0.74	0.71	0.56	0.74	0.93	0.93	0.88	0.88	0.93	0.010	<0.001	0.011	<0.001	0.022	<0.001	0.012	<0.001	0.026	<0.001
PUFA	0.83	0.83	0.79	0.68	0.75	0.93	0.93	0.87	0.85	0.83	0.011	<0.001	0.011	<0.001	0.026	**0.050**	0.021	<0.001	0.028	**0.056**

[1]Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of soybean oil (S6), palm fatty acid distillate (PA6), palm oil (P6), or oil blends with 4% soybean oil and 2% palm fatty acid distillate (S4-PA2) or 2% soybean oil and 4% palm fatty acid distillate (S2-PA4). AME = apparent metabolizable energy, 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 univariate ANOVA conducted for each dietary treatment to study whether the age affected the feed AME values, and the FA digestibility results ($n = 12$). $p < 0.05$ was considered significant.

Table S4. Contribution of each intestinal segment to FA absorption according to the age of the chicken.

	Age (days) [1]		*p*–Value	
Item	**11**	**35**	**SEM**	**Age**
Upper jejunum				
TFA	48.25	45.93	5.542	0.577
Palmitic	41.33	49.70	4.742	0.234
Stearic	-	-	-	-
Oleic	51.71	60.56	4.272	0.168
Linoleic	47.50	35.14	6.472	0.138
Lower jejunum				
TFA	36.24	37.85	5.09	0.735
Palmitic	39.38	36.87	4.753	0.521
Stearic	52.89	65.55	4.783	0.069
Oleic	33.00	29.31	3.957	0.342
Linoleic	37.68	40.06	5.283	0.872
Upper ileum				
TFA	5.52	12.36	1.299	<0.001
Palmitic	6.40	9.55	1.755	0.293
Stearic	18.80	24.73	3.863	0.380
Oleic	7.27	7.50	1.076	0.999
Linoleic	6.92	19.44	1.683	<0.001
Lower ileum				
TFA	9.99	3.86	1.206	<0.001
Palmitic	12.89	3.88	1.882	<0.001
Stearic	28.32	9.72	3.590	<0.001
Oleic	8.02	2.62	1.040	<0.001
Linoleic	7.90	5.36	0.984	0.089

[1]Values are means of 30 replicates from chickens fed diets at 11-day-old or 35-day-old. TFA = total fatty acids. SEM = standard error of the mean. *p*-Values were obtained from univariate ANOVA conducted for each intestinal segment to study whether the age affected the FA absorption ($n = 60$). $p < 0.05$ was considered significant.

4.SUPPLEMENTARY DOCUMENTATION

4.1 Palm oil replacement by soybean acid oil in broiler chicken diets: fat digestibility and lipid-class content along the intestinal tract

4.1.1 Simple Summary

Soybean acid oil is a by-product from the soybean oil refining industry rich in free fatty acids. Its inclusion in chicken diets is a way to upcycle it and reduce the feed cost. Its high unsaturation degree could enhance the absorption of saturated fatty acids of palm oil. The objective of this study was to assess the effect of palm oil replacement with increasing amounts of soybean acid oil on fat digestion and absorption in starter and grower chickens. The replacement of palm oil by soybean acid oil improved fat utilization in both 11 and 35-day-old broiler chickens. However, a better fat utilization of soybean acid oil was obtained at 35 days than at 11 days. Also, as age increased the contribution of the upper ileum to fat absorption increased. In grower chickens, soybean acid oil at 6% of total inclusion or a blend of palm oil with soybean acid oil (1:3, w/w) led to obtain an adequate fat utilization, similar to soybean oil at 6%. The results suggested that the use of soybean acid oil blended with palm oil is a good option to include this by-product in broiler chicken diets.

4.1.2 Abstract

Soybean acid oil (SA), by-product of soybean oil (S) refining, represents a sustainable and economically interesting alternative fat for chicken diets. Although it is rich in free fatty acids (FFA), its high unsaturated to saturated fatty acid ratio (UFA:SFA) could improve the absorption of fatty acids (FA) of palm oil (P). This study aimed to evaluate the effect of replacing P with increasing levels of SA on lipid-class content and FA digestibility along the intestine and in the excreta in chickens at 11 and 35 days (d). Five experimental diets were the results of a basal diet supplemented with 6% of P (P6), SA (SA6), two blends (P4-SA2 and P2-SA4) and S (S6). Replacing P with SA improved fat absorption at 11 and 35 d ($p < 0.05$), but not the feed AME values and the saturated FA (SFA) digestibility at 11 d. As

age increased, the absorption of SFA and FFA improved, and increased the contribution of the upper ileum to FA absorption ($p < 0.05$). At 35 d, SA6 (56% FFA) and P2-SA4 (40% FFA, 2.6 UFA:SFA) could replace S6 without impairing fat utilization. The replacement of P by SA represents a suitable strategy to use this by-product.

Keywords: fat digestibility; lipid classes; free fatty acid; fat by-product; acid oils; alternative energy source; broiler chickens; poultry; intestinal tract

4.1.3 Introduction

The inclusion of fat sources in poultry diets is a common practice because they are the ingredients with the highest energy value and also supply essential fatty acids. Among them, vegetable sources are widely used, being soybean oil (S) the most extensively included in broiler diets. The high and rising cost trend of S for the next years (Statista, 2018) is the main reason to search for alternative lipid sources at lower prices (Kierończyk et al., 2018; Schiavone et al., 2018; Viñado et al., 2020). Moreover, the use of ingredients non-suitable for human consumption in animal nutrition would allow animal food producers to move toward circular agroindustry. During the refining process of S, different by-products are generated which could be attractive alternative lipid sources to feed broiler chickens (Borsatti et al., 2018; Viñado et al., 2019a; Shahryari et al., 2021). One of them is soybean acid oil (SA), that derived from the chemical refining of S. It has a similar fatty acid (FA) profile to S, high unsaturated-to-saturated FA ratio (UFA:SFA), but due to its origin has a higher content of free fatty acid (FFA) (45 – 60%) and MIU (moisture, insoluble impurities, unsaponifiable matter) (4.4 – 10.8%) (Varona et al., 2021a).

It is well established that dietary fat utilization increases as age and UFA:SFA of the diet increase (Tancharoenrat et al., 2013; Vilarrasa et al., 2015; Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019a), but the different lipid-class (triacylglycerols, TAG; diacylglycerols, DAG; monoacylglycerols, MAG, and FFA) content of the fat sources is also important (Roll et al., 2018). A high level of FFA of a fat source has been negatively related to fat utilization (Wiseman and Salvador, 1991). However, subsequent studies have shown that blends of S+SA up to 15% FFA in starter chicks and up to 35% FFA in grower-finisher chickens have not a negative repercussion on fat utilization (Rodriguez-

Sanchez et al., 2019b, 2021). On the other hand, a recent study in 35-day-old broiler chickens reported the positive effect of blending a saturated acid oil (palm fatty acid distillate) with a conventional unsaturated oil (S), when the blend had less than 30% FFA and the UFA:SFA was higher than 2.6 (Jimenez-Moya et al., 2021a). Of these factors, saturation degree and FFA content, several studies suggest that the dietary saturation degree has a greater impact than the FFA content on the fat absorption process (Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019b; Jimenez-Moya et al., 2021a, 2021b). In this context, we hypothesized that the replacement of palm oil (P) with SA could improve the utilization of the former, being a potential strategy to use this by-product in broiler chicken diets. Nevertheless, its dietary inclusion could depend on the age of the bird. Up to date, only Blanch et al. (1996) have studied the addition of SA blended with P as a conventional saturated source (50:50, v/v) in 1-year roosters, who reported a positive synergism between both fat sources. However, to our knowledge, the effect of blending SA with P to feed broiler chickens remains to be addressed.

Therefore, the main objective was to study the effect of P replacement with increasing levels of SA on fat digestion and absorption process in broiler chickens at 11 days (d) and at 35 d. This was evaluated through the study of the dynamics of the lipid-class content and the FA digestibility along the intestinal tract and in the excreta. This study contributes to expanding knowledge about the relation of the UFA:SFA and the FFA content, and consequently the use of this by-product in broiler chickens diets.

4.1.4 Materials and Methods

4.1.4.1 Experimental Fats and Diets

Soybean oil (S) and soybean acid oil (SA) were obtained from Bunge (Wormerveer, North Holland). Palm oil (P) was sourced from Lipidos Santiga S.A. (Santa Perpetua de Mogoda, Barcelona, Spain). Oil samples were analyzed in triplicate for moisture and volatile matter (AOCS official Method Ca 2d-25., 2017) insoluble impurities (International Standard ISO 663:2017, 2017), unsaponifiable matter (AOCS official method Ca 6b-53, 2017), lipid-class composition (method 2508) (IUPAC, 1991), and total FA composition (Guardiola et al.,

1994). All methods were adapted to acid oils by Varona et al. (2021b). The chemical analyses of the experimental fats are presented in Table 4.1.

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

Item	P	SA	S
Moisture (g/100g)	ND	1.43	ND
Insoluble impurities (g/100g)	0.59	1.57	1.27
Unsaponifiable matter (g/100g)	0.21	2.34	0.99
Fatty acid composition (%) [2]			
C16:0	43.94	14.89	10.98
C18:0	4.64	3.46	3.47
C18:1 n-9	38.43	21.06	25.11
C18:2 n-6	9.70	51.71	51.70
C18:3 n-3	0.13	5.31	5.34
Minor fatty acids	3.15	3.58	3.40
SFA	50.64	19.88	15.86
cis-MUFA	39.44	23.06	27.06
trans-C18:1	0.08	0.04	0.04
PUFA	9.83	57.02	57.04
UFA:SFA	0.98	4.02	5.29
Lipid class composition (%) [3]			
TAG	92.46	25.32	96.27
DAG	7.54	13.48	3.23
MAG	ND	ND	ND
FFA	ND	61.20	0.50
T (mg/kg)	199.40	1464.25	1007.31
T3 (mg/kg)	431.87	8.78	ND

Abbreviations: P, palm oil; SA, soybean acid oil; S, soybean oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA:SFA, unsaturated to saturated fatty acid ratio, calculated as described by Varona et al. (2021a); TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids; T, sum of α-, β-, γ- and δ-tocopherols; T3, sum of α-, β-, γ- and δ-tocotrienols; ND, not detected. [1] Chemical composition analyzed as described by Varona et al. (2021a). [2] Percentage of total fatty acids (normalized data, area %); [3] Percentage of total lipid classes (normalized data, area %).

A basal diet based on wheat and soybean meal was formulated to meet or exceed FEDNA's (Fundación Española para el Desarrollo de la Nutrición Animal) requirements (FEDNA, 2008) and to minimize basal fat levels, as shown in Table 4.2. Two feeding periods were performed: starter from d 0 until d 22 and grower-finisher from d 23 to d 35, both in mash form. Titanium dioxide (TiO_2) was included (5 g/kg) in the diets as an indigestible marker. The basal diet was manufactured at Pinsos Molinet S.A. (Prats de Lluçanès, Barcelona, Spain).

Table 4.2. Ingredient composition of the experimental basal diet.

Ingredients, %	Starter Diet (from 0 d to 22 d)	Grower-Finisher Diet (from 23 d to 35 d)
Wheat	54.49	44.02
Soybean meal 47%	35.40	27.25
Barley	-	18.58
Experimental fats [1]	6.00	6.00
Calcium carbonate	1.44	1.39
Monocalcium phosphate	0.99	1.20
Titanium dioxide	0.50	0.50
Vitamin and mineral premix [2]	0.40	0.40
Sodium chloride	0.40	0.35
DL-Methionine	0.23	0.17
L-Lysine	0.15	0.12
L-Threonine	-	0.02

[1] Soybean oil, palm oil and palm fatty acid distillate in different proportions. [2] Provides per kg of feed: vitamin A (from retinol), 10,000 IU; vitamin D3 (from cholecalciferol), 4,800 IU; vitamin E (from alfa tocopherol), 45 mg; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 4.5 mg; vitamin B12, 40 μg; vitamin K3, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 150 μg; Fe (from FeSO4·7H2O), 54 mg; I (from Ca(I2O3)2), 1.2 mg; Cu (from CuSO4·5H2O), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na2SeO3), 0.18 mg; β-glucanase 150 U; xylanase 270 U.

The experimental diets were the result of a basal diet (94%) and the remaining 6% from one of the experimental fat sources (Table 4.1) or blends. The P was included at 6% (P6) and increasing amounts of SA were added in replacement of P: P4-SA2 (4% of P and 2% of SA), P2-SA4 (2% of P and 4% of SA), and SA6 (SA at 6%). The S was included at 6% (S6) as a positive control diet. The addition of the experimental fat sources or fat blends to a basal

diet to manufacture the experimental diets was performed at Lindo Pet Global S.A. (Castellar del Vallès, Barcelona, Spain). The composition of the experimental diets is shown in Table 4.3.

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

Item	Starter Diets (from 0 to 22 d)					Grower-Finisher Diets (from 23 to 35 d)				
	P6	P4-SA2	P2-SA4	SA6	S6	P6	P4-SA2	P2-SA4	SA6	S6
Macronutrient content										
Dry matter (g/100g)	90.93	91.19	90.70	91.00	91.00	90.02	90.27	90.25	91.16	90.14
Crude protein (g/100g)	23.15	23.49	23.95	23.81	23.61	20.84	21.34	20.57	21.77	21.04
Crude fat (g/100g)	7.70	7.62	7.43	7.38	7.51	7.49	7.71	7.69	7.56	8.18
Crude fiber (g/100g)	3.20	3.27	3.06	2.84	3.29	3.41	3.00	3.64	3.62	3.08
Ash (g/100g)	7.09	6.84	6.69	6.91	5.54	5.75	5.90	6.29	6.61	6.21
Gross energy, kcal/kg	4332	4345	4301	4325	4367	4324	4353	4320	4365	4339
Fatty acid composition (%)										
C14:0	0.87	0.65	0.41	-	-	0.85	0.63	0.38	-	0.06
C16:0	37.38	30.51	23.53	15.71	14.43	36.85	30.16	22.84	15.66	13.24
C18:0	4.29	4.00	3.70	3.37	3.48	4.17	3.91	3.62	3.33	3.35
C18:1 n-9	32.34	28.26	24.04	19.57	22.83	32.49	28.34	24.06	19.60	22.61
C18:1 n-7	0.83	1.06	1.30	1.57	1.46	0.80	1.05	1.30	1.55	1.50
C18:2 n-6	21.37	31.47	41.60	52.70	50.78	22.02	31.76	42.08	52.64	52.04
C18:3 n-3	1.46	2.70	3.98	5.35	5.27	1.60	2.82	4.12	5.42	5.50
Minor fatty acids	1.46	1.34	1.44	1.73	1.75	1.22	1.32	1.61	1.80	1.69
SFA	43.13	35.76	28.34	20.09	18.72	42.47	35.30	27.76	20.01	17.70
MUFA	34.04	30.06	26.08	21.87	25.24	33.92	30.12	26.05	21.93	24.76
PUFA	22.83	34.18	45.58	58.05	56.04	23.62	34.58	46.20	58.06	57.54
UFA:SFA	1.30	1.76	2.47	3.88	4.16	1.34	1.80	2.55	3.88	4.54
Lipid class composition (%)										
TAG	78.67	59.94	44.07	28.20	71.88	78.53	61.19	44.00	27.52	76.67
DAG	10.54	12.14	12.39	12.98	11.73	10.44	11.62	12.44	13.08	10.23
MAG	1.84	2.57	2.71	3.08	2.19	2.31	2.40	2.84	3.09	2.49
FFA	8.96	25.35	40.84	55.74	14.20	8.72	24.79	40.73	56.31	10.61

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA:SFA, unsaturated to saturated fatty acid ratio, calculated as described by Varona et al. (2021a); TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids. [1] All samples were analyzed at least in duplicate. [2] Dietary treatments supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). In all cases, fatty acids and lipid classes are expressed as internal area normalization (in %).

4.1.4.2 *Birds and Management*

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 protocol was reviewed and approved by the Animal Ethics Committee of the Universitat Autònoma de Barcelona (CEEAH; number code: 3938), in accordance with the European Union guidelines for the care and use of animals in research (2010/63/EU).

Day-old female broiler chickens (Ross 308) were obtained from a commercial hatchery (Pondex SAU; Lleida, Spain). On arrival, 480 birds with uniform BW were wing-banded, individually weighed and randomly allocated to cages (16 birds per cage). Each cage was assigned to one of the five experimental diets (six replicas per diet). Birds were housed in metabolic cages, with a grid floor and excreta collection tray, located in an environmentally controlled room. Throughout the study, feed and water were offered ad libitum. The temperature, humidity, ventilation, and illumination were automatically controlled, as recommended by the specifications in the Ross 308 lineage management handbook (Aviagen, 2014). The animals and housing facilities were inspected, at least twice a d.

4.1.4.3 *Controls and Sampling*

Individual body weight (BW) and feed intake by cage were recorded at 11, 22 and 35 d of age, to calculate the average daily gain (ADG), average daily feed intake (ADFI) and the feed conversion ratio (FCR) of each period, and the global result of the study. Daily mortality was recorded and weighed to correct these parameters.

Two digestibility balances were performed, in the starter period from 9 to 11 d and in the grower-finisher period from 33 to 35 d. A representative sample of excreta (free of contaminants, such as feed or feathers) of each cage was taken. At 11 d of age, 12 birds per cage were humanely killed by cervical dislocation, and the jejunum (from the distal-most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), and ileum (from the junction with Meckel's diverticulum to a point 1 cm proximal to the

ileocecal junction) were carefully excised. Then, both segments (jejunum and ileum) were divided into 2 equal portions, named as upper and lower. For each cage, samples were taken of the digestive content from the upper and lower jejunum and the upper and lower ileum. The samples were then homogenized and pooled. Samples were immediately frozen at -20ºC, and lyophilized. Samples of diets, digesta and excreta were ground to pass through a 0.5-mm sieve and kept at 4°C until further analyses. At the end of the study (35 d), 2 birds per cage were euthanized, and the same procedure described above was carried out for sampling. At 35 d, the abdominal fat pad (from the proventriculus surrounding the gizzard down to the cloaca) of each bird was removed and weighed. Abdominal fat pad weights were expressed in absolute values and as a percentage of BW.

4.1.4.4 Laboratory Analyses and Calculations

Analytical determinations of the diets were performed according to the methods of the AOAC International, (2005): dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fiber (Method 962.09), and ether extract (EE) by Soxhlet analysis (Method 920.39). Gross energy was determined by an adiabatic bomb calorimeter (IKA C-4000, Janke-Kunkel; Staufen, Germany). TiO_2 in feed, intestinal content and excreta was determined by spectrophotometry ICP-OES (Optima 3200 RL, Perkin Elmer, Waltham, MA) following the procedures of Short et al. (1996). Gross energy was determined in excreta samples by an adiabatic bomb calorimeter (IKA C-4000, Janke-Kunkel; Staufen, Germany). The FA content of the feed, intestinal content, and excreta was determined according to the method of Sukhija and Palmquist, (1988). The digestibility coefficients of FA in each segment of the intestinal tract and the excreta were determined using the TiO_2 ratio in the feed and digestive content or excreta as detailed in Jimenez-Moya et al. (2021a). The apparent metabolizable energy (AME) was calculated multiplying the apparent digestibility coefficient of the gross energy by its corresponding diet gross energy. Lipid-class composition (TAG, DAG, MAG, and FFA) of the extracted fat from the feed, digestive content, and excreta samples was determined as described by Jimenez-Moya et al. (2021a). To estimate the content of each lipid-class present in the intestinal tract and excreta of the chickens, the following formula (Rodriguez-Sanchez et al., 2019b) was used:

$$\text{Lipid-class content} = [LC] / [TiO_2], \quad (1)$$

where [LC] is the concentration of the lipid-class in the digesta of intestinal segment or excreta (mg/g DM) and [TiO_2] is the concentration of TiO_2 in the digesta of intestinal segment or excreta (mg/g DM). The macronutrient, FA and lipid-class composition of the experimental diets are presented in Table 4.3.

4.1.4.5 *Statistical Analysis*

The study consisted of 2 main factors: diet (5 diets) x intestinal segment (5 types, upper and lower jejunum, upper and lower ileum, and excreta). The effect of the age was also tested as described below (11 vs 35 d). The normality of the data and homogeneity of variance were verified. For each age, the effect of the diet on productive parameters, abdominal fat deposition, and feed AME values were statistically analyzed by one-way ANOVA using the GLM procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) (n=30; 5 diets x 6 replicas). For each age, and for each intestinal segment and excreta, the effect of the diet on the lipid-class content, FA digestibility, and its contribution on FA absorption was also evaluated by one-way ANOVA (n=30; 5 diets x 6 replicas).

For each age, the effect of the intestinal tract on the lipid-class content was also analyzed by one-way ANOVA with the intestinal segments and the excreta as the main factor (n=150; 30 samples x 5 types of digesta samples).

On each intestinal segment, the effect of the age (11 or 35 d) on FA absorption was statistically analyzed by one-way ANOVA using the age as the main factor (n=60; 5 dietary treatments replicated 6 times x 2 ages). Additionally, for each dietary treatment, one-way ANOVA was used to test the effect of the age on feed AME, and at lower ileum level on lipid-class content and FA digestibility, (n=12; 6 replicas of lower ileum x 2 ages).

The differences between treatments means were tested using Tukey's correction for multiple comparisons. The cage served as the experimental unit, so there were six units per diet.

The results shown in tables are reported as least-square means, and in all statistical analyses, differences were considered significant at $p < 0.05$.

4.1.5 Results

4.1.5.1 *Characterization of Experimental Oils and Diets*

The chemical analysis of the experimental fats is shown in Table 4.1. The highest value of MIU (moisture, impurities and unsaponifiable matter) content was obtained for SA (5.3%). Regarding FA composition, S and SA showed similar FA profile with high content in linoleic and oleic acids, while the major FA in P were palmitic and oleic acids. The UFA:SFA was higher for S (5.29) and SA (4.02) than for P (0.98). Regarding lipid-class content, S and P showed a higher TAG content (>92%), while SA was richer in FFA (61.2%).

The chemical analysis of the experimental diets is shown in Table 4.3. Both in starter and grower-finisher diets, the replacement of P by SA resulted in a progressive increase of the content of dietary FFA (from 9% to 56%), and consequently, a decrease in the TAG (from 79% to 28%). In parallel an increase in the UFA:SFA was also obtained, from 1.3 to 3.8.

4.1.5.2 *Growth Performance and Abdominal Fat Deposition*

The effect of dietary fat source on the growth-performance data and on abdominal fat deposition is shown in Table 4.4. The diet had no effect ($p > 0.05$) in any performance parameter in the starter period (from 0 to 22 d), nor in the global period (from 0 to 35 d). However, in the grower-finisher period (from 23 to 35 d), chickens fed P6 or P4-SA2 had higher FCR than chickens fed S6 ($p = 0.018$).

Regarding the effect of the diet on fat deposition, only differences were obtained between chickens fed diets containing fat blends. Abdominal fat depot was higher in those chickens fed P4-SA2 than in those birds fed P2-SA4 ($p = 0.018$).

Table 4.4. Growth performance and abdominal fat pad deposition of broiler chickens according to different fat sources in diet [1].

Item	Dietary Treatments [2]					SEM [3]	*p*-Value
	P6	P4-SA2	P2-SA4	SA6	S6		
From 0 to 22 d							
ADFI, g/d/bird	54.5	52.3	52.2	51.0	48.7	2.43	0.554
ADG, g/d/bird	40.6	40.5	39.1	38.7	37.2	1.43	0.422
FCR, g/g	1.34	1.29	1.34	1.32	1.31	0.037	0.833
BW at 22 d, g	933	930	898	890	856	31.4	0.416
From 23 to 35 d							
ADFI, g/d/bird	143	145	142	139	134	2.90	0.077
ADG, g/d/bird	89.9	90.3	90.9	88.3	87.8	1.96	0.768
FCR, g/g	1.60 ^{b}	1.61 ^{b}	1.57 ^{ab}	1.58 ^{ab}	1.53 ^{a}	0.017	0.018
BW at 35 d, g	2,101	2,104	2,079	2,038	1,997	45.6	0.425
From 0 to 35 d							
ADFI, g/d/bird	87.5	86.9	85.7	83.8	80.3	2.28	0.199
ADG, g/d/bird	58.9	59.0	58.3	57.1	56.0	1.30	0.426
FCR, g/g	1.49	1.47	1.47	1.47	1.43	0.018	0.383
Abdominal fat, g	32.36 ^{ab}	36.49 ^{a}	27.84 ^{b}	29.50 ^{ab}	29.62 ^{ab}	2.051	0.018
Abdominal fat, %	1.53 ^{ab}	1.73 ^{a}	1.33 ^{b}	1.43 ^{ab}	1.46 ^{ab}	0.091	0.019

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; BW, body weight. [1] Diets supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). [2] Values are pooled means of 6 replicates with 16 chickens/replicate from 0 to 11 d and 4 chickens/replicate from 11 to 35 d. In the case of BW, values are means of 24 chickens each treatment from 22 to 35 d. For abdominal fat, values are means of 2 chickens/replicate: 12 for each treatment at 35 d. 3 SEM, standard error of means of 6 observations per treatment (the experimental unit is the cage).

4.1.5.3 Lipid-class content along the Intestinal Tract

The lipid-class content (TAG, DAG, MAG, FFA) in the intestinal tract and excreta in 11-d-old broiler chicks (Table 4.5) and 35-d-old broiler chickens (Table 4.6) evidence that FFA was the main lipid-class present in the digesta and in the excreta. In starter chicks (11 d), a decrease of the TAG, DAG, and FFA content was obtained from the upper jejunum to lower jejunum ($p < 0.001$) (Supplementary Table 4.9). In grower-finisher chickens (35 d), the content of TAG DAG, MAG, and FFA decreased from the upper jejunum to upper ileum ($p < 0.001$) (Supplementary Table 4.9).

When P is replaced with graded levels of SA, higher TAG content was observed at 11 d (Table 4.5) in the lower jejunum ($p = 0.004$) and lower ileum ($p = 0.028$), and at 35 d (Table 4.6) in the upper and lower ileum and in the excreta ($p \leq 0.001$). Similarly, the replacement of P by SA increased MAG content in starter chicks from the lower jejunum on ($p < 0.001$) and in grower chickens in the jejunum, upper ileum, and in the excreta ($p \leq 0.013$).

Higher TAG content was observed in chickens fed SA6 compared to chickens fed S6 in the lower jejunum at 11 d ($p = 0.004$) and in the lower ileum and excreta at 35 d ($p \leq 0.001$). The content of MAG was also higher in chickens fed SA6 than those fed S6, being the values of the latter similar to those ones obtained in chickens fed P6; this was observed in starter chicks from the lower jejunum on ($p < 0.001$), and in grower chickens from the upper ileum on ($p \leq 0.029$).

Regarding FFA content in starter chicks (Table 4.5 and Figure 4.1.a) and grower-finisher chickens (Table 4.6 and Figure 4.1.b), lower FFA content was obtained as P was replaced by SA from the lower jejunum on at 11 d ($p \leq 0.001$) and at 35 d ($p \leq 0.007$). Those birds fed P6 diet showed the highest FFA content in the lower jejunum and upper ileum at 11 d, and from the upper ileum on at 35 d. In both starter and grower chickens, no differences were observed in the FFA content between SA6 and S6 diets in any intestinal segment, except in the excreta at 35 d. In grower chickens, no differences were observed between chickens fed SA6 diet and those animals fed blend diets (P4-SA2 or P2-SA4) in any intestinal segment nor in the excreta.

At lower ileum level, starter chicks showed higher content of all lipid classes compared to grower-finisher chickens fed the same dietary treatment ($p \leq 0.021$), except in MAG content for those birds fed P6, in which no differences were observed regarding of the age of the animal (Supplementary Table 4.10).

Table 4.5. Lipid-class content [1] along the intestinal tract and excreta according to different fat sources in the diet [2] in 11-d-old broiler chickens.

Item	Dietary Treatments					SEM [3]	*p*-Value
	P6	P4-SA2	P2-SA4	SA6	S6		
				Upper Jejunum			
TAG	0.49	0.52	0.46	0.85	0.53	0.136	0.261
DAG	1.99	1.98	2.72	2.60	1.30	0.445	0.193
MAG	0.15	0.31	0.24	0.36	0.18	0.052	0.052
FFA	18.20 ^{a}	14.64 ^{ab}	17.72 ^{a}	10.18 ^{ab}	6.58 ^{b}	2.045	0.001
				Lower Jejunum			
TAG	0.30 ^{b}	0.42 ^{ab}	0.45 ^{ab}	0.54 ^{a}	0.34 ^{b}	0.041	0.004
DAG	0.76	0.92	0.98	0.98	0.82	0.092	0.285
MAG	0.17 ^{c}	0.30 ^{b}	0.42 ^{ab}	0.46 ^{a}	0.18 ^{c}	0.029	<0.001
FFA	9.70 ^{a}	7.89 ^{b}	5.96 ^{c}	3.96 ^{d}	3.65 ^{d}	0.415	<0.001
				Upper Ileum			
TAG	0.24	0.30	0.32	0.34	0.25	0.050	0.522
DAG	0.62	0.58	0.73	0.69	0.64	0.099	0.843
MAG	0.13 ^{d}	0.27 ^{bc}	0.37 ^{ab}	0.40 ^{a}	0.16 ^{cd}	0.026	<0.001
FFA	8.69 ^{a}	6.90 ^{b}	5.15 ^{c}	3.22 ^{d}	3.05 ^{d}	0.414	<0.001
				Lower Ileum			
TAG	0.19 ^{b}	0.30 ^{ab}	0.36 ^{a}	0.38 ^{a}	0.32 ^{ab}	0.041	0.028
DAG	0.45	0.58	0.72	0.68	0.64	0.083	0.188
MAG	0.15 ^{d}	0.35 ^{bc}	0.45 ^{ab}	0.52 ^{a}	0.23 ^{cd}	0.033	<0.001
FFA	8.47 ^{a}	6.83 ^{a}	4.63 ^{b}	3.17 ^{b}	3.02 ^{b}	0.428	<0.001
				Excreta			
TAG	0.29 ^{b}	0.33 ^{ab}	0.53 ^{a}	0.37 ^{ab}	0.38 ^{ab}	0.044	0.034
DAG	0.80	1.08	1.07	0.88	0.98	0.167	0.515
MAG	0.21 ^{b}	0.28 ^{ab}	0.38 ^{a}	0.43 ^{a}	0.19 ^{b}	0.040	<0.001
FFA	10.24 ^{a}	9.01 ^{ab}	6.62 ^{bc}	4.05 ^{c}	4.16 ^{c}	0.631	<0.001

Abbreviations: TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids. [1] Lipid-class concentration (mg/g)/Ti concentration (mg/g) in each intestinal segment and excreta. [2] Values are pooled means of 6 replicates with 12 chickens/replicate fed diets supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). [3] SEM = standard error of the mean. a-d: means in a row not sharing a common letter are significantly different ($p < 0.05$).

Table 4.6. Lipid-class content [1] along the intestinal tract and excreta according to different fat sources in the diet [2] in 35-d-old broiler chickens.

Item	Dietary Treatments					SEM [3]	*p*-Value
	P6	P4-SA2	P2-SA4	SA6	S6		
				Upper Jejunum			
TAG	0.26	0.25	0.19	0.34	0.21	0.053	0.255
DAG	1.20	1.38	1.63	1.83	1.32	0.169	0.097
MAG	0.17 ^{b}	0.22 ^{ab}	0.33 ^{ab}	0.41 ^{a}	0.24 ^{ab}	0.047	0.011
FFA	7.40	7.56	7.99	8.59	8.28	0.816	0.830
				Lower Jejunum			
TAG	0.22	0.21	0.27	0.27	0.10	0.044	0.083
DAG	0.58	0.51	0.50	0.55	0.43	0.098	0.838
MAG	0.14 ^{b}	0.22 ^{ab}	0.25 ^{ab}	0.29 ^{a}	0.18 ^{ab}	0.030	0.013
FFA	5.08 ^{a}	3.97 ^{ab}	3.67 ^{ab}	3.18 ^{b}	3.10 ^{b}	0.403	0.007
				Upper Ileum			
TAG	0.09 ^{b}	0.18 ^{a}	0.19 ^{a}	0.21 ^{a}	0.14 ^{ab}	0.017	<0.001
DAG	0.17 ^{ab}	0.14 ^{b}	0.20 ^{ab}	0.27 ^{a}	0.16 ^{b}	0.024	0.007
MAG	0.11 ^{c}	0.14 ^{bc}	0.18 ^{ab}	0.21 ^{a}	0.11 ^{c}	0.015	<0.001
FFA	2.67 ^{a}	1.83 ^{b}	1.61 ^{bc}	1.71 ^{bc}	1.20 ^{c}	0.128	<0.001
				Lower Ileum			
TAG	0.07 ^{c}	0.11 ^{bc}	0.15 ^{ab}	0.18 ^{a}	0.09 ^{bc}	0.019	0.001
DAG	0.17	0.16	0.17	0.22	0.14	0.033	0.589
MAG	0.15 ^{ab}	0.17 ^{ab}	0.18 ^{ab}	0.24 ^{a}	0.13 ^{b}	0.023	0.029
FFA	2.92 ^{a}	1.62^{b}	1.33 ^{b}	1.40 ^{b}	0.87 ^{b}	0.241	<0.001
				Excreta			
TAG	0.15 ^{bc}	0.17 ^{bc}	0.19 ^{ab}	0.23 ^{a}	0.12 ^{c}	0.015	<0.001
DAG	0.15	0.13	0.17	0.22	0.13	0.026	0.111
MAG	0.13 ^{cd}	0.15 ^{bc}	0.19 ^{ab}	0.21 ^{a}	0.09 ^{d}	0.014	<0.001
FFA	2.66 ^{a}	1.50 ^{b}	1.59 ^{b}	1.66 ^{b}	0.96 ^{c}	0.119	<0.001

Abbreviations: TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids. [1] Lipid-class concentration (mg/g)/Ti concentration (mg/g) in each intestinal segment and excreta. [2] Values are pooled means of 6 replicates with 2 chickens/replicate fed diets supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). [3] SEM = standard error of the mean. a-d: means in a row not sharing a common letter are significantly different ($p < 0.05$).

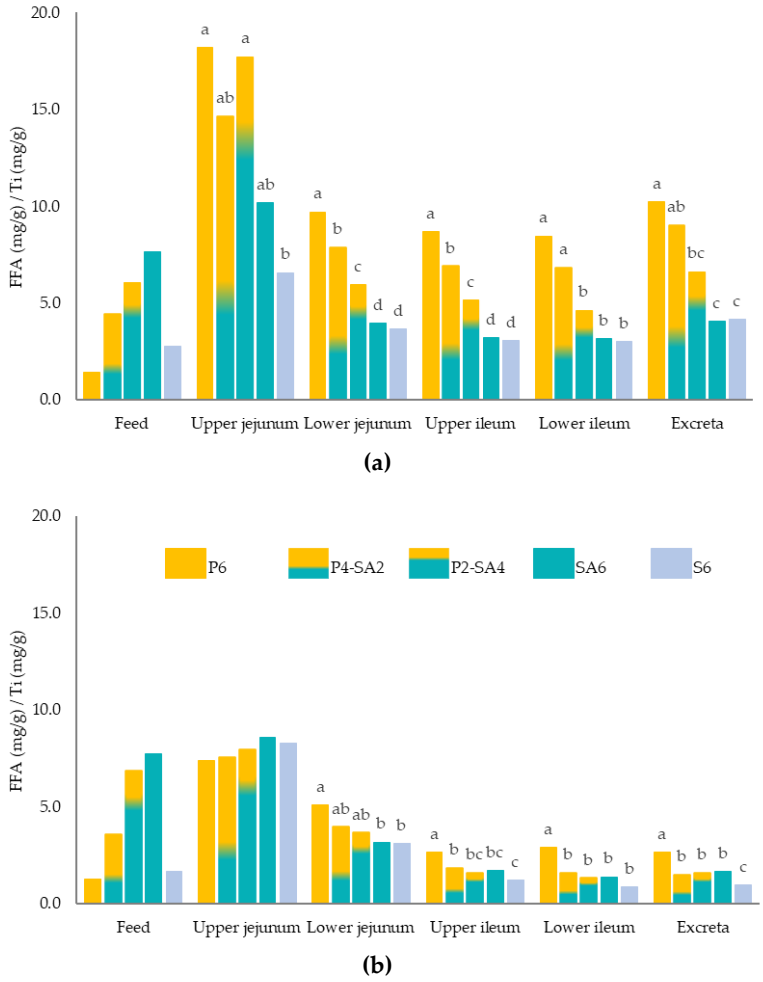

Figure 4.1. FFA content[1] in the feed, upper jejunum, lower jejunum, upper ileum, lower ileum, and excreta for the five different diets; with 6% of palm oil (P6), blend with 4% palm oil and 2% soybean acid oil (P4-SA2), blend with 2% palm oil and 4% soybean acid oil (P2-SA4), with 6% of soybean acid oil (SA6) and with 6% soybean oil (S6) in (a) 11-d-old broiler chickens and (b) 35-d-old broiler chickens. [1] FFA concentration (mg/g)/Ti concentration (mg/g) in each intestinal segment and excreta. Values are pooled means of 6 replicates per each diet with 12 chickens/replicate at 11 d, and 2 chickens/replicate at 35 d. a–d: columns not sharing a common letter within each intestinal segment are significantly different ($p \leq 0.01$).

4.1.5.4 Apparent Fatty-acid Digestibility along the Intestinal Tract

The apparent FA digestibility coefficients determined in the different intestinal segments and in the excreta, and the feed apparent metabolizable energy for the different dietary treatments are shown in Table 4.7 and Table 4.8, for 11-d-old and 35-d-old broiler chickens, respectively.

Considering the effect of the diet on the AME, differences were found among the different diets in both periods, from 0 to 21 d ($p < 0.001$) and from 22 to 35 d ($p = 0.008$). In the

starter phase, no differences were observed among P6, P4-SA2, and P2-SA4. Regarding the unsaturated diets, S6 had higher AME than SA6. In the grower-finisher phase, P2-SA4 showed higher AME value compared to SA6 and P6. The feed AME values of P6, blend diets, and SA6 were higher in the grower-finisher phase than in the starter phase ($p \leq 0.001$) (Supplementary Table 4.11).

Regarding the apparent FA digestibility results, replacing P with SA increased the digestibility of TFA for both starter chicks (11 d) from the lower jejunum on ($p < 0.001$) and grower-finisher chickens from the upper ileum on ($p \leq 0.001$). Similarly, the replacement of P by SA increased PUFA digestibility coefficients at 11 d in all intestinal segments ($p \leq 0.001$) and at 35 d from the lower jejunum on ($p < 0.001$). In contrast, no effect on SFA digestibility coefficients was observed in starter chicks, whereas in grower chickens replacing P with SA increased the digestibility of SFA at lower ileum level ($p < 0.001$). In the upper ileum, 35-d-old chickens fed P2-SA4 had the same SFA digestibility coefficients than those birds fed S6 and higher than those ones fed SA6 and P6 ($p < 0.001$).

Considering the results obtained at lower ileum level, at 11 d chick fed P2-SA4, SA6, and S6 did not differ for TFA, MUFA and PUFA, but for SFA those birds fed S6 showed the highest digestibility coefficients ($p < 0.001$). Grower chickens fed P4-SA2, P2-SA4, SA6, and S6 had no differences for TFA, SFA and MUFA values at lower ileum, however for the digestibility of PUFA the lack of differences was observed among chickens fed P2-SA4, SA6, and S6.

Regarding the effect of the age, the digestibility coefficients of TFA, SFA, and MUFA obtained in the lower ileum were higher in grower chickens than in starter chicks fed each dietary treatment ($p < 0.001$) (Supplementary Table 4.11). Similarly, higher digestibility of PUFA was observed at 35 d compared to 11 d in those animals fed S6 and blend diets ($p \leq 0.014$) (Supplementary Table 4.11).

Table 4.7. Feed apparent metabolizable energy values and apparent fatty acid digestibility coefficients along the intestinal tract and excreta according to different fat sources in the diet in 11-d-old broiler chickens.

Item	Dietary Treatments [1]					SEM [4]	*p*-Value
	P6	P4-SA2	P2-SA4	SA6	S6		
AME, kcal/kg [2]	3014 ^{bc}	3109 ^{bc}	3001 ^{c}	3119 ^{b}	3348 ^{a}	27.783	<0.001
Upper Jejunum [3]							
TFA	0.20 ^{b}	0.33 ^{ab}	0.12 ^{b}	0.39 ^{ab}	0.61 ^{a}	0.081	<0.001
SFA	0.19	0.22	-0.02	-0.19	0.20	0.111	0.050
MUFA	0.31 ^{ab}	0.36 ^{ab}	0.22 ^{ab}	0.20 ^{b}	0.51 ^{a}	0.078	0.028
PUFA	0.04 ^{d}	0.41 ^{bc}	0.15 ^{cd}	0.57 ^{ab}	0.78^{a}	0.092	<0.001
Lower Jejunum [3]							
TFA	0.48 ^{c}	0.54 ^{bc}	0.62 ^{b}	0.71 ^{a}	0.72 ^{a}	0.022	<0.001
SFA	0.37 ^{b}	0.37 ^{b}	0.40 ^{b}	0.46 ^{ab}	0.60 ^{a}	0.034	<0.001
MUFA	0.58 ^{b}	0.58 ^{b}	0.62 ^{ab}	0.65 ^{ab}	0.69 ^{a}	0.026	0.017
PUFA	0.56 ^{c}	0.69 ^{b}	0.77 ^{ab}	0.81 ^{a}	0.77 ^{ab}	0.024	<0.001
Upper Ileum [3]							
TFA	0.51 ^{c}	0.56 ^{c}	0.62 ^{bc}	0.72 ^{ab}	0.74 ^{a}	0.029	<0.001
SFA	0.36 ^{b}	0.36 ^{b}	0.40 ^{b}	0.48 ^{b}	0.65 ^{a}	0.037	<0.001
MUFA	0.62	0.61	0.62	0.67	0.73	0.033	0.112
PUFA	0.63 ^{b}	0.72 ^{ab}	0.74 ^{ab}	0.82 ^{a}	0.78 ^{a}	0.027	0.001
Lower Ileum [3]							
TFA	0.62 ^{c}	0.65 ^{bc}	0.72 ^{ab}	0.80 ^{a}	0.79 ^{a}	0.025	<0.001
SFA	0.49 ^{b}	0.46 ^{b}	0.51 ^{b}	0.55 ^{b}	0.69 ^{a}	0.037	<0.001
MUFA	0.74	0.70	0.73	0.75	0.76	0.028	0.552
PUFA	0.75 ^{c}	0.81 ^{bc}	0.85 ^{ab}	0.90 ^{a}	0.83 ^{abc}	0.023	0.001
Excreta [3]							
TFA	0.60 ^{c}	0.63 ^{bc}	0.72 ^{ab}	0.78 ^{a}	0.80 ^{a}	0.024	<0.001
SFA	0.39 ^{b}	0.40 ^{b}	0.43 ^{b}	0.47 ^{b}	0.64 ^{a}	0.035	<0.001
MUFA	0.73	0.70	0.72	0.74	0.79	0.022	0.093
PUFA	0.80	0.80	0.83	0.91	0.85	0.029	0.094

Abbreviations: AME, apparent metabolizable energy; TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [1] Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). [2] Values are pooled means of 6 replicates with 16 chickens/replicate. [3] Values are pooled means of 6 replicates with 12 chickens/replicate. [4] SEM = standard error of the mean. a–d: means in a row not sharing a common letter are significantly different ($p < 0.05$).

Table 4.8. Feed apparent metabolizable energy values and apparent fatty acid digestibility coefficients along the intestinal tract and excreta according to different fat sources in the diet in 35-d-old broiler chickens.

Item	Dietary Treatments [1]					SEM [4]	*p*-Value
	P6	P4-SA2	P2-SA4	SA6	S6		
AME, kcal/kg [2]	3279 [b]	3324 [ab]	3384 [a]	3274 [b]	3364 [ab]	23.350	0.008
Upper Jejunum [3]							
TFA	0.51	0.44	0.45	0.35	0.48	0.052	0.283
SFA	0.48 [a]	0.40 [a]	0.36 [ab]	0.11 [b]	0.21 [ab]	0.073	0.005
MUFA	0.67	0.60	0.59	0.49	0.60	0.043	0.059
PUFA	0.34	0.35	0.42	0.38	0.51	0.060	0.285
Lower Jejunum [3]							
TFA	0.69 [b]	0.74 [ab]	0.73 [ab]	0.72 [ab]	0.81 [a]	0.024	0.019
SFA	0.64	0.71	0.72	0.62	0.73	0.032	0.061
MUFA	0.83 [a]	0.83 [a]	0.79 [ab]	0.74 [b]	0.85 [a]	0.019	0.002
PUFA	0.56 [c]	0.69 [b]	0.70 [b]	0.74 [ab]	0.81 [a]	0.025	<0.001
Upper Ileum [3]							
TFA	0.82 [c]	0.84 [bc]	0.88 [a]	0.86 [ab]	0.89 [a]	0.009	<0.001
SFA	0.77 [b]	0.81 [ab]	0.85 [a]	0.77 [b]	0.85 [a]	0.015	<0.001
MUFA	0.91 [a]	0.87 [a]	0.90 [a]	0.86 [b]	0.91 [a]	0.007	<0.001
PUFA	0.78 [c]	0.82 [b]	0.89 [a]	0.89 [a]	0.90 [a]	0.008	<0.001
Lower Ileum [3]							
TFA	0.84 [b]	0.88 [ab]	0.91 [a]	0.91 [a]	0.92 [a]	0.010	<0.001
SFA	0.78 [b]	0.85 [a]	0.89 [a]	0.85 [a]	0.90 [a]	0.016	<0.001
MUFA	0.93	0.92	0.91	0.90	0.93	0.011	0.282
PUFA	0.83 [c]	0.88 [b]	0.92 [ab]	0.93 [a]	0.93 [a]	0.011	<0.001
Excreta [3]							
TFA	0.84 [c]	0.90 [b]	0.90 [ab]	0.89 [b]	0.93 [a]	0.006	<0.001
SFA	0.77 [b]	0.86 [a]	0.85 [a]	0.79 [b]	0.87 [a]	0.009	<0.001
MUFA	0.92 [ab]	0.92 [a]	0.91 [b]	0.87 [c]	0.93 [a]	0.004	<0.001
PUFA	0.84 [c]	0.91 [b]	0.93 [ab]	0.94 [ab]	0.94 [a]	0.007	<0.001

Abbreviations: AME, apparent metabolizable energy; TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [1] Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). [2] Values are pooled means of 6 replicates with 4 chickens/replicate. [3] Values are pooled means of 6 replicates with 2 chickens/replicate. 4 SEM = standard error of the mean. a-d: means in a row not sharing a common letter are significantly different ($p < 0.05$).

4.1.5.5 Contribution of each intestinal segment to FA digestibility

The contribution of each intestinal segment in the digestibility of TFA, palmitic acid, stearic acid, oleic acid and linoleic acid was calculated in relation to its maximum

digestibility reached at lower ileum, and results for the different dietary treatments for both 11-d- and 35-d-old broiler chickens are shown in Figure 4.2.

Regardless of the age, most of the TFA absorption toke place in the jejunum (84%), being the contribution of the upper jejunum and lower jejunum (on average of all diets at both ages) 49% and 35%, respectively. The jejunum was also the most important segment in the absorption of palmitic acid (≥ 72% at 11 d; ≥ 78% at 35 d), oleic acid (≥ 74% at 11 d; ≥ 83% at 35 d), and linoleic acid (≥ 77% at 11 d; ≥ 72% at 35 d). The absorption of stearic acid started at lower jejunum both in starter chicks and in grower chickens; the contribution of this segment, on average of all diets, was 57% at 11 d, and 61% at 35 d.

The contribution of the different intestinal segments to the absorption of TFA for both starter and grower-finisher chickens was not affected by the replacement of P by SA, nor by the FFA content when SA6 was compared to S6, however differences were observed for the absorption of individual FA. The results at 11 d (Figure 4.2.a) show that the inclusion of SA in replacement of P increased the absorption of linoleic acid at the upper jejunum level ($p < 0.001$). The absorption of linoleic acid in S6 occurred mainly in the upper jejunum, whereas this segment had lower contribution in those diets that included P, blend diets and P6 ($p < 0.001$). In grower-finisher broiler chickens (Figure 4.2.b) the replacement of P by SA delayed the absorption of palmitic acid, the absorption rate in SA6 compared to P6 was lower in the upper jejunum ($p = 0.004$) and inversely higher in the lower jejunum ($p = 0.022$). Similarly, the absorption of oleic acid in the upper ileum was higher as much P was replaced by SA ($p = 0.002$).

In general, the contribution of the lower ileum to the absorption of the TFA and individual FA was higher for starter chicks than for grower chickens ($p \leq 0.002$) (Supplementary Table 4.12). The contribution of the upper ileum was more important at 35 d than at 11 d, mainly to the absorption of stearic and linoleic acid ($p \leq 0.007$) (Supplementary Table 4.12). Differences between ages were also observed in the jejunum; the absorption of palmitic acid and oleic acid was higher in the upper jejunum at 35 d ($p \leq 0.019$), whereas the absorption of these FA was higher in the lower jejunum at 11d ($p \leq 0.005$) (Supplementary Table 4.12).

Figure 4.2. Contribution of each intestinal segment to the apparent fatty acid digestibility, calculated as a proportion of total digestibility reached at the lower ileum, along the intestinal tract for the five different diets; with 6% of palm oil (P6), blend with 4% palm oil and 2% soybean acid oil (P4-SA2), blend with 2% palm oil and 4% soybean acid oil (P2-SA4), with 6% of soybean acid oil (SA6) and with 6% soybean oil (S6) in (a) 11-d-old broiler chickens and (b) 35-d-old broiler chickens. TFA (Total Fatty Acids), Palmitic (C16:0), stearic (C18:0), oleic (C18:1n-9) and linoleic (C18:2n-6) acids. Values are means of 6 replicates per each diet with 12 chickens/replicate at 11 d, and 2 chickens/replicate at 35 d. a-c: columns with the same intestinal segment not sharing a common letter are significantly different ($p < 0.01$).

4.1.6 Discussion

The results obtained supported that the lipolysis process in starter chicks (11 d) took place until the lower jejunum, whereas in grower chickens (35 d) reached the upper ileum. The lower TAG content observed in the ileum at 35 d, confirms the improvement in the capacity of the hydrolysis process related to the age of the bird, in accordance with results obtained in our previous study (Jimenez-Moya et al., 2021a). This has been attributed to several factors: an insufficient emulsification process due to the immature gastrointestinal tract (GIT) in starter chicks compared to grower chickens (Krogdahl, 1985), an increase of the rate of bile secretion with age (Noy and Sklan, 1995) and a less efficient turnover of bile acids in young chicks than in older ones (Serafin and Nesheim, 1970). From the results obtained, no relation between TAG content in the feed and those obtained through the intestinal segments was observed. In this sense, Rodriguez-Sanchez et al. (2019b) determined the lipid classes in the duodenum, where the hydrolysis process mainly takes place (Rodriguez-Sanchez et al., 2019a), and reported that the dietary FFA% did not affect the hydrolysis process in 14-d-old chicks.

Regarding the absorption process, its evaluation is based on the results of the lipid classes, in particular, the decrease of the FFA content along the intestine together with the increase in the apparent FA absorption. It is well known that no FA absorption occurs after the lower ileum (Renner, 1965) and the results obtained in the excreta could be affected by the FA production from the microbiota (Lan et al., 2005) as has been suggested by Rodriguez-Sanchez et al. (2019b). Therefore, the results obtained from the lower ileum are understood as the maximum FA digestibility coefficients.

The results showed that the replacement of P by SA, increasing the UFA:SFA as well as the FFA level of the diet, had a positive effect on the dietary fat utilization, improving the digestibility of TFA and PUFA at both ages, and the digestibility of SFA at 35 d. In addition, the data showed that young broilers were not fully capable to utilize SA; the detriment was mainly obtained in the SFA absorption, being the digestibility coefficients of SA6 similar to P6. However, as the age of the chicken increased, the utilization of SA6

improved; 35-d-old chickens fed diets containing SA reached higher SFA digestibility in the lower ileum than those birds fed P6 diet.

On the other hand, the comparison between chickens fed SA6 and those birds fed S6 gives us information about the effect of the dietary FFA level, as both diets had a similar UFA:SFA. The dietary FFA level only had a negative repercussion on the feed AME values and the SFA absorption in 11-d-old broiler chicks obtained in the lower ileum. This could be associated with the formation of insoluble calcium soaps, between SFA in free form and Ca^{2+}, decreasing its availability to be absorbed and precipitating in the feces (Small, 1991), as it was obtained in a previous *in vitro* study, in which SA showed higher fat content precipitated than S (Jimenez-Moya et al., 2021b). Moreover, the poorly developed GIT in young chicks, mainly the limited bile salts content, and the lower utilization of MAG in SA6 compared to S6 (Table 4.5), both necessary to form DMM, could also explain the lower digestibility of SFA obtained. The negative effect of the dietary FFA disappeared with the age, suggesting that the use of SA (56% FFA) in grower-finisher diets could be a suitable and economical alternative energy source, in accordance with Borsatti et al. (2018). Results obtained of FFA absorption and FA digestibility at lower ileum using SA6 were similar than those obtained with S6, also for the performance parameters, abdominal fat deposition, and feed AME values. So, the potential inclusion of this acid oil depends on the age of the bird, which is in accordance with Rodriguez-Sanchez et al. (2019b), Jimenez-Moya et al. (2021a), and Rodriguez-Sanchez et al. (2021).

A synergism between P and SA was observed in P2-SA4 grower-finisher diet for the feed AME values; the feed AME values obtained were 107 kcal higher than the calculated ones from the value for the two separate sources at the proportion blended. The synergism obtained between P and SA is in accordance with the results reported by Blanch et al. (1996) adding a blend fat source (2% P + 2% SA) to a basal diet in 1-year-old roosters feeding. That synergism between the saturated oil and the unsaturated acid oil could be explained by the associative effect of blending a higher proportion of UFA with a lower proportion of SFA and the positive effect of increase the ratio of TAG and DAG to FFA, compared to SA, as is discussed in previous studies (Tancharoenrat et al., 2013; Roll et al., 2018; Viñado et al., 2019b; Jimenez-Moya et al., 2021a).

The results obtained using SA (56% FFA; 4 UFA:SFA) and the positive results obtained with the blend P2-SA4 (40% FFA; 2.6 UFA:SFA) in the grower-finisher broiler feeding phase suggest that it is possible to include a high FFA level in the diet if they come from an unsaturated source. In our previous study (Jimenez-Moya et al., 2021a) using a saturated acid oil (palm fatty acid distillate, PFAD) blended with S, a positive results was reported when the FFA content did not exceed 30% and the blend had also 2.6 UFA:SFA. From the results of both studies, it could be suggested that at the same UFA:SFA chickens can better absorb FFA provided by unsaturated sources rather than saturated ones. This could be explained because unsaturated FFA from SA would be easily included in the DMM being more bioaccessible (Jimenez-Moya et al., 2021b), whereas saturated FFA from PFAD could not be absorbed readily because they form more calcium soaps than the UFA (Small, 1991).

The positive effect of increasing the age of the bird on FA utilization, especially in the absorption of SFA, has been reported by Batal and Parsons (2002), Tancharoenrat et al. (2013), Roll et al. (2018), Rodriguez-Sanchez et al. (2019a), and Viñado et al. (2019a). This fact is evidenced by the higher feed AME values, FA digestibility coefficients, and the lower FFA content obtained in grower chickens compared to starter chicks. The results of the present study confirmed that the age of the chicken improved the dietary FFA utilization. This has been related to the improvement in the capacity of fat digestion, also when fats are rich in FFA as it has been reported using PFAD (Jimenez-Moya et al., 2021a). This fact could be associated with a longer feed passage time along the GIT in adult broilers compared to young birds (Angel et al., 2013). According to the results, the contribution of the upper ileum to FA absorption, in part, could explain the improvement in fat utilization in grower chickens as compared to starter ones, as it has previously suggested (Jimenez-Moya et al., 2021a).

Regarding the contribution of the intestinal segment to FA absorption, the results evidenced that, the jejunum was the most important segment for TFA absorption, in agreement with Tancharoenrat et al. (2014) and Rodriguez-Sanchez et al. (2019a). Its contribution ranged from 77% to 92%, in agreement with Jimenez-Moya et al. (2021a). Almost a half of the TFA absorption occurred in the upper jejunum (on average of all

diets: 48% at 11d; 50% at 35d), around one third was absorbed in the lower jejunum (37% at 11d; 33% at 35d), and the absorption of the residual FAs took place in the upper and lower ileum, which importance was affected by the age of the bird; upper ileum (6% at 11d; 13% at 35d) and lower ileum (9% at 11d; 4% at 35d). On the other hand, it is remarkable the delayed absorption of stearic acid, starting in the lower jejunum, as is described in Jimenez-Moya et al. (2021a). Although both palmitic acid and stearic acid are saturated FA, the greater molar volume of the latter probably causes the different behavior since it could affect the inclusion rate in the DMM (Freeman, 1969).

The results showed that the replacement of P by SA delayed the palmitic acid absorption in both starter and grower chicks. In contrast, the absorption of linoleic acid in 11-d-old chicks was faster as more SA was included in replacement of P. This suggests that the concentration of each FA in the diet could determine the preference for their inclusion into the DMM, being faster the absorption of a major FA than the absorption of a minor FA (palmitic acid, SA: 16% vs P6: 37%; linoleic acid, SA: 53% vs P6: 21%; Table 4.3). In addition, palmitic acid is less soluble in bile salts solution than linoleic acid (Freeman, 1969).

4.1.7 Conclusions

The current study evidenced that FA absorption determined in the lower ileum was essential to understand the utilization of different diets differing their UFA:SFA and FFA content. So, lower ileum content should be considered for sampling for future fat digestibility studies. The results demonstrated the strong effect of the age of the bird in the hydrolysis and the absorption process of fats. Increasing the age of the chicken led to improve the utilization of dietary SFA and FFA and increased the contribution of the upper ileum to FA absorption.

Blending palm oil with soybean acid oil is a potential strategy to use this by-product in both starter and grower-finisher diets, improving the absorption of palm oil, and obtaining in grower-finisher chickens a fat utilization similar to using soybean oil. In the starter phase, soybean acid oil improved palm oil digestibility, although it did not achieve the fat digestibility obtained with the use of soybean oil. In the grower-finisher phase, a

synergistic effect was obtained on AME blending 2% palm oil + 4% soybean acid oil. Soybean acid oil added at 6% to a basal diet or a blend between palm oil and soybean acid oil (1:3, w/w), with a dietary UFA:SFA of 2.6 and 40% FFA, could replace soybean oil in grower-finisher diets, without a negative repercussion in fat utilization.

The replacement of palm oil by soybean acid oil or the inclusion of soybean acid oil as a unique fat source to feed broiler chickens provides a potential strategy to use this by-product, improving the absorption of palm oil, and obtaining in grower-finisher chickens a fat utilization similar to using soybean oil.

4.1.8 References

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4.1.9 Supplementary Tables

Table 4.9. **Lipid-class content according to different intestinal segments and excreta in 11- and 35-day-old broiler chickens.**

	Intestinal Segment and Excreta [1]					*p*-Values	
Item	**Upper Jejunum**	**Lower Jejunum**	**Upper Ileum**	**Lower Ileum**	**Excreta**	**SEM**	**P**
11-day-old broiler chickens							
TAG	0.57 ^{a}	0.41 ^{b}	0.29 ^{b}	0.31 ^{b}	0.38 ^{b}	0.039	<0.001
DAG	2.12 ^{a}	0.89 ^{b}	0.65 ^{b}	0.61 ^{b}	0.96 ^{b}	0.103	<0.001
MAG	0.25	0.30	0.26	0.34	0.30	0.025	0.104
FFA	13.46 ^{a}	6.23 ^{b}	5.40 ^{b}	5.22 ^{b}	6.82 ^{b}	0.677	<0.001
35-day-old broiler chickens							
TAG	0.24 ^{a}	0.22 ^{ab}	0.16 ^{bc}	0.11 ^{c}	0.17 ^{bc}	0.016	<0.001
DAG	1.47 ^{a}	0.52 ^{b}	0.19 ^{c}	0.17 ^{c}	0.16 ^{c}	0.042	<0.001
MAG	0.27 ^{a}	0.22 ^{ab}	0.15 ^{c}	0.18 ^{bc}	0.15 ^{c}	0.016	<0.001
FFA	7.96 ^{a}	3.82 ^{b}	1.80 ^{c}	1.63 ^{c}	1.67 ^{c}	0.210	<0.001

[1] Values are pooled means of 30 replicates from chickens fed diets at 11-day-old (n = 150) or 35-day-old (n = 150). TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids. SEM = standard error of the mean. *p*-Values were obtained from univariate ANOVA conducted for each age to study whether the intestinal segment (or excreta) affected the lipid-class content. a-c: means in a row not sharing a common letter are significantly different ($p < 0.05$).

Table 4.10. **Lipid-class content in the lower ileum according to different fat sources in the diet in 11- and 35-day-old broiler chickens.**

Item	Dietary Treatments [1]										*p*-Values									
	11-day-old broiler chickens					35-day-old broiler chickens					P6		P4-SA2		P2-SA4		SA6		S6	
	P6	P4-SA2	P2-SA4	SA6	S6	P6	P4-SA2	P2-SA4	SA6	S6	SEM	P	SEM	P	SEM	P	SEM	P	SEM	P
TAG	0.19	0.30	0.36	0.38	0.32	0.07	0.11	0.15	0.18	0.09	0.021	0.002	0.019	<0.001	0.037	0.002	0.052	0.012	0.020	<0.001
DAG	0.45	0.58	0.72	0.68	0.64	0.17	0.16	0.17	0.22	0.14	0.071	0.021	0.045	<0.001	0.058	<0.001	0.070	<0.001	0.069	<0.001
MAG	0.15	0.35	0.45	0.52	0.23	0.15	0.17	0.18	0.24	0.13	0.019	1.000	0.023	<0.001	0.027	<0.001	0.047	0.002	0.014	<0.001
FFA	8.47	6.83	4.63	3.17	3.02	2.92	1.62	1.33	1.40	0.87	0.574	<0.001	0.298	<0.001	0.278	<0.001	0.208	<0.001	0.252	<0.001

[1]Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids. SEM = standard error of the mean. p-Values were obtained from univariate ANOVA conducted for each dietary treatment to study whether the age affected the lipid-class content (n = 12). $p < 0.05$ was considered significant.

Table 4.11. **Feed apparent metabolizable energy value and apparent fatty acid digestibility coefficients in the lower ileum according to different fat sources in the diet in 11- and 35-day-old broiler chickens.**

Item	Dietary Treatments [1]										*p*-Values									
	11-day-old broiler chickens					35-day-old broiler chickens					P6		P4-SA2		P2-SA4		SA6		S6	
	P6	P4-SA2	P2-SA4	SA6	S6	P6	P4-SA2	P2-SA4	SA6	S6	SEM	P	SEM	P	SEM	P	SEM	P	SEM	P
AME, kcal/kg	3014	3109	3001	3119	3348	3279	3324	3384	3274	3364	31.27	<0.001	26.57	<0.001	20.32	<0.001	24.26	0.001	24.64	0.656
FA digestibility																				
TFA	0.62	0.65	0.72	0.80	0.79	0.84	0.88	0.91	0.91	0.92	0.022	<0.001	0.016	<0.001	0.019	<0.001	0.017	<0.001	0.011	<0.001
SFA	0.49	0.46	0.51	0.55	0.69	0.78	0.85	0.89	0.85	0.90	0.026	<0.001	0.021	<0.001	0.034	<0.001	0.028	<0.001	0.011	<0.001
MUFA	0.74	0.70	0.73	0.75	0.76	0.93	0.92	0.91	0.90	0.93	0.026	<0.001	0.017	<0.001	0.024	<0.001	0.022	<0.001	0.010	<0.001
PUFA	0.75	0.81	0.85	0.90	0.83	0.83	0.88	0.92	0.93	0.93	0.028	0.056	0.013	0.002	0.017	0.014	0.016	0.234	0.011	<0.001

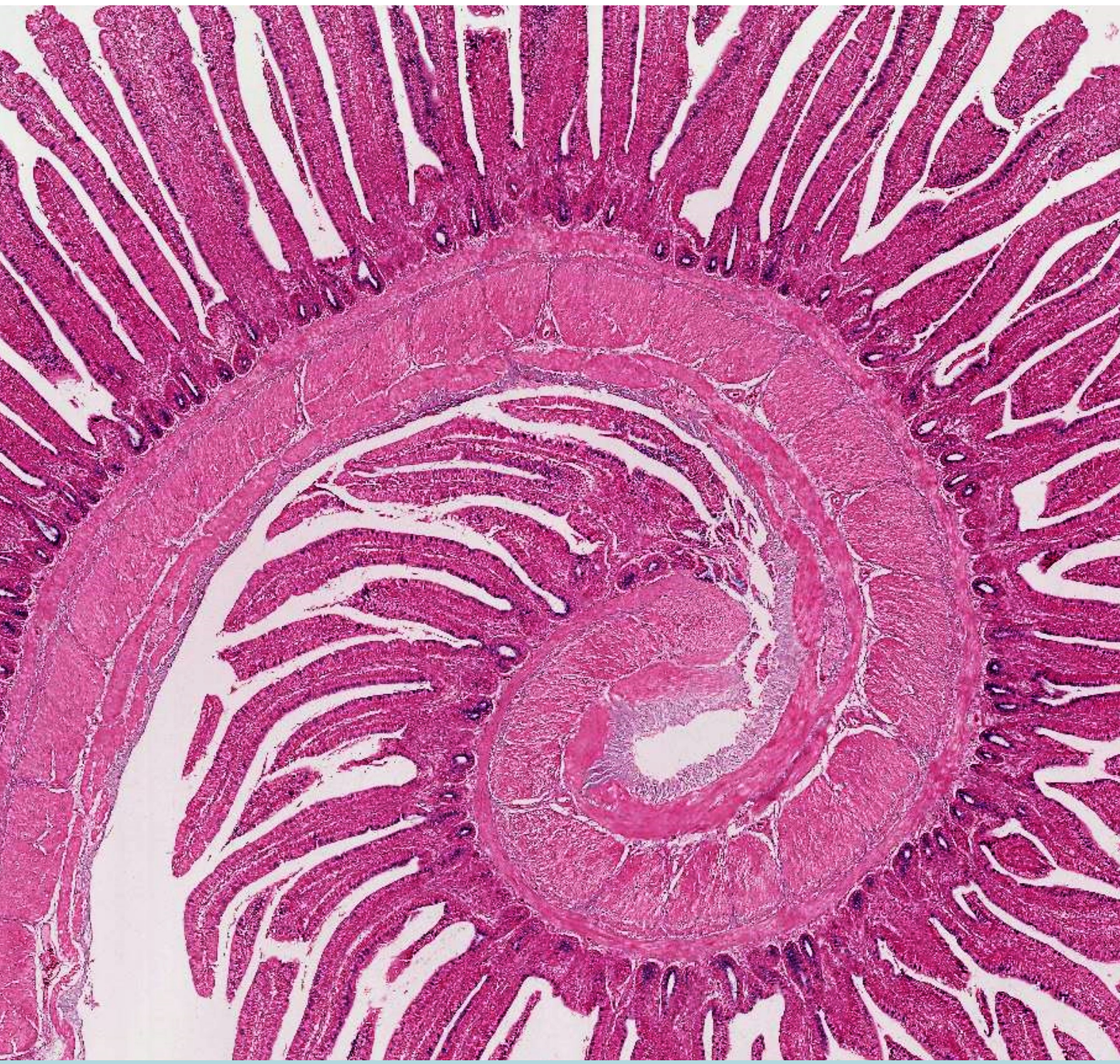
[1]Values are pooled means of 6 replicates from chickens fed diets supplemented with 6% of palm oil (P), soybean acid oil (SA), soybean oil (S), or oil blends with 4% palm oil and 2% soybean acid oil (P4-SA2) or 2% palm oil and 4% soybean acid oil (P2-SA4). AME = apparent metabolizable energy, TFA = 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 univariate ANOVA conducted for each dietary treatment to study whether the age affected the feed AME values, and the FA digestibility results (n = 12). $p < 0.05$ was considered significant.

Table 4.12. **Contribution of each intestinal segment to FA absorption according to the age of the chicken.**

	Age (days) [1]			*p*–Value
Item	**11**	**35**	**SEM**	**Age**
Upper jejunum				
TFA	54.96	49.83	4.198	0.347
Palmitic	34.24	49.36	4.736	0.019
Stearic	-	-	-	-
Oleic	47.89	64.45	3.731	0.002
Linoleic	54.93	44.55	4.999	0.124
Lower jejunum				
TFA	42.50	33.04	4.347	0.117
Palmitic	51.40	34.38	4.286	0.005
Stearic	58.31	59.05	4.160	0.896
Oleic	40.16	24.26	3.448	0.001
Linoleic	41.80	33.51	4.697	0.206
Upper ileum				
TFA	7.03	13.01	2.296	0.064
Palmitic	8.67	11.92	2.277	0.300
Stearic	16.17	27.34	2.880	0.007
Oleic	9.01	8.40	2.248	0.844
Linoleic	6.08	17.09	1.575	<0.001
Lower ileum				
TFA	10.02	4.11	0.720	<0.001
Palmitic	10.76	4.34	1.045	<0.001
Stearic	26.25	9.93	2.859	<0.001
Oleic	9.17	2.89	0.871	<0.001
Linoleic	8.39	4.84	0.790	0.002

[1]Values are means of 30 replicates from chickens fed diets at 11-day-old or 35-day-old. TFA = total fatty acids. SEM = standard error of the mean. p-Values were obtained from univariate ANOVA conducted for each intestinal segment to study whether the age affected the FA absorption (n = 60). $p < 0.05$ was considered significant.

5.GENERAL DISCUSSION



5.General discussion

Research in animal nutrition is essential in order to develop an optimal cost-efficient feed formulation. In recent years, because of the rising cost of conventional fats used in poultry diets and the increasing concern of moving toward circular agroindustry, there is greater interest in searching the use of by-products as alternative fat sources for feed formulation.

From the different diet-related factors, it is clear that the saturation degree of FA plays an important role in the utilization of fats by the animal. In contrast, there is a lack of consensus concerning the effect of the FFA content on FA utilization in broiler chickens. Although the FFA content has been negatively related to FA utilization, last studies using high-quality-controlled AO and FAD have provided promising results regarding their use in modern broiler chicken diets (Rodriguez-Sanchez et al., 2019a, 2021). However, to date, little was known about the use of these by-products blended with conventional fats varying the UFA:SFA and the FFA content.

Due to the need to better understand the complexity of the digestion and absorption process of fats, which implies different physicochemical steps, *in vitro* and *in vivo* studies to go deeper into the study of the hydrolysis and the absorption process of SA and PFAD have been performed. To attain this, *in vitro* studies were carried out to expand the basic knowledge about the lipolysis and the formation of the bioaccessible phase, namely the micellar phase (MP), containing the DMM, using both by-products and their corresponding conventional oils (section 3.1). Then, *in vivo* studies in broiler chickens were conducted, to test the potential strategy of using these by-products blended with conventional oils in young and adult broiler chickens. One, to study the use of PFAD replacing S (section 3.2) and the other to assess the effect of P replacement by SA (section 4.1). To gather a broader understanding of how the different fat combinations are absorbed by the animals, the *in vivo* digestibility studies were performed in different sections of the intestinal tract to determine its contribution to FA absorption. Overall, in

this general discussion, the most relevant findings obtained in this thesis and its implications are provided. Also, its limitations and future considerations are exposed.

5.1. *In vitro* model for lipid digestion assessment

In vitro techniques to provide results of the expected use of fats by broiler chickens should be reliable, reproducible, quick, and cheap (Mota de Carvalho et al., 2021). According to Mateos et al. (2019) fats are the most difficult ingredients to assess their energy content under *in vivo* trials. Moreover, although the results obtained from *in vivo* studies, mainly feed AME values or FA apparent digestibility coefficients (ADC), give practical information, it can be difficult from *in vivo* trials to obtain information about different physicochemical steps which are carried out in the process of digestion and absorption of fats. In this sense, the *in vitro* studies performed in this thesis were conducted to evaluate the potential use of these methods as a starting point to assess new fat ingredients, based on the determination of its hydrolysis and bioaccessibility, and to prove whether these techniques can be useful to expand the knowledge about the physicochemical processes to better understand the results obtained *in vivo*.

Regarding the different sequential steps that take place in the broiler lipid digestion and absorption (Section 1.3.2), Rodriguez-Sanchez et al. (2019a, 2019b) reported that the use of fats, determined under *in vivo* conditions, was more influenced by the absorption process than by the hydrolysis. Thus, both processes were evaluated under *in vitro* conditions, using the same experimental fat sources that previously were tested in *in vivo* conditions (Rodriguez-Sanchez et al., 2019a, 2021).

5.1.1. Hydrolysis

Figure 5.1 shows the hydrolysis as the evolution of the lipid-class composition of experimental oils (S, P, SA, and PFAD) during *in vitro* and *in vivo* digestion. In *in vivo* conditions, the hydrolysis mainly occurs in the duodenum, where the secretion of pancreatic lipase and colipase takes place. However, due to the retrograde movement of digesta (small intestinal flux), some lipolytic products reflux from the duodenum into the

gizzard, and it is in the gizzard where fat digestion starts. So, differences in lipid-class composition between feed and gizzard content could be expected as is reported by Rodriguez-Sanchez et al. (2019b). Therefore, to compare the behavior of the in *vitro* and the in *vivo* lipolysis of the experimental fats, the *in vitro* digestion data was from oils and aliquots taken at 10, 30, and 60 min of digestion. The *in vivo* data was from the lipid-class composition of the feed (represented at time 0), gizzard content (represented at time 10 min since the digestion starts in this segment and some changes can be obtained in lipid classes), and duodenum content (represented at time 60 min, where most of the hydrolysis takes place) of 37-d-old broiler chickens from the study conducted by Rodriguez-Sanchez et al., (2021).

As it can be observed, a similar pattern was obtained in both methodologies, *in vitro* and *in vivo* studies (Figure 5.1). **Regardless of the saturation degree and the FFA content of fats, the results showed that the hydrolysis is not limited**, and all experimental oils are hydrolyzed by the pancreatic lipase. Comparing each oil *in vitro* and *in vivo*, a similar content of each lipid-class was obtained at the end of the process (60 min or duodenum). This suggests that the *in vitro* hydrolysis method could be a good technique to predict the hydrolysis of these fats in broiler chickens. Nevertheless, it is important to note that this *in vitro* method has limitations because it is difficult to mimic the complex physicochemical and physiological processes that occur in the broiler GIT. Also, an important limitation is that the *in vitro* method is a static system that differs from the dynamic system *in vivo*, in which the concentration of lipolytic products from hydrolysis progressively increases but are not removed from de digestion media, and this causes a build-up of lipolytic products in the medium that can block further hydrolysis. In *in vivo* conditions this fact is counteracted by the absorption process. Overall, this *in vitro* method is a rapid, accurate, reproducible, and cheap technique, which may be useful to assess the lipolysis of these fats, being a potential tool to study fat sources as an ingredient for broiler nutrition or to study the release of encapsulated products in lipid matrices.

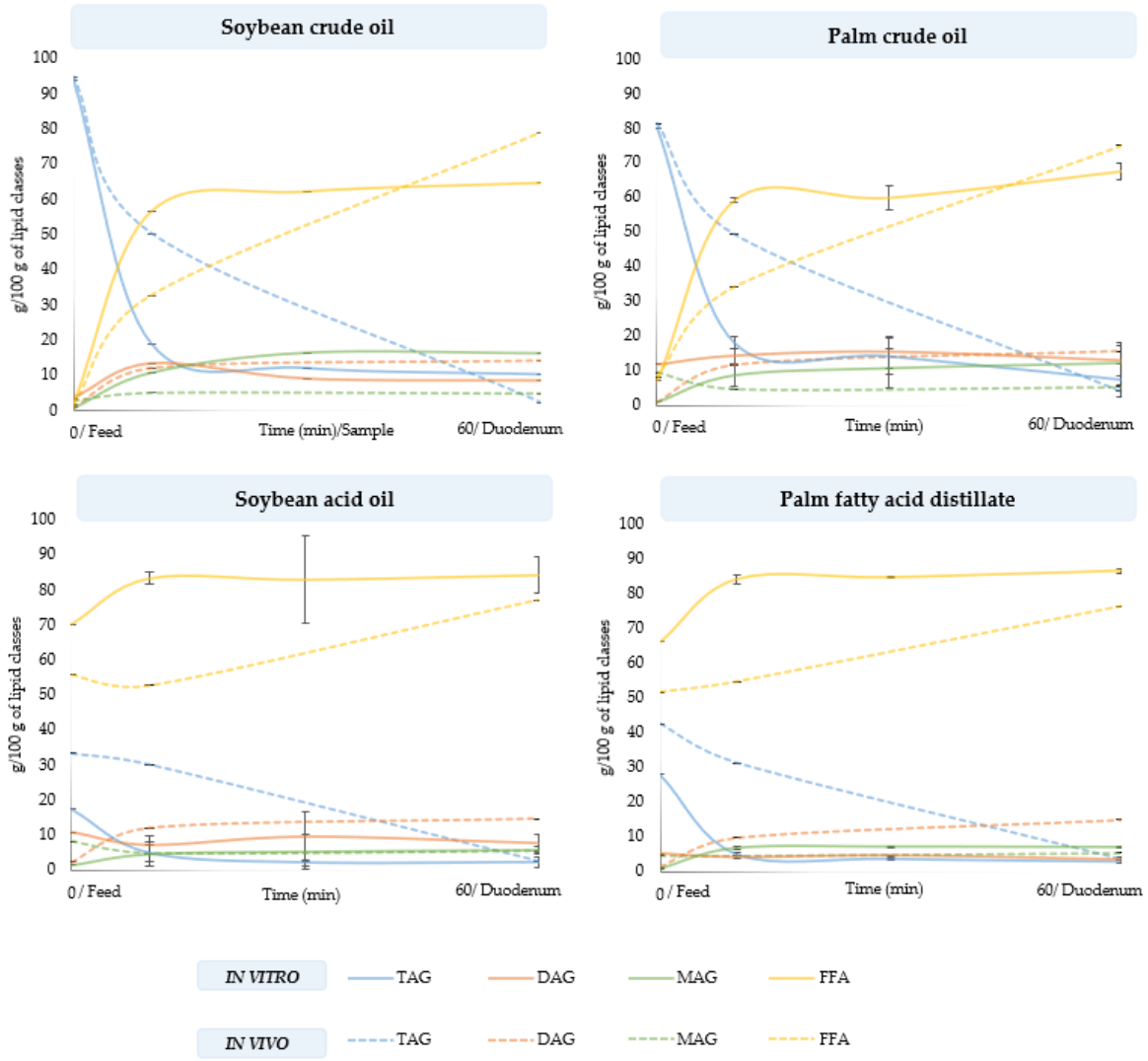


Figure 5.1. Evolution of lipid products (Triacylglycerol(s), TAG; Diacylglycerol(s), DAG; Monoacylglycerol(s), MAG; and Free fatty acid(s), FFA) (g /100g of lipid classes) during *in vitro* and *in vivo* intestinal digestion of experimental oils: soybean crude oil, palm crude oil, soybean acid oil, and palm fatty acid distillate (blend of 66% palm fatty acid distillate + 33% palm crude oil) or feed including 6% of each experimental oil. Lipid classes were identified and quantified by using standards for each lipid-class. Time 0 *in vitro* corresponds to lipid classes determined in oils, and time 10, 30, and 60 *in vitro* corresponds to minutes where samples of in *vitro* digested were collected. *In vivo* data are from the feed and digestive content of 37-day-old broiler chickens (Rodriguez-Sanchez et al., 2021). Value 0 *in vivo* corresponds to lipid classes determined in feed. Time 10 *in vivo* corresponds to lipid classes determined in the gizzard content. Time 60 *in vivo* corresponds to lipid classes determined in the duodenum content. Values of *in vitro* data are means (n = 3), and values of *in vivo* data are means (n = 6).

5.1.2. Absorption

In the *in vivo* studies of fats, the absorption was assessed based on the ADC of FA (Rodriguez-Sanchez et al., 2019a, 2019b). In the *in vitro* study, the bioaccessibility of fats

was evaluated, which is defined as the fraction of a compound that is released from its matrix in the GIT and thus becomes available for intestinal absorption. It includes the digestive transformation of feed into material potentially absorbable by the enterocytes (Fernández-García et al., 2009). In this sense, the bioaccessibility was assessed by the study of the formation of the MP and the precipitated phase (PP) (as the non-bioaccessible phase), and more specifically, by the study of the fraction including the DMM.

Considering the results reported in the *in vitro* study (section 3.1) and comparing with the available data from *in vivo* studies using the same experimental fats to feed broiler chickens at 37 d (Rodriguez-Sanchez et al., 2021), it seems that the assessment of the MP and PP, which is a rapid, easy, and broad method to determine the bioaccessibility, could give a preliminary approach. On the other hand, **the evaluation of the DMM is relatively tedious, is more time-consuming, but provides a more accurate result than studying the MP and PP** to understand the bioaccessibility and consequently the expected absorption in *in vivo* conditions, that can be obtained using these fats.

The results of the DMM suggest that **the bioaccessibility was more influenced by the saturation degree of the fats than by its FFA level,** in agreement with Rodriguez-Sanchez et al. (2021) who reported that the dietary fat saturation degree had a greater impact than the FFA content on FA absorption in 37-d-old broiler chickens. Similarly, the results obtained in this thesis in 35-d-old broiler chickens (sections 3.2 and 4.1), comparing the use of S, SA, P, and PFAD (Figure 5.2), showed an interaction between the saturation degree and the FFA level (p = 0.003); no differences were obtained between unsaturated sources (S and SA) for TFA digestibility coefficients in the lower ileum, being the highest values, whereas between saturated sources, P showed a higher value than PFAD. This suggests that **the saturation degree has a clear impact on FA digestibility and the effect of the FFA level depends on the saturation degree of the lipid source**.

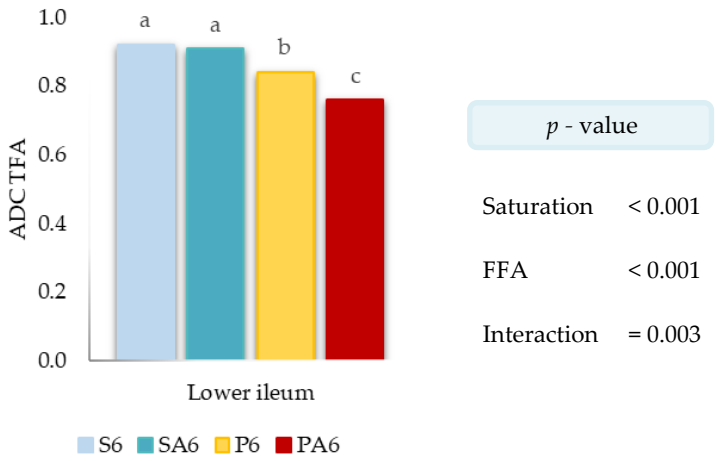


Figure 5.2. Apparent digestibility coefficients (ADC) of TFA determined in the lower ileum according to different diets; with 6% of soybean oil (S6); soybean acid oil (SA6); palm oil (P6); palm fatty acid distillate (PA6), in 35-day-old broiler chickens. Values are pooled means of 6 replicates per each diet with 2 chickens/replicate. *p*-values were obtained from a two-way ANOVA. a-c: columns not sharing a common letter are significantly different ($p < 0.05$) according to Tukey's correction for the multiple comparisons test.

The study of the lipid classes (TAG, DAG, MAG, FFA) in the PP (section 3.1) showed that the non-bioaccessible phase was mainly composed of FFA, and the same result was obtained in the lipid-class composition of the excreta in the *in vivo* studies (sections 3.2 and 4.1).

The lipid-class composition of the DMM phase (section 3.1) suggested that it was related to the available lipid classes in the digestate after digestion (60 min of *in vitro* hydrolysis); crude oils DMM have lower FFA and higher MAG content than acid oils DMM. The composition of the DMM could support the results of the contribution of the upper jejunum to TFA absorption obtained in 35-d-old broiler chickens since the upper jejunum is the first site where the absorption of FA can take place. The proportion of FFA and MAG obtained in the DMM phase of crude oils (about 64 g of FFA and 25 g of MAG / 100 g of lipid classes) might facilitate the absorption of FA, being faster its absorption than the absorption of FA from acid oils, which DMM phase has higher FFA and lower MAG content, mainly in PFAD (95 g of FFA and 3 g of MAG / 100 g of lipid classes). This could, in part, explain the different behavior in the absorption rate obtained in acid oils compared to conventional ones (Figure 5.3). Although the study of the lipid classes of

DMM phase could give complementary information, useful to understand the initial absorption carried out in the upper jejunum, it does not help to explain the results of final fat absorption obtained in the lower ileum. In this sense, the results of total lipids in the DMM, which agree with the fat utilization reported in broiler chickens, could be useful to predict the potential fat absorption expected in the lower ileum.

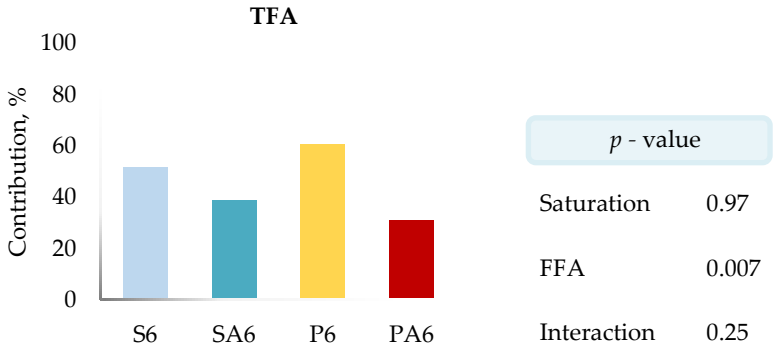


Figure 5.3. Contribution of the upper jejunum to the apparent total fatty acid (TFA) digestibility, calculated as a proportion of total digestibility reached at the lower ileum, along the intestinal tract for the different diets; with 6% of soybean oil (S6); soybean acid oil (SA6); palm oil (P6); palm fatty acid distillate (PA6), in 35-day-old broiler chickens. Values are means of 6 replicates per each diet with 2 chickens/ replicate. *p*-values were obtained from a two-way ANOVA.

In vitro studies are widely used in human research because the study of the processes that occurs during human digestion is technically difficult, costly, and limited by ethical issues (Lucas-González et al., 2018). In contrast, *in vitro* studies for animal nutrition are less common, the study of fat digestion in broiler chickens is easy and quick to perform and the number of animals used is limited. However, according to the ethics of animal testing, nowadays it is a major issue to minimize the use of experimental animals. Thus, the *in vitro* models in the animal nutrition field could gain importance in the next years. Nevertheless, as far as the authors' knowledge, there is still no standardized *in vitro* lipid digestion model that simulates and mimics the complete processes that take place during fat digestion, absorption, and cecal fermentation. Assuming the limitations of the *in vitro* model, especially the fact that is a static technique, it cannot replace *in vivo* studies. However, the *in vitro* model will enable us to obtain results that may provide valuable information in terms of the expected results that can be obtained *in vivo* and can be useful

to understand the physicochemical processes, which could explain *in vivo* data. In summary, ***in vitro*** **methodology can be a complementary technique, used in pre-experimental screening assays, for example, when the number of experimental fats is elevated, and this would involve a high number of animals, trials, time, and economic cost**.

5.1.3. Future considerations

Looking forward, future research should be conducted to establish optimized and standardized *in vitro* models mimicking the chicken GIT conditions; perform the *in vitro* digestion using bile salts of avian origin, simulate a dynamic model of chicken GIT (taken into account the variations in the environment of the different compartments), using a food matrix, and simulates the physical, chemical and microbiological conditions as realistically as possible. Furthermore, it would be interesting to study the DMM fraction and its lipid-class composition isolated from digestive content of broiler chickens.

Another interesting point could be to investigate the particle physicochemical characteristics of lipid emulsions, as the lipid type (FA profile) could influence the characteristics of lipid emulsion droplets, which in turn could affect the lipid digestion kinetics and the subsequent FA incorporation into DMM (Zhang et al., 2015; Salvia-Trujillo et al., 2017).

Moreover, the absorption process could be also assessed by *in vitro* studies using Caco-2 cells (Gil-Ramírez et al., 2014). It would be interesting to perform an *in vitro* study using the same fat blends as those tested in the *in vivo* studies (S+PFAD and P+SA), and apply the DMM fraction obtained from the digestion to Caco-2 cells monolayers to measure the transport of FFA and MAG through them. In addition, another interesting parameter is to evaluate the effect of these blends on epithelial barrier function in epithelial Caco-2 cells since interesting preliminary results were obtained when SA and PFAD were evaluated (Martín Venegas et al., 2019).

5.2 The effect of replacing conventional oils by soybean acid oil or palm fatty acid distillate on dietary fat utilization

As explained in the general introduction (section 1.4), the last contributions using SA and PFAD blended with their corresponding crude oil in broiler chicken diets, reported promising results (Rodríguez-Sánchez, 2018). The present thesis provides new insights into the understanding of the potential use of these by-products by blending them with conventional oils, varying the dietary UFA:SFA and the dietary FFA level.

Data reported in both *in vivo* studies (section 3.2 and 4.1) confirmed that **the use of these by-products depends on the age of the chickens**; the studies corroborated that **the hydrolysis and the absorption of fats are less efficient in starter birds compared to grower-finisher chickens**.

The present data proved that **the lower utilization of SA and PFAD by chickens is mainly due to limitations in the absorption process**, whereas the hydrolysis of these fats seems not to be a limiting factor that explains their use.

The limitations in the absorption process of the different combinations of P+SA and S+PFAD at both ages were mainly related to the absorption of SFA. The maximum FA ADC were obtained in the lower ileum, as it is the last segment where the absorption can take place. Therefore, the following discussion is focused on the results of the ADC of SFA obtained in the lower ileum in both starter and grower-finisher broiler chickens.

5.2.1. Fatty acid absorption in starter chicks

The studies conducted in 11-day-old broiler chickens confirmed that **young birds are more affected by the lower dietary UFA:SFA and the higher dietary FFA content than broilers at 35d.**

The representation of the regression analysis performed from the data of SFA ADC calculated at lower ileum level is presented in Figure 5.4. In S-PFAD diets, the best-fit regression equation for SFA ADC was quadratic, according to the dietary UFA:SFA (p = 0.001) and the dietary FFA% (p = 0.04). Decreasing the dietary UFA:SFA and increasing

the dietary FFA% led to a decrease in the SFA absorption. However, similar SFA absorption was obtained in chickens fed blends S4-PA2 (2.6 UFA; 33 FFA%) and S2-PA4 (1.7 UFA:SFA; 53 FFA%), regardless the differences in the UFA:SFA and the FFA%.

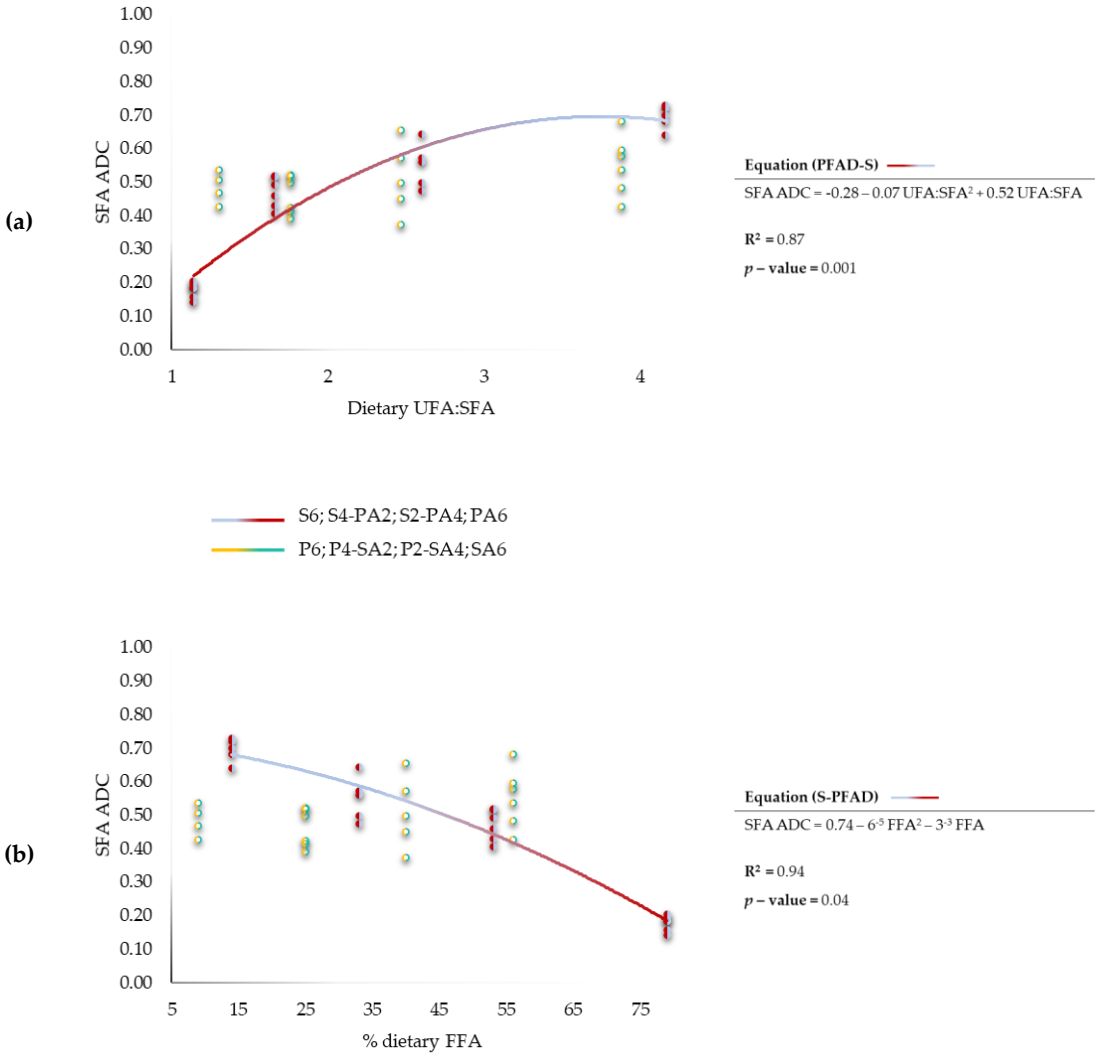


Figure 5.4. Apparent digestibility coefficients (ADC) of saturated fatty acids (SFA) calculated in the lower ileum in 11-day-old broiler chickens, according to (a) increasing the dietary unsaturated to saturated ratio (UFA:SFA) and to (b) increasing the dietary free fatty acid (FFA) %, achieved by blends of palm fatty acid distillate (PFAD) with soybean oil (S), or soybean acid oil (SA) with palm oil (P). Each point represents each replicate value (with 12 chickens/replicate).

In the case of P-SA diets, the SFA ADC did not fit any model. The addition of SA in replacement of P led to an increase in both the UFA:SFA and the FFA% of the diet, which

did not modify the SFA absorption; all combinations between P and SA showed similar SFA digestibility coefficients than those obtained in chicks fed both S-PFAD blend diets.

These results suggest that in starter chicks increasing at the same time the saturation degree and the FFA level of the diet cause a decrease in the SFA absorption, whereas increasing the FFA level, with an increase in the unsaturation degree of the diet does not cause an impairment in the absorption of SFA.

5.2.2. Fatty acid absorption in grower-finisher chickens

The improvement in the fat absorption capacity related to the age of the bird was reflected in the positive results obtained in both studies (sections 3.2 and 4.1) using PFAD and SA blended with S and P, respectively, in 35-d-old broiler chickens. The data evidenced that chickens at 35 d are more capable to absorb diets with lower UFA:SFA and higher FFA%.

Figure 5.5 shows how decreasing the dietary UFA:SFA and increasing the dietary FFA% (achieved by the addition of PFAD to S), decreased progressively the absorption of SFA when the UFA:SFA was lower than 2.6 and the FFA% exceeded 30%. However, when both factors (UFA:SFA and FFA%) increased at the same time (replacing P by increasing levels of SA), the SFA absorption, improved, obtaining similar ADC in chickens fed blends or SA6 (ranging UFA:SFA from 1.8 to 3.9, and the FFA% from 25% to 56%). Those values were in turn similar than those obtained in chickens fed S6 and S4-PA2.

As can be observed, both fat blends (S4-PA2 and P2-SA4) that had 2.6 UFA:SFA (Figure 5.5.a) reached the same SFA absorption, regardless of the difference in the FFA% (Figure 5.5.b; S4-PA2: 30% *vs.* P2-SA4: 41%). This points out that **chickens could better absorb FFA coming from the unsaturated acid oil than from the saturated by-product**.

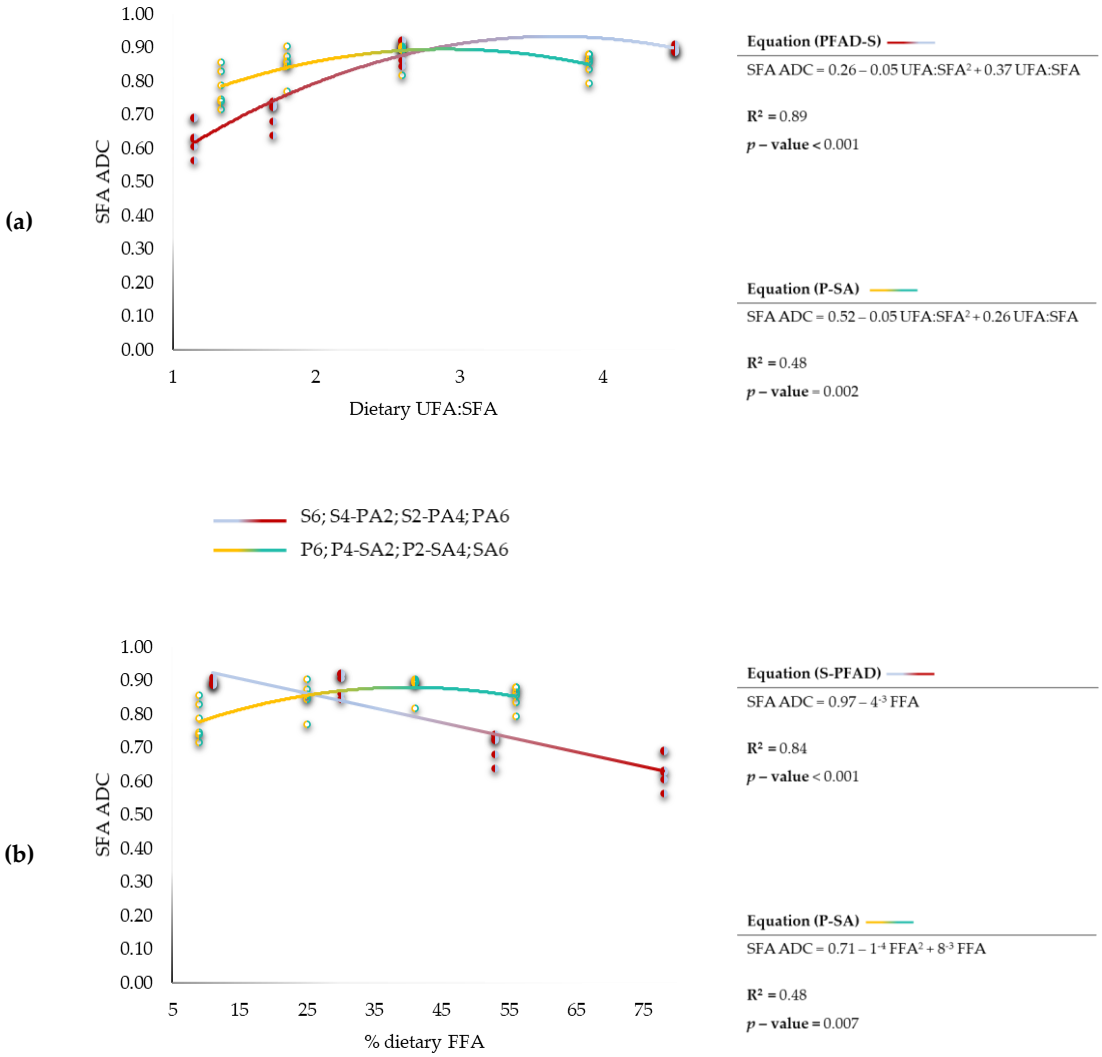


Figure 5.5. Apparent digestibility coefficients (ADC) of saturated fatty acids (SFA) calculated in the lower ileum in 35-day-old broiler chickens, according to (a) increasing the dietary unsaturated to saturated ratio (UFA:SFA) and to (b) increasing the dietary free fatty acid (FFA) %, achieved by blends of palm fatty acid distillate (PFAD) with soybean oil (S), or soybean acid oil (SA) with palm oil (P). Each point represents each replicate value (with 2 chickens/replicate).

Thus, to predict the absorption of different combinations between conventional oils and by-products rich in FFA it should be considered the age of the bird, the dietary UFA:SFA, the dietary FFA%, and also the origin of the by-product which determines the FA profile of the FFA.

One of the characteristics of these by-products is their high variability in their composition, which implies not only their FA composition, but also the presence of other compounds from the refining process. So, the PFAD and the SA used in this thesis were

previously characterized. For instance, the determination of the MIU compounds is advisable since their content could have a diluent effect on the energy content of the fat source (Varona et al., 2021). The analysis of MIU compounds in SA and PFAD (section 3.2 and 4.1) showed that the experimental by-products met those recommended by FEDNA's (Fundación Española para el Desarrollo de la Nutrición Animal) guidelines (5 g/100g) (FEDNA, 2002). Therefore, the results obtained in this thesis are mainly attributable to the UFA:SFA and the FFA content of the diets.

5.2.3. Contribution of intestinal segments to FA absorption

The study along the intestinal tract, dividing the jejunum and the ileum into upper and lower sections allowed us to better understand the dynamics of fat digestion. From the different studies, results corroborated that jejunum is the main site of fat absorption (> 73%) in both 11 and 35-d-old broiler chickens.

Regarding the effect of the diets on the TFA absorption rate, at 11 d the replacement of S by PFAD caused a delay in the TFA absorption (Figure 5.6.a), which could explain the lower fat digestibility obtained in the lower ileum as the dietary UFA:SFA decreased and the FFA level increased. In contrast, the addition of SA to replace P, increasing both the UFA:SFA and the FFA level of the diet, did not have a repercussion on the dynamics of fat absorption (Figure 5.6.b).

In grower-broiler chickens, no effect was observed in the TFA absorption rate related to the replacement of S by PFAD (Figure 5.7.a), neither with the addition of SA in substitution of P (Figure 5.7.b). Thus, the negative effect of increasing the saturation degree and the FFA content of the diet on the dynamics of fat absorption described in starter chicks disappeared in grower chickens. This suggests that the higher contribution of the upper jejunum to FA absorption of diets with higher SFA and FFA content, could in part explain the improvement observed in the fat digestibility obtained at 35 d compared to 11 d, in chickens fed diets containing PFAD.

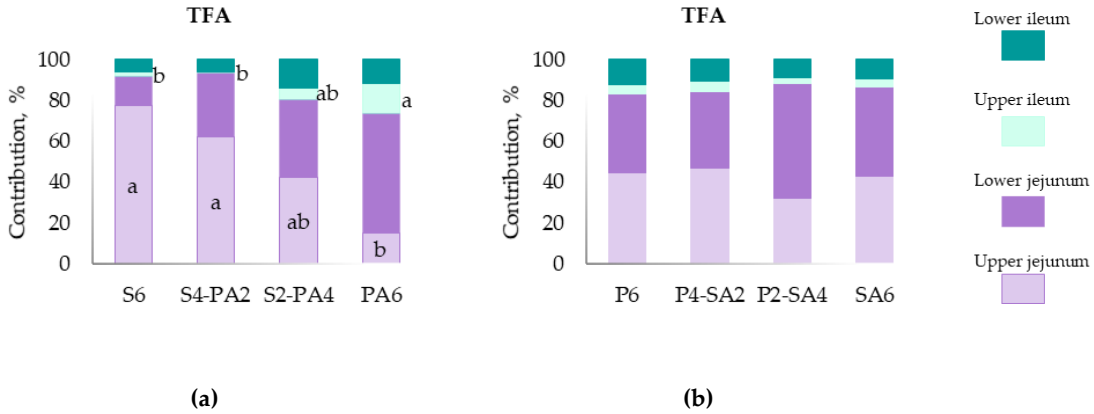


Figure 5.6. Contribution of each intestinal segment to the apparent TFA digestibility, calculated as a proportion of total digestibility reached at the lower ileum, along the intestinal tract in 11-day-old broiler chickens fed (a) S-PA diets and (b) P-SA diets. Values are means of 6 replicates per each diet with 12 chickens/replicate. a-b: columns with the same intestinal segment not sharing a common letter are significantly different ($p < 0.05$).

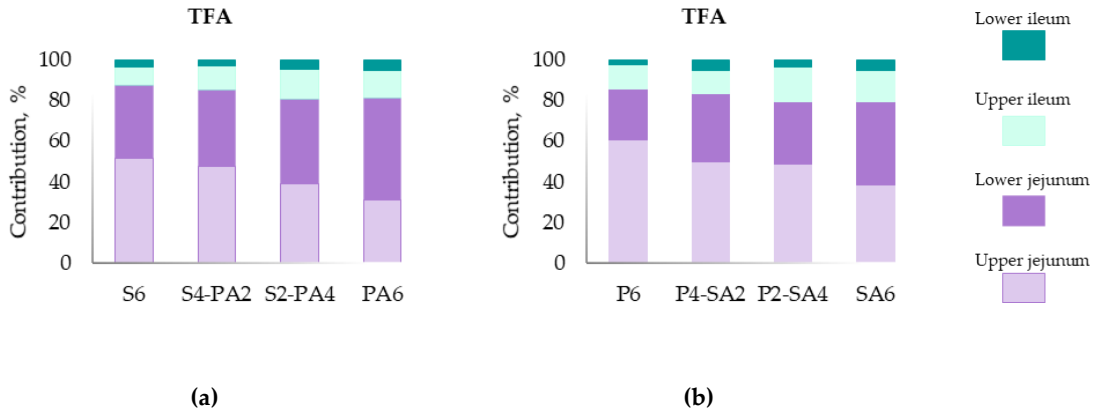


Figure 5.7. Contribution of each intestinal segment to the apparent TFA digestibility, calculated as a proportion of total digestibility reached at the lower ileum, along the intestinal tract in 35-day-old broiler chickens fed (a) S-PA diets and (b) P-SA. Values are means of 6 replicates per each diet with 2 chickens/replicate.

Considering the results of ADC of SFA obtained in the upper jejunum (Figure 5.8), it is notable that the pattern of the digestibility coefficients obtained in this segment was quite different, and had higher variability among replicas, compared with the pattern of those obtained in the rest of the intestinal tract. In fact, no absorption was reported for stearic acid at this level since negative ADC of stearic acid were calculated in the upper jejunum, which is in accordance with the negative ADC for stearic acid of S calculated in the upper jejunum by Tancharoenrat et al. (2014).

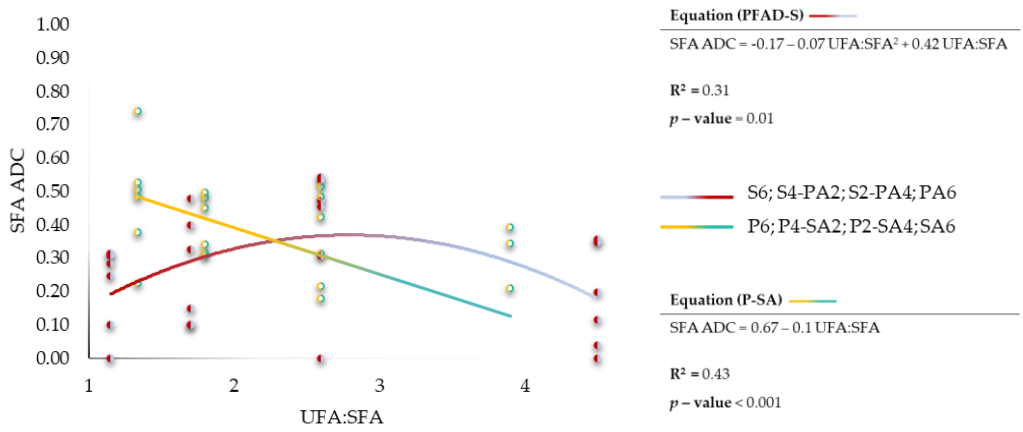


Figure 5.8. Apparent digestibility coefficients (ADC) of saturated fatty acids (SFA) calculated in the upper jejunum in 35-day-old broiler chickens, according to increasing the dietary unsaturated to saturated ratio (UFA:SFA), achieved by blends of palm fatty acid distillate (PFAD) with soybean oil (S), or soybean acid oil (SA) with palm oil (P). Each point represents each replicate value (with 2 chickens/replicate).

The results obtained at this level may be influenced by the net lipid secretion that occurred at the duodenum level (Hurwitz et al., 1973). This has been related to the FA composition of bile (mainly linoleic, stearic, palmitic, arachidonic, and oleic acids) that has been reported to be the main source of lipid net secretion (Tancharoenrat et al., 2014). In fact, Tancharoenrat et al. (2014) and Rodriguez-Sanchez et al. (2019b) reported a negative ADC of fat in the duodenum, which was higher for unsaturated sources than for saturated ones. It is noteworthy that the determination of FA digestibility with total jejunum content without the partition into two segments (upper and lower) could underestimate the results. Therefore, **for further studies might be recommendable the determination of the FA digestibility from the lower jejunum on**.

Dividing the ileum into the upper and lower segments proved that the **upper ileum was crucial for fat absorption in grower-finishers chickens**, and its implications could be associated with the improvement in fat absorption observed as age increases. Moreover, the maximum values of fat ADC obtained in the lower ileum confirmed that for future studies the content of this segment might be sampled instead of excreta, to obtain more accurate results, without the possible interference of the FA production from the cecal microbiota.

5.2.4. Future considerations

This thesis was conducted in order to obtain information regarding the digestion and the absorption process of FA and lipid classes. So, *in vitro* (section 3.1) and *in vivo* studies (sections 3.2 and 4.1) were performed. In *in vivo* studies, the performance parameters recorded indicated that the study was successfully carried out, but no significant effect was observed regarding the use of the different diets. In this thesis, the number of chickens used in the trials and the experimental facilities (metabolic cages) was probably limited to obtain significant results regarding the effect of the diets. Therefore, it would be interesting to perform an *in vivo* trial with a higher number of birds, allocated in pens instead of cages, which is more similar to commercial conditions, to obtain valuable information regarding the potential effect of adding these by-products on performance parameters. Furthermore, several questions may be clarified in future experiments; the effect of these by-products on the intestinal morphology and on the microbiota remains unanswered. The lower absorption of PFAD and SA in starter chicks and the improvement in their utilization in grower birds could be observed in changes in the morphology of the intestinal epithelium, as it has been reported that an increase in villus height may be a consequence of an increasing need for digestive capacity (Svihus, 2014). This is being carried out in parallel to this thesis and the preliminary results in 35-d-old broiler chickens point out that the level of dietary FFA increased the length of the villi, whereas did not affect the number of goblet cells. On the other hand, the saturation degree of the diet affected both the morphometry of the intestinal epithelium and the number of goblet cells (Jimenez-Moya et al., 2019).

Another interesting point would be to study the effect of SA and PFAD on the final lipid composition and quality of meat. In addition, due to the different feed forms that can be used to feed broiler chickens, the study of the effect of the manufacturing process and conditions of storage on lipid hydrolysis in feed containing these free fatty acid -rich oils could provide important recommendations regarding the feed quality and shelf live (Varona et al., 2018, 2019). All these studies are part of the global project, and the results derived from them together with the results from this thesis will provide valuable

information regarding the potential use of these by-products to feed broiler chickens from farm to fork.

Finally, in order to improve the use of these by-products, mainly in starter chicks, future studies could examine the potential strategy of the addition of cost-effective emulsifiers, such as soybean lecithin, a co-product from the degumming of soybean crude oil during the refining process, from which interesting results have been obtained in broiler chickens (Viñado, 2019).

Altogether, this thesis provides further information in understanding the physiological limitations in the use of these by-products by broiler chickens. This work gives valuable data about the potential strategy to use SA and PFAD to feed broiler chickens, and the importance of the age of the bird to make the recommendations. Although several recommendations for future research are given, the results obtained in this thesis will help to increase animal nutritionist's knowledge of the use of these by-products, increasing its confidence in them, being a way to move toward a circular agroindustry.

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6.FINAL CONCLUSIONS

6.Final Conclusions

From the results obtained in this dissertation, by using *in vitro* conditions and *in vivo* trials with broiler chickens fed diets with 6 % of experimental fat sources, the following conclusions can be drawn:

1. The *in vitro* study supports the findings obtained in *in vivo* studies; the absorption process is a more limiting step than hydrolysis. The assessment of the bioaccessibility corroborates that the free fatty acid level of dietary fat influences fat utilization less than its saturation degree.

2. The study of the evolution of the lipid-class content and apparent fatty acid digestibility throughout the intestinal tract is essential to understand the utilization of different fat sources in broilers, being the lower ileum the most important segment to evaluate fat utilization.

3. As the saturation degree and the free fatty acid content of the diet increase, the absorption of the dietary fatty acids is reduced and delayed in **starter broiler chicks.**

4. As the age of the chicken increases, the efficiency of both hydrolysis and absorption improves. There is an improvement in the absorption of saturated and free fatty acids in the jejunum, with an increase in the contribution of the upper ileum to fatty acid absorption.

5. Palm fatty acid distillate blended with soybean oil represents a suitable alternative fat source for grower-finisher diets without impairing fatty acid utilization, as long as the blend has at least 2.6 UFA:SFA ratio and the FFA content does not exceed 30%.

6. Replacing palm oil with increasing levels of soybean acid oil improves dietary fat utilization in **starter broiler chickens**, although fatty acid digestibility levels do not reach those of soybean oil.

7. The replacement of palm oil with increasing levels of soybean acid oil improves dietary fat utilization in **grower-finisher broiler chickens** with a fat digestibility similar to soybean oil.

8. The absorption of dietary free fatty acids is higher when the free fatty acids come from unsaturated rather than saturated fat sources.