Universitat Autònoma de Barcelona

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Gerard Verge Mèrida

PhD THESIS *2022*

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Doctoral Thesis

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A thesis presented for the degree of Doctor of Philosophy in Animal Production

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FACULTAT DE VETERINÀRIA

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Certifiquen:

Que la memòria titulada "**Strategies for the inclusion of olive pomace oil and acid oil in monogastric animal diets**", presentada per Gerard Verge Mèrida amb la finalitat d'optar al grau de Doctor en Veterinària, ha estat realitzada sota la seva direcció i, considerant-la acabada, autoritzen la seva presentació per a que sigui jutjada per la comissió corresponent.

I, perquè consti, a efectes oportuns, signen la present a Bellaterra, 1 de setembre de 2022,

Dr. David Solà Oriol

Dra. Roser Sala Pallarès

Dra. Ana Cristina Barroeta Lajusticia

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"If I were to lose you, I'd surely lose myself"

Joel Miller, The Last of Us.

Resum

La inclusió de greixos i olis en l'alimentació d'animals monogàstrics és una pràctica molt extesa degut al seu alt contingut energètic i a l'aport d'àcids grassos (**AG**) essencials. No obstant, la necessitat d'una producció animal més eficient i sostenible exigeix una cerca contínua de fonts de greix alternatives i competitives. En aquest sentit, l'oli de pinyolada d'oliva i l'oli àcid de pinyolada d'oliva son dos fonts de greix amb gran potencial per a ser incloses en les dietes d'animals monogàstrics, que poden ajudar a desenvolupar una economia circular y contribuir a una producció animal més eficient i sostenible. A més, l'ús d'oli àcid de pinyolada d'oliva podria ajudar a reduir els costs d'alimentació, ja que sol tenir un preu més competitiu. Ambdós olis són rics en AG monoinsaturats (**AGMI**) i àcid oleic (C18:1 n-9), però difereixen en l'estructura molecular. L'oli de partida és ric en triglicèrids, mentre que l'oli àcid, derivat de la seva refinació química, és ric en AG lliures (**AGL**) i tendeix a acumular un major contingut d'humitat, impureses i matèria insaponificable (**MIU**), el que pot perjudicar la seva utilització per part de l'animal. L'objectiu global de la present tesi doctoral va ser investigar l'ús potencial de l'oli de pinyolada d'oliva i de l'oli àcid de pinyolada d'oliva en les dietes per a porcs, pollastres de carn i llobarro, focalitzant en els efectes sobre el rendiment productiu, l'eficiència alimentària, la digestibilitat, els paràmetres de la canal, el dipòsit de greix i el perfil d'AG del greix de dipòsit i dels productes carnis finals.

Al primer estudi (**Capítol 3**), es van incloure l'oli de pinyolada d'oliva i l'oli àcid al 5% (en matèria fresca) a les dietes de 224 porcs mascles i femelles d'engreix ([Landrace x Large White] x Duroc), de 58 a 130 kg de pes viu (**PV**), i es van comparar amb l'oli de palma, una font de greix convencional. A més, es va utilitzar una dieta que incloïa una barreja d'oli de palma amb oli de pinyolada d'oliva (proporció 1:1). Els resultats van mostrar que l'oli de pinyolada d'oliva no afectava el rendiment, l'eficiència d'utilització de l'aliment o la digestibilitat, però sí que millorava la deposició de greix intramuscular del llom (+24% en comparació amb la dieta d'oli de palma). D'altra banda, l'oli àcid de pinyolada d'oliva va mostrar un millor rendiment quan es va barrejar amb l'oli de palma que quan es va incloure sol, encara que la digestibilitat no es va veure afectada en cap dels dos casos, fet que revela la importància del grau de insaturació i del valor MIU de la font de greix. Quan es va substituir l'oli de palma per l'oli de pinyolada o l'oli

àcid, es va modificar el perfil d'AG de la carn de llom, reduint el contingut d'AG saturats (**AGS**) i augmentant els AGMI.

Al segon assaig (**Capítol 4**) es va alimentar 3.048 pollastres de carn (Ross 308) amb una de les 3 dietes experimentals que incloïen un 6% d'oli de pinyolada d'oliva (O), oli àcid de pinyolada d'oliva (OA) o oli de palma (PO) des dels 22 fins als 39 dies d'edat. Els pollastres que van consumir el pinso amb oli de pinyolada d'oliva van obtenir els millors rendiments i índex de transformació de l'aliment (O: 1,622 vs PO: 1,668 i OA: 1,673; $P < 0.05$), assolint alts valors de digestibilitat dels AG totals (95,75%). D'altra banda, la inclusió d'oli àcid de pinyolada d'oliva en comparació amb l'oli de palma, no va modificar el rendiment ni l'eficiència d'utilització de l'aliment i va millorar la digestibilitat dels AG totals (OA: 93,88% vs PO: 85,92%; $P < 0.001$), el que suggereix que es una bona font de greix alternativa a l'oli de palma per a les dietes de pollastres de carn. Igual que en el cas dels porcs, quan l'oli de palma es va substituir per l'oli o l'oli àcid de pinyolada d'oliva, es van obtenir uns pits de pollastre més saludables, enriquits amb AGMI i menor contingut en AGS. Tot i això, malgrat tenir un perfil d'AG similar, l'oli àcid de pinyolada d'oliva va donar lloc a pitjors índex de transformació de l'aliment i a menors valors de digestibilitat dels AGS (reducció del 5%) que l'oli de pinyolada d'oliva, fet que indica l'efecte negatiu de la presència d'alts nivells d'AGL i/o MIU.

El tercer assaig (**Capítol 5**) es va dur a terme en 480 llobarros (101 g de pes corporal inicial), que van ser alimentats durant 100 dies amb una de les vuit dietes experimentals que contenien un 15,4% de greix afegit, consistent en un 25% d'oli de peix i un 75% de diferents fonts de greix: oli de peix, oli de soja, oli de pinyolada d'oliva, oli àcid de soja i gira-sol, oli àcid de pinyolada oliva i 3 barreges (proporció 1:1) que contenien dos dels anteriors olis. Els resultats van diferir segons l'origen botànic i el contingut en AGL dels olis experimentals. L'oli de soja i el de pinyolada d'oliva, rics en triglicèrids, van donar lloc a uns rendiments productius similars als obtinguts amb l'oli de peix i van assolir alts valors de digestibilitat (93,93% i 93,79% per a la digestibilitat dels AG totals, respectivament). Tot i això, els peixos alimentats exclusivament amb oli àcid de pinyolada d'oliva van mostrar un pitjor rendiment (un 8,6% menys de PV final), fet que no es va observar quan es va utilitzar oli àcid de soja i gira-sol o barreges malgrat un contingut d'AGL i un grau de saturació similar, el que suggereix la importància del de MIU del greix. D'altra banda, la inclusió d'olis àcids a la dieta va donar lloc a un efecte negatiu sobre la digestibilitat dels lípids i els AGS (un 3% i un 5% de reducció, respectivament).

De forma global, aquests resultats suggereixen que, quan s'utilitzen olis àcids, no només s'ha de tenir en compte el contingut d'AGL sinó també el contingut a MIU, ja que una correcta caracterització de la font de greix afegida és clau per a una òptima utilització en la formulació de les dietes. Tant l'oli de pinyolada d'oliva com l'oli àcid de pinyolada d'oliva son dues fonts de greix alternatiu adequades per a la seva incorporació en les dietes d'animals monogàstrics, essent necessàries diferents estratègies d'inclusió en funció de l'espècie i de les característiques de qualitat i composició de l'oli utilitzat.

Resumen

La inclusión de grasas y aceites en la alimentación de los animales monogástricos es una práctica muy extendida debido a su alto contenido energético y al aporte de ácidos grasos (**AG**) esenciales. Sin embargo, la necesidad de una producción animal más eficiente y sostenible exige una búsqueda continua de fuentes de grasa alternativas y competitivas. En este sentido, el aceite de orujo de oliva y el aceite ácido de orujo de oliva son dos fuentes de grasa con gran potencial para su inclusión en las dietas de animales monogástricos, que pueden ayudar a desarrollar una economía circular y contribuir a una producción más eficiente y sostenible. Además, el uso de aceite ácido de orujo de oliva podría ayudar a reducir los costes de alimentación, ya que suele tener un precio competitivo. Ambos son ricos en AG monoinsaturados (**AGMI**) y ácido oleico (C18:1 n-9), pero difieren en la estructura molecular. El aceite de partida es rico en triglicéridos, mientras que el aceite ácido, derivado de su refinación química, es rico en AG libres (**AGL**) y tiende a acumular mayor humedad, impurezas y contenido insaponificable (**MIU**), lo que puede perjudicar su utilización por el animal. El objetivo global de la presente tesis doctoral fue investigar el uso potencial del aceite de orujo de oliva y del aceite ácido de orujo de oliva en dietas para cerdos, pollos de carne y lubinas, centrándose en los efectos sobre el rendimiento, la eficiencia alimentaria, la digestibilidad, los parámetros de la canal, la deposición de grasa y el perfil de AG de la grasa de depósito y los productos cárnicos finales.

En el primer estudio (**Capítulo 3**) se incluyeron el aceite de orujo de oliva y el aceite ácido al 5% (en materia fresca) en las dietas de 224 cerdos machos y hembras de engorde ([Landrace x Large White] x Duroc) de 58 a 130 kg de peso vivo (**PV**) y se compararon con el aceite de palma, una fuente de grasa convencional. Además, se utilizó una dieta que incluía una mezcla de aceite de palma con aceite de orujo de oliva (proporción 1:1). Los resultados mostraron que el aceite de orujo de oliva no afectó al rendimiento, la eficiencia de utilización del alimento o la digestibilidad, pero sí mejoró la deposición de grasa intramuscular del lomo (+24% en comparación con la dieta de aceite de palma). Por otra parte, el aceite ácido de orujo de oliva mostró un mejor rendimiento cuando se mezcló con el aceite de palma que cuando se incluyó solo, aunque la digestibilidad no se vio afectada en ninguno de los dos casos, lo que revela la importancia del grado de insaturación y el contenido de MIU de la fuente de grasa. Cuando se

sustituyó el aceite de palma por aceite o aceite ácido de orujo de oliva, se modificó el perfil de AG del lomo, reduciéndose el contenido de AG saturados (**AGS**) y aumentándose el de AGMI en comparación con la dieta de aceite de palma.

En el segundo ensayo (**Capítulo 4**) se alimentaron a 3.048 pollos de carne (Ross 308) con una de las 3 dietas experimentales que incluían un 6% de aceite de orujo de oliva (O), aceite ácido de orujo de oliva (OA) o aceite de palma (PO), desde los 22 hasta los 39 días de edad. Los pollos que consumieron el pienso con aceite de orujo de oliva obtuvieron los mejores rendimientos e índices de transformación del alimento (O 1,622 vs PO: 1,668 y OA: 1,673; $P < 0.05$), alcanzando altos valores de digestibilidad de los AG totales (95,75%). Por otro lado, la inclusión de aceite ácido de orujo de oliva en comparación con el aceite de palma, no modificó el rendimiento ni la eficiencia de utilización del alimento, pero mejoró la digestibilidad de los AG totales (OA: 93,88% vs PO: 85,92%; $P < 0.001$), lo que sugiere que es una buena fuente de grasa alternativa al aceite de palma para las dietas de pollos de carne. Al igual que en el caso de los cerdos, cuando el aceite de palma se sustituyó por el aceite o aceite ácido de orujo de oliva, se obtuvieron pechugas de pollo más saludables, enriquecidas con AGMI y con menor contenido en AGS. Sin embargo, a pesar de tener un perfil de AG similar, el aceite ácido de orujo de oliva dio lugar a peores índices de transformación del alimento y menores valores de digestibilidad de los AGS (reducción del 5%) que el aceite de orujo de oliva, lo que indica el efecto negativo de la presencia de altos niveles de AGL y/o MIU.

El tercer ensayo (**Capítulo 5**) se llevó a cabo con 480 lubinas (101 g de peso corporal inicial), que fueron alimentadas durante 100 días con una de las ocho dietas experimentales que contenían un 15,4% de grasa añadida, consistente en un 25% de aceite de pescado y un 75% de diferentes fuentes de grasa: aceite de pescado, aceite de soja, aceite de orujo de oliva, aceite ácido de soja-girasol, aceite ácido de orujo de oliva y 3 mezclas (proporción 1:1) que contenían dos de estos aceites. Los resultados difirieron según el origen botánico y el contenido en AGL de los aceites experimentales. El aceite de soja y el aceite de orujo de oliva, ricos en triglicéridos, dieron lugar a unos rendimientos productivos similares a los obtenidos con el aceite de pescado y alcanzaron altos valores de digestibilidad (93,93% y 93,79% para la digestibilidad de los AG totales, respectivamente). Sin embargo, los peces alimentados exclusivamente con aceite ácido de orujo de oliva mostraron un peor rendimiento (un 8,6% menos de peso corporal final), aunque esta disminución no se observó cuando se utilizó aceite ácido de soja-girasol o mezclas,

con un contenido de AGL y grado de saturación similar, lo que sugiere la importancia del contenido de MIU de las grasas. Por otra parte, la inclusión de aceites ácidos en la dieta dio lugar a un efecto negativo sobre la digestibilidad de los lípidos y los AGS (alrededor de un 3% y un 5% de reducción, respectivamente).

De forma global, estos resultados sugieren que cuando se utilizan aceites ácidos no sólo se debe tener en cuenta el contenido en AGL, sino también el contenido en MIU, ya que una correcta caracterización de la fuente de grasa añadida es clave para su óptima utilización en la formulación de las dietas. Tanto el aceite de orujo de oliva como el aceite ácido de orujo de oliva son fuentes de grasa alternativa adecuadas para su incorporación en las dietas de animales monogástricos, siendo necesarias diferentes estrategias de inclusión en función de la especie y de las características de calidad y composición del aceite utilizado.

Summary

The inclusion of fats and oils in monogastric animal feeding is a widespread practice due to their high energy content and supply of essential fatty acids (**FA**). However, the need for a more efficient and sustainable animal production requires a continuous search for alternative and competitive fat sources. In this sense, olive pomace oil and olive pomace acid oil are two interesting alternative fat sources with high potential for inclusion in monogastric animal diets, that could help to develop a circular economy and contribute to more efficient and sustainable animal production. In addition, the use of olive pomace acid oil could help to reduce feeding costs as it is usually competitively priced. Both are rich in monounsaturated FA (**MUFA**) and oleic acid (C18:1 n-9), but differ in the molecular structure. The crude oil is rich in triacylglycerols, whereas the acid oil, derived from chemical refining, is rich in free FA (**FFA**) and tend to accumulate higher moisture, impurities and unsaponifiable content (**MIU**), which may impair their utilisation by the animal. The global aim of the present PhD thesis was to investigate the potential use of olive pomace oil and olive pomace acid oil in pigs, broiler chickens and European seabass diets, focusing on the effects on performance, feed efficiency, digestibility, carcass parameters, fat deposition and FA profile of depot fat and final meat products.

In the first study (**Chapter 3**), olive pomace oil and acid oil were included at 5% (as-fed basis) in the diets of 224 male and female growing-finishing pigs ([Landrace x Large White] x Duroc) from 58 to 130kg of body weight (**BW**) and compared to palm oil, a conventional fat source. In addition, a diet including a blend of palm oil with olive pomace oil was used (1:1 ratio). The results showed that olive pomace oil did not affect performance, feed efficiency or digestibility, but did improve (+24% compared to palm oil diet) intramuscular fat deposition in loin meat. On the other hand, olive pomace acid oil showed better performance when blended with palm oil than when included alone, although digestibility was not affected in either case, revealing the importance of the degree of unsaturation and the MIU value of the fat source. When palm oil was replaced with olive pomace oil or acid oil, the FA profile of the loin meat was modified, reducing saturated FA (**SFA**) and increasing MUFA.

In the second trial (**Chapter 4**) 3,048 broiler chickens (Ross 308) were fed one of the three experimental diets including 6% of olive pomace oil (O), olive pomace acid oil (OA) or palm oil (PO) from 22 to 39 days of age. Broilers that were fed the olive pomace oil diet showed the best performance and feed conversion ratio (O: 1.622 vs PO: 1.668 and OA: 1.673; $P < 0.05$), reaching high digestibility of total FA (95.75%). On the other hand, the inclusion of olive pomace acid oil compared to palm oil, did not modify performance or feed efficiency, but improved digestibility of total FA (OA: 93.88% vs PO: 85.92%; $P < 0.001$), suggesting that it is a good alternative fat source to palm oil in broiler diets. As in the case of pigs, when palm oil was replaced with olive pomace oil or acid oil, a healthier breast meat was obtained, enriched with MUFA and with low SFA. However, despite having a similar FA profile, olive pomace acid oil led to worse feed efficiency and lower values for SFA digestibility (5% reduction) than olive pomace oil, indicating the negative effect of high levels of FFA and/or MIU.

The third trial (**Chapter 5**) was conducted in 480 European seabass (101g of initial BW), fed for 100 days with one of the eight experimental diets containing 15.4% added fat consisting of 25% of fish oil and 75% of different fat sources: fish oil, soybean oil, olive pomace oil, soybean-sunflower acid oil, olive pomace acid oil and 3 blends (1:1 ratio) containing two of these oils. The results differed according to the botanical origin and the FFA content of the experimental oils. Soybean and olive pomace oil, rich in triacylglycerols, performed as well as fish oil and achieved high digestibility values (93.93% and 93.79% for total FA digestibility, respectively). However, fish fed olive pomace acid oil alone showed worse performance (8.6% less final BW), but this was not observed when soybean-sunflower acid oil or blends were used despite having a similar FFA content and saturation degree, suggesting the importance of MIU content of the fat. On the other hand, the inclusion of acid oils showed a negative effect on the lipid and SFA digestibility (about 3% and 5% of reduction, respectively).

Overall, these results suggest that, when using acid oils, not only the FFA content but also the MIU content should be taken into account, as a correct characterisation of the added fat source is key for an optimal formulation of diets. Both olive pomace oil and olive pomace acid oil are two suitable alternative fat sources for inclusion in monogastric animal diets, with different inclusion strategies needing to be adapted depending on the species and the quality and compositional characteristics of the oil used.

- "That day, for no particular reason, I decided to go for a little run. So I ran to the end of the road. And when I got there, I thought maybe I'd run to the end of town (...). I ran clear to the ocean. And when I got there, I figured, since I'd gone this far, I might as well turn around, just keep on going. When I got to another ocean, I figured, since I'd gone this far, I might as well just turn back, keep right on going. When I got tired, I slept. When I got hungry, I ate. When I had to go, you know, I went"

- "And so, you just ran?"

- "Yeah"

Forrest Gump.

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Abbreviations

FA	Fatty acid	**OA**	Olive pomace acid oil diet
FFA	Free fatty acid	**FO**	Fish oil
TAG	Triacylglycerol	**BW**	Body weight
DAG	Diacylglycerol	**ADG**	Average daily gain
MAG	Monoacylglycerol	**ADFI**	Average daily feed intake
TFA	Total fatty acids	**FCR**	Feed conversion ratio
SFA	Saturated fatty acids	**G:F ratio**	Gain to feed ratio
UFA	Unsaturated fatty acids	**DE**	Digestible energy
MUFA	Monounsaturated fatty acids	**AME**	Apparent metabolizable energy
PUFA	Polyunsaturated fatty acids	**AID**	Apparent ileal digestibility
n-3 HUFA	n-3 Highly unsaturated fatty acids	**ATTD**	Apparent total tract digestibility
MIU	Moisture, impurities and unsaponifiable	**ADC**	Apparent digestibility coefficient
AO	Acid oil	**BF**	Backfat
FAD	Fatty acid distillate	**AFP**	Abdominal fat pad
PO	Palm oil diet	**IMF**	Intramuscular fat
O	Olive pomace oil diet		

Chapter 1.

General introduction

"It's like everyone tells a story about themselves inside their own head. Always. All the time. That story makes you what you are. We build ourselves out of that story"

Patrick Rothfuss, The Name of the Wind.

1. General introduction

The present PhD dissertation is part of a larger project entitled "Use of acid oils in monogastric animals: characterisation, comparative nutrition and meat quality repercussions" (ref. AGL2015-64431-C2-1-R) and constitutes the following step after the results presented in the previous thesis "Use of acid oils in broiler chicken diets" (Rodríguez-Sánchez, 2018), "Use of acid oils and fatty acid distillates in animal feeding: characterisation of these by-products and the repercussions of their use on the oxidative stability of poultry feed and meat" (Varona, 2021) and "Use of soybean acid oil and palm fatty acid distillate in broiler chicken diets" (Jiménez-Moya, 2021). This project aims to investigate the search for new alternative fat by-products for monogastric animal diets.

The following general introduction aims to provide sufficient information and background preceding the three studies carried out during this thesis. Therefore, the general characteristics of fats (from a nutritional point of view) and the main sources of fats used in monogastric animal feed are described first. Then, the nutritional value of the added fat sources is explained, focusing on digestion processes, factors affecting fat utilization, assessment of digestibility, essential fatty acids supply and effects on fat deposition, comparing the critical points between the three species studied (pigs, broiler chickens and European seabass). Finally, a literature review about the studies evaluating the use of acid oils in swine, poultry and farmed fish diets is presented.

1.1. Fats in animal nutrition: characteristics and benefits

1.1.1. General characteristics of fats

Lipids are a group of organic compounds, found in plant and animal tissues, which are relatively insoluble in water but soluble in common organic solvents such as benzene, ether and chloroform (Gurr et al., 2002; Pond et al., 2005). Lipids can carry out several functions, such as electron carriers, substrate carriers in enzymatic reactions, components of biological

membranes and sources and stores of energy (Larsson et al., 2006; Bhagavan and Ha, 2011). There are different kind of lipids depending on their chemical structure and biological activity they have, and a schematic classification has been given in **Figure 1.1**. Because of the importance they have in monogastric animal nutrition, main attention will be paid at glycerol-based simple lipids: fats and oils.

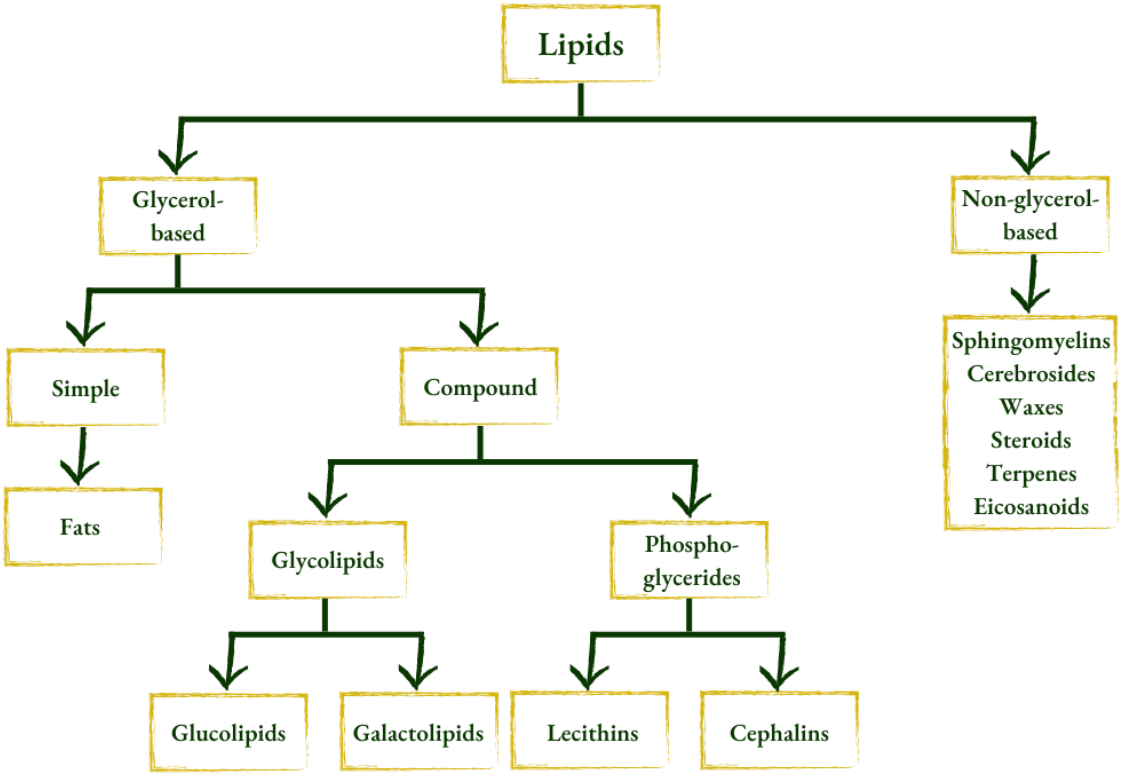

Figure 1.1. Classification of the lipids (adapted from McDonald et al., 2011). Graph by author.

Fats and oils are commonly interchanged terms, although the difference in terminology is due to the different melting point of fat and oil, which conditions their physical state in room temperature. Oils are those fats that have a lower melting point, so they appear as liquid at room temperature while fats (if the term is used in strict sense) appear solid. Furthermore, fats usually have an animal origin while oils stem from vegetal sources. The term "fat", nevertheless, is frequently used to define both groups, and so will be used indistinctly throughout the present PhD thesis.

Fats are, structurally, esters composed of glycerol-bound fatty acids (**FA**). When all three alcohol groups are esterified by fatty acids, the compound is called triacylglycerol (**TAG**). Triglycerides are the predominant fraction in fats. However, mono- and diacylglycerols (**MAG**

and **DAG**, respectively) may also be present in fats, even free fatty acids (**FFA**), but in smaller amounts (< 5%). Fatty acids are aliphatic chains of carbon atoms of variable length, usually even-numbered, ranging from 2 carbon atoms to 24 or more, one ending with a carboxyl group and the other with a methyl group. Depending on the aliphatic-chain length, fatty acids can be classified as short-chain fatty acids (**SCFA**), containing less than 6 carbon atoms; medium-chain fatty acids (**MCFA**), containing between 8 to 12 carbon atoms; long-chain fatty acids (**LCFA**), containing between 14 to 20 carbon atoms; and very-long-chain fatty acids (**VLCFA**), containing more than 20 carbon atoms (Stillwell, 2016). FA can be also classified according to whether they have double bonds or not. FA that do not have double bonds are termed saturated fatty acids (**SFA**), while those with any double bond are known as unsaturated fatty acids (**UFA**). UFA, at the same time, are divided into monounsaturated fatty acids (**MUFA**) if they have one double bond, or polyunsaturated fatty acids (**PUFA**) if they have more than one double bond. The carbon chain length and the level of saturation of FA will determine their physical-chemical properties. So, those with a higher proportion of SCFA and UFA will have lower melting points. The most common FA of natural fats and oils are presented in **Table 1.1**.

In the FA carbon chains, the methyl carbon located on the distal end of the chain is named omega (ω) carbon. This ω carbon atom acts as a reference in nutritional work practice and is known as carbon 1, which will enable to identify the position of double bonds in unsaturated FA. For example, linoleic acid is named ω-6,9-18:2, which means that the two double bonds are located on carbons 6 and 9 counting from the ω carbon atom. Usually, the letter ω is substituted by letter n, so we then have n-6,9-18:2, and in a compacted notation, C18:2 n-6. Based on this nomenclature, UFA are grouped into families depending on which precursor they have. These families are omega-9 (ω-9; based on oleic acid, C18:1 n-9), omega-6 (ω-6; based on linoleic acid, C18:2 n-6) and omega-3 (ω-3; based on α-linolenic acid C18:3 n-3). When naming these families, it is also common to use the letter n instead of ω. Also, mainly in fish nutrition, it is usually common to refer to C20:5 n-3 and C22:6 n-3 fatty acids to n-3 highly-unsaturated FA (**HUFA**), as a subgroup of the n-3 family.

Table 1.1. Most common fatty acids nomenclature (adapted from Pond et al. 2005; McDonald et al. 2011; Bowen-Forbes and Goldson-Barnaby 2017).

Systematic nomenclature	Trivial nomenclature	Notation	Food sources
Saturated fatty acids			
Butanoic	Butyric	C4:0	
Hexanoic	Caproic	C6:0	
Octanoic	Caprylic	C8:0	Palm oil, coconut oil
Decanoic	Capric	C10:0	Goat and cow butter
Dodecanoic	Lauric	C12:0	Coconut and palm kernel oil
Tetradecanoic	Myristic	C14:0	Coconut oil, dairy fat
Hexadecanoic	Palmitic	C16:0	Palm oil, meat, dairy fats
Octadecanoic	Stearic	C18:0	Meat, poultry, fish and grain products
Eicosenoic	Arachidic	C20:0	
Tetracosanoic	Lignoceric	C24:0	
Unsaturated fatty acids			
cis-9-hexadecanoic	Palmitoleic	C16:1 n-9	
cis-9-octadecanoic	Oleic	C18:1 n-9	Olive, canola, sunflower oil
cis-11-octadecanoic	Vaccenic	C18:1 n-7	
All-cis-9,12-octadecadienoic	Linoleic	C18:2 n-6	Corn, safflower, grape oil
All-cis-9, 12, 15-octadecatrienoic	α-Linolenic	C18:3 n-3	Canola oil, walnuts, flaxseed, flax oil
All-cis-6, 9, 12-octadecatrienoic	γ-Linolenic	C18:3 n-6	
cis-11-eicosenoic	Gondoic	C20:1 n-9	
All-cis-11, 14-eicosadienoic	Eicosadienoic	C20:2 n-6	
All-cis-5, 8, 11, 14-eicosatetraenoic	Arachidonic	C20:4 n-6	Chicken, eggs
All-cis-5, 8, 11, 14, 17-eicosapentaenoic	Eicosapentaenoic (EPA)	C20:5 n-3	Marine algae, fish oils
All-cis-5, 8, 11, 14, 17, 20-docosahexaenoic	Docosahexaenoic (DHA)	C22:6 n-3	Fish oils and algae oils

1.1.2. Benefits of the use of fat in monogastric animal nutrition

Fats and oils are widely used in monogastric animal nutrition since there are many benefits, encompassing nutritional and non-nutritional aspects, of including them in the diets:

Nutritional aspects:

a) ***High-energy input to the diet***, as fats and oils have an energy level that is unattainable for other ingredients (Ravindran et al., 2016), since lipids contain about the double amount of kcal/kg than carbohydrates or proteins, and can represent up to the 18% of the energy fraction of feed.

b) ***Supply of essential fatty acids***, which are crucial for many vital functions such as being components of cell-membranes or modulating immune response (Wiseman and Whitehead, 1984). This aspect is detailed below in a separate section.

c) ***Improve absorption of fat-soluble vitamins A, D, E and K.*** The presence of fat in the intestine facilitates the solubilisation of these vitamins into the mixed micelles, a crucial step for their absorption.

d) ***Reduce the rate of food passage*** through the gut, allowing a better nutrient absorption, which is referred to as extra-caloric effect of fat (Mateos and Sell, 1981). Ileal digestibility of amino acids and protein increases when increasing the level of dietary fat, what seems to be due to the major time of contact with absorptive cells (Li and Sauer, 1994; Albin et al., 2001; Cervantes-Pahm and Stein, 2008). The same occurs in the digestibility of fiber, increased because of the more time allowed for microbial fermentation (Cho and Kim, 2012). In addition, the extra-caloric effect of fat may be enhanced by a synergistic effect when unsaturated and saturated fats are mixed, since UFA help SFA to enter the mixed micelles and thus their absorption (Young and Garrett, 1963; Jimenez-Moya et al., 2021b). This synergism results in higher apparent digestible (**DE**) or metabolisable energy (**AME**) values than the arithmetically predicted ones (Powles et al., 1993; Wiseman et al., 1998).

e) Increase the efficiency of utilization of consumed energy due to the ***lower heat increment*** when metabolising fat compared to other ingredients. The lower heat increment can also be an

advantage in warm climates where feed intake is compromised (Stahly and Cromwell, 1979; Coffey et al., 1982; Wiseman and Stahly, 1984; Noblet and Etienne, 1987).

Non-nutritional aspects:

f) The inclusion of fats may ***affect the manufacturing process of the feed***, reducing wastes due to dust production or avoiding the wear and tear of machinery. However, it should be noted that high levels of fat inclusion can impair the pelleting process, reducing the pellet yield and quality. In fact, the limit of incorporation of fat in feeds is due to technological issues. Depending on the feed manufacturing process, added fat inclusion levels can reach up to 15% added fat when pelleted or up to 35% added fat when extruded. Usually, pelleted diets are used for swine or poultry (containing 2-6% of added fat, as-fed basis) and extruded diets for farmed fish (containing 18-24% of added fat, as-fed basis).

g) ***Reduce dust***. Dietary fat helps to bond small particles of feed together, playing an important role in reducing dust in farmed animal facilities. For example, Chiba et al. (1985) observed that aerial and settled dust were reduced by 49 and 10%, respectively, when a 5% of dietary added fat was included in growing pigs diet.

h) ***Increase palatability.*** In both pig and poultry nutrition, fats and oils are generally known to improve palatability of feed by changes in taste perception, texture, dustiness or the release of liposoluble flavor components, although it is always subject to the nature, inclusion rate and quality of the fat source used (Mizushige et al., 2007; Solà-Oriol et al., 2011; Ravindran et al., 2016). However, fish are mainly attracted by water-soluble compounds nitrogen-based (e.g. free amino acids, betaine or amines) and to a minor extent by other non-nitrogenous compounds such as glucose, lactic acid and some alcohols (Kasumyan and Doving, 2003), so dietary lipids play a minor role in determining palatability in farmed fish (Turchini et al., 2009).

i) ***Affects meat and flesh quality.*** Dietary fat will highly affect fat composition of meat and flesh fat, in terms of quantity but also of quality, since the FA profile of meat and flesh reflects that from the diet (Wood et al., 1999; Duran-Montgé et al., 2010; Nasopoulou et al., 2011; Vilarrasa et al., 2015b). This effect will be detailed later in a separate section.

1.2. Added fats in monogastric animal nutrition

1.2.1. Native oils

Native (or crude) oils are widely used in animal nutrition as a source of energy and FA, and their composition and nutritional values are well known in the literature. Native oils are mainly composed by TAG, with lower proportions of DAG, MAG and FFA. A, mentioned above, a TAG consists of a glycerol molecule with three FA esterified to the carbon-hydroxyl groups of the glycerol. The principal FA of the common vegetable and animal sources are presented in **Table. 2.1.**, as well as some of the acid oils obtained from the chemical refining industry. In general terms, vegetable and marine (especially those of fish) fat sources are more highly unsaturated than those of mammalian origin. Vegetable oils have considerable amounts of linoleic and linolenic acids, in addition to oleic acid (which is the major fatty acid in most natural fats), and fish oils present notable concentrations of EPA and DHA. Conversely, fats of mammalian origin have a lower proportion of the more unsaturated acids but a higher proportion of high-molecular-weight saturated acids such as palmitic and stearic acids (McDonald et al., 2011).

Table 1.2. Principal fatty acids (%) of the common vegetable, animal and acid oil sources used in pig, poultry and farmed fish diets (adapted from FEDNA, 2021).

	C<14	C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:2 n-6	C18:3 n-3	C>20	C20:5 n-3	C22:6 n-3
Animal fat											
Tallow	-	3.2	25.0	3.2	21.1	38.3	2.2	-	-	-	-
Lard	-	1.5	23.7	3.0	13.0	44.0	10.0	0.8	1.3	-	-
Nordic fish oil	-	6.0	11.0	7.2	1.2	11.0	1.0	0.5	>45.0	8.1	7.8
Spanish fish oil	0.1	4.3	15.7	4.1	4.3	13.5	1.8	1.1	>47.0	11.0	11.0
Vegetable oil	-									-	-
Soybean oil	-	-	9.5	0.2	4.0	22.0	54.0	7.3	1.1	-	-
Sunflower oil	-	-	6.4	-	5.0	22.6	63.0	0.5	1.1	-	-
Palm oil	-	1.0	43.0	0.3	4.8	40.0	10.0	-	-	-	-
Rapeseed oil	-	-	5.0	0.3	2.2	57.0	20.5	9.0	4.4	-	-
Olive oil	-	-	10.0	0.2	3.5	78.0	6.5	0.3	-	-	-
Coconut oil	>55.0	17.0	9.0	-	2.5	7.0	1.5	-	-	-	-
Palm kernel oil	>50.0	15.0	8.5	-	1.7	17.1	1.1	-	-	-	-
Linseed oil	-	-	6.0	-	4.5	19.0	16.0	54.0	1.0	-	-
Acid oils										-	-
Soybean acid oil	-	-	9.5	0.2	4.0	22.0	54.0	7.3	9.5	-	-
Sunflower acid oil	-	-	6.4	-	5.0	22.6	63.0	0.5	1.1	-	-
Olive acid oil	-	-	10.0	0.2	3.5	78.0	6.5	0.3	-	-	-
Coconut acid oil	>55.0	17.0	9.0	-	2.5	7.0	1.5	-	-	-	-

Considering the global production of the major vegetable oils, a total of 215.36 million metric tons (**Mmt**) are produced annually (USDA, 2022). The top four vegetable oils produced are palm oil (76.52 Mmt), soybean oil (61.86 Mmt), rapeseed oil (27.73 Mmt) and sunflower oil (22.10 Mmt), which represents an 87.4% of total world production. Since 2012, the total vegetable oils production has increased 86.89 Mmt (+36.3%). This is owing to the increase of the top four vegetable oils, which has been of +40.9% for palm oil, +41.1% for soybean oil, +18.7% for rapeseed oil and +35.0% for sunflower oil.

Palm oil and soybean oil are, therefore, the two most commonly used vegetable oils in animal feeding. Palm oil is rich in SFA, concretely in palmitic acid (C16:0), and hence a saturated fat source. Additionally, palm oil contains some other valuable components, such as tocotrienols or β- and α-carotenes, the latter being responsible of the characteristic orange color of the oil (Nagendran et al., 2016). Despite of being a commonly used fat source in animal feeding worldwide, palm oil faces negative public perception due to the environmental impact of its production (i.e. deforestation, consequences for biodiversity or greenhouse gas emissions) and the implication of cardiovascular diseases because of the high content in SFA (Briggs et al., 2017). On the other hand, soybean oil is rich in PUFA, concretely in linoleic (C18:2 n-6) and in a lesser extent in linolenic (C18:3 n-3) acids. Additionally, is a source of desirable minor components such as phospholipids, sterols and tocopherols.

In the European Union, the most widely used native vegetable oils in the animal feed industry are the four most produced and available worldwide (palm, soybean, sunflower and rapeseed oil) and, in addition, olive oil. Although the global production of olive oil is relatively low in comparison to other vegetable oils (1.47 Mmt), the 80% of it is achieved in the Mediterranean arc, so the availability of olive oil and their derivates is high for animal feed manufacturers at European level. Olive pomace oil is an interesting co-product generated from the milling process for obtaining olive oil. Olive pomace consists on the remaining olive pulp, skin and pits with the major part of oil removed after the olive milling. Then, the remaining oil from olive pomace is extracted with solvents, and olive pomace oil is obtained. Olive pomace oil is rich in MUFA, particularly in oleic acid (C18:1 n-9). Oleic acid consumption, together with other compounds coming from olive oil such as sterols, tocopherols or some hydrocarbons (e.g. squalene and β-carotene) have been linked to many beneficial health traits (Foscolou et al., 2018; Gavahian et al., 2019). Then, the inclusion of olive pomace oil in animal feeding could

lead to high quality meat products, since dietary fat can modify the FA profile of meat and flesh as it will be detailed below in a separate section.

Regarding the economics of palm and soybean oil, the two major vegetable oils available worldwide, they had an average price in 2019 of about 486 € / Mmt and 730 € / Mmt, respectively (USDA, 2022). On the other hand, olive pomace oil had a price about 714 € / Mmt in 2019 (Olimerca, 2022). However, these prices are merely indicative, since market prices are constantly fluctuating and subjected to many variable factors that affect the availability and profitability of such products, therefore altering their usage in animal feeding (e.g. the recent conflict between Russia and Ukraine has considerably increased the prices of feedstuffs, as did Covid-19 pandemic). The evolution of European market prices for crude vegetable oils is represented in **Figure 1.2.**

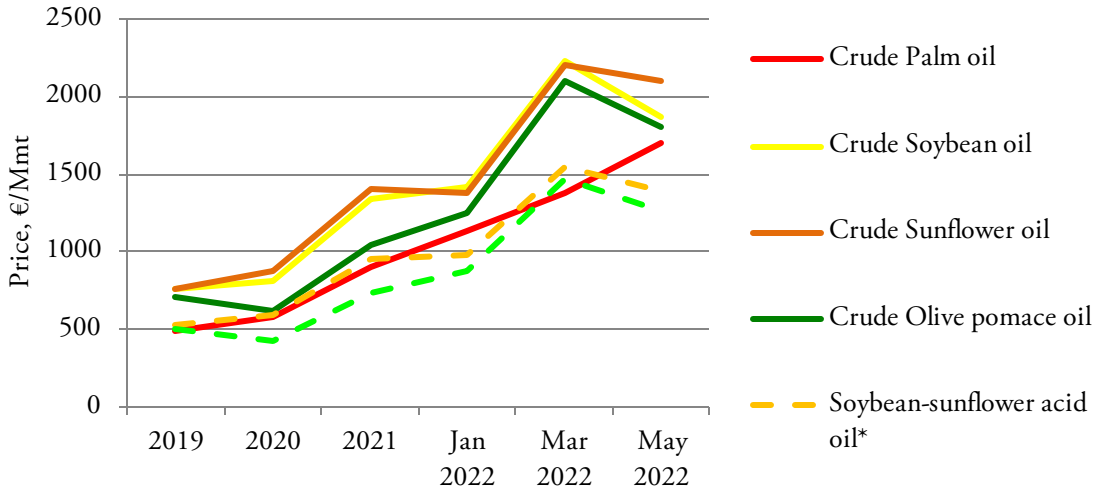

Figure 1.2. European market prices for vegetable oils in the recent years (€ / Mmt). Data extracted from USDA (2022) and Olimerca (2022). *Prices of acid oils have been estimated from calculating the 70% of the price of their respective crude oils.

1.2.2. By-products from the edible oil refining

Crude or unrefined native oils are composed mainly by TAG and lower proportions of DAG, MAG and FFA, but also by phosphatides, pigments, sterols, tocopherols, glycerol, hydrocarbons and vitamins, among other compounds (Cheryan, 1998). The main objective of the refining of crude native oils is to reduce the proportion of FFA as well as other impurities and undesired flavors, in order to make refined oil suitable for human consumption. Conventional processing steps of oil refining are summarized on **Figure 1.3.** There are two

types of oil refining that can be performed: chemical and physical refining, which generate acid oils (**AO**) and fatty acid distillates (**FAD**), respectively. Chemical refining is the most extended method, since physical refining is destined only to those oils that have low phospholipid content, such as palm oil (Ruíz-Méndez and Dobarganes, 2011). Both processes have common steps, such as degumming, bleaching or deodorization, but they differ in the phase where the major part of undesired substances is removed, including FFA. In chemical refining this stage is known as alkali neutralization, where an insoluble soap is formed when the added NaOH contact the FFA present in the crude oil. Sulfuric acid is later added to the sodium paste that results from alkali neutralization, giving rise to those commonly known as acidulated soapstocks. Acidulated soapstocks are then washed to drag the excess of sulfuric acid and dried by decantation leading to the commercial acid oils from chemical refining, AO (Nuchi et al., 2009).

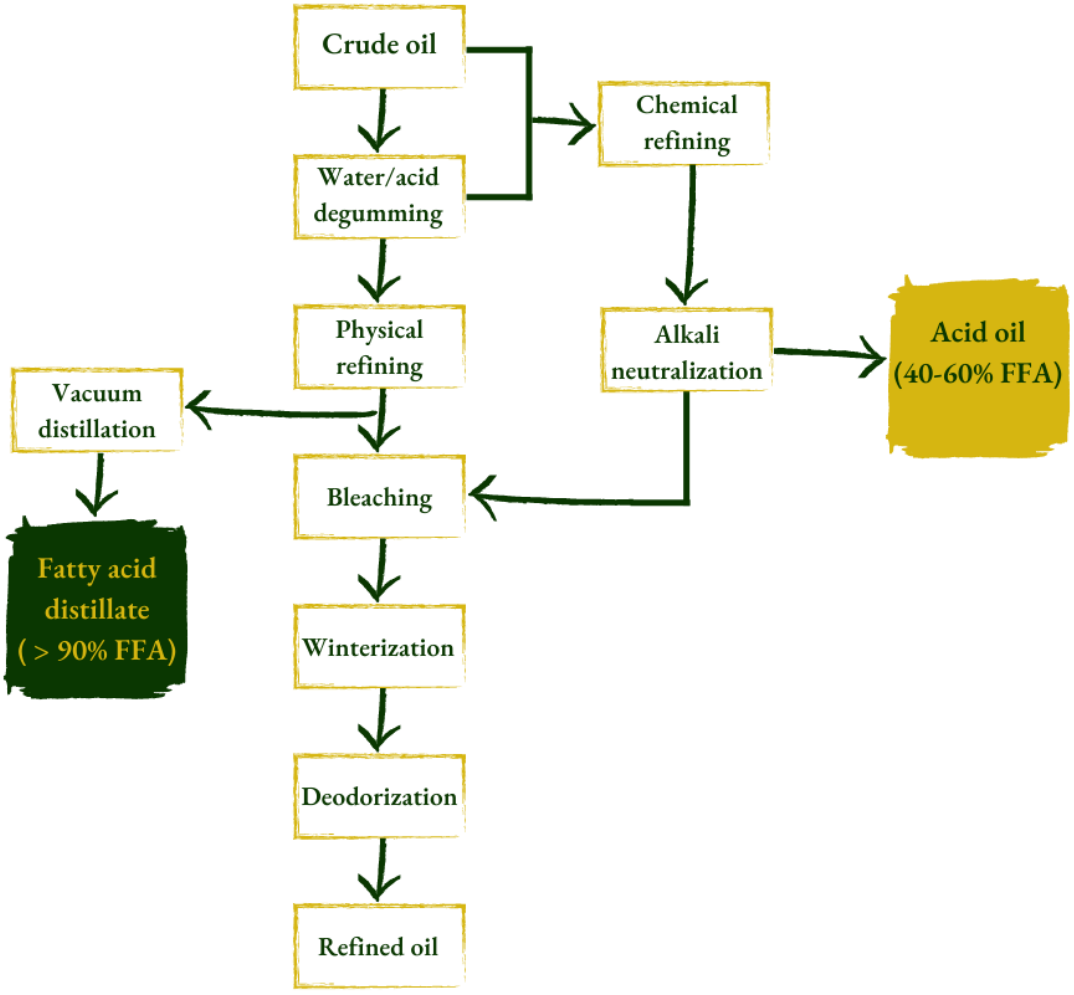

Figure 1.3. Main conventional steps of the oil refining process (adapted from Dumont and Narine 2007). Graph by author.

On the other hand, physical refining process consists on a vacuum distillation at high temperatures, also called as physical neutralization or by-steam, where FFA are removed from the oil generating a by-product known as fatty acid distillates from physical refining, FAD (Nuchi et al., 2009). An important fact to keep in mind regarding the obtaining method of these by-products is that AO and FAD do not present the same amount of FFA, so that the former have a 40-60% and the latter a > 90% content in FFA, approximately. Physical refining has some advantages against traditional chemical refining, consisting on improved yield, lower investment cost, less environmental impact (since soapstocks to be treated are not formed) and mild refining with fewer chemicals used (Kovari, 2004; Dumont and Narine, 2007). However, the use of this method still has a major drawback, which is that it is not suitable for oils with a high phospholipid content (Pawar and Marathe, 2015). Otherwise, it has been shown that the quality of the initial crude oil has a key role on the quality of the refined product (Li et al., 2016; Sampaio et al., 2017), so if greater developments are made in this area it could be possible to modify the process, probably including a pre-treatment on the crude oil (Dumont and Narine, 2007), in order to widespread the use of physical refining instead of chemical refining.

By-products from the edible oil refining are therefore characterized by presenting high amounts of FFA (instead the high TAG content of native oils) but with a similar FA profile to that of their corresponding crude oil. Additionally, although the refining process is oriented mainly to the extraction of the FFA from the crude oil, other desirable components are concomitantly removed and therefore accumulated in AO and FAD, such as lipid soluble vitamins, tocopherols, polyphenols, or sterols (Varona et al., 2021). However, AO and FAD also tend to accumulate a high content in moisture, impurities and unsaponifiable matter (globally known as **MIU**), which include compounds that act as diluents of the final energy content and hence determining the quality of the fat. Moisture is the amount of water present in the fat. Impurities are determined as the insoluble fraction of the fat in petroleum ether, which includes several different compounds (mechanical particles, minerals, carbohydrates, nitrogen-based compounds, calcium soaps, and oxidized FA, among others). On the other hand, unsaponifiable matter are those substrates that are not saponified after a treatment with caustic soda (including sterols, tocopherols, carotenoids and pigments). The guidelines of the Spanish Foundation for the Development of Animal Nutrition (FEDNA) states that MIU content in

AO and FAD should be below 5% (FEDNA, 2019). However, values of MIU content have been reported to be 7.62 ± 3.22% (mean ± SD) for AO and 5.37 ± 3.00% (mean ± SD) for FAD (79 AO and 13 FAD samples from the Spanish market; Varona et al., 2021), which shows that these by-products may often have higher MIU values than the recommended ones. Also, since the composition and quality of edible oil refining by-products has been reported to be very variable (Nuchi et al., 2009; Varona et al., 2021), the lack of standardization of AO and FAD, as well as the scarce information available about their nutritional value for animal feeding, could explain why many nutritionists and feed manufacturers are still reluctant to include them in animal diets.

In the European context, two interesting acid oils to be used in animal feeding are olive pomace and soybean-sunflower acid oils. Olive pomace acid oil is obtained from the chemical refining of olive pomace oil and, as well as olive pomace oil, it is rich in MUFA, particularly in oleic acid (C18:1 n-9; 55-83%). On the other hand, soybean and sunflower acid oils are generated by the chemical refining process of soybean and sunflower oils. Both AO are commonly commercialized together in a blend, representing one of the principal AO sources available on the European market since both soybean and sunflower oils are two of the most refined vegetable oils in European Union (USDA, 2022). Soybean and sunflower oils are rich in PUFA, in particular in linoleic acid (C18:2 n-6; 48-63%). The high content in PUFA in soybean and sunflower AO and the high content in MUFA in olive pomace AO could result in a reduction in the SFA content of the meat products generated and hence representing a health benefit for consumers (Briggs et al., 2017).

In terms of costs, AO are usually sold at 65-75% of the price of their respective crude oil from they were obtained (Francesc Guardiola, personal communication). So, olive pomace acid oil could be estimated at 646-535 € / Mmt and soybean-sunflower acid oil at 495-572 € / Mmt in 2019 (Olimerca, 2022; USDA, 2022) (Figure 1.3). Then, these AO are usually competitively priced and readily available for feed manufacturers, which makes them potentially interesting alternative fat sources to be included in animal feeds that can contribute to both a circular economy system and a more efficient and environmentally sustainable animal production.

1.3. Nutritional value of fats

1.3.1. Digestion of fat in pigs, poultry and fish

Digestion is the set of physical, chemical and microbial processes that allow the degradation of dietary nutrients so that they can pass through the intestinal mucosal membrane and be used by the animal. In general terms, the lipid fraction of the diet of monogastric animals is mainly composed by TAG. Then, digestion of fat consists of a three step process that begins with an emulsification of dietary fat into fat globules, a posterior hydrolysis of the TAG by the enzyme pancreatic lipase and a final aqueous dispersion of lipolytic products in bile-salt micelles. A schematic view of the digestion process in monogastric animals is showed in **Figure 1.4.**

Digestion process of nutrients initiates in the mouth. Mouth digestion consists mainly on the mechanical process of mastication. However, mouth digestion in pig is doubtful since feed is quickly swallowed by the animal and there is a short chewing time (McDonald et al., 2011). On the other hand, poultry do not have teeth, so feed is swallowed directly to the crop without there being any type of mouth digestion process (Leeson and Summers, 2001). In fish, although mouth act as the start of physical processes by puncturing or crushing feed, no mastication occurs and feed is swallowed and reaches the stomach, were digestion starts (Halver and Hardy, 2002).

Stomach (or proventriculus and gizzard in poultry) has a primer role in fat digestion, producing shear forces that enable fat emulsification, which consists on the breakdown of fat globules into fat droplets, providing a larger surface area for the enzymatic hydrolysis of FA. Hydrolysis of fat has been described to start at a gastric level, where up to 30% of the total dietary TAG may be digested by lipases secreted from cells that are located along the tongue, pharynx or stomach, known collectively as the pre-duodenal lipases. While in some species the predominant pre-duodenal lipases are lingual lipases (i.e. rat and mouse) or pharyngeal lipases (i.e. calf, lamb and sheep), in pigs enzymatic digestion of fat starts with gastric lipases (DeNigris et al., 1988; Armand et al., 1992; Miled et al., 2000).

In birds, the gizzard has the analogue role to the stomach in pigs in terms of mechanical forces that induce a prior fat emulsification. Concerning enzymatic digestion, although some carnivore avian species have lipase secretion from the walls of the stomach, no significant pre-

duodenal lipase activity was found in chicken (Moreau et al., 1988), so, in them, hydrolysis of fats starts in the small intestine (Leeson and Summers, 2001). In some species of fish, although gastric lipases are present, the primary site of lipid hydrolysis appears to be in anterior intestine and pyloric ceca (Halver and Hardy, 2002). Pyloric ceca are extensions of the upper intestine present in some species of fish, histologically similar to the anterior intestine, which are characterised by digestive and absorptive functions, and are completely different from the distally located ceca of birds and mammals, which have fermentation functions (Buddington and Diamond, 1987; Halver and Hardy, 2002). The number and shape of pyloric ceca varies between species, ranging from a couple to several hundred in some salmonid species. When the number of pyloric ceca is low (eg. European seabass), the major part of digestion and absorption processes described below take part in the anterior intestine.

Once the gastric chyme leaves the stomach, fat reaches the duodenum, where the main part of its digestion will take place. When the food bolus enters the duodenum, it stimulates the secretion of two hormones: secretin and cholecystokinin. The former activates the pancreatic mucosa for the secretion of an aqueous fluid that will act as buffer of the gastric chyme, while the latter promotes the secretion of several enzymes, including pancreatic lipase. However, fat arrives at duodenum in the form of large fat globules, some of them partially emulsified into droplets by the previous mechanical forces, but not suitable enough for their absorption due to their fairly large structure (about 5000 Å). For this reason, the degradation of TAG by pancreatic lipase action is required. However, pancreatic lipase is not able to act without the presence of the cofactor called colipase, which acts as an anchor for the lipase on the oil/water interface of emulsified droplets, and also protects it from denaturation. Moreover, pancreatic lipase is assisted by the bile salts, which are produced by the liver and stored at the gall bladder since the presence of the food bolus at the small intestine promotes its secretion to the duodenum. Bile salts emulsify fat droplets and prevent them from re-associating helping to stabilize them and let the pancreatic lipase act on the oil/water interface, thanks to their modification on it (Salentinig et al., 2011).

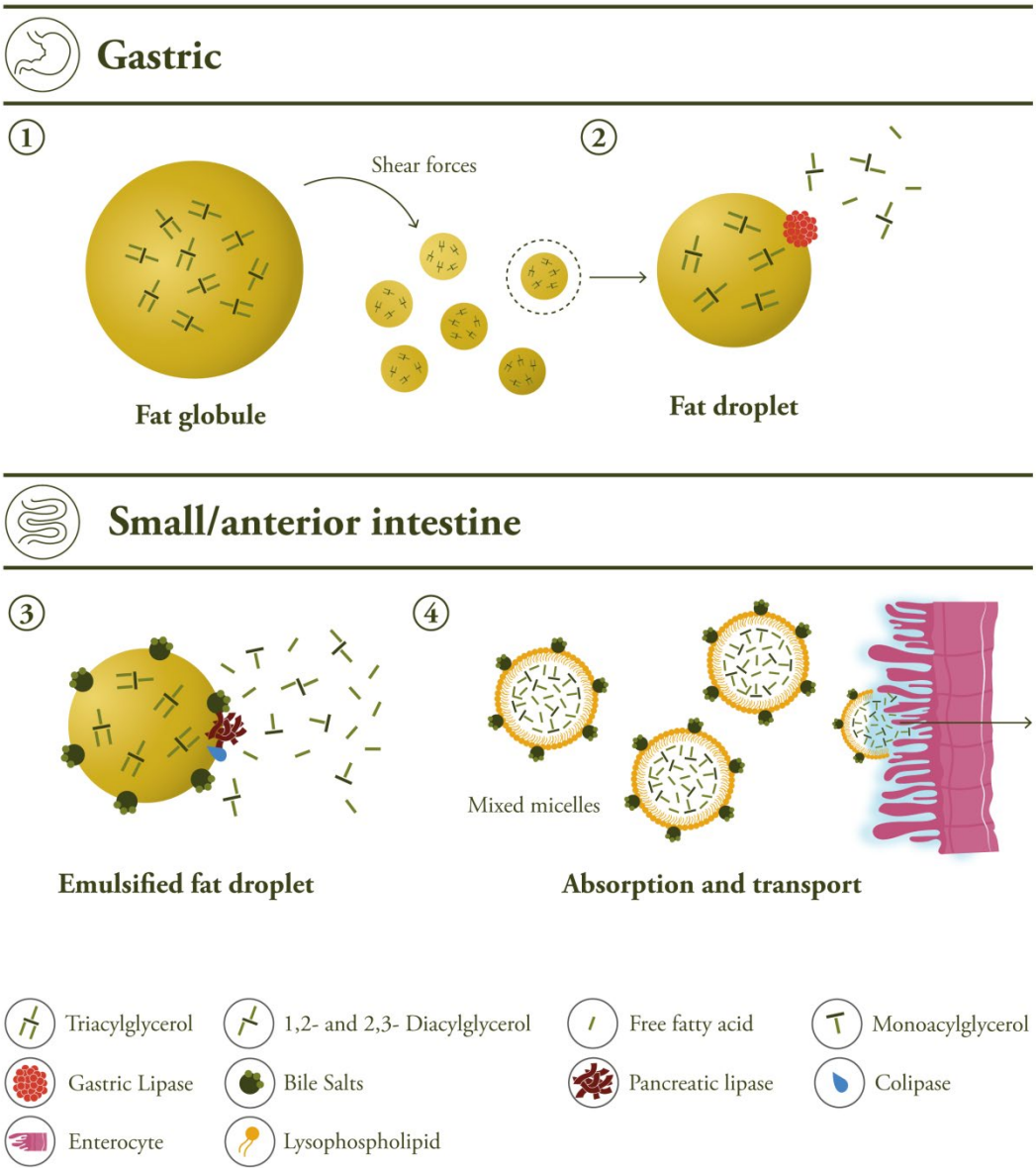

Figure 1.4. Simplified schematic view of the digestion process of fat in monogastric animals. 1) Fat globules are reduced to fat droplets due to shear forces in the stomach (or gizzard in poultry). 2) Gastric lipase acts in the stomach and hydrolyses some of the triacylglycerols present in the fat droplet. 3) Bile salts facilitate the emulsification of fat droplets and the pancreatic lipase-colipase system hydrolyses the triacylglycerols, generating diacylglycerols, monoacylglycerols and free fatty acids. 4) Lipolysis end-products form mixed micelles, which disaggregate on contact with the unstirred water layer and are absorbed through enterocytes. Graph by author.

Once the droplet is stabilized and the bile salt-lipase-colipase system is formed, hydrolysis of TAG takes place. Pancreatic lipase acts between pH 4.5 and 7.5, with its maximum activity at pH 6.5 (Salentinig et al., 2011). Enzymatic hydrolysis of TAG occurs preferentially on the FA located at external positions sn-1 and sn-3 rather than those located at sn-2 internal position (Dryden, 2008). Thus, the first step of the hydrolysis transforms a TAG into a 1,2-DAG (or

2,3-DAG) and a FFA, while the second step hydrolyses a 1,2-DAG (or 2,3-DAG) into a 2-MAG and another FFA. Other pancreatic enzymes are secreted into the duodenum and play a role on the digestion of other dietary lipid products, such cholesterol esterase or phospholipase A2. The MAG and some of the FFA, due to the amphipathic activity of bile salts, aggregate together and form primary micelles. At first, these micelles are composed of MAG, medium-chain FA, long-chain UFA and lysophospholipids, all of them characterized by having amphiphilic properties. The hydrophobic core of these primary micelles act as liquid crystal with the ability to solubilize more lipophilic compounds, such as long-chain SFA, DAG, fat-soluble vitamins and cholesteryl esters, forming the secondary or mixed micelles (Krogdahl, 1985). In contrast to large oil-water emulsion droplets, micelles are only 30-100 Å in diameter, being stable solutions that are readily brought into contact with the microvillus (Leeson and Summers, 2001), allowing the absorption process to start. For this, a disaggregation of the micelle occurs (due to the low pH value of the unstirred water layer), so the lipolysis end-products are released and can be absorbed (Krogdahl, 1985). Absorption process could be done by passive diffusion across the enterocyte membrane (for MAG, SCFA or MCFA) or by active protein-mediated process (for LCFA). Once in the enterocyte, SCFA and MCFA pass directly to the portal blood bounded to albumin, while LCFA and MAG are re-esterified to TAG and then included into lipoproteins for their further transport and utilisation by the animal (Wang et al., 2013). In poultry, the absorption processes of dietary FA mainly take place at the jejunum (73-92%), with ileum (from 8-27%) playing also a key role (Rodriguez-Sanchez et al., 2019b; Jimenez-Moya et al., 2021b). As described in mammals (Rechkemmer et al., 1988; Jorgensen et al., 2001), similar may occur in pigs, were the major part is absorbed in the jejunum and ileum, and some SCFA and MCFA can be absorbed by the colonic epithelium (Wealleans et al., 2021). In fish, lipid absorption processes is thought to follow those described in other vertebrates, although there is limited direct knowledge about the absorption processes in the fish intestine. Lipid absorption process mainly occur in the pyloric ceca and anterior intestine, although long-chain SFA may not be readily absorbed as SCFA, MCFA or long-chain UFA and therefore absorbed in more distal parts (Røsjø et al., 2000; Halver and Hardy, 2002; NRC, 2011).

On the large intestine of pigs and poultry, specially on the caecum, an intense microbial activity is responsible of the metabolization of a wide variety of nitrogen and hydrocarbon products

from feed and endogenous waste. As a result of this activity, other products are generated, mostly volatile FA, which are acetic (C2:0), propionic (C3:0) and butyric (C4:0), but also other medium and long chain FA such as capric (C10:0), pentadecanoic (C15:0), margaric (C17:0) or C18:1 trans acids can be generated (Rodriguez-Sanchez et al., 2019a, 2021). The short-chain volatile FA can be absorbed and contribute to the pig's energy intake (McDonald et al., 2011). In poultry, microbial activity has a lower influence, so volatile fatty acids produced by this activity have a minor contribution on energetic aport. In fish, microbial activity is even lower than in birds, being practically negligible especially in marine carnivorous species (Clements, 1997). It is important to note that, in comparison to mammals, poultry and fish have a shorter gastrointestinal tract, which causes a short retention time of feed that can affect their exposure to enzymes and microvillus and hence their digestion and absorption (Angel et al., 2013). However, in birds and most fish, the decreased time of food retention is compensated by reverse peristalsis or reflux (Sklan et al., 1978; Kikuchi et al., 2020). In birds, it has been described between the gizzard and proventriculus, between the upper ileum and gizzard and between the cloacae to the caecum (Sklan, 1979; Leeson and Summers, 2001).

1.3.2. Factors affecting fat utilization

Digestion of fat can be affected by many factors, and the most relevant of them have been summarized in **Figure 1.5.** and detailed below.

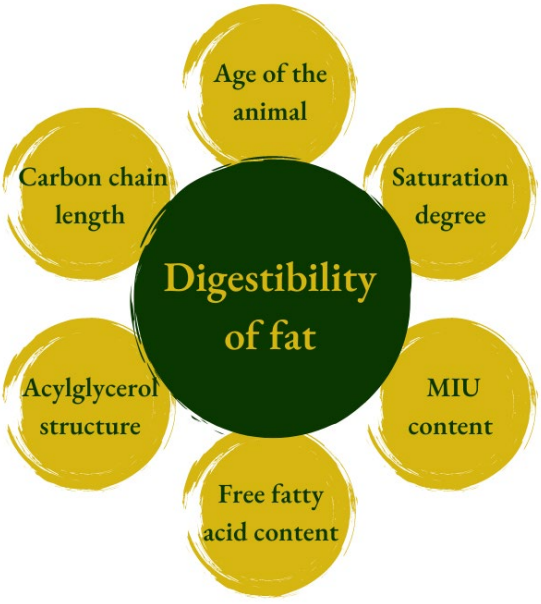

Figure 1.5. Factors affecting digestibility of fat in monogastric animals. Graph by author.

Factors regarding dietary fat composition:

- The **saturation degree** of the fat. As saturation degree increases, digestibility of fat decreases (Sklan, 1979; Wiseman et al., 1991; Jimenez-Moya et al., 2021a; b). However, when unsaturated and saturated fats are blended, a synergic effect is observed due to the assistance in the absorption of SFA by UFA, and digestibility values for SFA are increased (Young and Garrett, 1963; Wiseman et al., 1998).

- The **carbon chain length**. Similarly to the degree of saturation, as carbon chain length increases, digestibility deceases (Wiseman et al., 1991).

- The **low- or non-energetic fraction (i.e. MIU)** content. The MIU content of a fat, which is an estimator of its quality, includes moisture, impurities and unsaponifiable content that can negatively affect fat digestibility values.

Factors regarding lipid-class composition of dietary fat:

- The **FA distribution** in the glycerol backbone. Long chain SFA are better absorbed when bound to a glycerol backbone than when they are in FFA form (Renaud et al., 1995). Then, as pancreatic lipase has preference for hydrolyzing the sn-1 and sn-3 FA (Dryden, 2008), SFA located at sn-2 position will be absorbed more efficiently (Vilarrasa et al., 2015a; Ravindran et al., 2016).

- The **FFA** content. High levels of dietary FFA have been associated with a decrease on digestibility values (Powles et al., 1993). This could be related to the formation of insoluble soaps when FFA interact with some ionised minerals such as calcium or magnesium, becoming both the FFA and the mineral unavailable for absorption (Small, 1991). However, this effect has been mainly attributed to long-chain SFA rather than to MUFA or PUFA, which suggests that the negative effects of FFA on fat digestibility are mainly limited to saturated sources of FFA (Atteh and Leeson, 1985; Wiseman et al., 1998; Jimenez-Moya et al., 2021b), and especially in young animals (Leeson and Summers, 2001). On the other hand, although dietary FFA content may affect fat digestibility, many studies observed no negative effects in performance, feed intake or digestibility of fat in pigs (DeRouchey et al., 2004; Rojas-Cano et al., 2014; Vilarrasa et al., 2015a), in broiler chickens (Zumbado et al., 1999; Jimenez-Moya et

al., 2021b; Rodriguez-Sanchez et al., 2021) or in farmed fish (Ng et al., 2010). However, further studies are needed to better understand the effects of dietary FFA in fat digestibility, especially including unsaturated fat sources rich in MUFA that can potentially avoid the negative impact of a high dietary FFA content.

- The presence of **MAG**, which could enhance the incorporation of FFA into the mixed micelles facilitating their absorption and minimizing the negative effect of FFA on fat digestibility (Ravindran et al., 2016; Roll et al., 2018; Jimenez-Moya et al., 2021b).

Factors regarding the animal:

- The **age** of the animal. It has been stated that digestion of fat is limited in young animals, since they have a low secretion of bile salts and hence a poor emulsification of fat (Noy and Sklan, 1995). Then, fat is better digested in growing-finishing stages than in starter periods (Rodriguez-Sanchez et al., 2019a, 2021; Jimenez-Moya et al., 2021b).

The factors affecting fat digestion and utilisation have been extensively reviewed by Wealleans et al. (2021) in pigs, Ravindran et al. (2016) in poultry and Turchini et al. (2021) in fish.

1.3.3. Assessing the nutritional value of fats: digestibility

Digestibility of a nutrient can be defined as the amount of that nutrient that is not excreted after digestion and, then, it is considered absorbed by the animal (McDonald et al., 2011). In most of published literature, digestibility of fat in pigs and poultry is reported as apparent total tract digestibility (**ATTD**; losses of fat measured at faeces or excreta), and less frequently as apparent ileal digestibility (**AID**; losses of fat measured at the terminal ileum). In poultry, although measurements at excreta include urine collection (and hence it would be better to talk about metabolicity instead of digestibility), fat losses in urine are practically negligible so the term digestibility is accepted and most commonly used. In fish, digestibility is usually assessed by faeces collection and hence an apparent digestibility coefficient (**ADC**) is reported. In many cases, direct determination of digestibility can be difficult to assess, because the total collection of faeces or excreta is needed and sometimes the required equipment may be not available or the study characteristics might not fit. In these cases, determination of digestibility is possible if an inert marker is included in the feed, where the relationship between marker concentration in the feed and in the faeces or excreta gives an estimation of digestibility. Commonly used

markers in pigs and poultry digestibility studies are titanium dioxide (TiO2) or HCl-insoluble ash (with added silicate in the diet), among others (McDonald et al., 2011), while yttrium oxide (Y2O3) has been traditionally used for fish (Bai et al., 2021).

There are some points that need to be taken into account when assessing digestibility at ileal or total tract level. In both pigs and poultry, the measurement of total tract digestibility may be misleading due to the presence of "endogenous" fat. These endogenous fat include the fat that is synthesized and secreted by the animal (i.e. sloughed-off epithelial compounds), but also the microbial fat that may be synthesized during fermentation processes in the hindgut of pigs or, to a lesser extent, in the caecum of birds (Jørgensen et al., 1993). Additionally, microbial activity and fat synthesis can be enhanced by high levels of undigested carbohydrates or amino acids entering the hindgut or caecum (Kil et al., 2010). In this sense, a comparative study assessing AID and ATTD of fat in pigs, noted that the former showed higher values than the latter (73.6% and 71.5%, respectively) due to the microbial synthesis of fat in the hindgut (Kil et al., 2010). The same effect has been observed in broiler chickens (Jimenez-Moya et al., 2021a; Rodriguez-Sanchez et al., 2021). However, only few studies reporting both the AID and ATTD values of fat are available and information about these comparison is limited. Some of the main characteristics of determining AID vs ATTD are summarized in **Table 1.3**.

Table 1.3. Comparison between determining apparent ileal digestibility (AID) vs apparent total tract digestibility (ATTD).

AID	ATTD
Microbial synthesis of fats is avoided	Microbial synthesis of fats devaluates the value of ATTD
Better for high inclusion levels of fiber and carbohydrates	Very devalued on high inclusion levels of fiber and carbohydrates
There is still an error associated with endogenous secretions of fat in small intestine, which is higher when low levels of inclusion of dietary fat	The main affected by endogenous fat losses and in general, lower than AID
Extra cost of animal euthanize/cannulation	Cheaper
Difficult sample collection	Easy sample collection

In sum, it seems to be more accurate to determine the value of AID than the ATTD, although it suppose an extra costs that should be taken into account (need to euthanize the animals for obtaining sample, or cannulation in pigs).

1.3.4. Essential fatty acids

Essential FA are known to be crucial in numerous vital functions, as they appear to play an important part of the lipid-protein structure of cell-membranes and lipoprotein enzymes and on lipid transport. In addition, essential FA are the source materials for the synthesis of eicosanoids, a group of hormone-like substances that regulate many functions, including blood clotting, blood pressure, smooth muscle contraction and immune response (Shireman, 2003; Glencross, 2009; Rosenberg and Asbell, 2010). Both linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids are considered dietary essential FA because animals lack the capacity for inserting double bonds in the n-6 and n-3 positions (Shireman, 2003). From dietary linoleic and linolenic acids, most animal species are able to synthesize other long-chain FAs essential for cellular growth and function such as arachidonic acid (C20:4 n-6), eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) by the action of elongase and desaturase enzymes. However, marine carnivorous fish have lost the ability to convert linolenic acid into EPA and DHA, probably as the result of adapting to a n-3 HUFA-rich environment, and therefore both EPA and DHA become dietary essential FAs for these species (Sargent et al., 2002; Turchini et al., 2009).

In terms of requirements, C18:2 n-6 and C18:3 n-3 do not usually suppose a problem in swine or poultry nutrition. For piglets, requirement of linoleic acid is of 0.1%, and ranged from 0.1% to 1.5% for growing pigs over 60kg of bodyweight (NRC, 2012; FEDNA, 2013). No advantage has been observed with an extra input of C18:2 n-6 in the diet of growing pigs. However, several studies have shown a benefit in the supplementation of sources rich in n-3 HUFA (mainly fish oils) during gestation and lactation periods of sows on the vitality and viability of piglets and on their productive efficiency (Edwards, 2005; Kim et al., 2007; Leonard et al., 2011; Spencer, 2011). In broiler chickens, recommended level of C18:2 n-6 is of 1% (NRC, 1994; FEDNA, 2008). An excess of C18:2 n-6 in broiler finishing diets (>28 days of age) may suppose a problem in relation to carcass quality, as high levels of UFA may lead to excessive fluidity of the carcass fat, so it is recommended not to exceed the 2% of C18:2 n-6 in these diets (FEDNA, 2018). In general, vegetable oils and oilseeds products, which conventionally are included in pigs and poultry diets, are good sources of C18:2 n-6, so they will normally receive an adequate supply of essential FA.

On the other hand, aquaculture nutrition includes an extensive range of numerous farmed species, involving those presenting a "freshwater" pattern (i.e. freshwater fish that are capable of converting linoleic and linolenic acid to n-3 HUFA) and those presenting a "marine" pattern (i.e. marine carnivorous species, such as European seabass, that are not able to convert linoleic and linolenic acid and therefore require n-3 HUFA as essential FA). Then, the reported requirements of essential FA in farmed fish covers a relatively wide range, being 5.5% (dry diet) of n-3 HUFA for the larval stage of some marine carnivorous species and 0.5% (dry diet) of linoleic and linolenic acid for adults of freshwater species (Sargent et al., 2002; Turchini et al., 2009). In European seabass, essential FA requirements have been reported to be about 1% (dry diet) of n-3 HUFA in older juvenile and pre-adult stages (Coutteau et al., 1996; Sargent et al., 2002). Then, in farmed fish diets, fish oil had traditionally been used as the only dietary fat source, partly due to its high energy content but mainly to its high content in EPA and DHA. However, since the global supply is insufficient to cover the increasing demand for fish oil in aquaculture, fish oil is being replaced by vegetable oils in farmed fish diets, leading to a major research effort in recent years in studying different strategies and alternatives to address this issue (see the reviews by Turchini et al., 2010 and Tocher et al., 2019). When fish oil is completely replaced by vegetable oils, the essential FA requirements can be fulfilled by a high inclusion of fish meal, which contains 8-10% of residual fat, of which typically a 20 to 35% are n-3 HUFA (Bimbo, 2000) or alternative appropriate sources of these FA (e.g. microalgae oil) to avoid a risk of an essential FA deficiency.

1.4. Effects of dietary fat on fat deposition

When dietary FA are absorbed, they can be either oxidized and provide energy to the animal or deposited as energy storage, depending on the physiological requirements of the animal. Generally, pigs, poultry and farmed fish are in positive energy balance, since they are feed *ad libitum* (or to satiety in fish), and then some of the dietary FA are expected to be esterified and deposed into adipocytes. Then, although *de novo* FA synthesis or elongation/desaturation of dietary FA must be taken into account, deposed fat usually is a reflection of dietary FA composition. This is indeed the case for pigs (Miller et al., 1990; Vilarrasa et al., 2015a), broiler chickens (Ferrini et al., 2008; Vilarrasa et al., 2015b; Skřivan et al., 2018) and farmed fish (Kestin and Warriss, 2001; Izquierdo et al., 2003; Mourente et al., 2005; Álvarez et al., 2020).

Dietary FA profile not only modifies the fat and muscle FA profile but also can vary the amount of fat deposition in the animal. In fact, an increase of dietary PUFA has been associated with a decrease in fat deposition (Crespo and Esteve-Garcia, 2002a; Ferrini et al., 2008; Vilarrasa et al., 2015b). Once absorbed, dietary PUFA are preferentially β-oxidized with respect to SFA or MUFA. This, together with a decreased rate of FA de novo synthesis and insulin and very low density lipoprotein levels in serum, may explain the lower fat deposition on animals fed high levels of PUFA (Crespo and Esteve-Garcia, 2002b, 2003). On the other hand, it is not recommended to include high levels of PUFA in finishing diets as it could lead to fat dripping on the carcasses due to the lower melting point of these FA. Although the effect of PUFA seems to be clear in the literature, little is known about the effect of MUFA and further studies are required to better understand the role of dietary fat in fat deposition.

Apart from deposed fat (usually measured as backfat or ham fat in pigs, abdominal fat pad in broilers or perivisceral fat in fish), dietary fat may affect also the fat content and composition in meat products generated. Then, many quality characteristics of the final meat product can be affected by dietary fat, including firmness (or hardness), shelf life (lipid and pigment oxidation), flavor, tenderness and juiciness, directly affecting the consumers' acceptability of the product and its stability. In this sense, as the degree of unsaturation of meat fat increases, so does the propensity to oxidation and dripping (Bou et al., 2009). In broiler industry, genetic improvements based on better performance and breast yield have lead to modern broiler chickens that generate a breast meat with very low content of intramuscular fat (**IMF**), usually <1.5% (Chmiel et al., 2019; Chodová et al., 2021). In the case of the pig industry, a higher IMF content levels have been linked with a better sensory quality of pork (Font-i-Furnols et al., 2012), and it is considered one of the most important traits in sensory differentiation (Ngapo et al., 2012). In this sense, although high IMF is associated with a high levels of carcass fat, a 5% of increase in carcass fat generally corresponds only to a 1% of increase in IMF content (Goutefongea and Dumont, 1990; Hocquette et al., 2010), with a correlation between loin or ham fatness and IMF content varying from 0.28 and 0.49 (Font-i-Furnols et al., 2019).

In pigs, the IMF content of *longissimus* muscle, at a commercial BW of 100 kg, generally varies from 0.5-2.5% of muscle wet weight (Cagnazzo et al., 2006; Rincker et al., 2008), but this range can be extended up to about 4% in some breeds and ages, depending on several factors such as breed, gender, diet and age/weight (Font-i-Furnols et al., 2019). Then, high-fat content

crossbreeds (eg. Duroc lines), females, and older/bigger pigs have a higher IMF content (Hocquette et al., 2010). Regarding genetic effects, early differences in gene expression involving fatty acid metabolism were found between Duroc and Pietrain pigs, both extremes of fat and lean pig breeds, respectively. In this sense, an opposite expression profile on fatty acid metabolism genes in relation to energy metabolism genes was found, being the former greater in early Duroc embryonic tissues than in Pietrain (Cagnazzo et al., 2006). Regarding nutritional strategies to improve IMF content in pork, Isabel et al. (2004) reported a higher concentration of IMF at slaughter on pigs fed with a higher MUFA:PUFA ratio in their diets, indicating that probably MUFA play a role, in some way, on the level of IMF in pork. In this sense, Gerfault et al. (2000) associated a higher lipogenic enzyme activity in muscle with higher concentrations of oleic acid and lower of linoleic acid (and therefore a higher MUFA:PUFA ratio) in the diet. Recently, Zhang et al. (2019) found negative genetic correlations with linoleic acid and positive genetic correlations with oleic acid. In sum, it is assumed that it is not so easy to increase IMF of pork just with dietary strategies, but when combined with genetics, nutrition may be very advantageous.

In farmed fish, there are many benefits of n-3 HUFA consumption for human health, including prevention of cardiovascular or neuropsychological pathologies (Delgado-Lista et al., 2012; Fernandes et al., 2012; McBurney et al., 2021). However, dietary replacement of dietary FO by VO decreases the n-3 HUFA content in the fillets, which may devaluate the final product obtained. For this, many strategies are being studied to increase the n-3 HUFA content in flesh of farmed species, such as the inclusion of oils obtained from microalgae, some Boraginaceae plants (i.e. Echium sp.) or genetically modified n-3 HUFA enriched oilseed crops (eg. rapeseed or camelina).

1.5. Use of acid oils in monogastric animal diets

This section reviews studies in the literature over the last 30 years on the effect of high FFA content in the diets of pigs, poultry and fish on production parameters, digestibility and fat deposition. This topic has been studied somewhat more in poultry, and information concerning pigs and especially farmed fish is really scarce. However, many of the studies available in the literature have focused on assessing effects on digestibility, and few results have been reported concerning effects on performance or fat deposition. In general, differences

between ages, production periods, crossbreeds and the lack of characterisation of the experimental fat sources studied make comparison between studies difficult. In this regard, the reported results are unclear and controversial, with some reporting positive effects on performance parameters and dietary DE or AME, others reporting negative effects on fat DE or AME and fat digestibility, and others reporting no significant effects on either performance, digestibility or fat deposition due to dietary FFA content. The results reported in the literature suggest that there are many factors that play a role and somehow affect these studied variables despite FFA content, which may likely include the degree of saturation, the level of fat inclusion or the quality of the fat source (i.e. MIU content), among others. In addition, very few studies have evaluated the effects of FFA content in diets of pigs, poultry or fish using MUFA fat sources or growing large numbers of animals under commercial conditions, so information regarding these two factors is scarce.

1.5.1. Use of acid oils in swine diets

The studies evaluating the effects of dietary free fatty acid content in pig diets are summarized in **Table 1.4.** The research group of **Powles et al. (1993)** investigated the effect of dietary FFA content on dietary DE and added fat digestibility. This was assessed by replacing soybean oil and tallow for their respective acid oils in growing gilts (Landrace x [Landrace x Large White]; 25kg of BW). For this, two trials were designed with increasing inclusion levels of fat (4, 8 and 12%) and increasing levels of replacement of the native oil by its respective acid oil (0, 25, 50, 75 and 100% of replacement). Results found in both trials indicated that the DE and the apparent digestibility of added fat decreased when increasing the dietary FFA content. However, this effect was more pronounced for tallow acid oil than for soybean acid oil. Later, following the same experimental design, **Powles et al. (1994)** reported similar results for young weaning piglets (12kg of BW). The datasets from these two studies were treated and analysed to generate prediction equations for the DE on the study of Wiseman et al. (1998), who concluded that the dietary FFA content has a crucial (and adversely) effect on the DE for pigs, despite of the age of the animal.

Jørgensen and Fernández (2000) determined, in growing pigs (from 50 to 70kg of BW), the digestibility and energy value of two oil products rich in FFA: "palm oil mixture", with 79% of FFA, and "vegetable oil by-product", with a 55% of FFA. However, no further details were

provided on their obtaining processes. Results reported showed low digestibility values for these two oil products (62.3-71.5%) when compared to palm oil or soybean oil (85.3-91.4%). Similar was obtained for the ME values, where 23.8-25.9 MJ/kg (dry matter) were found for "palm oil mixture" and "vegetable oil by-product" while they were 33.7-36.2 MJ/kg (dry matter) for palm and soybean oil. Therefore, the authors concluded that a high level of FFA is negatively related to digestibility and consequently to energy value of the added fat source.

DeRouchey et al. (2004) evaluated the effects of increasing levels of FFA in choice white grease (ratio UFA:SFA = 1.5) in weaning piglets (from 21 to 54d old). To obtain the increasing levels of FFA in choice white grease, it was treated with 0, 872, 1,752 or 2,248 lipase units/g of fat, and thus concentrations of 2, 18, 35 and 53% of FFA were obtained, respectively. In these diets, the authors noted that MIU concentrations increased as FFA did (from 1.2 to 3.3%). However, these MIU values were lower than those frequently reported in other studies for acid oils obtained during the refining process, which are typically 2 to 4 times higher (Varona et al., 2021). No major differences were reported throughout the study regarding performance parameters, DE or fat digestibility due to the increasing FFA content. However, there was a trend for G:F ratio to decrease in pigs fed the diet containing 35% of FFA, but then to increase as FFA concentrations were increased to 53%. With this, the authors concluded that FFA concentrations up to 53% do not negatively affect utilization of choice white grease in weaning pigs.

Rojas-Cano et al. (2014) replaced a basal diet based on barley, corn and soybean meal by increasing levels of olive oil soapstocks (0 to 7.5%), rich in FFA, in diets for growing-finishing Iberian x Duroc barrows (from 27.3 to 90.2kg of BW). With this progressive levels of replacement, the dietary gross energy increased from 16.2 to 17.8 MJ/kg of feed, so the diets were not isoenergetic. Olive oil soapstock is a by-product obtained from the olive oil extraction industry by means of physical refinement processes. This product had a high content in FFA (59.8%) but also a high MIU content (12.09%). Body-weight gain improved in pigs fed the highest levels of olive soapstocks, and the gain to feed ratio showed a tendency to increase. Similarly, a positive effect was observed in the DE values, which increased as the proportion of olive soapstocks did.

Vilarrasa et al. (2015a) studied the effect of replacing palm native oil by palm fatty acid distillate or re-esterified palm oil. The three fat sources were included at 4% to the diet of growing-finishing pigs (from 24.7 to 107kg of BW, 100 days of study). Palm acid oil showed relative low MIU values (< 1.81%) and a high FFA content (53.2%). No differences were observed regarding any performance, DE of the feed or digestibility of fat between pigs fed palm native oil or those fed palm acid oil. In addition, fat deposition, measured as backfat thickness, showed similar values between pigs fed the two different fat sources.

Kerr and Shurson (2017) compared the digestibility and energy value of an animal-based lipid product (ratio UFA:SFA = 0.11) and soybean oil with their respective high-FFA source in weaning piglets. No differences were reported on final BW of piglets. However, diets fed the animal-based lipid product with high FFA content showed higher DE and fat digestibility values than those fed the animal-based product, although fat digestibility values of both sources were very low (50.45 and 33.09%, respectively). In contrast, soybean oil rich in FFA showed similar DE and fat digestibility values than soybean oil. From those results, the authors suggested that the FFA content has minimal effect on fat digestibility or energy utilisation of a fat source when they are unsaturated.

The studies carried out in pigs report very different results, including positive, negative, or no significant effects on dietary DE or fat digestibility. Additionally, many of them focused on the study of dietary DE or fat digestibility, and housed pigs in metabolic cages or used a small number of animals. Most of them reported no significant effect of dietary FFA content on performance parameters or feed efficiency, although they do not provide fully representative results in terms of performance or feed efficiency due to housing conditions. On the other hand, there are many factors that can influence the results found in these studies, such as the degree of saturation of the fat source evaluated, the level of inclusion, the quality of the fat or the breed/genetic line of the animals. Additionally, final digestibility values can be also affected by the interaction between ingredients, feed manufacturing and processing, which can even differ between countries). In this sense, the lack of a good characterisation of the experimental fats makes it difficult to establish a proper comparison between them. On the other hand, negative effects are linked to older studies, while in more recent trials no significant or even positive results were observed when including a high content of FFA in the diet. Therefore, modern genetic breeds may be more efficient and less affected by FFA content in the diet.

Furthermore, there do not appear to be notable differences between weaning piglets and growing-finishing pigs, suggesting that age may not be a key factor for pigs in utilising FFA. Additionally, it is important to note that few studies have assessed the FFA effect on fat AID, since many of them are focused on fat ATTD. On the other hand, information about the effects of FFA on fat deposition is really scarce, as values were reported in only one of the studies. Moreover, little information is provided about the effects of FFA using MUFA fat sources. In summary, it is concluded that more studies should be conducted with established quality fat sources in modern genetic lines of pigs reared under more commercial conditions to better understand the effects of dietary FFA and to establish practical strategies for the use of acid oils in pig diets.

Table 1.4. Studies evaluating the effects of dietary free fatty acid content in pigs diets.

Reference	FFA fat source (% of FFA)	Control	Inclusion (%)	MIU (%)[1]	Breed - Strain	Period	Effects of FFA source on:				
							BW	FCR	Feed digestible energy	Digestibility of dietary fat	Fat deposition
Powles et al. (1993)	Soybean acid oil (62.6%) and tallow acid oil (81.8%)	Soybean oil and tallow	4-12	-	Landrace x (Landrace x Large White)	Growing (25kg of BW)	-	-	Negative *** (added fat DE)	Negative *** (added fat)	-
Powles et al. (1994)	Soybean acid oil (69.8%) and tallow acid oil (75.6%)	Soybean oil and tallow	4-12	-	Landrace x (Landrace x Large White)	Weaning (12kg of BW)	-	-	Negative *** (added fat DE)	Negative *** (added fat)	-
Jørgensen and Fernández (2000)	Palm oil mixture (79%) and vegetable oil by-product (55%)	Palm oil and soybean oil	5-30	-	Not specified	Growing (50 to 70kg of BW)	-	-	Negative *** (dietary ME)	Negative ***	-
DeRouchey et al. (2004)	Choice white grease (18, 35 and 53% of FFA)	Choice white grease	6	1.2-3.3	Lines 326 boars x C22 sows (PIC, Franklin, KY)	Weaning (6kg of BW)	NS	NS	NS	NS	-
Rojas-Cano et al. (2014)	Olive oil soapstocks (59.8%)	Basal diet	0-7.5	12.09	Iberian x Duroc barrows	Growing-finishing (27 to 90 kg of BW)	Positive *	NS	Positive ***	NS	-
Vilarrasa et al. (2015a)	Palm acid oil (53.2%)	Palm oil	4	< 1.81	(Landrace x Duroc) x Pietrain), boars and gilts	Growing-finishing (25 to 107 kg of BW)	NS	NS	NS	NS	NS
Kerr and Shurson (2017)	Animal lipid product high FFA (98.1%) and soybean oil high FFA (89.6%)	Animal-based lipid product and soybean oil	10	2.02 (animal lipid) and 8.13 (soybean oil)	Not specified	Weaning (14kg of BW)	NS	-	Positive *** (animal lipid) or NS (soybean oils)	Positive *** (animal lipid) or NS (soybean oils)	-

Abbreviations: FFA = free fatty acid; MIU = moisture, impurities and unsaponifiable content; BW = body weight; FCR = feed conversion ratio; ME = metabolizable energy; NS = non significant; "-" = non determined. Significance is indicated as * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$). [1]MIU refers to the FFA fat source.

1.5.2. Use of acid oils in poultry diets

The studies evaluating the effects of dietary free fatty acid content in poultry diets are summarized in **Table 1.5.** The research group of Wiseman et al. investigated the effect of dietary FFA content on the fat AME value and fat digestibility in broiler chickens. For this, **Wiseman and Salvador (1991)** designed different dietary treatments blending palm fatty acid distillate, soybean acid oil or tallow acid oil with their respective native oils, to obtain increasing FFA dietary contents, and the blends were included into a basal diet at 4, 8 or 12%. Results reported showed that the increase of dietary FFA content lead to a decrease in the AME value and the ATTD of fats in both starter (11d of age) and growing (39d of age) broiler chickens. However, this effect was more pronounced in starter than in grower broiler chickens, as well as for palm oil in comparison to soybean oil, which suggested that the negative effect of FFA content in added fat has a greater impact in young broilers fed saturated fat sources. Later, **Wiseman et al. (1992)** calculated the AME and the ATTD of fat using blends of sunflower oil and sunflower acid oil in 32d old broiler chickens, obtaining similar results than for the previous study. In this case, the MIU value was reported, which was high for sunflower acid oil (9.03%).

Zumbado et al. (1994) studied, in broiler chickens, the effect of increasing levels of dietary FFA (blending palm oil with palm fatty acid distillate) on BW, FCR, dietary AME and ATTD of dietary fat. No differences were found for BW or FCR among different dietary FFA inclusion levels. Dietary AME showed similar values for diets including a <5% (control diet), 20%, 60% and >85% of FFA but lower values for diet including a 40% of FFA. In contrast, the ATTD of dietary fat showed no significant differences between dietary treatments.

The research group of Blanch et al. investigated the effects of including soybean acid oil in poultry diets. For this, **Blanch et al. (1995)** evaluated the effect of FFA on dietary AME and ATTD of fat comparing the inclusion of 4% of a blend of tallow + soybean oil (1:1) or of tallow + soybean acid oil (1:1; 34.2% FFA) in young broiler chickens (14d-old). Results indicated no effect of FFA on dietary AME, but ATTD of fat was decreased in those chicks fed the diet including a higher content in FFA (89.4 vs 81.5% of ATTD). Later, **Blanch et al. (1996)** reported similar results when comparing the inclusion of 4% of soybean oil or soybean

acid oil (64.7% of FFA) in Warren roosters diets (1-year-old), which showed no effect on dietary AME but a decrease on the ATTD of fat.

Zumbado et al. (1999) studied, in 25d-old broiler chickens, the effects of dietary inclusion of palm fatty acid distillate and "soybean FFA" (a mixture of acidulated soapstocks, gums and distilled FFA) in comparison to a control diet including palm oil. Diet including soybean FFA showed a similar BW and a better FCR, dietary AME and ATTD of fat than the control diet including palm oil. However, the diet including palm fatty acid distillate showed lower BW , dietary AME, ATTD of fat and a negative effect on FCR when compared to the control diet. The MIU values of experimental fats were not reported in this study.

Balevi et al. (2001) reported no effect on performance when using sunflower acid oil instead of sunflower oil in the diet of broiler chickens (Peterson x Avian; from 1 to 49d old). Moreover, no differences were observed in abdominal fat deposition of chickens, while the FA profile of abdominal fat reflected that of the diet. However, no percentage of dietary/oil FFA content is reported in this study.

In recent years, our research group at the Animal Nutrition and Welfare Service of the Universitat Autònoma de Barcelona has carried out several studies evaluating the inclusion of by-products from the oil industry in poultry diets, focusing especially on those rich in FFA. In this sense, **Vilarrasa et al. (2015b)** investigated the effects of dietary inclusion of palm fatty acid distillates (diet with 55.8% of FFA) and soybean acid oil (diet with 55.0% of FFA) in female broiler chicken diets in broiler chickens (Ross 308) from 0 to 40d of age. Regarding performance, diets rich in FFA did not show differences in any performance parameter when compared to control diets (palm oil or soybean oil diets). Moreover, diets rich in FFA showed lower AME values to their respective control diets in the starter (12d of age) period, but not for the finisher (36d of age) period where similar values were obtained among diets. However, the ATTD of dietary TFA showed lower values in FFA diets in both the starter and finisher periods, although this effect was more pronounced in the starter period. In both the dietary AME and fat ATTD, differences were more pronounced in treatments including palm oils than in those including soybean oils, since saturation degree played an important role. In addition, authors found that the FA profile of the abdominal fat was a clear reflection of the dietary FA profile regardless the dietary FFA content. Later, **Roll et al. (2018)** assessed the effects of

dietary inclusion (6%) of palm fatty acid distillate in comparison to palm oil. No effects were observed due to the dietary FFA content in any performance parameter throughout the study (from 0 to 42d of age). Dietary AME showed lower values for the diet including palm fatty acid distillate than for the palm oil diet in the starter (10d) period, although no differences were obtained in the finisher (39d) period. In this sense, ATTD of fat showed a decrease in the diet including palm fatty acid distillate at both ages, although differences were only statistically significant in the finisher period. However, differences were found at both periods for the ATTD of SFA, showing a clear negative impact of FFA on the ATTD of SFA.

The latest studies carried out by our research group, which are a part of the same project were the current PhD thesis has been developed, have focused on the utilisation of acid oils along the gastrointestinal tract. For this, the effect of the dietary FFA content on performance and FA digestibility along the gastrointestinal tract has been evaluated in starter (0 to 21d of age) (**Rodriguez-Sanchez et al., 2019a**) and in growing-finishing broiler chickens (from 22 to 37d of age) (**Rodriguez-Sanchez et al., 2021**). It is important to note that this was the first time the group assessed fat AID, since then it has only been studied as ATTD. For this, 8 dietary treatments (6% of added fat) were designed from blending soybean oil and soybean acid oil or palm oil and palm fatty acid distillate, varying in their FFA content (5, 15, 35 or 50% of FFA). In both starter and growing-finishing broiler chickens, no negative effect was observed in any performance parameter despite FFA content in the diet, neither for the palm oil nor for the soybean oil diets. Regarding dietary AME in young chicks, a linear decrease was found for soybean oil diets, which was up to approximately 500kcal/kg between extreme diets. However, similar AME values were obtained among the palm oil diets. In growing-finishing broiler chickens, no differences were observed in dietary AME neither for the soybean oil nor for the palm oil diets, indicating that the negative effect of FFA on dietary AME is limited to young ages. When digestibility of fat was measured along the gastrointestinal tract in 14d-old chicks, no negative effect was found on the AID of total FA, MUFA or PUFA digestibility when increasing dietary FFA content. However, high levels of dietary FFA (50%) had a negative impact on the AID of SFA when compared to low dietary FFA content (5%). In adult broilers, no differences were observed in the AID of TFA, SFA, MUFA, PUFA, so the negative impact of FFA on the AID of SFA may be also limited to young chicks. However, when assessing the FA ATTD, a clear negative effect of dietary FFA was reported in both starter and growing-

finishing broiler chickens. Since the ileum has been described to be the last gastrointestinal segment were FA absorption takes place (Jimenez-Moya et al., 2021a; b), the negative effect of dietary FFA found in the excreta may be influenced by bacterial activity or endogenous losses of fat, misleading the true impact of dietary FFA on FA digestibility. The authors concluded that the inclusion of acid oils in growing-finsihing broiler diets does not have a negative impact on FA absorption process as it has in young animals. Additionally, they argued that the degree of saturation affected the FA absorption to a greater extent than did the FFA content, being more efficient in unsaturated than in saturated diets irrespective of their FFA content.

Jimenez-Moya et al. (2021a) studied the effects of dietary replacement of palm oil with soybean acid oil in broiler chicken diets (from 0 to 35d of age). No effect was found due to dietary FFA content throughout the entire experimental period. In 11d old chicks, dietary AME was similar for palm oil diet and the blends of palm oil with soybean acid oil at different levels (up to 56% of dietary FFA), although it showed higher values for the diet including soybean oil. In contrast, the AID and ATTD of TFA increased with higher level of replacement of palm oil by soybean acid oils, and the diet including soybean acid oil (56% of dietary FFA) showed similar values than soybean oil diet. In growing broiler chickens (35d old), no negative effect of FFA on dietary AME or fat digestibility was reported. Authors concluded that soybean acid oil can replace palm oil in grower broiler chicken diets without impairing growth performance or fat utilization. In a parallel study with the same experimental design, **Jimenez-Moya et al. (2021b)** evaluated the effects of soybean oil replacement by palm fatty acid distillate in broiler chicken diets. In comparison with the previous study, the FFA source was saturated instead of unsaturated. No differences in any performance parameter were reported throughout the study due to the inclusion of high content of dietary FFA (up to 79%), but a tendency to decrease the abdominal fat deposition was reported. Regarding fat utilization, as dietary FFA increased, authors found that dietary AME decreased, as well as both the AID and ATTD of TFA, in 11d old chicks. In contrast, for 35d old chickens, these effect was less marked, and dietary AME, AID and ATTD of TFA decreased only in diets including a very high proportion of FFA (>53%), while diets with lower FFA content (<30%) showed no effect on AME or digestibility of fat. In agreement with Rodriguez-Sanchez et al. (2021), authors concluded that the effect of dietary saturation degree on dietary fat utilization is higher than that of the dietary FFA level. Furthermore, they comment that, as with SFA, the digestibility of

FFA increases with age, so the use of high FFA fat sources is recommended for growing broiler chicken diets, but not for starter diets.

Numerous studies have evaluated the effects of including different fat sources rich in FFA in poultry diets. However, there is a lack of consensus regarding the possible negative impact of FFA on dietary AME and fat digestibility. Most of the results that pointed out a negative effect are old, while more recent studies showed that the negative effect of FFA content is more limited to saturated fat sources and young ages. However, as observed in recent studies, this may be conditional on several factors, including the age of the animals, the degree of saturation or the quality of the added fat. On the other hand, many of the studies showed that a high dietary FFA content does not impair performance, so it could be assumed that the relevance of the possible negative impact on AME and digestibility is not sufficient to reveal a clear negative effect on performance. However, most studies were conducted in metabolic cages and with small numbers of animals, so reported performance parameters may not be fully representative of what occurs under commercial conditions. Additionally, it is important to mention that more attention needs to be paid to fat AID, as most studies only assessed fat digestibility as ATTD and microbial activity may play a role in the results reported.

Table 1.5. Studies evaluating the effects of dietary free fatty acid content in poultry diets.

Reference	FFA fat source (% of FFA)	Control	Inclusion (%)	MIU (%)	Breed - Strain	Period	Effects of FFA source on:				
							BW	FCR	Dietary AME	Digestibility of dietary fat	Fat depot
Wiseman and Salvador (1991)	Palm fatty acid distillate (91.8%), soybean acid oil (68.3%) and tallow acid oil (95.2%)	Palm oil, soybean oil and tallow, respectively	4, 8 or 12	-	Not specified (broiler chickens)	11d and 39d	-	-	Negative *** (fat AME)	Negative ***	-
Wiseman et al. (1992)	Sunflower acid oil (38.8%)	Sunflower oil	4, 8 or 12	9.03	Not specified (broiler chickens)	36d	-	-	Negative *** (fat AME)	Negative ***	-
Zumbado et al. (1994)	Blend of palm oil with palm fatty acid distillate (5-85% of FFA)	Palm oil	6	-	Not specified (broiler chickens)	Not specified	NS	NS	20, 60 or >85% FFA): NS 40% FFA: Negative **	NS	-
Blanch et al. (1995)	Blend of tallow + soybean acid oil at 1:1 (34.2%)	Blend of tallow + soybean oil at 1:1	4	1.05	Not specified (broiler chickens)	14d	-	-	NS	Negative **	-
Blanch et al. (1996)	Soybean acid oil (64.7%	Soybean oil	4	5.88	Warren roosters	1 year	-	-	NS	Negative **	-
Zumbado et al. (1999)	Palm fatty acid distillates (PFAD; 91.7%) and soybean FFA (SFFA; 50.6%)	Palm oil (PO)	10	0.85 (PFAD) and 0.98 (SFFA)	Not specified (broiler chickens)	25-29d	SFFA: NS PFAD: Negative *	SFFA: Positive * PFAD: Negative *	SFFA: Positive * PFAD: Negative *	SFFA: Positive * PFAD: Negative *	-
Balevi et al. (2001)	Sunflower acid oil (% of FFA not specified)	Sunflower oil	5	-	Peterson x Avian broiler chickens	0-49d	NS	NS	-	-	NS

Vilarrasa et al. (2015b)	Palm fatty acid distillate (PFAD; 55.8%) and soybean acid oil (SAO; 55.0%)	Palm oil (PO) and soybean oil (SO)	6	2.44 (PFAD) and 0.26 (SAO)	Ross 308 broiler chickens	0-40d	NS	NS	12d: Negative * 36d: NS	12d: Negative *** 36d: Negative *	NS
Roll et al. (2018)	Palm fatty acid distillate (88.6%)	Palm oil	6	-	Ross 308 broiler chickens	0-42d	NS	NS	10d: Negative *** 36d: NS	10d: Negative *** (only SFA) 36d: Negative ***	-
Rodriguez-Sanchez et al. (2019)	Blends of soybean oil and soybean acid oil or palm oil and palm fatty acid distillate (5, 15, 35 or 50%)	-	6	-	Ross 308 broiler chickens	0-21d	NS	NS	Palm acid oil: NS Soybean acid oil: Negative *	TFA, MUFA, PUFA (AID): NS SFA (AID): Negative *	-
Rodriguez-Sanchez et al. (2021)	Blends of soybean oil and soybean acid oil or palm oil and palm fatty acid distillate (5, 15, 35 or 50%)	-	6	4.32 (soybean acid oil) and 1.73 (palm fatty acid distillate)	Ross 308 broiler chickens	22-37d	NS	NS	NS	NS (AID)	-
Jimenez-Moya et al. (2021a)	Soybean acid oil (61.20%)	Soybean oil	6	5.34	Ross 308 broiler chickens	0-35d	NS	NS	11d: Negative *** 35d: NS	NS (AID)	NS
Jimenez-Moya et al. (2021b)	Palm fatty acid distillate (92.94%)	Palm oil	6	5.11	Ross 308 broiler chickens	0-35d	NS	NS	11d and 35d: Negative ***	11d and 35d: Negative *** (AID)	NS

Abbreviations: FFA = free fatty acid; MIU = moisture, impurities and unsaponifiable content; BW = body weight; FCR = feed conversion ratio; AME = apparent metabolizable energy; NS = non significant; "-" = non determined. Significance is indicated as * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$).

1.5.3. Use of acid oils in farmed fish diets

The studies evaluating the effects of dietary free fatty acid content in farmed fish diets are summarized in **Table 1.6. Ng et al. (2010)** evaluated the effect of replacing fish oil with palm fatty acid distillate in the diets of rainbow trout (133g of initial BW). Reported results indicate a positive effect on SFA digestibility when fish oil is replaced with palm fatty acid distillate, and a non-significant effect on MUFA, PUFA or total lipid digestibility. However, the results two sources with different FA profile, so the effect could not be directly attributed to dietary FFA content since no diet containing palm oil as a control was fed to fish. Therefore, results lack of a clear interpretation regarding the FFA effect.

As far as we know, the rest of the published studies evaluating the effects of acid oils in farmed fish species have been conducted in our research group by Trullàs et al. In this sense, **Trullàs et al. (2015)** studied the effects of including 21% dietary crude or acid rapeseed and palm oils on the FA digestibility of rainbow trout (412g of initial BW). Results reported indicate a decrease on FA digestibility for TFA, SFA, MUFA and PUFA when rapeseed or palm acid oils are fed to rainbow trout, which suggested that a high dietary content of FFA (47-49%) negatively affect FA digestibility. In a similar experimental design, **Trullàs et al. (2017a)** reported the same negative effect when rapeseed and palm acid oils (53.4% and 55.4% of FFA, respectively) were fed to gilthead sea bream (296g of initial BW), with this effect being more pronounced for SFA digestibility.

The results reported by **Trullàs et al. (2016)** and **Trullàs et al. (2017b)** investigated the effect of feeding for 72d rainbow trout (initial BW of 101g) with a diet including 15% of added rapeseed oil, rapeseed acid oil or a blend between both fat sources (at 2:1 or 1:2 ratio). Results reported showed that dietary FFA content had no significant effect on any performance parameter. Regarding digestibility of fat, diet containing rapeseed acid oil alone had lower digestibility values. In contrast, blends of rapeseed acid oil and rapeseed oil showed similar values than the control diet. Regarding quality characteristics of fillets, results observed showed that dietary FFA seemed to have no effect on fillet fat content, so the authors concluded that including a 15% of rapeseed oil in rainbow trout diets do not seem to produce relevant changes in flesh quality.

Very few studies have been performed with the aim to evaluate the effects of including a high dietary FFA content in fish diets, all of them focusing on the use of fat sources rich in SFA or PUFA. Therefore, no information regarding the effects of FFA using MUFA fat sources has been reported. On the other hand, none of the publications reported MIU values of the experimental fats. Moreover, many of the published literature is about rainbow trout (Oncorhynchus mykiss), and only the study of Trullàs et al. (2017a) evaluated the effect of acid oils in a marine species, gilthead seabream (Sparus aurata). As far as we know, no studies evaluating the effects of acid oils have been carried out in European sea bass (Dicentrarchus labrax), although it is one of the most important farmed marine fish species in Mediterranean aquaculture. Results reported seem to indicate a negative effect on FA digestibility when acid oils are fed to fish, especially for SFA. However, more studies are needed to clearly understand the role of dietary FFA on fish performance, fat digestibility and carcass and flesh parameters.

Table 1.6. Studies evaluating the effects of dietary free fatty acid content in farmed fish diets.

Reference	FFA fat source (% of FFA)	Control	Inclusion (%)	MIU (%)	Species	Experimental length (initial BW)	Effects of FFA source on:				
							BW	FCR	Feed digestible energy	Digestibility of dietary fat	Fat deposition and fillet quality
Ng et al. (2010)	Palm fatty acid distillate (31.5-60.3% dietary FFA)	Fish oil	10 or 15	-	Rainbow trout (*Oncorhynchus mykiss*)	42d (133g)	-	-	-	SFA: Positive * MUFA, PUFA and total lipid: NS	-
Trullàs et al. (2015)	Palm acid oil (55.4%) and rapeseed acid oil (53.4%)	Palm oil and rapeseed oil	21	-	Rainbow trout (*Oncorhynchus mykiss*)	21d (412g)	-	-	-	Negative *	-
Trullàs et al. (2016)	Rapeseed acid oil (64.3%) and blends with rapeseed oil (at 2:1 and 1:2)	Rapeseed oil	15	-	Rainbow trout (*Oncorhynchus mykiss*)	72d (101g)	NS	NS	-	Rapeseed acid oil: Negative * Blends: NS	-
Trullàs et al. (2017a)	Palm acid oil (55.4%) and rapeseed acid oil (53.4%)	Palm oil and rapeseed oil	21	-	Gilthead sea bream (*Sparus aurata*)	28d (296g)	-	-	-	Negative *	-
Trullàs et al. (2017b)	Rapeseed acid oil (64.3%) and blends with rapeseed oil (at 2:1 and 1:2)	Rapeseed oil	15	-	Rainbow trout (*Oncorhynchus mykiss*)	72d (101g)	-	-	-	-	NS

Abbreviations: FFA = free fatty acid; MIU = moisture, impurities and unsaponifiable content; BW = body weight; FCR = feed conversion ratio; NS = non significant; "-" = non determined. Significance is indicated as * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$).

1.6. References

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Chapter 2.

Hypotheses and objectives

"If you believe in nothing else, just keep believing in yourself. There will be times of trouble, it's gonna hurt like hell. This much I now. All ends well"

Alter Bridge.

Chapter 2. Hypotheses and objectives

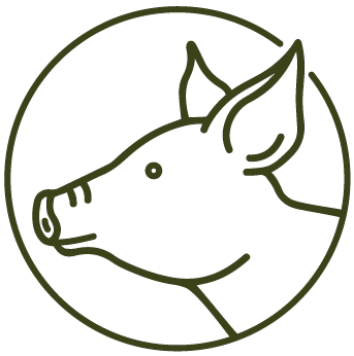
The present PhD dissertation is part of a public project (ref. AGL2015-64431-C2) aimed at improving knowledge on characterisation, nutritional value, effects on the final product quality and practical inclusion of acid oils in monogastric animal diets. This project is in the priority line of our research group in the search for new alternative sustainable, efficient and cost-effective fat by-products as energy sources for monogastric animal diets, following the research carried out in previous projects (ref. FP6 FOOD-CT-2004-007020; ref. AGL2010-2208-C02).

In the recent context, the price and availability of the commonly used ingredients in animal diets are extremely volatile, so the continuous search for competitive alternative fat sources is of high interest for feed manufacturers. As seen in the literature review, both olive pomace oil and olive pomace acid oil are two alternative fat sources that can be potentially interesting for their inclusion in monogastric animal diets. The main benefits of including these co-products of the olive oil industry in the diets are their high nutritional value (as they are monounsaturated fat sources, rich in oleic acid), their high availability for European feed manufacturers, the contribution to the circular economy system and to a potentially more efficient and sustainable production. However, the lack of information about the use of these two fat sources and the unclear results regarding the effect of dietary FFA in monogastric animal diets prompted the interest in developing this PhD thesis.

In this context, it was hypothesised that both olive pomace oil and acid oil could be suitable fat sources to be included in swine, poultry and fish diets. Therefore, the aim of the present thesis was to investigate the potential inclusion of crude and acid oils from olive pomace, rich in MUFA but differing tin the FFA content, as alternative fat sources for pig, broiler chicken and European seabass diets. The specific objectives were:

- To evaluate the effect on performance and feed efficiency of the animals.
- To assess the dietary energy value and FA digestibility of the diets.
- To measure the level of fat deposition and the FA profile of depot fat and final meat products obtained.

In order to approach the above mentioned objectives, three in vivo trials were performed in growing-finishing pigs, broiler chickens and European seabass. In these trials, the inclusion of olive pomace oil and acid oils was compared to a conventional fat source, which was palm oil for pigs and broilers, and fish oil for European seabass. In addition, some blends were designed in order to establish the best nutritional strategy to be used in feed formulation.



Chapter 3.
Inclusion of olive pomace oil and acid oil in pig diets

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"For my whole life, I didn't know if I even really existed. But I do, and people are starting to notice"

Arthur Flake, The Joker.

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Crude and acid oils from olive pomace as alternative fat sources in growing-finishing pigs

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ABSTRACT

The inclusion of crude and acid oils from olive pomace can lead to more unsaturated meat products and, especially in the case of olive pomace acid oil, achieve a more economically and environmentally sustainable swine production. The objective of this trial was to study the effect of dietary supplementation with crude and acid oils from olive pomace, which are rich in monounsaturated fatty acids (FAs) and have differing free FA content, on growth performance, digestibility, carcass parameters and FA profile of *Longissimus* muscle (LM) and backfat in growing-finishing pigs compared to the conventional crude palm oil. A total of 224 male and female pigs [(Landrace × Large White) × Duroc] were randomly distributed into 48 pens according to initial BW (58.7 ± 9.71 kg, mean ± SD) and sex. Four experimental treatments were randomly assigned (n = 12 pens/treatment; 4–5 pigs/pen) for the growing (0–42 days) and finishing (40–62 days) phases. Treatments consisted of a basal diet supplemented with 5% (as-fed basis) palm oil (PO), olive pomace oil (O), olive pomace acid oil (OA) or a mixture (M) of PO and OA at 50/50. No differences were found in the growth performance results between PO, O or M, but animals fed OA showed a lower gain to feed ratio than M (P = 0.008). No differences were found in apparent ileal digestibility among treatments, however, animals fed O and OA showed the highest values of total FA apparent total tract digestibility, while those fed PO had the lowest values, and M had intermediate values (P < 0.001). No differences were observed in carcass composition among treatments. In relation to backfat and the LM FA profile, O and OA treatments led to a higher unsaturated FA to saturated FA ratio and a lower content in saturated FA than PO. Moreover, O showed a higher intramuscular fat (IMF) content in LM than PO (P = 0.037). It is concluded that olive pomace oil is an interesting alternative fat source that can be included at 5% in growing-finishing pig diets, leading to meat products with more IMF, rich in monounsaturated FA, reaching high FA digestibility values and good pig performance parameters. Alternatively, olive pomace acid oil blended with conventional palm oil did not negatively impact fat utilisation nor performance. Including these fat by-products reduced feeding costs and led to a more efficient and environmentally sustainable production.

Implications

The use of crude and acid oils from olive pomace can increase the ratio of monounsaturated to saturated fatty acids in meat products. Moreover, the inclusion of olive pomace acid oil, by-product from the refining industry, in swine diets results in a more efficient and environmentally sustainable swine production. Our results indicate that crude and acid oils from olive pomace may be suitable alternative fat sources to commonly used saturated fats, such as palm oil. Moreover, including olive pomace oil may be a good nutritional strategy to both increase intramuscular fat content in *Longissimus* muscle and achieve good performance.

Introduction

The inclusion of fats and oils in monogastric animal feeding is a widespread practice due to their high energetic input and their supply of essential fatty acids (FAs), which contribute to efficient production. Moreover, dietary fat modifies lipid quality, and the nutritional and organoleptic properties of meat, which is of partic-

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ular interest in high-fat content crossbred pigs, such as those finished with Duroc lines.

Crude olive pomace oil is obtained by extraction from olive pomace, and olive pomace acid oil is a by-product generated from the soapstocks obtained during the chemical refining of the crude olive pomace oil. The FA profile of these two oils is rich in monounsaturated FA, particularly in oleic acid (C18:1 n-9; 55–83%), which consumption has been widely linked to many beneficial health traits (Isabel et al., 2004; Foscolou et al., 2018). The growing trend towards healthy meat products makes olive oil and its by-products interesting for use in pig feeding, as they can lead to high quality meat products, such as loin or cured ham with a lower saturated fat content and enriched in oleic acid. Olive pomace acid oil is rich in free FA (50–70%). Although it is well known that the degree of saturation, chain length and positional distribution in the triglycerides of the dietary FA affect their digestibility (Cho and Kim, 2012; Vilarrasa et al., 2015), there is some controversy in relation to the effects of dietary free FA content. Thus, while some authors have reported negative effects of free FA on digestible energy, which can impair performance (Powles et al., 1993; Wiseman et al., 1998; Jørgensen and Fernández, 2000), other authors have observed no effects on either digestible energy, FA digestibility or the performance of pigs (DeRouchey et al., 2004; Rojas-Cano et al., 2014; Vilarrasa et al., 2015) when including sources rich in free FA in pigs' diets.

Current animal production is expected to be efficient and environmentally sustainable. In this context, using food-chain fat by-products as alternative fat sources in swine feeding could be a good opportunity to reduce both the feeding costs and the environmental impact. Therefore, the aim of the present study was to research the potential of using crude and acid oils from olive pomace, rich in monounsaturated FA and differing in free FA content, as fat sources in growing-finishing pig diets. This was assessed by studying the effect of dietary supplementation with crude olive pomace oil and olive pomace acid oil on growth performance, digestibility, carcass parameters and the FA profile of the L*ongissimus* muscle (LM) and backfat in growing-finishing pigs compared to conventional crude palm oil as a conventional dietary fat.

Material and methods

Experimental fats

Crude olive pomace oil and olive pomace acid oil were supplied by RIOSA S.A. (Jaén, Spain), and crude palm oil was provided by bonÀrea Agrupa (Guissona, Spain). All samples were analysed in duplicate for FA composition, lipid class composition, moisture, impurities and unsaponifiable matter as described by Varona et al. (2021a).

Experimental design and diets

The study was performed at the animal experimental facilities of bonÀrea AGRUPA (Nial farm, Guissona, Lleida, Spain). All animal housing and husbandry were in accordance with the European Union Guidelines (2010/63/EU). The experiment was planned to cover the BW range from 60 to 130 kg BW. Therefore, the feeding programme consisted of two diets (in pelleted form): a grower diet (from 60 to 103 kg average BW; from 0 to 40 days of the experimental period) and a finisher diet (from 103 to 130 kg BW; from 41 to 62 days of the experiment). The ingredients of the experimental diets are shown in Table 1. Basal diets were formulated to meet or exceed requirements (FEDNA, 2013) and to minimise basal fat levels. Silicate (Ibersil D-100 M; IQESIL S.A., Zaragoza, Spain) was added to the diets (2.74% as-fed basis) to increase the amount of hydrochloric acid-insoluble ash as an inert digestibility marker. Four experimental treatments were obtained as the result of adding, to the same basal diet, 5% (as-fed basis) of different fat sources: crude palm oil (PO), crude olive pomace oil (O), olive pomace acid oil (OA) and a mixture (M) of PO + OA 50/50. Thus, 12 replicates per treatment were obtained, six per sex and four per block of BW.

Table 1
Ingredient composition of the experimental diets (as-fed basis) for growing-finishing pigs.

Diet composition	Experimental period	
	Grower (from 0 to 40 days)	Finisher (from 41 to 62 days)
Ingredients, %		
Corn meal	30.00	17.98
Barley	8.00	18.07
Sorghum	11.70	16.15
Wheat	10.00	10.00
Soybean meal 47%	15.95	10.96
Wheat bran	5.75	8.00
Experimental fat[1]	5.00	5.00
Silicate	2.74	2.74
Cane molasses	2.00	–
Sunflower meal 30%	5.00	7.04
Calcium carbonate	0.90	1.12
Di-calcium phosphate	0.65	0.64
Sodium chloride	0.55	0.60
Vitamin and mineral premix[2]	0.61	0.61
DL-Methionine[3]	0.17	0.13
L-Lysine[4]	0.73	0.74
L-Tryptophan	0.02	0.02
L-Threonine	0.19	0.18
L-Valine	0.04	0.02
Predicted values[5]		
Net energy, MJ/kg	10.21	10.13
CP, %	15.81	15.12
Standardised ileal digestible lysine, %	0.99	0.92
Ca, %	0.74	0.81
P, %	0.50	0.52

[1] Crude palm oil (PO), crude olive pomace oil (O), olive pomace acid oil (OA) or a mixture (M) of PO + OA at 50/50.
[2] Provides per kg of feed: vitamin A, 5 995 IU; Vitamin D3, 1 497 IU; Vitamin E, 15 ppm; Fe, 100 ppm ($FeSO_4 \cdot H_2O$); I, 0.3 ppm (KI); Cu, 18 ppm ($CuSO_4 \cdot 5H_2O$); Mn, 40 ppm (MnO_2); Zn, 94 ppm (ZnO); Se, 0.34 ppm (Na2SeO3).
[3] DL-2-hydroxy-4-methylthiobutanoic acid (HMTBa), the hydroxy analogue of DL-methionine.
[4] L-Lysine sulphate.
[5] Ted values from the theoretical formulation of the diets.

A total of 224 boars and gilts [(Landrace × Large White) × Duroc] were obtained from the swineherd of the same facility. Pigs (age of 103 ± 3 days) were individually weighed (58.7 ± 9.71 kg of BW, mean ± SD) and randomly allocated to 48 pens and two different barns according to four dietary treatments and sex. There were a total of 12 pens per treatment, six for males and six for females. Pens were classified in one of three BW blocks (light 48.4 ± 8.19 kg, medium 60.2 ± 6.18 kg, and heavy 68.3 ± 5.91 kg BW, mean ± SD, respectively) balanced within sex (boars 59.0 ± 9.39 kg and gilts 59.7 ± 8.27 kg) and treatment (PO 59.0 ± 8.43 kg, O 59.4 ± 9.35 kg, OA 58.7 ± 8.43 kg, and M 59.3 ± 9.82 kg BW, mean ± SD, respectively; according to two pens of light, medium and heavy BW per sex within each treatment). There were four pigs per pen for heavy animals and five pigs per pen for medium and light animals. Each pen (1.13 m^2 per animal for heavy pigs and 0.91 m^2 per animal for medium and light pigs) had a half slatted concrete floor, a feeder and a nipple waterer. All the animals had ad libitum access to feed and water during the entire trial. No mortality or sick animals were observed throughout the experimental period.

Controls and sampling

Feed consumption (pen basis) and individual BW of the animals were recorded at days 0, 40 and 62 of the experiment. This was used to calculate the average daily feed intake, average daily gain and gain to feed ratio per pen for each period and for the overall study. The digestibility balance was determined from day 40 to day 62 in a subset of 64 pigs (n = 16 animals/treatment). Animals selected for the digestibility balance were those closest to the average BW for each group of each sex within each treatment (at least one pig from each pen). Faecal samples were collected on the last two days of the study (days 61 and 62) from selected animals (average marketing weight of 130.00 ± 16.73 kg BW, mean ± SD) by rectal stimulation and samples were pooled immediately after the second day of collection. Then, all the animals were slaughtered (same day, at 166 ± 3 days of age), and the ileal content was collected at the slaughterhouse from the same selected animals for faces collection. All samples were immediately homogenised, freeze-dried (LyoAlpha 10/15; Telstar, Barcelona, Spain), ground (1 mm screen diameter) and kept at 5 °C until further analysis.

Backfat thickness was individually measured by ultrasound (Future-1; Inserbo, Lleida, Spain) at the midline between the last thoracic and first lumbar vertebrae (P2) at the start (5.49 ± 1.09 mm) and at the end of the study. In addition, carcass quality parameters were monitored in a subset of 100 pigs at the slaughterhouse. Animals selected for carcass quality assessment were those closest to the average BW within each group of BW (eight heavy, nine medium and eight light) for each treatment, equally for each pen (two animals per pen) and sex (in medium block of BW, five males and four females were selected). Pigs were fasted (deprived of feed but not water) for 20 h (except in pigs selected for digestibility balance, which were not fasted), and weighed the following morning to obtain the fasted live weight. Animals were stunned with 85% CO_2 for 120 s and immediately exsanguinated at the commercial slaughterhouse of bonÀrea Agrupa (La Closa; Guissona, Spain). Ham fat thickness and lean meat percentage were obtained with an AutoFom III ultrasonic system (Frontmatec A/C; Herlev, Denmark). Backfat and LM samples were obtained (n = 18 samples/treatment) from the dorsal midline between the last rib and the first lumbar vertebrae. Animals selected for backfat and muscle sampling were the 18 females that were closest to the average BW within each treatment (at least one from each pen). Samples were homogenised and stored at 20 °C until the chemical analyses were performed.

Chemical analysis

Analytical determinations of the feeds were performed according to AOAC International (2005) methods: DM (Method 934.01), ash (Method 942.05), CP (Method 954.01), ether extract (Method 920.39) and crude fibre (Method 962.09). The gross energy was determined with an adiabatic calorimeter (Parr 6300 Calorimeter, Parr Instrument Company, Moline, IL, USA) according to the Standard UNE-EN ISO 9831:2004. Lipid class composition was analysed by size exclusion HPLC with refractive index detection, following the method described by Varona et al. (2021a). The FA content of feed, ileal content and faeces were analysed following the method described by Sukhija and Palmquist (1988). Backfat and LM FAs were analysed by gas chromatography following the method described by Carrapiso et al. (2000). Nonadecanoic acid (C19:0; Sigma-Aldrich Chemical Co.; St. Louis, MO, USA) was added as internal standard. The final extract obtained was injected in a gas chromatograph (HP6890, Agilent Technologies; Waldbronn, Germany) following the method conditions described by Cortinas et al. (2004). The intramuscular fat content (IMF) of the LM minced and homogenised samples was determined by near infrared transmittance spectroscopy (FoodScan TM; Foss Analytical, Hillerød, Denmark), previously validated (Font-i-Furnols et al., 2012), at wavelengths between 850 and 1048 nm. Hydrochloric acid-insoluble ash was determined in feeds, ileal content and faeces according to the methods of the European Commission Regulation no. 152/2009.

Calculations

The apparent digestibility of a particular FA (X) was calculated as follows:

$$\%\ apparent\ digestibility\ of\ X = \left[1 - \left(\frac{Xf}{Mf} \div \frac{Xd}{Md}\right)\right] \times 100$$

where Xf is the concentration of a particular FA in faeces or ileal content, Mf is the concentration of the inert marker in faeces or ileal content, Xd is the concentration of a particular FA in the diet, and Md is the concentration of the inert marker in the diet. The digestible energy of feeds was calculated from the product of energy apparent digestibility and its corresponding feed gross energy.

Statistical analysis

The normality of the data and homogeneity of variance were verified using the CAPABILITY procedure of SAS (version 9.4, SAS Inst. Inc.; Cary, NC, USA). Performance parameters were analysed using the MIXED procedure of SAS. Diet, block and sex were defined as the main factors and room was defined as a random effect. Digestibility coefficients, FA composition of LM and backfat, carcass composition and IMF were analysed by using the GLM procedure of SAS. For digestibility balance, diet and sex were defined as the main factors. For carcass composition and IMF, diet, sex and block were defined as the main factors. For FA composition of LM and backfat, diet was defined as the main factor. No interactions between diet, sex and block were observed for any of the variables studied. For the performance parameters, the experimental unit was the pen. For digestibility balance, FA composition, carcass parameters and IMF, the experimental unit was the individual. For all statistical analyses, differences between means were tested using Tukey's adjust correction for multiple comparisons. The results in the tables are reported as least square means. For all statistical analyses, significance was declared at $P < 0.05$ and tendencies were discussed at $0.05 > P > 0.10$.

Results

Experimental fats and diets

Composition of the experimental fats is presented in Table 2. Crude olive pomace oil and olive pomace acid oil were rich in monounsaturated FA, in particular in oleic acid (70.5 and 65.0%, respectively), while crude palm oil was composed mainly of palmitic (42.4%) and oleic (41.6%) acids in similar proportions. Both crude palm oil and crude olive pomace oil, as crude oils, were composed of triacylglycerols (>80%). In contrast, olive pomace acid oil, a by-product of the olive pomace oil refining process, had the highest amount of free FA (53.98%). Olive pomace acid oil had the highest moisture, impurities and unsaponifiable values (MIU; 12.67%), while crude olive pomace oil (4.65%) and crude palm oil (0.49%) had the lowest values. Composition of the experimental diets is presented in Table 3. All diets showed a similar content in gross energy and all macronutrients analysed. The fatty acid and lipid class composition of the experimental diets resemble those of the added fats, and M was at the midpoint between PO and OA for all analysed parameters.

Table 2
Composition of experimental fats[1] included in the diets of growing-finishing pigs.

	Experimental fats		
Item	PO	O	OA
Fatty acid composition (g/100 g of FA)			
C14:0	0.85	–	–
C16:0	42.43	12.95	13.59
C18:0	4.57	2.60	3.62
C18:1 n-9	41.62	70.47	64.97
C18:2 n-3	0.28	0.89	0.97
C18:2 n-6	9.73	12.03	15.03
C20:0	0.32	0.41	0.56
C20:1 n-9	0.13	0.32	0.29
C22:0	–	–	0.45
C24:0	–	–	0.44
Saturated FA	48.25	16.14	18.67
Monounsaturated FA	41.75	70.79	65.25
Polyunsaturated FA	10.01	12.93	16.00
Unsaturated FA to Saturated FA ratio	1.07	5.19	4.35
Lipid class composition (g/100 g of FA)			
Triacylglycerols	86.67	82.36	23.98
Diacylglycerols	8.36	8.25	19.76
Monoacylglycerols	0.63	0.56	2.29
Free FA	4.34	8.82	53.98
MIU (g/100 g of fat)			
Moisture	0.14	0.28	1.27
Impurity	0.13	0.79	7.84
Unsaponifiable	0.22	3.58	3.56

Abbreviations: PO = crude palm oil; O = crude olive pomace oil; OA = olive pomace acid oil; FAs = fatty acids; MIU = moisture, impurities and unsaponifiable.
[1] All samples were analysed in duplicate.

Performance and feed intake

The effects of the dietary fat sources on growth performance are shown in Table 4. No differences were observed among dietary treatments during the grower period (from day 0 to day 40 of the experiment; 59.3 ± 8.74–104 ± 10.92 kg BW) in any performance parameter (P > 0.10). During the finishing period (from day 40 to day 62 of the experiment; 104 ± 10.92–131.7 ± 12.67 k g BW), animals fed M showed a higher average daily gain than those fed OA (P = 0.005). Concerning the entire experimental period (from 0 to 62d), animals fed OA showed a lower average daily gain than those fed M or PO (P = 0.006). In addition, the gain to feed ratio in animals fed OA was lower than in those fed M (P = 0.008), and tended to be lower than in those fed O (P = 0.074) or PO (P = 0.076). No differences were observed in the final BW or in the global average daily feed intake among treatments (P > 0.10). Regarding sex, gilts showed a lower BW, average daily gain and gain to feed ratio than boars considering the entire experimental period (P < 0.001). Concerning block of BW, differences between heavy, medium and light animals were maintained throughout the experimental period for BW, average daily feed intake and gain to feed ratio (P < 0.001). However, no differences between blocks of BW were observed on the average daily gain when considering the entire experimental period (P = 0.807) although heavy animals showed a higher average daily gain than light animals in the growing period (from 0 to 40 days; P = 0.022).

Digestibility balance

Feed digestible energy and FA apparent ileal (AID) and total tract (ATTD) digestibility values are presented in Table 5. Regarding dietary treatments, no significant differences were observed in AID (P > 0.10); however, pigs fed O showed a tendency to have higher values than PO in the AID of total FA (P = 0.078), monounsaturated FA (P = 0.066) and oleic acid (P = 0.071). In addition, M showed a tendency to have higher values than PO in the AID of palmitic acid (P = 0.092). Regarding ATTD, OA and M showed a higher DE than PO (P = 0.001). In addition, O and OA showed the highest values for total FA (P < 0.001). OA had the highest values in the ATTD of monounsaturated FA and polyunsaturated FA, while PO had the lowest (P < 0.001). M showed higher values for saturated FA ATTD than O (P = 0.032). Concerning individual FA, OA showed the highest ATTD values for palmitic, oleic and linoleic acids (P < 0.001). However, very low values were obtained in the ATTD of stearic acid, which were even negative in the case of O and OA. In terms of sex, no differences were obtained in the AID (P > 0.10), but higher values were obtained for ATTD of monounsat-

Table 3
Analysed[1] macronutrient content and fatty acid and lipid class composition of the experimental diets of growing-finishing pigs.

	Grower diets				Finisher diets			
Item	PO	O	OA	M	PO	O	OA	M
Macronutrient content (g/100 g of feed)								
DM	88.43	87.28	87.75	87.40	89.25	88.85	89.07	89.04
CP	16.05	15.53	15.61	16.65	15.60	15.33	15.11	15.45
Ether extract	6.55	6.32	6.63	6.43	6.79	6.29	6.44	6.44
Crude fibre	4.45	4.15	4.13	4.31	4.65	4.84	4.33	4.67
Ash	6.38	6.66	7.06	6.77	6.59	6.74	6.10	6.67
Gross energy (MJ/kg)	17.28	16.97	16.89	17.03	17.18	17.12	17.33	17.27
Fatty acid composition (g/100 g of FA)								
C16:0	30.02	13.68	13.24	23.07	30.56	15.11	14.40	24.27
C18:0	4.05	2.74	2.99	3.60	3.86	2.78	2.97	3.55
C18:1 n-9	35.21	46.51	46.00	39.03	34.10	45.94	44.96	38.86
C18:2 n-6	27.43	31.62	32.33	29.97	27.83	30.58	31.91	28.59
C18:3 n-3	1.42	1.87	1.86	1.66	1.66	2.04	2.09	1.72
Minor fatty acids	1.87	3.58	3.58	2.67	1.99	3.55	3.67	3.01
Saturated FA	34.97	17.50	17.55	27.71	35.38	18.93	18.72	29.19
Monounsaturated FA	36.19	49.01	48.26	40.65	35.14	48.45	47.28	40.50
Polyunsaturated FA	28.85	33.49	34.19	31.64	29.49	32.62	34.00	30.31
Lipid class composition (g/100 g of FA)								
Triacylglycerols	83.69	70.72	49.26	68.21	81.19	67.13	44.77	64.37
Diacylglycerols	8.89	11.12	14.90	11.77	9.55	11.91	15.05	12.39
Monoacylglycerols	0.93	1.27	1.66	1.27	1.09	1.40	1.63	1.54
Free FA	6.49	16.88	34.19	18.75	8.17	19.56	38.55	21.70

Abbreviations: PO = crude palm oil; O = crude olive pomace oil; OA = olive pomace acid oil; M = mixture of PO + OA at 50/50; FAs = fatty acids.
[1] All samples were analysed in duplicate.

Table 4
Performance parameters in growing–finishing pigs fed different dietary fat sources.

	Dietary treatments					*P*-values		
Item[1]	PO	O	OA	M	SEM[2]	Diet	Sex[3]	Block[4]
From 0 to 40 days								
BW at 0d (kg)	59.0	59.4	58.7	59.3	0.89	0.942	0.175	<0.001
Average daily feed intake (g)	2 593	2 561	2 594	2 581	37.00	0.930	0.487	<0.001
Average daily gain (g)	1 151	1 130	1 098	1 121	28.79	0.257	<0.001	0.022
Gain to feed ratio (g/kg)	444	443	424	436	1.19	0.178	<0.001	<0.001
From 40 to 62 days								
BW at 40d (kg)	105.1	104.5	102.6	104.1	1.69	0.633	0.009	<0.001
Average daily feed intake (g)	3 168	3 115	3 045	3 114	170.70	0.491	0.016	0.002
Average daily gain (g)	1 235^{ab}	1 185^{ab}	1 155^{b}	1 273^{a}	51.53	0.005	<0.001	0.056
Gain to feed ratio (g/kg)	391	386	380	412	0.96	0.110	<0.001	<0.001
From 0 to 62 days								
BW at 62d (kg)	133.0	131.5	128.8	133.5	4.69	0.119	<0.001	<0.001
Average daily feed intake (g)	2 802	2 737	2 785	2 768	72.53	0.748	0.352	<0.001
Average daily gain (g)	1 181^{a}	1 154^{ab}	1 119^{b}	1 186^{a}	51.18	0.006	<0.001	0.807
Gain to feed ratio (g/kg)	422^{ab}	423^{ab}	403^{b}	429^{a}	1.02	0.008	<0.001	<0.001

Abbreviations: PO = crude palm oil; O = crude olive pomace oil; OA = olive pomace acid oil; M = mixture of PO + OA at 50/50.
^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] Values of average daily feed intake and gain to feed ratio expressed as-fed basis.
[2] $n = 12$.
[3] Boars vs gilts.
[4] Three blocks of BW: light 48.37 ± 8.19 kg, medium 60.22 ± 6.18 kg, and heavy 68.33 ± 5.91 kg BW, mean ± SD of initial BW.

Table 5
Feed digestible energy (MJ/kg) and fatty acid apparent digestibility (%, ileal and total tract) in finishing pigs fed different dietary fat sources.

	Dietary treatments					*P*-values	
Item	PO	O	OA	M	SEM[1]	Diet	Sex[2]
Apparent ileal digestibility							
Digestible energy, MJ/kg	12.55	12.32	11.82	12.04	0.34	0.319	0.489
Total FA	76.21	83.07	81.56	82.50	2.11	0.078	0.922
Saturated FA	54.19	58.50	60.63	66.94	4.27	0.177	0.926
Monounsaturated FA	85.56	90.29	89.38	90.06	1.50	0.066	0.991
Polyunsaturated FA	80.56	86.36	82.19	87.01	3.13	0.336	0.749
C16:0	61.13	68.93	70.25	71.33	3.36	0.092	0.866
C18:0	22.64	23.69	19.44	41.96	8.62	0.236	0.405
C18:1 n-9	86.13	90.43	89.56	90.30	1.40	0.071	0.964
C18:2 n-6	82.38	87.14	83.56	87.63	2.85	0.421	0.685
C18:3 n-3	87.25	92.25	88.29	89.69	1.19	0.233	0.997
Apparent total tract digestibility							
Digestible energy, MJ/kg	13.88^{b}	14.07^{ab}	14.20^{a}	14.20^{a}	0.06	0.001	0.097
Total FA	79.63^{c}	87.94^{a}	89.31^{a}	85.26^{b}	0.54	<0.001	0.237
Saturated FA	51.81^{ab}	50.56^{b}	54.19^{ab}	58.81^{a}	2.08	0.032	0.222
Monounsaturated FA	93.81^{c}	95.94^{b}	96.94^{a}	94.63^{c}	0.25	<0.001	0.031
Polyunsaturated FA	96.66^{c}	97.56^{b}	98.19^{a}	97.50^{b}	0.12	<0.001	0.026
C16:0	60.75^{c}	73.50^{b}	81.13^{a}	69.31^{b}	1.37	<0.001	0.003
C18:0	2.31^{a}	—26.79^{b}	—49.31^{b}	0.94^{a}	6.56	<0.001	0.551
C18:1 n-9	94.13^{d}	96.19^{b}	97.19^{a}	95.06^{c}	0.24	<0.001	0.045
C18:2 n-6	96.50^{c}	97.44^{b}	98.13^{a}	97.50^{b}	0.12	<0.001	0.002
C18:3 n-3	100.00	100.00	100.00	100.00	.	.	.

Abbreviations: PO = crude palm oil; O = crude olive pomace oil; OA = olive pomace acid oil; M = mixture of PO + OA at 50/50; FAs = fatty acids.
$^{a-d}$Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] $n = 16$.
[2] Boars vs gilts.

urated FA and polyunsaturated FA in females ($P < 0.05$). When considering individual FA, females had a higher ATTD for palmitic acid (C16:0; $P = 0.003$), oleic acid (C18:1 n-9; $P = 0.045$) and linoleic acid (C18:2 n-6, $P = 0.002$).

Carcass quality and intramuscular fat

The effects of dietary treatments on carcass yield, composition and LM IMF are shown in Table 6. No differences were observed in carcass weight, yield and lean percentage or ham and backfat thickness among dietary treatments ($P > 0.10$). However, some differences were found in terms of BW block. As expected, heavy animals (140.8 ± 15.5 kg BW) had the highest carcass weight while light animals (119.2 ± 12.0 kg BW) had the lowest (111.2 vs 90.0 kg carcass weight for heavy and light animals, respectively; $P < 0.001$). Moreover, in contrast to light animals, heavy animals showed a greater ham fat thickness (13.29 vs 10.93 mm of ham fat thickness for heavy and light animals, respectively; $P = 0.004$)

Carcass yield, composition and *Longissimus* muscle intramuscular fat of fattening pigs according to different dietary fat sources.

	Dietary treatments					*P*-values		
Item	PO	O	OA	M	SEM[1]	Diet	Sex	Block
Carcass weight (kg)	101.7	99.9	100.4	102.2	2.52	0.900	0.230	<0.001
Carcass yield (%)	73.59	73.95	73.90	72.71	0.91	0.743	0.109	0.555
Carcass lean percentage (%)	60.26	60.78	61.45	61.06	0.57	0.495	0.734	0.916
Backfat thickness[2] (mm)	10.85	10.77	9.93	10.80	0.72	0.198	0.223	0.053
Ham fat thickness (mm)	12.84	11.95	11.80	12.18	0.58	0.585	0.845	0.004
Intramuscular fat[3] (%)	1.78^{b}	2.21^{a}	2.15^{ab}	2.07^{ab}	0.107	0.037	<0.001	0.844

Abbreviations: PO = crude palm oil; O = crude olive pomace oil; OA = olive pomace acid oil; M = mixture of PO + OA at 50/50.
^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] $n = 25$. For intramuscular fat, $n = 18$.
[2] Backfat thickness measurement made by ultrasound between the last thoracic and first lumbar vertebrae (P2) in live pigs.
[3] Measured by near infrared transmittance spectroscopy (FoodScan TM; Foss Analytical, Hillerød, Denmark).

and tended to have a thicker backfat thickness (11.10 vs 10.03 mm of backfat thickness for heavy and light animals, respectively; $P = 0.053$). Animals fed O showed a higher IMF value than those fed PO ($P = 0.037$). In addition, a sex effect was observed, since females showed a higher IMF level (2.27 mm) than males (1.88 mm; $P < 0.001$).

Fatty acid composition of backfat and Longissimus muscle

The fatty acid composition of backfat and LM are presented in Tables 7 and 8, respectively. The FA profiles of backfat and LM resemble the profile of the fat sources supplemented in the feed. Animals fed O or OA had a higher unsaturated FA to saturated FA ratio than animals fed PO. In relation to backfat, animals fed PO had the highest level of saturated FA while those fed O or OA had the lowest ($P = 0.034$). Animals fed O had the highest monounsaturated FA levels and those fed PO had the lowest ($P < 0.001$). Therefore, animals fed O had the highest unsaturated FA to saturated FA ratio while animals fed PO had the lowest, and diets OA and M presented intermediate values ($P < 0.001$). In terms of individual FA, oleic acid showed the highest values for O or OA and linolenic acid showed the highest values for O, OA or M. Palmitic and stearic acid showed the highest values for PO ($P < 0.001$). No differences were observed for linoleic acid.

In relation to LM, the lowest levels of saturated FA ($P < 0.001$) were observed in animals fed O or OA. Moreover, PO showed higher values of palmitic acid than OA ($P = 0.026$) and higher values of stearic acid than O ($P = 0.001$). Animals fed O had a higher monounsaturated FA content, in particular oleic acid, than those fed PO ($P = 0.003$). No differences were observed for linoleic or linolenic acids. In terms of unsaturated FA to saturated FA ratio, the highest values were obtained with the O and OA diets, while the lowest value was obtained with the PO diet ($P = 0.001$).

Discussion

Performance and feed intake

The study results show that including crude olive pomace oil, rich in monounsaturated FA and triacylglycerols, as an alternative fat source to conventional crude palm oil did not modify the performance parameters in any phase or in the overall trial. On the other hand, the biological response obtained regarding the effects of sex and block of BW were as expected. These results are in agreement with previous studies that include olive oil in the diets of growing-finishing pigs, which are similar in FA composition to O, and which did not find differences in growth performance compared to other commonly used fat sources, such as tallow or soybean oil (Park et al., 2012). Other studies have reported no negative effects on growth performance when olive pomace cake (with an approximate content of 28% fat) was added to growing-finishing pig diets (Ferrer et al., 2020).

Table 7
Fatty acid composition (g/100 g of FA) of backfat from finishing pigs according to different dietary fat sources.

	Dietary treatments					
Item	PO	O	OA	M	SEM[1]	*P*-value
C14:0	1.11	1.05	1.11	1.09	0.03	0.212
C16:0	22.71^{a}	19.82^{c}	20.25^{c}	21.20^{b}	0.22	<0.001
C16:1	1.51	1.56	1.62	1.44	0.06	0.159
C17:0	0.22	0.20	0.23	0.22	0.23	0.093
C18:0	12.56^{a}	10.39^{c}	10.96^{bc}	11.64^{b}	0.20	<0.001
C18:1 n-9	42.20^{c}	46.31^{a}	44.12^{a}	43.33^{bc}	0.36	<0.001
C18:1 n-7	1.93^{b}	2.31^{a}	2.26^{a}	1.99^{b}	0.04	<0.001
C18:2 n-6	15.06	15.29	16.22	16.16	0.43	0.117
C18:3 n-3	0.67^{b}	0.80^{a}	0.83^{a}	0.79^{a}	0.02	<0.001
C20:0	0.19	0.15	0.19	0.19	0.01	0.060
C20:1 n-9	0.73	0.79	0.80	0.76	0.03	0.289
C20:2	0.58	0.62	0.65	0.63	0.02	0.096
C20:4 n-6	0.23^{b}	0.25^{ab}	0.28^{a}	0.28^{a}	0.01	0.007
Saturated FA	37.14^{a}	31.67^{c}	32.79^{c}	34.41^{b}	0.39	0.034
Monounsaturated FA	46.37^{c}	51.24^{a}	48.78^{b}	47.58^{bc}	0.43	<0.001
Polyunsaturated FA	16.12	16.47	17.50	17.38	0.46	0.083
Unsaturated FA to Saturated FA ratio	1.68^{c}	2.14^{a}	2.01^{b}	1.89^{b}	0.04	<0.001

Abbreviations: PO = crude palm oil; O = crude olive pomace oil; OA = olive pomace acid oil; M = mixture of PO + OA at 50/50; FAs = fatty acids.
$^{a-c}$Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] $n = 18$.

Table 6
Fatty acid composition (g/100 g of FA) of *Longissimus* muscle from finishing pigs according to different dietary fat sources.

	Dietary treatments					
Item	PO	O	OA	M	SEM[1]	*P*-value
C14:0	1.05	1.06	1.06	1.06	0.03	0.988
C16:0	22.57[a]	21.70[ab]	21.60[b]	22.17[ab]	0.25	0.026
C16:1	2.50	2.68	2.57	2.58	0.10	0.642
C17:0	0.13	0.13	0.20	0.15	0.02	0.131
C18:0	12.51[a]	11.58[b]	11.99[ab]	12.04[ab]	0.15	0.001
C18:1 n-9	38.41[b]	41.32[a]	39.64[ab]	40.17[ab]	0.53	0.003
C18:1 n-7	3.18[b]	3.48[a]	3.30[ab]	3.27[ab]	0.07	0.024
C18:2 n-6	14.32	13.31	14.30	13.55	0.61	0.531
C18:3 n-3	0.38	0.41	0.41	0.39	0.02	0.485
C20:0	0.13	0.14	0.08	0.13	0.02	0.249
C20:1 n-9	0.62	0.63	0.63	0.64	0.01	0.687
C20:2	0.54	0.51	0.56	0.54	0.02	0.320
C20:4 n-6	3.13	2.81	3.19	2.99	0.19	0.487
C21:0	0.35	0.34	0.36	0.35	0.02	0.836
Saturated FA	36.75[a]	34.70[b]	35.32[b]	35.74[ab]	0.33	<0.001
Monounsaturated FA	44.71[b]	48.10[a]	46.16[ab]	46.69[ab]	0.66	0.006
Polyunsaturated FA	18.00	16.52	17.96	16.85	0.80	0.442
Unsaturated FA to Saturated FA ratio	1.71[b]	1.86[a]	1.82[a]	1.79[ab]	0.03	0.001

Abbreviations: PO = crude palm oil; O = crude olive pomace oil; OA = olive pomace acid oil; M = mixture of PO + OA at 50/50; FAs = fatty acids.
[a–b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] $n = 18$.

However, these results showed that including olive pomace acid oil, rich in monounsaturated FA and free FA, at 5% in the growing-finishing diets decreased the average daily gain and therefore reduced the gain to feed ratio. Despite this, there are few studies that assess the effect of including fat by-products rich in free FA on the growth performance of growing pigs. DeRouchey et al. (2004) observed a linear increase in average daily feed intake as the free FA concentration increased (up to 53% free FA; MIU < 3.3) when choice white grease was included in weaning pigs' diets. Rojas-Cano et al. (2014) did not find an improvement in the performance of growing pigs when the dietary energy level was increased by including increasing levels of olive acid oil up to 75 g/kg. However, Vilarrasa et al. (2015) observed no differences in the growth performance of growing pigs fed palm fatty acid distillates (53% of free FA) in contrast to palm oil added at 4%. The inconsistent results among studies that include free FA rich sources in swine diets might be explained by the high variability in the composition and quality of available fat by-products. It has been stated that the MIU value of the added acid oils and fatty acid distillates could lead to a decrease in the DE of pigs (Wiseman et al., 1992; Varona et al., 2021b). In agreement with this, the present study found the highest MIU values for olive pomace acid oil (12.67%), while crude olive pomace oil or crude palm oil had values of 4.64 and 0.49%, respectively. Although most of the studies available in the literature did not report values of MIU, it is important to highlight the significance of knowing the non-energetic fraction of dietary added fats for a correct assessment of their DE value.

The present results also show that including olive pomace acid oil at 2.5% in a blend with 2.5% crude palm oil achieved similar growth performance results as PO or O. It is well established that blending different fats and oils with different chemical compositions (differing in saturation degree, chain length or molecular structure) produces positive interactions in terms of energy and FA utilisation (Zumbado et al., 1999; Roll et al., 2018). Moreover, blending olive pomace acid oil with a high quality fat source with low MIU content, such as crude palm oil, dilutes the final MIU content of the blend. With this, the use of the fat blend did not impair performance parameters, so our results suggest that olive pomace acid oil can be included in growing-finishing swine diets, blended with conventional crude palm oil, achieving a combination of triacylglycerols and free FA and a moderate level of MIU.

Digestibility balance

In the present study, OA and M diets showed higher digestible energy at the faecal level than the PO diet. However, this effect was not observed in the dietary digestible energy determined at ileal level. The different results between the digestible energy measured in faeces or in ileum could be explained by the effect of microbiota present in the hindgut (Stein, 2017); therefore, the results obtained at ileal level are more representative. Although no statistical differences were observed in this study, a numerical reduction of digestible energy at ileal level for OA and M could be explained by the higher MIU content in these fat sources. Studying the effect of dietary treatments on digestibility of FA, and concerning ATTD, the present results showed that PO had the lowest FA digestibility among the dietary treatments under study. As expected, as dietary unsaturation increases, digestibility also increases (Cho and Kim, 2012; Duran-Montgé et al., 2007). Otherwise, no differences were observed between O and OA in total FA, despite the higher free FA content in the latter. There are controversial results in the literature about the effects of free FA on FA digestibility. The results from the present study are in agreement with those found by DeRouchey et al. (2004), who observed no differences in FA digestibility when free FA levels were increased in choice white grease fed to weaning pigs. In addition, Vilarrasa et al. (2015) did not obtain different results in FA digestibility when they compared palm fatty acid distillates with crude palm oil in growing-finishing pigs. On the contrary, Powles et al. (1993) concluded that the level of free FA in the diet of growing pigs appeared to be determinant for the digestible energy value of fats. It has been proposed that the negative effects of free FA on FA digestibility are related to its reaction with ionised minerals, such as calcium and magnesium, which form insoluble soaps that cause both the free FA and the mineral to become unavailable for absorption (Small, 1991). However, this effect is mainly related to long-chain saturated FA rather than to unsaturated FA, as the former has a greater ability to form insoluble soaps than the latter (Atteh and Leeson, 1985). This suggests that the negative effect of free FA on FA digestibility is restricted to saturated sources of free FA (Wiseman and Salvador, 1991).

Blending 2.5% olive pomace acid oil and 2.5% crude palm oil is a suitable option in terms of FA utilisation. In general, M showed AID and ATTD values above the arithmetically predicted values

obtained from the two individual fats. This phenomenon is frequently referred to as synergism (Powles et al., 1993; Wiseman et al., 1998). First, a synergic effect occurs when unsaturated and saturated fats are blended, since long-chain unsaturated FAs are more able to form mixed micelles than long-chain saturated FAs are. Therefore, the presence of unsaturated FA can increase the capacity to take up saturated FA in the core of mixed micelles, improving their absorption (Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019). Second, it has been suggested that increasing amounts of diacylglycerols or monoacylglycerols have a positive effect on free FA digestibility because their emulsifying effect enhances the inclusion of free FA in mixed micelles (Roll et al., 2018). In agreement with our results, Zumbado et al. (1999) described a synergistic effect on digestibility and dietary apparent metabolisable energy values of broiler chickens when unsaturated and saturated fats rich in free FA were blended. The synergic effect observed between 2.5% olive pomace acid oil and 2.5% crude palm oil could be due to it being an adequate unsaturated FA to saturated FA ratio, with the presence of monounsaturated FA from OA and a high proportion of monoacylglycerols from the lipolysis of palm triacylglycerols, capable of better solubilising free FA in the mixed micelle and facilitating its absorption.

In our study, ATTD showed higher values than AID for digestible energy and for all FA except for saturated FA, which means that energy and unsaturated FA that were not absorbed at the end of the ileum disappeared in the hindgut. Duran-Montgé et al. (2007) also reported higher values for unsaturated FA ATTD than the corresponding AID, and lower values for saturated FA. This effect may be explained by a biohydrogenation of oleic, linoleic and linolenic acids that are saturated by the microflora and, in part, converted into stearic acid (Jørgensen and Fernández, 2000; Duran-Montgé et al., 2007). Thus, the low values (or even negative for O and OA) obtained in the ATTD of stearic acid could be explained due to this microbial activity. Moreover, C15:0 and C17:0, which were not present in the diet, were observed in faecal samples, and were probably generated by microbial synthesis. In relation to this, the differences between sexes in the ATTD of FA obtained in the present study may be affected by these effects, since no differences were observed in any FA in the AID. Although it is generally accepted to report digestibility of fat as total tract digestibility, these findings suggest that using ileal digestibility is more accurate for the FA digestibility and estimates the nutritive value of fats better than faecal digestibility. Moreover, the interaction generated by microbial biohydrogenation of unsaturated FA in the hindgut can be avoided in this way (Stein, 2017).

Carcass quality and intramuscular fat

The dietary supplementation of crude and acid oils from olive pomace oils did not modify carcass weight, yield and lean percentage, or ham fat and backfat thickness. However, supplementation with crude olive pomace oil lead to an increase in IMF content in the LM. Although there is a relationship between loin and ham fatness and IMF content, the correlation is not very high, varying between 0.28 and 0.49 (Font-i-Furnols et al., 2019). Generally, a 5% increase in carcass fat corresponds only to a 1% increase in IMF (Hocquette et al., 2010) due to the lower proliferative potential or low activity levels of lipogenesis enzymes in intramuscular preadipocytes compared to subcutaneous adipocytes (Gardan et al., 2006). Consequently, changes in IMF content are difficult to note, especially in commercial lean breeds such as Landrace or Pietrain. However, in high-fat content crossbreed pigs such as Duroc, changes in IMF content may be accentuated. Samples of LM from the present study (Duroc-finished pigs) showed a higher IMF content (an average of 2.05 ± 0.65%) than other values recorded in commercial lean breeds such as Landrace or Pietrain (0.5–2.5% of wet weight; Cagnazzo et al., 2006). Higher IMF content levels have been linked with a better sensory quality of pork (Font-i-Furnols et al., 2012). The use of Duroc-finished lines in a commercial environment is an alternative for obtaining meat products with a higher IMF content and therefore fulfilling sensory acceptance by consumers.

Our results confirm that the IMF content can be modified through changes in the fat source added to growing-finishing pig diets, as supplementation of crude olive pomace oil increased the IMF content above the other diets of the study. Present results were consistent with data reported by Miller et al. (1990), who observed higher IMF levels in pigs fed diets rich in oleic acid. In addition, Isabel et al. (2004) found a higher IMF content in growing-finishing pigs when the monounsaturated FA to polyunsaturated FA ratio was increased. In the work of Gerfault et al. (2000), higher concentrations of oleic acid and lower of linoleic acid (therefore, a higher monounsaturated FA to polyunsaturated FA ratio) in porcine skeletal muscle and adipose tissue had been associated with higher lipogenic enzyme activity. In agreement with this, in the present study, O had the highest monounsaturated FA to polyunsaturated FA ratio both in the diet (1.46) and in LM (2.91) among treatments, while PO had the lowest (1.25 in the diet; 2.48 in LM), and hence the lowest IMF values. Alternatively, other nutritional strategies have been applied to reach high IMF levels in pork. Some authors have observed an increase in IMF levels when the protein to digestible energy ratio is decreased; however, this also may have a negative effect on performance (Kerr et al., 1995; D'Souza et al., 2003). Higher IMF content (+53%) has been reported when growing-finishing pigs are fed a diet deficient in vitamin A, as retinoic acid, a derivative of vitamin A, could inhibit the terminal differentiation of intramuscular adipose tissue (D'Souza et al., 2003). However, although these nutritional manipulations increased the IMF content, they also led to other detriments and disadvantages in the pigs' performance, which made their application unviable in a commercial environment. Alternatively, the work of Katsumata et al. (2005) showed that a defined lysine deficiency (about a 40% of reduction in recommended dietary lysine levels) in finishing pigs' diets was an effective way of increasing IMF without affecting performance. Our results suggest that increasing dietary monounsaturated FA by including crude olive pomace oil may be another good nutritional strategy to both increase IMF content in the LM of pigs and achieve a good performance.

Fatty acid composition of backfat and Longissimus muscle

The FA profile of both the backfat and LM was affected by the dietary fat, which is in agreement with the literature (Miller et al., 1990; Vilarrasa et al., 2015). In particular, the use of O or OA as an alternative to PO reduced saturated FA and increased the unsaturated FA to saturated FA in both the backfat and LM. Other studies have reported that reducing saturated FA while increasing monounsaturated FA content in meat has health benefits for consumers (Mateos et al., 2019). Specifically, it has been observed that including olive pomace cake in growing-finishing pig diets makes it possible to reduce saturated FA in porcine adipose tissue (Ferrer et al., 2020). Overall, animals fed O lead to meat products with more IMF, rich in monounsaturated FA, with high FA digestibility values and good performance parameters. Thus, this pork should result in both better sensory-perceived and healthier products. Similar effects were obtained when OA was used blended with a common saturated fat source such as PO. In addition, it is important to note that the use of fat by-products could also reduce feeding costs, as they usually have competitive prices.

In conclusion, crude olive pomace oil can be included at 5% in growing-finishing pig diets to obtain a meat rich in monounsatu-

Table 6
fated FA and low in saturated FA content with high fatty acid digestibility values and performance. Moreover, the inclusion of crude olive pomace oil lead to a higher IMF content in the *Longissimus* muscle. Another dietary strategy is to include olive pomace acid oil blended with crude palm oil in feed formulation, which did not negatively impact fat utilisation or performance. In addition, including this by-product reduced feed costs, resulting in a swine production of high-fat content crossbreeds that is more efficient and environmentally sustainable.

Ethics approval

Not applicable. The staff at Nial farm facilities looked over the animal continuously.

Data and model availability statement

None of the data was deposited in an official repository. Available upon request.

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Declaration of interest

The authors declare that partial funding was received from bonÀrea AGRUPA. M. Balart and M. Verdú both work for bonÀrea AGRUPA. All authors contributed to analysing and interpreting the data and therefore declare no conflict of interest.

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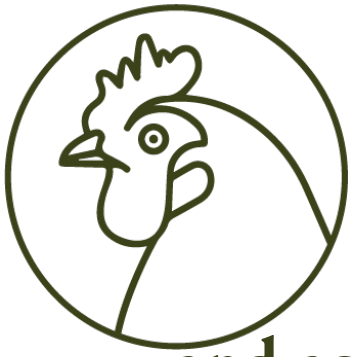

Chapter 4.

Inclusion of olive pomace oil and acid oil in broiler chicken diets

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"A wizard is never late. Nor is he early. He arrives precisely when he means to"

Gandalf, The Lord of the Rings.

Olive pomace oil and acid oil as alternative fat sources in growing-finishing broiler chicken diets

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ABSTRACT The aim of the present study was to investigate the effect of dietary supplementation of olive pomace oil and olive pomace acid oil, which are rich in monounsaturated fatty acids (FA) but differ in free FA content, on growth performance, digestibility and FA profile of abdominal fat and breast meat. A total of 3,048 one-day-old mixed-sex broiler chickens (Ross 308) were randomly distributed into 24 pens and 3 dietary treatments (8 replicates per treatment). Experimental diets were administered for growing (from 22 to 29 d) and finishing (from 30 to 39 d) periods, consisting of a basal diet supplemented with 6% (as-fed basis) palm oil (PO), olive pomace oil (O), or olive pomace acid oil (OA). Animals fed O achieved the lowest feed conversion ratio ($P < 0.01$), together with the highest AME value ($P = 0.003$), but no differences were observed between OA and PO. Regarding FA digestibility, O and OA showed higher values than PO for all FA in both apparent ileal digestibility (AID) and apparent total tract digestibility. Comparing the AID between O and OA, no differences were observed for total FA, monounsaturated FA, or polyunsaturated FA, but animals fed OA showed lower AID values for saturated FA than those fed O ($P < 0.001$). The FA profile of abdominal fat and breast meat reflected that of the diet, with higher monounsaturated FA and lower saturated FA in animals fed O and OA compared to those fed PO. In sum, the inclusion of both olive pomace oil and acid oil in growing-finishing broiler chicken diets led to great performance parameters and high FA digestibility values, together with an enrichment with monounsaturated FA in abdominal fat and breast meat compared to the use of palm oil. However, a better AID of saturated FA and feed conversion ratio is achieved with O compared to OA.

Key words: olive pomace oil, acid oil, digestibility, fatty acid, by-product

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INTRODUCTION

Fats and oils are widely included in broiler chicken diets due to their high energy content and supply of essential fatty acids (FA). However, the need for more efficient and sustainable animal production necessitates a continuous search for alternative fat sources to achieve these goals. In this regard, olive pomace oil and olive pomace acid oil could be 2 potential alternative fat sources for use in broiler chicken diets. Both are rich in monounsaturated FA (MUFA), and particularly in oleic acid (C18:1 n-9), which is of special interest since unsaturated FA (UFA) are known to be better absorbed than saturated FA (SFA; Ravindran et al., 2016). Moreover, the inclusion of olive-derived oils in broiler chicken diets could enrich the FA profile in meat and deposition fat with UFA (Skřivan et al., 2018).

In terms of molecular structure, olive pomace oil is mainly composed of triacylglycerols, while olive pomace acid oil, a by-product generated from the chemical refining of olive pomace oil, accumulates a high content of free FA (FFA, 40−60%; Varona et al., 2021a). There are controversial results in the literature regarding the effects of dietary FFA content on fat utilization and AME. Some authors have reported a decrease in the AME value and lower digestibility values when dietary FFA content increases (Wiseman and Salvador, 1991; Blanch et al., 1995,1996; Wiseman et al., 1998), while other studies observed no negative impact on

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performance or feed efficiency (Zumbado et al., 1994; Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019a,2021; Jimenez-Moya et al., 2021a). In this regard, and according to the results of previous studies, it may be better to use acid oils from unsaturated sources and include them in growing-finishing broiler chicken diets rather than in earlier stages, as age has a positive effect on FFA absorption and fat utilization (Tancharoenrat et al. 2013; Roll et al. 2018; Rodriguez-Sanchez, et al. 2019b; Viñado et al. 2019; Jimenez-Moya et al. 2021a). Additionally, the inclusion of olive pomace acid oil, a food-chain by-product, could help to reduce feeding costs as it is usually competitively priced, and its direct application might contribute to a circular bioeconomy system and more sustainable production compared to other uses of these by-products that require further processing. Therefore, olive pomace oil and acid oil, which are rich in MUFA but differ in FFA content, could be suggested as feeding fats for growing-finishing broilers. However, before recommending them, it is essential to evaluate their effects to assure suitable animal production performance, especially under commercial production practices.

Therefore, the aim of the present study was to investigate the potential use of olive pomace oil and olive pomace acid oil as fat sources in growing-finishing broiler diets. This was assessed by studying the effect of dietary supplementation of olive pomace oil and olive pomace acid oil on the growth performance, carcass parameters, digestibility, abdominal fat deposition, and FA profile of abdominal fat and breast meat.

Table 1. Composition of experimental fats included in the grower and finisher diets of broiler chickens.

	Experimental fats		
Item[1]	POO	OO	OAO
Fatty acid composition, %			
C 14:0	0.96	0.03	0.06
C 16:0	42.56	12.66	12.43
C16:1 n-7	0.16	0.91	1.04
C 18:0	4.53	2.69	2.69
C18:1 n-9	40.80	70.06	63.24
C18:1 n7	0.61	1.59	1.68
C18:1 trans	0.02	0.12	0.43
C18:2 n-6	9.50	10.09	15.83
C18:3 n-3	0.30	0.68	0.90
C20:0	0.40	0.48	0.52
C20:1 n-9	0.15	0.35	0.28
C22:0	-	0.23	0.46
C24:0	-	0.11	0.44
SFA	48.61	16.20	16.60
MUFA	41.59	73.03	66.67
PUFA	9.80	10.77	16.73
UFA:SFA	1.06	5.17	5.02
n-6:n-3	31.89	14.73	17.61
Lipid class composition, %			
TAG	87.98	91.09	24.47
DAG	8.67	8.38	18.60
MAG	0.05	0.32	2.34
FFA	3.31	0.21	54.59
MIU, %			
Moisture	-	-	0.73
Impurities	0.49	0.28	1.37
Unsaponifiable	0.22	1.44	4.53

Abbreviations: POO, palm oil; OO = olive pomace oil; OAO, olive pomace acid oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids; MIU, moisture, impurities and unsaponifiable.

[1]All samples were analyzed in duplicate.

MATERIALS AND METHODS

Experimental Fats

The chemical composition of experimental fats is shown in Table 1. Olive pomace oil and olive pomace acid oil were supplied by RIOSA S.A. (Jaén, Spain), and palm oil was provided by bonÀrea Agrupa (Guissona, Spain). All samples were analyzed in duplicate for FA composition, lipid class composition, moisture, impurities, and unsaponifiable matter as described by Varona et al. (2021b).

Experimental Design and Diets

The study was conducted on the experimental facilities of bonÀrea Agrupa (Nial Farm, Guissona, Spain). All animal housing and husbandry was in accordance with the European Union Guidelines (2010/63/EU), and all management practices and procedures were approved by the Animal Ethics Committee (CEEAH) of the Universitat Autònoma de Barcelona (code number: 3938). A total of 3,048 newly hatched mixed-sex broiler chickens (Ross 308; 40.9 § 0.26 g of BW, mean § SD) were obtained from the commercial hatchery of bonÀrea Agrupa. On arrival, birds were distributed into 24-floor pens (12 m^2, 127 animals per pen), balanced by body weight and assigned to 1 of the 3 dietary treatments (8 replicates per treatment). Each pen was provided with feed and water that could be accessed ad libitum throughout the study. Environmental conditions were automatically controlled, following the recommendations and specifications of the Ross 308 management handbook (Aviagen, 2018).

A 4-phase feeding program was used, consisting of a pre-starter diet from 0 to 7 d, a starter diet from 8 to 21 d, a grower diet from 22 to 29 d and a finisher diet from 30 to 39 d, all in pelleted form. The ingredients of the experimental diets are shown in Table 2, and these were formulated to meet or exceed requirements (FEDNA, 2018). Pre-starter and starter diets were common to all animals. For grower and finisher diets, 3 experimental treatments were obtained as the result of adding 6% (as-fed basis) of different fat sources: palm oil (PO), olive pomace oil (O), and olive pomace acid oil (OA). Silicate (Ibersil D-100M; IQESIL S.A., Zaragoza, Spain) was added to the finisher diets (1.00% as-fed basis) to increase the amount of HCl-insoluble ash as an inert digestibility marker.

Controls and Sampling

Feed consumption and BW (pen-basis) were recorded at 7, 21, 29, and 39 d of age. This was used to calculate the ADG, the ADFI and the feed conversion ratio

Table 2. Ingredient composition of the pre-starter, starter, grower and finisher diets (as-fed basis) for broiler chickens.

	Common period		Experimental period	
Ingredients, %	Pre-Starter	Starter	Grower	Finisher
Corn	24.01	35.07	35.11	34.99
Soybean meal 47%	36.20	30.38	23.79	19.74
Wheat	30.04	24.86	10.85	15.01
Sorghum	-	-	10.00	10.00
Sunflower meal	-	-	10.00	10.00
Soybean oil	4.69	4.72	-	-
Experimental fat[1]	-	-	6.00	6.00
Silicate	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.36	1.12	0.98	0.73
Calcium carbonate	1.04	1.12	0.69	1.02
Vit-Min. premix[2]	0.45	0.45	0.45	0.45
Sodium chloride	0.32	0.30	0.29	0.28
DL-Methionine[3]	0.36	0.34	0.28	0.24
L-Lysine[4]	0.39	0.46	0.46	0.45
L-Threonine	0.12	0.14	0.09	0.08
L-Valine	0.02	0.04	0.01	0.01

[1]Palm oil, olive pomace oil or olive pomace acid oil.

[2]Provides, per kg of feed: vitamin A (retinyl acetate), 10,000 IU; vitamin D, 4,700 IU; vitamin E (dl-alpha-tocopheryl acetate), 100 IU; vitamin K, 4 mg; vitamin B1, 4 mg; vitamin B2, 8 mg; vitamin B6, 5 mg; vitamin B12, 18 mg; biotin, 0.25 mg; Cu, 13.12 mg (from $CuSO_4$); I, 1.25 mg (from KI); Mn, 121.5 mg (from MnO_2); Se, 0.3 mg (from Na_2SeO_3); Zn, 67.5 mg (from ZnO); Fe, 141.75 mg (from $FeSO_4$); phytase, 1,500 FYT (Ronozyme Trade mark; DSM, Herleen, The Netherlands).

[3]DL-2-hydroxy-4-methylthiobutanoic acid (HMTBa), the hydroxyl analogue of DL-methionine.

[4]L-Lysine sulphate.

(FCR) for each period and for the overall study. Mortality was recorded and weighed to adjust and correct these parameters. The digestibility balance was determined from 30 to 36 d of age in a subset of 144 animals (n = 48 animals/treatment). Animals selected for the digestibility balance were closer to the average BW (mean§ 0.5 SD) of each sex within each pen (3 males and 3 females were selected from each pen). At d 36, excreta samples were collected from selected animals by abdominal-massage stimulation and then these animals were electrically stunned (Reference: 105523; FAF, Saint-Sernin-sur-Rance, France) and immediately exsanguinated to obtain ileal content. The ileal digestive contents (from the junction with Meckel's diverticulum to a point 1 cm proximal to the iliocecal junction) of samples from each sex group in each pen were pooled, homogenized, freeze dried (LyoAlpha 10/15; Telstar, Barcelona, Spain), ground (1 mm screen diameter) and kept at 5°C until further analyses. Animals euthanized for digestibility balance were adjusted as mortality for the performance parameters calculations.

At d 39, animals were fasted for 10 h and slaughtered at the bonÀrea Agrupa commercial slaughterhouse (Guissona, Spain). All carcasses were processed (blood, feathers, viscera, head, and feet were removed) and weighed to obtain the carcass yield. Breast meat and abdominal fat pad were obtained from the 5 female broilers per pen (n = 40 animals/treatment) that were closest to the average BW (mean§0.5 SD). Samples of breast meat were homogenized, minced, pooled for each pen, freeze-dried, ground (1-mm screen diameter), and kept at 5°C until further analyses. Samples of abdominal fat pad were homogenized, pooled for each pen and kept at —20°C until further analyses.

Chemical Analyses

The composition of feeds is shown in Table 3. Analytical determinations of the feeds were performed according to AOAC International (2005) methods: dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 954.01), ether extract (Method 920.39), and crude fiber (Method 962.09). The gross energy was determined with an adiabatic calorimeter (Parr 6300 Calorimeter, Parr Instrument Company, Moline, IL) according to the Standard UNE-EN ISO 9831:2004. Lipid class composition was analyzed by size exclusion HPLC with refractive index detection, following the method described by Varona et al. (2021b). The FA content of feed, ileal content and excreta were analyzed following the method described by Sukhija and Palmquist (1988). Abdominal fat pad and breast meat FA were analyzed following the method described by Carrapiso et al. (2000). Nonadecanoic acid (C19:0, Sigma Aldrich Chemical Co.; St. Louis, MO) was added as an internal standard. The FA composition of the final extract was injected in a gas chromatograph (HP6890, Agilent Technologies; Waldbronn, Germany) following the method conditions described by Cortinas et al. (2004). HCl-insoluble ash was determined in feeds, ileal content and feces according to the methods of European Commission Regulation n° 152/2009("European Commission Regulation No 152/2009 of 27 January 2009. Laying down the methods of sampling and analysis for the official control of feed - Publications Office of the EU,").

Calculations

The apparent digestibility of a particular FA (X) was calculated as follows:

$$\%\ apparent\ digestibility\ of\ X = \left[1 - \left(\frac{Xf/Mf}{Xd/Md}\right)\right] \times 100$$

where Xf is the concentration of a particular FA in excreta or ileal content, Mf is the concentration of the inert marker in excreta or ileal content, Xd is the concentration of a particular FA in the diet, and Md is the concentration of the inert marker in the diet. The ileal digestible energy and AME of feeds was calculated from the product of energy apparent digestibility and its corresponding feed gross energy.

Statistical Analysis

The normality of the data and homogeneity of variance were verified using the CAPABILITY procedure of SAS (version 9.4, SAS Inst. Inc.; Cary, NC). All data were analyzed using the GLM procedure of SAS. For performance, carcass parameters and FA profile of

Table 3. Macronutrient and energy content, fatty acid, and lipid class composition of the diets fed to broiler chickens.

			Grower diets			Finisher diets		
Item[1]	Pre-starter diet	Starter diet	PO	O	OA	PO	O	OA
Macronutrient and energy content, %								
Dry matter	89.19	89.42	89.70	90.14	89.77	90.27	90.34	90.36
Crude protein	20.15	20.16	18.98	19.11	19.68	18.53	18.19	18.45
SID Methionine*	0.65	0.61	0.56	0.56	0.56	0.51	0.51	0.51
SID Lysine*	1.24	1.13	1.04	1.04	1.04	0.94	0.94	0.94
Ether extract	5.91	5.40	7.71	7.76	7.49	8.06	7.71	8.07
Crude fiber	3.24	3.09	4.47	4.48	4.98	5.06	4.43	4.6
Ash	6.13	5.45	5.38	5.17	5.46	5.62	5.54	5.81
Calcium*	1.08	1.04	0.85	0.85	0.85	0.90	0.90	0.90
Phosphorus*	0.63	0.56	0.60	0.60	0.60	0.54	0.54	0.54
Digestible phosphorus*	0.48	0.43	0.42	0.42	0.42	0.37	0.37	0.37
Chloride*	0.24	0.23	0.24	0.24	0.24	0.23	0.23	0.23
Gross energy, kcal/kg	4,059	4,162	4,231	4,243	4,203	4,232	4,221	4,225
Fatty acid composition, %								
C16:0	15.99	20.03	30.62	13.67	15.49	30.89	13.89	17.50
C18:0	3.75	3.73	3.87	2.81	3.25	3.81	2.82	3.34
C18:1 n-9	18.65	24.41	33.93	51.42	43.80	34.71	51.72	43.44
C18:1 n-7	1.35	1.22	1.06	1.91	1.67	1.06	1.56	1.83
C18:2 n-6	54.12	45.71	27.71	27.14	32.25	26.84	26.72	30.42
C18:3 n-3	5.46	4.20	1.32	1.51	1.68	1.19	1.42	1.50
Minor fatty acids	0.69	0.69	1.50	1.53	1.86	1.50	1.87	1.96
SFA	20.59	24.67	36.22	17.21	19.94	36.21	17.46	22.43
MUFA	19.83	25.42	34.75	54.14	46.13	35.75	54.40	45.64
PUFA	59.58	49.91	29.03	28.65	33.93	28.03	28.14	31.93
UFA:SFA	3.86	3.05	1.76	4.81	4.02	1.76	4.73	3.46
Lipid class composition, %								
TAG	58.93	70.04	82.54	84.14	54.84	84.21	84.98	56.38
DAG	15.85	12.18	8.83	9.17	13.19	8.27	8.84	12.69
MAG	1.99	1.14	0.43	0.58	1.27	0.45	0.13	1.10
FFA	23.23	16.64	8.19	6.11	30.70	7.08	0.18	29.83

Abbreviations: DAG, diacylglycerols; FFA, free fatty acids; MAG, monoacylglycerols; MUFA, monounsaturated fatty acids; O, olive pomace oil diet; OA, olive pomace acid oil diet; PO, palm oil diet; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TAG, triacylglycerols; UFA, unsaturated fatty acids.

*Calculated values from the theoretical formulation of the diets

[1]All samples were analyzed in duplicate.

breast meat and abdominal fat pad, diet was defined as the main factor. For digestibility balance, diet and sex were defined as the main factors. No interactions were found between diet and sex for any of the variables studied. For all analyses, the experimental unit was the pen (n = 8 for each treatment), and differences between means were tested using Tukey's adjust correction for multiple comparisons. The results in the tables are reported as least square means. For all statistical analyses, significance was declared at $P < 0.05$ and tendencies were discussed at $0.05 < P < 0.10$.

RESULTS

Characterization of Experimental Oils and Diets

The composition of the experimental oils is presented in Table 1. Regarding the FA composition, olive pomace oil, and olive pomace acid oil were rich in monounsaturated FA (73.03 and 66.67%, respectively), while palm oil was rich in saturated FA (48.45%). Of the experimental oils, olive pomace acid oil had the highest content of polyunsaturated FA (PUFA; 16.73%). The main FA was oleic acid in the cases of olive pomace oil and olive pomace acid oil (70.06 and 63.24%, respectively), while for palm oil, palmitic (42.56%), and oleic (40.80%) appeared in similar proportions. Both olive pomace oil and palm oil were composed mainly of triacylglycerols (>90%), while olive pomace acid oil had a higher amount of free FA (54.59%). Additionally, the highest values for moisture, impurities, and unsaponifiable (MIU) were found for olive pomace acid oil (6.63%), while olive pomace (1.72%) and palm oil (0.71%) had lower values. The composition of experimental diets is shown in Tables 2 and 3. The gross energy and all macronutrient content values were similar among dietary treatments. The FA and lipid class composition mirrored that of the added experimental oils, the O and OA diets being richer in MUFA while PO was richer in SFA. Also, the OA diet showed a higher content in FFA.

Growth Performance and Feed Intake

The effects of the dietary experimental oils on growth performance and feed intake are shown in Table 4. No differences were found between OA and PO in any performance parameter ($P > 0.10$). Considering the grower period, from d 22 to 29, animals fed O had a higher ADG than those fed OA ($P = 0.017$) and the lowest FCR among dietary treatments ($P = 0.004$). No differences were observed in BW at d 29 or in ADFI during this period. For the finishing period, from d 30 to 39, animals fed O had the lowest ADFI and FCR among

Table 4. Growth performance and carcass parameters of broiler chickens fed different dietary fat sources.

Item[1]	Common diets		Dietary treatments				
	Mean	SD	PO	O	OA	SEM[2]	*P*-value
From 0 to 21 d							
BW 0 days, g	40.85	0.26					
BW 7 days, g	181.88	3.33					
ADFI, g/d	59.0	1.82					
ADG, g/d	43.0	0.56					
FCR, g/g	1.370	0.04					
From 22 to 29 d							
BW 21 days, g			944.3	945.3	944.4	4.44	0.985
BW 29 days, g			1,679	1,692	1,670	7.73	0.161
ADFI, g/d			135.2	133.6	134.8	1.11	0.577
ADG, g/d			91.8[ab]	93.3[a]	90.7[b]	0.67	0.017
FCR, g/d			1.478[a]	1.432[b]	1.487[a]	0.012	0.004
From 30 to 39 d							
BW 39 days, g			2,674	2,701	2,647	15.68	0.071
ADFI, g/d			189.0[ab]	183.2[b]	191.5[a]	1.81	0.013
ADG, g/d			100.2	101.0	98.9	0.95	0.296
FCR, g/g			1.897[a]	1.815[b]	1.935[a]	0.019	< 0.001
From 0 to 39 d							
ADFI, g/d			112.6	110.6	111.8	0.99	0.385
ADG, g/d			67.5	68.2	66.8	0.40	0.071
FCR, g/g			1.668[a]	1.622[b]	1.673[a]	0.012	0.016
Carcass parameters							
Carcass weight, g			2,016	2,007	1,968	20.15	0.210
Carcass yield, %			75.42	74.29	73.72	0.61	0.154

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; FCR, feed conversion ratio; O, olive pomace oil diet; OA, olive pomace acid oil diet; PO, palm oil diet; SEM, standard error of the mean.

[1]Values of ADFI and ADG are expressed as-fed basis.

[2]n= 8.

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

Table 5. Feed apparent digestible and metabolizable energy (kcal/kg) and fatty acid apparent ileal and total tract digestibility in 36-day-old broiler chickens fed different dietary fat sources.

Item	Dietary treatments			SEM[1]	*P*-value[2]
	PO	O	OA		
AID, %					
Apparent ileal digestible energy, kcal/kg	3,024.97[b]	3,288.03[a]	3,052.98[b]	45.96	0.003
Total FA	85.92[b]	95.75[a]	93.88[a]	0.64	< 0.001
SFA	71.74[c]	89.75[a]	84.75[b]	1.43	< 0.001
MUFA	92.61[b]	96.44[a]	95.25[a]	0.44	< 0.001
PUFA	95.88[b]	97.88[a]	97.81[a]	0.20	< 0.001
C16:0	72.82[b]	90.50[a]	86.31[a]	1.33	< 0.001
C18:0	66.04[c]	86.31[a]	79.94[b]	1.59	< 0.001
C18:1 n-9	93.27[b]	96.44[a]	95.31[a]	0.38	< 0.001
C18:1 n-7	89.37[b]	95.88[a]	94.44[a]	0.64	< 0.001
C18:2 n-6	95.67[b]	97.75[a]	97.69[a]	0.20	< 0.001
C18:3 n-3	97.73[b]	99.56[a]	100.00[a]	0.47	0.028
ATTD, %					
AME, kcal/kg	3,087.56[b]	3,281.07[a]	3,153.88[b]	39.25	0.003
Total FA	85.06[c]	95.25[a]	93.06[b]	0.47	< 0.001
SFA	70.69[c]	90.75[a]	85.59[b]	0.95	< 0.001
MUFA	92.25[c]	95.56[a]	93.81[b]	0.36	< 0.001
PUFA	93.94[b]	96.88[a]	97.19[a]	0.26	< 0.001
C16:0	71.81[c]	91.69[a]	87.25[b]	0.88	< 0.001
C18:0	63.00[c]	87.25[a]	79.44[b]	1.28	< 0.001
C18:1 n-9	92.25[c]	95.56[a]	93.81[b]	0.36	< 0.001
C18:1 n-7	88.63[c]	94.63[a]	92.06[b]	0.45	< 0.001
C18:2 n-6	94.13[b]	96.88[a]	97.19[a]	0.24	< 0.001
C18:3 n-3	93.00[b]	99.00[a]	99.75[a]	0.38	< 0.001

Abbreviations: AID , apparent ileal digestibility; ATTD, apparent total tract digestibility; MUFA, monounsaturated fatty acids; O, olive pomace oil diet; OA, olive pomace acid oil diet; PO, palm oil diet; PUFA, polyunsaturated fatty acids; SEM, standard error of the mean; SFA, saturated fatty acids.

[1]n = 16 for each treatment (8 replicates £2 sex).

[2]No effect of sex or interactions between diet and sex were detected for any of the variables studied and therefore, only diet effects are presented.

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

dietary treatments ($P < 0.05$) and showed a tendency to reach a higher BW at d 39 than those fed OA. Concerning the entire production cycle, animals fed O had the lowest FCR among dietary treatments ($P = 0.016$) and a tendency to have a higher ADG than those fed OA ($P = 0.071$). No differences were found in carcass weight or carcass yield among dietary treatments ($P > 0.10$).

Digestibility Balance

The ileal apparent digestible energy, AME and apparent ileal (AID), and total tract (ATTD) FA digestibility of the feeds are presented in Table 5. No effect of sex or interactions between diet and sex were detected for any of the variables studied and therefore, only diet effects are presented. For apparent ileal digestible energy and AME, O showed the highest values among dietary treatments, while no differences were observed between OA and PO ($P < 0.01$). Considering the digestibility of FA, O and OA showed higher values than PO for all analyzed FA in both AID and ATTD ($P < 0.001$). When comparing O and OA, no differences were observed for the AID of total FA, MUFA, and PUFA, but O had higher values for SFA than OA ($P < 0.001$). For ATTD, O showed higher values for total FA, SFA and MUFA than OA ($P < 0.001$), and no differences were observed for PUFA.

Fatty Acid Composition of Abdominal Fat Pad and Breast Meat

The FA composition of abdominal fat pad and breast meat are presented in Tables 6 and 7, respectively. The FA profiles of abdominal fat pad and breast meat resembled that of the diet. Animals fed O had a higher abdominal fat deposition than those fed OA ($P = 0.023$). In relation to abdominal fat pad, O had the highest MUFA content and the highest UFA:SFA ratio, together with the lowest PUFA content among dietary treatments ($P < 0.01$). Also, O had the lowest SFA content while PO had the highest ($P < 0.001$). For individual FA, oleic acid showed the highest values for O while palmitic acid did for PO ($P < 0.001$). Moreover, O had the lowest content in linoleic acid among dietary treatments ($P < 0.001$). In relation to breast meat, differences were similar to those obtained for abdominal fat pad. Breast meat from animals fed O was the richest in MUFA, while that from animals fed OA was richest in PUFA and that from animals fed PO was richest in SFA ($P < 0.001$).

Table 6. Fatty acid composition (%) of abdominal fat pad from female broiler chickens according to different dietary fat sources.

	Dietary treatments				
Item	PO	O	OA	SEM[1]	*P*-value
Abdominal fat pad, %	1.31[ab]	1.44[a]	1.25[b]	0.05	0.023
Sum of FA, mg/g	717.80	744.85	708.91	13.28	0.150
SFA	30.73[a]	25.64[c]	27.90[b]	0.50	< 0.001
MUFA	47.38[c]	54.78[a]	49.71[b]	0.64	< 0.001
PUFA	21.89[a]	19.58[b]	22.40[a]	0.53	0.002
UFA:SFA	2.26[c]	2.90[a]	2.60[b]	0.06	< 0.001
n-6:n-3	19.53[b]	17.91[a]	18.82[ab]	0.61	0.049
C16:0	24.44[a]	20.13[c]	21.85[b]	0.48	< 0.001
C16:1	4.00	3.73	3.96	0.18	0.507
C18:0	5.02	4.76	5.11	0.13	0.157
C18:1 n-9	41.44[c]	48.17[a]	43.61[b]	0.58	< 0.001
C18:1 n-7	1.60[b]	1.97[a]	1.74[b]	0.05	< 0.001
C18:2 n-6	20.14[a]	18.21[b]	20.57[a]	0.49	0.005
C18:3 n-3	1.07	1.07	1.13	0.04	0.464
Minor FA	2.21[a]	1.86[b]	2.04[ab]	0.06	0.001

Abbreviations: FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; O, olive pomace oil diet; OA, olive pomace acid oil diet; PO, palm oil diet; PUFA, polyunsaturated fatty acids; SEM, standard error of the mean; UFA, unsaturated fatty acids.

[1]n = 8.

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

Hence, both O and OA had a higher unsaturated-to-saturated FA (UFA:SFA) ratio than PO ($P < 0.001$). In terms of individual FA, palmitic acid showed the highest values for PO, oleic acid did for O and linoleic acid did for OA ($P < 0.001$).

DISCUSSION

Growth Performance and Feed Intake

The present results show that the inclusion of olive pomace oil, which is rich in monounsaturated FA and mainly composed of triacylglycerols, improved performance parameters and feed efficiency in both growing (from d 22 to 29) and finishing (from d 30 to 39) periods, which resulted in a better FCR when considering each period and the overall trial. As far as we know, no other studies have assessed the effects of olive pomace oil or olive pomace acid oil in broiler chickens. In agreement with the present results, Crespo and Esteve-Garcia (2001) reported an increase in feed efficiency in growing-finishing broilers fed olive oil, which has a similar FA profile to olive pomace oil, compared to those fed tallow, which is rich in SFA. However, Zhang et al. (2013) did not observe differences in broilers fed olive oil compared to those fed tallow. The improved feed efficiency in animals fed O compared to those fed PO may be explained by the higher degree of unsaturation, since many authors have reported that digestibility increases as the degree of unsaturation does (Tancharoenrat et al., 2014; Rodriguez-Sanchez et al., 2019a,2021; Jimenez-Moya et al., 2021b). In contrast, OA performed more poorly than O despite having a similar FA composition. This could be related either to the higher FFA or MIU content, or both. It has been described that high FFA content could lead to a lower AME (Wiseman et al., 1992; Powles et al., 1993), which is in agreement with the present results as OA showed a lower AME than O. Similarly, a higher MIU content also dilutes the energy content of the added fat source (Varona et al., 2021a). Hence, the higher OA FFA and MIU contents than O could explain the lower AME values. In turn, this could be related to the higher ADFI found in the finishing period (from 30 to 39 d) for animals fed OA in comparison to O, since broilers tend to vary their feed intake in order to cover their energy requirements (NRC, 1994).

Table 7. Fatty acid composition (%) of breast meat from female broiler chickens according to different dietary fat sources.

	Dietary treatments				
	PO	O	OA	SEM[1]	*P*-value
Sum of FA, mg/g	13.63	13.53	13.52	1.06	0.994
SFA	32.49[a]	27.03[c]	28.92[b]	0.24	< 0.001
MUFA	41.92[c]	48.47[a]	44.42[b]	0.37	< 0.001
PUFA	25.58[b]	24.50[c]	26.65[a]	0.23	< 0.001
UFA:SFA	3.68[b]	4.89[a]	4.39[a]	0.19	< 0.001
n-6:n-3	14.37[a]	19.94[b]	16.46[a]	0.10	< 0.001
C16:0	23.83[a]	18.85[c]	20.30[b]	0.13	< 0.001
C16:1	3.09[a]	2.50[b]	2.68[b]	0.10	0.002
C18:0	8.07	7.82	8.16	0.19	0.433
C18:1 n-9	36.26[c]	42.84[a]	38.93[b]	0.33	< 0.001
C18:1 n-7	2.18[c]	2.64[a]	2.40[b]	0.06	< 0.001
C18:2 n-6	19.33[b]	18.30[c]	20.24[a]	0.19	< 0.001
C18:3 n-3	0.74[b]	0.82[a]	0.86[a]	0.02	< 0.001
C20:1 n-9	0.39[c]	0.48[a]	0.42[b]	0.01	< 0.001
C20:4 n-6	4.08	3.98	4.11	0.20	0.888
Minor FA	2.04[a]	1.77[b]	1.91[ab]	0.05	0.004

Abbreviations: FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; O, olive pomace oil diet; OA, olive pomace acid oil diet; PO, palm oil diet; PUFA, polyunsaturated fatty acids; SEM, standard error of the mean; UFA, unsaturated fatty acids.

[1]n = 8.

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

Digestibility Balance

In the present study, O and OA showed higher FA digestibility than PO, both in AID and ATTD. This was expected because, as mentioned earlier, digestibility increases as the degree of unsaturation does. When comparing FA AID of OA with O, similar results were obtained for total and UFA. However, for SFA and stearic acid, lower AID values were obtained in OA compared to O. The lower digestibility of SFA in OA could be explained by the higher content of FFA. Some authors agree that the explanation for the different fat utilization in diets rich in FFA is found in the absorption processes (Rodriguez-Sanchez et al., 2019a; Jimenez-Moya et al., 2021a). First, due to a lower monoacylglycerol content and bile acid secretion in the duodenum (Sklan, 1979; Atteh and Leeson, 1985), which are considered essential for the formation of mixed micelles and hence the absorption of lipolysis end-products (Krogdahl, 1985; Ravindran et al., 2016). On the other hand, FFA can interact with ionized minerals, forming insoluble soaps that are unavailable for absorption (Small 1991; Jimenez-Moya et al., 2021a). Concretely, and in accordance with the present results, this effect has been found to be much more pronounced in SFA rather than

UFA (Atteh and Leeson, 1985; Wiseman and Salvador, 1991). In agreement with this, other studies in broiler chickens have found that the ileal digestibility of SFA decreases as dietary FFA content increases (Rodriguez-Sanchez et al., 2019a). However, although absorption of SFA could have been compromised by the presence of FFA in OA, the values obtained for these animals were much higher than those obtained for PO. These results suggest that the saturation degree had more influence on FA digestibility than the dietary FFA content did, which is in agreement with other previous studies (Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019a; Jimenez-Moya et al., 2021a; Rodriguez-Sanchez et al., 2021).

The present study showed that when acid oils are included in growing-finishing diets (from 22 to 39 d) only SFA is affected, and no changes in TFA, MUFA or PUFA AID are observed, which may be explained by the fact that saturated FFA are more prone than unsaturated FFA to form insoluble soaps. In agreement with the present results, recent studies showed that adding a moderate content of dietary FFA does not negatively affect TFA digestibility. Rodriguez-Sanchez et al. (2021) and Jimenez-Moya et al. (2021a) did not find negative effect on the digestibility of TFA in growing-finishing broiler chickens that were fed diets containing up to 35% and 30% of FFA, respectively. Furthermore, the use of olive pomace acid oil (38.6% of dietary FFA) in growing-finishing pigs did not affect the digestibility of TFA compared to olive pomace oil (Verge-Mèrida et al., 2021). In fact, other authors have found that the effect of dietary FFA is limited to SFA, and especially in young animals, that have lower production and secretion of bile acids, which hinders their dietary fat assimilation (Wiseman et al., 1991; Rodriguez-Sanchez et al., 2019a; Jimenez-Moya et al., 2021a). Hence, in agreement with previous studies, the present results suggest that acid oils could be included in growing-finishing diets without major impairment of FA digestibility, at least when dietary FFA content does not exceed 30% and the dietary UFA:SFA ratio is above 3.46.

In general, similar values were obtained for ATTD to those obtained for AID. This was expected since the absorption of fat is practically negligible in the hindgut of poultry (Renner, 1965; Ravindran et al., 2016). However, in contrast to what was observed in the AID of FA, values for ATTD were lower in OA when compared to O for most of the FA, with the exception of PUFA. This is in accordance with other studies that described how high dietary FFA has negative effects on fat digestibility measured at fecal level (Blanch et al., 1995,1996; Vilà and Esteve-Garcia, 1996; Wiseman et al., 1998). This effect could be caused by bacterial activity, mainly in the cecum. In this regard, bacteria biohydrogenation of oleic, linoleic, and linolenic acids would convert them into stearic acid and other FA that originate from this activity (Duran-Montgé et al., 2007; Rodriguez-Sanchez et al., 2019a, 2021), which therefore affects the ATTD values, especially those of SFA and MUFA. Previous studies (Rodriguez-Sanchez et al. 2019a; Rodriguez-Sanchez et al. 2021) found increasing concentrations of FA produced by bacterial activity (capric acid, C10:0; margaric acid, C17:0; *trans* C18:1; and vaccenic acid, C18:1 n-7) as dietary FFA content increased, suggesting that the higher the dietary FFA content, the greater the bacterial activity. These results indicate that AID data are more accurate and should be used instead of ATTD data for FA digestibility, since the microbial effect and other confounding factors such as endogenous losses are thus avoided (Stein, 2017).

Fatty Acid Composition of Abdominal Fat and Breast Meat

In the present study, animals fed O showed a higher abdominal fat deposition than those fed OA. In fact, olive pomace acid oil used in this study had a higher content in PUFA, which previous studies have associated with a decrease in abdominal fat deposition (Crespo and Esteve-Garcia, 2002a,b; Ferrini et al., 2008; Vilarrasa et al., 2015). Preferential b-oxidation of PUFA with respect to SFA or MUFA and a decreased rate of FA synthesis could explain this (Crespo and Esteve-Garcia, 2002c). Moreover, dietary PUFA seem to reduce serum levels of insulin and of very low density lipoproteins (Crespo and Esteve-Garcia, 2003), which also limits fat deposition.

The FA profile of both abdominal fat pad and breast meat reflected that of the diet, depending on the added fat source, which is in agreement with the results reported in the literature (Ferrini et al., 2008; Vilarrasa et al., 2015; Skřivan et al., 2018; Viñado et al., 2020). Concretely, in O and OA treatments, SFA were reduced in both tissues, increasing the content in MUFA and the UFA:SFA ratio when compared to PO. The reduction of SFA in breast meat contributes to the global trend towards producing healthy meat products, since consumption of saturated fat have been related to many health concerns (Islam et al., 2019; López-Pedrouso et al., 2021).

In conclusion, the inclusion of olive pomace oil at 6% in growing finishing broiler diets achieved better performance, feed efficiency, and digestibility compared to a conventional source such as palm oil. On the other hand, when olive pomace acid oil (fat by-product rich in FFA) is used instead of palm oil, no negative effect was observed in performance or feed efficiency. However, olive pomace acid oil showed lower digestibility of SFA than olive pomace oil, although no changes in TFA, MUFA, or PUFA ileal digestibility were observed. Additionally, the inclusion of olive pomace oil or olive pomace acid oil leads to a reduction in saturated fatty acids in both abdominal fat and breast meat compared to palm oil. Hence, the present results suggest that olive pomace oil and acid oil are interesting sources for inclusion in growing-finishing broiler chicken diets, which may potentially reduce feeding costs and contribute to more efficient production and the circular economy.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter 5.

Inclusion of olive pomace oil and acid oil in European seabass diets

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"I'd rather you hate me for everything I am than have you love me for something that I can't"

Five Finger Death Punch.

animals

MDPI

Article

Olive Pomace and Soybean-Sunflower Acid Oils as Alternative Fat Sources in European Seabass (*Dicentrarchus labrax*) Diets: Effects on Performance, Digestibility and Flesh Fatty Acid Composition and Quality Parameters

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Simple Summary: Acid oils, by-products of edible oil refining, are potentially interesting fat sources for farmed fish diets because of their high energy content and usually competitive price. Their use and revaluation may contribute to more efficient and sustainable fish production. They are characterised by presenting a similar fatty acid profile to their respective crude oils, but with a high content of free fatty acids. The present study aimed to investigate the effects of including soybean-sunflower and olive pomace acid oils in European seabass diets, as a preliminary step to determine whether they might be suitable energy sources for fish diets. The results showed that growth was only impaired in animals fed the diet containing olive pomace acid oil, which had the highest moisture, impurities and unsaponifiable matter. They also suggest that dietary free fatty acid content affects digestibility, but not the fatty acid profile of flesh and perivisceral fat. Notwithstanding, further studies assessing the effects of the inclusion of these oils are needed before recommending their use.

Abstract: The effects of dietary inclusion of soybean-sunflower and olive pomace acid oils on growth, digestibility and flesh composition were studied in European seabass. Eight diets were fed for 100 days (101.37 ± 0.33 g initial weight, mean ± SD), differing in the added fat source (25% fish oil, 75% experimental oil): S (crude soybean oil), SA (soybean-sunflower acid oil), O (crude olive pomace oil) or OA (olive pomace acid oil); 3 blends: S-O, S-OA, SA-OA at a 1:1 ratio; and a diet containing only fish oil (F) as a control. Animals fed OA showed the worst performance among dietary treatments, with the lowest weight, specific growth ratio, average daily gain and the highest feed conversion ratio ($p < 0.01$). In contrast, other diets including acid oils did not impair performance. Acid oil diets did not affect the apparent digestibility of dry matter, crude protein or total fatty acids ($p > 0.05$), but a lower digestibility of lipids and saturated fatty acids was observed ($p < 0.001$). Flesh composition and fatty acid profile were not affected by the high dietary free FA content ($p > 0.05$). Hence the results suggest that the studied acid oils may potentially be used in fish diets although further studies are needed.

Keywords: acid oil; free fatty acid; fat by-product; alternative energy source; dietary fat; flesh quality; fish nutrition

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1. Introduction

The increasing importance of aquaculture, linked to factors such as population growth, the increasing demand for aquatic food products and the bioaccumulation of toxic compounds in wild marine species, has raised the need for safe and efficient production of aquatic species. In Mediterranean aquaculture, European seabass (*Dicentrarchus labrax*) is one of the most important farmed marine fish species, Turkey, Greece, Spain and Egypt being the countries that account for 88% of total production [1].

In farmed fish diets, fish oil (FO) had traditionally been used as the only dietary fat source, partly due to its energy content but mainly to its contribution of eicosapentaenoic (EPA; C20:5 n−3) and docosahexaenoic (DHA; C22:6 n−3) acids. These n−3 highly unsaturated fatty acids (n−3 HUFA) are considered essential for marine fish [2]. However, since the global supply is insufficient to cover the increasing demand for FO in aquaculture, the need arose to find sustainable alternative oil sources, and major research efforts in recent years have studied different strategies and alternatives for the replacement of FO with vegetable oils (VO) in fish diets [3,4]. Aquaculture production is expected not only to be efficient but also sustainable, so circularity should be one of the cornerstones of future aquaculture feeds [5]. Results of studies carried out in broiler chickens and pigs suggest that soybean-sunflower and olive pomace acid oils are by-products of edible oil refining that can be used as potential alternative fat sources [6–8]. These acid oils are obtained by chemically refining crude oils, which consists of many steps, including degumming, bleaching, deodorization and alkali neutralization. Essentially, the refining process of crude oils consists of removing free FA (FFA) and other non-desirable compounds in order to obtain a refined oil suitable for human consumption, and acid oils are generated as by-products [9]. Hence, acid oils are characterised by a similar fatty acid (FA) profile to their respective crude oils, but with a higher content of free FA (FFA) (40–60%; [8,10]). They are also cost-effective as they are usually competitively priced, and are readily available to fish feed manufacturers at the European level since soybean and sunflower oils are two of the most produced VO worldwide and the extraction of olive pomace oil is mainly concentrated in the Mediterranean arc [11]. In fact, soybean-sunflower acid oil is the most widely available in the European market. Information about the effects of oils rich in FFA on farmed fish species is scarce, and only a few studies using palm fatty acid distillate (90% of FFA) or rapeseed acid oil (47% of FFA) in rainbow trout (*Oncorhynchus mykiss*) [12–15] and gilthead seabream (*Sparus aurata*) [16] have been found in the literature.

The potential use of a new ingredient requires the assessment of its quality and composition. According to Glencross [17], the characterization of ingredients is a critical step in the evaluation process. In this sense, the characterization of soybean-sunflower and olive pomace acid oils has been reported by [10,18]. Therefore, the objective of the present study was to investigate the effects of including soybean-sunflower and olive pomace acid oils in European seabass diets on growth performance, digestibility and flesh composition, as a preliminary step to determining whether they might be suitable energy sources for fish diets.

2. Materials and Methods

2.1. Experimental Fats and Diets

Eight experimental diets were formulated to be isoproteic and isolipidic using the same ingredient composition except for the added fat source (15.4% of the diet, as-fed basis). The added fat consisted of 25% FO and 75% experimental oil. Then, four diets including experimental oils, namely S (crude soybean oil diet), SA (soybean-sunflower acid oil diet), O (crude olive pomace oil diet) and OA (olive pomace acid oil diet); and three blends at a 1:1 ratio (diet S-O; diet S-OA; diet SA-OA) were formulated. A diet was formulated including only commercial fish oil for use as a control (F). Diets were formulated according to the nutritional requirements of the species [19]. Ingredients and proximate composition of the experimental diets are shown in Table 1. Yttrium oxide (Y_2O_3) was added to the diets as an inert marker for digestibility balance.

Table 1. Ingredients and approximate composition of experimental diets.

Item, g/kg	Experimental Diets							
	F	S	SA	O	OA	S-O	S-OA	SA-OA
Ingredient composition								
Wheat meal	110.34	110.34	110.34	110.34	110.34	110.34	110.34	110.34
Wheat gluten	155.94	155.94	155.94	155.94	155.94	155.94	155.94	155.94
Soya protein concentrate	265.99	265.99	265.99	265.99	265.99	265.99	265.99	265.99
Fish meal	202.45	202.45	202.45	202.45	202.45	202.45	202.45	202.45
Hydrolysed fish protein	25.31	25.31	25.31	25.31	25.31	25.31	25.31	25.31
Krill meal	25.52	25.52	25.52	25.52	25.52	25.52	25.52	25.52
Soybean lecithin	9.62	9.62	9.62	9.62	9.62	9.62	9.62	9.62
Fish oil	153.87	38.47	38.47	38.47	38.47	38.47	38.47	38.47
Experimental oil	-	115.40	115.40	115.40	115.40	115.40	115.40	115.40
L-lysine	2.88	2.88	2.88	2.88	2.88	2.88	2.88	2.88
DL-methionine	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Choline chloride	4.81	4.81	4.81	4.81	4.81	4.81	4.81	4.81
Betaine	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Vitamin and mineral premix [1]	19.23	19.23	19.23	19.23	19.23	19.23	19.23	19.23
Vitamin C	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Guar gum	19.23	19.23	19.23	19.23	19.23	19.23	19.23	19.23
Yttrium	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92
Proximate composition (as-fed basis)								
Dry matter	927.40	928.80	929.70	927.40	928.60	932.50	923.60	926.80
Crude protein	418.30	405.30	396.20	413.10	414.30	419.60	410.90	414.70
Ether extract	190.50	190.40	182.80	186.90	180.00	182.90	186.10	184.20
Ash	72.20	72.40	73.20	72.40	73.40	71.00	71.10	72.10
Gross energy (MJ/kg)	21.72	21.80	21.78	21.69	21.95	21.85	21.75	21.65

Abbreviations: F = fish oil diet (control); S = soybean oil diet; SA = soybean-sunflower acid oil diet; O = olive pomace oil diet; OA = olive pomace acid oil diet; S-O = S and O at 1:1 ratio; S-OA = S and OA at 1:1 ratio; SA-OA = SA and OA at 1:1 ratio. [1] Provides, per kg: vitamin A (2,000,000 UI); vitamin D3 (200,000 UI); vitamin E (10,000 mg); vitamin K3 (2500 mg); vitamin B1 (3000 mg); vitamin B2 (3000 mg); calcium pantothenate (10,000 mg); nicotinic acid (20,000 mg); vitamin B6 (2000 mg); vitamin B9 (1500 mg); vitamin B12 (10 mg); vitamin H (300 mg); inositol (50,000 mg); betaine (50,000 mg); cobalt carbonate (65 mg); cupric sulfate (900 mg); iron sulfate (600 mg); potassium iodide (50 mg); manganese oxide (960 mg); sodium selenite (1 mg); zinc sulphate (750 mg); calcium carbonate (186,000 mg); potassium chloride (24,100 mg); sodium chloride (40,000 mg).

Crude soybean oil and soybean-sunflower acid oil (approximately 55:45, *w*/*w*) were supplied by Bunge Ibérica S.A.U. (Sant Just Desvern, Spain). Crude olive pomace oil was supplied by General d'Olis i Derivats S.L. (Borges Blanques, Spain) and olive pomace acid oil was supplied by RIOSA S.A. (Refinación Industrial Oleícola S.A., Ibros, Spain). Comercial FO was obtained from AFAMSA (Agrupación de Fabricantes de Aceites Marinos, S.A., Mos, Spain). The experimental diets were manufactured as extruded pellets by Ceimar-University of Almeria (Experimental Diets Service, Almeria, Spain) using standard aquafeed procedures. Briefly, feed ingredients were finely ground and mixed in a vertical helix ribbon mixer (Sammic BM–10, 10-L capacity, Sammic, Azpeitia, Spain) before oil and diluted choline chloride were added. All the ingredients were mixed together for 20 min, and then water (350 mL/kg) was added to the mixture to obtain a homogeneous dough. The dough was passed through a single screw laboratory extruder (Miltenz 51SP, JSConwell Ltd., New Zealand). The extruder barrel consisted of four sections, and the temperature profile in each section (from inlet to outlet) was 95 °C, 98 °C, 100 °C, and 110 °C, respectively. Finally, pellets were dried at 27 °C in a drying chamber (Airfrio, Almeria, Spain) for 24 h and feeds were kept in sealed plastic bags at −20 °C until use.

2.2. Fish Husbandry and Sampling

All the procedures were conducted following the European Union Guidelines for the ethical care and handling of animals under experimental conditions (2010/63/EU) and in accordance with the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona (CEEAH). The trial was carried out at the Aquaculture Center facilities of the

Institute of Agrifood Research and Technology (IRTA, Sant Carles de la Ràpita, Spain). A total of 480 European seabass (with an average of 101.37 0.33 g body weight, mean ± SD) were randomly allocated into 24 cylindroconical tanks with a capacity of 500 L (20 fish per tank) in a sea water recirculation system (IRTAmar®; IRTA, Sant Carles de la Ràpita, Spain). This system allows for water recirculation of between 1 and 1.5 tank volumes per hour (15 m^3/h), and is equipped with an aerobic biofilter for the removal/transformation of ammonia to nitrite and nitrite to nitrate. The supply of fresh water to the system consists of 5–15% of the total volume per day. Each experimental diet was randomly assigned to three tanks and was administered twice a day by automatic feeders (adjusted to provide 2–2.5% average BW daily; at 8.00 am and 2.00 pm) for 100 days. Uneaten feed was collected by filtering effluent water from each tank and collectors were emptied at the end of each meal, so the average feed intake per tank was recorded daily. Water temperature (22.5 ± 0.84 °C), dissolved oxygen levels (7.30 ± 0.66 mg/L), pH (7.9 ± 0.2) and salinity (35.5 ± 0.50‰) were maintained throughout the study. The levels of ammonia (0–0.5 ppm) and nitrites (0–2 ppm) were maintained within the safe levels for the species. During the experimental period (from July to October), the tanks were subjected to natural photoperiod.

All animals were weighed and measured individually at the beginning (day 0) and at the end of the experimental period (day 100). Each tank had a removable faecal settling system for the collection of faecal samples where feed and faeces are separated on the basis of their different densities. Faecal collection was carried out during the last two weeks of the experimental period and then stored at − 20 °C until further analyses. At the end of the experimental period and after 24 h of fasting, six fish from each tank (18 animals per treatment) were euthanized by hypothermia in a mix of water and ice (1:3) and individually gutted. Viscera and abdominal fat pad were removed and weighed. The entire left and right muscles were also removed and weighed. The left muscle was immediately used for fresh colorimetric determination. The right muscle was cut into two different sections (dorsal and ventral, according to horizontal septum) and weighed. All samples were bagged individually and stored at −20 °C until chemical analyses.

2.3. *Chemical Analyses*

Prior to chemical analyses, samples of each experimental oil and feed were pooled, homogenized and kept at 5 °C. Faeces, skinned muscle (whole left muscle and the dorsal and ventral portions of the right muscle) and perivisceral fat samples were homogenized, freeze-dried (LyoAlpha 10/15; Telstar, Terrassa, Spain) and kept at 5 °C. Fatty acid composition, lipid class composition and MIU (moisture, impurities and unsaponifiable matter) content of experimental oils were analysed in duplicate as described by Varona et al. [18]. Analytical determinations for the chemical composition of the feeds, faeces and left muscle were performed according to AOAC International [20] methods: dry matter (934.01), ash (942.05), crude protein (954.01), ether extract by Soxhlet analysis (920.39) and crude fibre (962.09). The gross energy of feed and faeces was determined using an adiabatic bomb calorimeter (Parr 6300 Calorimeter, Parr Instrument Company, Moline, IL, USA) according to the UNE-EN ISO 9831:2004 standard. Liquid holding capacity analysis was carried out as described in Trullàs et al. [14]. Triplicate muscle samples of 3–4 cm were taken, weighed and placed in a tube with a weighed filter paper (Filter-Lab, Filtros Anoia, Sant Pere de Riudebitlles, Spain). Tubes were then placed in a centrifuge (Sigma 4K15, St. Louis, MO, USA) at 500 g for 10 min at 10 °C. Finally, the filter paper was dried at 50 °C until constant weight and drip, water and fat loss values were obtained. Liquid holding capacity assessment parameters were calculated as follows: water retained = (% total moisture % water loss)/% total moisture; fat retained = (% total fat % fat loss)/% total fat. Values were expressed as % of water and fat retained.

The FA content of feed and faeces were analysed following the method described by Sukhija and Palmquist [21]. Perivisceral fat and dorsal and ventral sections of the right muscle were analysed following the method described by Carrapiso et al. [22]. Nonadecanoic acid (C19:0; Sigma-Aldrich Chemical Co.; St. Louis, MO, USA) was added as an internal

standard. The final extract obtained was injected in a gas chromatograph (HP6890, Agilent Technologies; Waldbronn, Germany) following the method and conditions described by Cortinas et al. [23].

2.4. Characterization of Experimental Oils and Diets

Lipid class composition and MIU values of experimental oils are shown in Table 2. Crude oils (FO, soybean oil, olive pomace oil) were mainly composed of triacylglycerols (TAG; >77%), while the main lipid class component in soybean-sunflower acid oil and olive pomace acid oil was FFAs (53.25% and 44.95%, respectively). Furthermore, acid oils showed higher MIU values than their respective crude oils, with olive pomace acid oil having the highest total MIU value (6.15%), due to its higher values for both impurities and unsaponifiable matter.

Table 2. Fatty acid and lipid class composition and MIU values of experimental oils.

Item, %	Experimental Oils				
	FO	SO	SAO	OO	OAO
Fatty acid composition					
SFA	34.93	14.95	16.34	16.84	15.96
MUFA	28.68	25.83	32.33	71.82	66.73
PUFA	36.39	59.22	51.33	11.34	17.31
UFA:SFA	1.86	5.69	5.12	4.94	5.27
Individual fatty acids					
C16:0	21.76	10.73	11.24	13.26	11.54
C18:0	6.41	3.35	3.45	2.73	3.12
C18:1 n − 9	15.96	23.49	30.53	68.65	62.96
C18:2 n − 6	1.88	53.12	48.29	10.43	16.54
C18:3 n − 3	0.89	6.07	3.02	0.85	0.73
C20:5 n − 3	6.32	ND	ND	ND	ND
C22:6 n − 3	26.02	ND	ND	ND	ND
Lipid class composition					
TAG	85.67	93.88	29.31	77.47	36.27
DAG	6.85	4.16	16.10	8.42	17.35
MAG	4.35	0.50	1.34	0.87	1.43
FFA	3.13	1.46	53.25	13.24	44.95
MIU, g/100					
Moisture	0.24	0.05	0.40	0.36	0.31
Impurities	0.30	0.21	0.89	0.44	1.94
Unsaponifiable	2.01	0.53	2.35	1.64	3.90
Total MIU	2.55	0.80	3.64	2.44	6.15

Abbreviations: FO = fish oil; SO = crude soybean oil; SAO = soybean-sunflower acid oil; OO = crude olive pomace oil; OA = olive pomace acid oil; TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids; MIU = moisture, impurities and unsaponifiable; ND = non-detected.

The FA composition of experimental oils and diets are shown in Tables 2 and 3, respectively. Experimental diets showed a FA profile in accordance with the added experimental oils. Soybean diets (S and SA) were the richest in polyunsaturated fatty acids (PUFA; 53.61% and 48.62%, respectively) mainly due to their high linoleic acid content (C18:2 n − 6). In contrast, olive oil diets (O and OA) were the richest in monounsaturated fatty acids (MUFA; 52.84% and 48.22%, respectively), oleic acid (C18:1 n − 9) being the most abundant. Comparing acid with its corresponding crude oil diets, slightly higher MUFA and lower PUFA content were obtained for SA compared to S, while slightly lower MUFA and higher PUFA content were obtained for OA with respect to O. Diets with the experimental oil blends showed values close to the mean of those between the corresponding single oil diets. Among dietary treatments, the control diet (F) showed the highest percentage for saturated fatty acids (SFA; 33.11%), and n−3:n−6 ratio (2.09) due to having the highest EPA (6.98%) and DHA (23.21%) content.

Table 3. Fatty acid profile of experimental diets.

Item, %	Experimental Diets							
	F	S	SA	O	OA	S-O	S-OA	SA-OA
Fatty acid composition								
SFA	33.11	21.82	22.89	22.03	22.75	22.30	21.86	22.81
MUFA	24.45	24.57	28.49	52.84	48.22	36.26	38.60	38.41
PUFA	42.44	53.61	48.62	25.12	29.03	41.44	39.54	38.78
n − 3	31.55	14.06	12.35	11.01	11.40	12.70	12.43	11.92
n − 6	10.56	39.42	36.14	14.00	17.52	28.60	26.97	26.73
n − 3:n − 6	2.99	0.36	0.34	0.79	0.65	0.44	0.46	0.45
UFA:SFA	1.41	2.18	2.15	2.26	2.28	2.22	2.25	2.20
MUFA:PUFA	0.52	0.68	0.79	1.53	1.42	1.03	1.11	1.09
Individual fatty acids								
C16:0	20.73	14.52	14.96	15.11	15.17	14.85	14.77	15.02
C18:0	6.00	4.24	4.31	3.89	4.11	4.18	4.05	4.22
C18:1 n − 9	15.36	20.19	24.21	47.47	42.34	31.14	33.78	33.33
C18:2 n − 6	8.62	38.85	35.55	13.42	16.92	28.01	26.40	26.13
C18:3 n − 3	1.16	4.61	2.60	1.29	1.24	2.96	3.01	1.94
C20:4 n − 6	1.83	0.57	0.58	0.59	0.60	0.59	0.57	0.60
C20:5 n − 3	6.98	2.47	2.60	2.55	2.72	2.55	2.48	2.63
C22:6 n − 3	23.21	6.97	7.15	7.18	7.44	7.19	6.95	7.36

Abbreviations: F = fish oil diet (control); S = soybean oil diet; SA = soybean-sunflower acid oil diet; O = olive pomace oil diet; OA = olive pomace acid oil diet; S-O = S and O at 1:1 ratio; S-OA = S and OA at 1:1 ratio; SA-OA = SA and OA at 1:1 ratio; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids.

2.5. Colour Evaluation of Flesh

Colorimetric determinations were performed on the fresh and thawed left muscle using a Minolta chroma meter (Model CR_410, Minolta Co., Osaka, Japan) on the Norwegian Quality Cut (NQC) section [24]. Thawed muscles were stored for six months at_ 20 °C and defrosted overnight at 4 °C the day prior to colorimetric assessment. Determinations were carried out in the colour space L^*, a^*, b^* [25], where L^* represents the lightness of the sample, a^* defines the position between red/magenta and green and b^* defines the position between yellow and blue. Then, C^* (chroma, colour saturation) and h (hue angle) values were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and h = arctan (b^*/a^*), respectively [26]. Three measurements were performed on each of the six muscles per tank, and the mean value was used for statistical analysis.

2.6. Digestibility and Performance Parameter Calculations

All calculations were in accordance with standard formulae [27,28]. The apparent digestibility coefficient (ADC) of a particular nutrient or FA (X) was calculated as follows:

$$\% \text{ ADC of X} = \{1 - [(Xf/Mf)/(Xd/Md)]\} \times 100, \quad (1)$$

where Xf is the concentration of a particular nutrient or FA in faeces, Mf is the concentration of the inert marker in faeces, Xd is the concentration of a particular nutrient or FA in the diet, and Md is the concentration of the inert marker in the diet. The digestible energy of feeds was calculated from the product of energy ADC and its corresponding feed gross energy.

Growth performance and carcass parameters were calculated according to standard formulae. The average daily gain was calculated from:

$$\text{ADG (g)} = (\text{final weight} - \text{initial weight})/\text{numbers of days}; \quad (2)$$

average daily feed intake from:

$$\text{ADFI} = \text{total feed intake (as-fed basis)}/(\text{number of fish} * \text{number of days fed}); \quad (3)$$

feed conversion ratio from:

$$FCR = \text{total feed fed (as-fed basis)/wet weight gain;} \quad (4)$$

specific growth rate from:

$$SGR = [(\ln \text{final weight} - \ln \text{initial weight})/(\text{number of days})] * 100; \quad (5)$$

condition factor from:

$$CF = (\text{final weight/fork length})^3 \times 100; \quad (6)$$

carcass yield from:

$$\text{Carcass yield} = [(\text{body weight (BW)} - \text{visceral weight})/BW] * 100; \quad (7)$$

gross flesh yield from:

$$\text{Gross flesh yield} = (\text{entire left and right muscle weight/BW}) * 100; \quad (8)$$

net flesh yield from:

$$\text{Net flesh yield} = (\text{entire left and right muscle weight/eviscerated carcass weight}) * 100; \quad (9)$$

and perivisceral fat percentage from:

$$\text{Perivisceral fat percentage} = (\text{perivisceral fat weight/BW}) * 100. \quad (10)$$

2.7. Statistical Analysis

The normality of the data and homogeneity of variance were verified using the CAPABILITY procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). All data were analysed using the GLM (general linear model) procedure of SAS. Differences between means were tested using Tukey's adjust correction for multiple comparisons. For growth performance, digestibility balance, colorimetric and quality assessment of flesh and FA profile of muscle and perivisceral fat, the experimental unit was the tank. For carcass parameters, the experimental unit was the individual. The results in the tables are reported as the least square means, and differences were considered significant at $p < 0.05$.

3. Results

3.1. Performance and Carcass Parameters

The effects of added oils on growth performance and carcass parameters are shown in Table 4. Differences were obtained for all performance parameters among experimental diets throughout the experimental period, except for CF. At the end of the experimental period, animals fed OA showed the lowest BW ($p = 0.002$) ADG ($p = 0.003$) and SGR ($p = 0.002$) values among dietary treatments. Additionally, they had the highest FCR, which was significantly different to those fed F or SA ($p = 0.004$). In terms of ADFI, the lowest value was observed in animals fed F ($p = 0.005$).

Regarding carcass parameters, no differences among dietary treatments were observed in carcass weight or in the percentages of carcass yield, flesh yield and perivisceral fat ($p > 0.05$). Mean values of carcass yield and gross and net flesh yields were about 89%, 41% and 46%, respectively.

3.2. Digestibility Balance

Feed digestible energy and the ADC of macronutrients and FA are shown in Table 5. Experimental diets were well digested with an ADC for dry matter of about 96%. No differences were obtained for feed digestible energy or for the ADC of dry matter or crude protein ($p > 0.05$) among dietary treatments. In contrast, differences were observed in the

ADC of lipids. Acid oil diets (SA, OA and SA-OA) showed a lower ADC for crude fat than their corresponding crude oil diets (S, O and S-O, respectively; $p < 0.001$). When comparing to F, no differences were observed for diets including crude oils (alone or in a blend), while lower values of crude fat ADC were obtained for diets composed only of acid oils (alone or in a blend) ($p < 0.001$).

Table 4. Performance, feed efficiency and carcass parameters in European seabass fed different dietary fat sources.

Item	Experimental Diets								SEM [1]	*p*-value
	F	S	SA	O	OA	S-O	S-OA	SA-OA		
Performance parameters										
BW 0 days (g)	101.29	101.50	101.33	101.36	101.37	101.47	101.34	101.31	0.22	0.997
BW 100 days (g)	250.20 ^{a}	245.62 ^{a}	244.57 ^{a}	247.40 ^{a}	226.22 ^{b}	244.45 ^{a}	244.54 ^{a}	246.00 ^{a}	3.37	0.002
ADFI (g)	3.34 ^{b}	3.57 ^{a}	3.42 ^{ab}	3.57 ^{a}	3.41 ^{ab}	3.60 ^{a}	3.55 ^{ab}	3.59 ^{a}	0.05	0.005
ADG (g)	1.49 ^{a}	1.44 ^{a}	1.43 ^{a}	1.46 ^{a}	1.25 ^{b}	1.43 ^{a}	1.43 ^{a}	1.45 ^{a}	0.03	0.003
FCR	2.246 ^{b}	2.480 ^{ab}	2.388 ^{b}	2.443 ^{ab}	2.735 ^{a}	2.517 ^{ab}	2.480 ^{ab}	2.481 ^{ab}	0.074	0.004
SGR (%/d)	0.90 ^{a}	0.88 ^{a}	0.88 ^{a}	0.89 ^{a}	0.80 ^{b}	0.88 ^{a}	0.88 ^{a}	0.90 ^{a}	0.016	0.002
CF	1.94	1.95	1.95	1.99	1.88	2.00	1.93	1.99	0.034	0.206
Carcass parameters										
Carcass weight (g)	219.40	216.33	218.62	215.58	200.19	222.05	225.45	230.43	7.85	0.233
Carcass yield (%)	90.03	90.20	89.12	88.96	89.36	89.14	88.59	88.97	0.53	0.349
Gross flesh yield (%)	42.99	41.96	41.62	41.14	41.47	43.12	40.03	39.66	1.63	0.761
Net flesh yield (%)	47.82	46.70	46.88	46.42	45.16	48.74	45.40	44.56	1.99	0.826
Perivisceral fat (%)	6.22	5.98	6.93	6.76	6.29	7.12	7.10	6.87	0.43	0.341

Abbreviations: F = fish oil diet (control); S = soybean oil diet; SA = soybean-sunflower acid oil diet; O = olive pomace oil diet; OA = olive pomace acid oil diet; S-O = S and O at 1:1 ratio; S-OA = S and OA at 1:1 ratio; SA-OA = SA and OA at 1:1 ratio; BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; SGR = specific growth rate; CF = condition factor; SEM = standard error of the mean. [1] n = 3. ^{a,b} Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 5. Feed digestible energy, macronutrient and fatty acid apparent digestibility coefficients in European seabass fed different dietary fat sources.

Item, %	Dietary Treatments								SEM [1]	*p*-value
	F	S	SA	O	OA	S-O	S-OA	SA-OA		
DE and macronutrient ADC										
Digestible energy (kcal/kg)	4335.11	4335.05	4234.40	4336.50	4308.66	4485.63	4387.59	4303.19	55.60	0.114
Dry matter	96.34	96.08	95.81	96.02	95.91	96.67	96.21	96.14	0.28	0.388
Crude protein	91.63	89.45	89.16	90.05	89.52	91.96	90.47	90.74	0.89	0.167
Lipids	96.87 ^{a}	96.66 ^{ab}	93.96 ^{cd}	96.26 ^{abc}	93.38 ^{d}	97.37 ^{a}	95.79 ^{abc}	94.41 ^{bcd}	0.59	<0.001
Fatty acid ADC										
Total fatty acids	90.73 ^{c}	93.93 ^{ab}	91.77 ^{bc}	93.79 ^{abc}	91.66 ^{bc}	95.56 ^{a}	94.26 ^{ab}	93.32 ^{abc}	0.78	0.003
SFA	79.59 ^{cd}	84.37 ^{abc}	78.77 ^{d}	86.20 ^{ab}	81.11 ^{bcd}	88.96 ^{a}	86.56 ^{ab}	83.02 ^{abcd}	1.37	<0.001
MUFA	92.31 ^{b}	93.92 ^{ab}	93.14 ^{ab}	95.31 ^{ab}	93.65 ^{ab}	96.43 ^{a}	94.96 ^{ab}	95.03 ^{ab}	0.83	0.023
PUFA	98.50	97.81	97.09	97.23	96.61	98.37	97.79	97.68	0.52	0.104
n − 3	99.27	98.79	98.39	98.25	98.04	99.15	98.66	98.65	0.36	0.141
n − 6	96.16	97.46	96.63	96.40	95.65	98.01	97.39	97.23	0.58	0.072
Individual fatty acids										
C16:0	80.13 ^{d}	85.7 ^{abc}	81.52 ^{cd}	87.48 ^{ab}	83.24 ^{bcd}	89.81 ^{a}	88.05 ^{ab}	85.23 ^{abcd}	1.31	<0.001
C18:0	75.17 ^{cd}	81.04 ^{abc}	74.30 ^{d}	82.85 ^{ab}	76.85 ^{bcd}	86.35 ^{a}	83.79 ^{a}	79.41 ^{abcd}	1.66	<0.001
C18:1 n − 9	92.99 ^{b}	94.71 ^{ab}	93.86 ^{ab}	95.71 ^{ab}	94.16 ^{ab}	96.77 ^{a}	95.50 ^{ab}	95.58 ^{ab}	0.82	0.040
C18:2 n − 6	95.30 ^{b}	97.42 ^{ab}	96.58 ^{ab}	96.24 ^{ab}	95.50 ^{ab}	97.96 ^{a}	97.34 ^{ab}	97.17 ^{ab}	0.60	0.020
C18:3 n − 3	98.03	98.27	97.33	96.49	95.86	98.67	98.10	97.55	0.64	0.034
C20:5 n − 3	99.28	99.48	99.16	98.49	98.34	100.00	99.08	99.09	0.54	0.348
C22:6 n − 3	99.33	98.90	98.50	98.48	98.30	99.05	98.75	98.78	0.27	0.091

Abbreviations: DE = digestible energy; ADC = apparent digestibility coefficient; F = fish oil diet (control); S = soybean oil diet; SA = soybean-sunflower acid oil diet; O = olive pomace oil diet; OA = olive pomace acid oil diet; S-O = S and O at 1:1 ratio; S-OA = S and OA at 1:1 ratio; SA-OA = SA and OA at 1:1 ratio; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SEM = standard error of the mean. [1] n = 3. $^{a–d}$ Values within a row with different superscripts differ significantly at $p < 0.05$.

Concerning ADC of FA, significant differences were observed among dietary treatments for total FA (TFA), SFA and MUFA, but not for PUFA, n − 3 or n − 6 FA. No

significant differences were obtained for TFA and MUFA digestibility between diets containing acid oils and their corresponding crude oil ($p > 0.05$). For SFA, only SA showed lower digestibility than S ($p < 0.05$).

When acid oil diets (SA, OA and SA-OA) were compared to F, no significant differences in the ADC of FA were obtained ($p > 0.05$). In contrast, higher TFA digestibility was obtained for diets including crude soybean oil (S, S-O and S-OA) ($p < 0.01$). Similarly, diets with crude olive pomace oil (O and S-O) showed higher SFA digestibility than F ($p < 0.001$).

3.3. Flesh Composition and Quality Parameters

The colorimetric assessment, chemical composition and liquid holding capacity of the flesh are shown in Table 6. In fresh muscle, differences were only observed for the parameter L^*, S-OA showing the lowest value among dietary treatments ($p = 0.001$). In thawed muscle, differences were observed for both C^* and b^* parameters, the flesh from diets O and S-O being those that showed the lowest values among dietary treatments ($p < 0.01$). When comparing fresh to thawed muscle, L^* increased, while h and a^* decreased ($p < 0.001$).

Table 6. Colorimetric assessment, chemical composition and liquid holding capacity of European seabass flesh according to different dietary treatments.

Colour Parameters [1]	Dietary Treatments								SEM [2]	*p*-value
	F	S	SA	O	OA	S-O	S-OA	SA-OA		
Fresh muscle										
L^*	40.21 [a]	40.24 [a]	41.63 [a]	39.62 [ab]	41.73 [a]	39.84 [a]	32.25 [b]	35.48 [ab]	1.74	0.001
C^*	3.02	3.43	2.99	3.08	3.21	3.55	2.53	2.54	0.26	0.093
h	1.26	1.26	1.25	1.26	1.27	1.21	0.89	1.22	0.10	0.062
a^*	0.94	1.22	0.90	0.97	0.99	1.33	0.70	0.90	0.19	0.498
b^*	2.82	3.20	2.62	2.87	2.97	3.06	2.37	2.31	0.23	0.058
Thawed muscle										
L^*	48.94	47.46	48.61	48.00	49.51	48.16	49.51	47.78	0.77	0.435
C^*	4.16 [a]	3.52 [ab]	3.68 [ab]	3.08 [b]	3.93 [ab]	2.98 [b]	3.60 [ab]	3.25 [ab]	0.23	0.006
h	−1.30	−1.09	−1.18	−1.04	−1.02	−0.93	−0.84	−0.99	0.11	0.109
a^*	−0.98	−1.35	−1.27	−1.48	−1.24	−1.51	−1.52	−1.50	0.13	0.057
b^*	3.99 [a]	3.15 [abc]	3.39 [abc]	2.64 [bc]	3.67 [ab]	2.39 [c]	3.08 [abc]	2.74 [abc]	0.29	0.002
Chemical composition (%) [3]										
Moisture	68.23	68.44	69.13	69.56	68.99	68.42	68.02	68.17	0.51	0.393
Organic matter	96.25	96.39	95.99	95.86	95.81	96.25	96.39	95.70	0.39	0.820
Crude protein	63.73	61.47	63.66	65.22	63.56	62.22	62.46	65.05	1.13	0.286
Ash	3.75	3.61	4.01	4.14	4.19	3.75	3.61	4.30	0.39	0.820
Lipid content in dorsal muscle	17.19	17.75	16.93	16.69	16.95	17.04	20.42	16.66	1.95	0.747
Lipid content in ventral muscle	30.12	37.11	35.20	35.48	33.88	38.12	37.93	34.94	2.12	0.235
Liquid holding capacity (as % retained) [4]										
Drip loss	21.91	23.39	21.68	22.84	23.18	24.83	21.37	23.53	1.10	0.420
Water retained	72.70	72.22	72.27	72.30	76.08	69.73	73.15	70.41	2.02	0.537
Fat retained	86.68	83.80	85.35	84.16	85.67	83.33	88.44	85.11	2.05	0.704

Abbreviations: F = fish oil diet (control); S = soybean oil diet; SA = soybean-sunflower acid oil diet; O = olive pomace oil diet; OA = olive pomace acid oil diet; S-O = S and O at 1:1 ratio; S-OA = S and OA at 1:1 ratio; SA-OA = SA and OA at 1:1 ratio; SEM = standard error of the mean. [1] L^* = lightness; C^* = Chroma = $(a^{*2} + b^{*2})^{1/2}$ (Wyszeki and Stiles, 1967); h = hue = arctan (b^*/a^*) (Wyszeki and Stiles, 1967); a^* = position between red/magenta and green; b^* = position between yellow and blue. [2] n = 3. [3] Values expressed as % of dry matter. [4] Measured in thawed muscles. [a–c] Values within a row with different superscripts differ significantly at $p < 0.05$.

No differences were observed in the chemical composition of flesh. Regarding lipid content, no statistical differences were found among dietary treatments either for the dorsal or ventral sections. However, the ventral section of the muscle showed a higher lipid content than the dorsal (about 35% vs. 17% on average, respectively; $p < 0.001$). On the other hand, dietary treatments showed no differences in terms of the liquid holding capacity of thawed muscle.

3.4. Fatty Acid Profile of Flesh and Perivisceral Fat

The FA profiles of the dorsal and ventral sections of the muscle and perivisceral fat are presented in Tables 7 and 8, respectively. In both tissues, differences observed in the

FA profile among dietary treatments mirrored those of the FA profile of the experimental diets. Animals fed soybean oil diets (S or SA) had the highest PUFA ($p < 0.001$) content, while those fed olive pomace oil diets (O or OA) did for MUFA ($p < 0.001$). However, the differences in MUFA and PUFA composition observed between acid oil and their corresponding crude oil diets are more clearly reflected in the FA profile of perivisceral fat than in the two sections of muscle (dorsal and ventral). When compared with animals fed F, higher UFA: SFA and lower n −3: n − 6 ratios ($p < 0.001$) were observed in animals fed VO diets. In perivisceral fat, the EPA and DHA contents in treatments including VO were about 30–33% and 43–52%, respectively, of those of F. Higher content (with respect to F) was obtained for the dorsal (56–62% for EPA; 45–52% for DHA) and ventral (54–60% for EPA; 42–57% for DHA). Moreover, higher DHA content was obtained for the dorsal muscle compared to the ventral muscle ($p = 0.046$), but no other significant differences were observed in the FA profile of the dorsal and ventral sections.

Table 7. Fatty acid composition of dorsal and ventral muscle from European seabass according to different dietary treatments.

Item, %	Dietary Treatments								SEM [1]	*p*-value
	F	S	SA	O	OA	S-O	S-OA	SA-OA		
Dorsal muscle										
Fatty acid composition										
SFA	26.46 ^{a}	22.38 ^{b}	22.61 ^{b}	22.40 ^{b}	22.49 ^{b}	22.46 ^{b}	22.33 ^{b}	22.36 ^{b}	0.16	<0.001
MUFA	32.80 ^{c}	32.88 ^{c}	34.15 ^{c}	48.56 ^{a}	46.01 ^{a}	39.15 ^{b}	40.93 ^{b}	40.07 ^{b}	0.56	<0.001
PUFA	40.74 ^{bc}	44.74 ^{a}	43.25 ^{ab}	29.04 ^{e}	31.51 ^{e}	38.39 ^{cd}	36.74 ^{d}	37.56 ^{d}	0.58	<0.001
UFA:SFA	2.78 ^{b}	3.47 ^{a}	3.42 ^{a}	3.46 ^{a}	3.45 ^{a}	3.45 ^{a}	3.48 ^{a}	3.47 ^{a}	0.03	<0.001
MUFA:PUFA	0.81 ^{d}	0.74 ^{d}	0.79 ^{d}	1.67 ^{a}	1.46 ^{b}	1.02 ^{c}	1.11 ^{c}	1.07 ^{c}	0.03	<0.001
n − 3	26.93 ^{a}	15.84 ^{b}	15.82 ^{b}	14.72 ^{b}	15.05 ^{b}	14.96 ^{b}	14.57 ^{b}	15.47 ^{b}	0.44	<0.001
n − 6	12.71 ^{d}	27.52 ^{a}	26.21 ^{a}	13.61 ^{d}	15.61 ^{c}	22.42 ^{b}	21.08 ^{b}	21.31 ^{b}	0.36	<0.001
n − 3:n − 6	2.13 ^{a}	0.58 ^{c}	0.60 ^{c}	1.08 ^{b}	0.96 ^{b}	0.67 ^{c}	0.69 ^{c}	0.73 ^{c}	0.04	<0.001
Individual fatty acids										
C16:0	17.82 ^{a}	15.30 ^{b}	15.52 ^{b}	17.73 ^{b}	15.66 ^{b}	15.42 ^{b}	15.56 ^{b}	15.44 ^{b}	0.14	<0.001
C18:0	4.74 ^{a}	4.55 ^{ab}	4.52 ^{ab}	4.18 ^{d}	4.20 ^{cd}	4.48 ^{abc}	4.22 ^{cd}	4.36 ^{bcd}	0.06	<0.001
C18:1 n − 9	24.05 ^{e}	26.59 ^{de}	27.72 ^{d}	41.71 ^{a}	38.63 ^{b}	32.55 ^{c}	34.14 ^{c}	33.53 ^{c}	0.53	<0.001
C18:2 n − 6	11.15 ^{d}	26.76 ^{a}	25.41 ^{a}	12.80 ^{d}	14.83 ^{c}	21.66 ^{b}	20.37 ^{b}	20.48 ^{b}	0.36	<0.001
C18:3 n − 3	1.85 ^{bc}	3.18 ^{a}	2.74 ^{ab}	1.77 ^{c}	1.93 ^{bc}	2.69 ^{ab}	2.72 ^{ab}	2.06 ^{bc}	0.19	<0.001
C20:4 n − 6	1.56 ^{a}	0.77 ^{b}	0.80 ^{b}	0.81 ^{b}	0.78 ^{b}	0.76 ^{b}	0.71 ^{b}	0.83 ^{b}	0.03	<0.001
C20:5 n − 3	5.46 ^{a}	3.10 ^{bc}	3.21 ^{bc}	3.19 ^{bc}	3.39 ^{b}	3.08 ^{c}	3.07 ^{c}	3.22 ^{bc}	0.06	<0.001
C22:6 n − 3	19.62 ^{a}	9.56 ^{b}	9.87 ^{b}	9.76 ^{b}	9.73 ^{b}	9.19 ^{b}	8.78 ^{b}	10.18 ^{b}	0.42	<0.001
Ventral muscle										
Fatty acid composition										
SFA	26.00 ^{a}	21.97 ^{b}	21.99 ^{b}	22.04 ^{b}	21.86 ^{b}	21.86 ^{b}	21.90 ^{b}	21.74 ^{b}	0.21	<0.001
MUFA	35.41 ^{c}	33.47 ^{c}	35.82 ^{c}	50.21 ^{a}	48.08 ^{a}	40.70 ^{b}	42.16 ^{b}	41.99 ^{b}	0.65	<0.001
PUFA	38.57 ^{b}	44.50 ^{a}	42.09 ^{a}	27.69 ^{c}	29.96 ^{c}	37.34 ^{b}	35.86 ^{b}	36.16 ^{b}	0.56	<0.001
UFA:SFA	2.85 ^{b}	3.55 ^{a}	3.54 ^{a}	3.53 ^{a}	3.57 ^{a}	3.57 ^{a}	3.56 ^{a}	3.60 ^{a}	0.04	<0.001
MUFA:PUFA	0.92 ^{de}	0.75 ^{e}	0.85 ^{e}	1.82 ^{a}	1.61 ^{b}	1.09 ^{cd}	1.18 ^{c}	1.16 ^{c}	0.04	<0.001
n − 3	24.31 ^{a}	13.91 ^{b}	13.63 ^{b}	12.83 ^{b}	12.84 ^{b}	12.84 ^{b}	12.98 ^{b}	12.92 ^{b}	0.52	<0.001
n − 6	13.13 ^{f}	29.12 ^{a}	27.21 ^{b}	14.03 ^{f}	16.18 ^{e}	23.41 ^{c}	21.77 ^{d}	22.18 ^{cd}	0.27	<0.001
n − 3:n − 6	1.85 ^{a}	0.48 ^{d}	0.50 ^{d}	0.91 ^{b}	0.79 ^{bc}	0.55 ^{d}	0.60 ^{cd}	0.58 ^{cd}	0.05	<0.001
Individual fatty acids										
C16:0	17.45 ^{a}	15.04 ^{b}	15.08 ^{b}	15.41 ^{b}	15.23 ^{b}	15.03 ^{b}	15.24 ^{b}	15.00 ^{b}	0.14	<0.001
C18:0	4.40 ^{a}	4.23 ^{ab}	4.09 ^{bc}	3.83 ^{d}	3.82 ^{d}	4.08 ^{bc}	3.97 ^{cd}	3.95 ^{cd}	0.05	<0.001
C18:1 n − 9	26.22 ^{c}	26.75 ^{c}	29.23 ^{c}	42.91 ^{a}	40.36 ^{a}	33.82 ^{b}	35.19 ^{b}	35.12 ^{b}	0.72	<0.001
C18:2 n − 6	11.87 ^{g}	28.57 ^{a}	26.60 ^{b}	13.42 ^{f}	15.59 ^{e}	22.85 ^{c}	21.22 ^{d}	21.59 ^{cd}	0.28	<0.001
C18:3 n − 3	1.93 ^{e}	3.73 ^{a}	2.55 ^{c}	1.88 ^{e}	2.01 ^{de}	2.84 ^{b}	2.83 ^{b}	2.22 ^{d}	0.05	<0.001
C20:4 n − 6	1.26 ^{a}	0.55 ^{b}	0.61 ^{b}	0.61 ^{b}	0.59 ^{b}	0.55 ^{b}	0.55 ^{b}	0.59 ^{b}	0.02	<0.001
C20:5 n − 3	5.15 ^{a}	2.86 ^{b}	2.98 ^{b}	2.96 ^{b}	3.10 ^{b}	2.77 ^{b}	2.84 ^{b}	2.90 ^{b}	0.10	<0.001
C22:6 n − 3	17.20 ^{a}	7.33 ^{b}	8.09 ^{b}	7.99 ^{b}	7.73 ^{b}	7.23 ^{b}	7.32 ^{b}	7.80 ^{b}	0.42	<0.001

Abbreviations: F = fish oil diet (control); S = soybean oil diet; SA = soybean-sunflower acid oil diet; O = olive pomace oil diet; OA = olive pomace acid oil diet; S-O = S and O at 1:1 ratio; S-OA = S and OA at 1:1 ratio; SA-OA = SA and OA at 1:1 ratio; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids; SEM = standard error of the mean. [1] n = 3. $^{a–g}$ Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 8. Fatty acid composition of perivisceral fat from European seabass according to different dietary fat sources.

Item, %	Dietary Treatments								SEM [1]	*p*-value
	F	S	SA	O	OA	S-O	S-OA	SA-OA		
Fatty acid composition										
SFA	26.33 [a]	21.58 [b]	22.04 [b]	21.25 [b]	21.35 [b]	21.57 [b]	21.94 [b]	21.51 [b]	0.38	<0.001
MUFA	35.58 [e]	34.71 [e]	40.01 [d]	54.35 [a]	50.24 [b]	44.17 [c]	42.04 [cd]	44.34 [c]	0.57	<0.001
PUFA	37.37 [b]	42.77 [a]	37.20 [b]	23.93 [e]	27.92 [d]	33.66 [c]	35.38 [bc]	33.60 [c]	0.54	<0.001
UFA:SFA	2.77 [b]	3.59 [a]	3.51 [a]	3.69 [a]	3.66 [a]	3.61 [a]	3.53 [a]	3.63 [a]	0.08	<0.001
MUFA:PUFA	0.96 [ef]	0.81 [f]	1.08 [de]	2.27 [a]	1.80 [b]	1.32 [c]	1.19 [cd]	1.32 [c]	0.04	<0.001
n − 3	22.99 [a]	11.06 [b]	10.68 [bc]	9.26 [c]	9.70 [bc]	10.45 [bc]	10.28 [bc]	10.04 [bc]	0.31	<0.001
n − 6	14.38 [e]	31.71 [a]	26.52 [b]	14.67 [e]	18.22 [d]	23.21 [c]	25.09 [bc]	23.56 [c]	0.41	<0.001
n − 3:n − 6	1.60 [a]	0.35 [d]	0.40 [cd]	0.63 [b]	0.53 [bc]	0.45 [cd]	0.41 [cd]	0.42 [cd]	0.03	<0.001
Individual fatty acids										
C16:0	17.44 [a]	14.70 [b]	15.14 [b]	14.97 [b]	14.73 [b]	15.05 [b]	15.12 [b]	14.93 [b]	0.26	<0.001
C18:0	4.14 [a]	3.90 [ab]	3.76 [ab]	3.33 [b]	3.43 [b]	3.64 [ab]	3.86 [ab]	3.41 [b]	0.14	0.009
C18:1 n − 9	25.75 [e]	28.01 [e]	32.56 [d]	46.97 [a]	42.40 [b]	37.17 [c]	35.29 [c]	37.10 [c]	0.50	<0.001
C18:2 n − 6	12.99 [e]	30.99 [a]	25.77 [b]	14.04 [e]	17.54 [d]	22.55 [c]	24.43 [bc]	22.83 [c]	0.41	<0.001
C18:3 n − 3	2.20 [cde]	4.05 [a]	2.75 [bc]	1.91 [e]	2.15 [de]	3.01 [b]	2.93 [b]	2.54 [bcd]	0.11	<0.001
C20:4 n − 6	1.10 [a]	0.37 [b]	0.42 [b]	0.38 [b]	0.39 [b]	0.38 [b]	0.36 [b]	0.38 [b]	0.02	<0.001
C20:5 n − 3	5.12 [a]	2.31 [b]	2.65 [b]	2.30 [b]	2.43 [b]	2.41 [b]	2.20 [b]	2.38 [b]	0.10	<0.001
C22:6 n − 3	15.48 [a]	4.59 [b]	5.17 [b]	4.96 [b]	5.02 [b]	4.93 [b]	5.05 [b]	5.01 [b]	0.35	<0.001

Abbreviations: F = fish oil diet (control); S = soybean oil diet; SA = soybean-sunflower acid oil diet; O = olive pomace oil diet; OA = olive pomace acid oil diet; S-O = S and O at 1:1 ratio; S-OA = S and OA at 1:1 ratio; SA-OA = SA and OA at 1:1 ratio; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids; SEM = standard error of the mean. [1] n = 3. Minor fatty acids were considered those that were in <1% proportion. [a–f] Values within a row with different superscripts differ significantly at $p < 0.05$.

4. Discussion

4.1. Performance and Carcass Parameters

In the present study, the level of replacement of FO was formulated according to previous studies in rainbow trout and gilthead seabream [12,15,16,29] and to ensure the reported requirements of n-3 HUFA for European seabass in older juvenile and pre-adult stages [19,30,31]. This level of replacement of FO (75%) with crude vegetable oils (soybean and olive pomace oils) did not affect the performance, achieving similar final weights and FCR. This is in agreement with other studies in European seabass, in which no differences in either SGR or feed utilisation efficiency were found with up to 60–80% of FO replacement in diets with 16–20% added dietary fat [32–35]. Nevertheless, when acid oils rich in FFA were used, differences were observed between the two oil sources of different botanical origin. Animals fed SA (53.25% of FFA) achieved a similar performance to that obtained for animals fed crude soybean oil. However, the opposite was obtained for olive pomace acid oil, since animals fed OA (44.95% of FFA) showed the worst performance (lowest SGR and final BW, together with the highest FCR value). In the study by Trullàs et al. [12], where another MUFA-rich acid oil such as rapeseed acid oil was used in rainbow trout diets, no differences were observed in SGR or FCR (42.4–47.3% of FFA) compared to diets containing crude or re-esterified rapeseed oils. A high dietary content of FFA has been associated with lower feed DE values [36,37], which might affect growth. However, in the present study, no differences in digestible energy (DE) of feed among dietary treatments were observed. In addition, a higher MIU value, which estimates the non-energetic fraction of fats and oils, has been associated with a decrease in the DE content of dietary fats [10,38]. The MIU value observed in olive pomace acid oil (6.15%) was 1.7 to 2.5 times higher than those obtained for soybean-sunflower acid oil and the other experimental crude oils, so the MIU content could explain the lower performance observed by fish fed OA. On the other hand, the negative effect of the inclusion of olive pomace acid oil on performance was not observed when this acid oil was blended with soybean oils (crude or acid oil). In this case, a decrease in the MIU content as a result of the blend between oils might contribute to this effect. However, although Trullàs et al. [12] described a lack of negative effects on performance in rainbow

trout fed rapeseed acid oil, the MIU values of dietary added fats are not reported in this study. It is therefore important to highlight the need to assess the non-energetic fraction of dietary added fats, especially in the case of acid oils, since as by-products from the edible oil refining industry they can present high variability in their composition depending on the amount and type of compounds removed from crude oil [10].

Both carcass and muscle yields are parameters that may be useful for the industry, as the main valuable final product of aquaculture production is the fillet. However, as far as we know, no studies have assessed the effect of different VO or dietary FFA content on carcass parameters or flesh yield. In the present study, the percentage of FO replacement (75%) with VO (crude or acid, alone or in blends) had no effect on carcass parameters or flesh yield. In addition, lower gross flesh yields were obtained in the present study (40% to 43% of BW) than those reported by Lanari et al. [39] and Vandeputte et al. [40] (44.5% and 57.4% of BW, respectively), which may be related to the smaller size of the animals obtained at the end of the study (226–250 g vs. 316–395 g of BW). On the other hand, similar values of perivisceral fat deposition were obtained for diets including acid oils and their respective crude oils, and also for the other experimental oils or blends, so dietary FFA content and the different compositions of the two added oils of different botanical origin do not seem to be relevant factors in determining perivisceral fat deposition.

4.2. Digestibility Balance

The replacement of FO with crude or soybean-sunflower and olive pomace acid oils or blends had no effect on the digestibility of dry matter and crude protein. These results are in agreement with other studies that found no differences in digestibility of nutrients when FO was replaced with different VO [15]. In contrast, the results suggest that the digestibility of lipids decreases when acid oils are included in European seabass diets, as a lower digestibility was obtained in diets containing acid oils (SA, OA and SA-OA) when compared to those containing their corresponding crude oil (alone or in blends). It has been described that increasing the FFA content of the added lipid source has a negative effect on lipid digestibility [36,37] due to the higher melting point and the ability to form insoluble soaps that are unavailable for absorption [41,42]. However, the negative effects of FFA on fish lipid digestibility are controversial in the literature. In contrast to the observations in the present results, Ng et al. [13] described an increase in the ADC values of lipids when replacing FO with palm fatty acid distillates (which are mainly composed of FFA; >90%) in rainbow trout diets. As described above, the higher non-energetic fraction (MIU, especially of unsaponifiable matter) content of the acid oils used in the present study might negatively affect lipid digestibility, leading to a more pronounced decrease in the ADC values of lipids in SA and OA diets [10].

Similar to the observation for ADC of lipids, a decrease in total FA digestibility was observed for the use of acid oil diets, although it was not significant. Trullàs et al. [15] reported a significant decrease in total FA digestibility in rainbow trout fed diets including rapeseed acid oil in comparison to its respective crude oil. In the present study, the lower values for total FA digestibility seem to be related to a lower SFA digestibility in acid oils, as no differences were observed either for MUFA or PUFA. In agreement with this, it is well known that saturated FFA have a greater ability to form insoluble soaps as opposed to unsaturated FFA [43]. In contrast, when diets included acid oils in blends, higher values for ADC of FA were obtained. This effect could be explained by the decrease in the MUFA:PUFA ratio (increasing the level of unsaturation) and/or by the presence of a higher content of other lipid classes such as DAG or MAG, generated from the hydrolysis of TAG (in the case of S-OA), which may enhance the inclusion of FFA from olive pomace acid oil in mixed micelles facilitating their absorption [44,45]. It is important to note that the ADCs of lipids and total FA were high for all dietary treatments (90.7–97.4%), in agreement with results reported by other authors using VO as FO replacers in fish diets [46–48].

4.3. Flesh Composition and Quality Parameters

In species with a white flesh colour, such as European seabass, preservation of the expected whiteness is a key attribute for determining sensory quality with regard to consumer acceptance [49]. Acid oils can concentrate a higher content of unsaponifiable matter during the refining process of the crude oil that they come from, which consists of many components, including compounds such as sterols, tocopherols, tocotrienols and hydrocarbons, and also pigments that could modify the flesh colour [14]. However, diets including acid oils alone did not show different values to those including crude oils despite the higher content of unsaponifiable matter, especially in the case of olive pomace acid oil, which is characterised by a notable dark colour. In fact, the slight differences in lightness of fresh muscles obtained in the present study did not show a consistent pattern in relation to the dietary treatments. In general, the replacement of crude VO with its acid oils (soybean-sunflower or olive pomace acid oils) did not modify the colour parameters of either fresh or thawed muscle. When comparing fresh to thawed muscle, the present results showed higher L^* values in thawed muscles, in agreement with other authors who have confirmed that freezing and storage generally increases flesh brightness [14,50,51].

When the chemical composition and liquid holding capacity of thawed muscle are considered, a non-significant effect of replacing FO with crude and soybean-sunflower and olive pomace acid oils was obtained. These results are in agreement with those found when replacing FO with crude VO such as soybean, rapeseed, linseed and olive oils [34,35,52,53], or with rapeseed acid oil [14]. Similarly, the lipid content of the dorsal and ventral muscle sections was not affected by the botanical origin of the added oil or by FFA content. However, it is important to note that the ventral section of the muscle had approximately twice the amount of lipid content compared to the dorsal section, in agreement with the literature, as it is well known that there is higher fat deposition in the ventral section of the muscle in fish [54,55].

4.4. Fatty Acid Profile of Flesh and Perivisceral Fat

The results of the present study suggest that there is no effect of dietary FFA content on the FA profile of flesh and perivisceral fat, but it is affected by the dietary FA profile. The slight differences between diets containing crude or acid oils were those present in the FA profile of the diets. Animals fed S or SA produced flesh that was richer in PUFA and the flesh of those fed O or OA was richer in MUFA. Hence, the inclusion of acid oils in the diets helped to obtain a final product with a similar FA profile to that obtained with animals fed their respective crude oils.

Although the FA composition of flesh and perivisceral fat reflected that of the diet, differences in n–3 HUFA and C18 FA concentrations were less marked in the FA composition of flesh than that of perivisceral fat. The same effect was observed in other studies performed in European seabass [34,35], in Atlantic salmon (*Salmo salar*) [56,57], in rainbow trout [58] and in gilthead seabream [29]. This could be related to preferential–n 3 HUFA retention in the muscle to maintain an adequate level of fluidity in cell membranes [57–59], while the main C18 FA present in VO diets are preferentially used for oxidation processes or are accumulated in the liver [34,56]. Regarding the two sections of flesh, the dorsal section of the muscle showed a higher DHA content than the ventral, which is consistent with the results obtained in European seabass by Campos et al. [60], and could be explained by the higher lipid content in the ventral section of the flesh.

5. Conclusions

In conclusion, the substitution of fish oil with different vegetable oils (75%) showed different results depending on the botanical origin and free fatty acid content of the experimental oils. The inclusion of soybean-sunflower acid oil as a replacement for soybean oil does not have a negative effect on performance, feed efficiency or the studied flesh parameters. In contrast, the inclusion of olive pomace acid oil as a replacement for olive pomace oil impaired performance and feed efficiency. However, the negative effects observed for

the inclusion of olive pomace acid oil alone disappeared when acid oils were included in a blend with soybean oil or soybean-sunflower acid oil. It is important to note that although similar performance and feed efficiency results could be achieved by including acid oils instead of their respective crude oils, the digestibility of lipids decreased. Hence, the correct evaluation of acid oil quality parameters such as MIU would help to incorporate acid oils in aquaculture diets, since they are highly variable sources in terms of composition.

The present results offer a view on the preliminary step for the potential use of acid oils in farmed fish species. However, further studies assessing the effects of the inclusion of these oils on metabolism, immunology, intestinal health and product quality are needed before recommending their use.

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Chapter 6.

General discussion

"It's not so important what people think when you come in. It's much more important what people think when you leave"

Jürgen Klopp.

6. General discussion

6.1. Inclusion of olive pomace oil and acid oil in pig, broiler chicken and European seabass diets

In this part of the general discussion results obtained in the three studies carried out during this PhD thesis will be compared and discussed among species (pigs, broiler chickens and European seabass), evaluating the results of performance and feed efficiency, digestibility, and fat deposition and the FA profile of the final products obtained. In all three studies, experimental treatments were designed consisting of a basal diet with different experimental added fat sources, where the fat sources and the level of inclusion varied according to species. However, this discussion focuses on diets that included olive pomace oil (**O**) and olive pomace acid oil (**OA**) as the sole source of added fat, comparing them with each other and with a control diet that included a commonly used fat source for each species (palm oil [**PO**] for pigs and broilers; fish oil [**FO**] for European seabass). In this sense, two main effects will be evaluated, that of the degree of saturation (PO, rich in SFA; FO, rich in long-chain PUFA; O/OA, rich in MUFA) and that of the molecular structure of the FA (O, rich in TAG; OA, rich in FFA).

In general, the diet including olive pomace oil, rich in MUFA and TAG, showed great results in the three studied species, achieving good performance and feed efficiency, high digestibility values and elevated levels of fat deposition in final meat products. On the other hand, the diet including olive pomace acid oil, rich in MUFA and FFA, showed controversial results according to the animal species: similar results than the control diet (PO, rich in SFA and TAG) were obtained in both pigs and broiler chickens, but an impairment on performance and feed efficiency was observed in European seabass compared to FO (rich in PUFA and TAG). However, the usually competitive prices of acid oils may make olive pomace acid oil cost-effective as an alternative fat source, offsetting the drop in performance and feed efficiency.

6.1.1. Performance and feed efficiency

The effects of dietary inclusion of olive pomace oil and acid oil on the BW and the FCR of pigs, broiler chickens and European seabass are shown in **Figure 6.1.** Olive pomace acid oil,

rich in MUFA and FFA, showed a significant effect in European seabass, where fish fed OA had the lowest final BW and the highest FCR among dietary treatments. Additionally, a tendency to decrease BW was also observed in broiler chickens (statistically) and in pigs (numerically) compared to O. On the other hand, olive pomace oil had the lowest feed conversion ratio in broiler chickens.

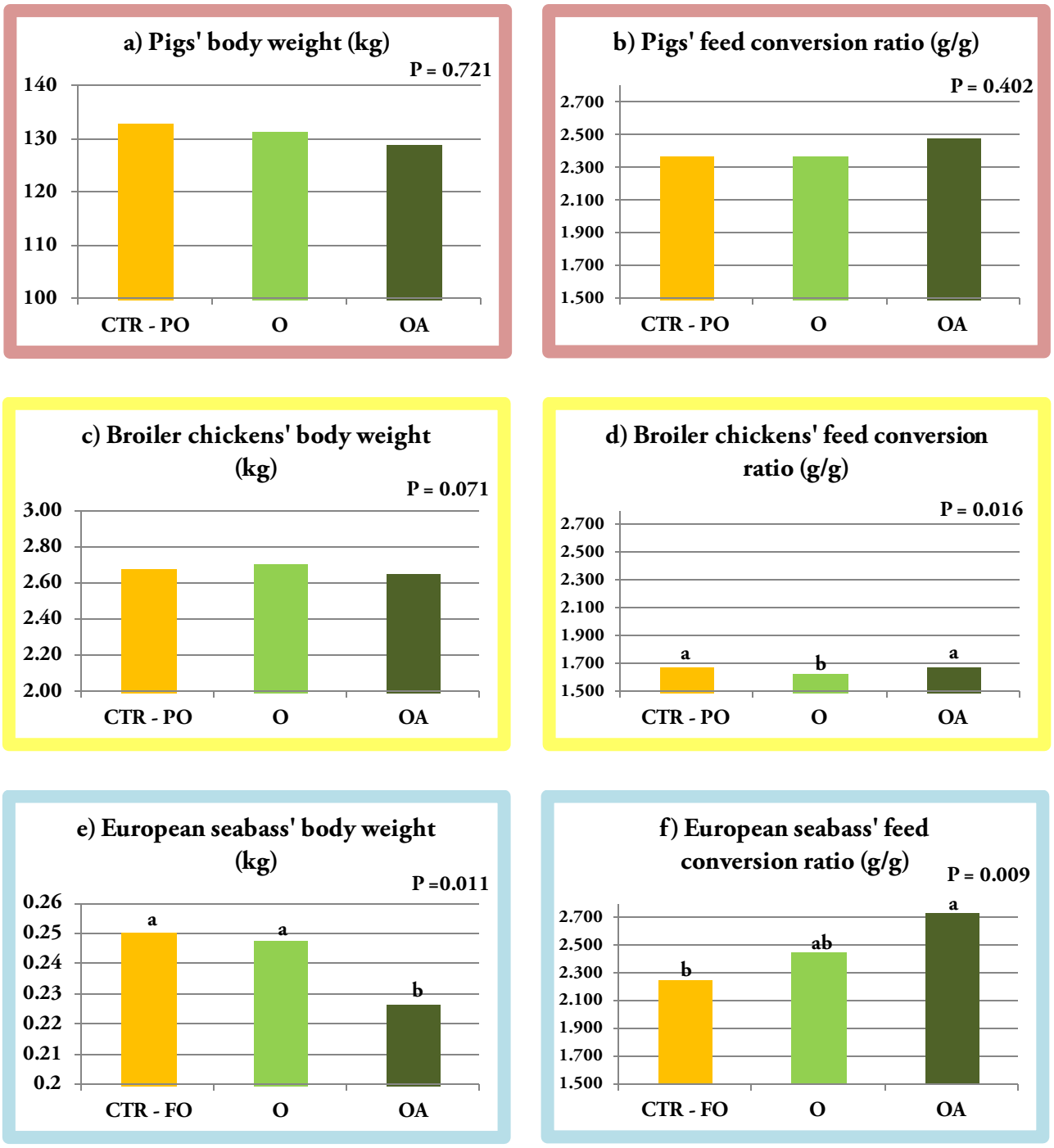

Figure 6.1. Effect of dietary inclusion of olive pomace oil and acid oil on final body weight and feed conversion ratio of pigs (a, b), broiler chickens (c, d) and European seabass (e, f). Abbreviations: PO = palm oil; FO = fish oil; O = olive pomace oil; OA = olive pomace acid oil.

In line with what was observed for BW, a worse FCR was found for broiler chickens and European seabass fed the diet containing olive pomace acid oil compared to O and the control diet, and a numerical increase for pigs. Differences were more notable in the case of European seabass, where animals fed olive pomace acid oil increased FCR by +17.9% when compared to the animals fed fish oil. This effect was less marked in pigs and broiler chickens where it was +4.5% and +0.3%, respectively, compared to the animals fed palm oil. Therefore, it could be suggested that the molecular structure of the FA affected performance parameters and feed efficiency, especially in the case of European seabass. However, the effect of FFA in performance seems to remain unclear due to the controversial results reported in the literature, as discussed in Chapter 1. On the one hand, a high level of FFA have been associated with a poor digestibility and therefore to a decrease in DE (Wiseman et al., 1991; Powles et al., 1993; Jørgensen and Fernández, 2000). On the other hand, a high MIU content reduces the energy value of the added fat source (Wiseman et al., 1992; Varona et al., 2021). In this sense, olive pomace acid oil showed a higher MIU value than olive pomace oil in the three studied species (pigs: 12.67 vs 4.65%; broiler chickens: 6.63 vs 1.72%; European seabass: 6.15 vs 2.44%). The composition of MIU contents of olive pomace acid oil in the diets of the three studied species are presented in **Table 6.1.** The effect of MIU, FFA, or the combination of both, would explain the negative effect observed on performance and feed efficiency in the three species.

Table 6.1. Composition of the MIU content of olive pomace acid oil included in pig, broiler chicken and European seabass diets.

	Pigs	Broiler chickens	European seabass
Moisture, %	1.27	0.73	0.31
Impurities, %	7.84	1.37	1.94
Unsaponifiable, %	3.56	4.53	3.90
Total MIU, %	12.67	6.63	6.15

Abbreviations: MIU = moisture, impurities and unsaponifiable matter.

By definition, MIU is composed by moisture, impurities and unsaponifiable matter, so three different groups of energy diluents are established. One possible hypothesis could be that different MIU compositions regarding these groups might reflect different impacts on dietary energy dilution. In this sense, olive pomace acid oil included in pig diets had a higher MIU content, especially of impurities, than that included in broiler chicken diets. However, the effects on performance and feed efficiency were quite similar. In contrast, olive pomace acid oil

included in European seabass diets had a lower MIU content and similar proportions of moisture, impurities and unsaponifiable matter than tat included in broiler chicken diets, but the effect was significantly different between these two species, with a much greater impairment observed for European seabass. It could be suggested that the different MIU fractions maintain similar effects on the dilution of energy content, and that the differences in performance response and feed efficiency observed between species are based on their ability to modulate consumption to meet energy requirements, which could have been quite efficient for pigs and broilers, but insufficient for European seabass. In relation to this extra consumption, pigs and broilers fed with olive pomace acid oil had a numerically higher ADFI than those fed with olive pomace oil, while this effect was not observed in European seabass, where fish fed with olive pomace acid oil had a numerically lower ADFI than those fed with olive pomace oil.

Notwithstanding the above mentioned, the components of each MIU fraction may also have played different roles in the fat utilisation by the animals, but they were not analysed. Furhtermore, the experimental design of these studies does not allow to distinguish whether the negative impact of diets including olive pomace acid oil is due to the molecular structure of its FA or to the content and composition of its MIU. In fact, MIU content of acid oils (and experimental fats used) could be one of the factors that have generated controversial results in the literature, as the MIU values are often not reported and therefore, if any negative effect has been observed when including an acid oil, it has been attributed to its high FFA content.

In contrast to OA, animals fed the diets supplemented with olive pomace oil, rich in MUFA and low in FFA, showed a similar BW than those fed the control diet, and a similar or better (broiler chickens) FCR. In the case of pigs and broiler chickens, where the control diet was PO, this may be explained by the higher degree of unsaturation of olive pomace oil compared to palm oil (UFA:SFA ratio of 5.2 and 1.1, respectively). It is well known that digestibility increases as the degree of unsaturation does (Wiseman and Stahly, 1984; Tancharoenrat et al., 2014; Jimenez-Moya et al., 2021a). Overall, it can be stated that olive pomace oil showed a great performance and feed efficiency when compared to the control added fat source in the three species studied.

Finally, it is important to mention that blending olive pomace acid oil with other crude oils (palm oil, soybean oil or olive pomace oil) in pig and European seabass diets had better

performance and feed efficiency than using olive pomace acid oil alone, suggesting some kind of synergism. The synergism of blending a saturated fat source with an unsaturated one is well described (Sibbald and Slinger, 1963; Ravindran et al., 2016), but this fact may not explain the improve on the use of olive pomace acid oil, as it already has a high UFA:SFA ratio (4.3-5.3).In contrast, a reduction on MIU and FFA content in the diet could have diluted the negative effects and avoid impairment in performance or feed efficiency due to the inclusion of olive pomace acid oil. When olive pomace acid oil was the only added fat source, the lack of glycerides and MAG in the intestinal lumen could have difficult the solubilisation of FFA in mixed micelles, thus requiring a higher amount of bile salt secretion for emulsification. Although bile salt and lipase secretion is known to increase as the animal ages (Noy and Sklan, 1995; Sargent et al., 2002), it has been suggested to be insufficient for a maximum fat digestion, which could be especially limiting at high inclusion levels of added fat (Sklan, 2001). It could be hypothesised that reducing the inclusion level of olive pomace acid oil (which is highly dependent on bile salt for an optimal absorbability) together with the presence of MAG from TAG lipolysis may have resulted in improved emulsification and absorption of FA, suggesting that acid oils may perform well under a dietary inclusion threshold that does not limit the animal's capacity to emulsify and absorb their FA.

6.1.2. Impact of high-oleic oils on the digestibility balance and the dietary energy value

The comparison of AID and ATTD for TFA and SFA in pigs and broiler chickens is shown in **Figure 6.2.** Regarding TFA and pigs, higher digestibility values could be observed for all dietary treatments when comparing AID to ATTD. In contrast, for SFA, ATTD values were clearly lower than those of AID. These results indicate that some UFA disappeared and SFA were generated in the hindgut. This could be explained by the biohydrogenation of oleic, linoleic and linolenic acids that are saturated by the microbiota and converted into SFA such as stearic acid or other FA from bacterial origin (i.e. that were not present in the diet: C15:0; C17:0) (Jørgensen and Fernández, 2000; Duran-Montgé et al., 2007). In broiler chickens, differences between AID and ATTD were lower. This was expected as the fermentation capacity of broiler chickens is wide lower than that of pigs, mainly due to shorter gastrointestinal tract and a faster rate of passage (Ravindran et al., 2016).

Among dietary treatments, this effect in digestibility of TFA was more pronounced in animals fed OA than in those fed O and was much more pronounced in the case of pigs. In agreement with this, Rodriguez-Sanchez et al. (2019, 2021), found increasing concentrations of FA from bacterial origin when increasing dietary FFA content. These finding suggest that a high dietary FFA content could promote bacterial activity, and therefore might potentially generate confounding results when acid oils are fed and digestibility is assessed as ATTD. Then, AID data should be used instead of ATTD data for FA digestibility, especially in the case of pigs, as it seems to be more accurate since it avoids the interaction generated by the microbiota (Stein, 2017).

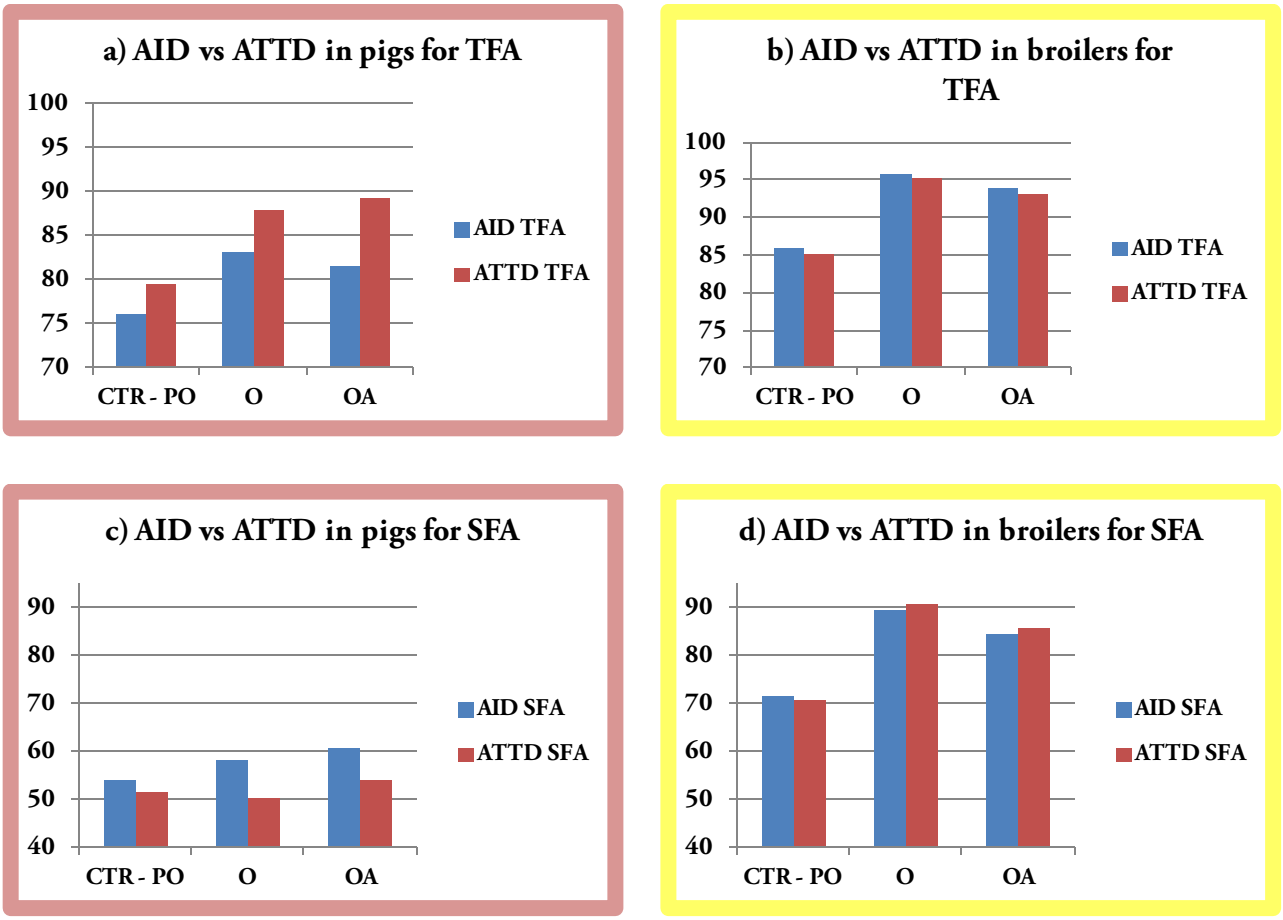

Figure 6.2. Comparison between the apparent ileal digestibility (AID) and the apparent total tract digestibility (ATTD) for total fatty acids (TFA) and saturated fatty acids (SFA) in pigs (a, c) and broilers (b, d). Abbreviations: PO = palm oil; O = olive pomace oil; OA = olive pomace acid oil.

In accordance with the above statement, the data on FA digestibility and digestible energy of the feed in the three species at the AID level are compared. For European seabass, the apparent digestibility coefficient (ADC) is presented. Despite being a measure of the total tract, from a

nutritional point of view the ADC in fish could be considered equal to the AID for pigs and poultry as no fermentation occurs in the posterior intestine of fish (Turchini et al., 2021). The effects of dietary inclusion of olive pomace oil and acid oil regarding FA digestibility are shown in **Figure 6.3.** In general, the highest AID values were obtained for animals fed O, rich in MUFA and low in FFA. It is important to note that these values were higher than those obtained for the control diet in the three species (despite not being significant in pigs), revealing that O had a great FA digestibility. In contrast, when animals were fed OA, the AID of FA tended to decrease in the three species, although statistically significant differences were only found in European seabass. This effect could be explained by the higher content in dietary FFA, which has been related with an impairment of the absorption processes. On the one hand, FFA can interact with ionised minerals such as calcium or magnesium forming insoluble soaps, making both unavailable for absorption (Small, 1991). However, this effect seems to be more pronounced in SFA rather than in UFA, as SFA have a greater capacity to form insoluble soaps (Atteh and Leeson, 1985; Wiseman and Salvador, 1991). On the other hand, the decrease in FA digestibility values in OA could be due to lower content in MAG and bile acid secretion in the duodenum, both of which are essential for the formation of mixed micelles and thus for the absorption of FA (Sklan, 1979; Ravindran et al., 2016).

Despite slightly reduced AID values for OA compared to O in pigs and broiler chickens, OA showed higher values than PO in both species, being statistically significant in the case of broiler chickens. These results suggest that the saturation degree had a greater influence on FA digestibility than the dietary FFA content (UFA:SFA ratio of 1.07/1.76 for PO and 4.35/3.46 for OA in pigs/broiler chickens, respectively). In contrast, as far as the ADC for FA in European seabass is concerned, this effect was not observed, and OA showed similar (not higher) values than the control diet (FO) despite having a higher UFA:SFA ratio (1.86 fish oil vs 5.27 olive pomace acid oil). It is important to note that the high content in n-3 HUFA of fish oil gives it a high digestibility, mainly due to a higher degree of unsaturation and thus a lower melting point than other fat sources that may have a similar UFA:SFA ratio. However, these results indicate that the negative effect of FFA was higher in fish than in pigs or broiler chickens. In this sense, the formation of insoluble soaps between dietary FFA and ionised minerals might be higher in marine fish species, as marine fish drink seawater for osmoregulation purposes (Lall, 2021). Seawater is rich in calcium and magnesium and

therefore these minerals are more available to interact with dietary FFA in the gut (Olsen et al., 1998), which could explain the more pronounced negative effect of FFA observed in European seabass compared to pigs or broilers.

Finally, a comparison of absolute values between species shows that the digestibility of FA was higher in broiler chickens and European seabass (especially in the latter) than in pigs. This could be because, as digestion processes in pigs involve further fermentation in the hindgut, the adaptation of this species has made the processes of lipase hydrolysis, bile salt secretion and emulsification less efficient in the small intestine of pigs than in broilers or in the digestive tract of European seabass. Moreover, fish are known to be highly efficient in the digestion of lipids, as their diet is usually composed by a high level of fat, although this vary between fish species (Turchini et al., 2021).

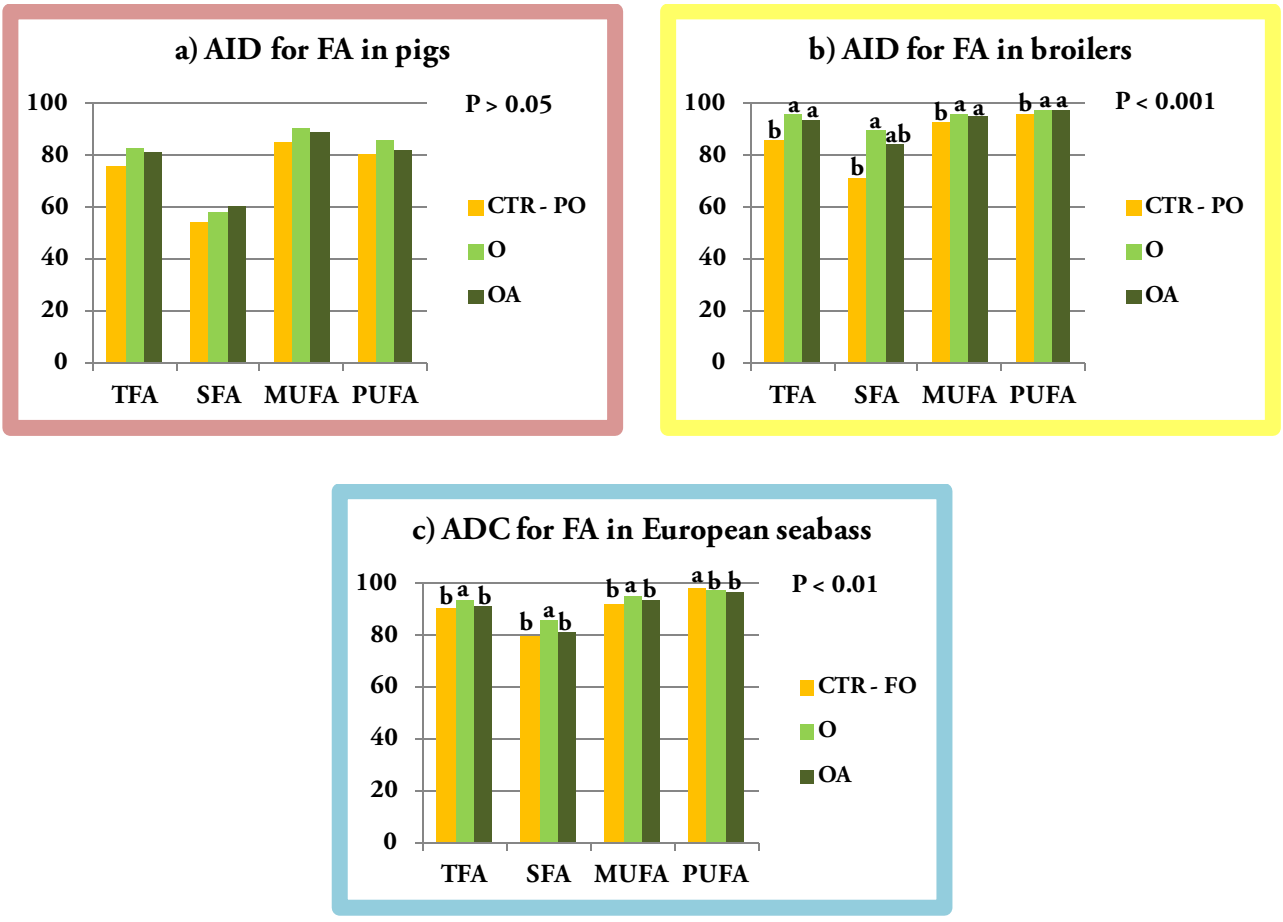

Figure 6.3. Effect of dietary inclusion of olive pomace oil and acid oil on the apparent digestibility of fatty acids (FA) in pigs (a), broiler chickens (b) and European seabass (c). Abbreviations: PO = palm oil; FO = fish oil; O = olive pomace oil; OA = olive pomace acid oil; AID = apparent ileal digestibility; ADC = apparent digestibility coefficient; TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

The effects of dietary inclusion of olive pomace oil and acid oil on feed DE are presented in **Figure 6.4.** Regarding the two alternative fat sources studied, O showed a higher DE for broiler chickens than OA (+127kcal/kg) despite having a similar FA profile. The same trend, although not statistically significant, was observed for pigs (+120kcal/kg) and European seabass (+30kcal/kg). These results are in agreement with other studies that found a decrease in the dietary energy value when acid oils are fed to monogastric animals (Jørgensen and Fernández, 2000; Vilarrasa et al., 2015b; Jimenez-Moya et al., 2021a). The decrease in energy value could be related to a higher FFA content (Wiseman et al., 1992; Powles et al., 1993) or to a higher MIU value (Wiseman et al., 1992; Varona et al., 2021), both of which were higher in OA diets than in O diets. However, diets including olive pomace acid oil showed a similar DE value than the control diets (palm oil for pigs and broiler chickens, fish oil for European seabass), so the decrease on the DE caused by the higher FFA or MIU content might have been compensated by the higher degree of unsaturation.

In practical feed formulation, energy can be addressed as metabolisable energy (ME) or net energy (NE) in order to make an accurate estimation of the energy values of feed ingredients for optimal animal growth and to minimize feed costs (Kil et al., 2013). However, an estimate of NE values for ingredients is often calculated from prediction equations or ME:NE ratios, as the NE of ingredients is difficult and costly to determine (Wealleans et al., 2021a). In the case of pigs, the ME of lipids is considered to be approximately 98% of the DE, and NE is considered to be 90% of the ME (Noblet et al., 1994). In this regard, a general prediction equation, known as Wiseman's equation, was developed more than 20 years ago to determine DE and ME of fats for pigs and poultry, respectively. These equations consider a constant (that depends on the species, pigs or poultry, and the age), degree of saturation and FFA content of the fat source (Wiseman et al., 1998). The fats and oils used for the development of these prediction equations had a maximum content of 2% MIU (Wiseman and Salvador, 1991; Wiseman et al., 1992, 1998). However, these assumption is not always true for fat sources commonly used in monogastric animal diets, especially for acid oils, as has been reported by Wealleans et al. (2021b) and Varona et al. (2021). Indeed, predicting the energy value of fat is challenging as it is affected by many different factors, such as the age and breed of the animal, the degree of saturation, the level of inclusion, the molecular position of FA in the glycerol backbone or the FFA content, but also the MIU content (Ravindran et al., 2016). For this reason, a modified

version of the Wiseman's original equation should be proposed taking into account the MIU value of fats and oils used for animal feeding. Thus, when applying the modified Wiseman equation, the average energy values of common fat sources may differ considerably from previously published estimates (Wealleans et al., 2021b). In this sense, when the energy content of olive pomace acid oil used in broiler chickens with and without taking into account the MIU value of the fat is calculated using the Wiseman equation, the prediction of ME varies from 8379 kcal/kg and 8974 kcal/kg, respectively, thus generating an overestimation of +595 kcal/kg ME if MIU is not considered. On the other hand, in the case of olive pomace acid oil used in pigs, the differences are even greater, as the MIU value was much higher, leading to an overestimation of +1,140 kcal/kg when MIU is not considered (7610 vs 8715 kcal/kg with and without considering MIU, respectively). The variation of the FFA and MIU contents of olive pomace oil and acid oil between the studies carried out in the present PhD thesis evidence the variability present in the available fat sources and the need to characterise them well before their use in the formulation of monogastric animal diets.

Despite the influence of FFA and MIU contents of the added fats, the level of inclusion of the added fat in the diet and feed processing also varied between studies, and other factors such as the molecular distribution of FAs were not analysed. These several factors may have influenced the final energy value of the added fats and thus affected the dietary energy values assessed, leading to different responses between species. Thus, while it is well recognised that predicting the energy value of fat is difficult to address in practice, it seems clear that if a more accurate prediction is sought, a better characterisation of fat sources should be made. In this sense, the present results support that the inclusion of MIU content in energy prediction equations would be useful when implemented together with other factors such as degree of saturation and FFA content, already considered in Wiseman's equations (Varona et al., 2021; Wealleans et al., 2021b).

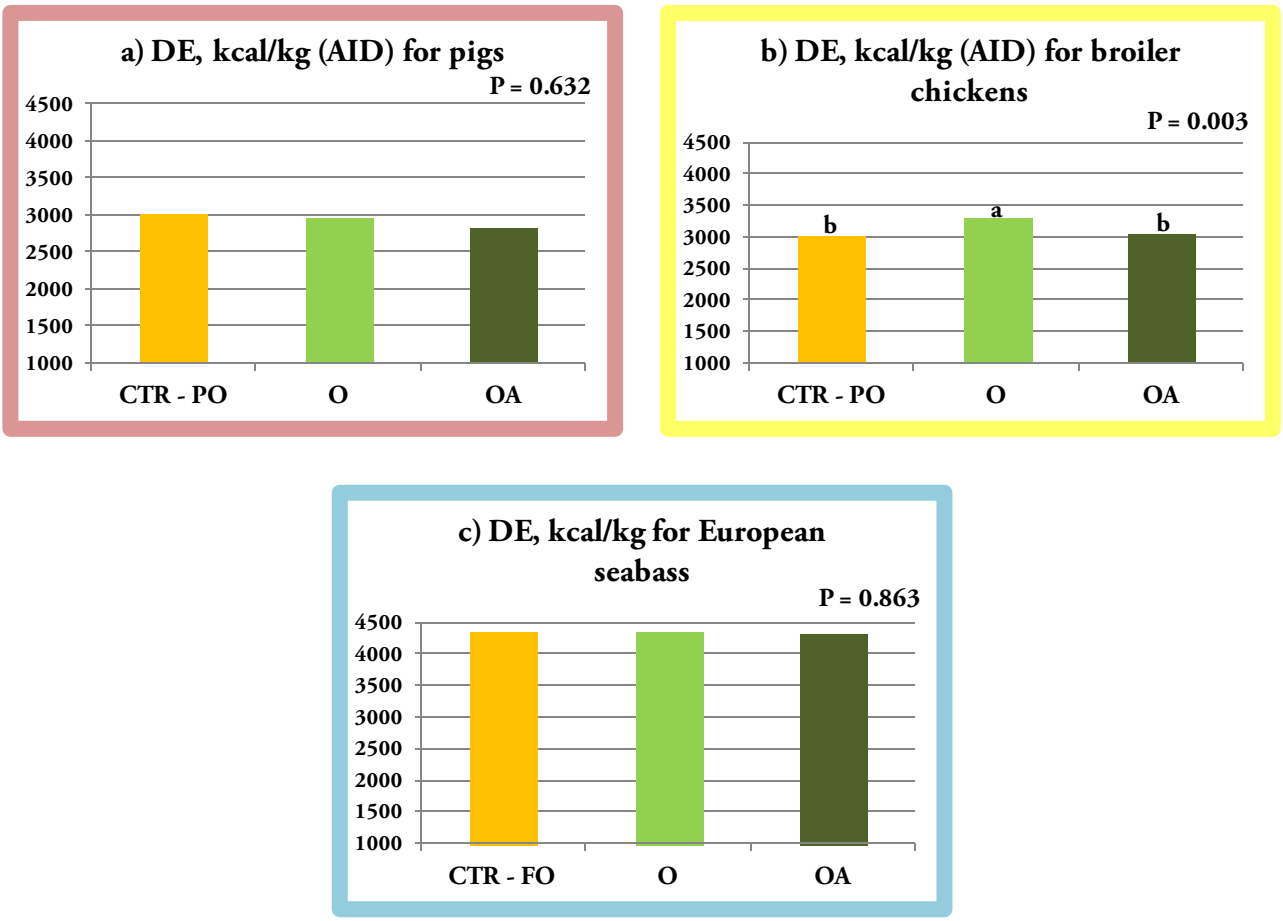

Figure 6.4. Effect of dietary inclusion of olive pomace oil and acid oil on feed digestible energy (DE) in pigs (a), broiler chickens (b) and European seabass (c). Abbreviations: PO = palm oil; FO = fish oil; O = olive pomace oil; OA = olive pomace acid oil.

6.1.3. Fat deposition and fatty acid profile of meat products

The effects of dietary inclusion of olive pomace oil and acid oil on fat deposition are presented in **Figure 6.5**. Significant differences were only observed in broiler chickens, where animals fed O deposed a higher amount of abdominal fat than those fed OA. These differences could be explained by the slight changes in the dietary FA profile, since OA had a higher content in PUFA (32%) than O (28%). It has been widely described in broiler chickens that a higher content of dietary PUFA lead to a decrease in the abdominal fat depot, mainly because a preferential β-oxidation of PUFA in respect to MUFA or SFA, a decreased rate of FA synthesis and a reduction of levels of insulin and very low density lipoproteins (Crespo and Esteve-Garcia, 2002a; b; c, 2003; Ferrini et al., 2008). This effect has also been described in pigs (Zhang et al., 2019), and although no significant differences were found in the present study, a numerical decrease on backfat thickness in pigs fed OA was observed. On the other hand, this

effect was not observed in European seabass, as OA deposed a similar perivisceral fat content than the control diet (FO), despite the latter having a much higher PUFA content (42 vs 29%), indicating that PUFA in fish are not preferentially β-oxidised, but are stored to adapt to the environment and cold water temperature to maintain an optimal cell membrane fluidity (Farkas et al., 2001).

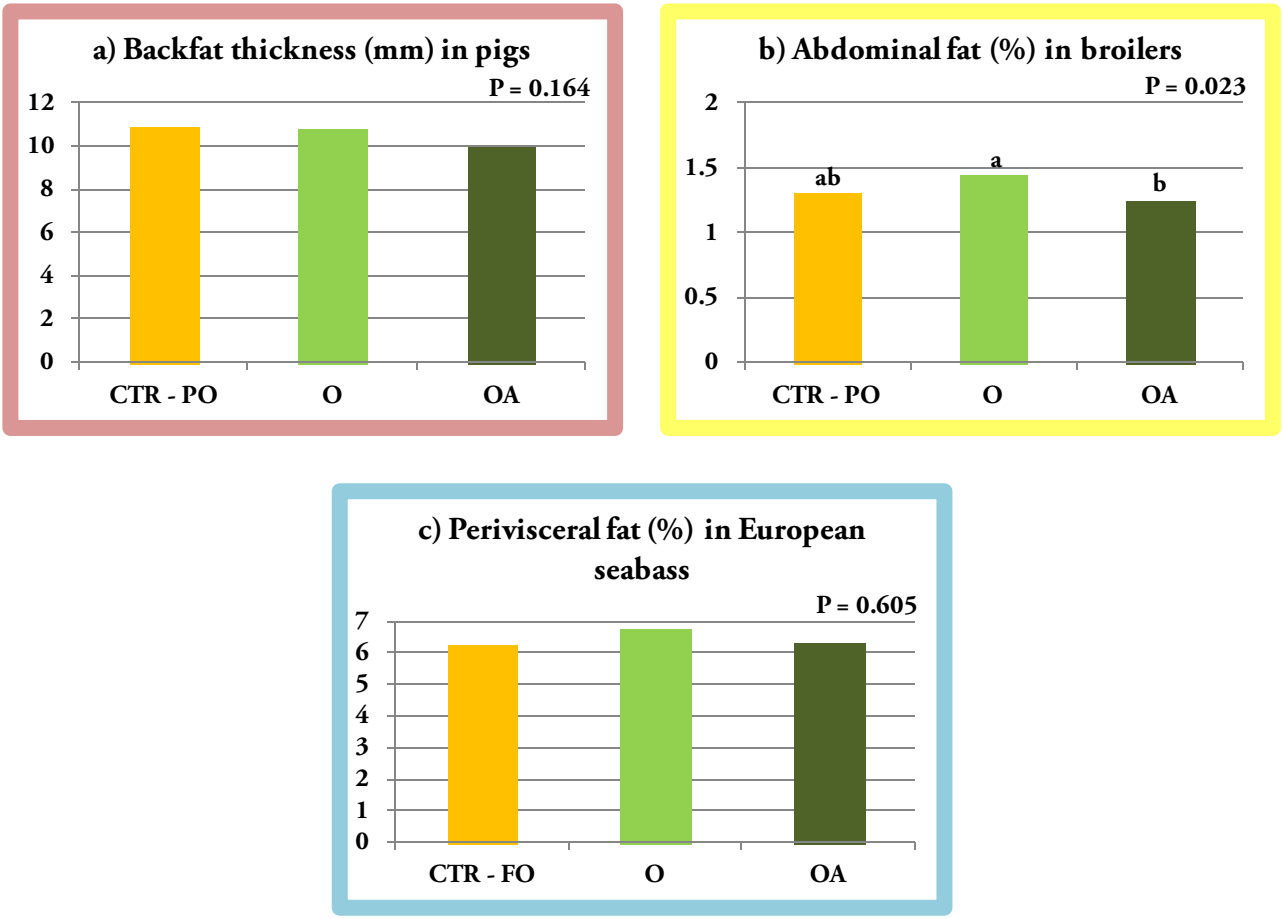

Figure 6.5. Effect of dietary inclusion of olive pomace oil and acid oil on fat deposition in pigs (a), broiler chickens (b) and European seabass (c). Abbreviations: PO = palm oil; FO = fish oil; O = olive pomace oil; OA = olive pomace acid oil.

On the other hand, differences in fat deposition could be related not only to the dietary FA profile, but to the DE content of the diets. As described above, OA diet had lower DE than O in broilers, and the former deposed lower abdominal fat than the latter. Additionally, a similar trend was observed for pigs. In this sense, when dietary energy supply is decreased, fat deposition also decreases (Beaulieu et al., 2009; Cámara et al., 2014; Aymerich et al., 2020). In European seabass, however, a slight increase in perivisceral fat deposition was observed in animals fed olive pomace oil compared to those fed fish oil or olive pomace acid oil, despite

having similar DE values. A possible hypothesis would be that the higher MUFA content of the olive pomace oil diet could have led to a better efficiency of metabolic energy utilization of dietary fat, as MUFA have been described as the preferred substrates for β-oxidation in fish (Menoyo et al., 2003), whereas PUFA are the preferred substrates for pigs and broiler chickens and therefore this effect was not observed (Crespo and Esteve-Garcia, 2002a; Zhang et al., 2019).

The effects of dietary inclusion of olive pomace oil and acid oil on intramuscular fat deposition are presented in **Figure 6.6**. In broiler chickens, the total amount of intramuscular lipid content was not analysed, and therefore the sum of FA has been given as a measure of intramuscular fat content. No differences were observed in the amount of intramuscular fat of breast meat or fish flesh. However, in loin meat, animals fed O showed the highest intramuscular fat deposition. This fact could be explained by the high content in MUFA present in O, especially oleic acid (C18:1 n-9), which has been associated with a higher IMF content (Miller et al., 1990; Ruiz-Carrascal et al., 2000; Isabel et al., 2004). In this sense, when the MUFA:PUFA ratio of the diet increases, the levels of expression of lipogenic enzyme activity increase (Gerfault et al., 2000; Zhang et al., 2019). In agreement, O had the highest MUFA content and MUFA:PUFA ratio among dietary treatments in pigs (49.01% and 1.46, respectively), which was reduced in OA (48.26% and 1.39) and PO(36.19% and 1.25), reaching lower levels of IMF. In contrast, this effect was not observed in the IMF content of flesh, where the control diet (FO) had a considerably lower MUFA content and MUFA:PUFA ratio (24.5% and 0.52, respectively) than O (52.8% and 1.53) or OA (48.2% and 1.42) and achieved a similar IMF content.

Increased dietary intake of oleic acid could lead not only to a pork with higher IMF content but also to healthier and more sensory-accepted meat products (Ruiz-Carrascal et al., 2000; Jiménez-Colmenero et al., 2010). An increase in MUFA (and in particular oleic acid) have been found in dry-cured ham from Iberian pigs when compared to modern conventionally reared breeds (Ruiz-Carrascal et al., 2000; Čandek-Potokar and Škrlep, 2012), as a direct consequence of the high oleic acid content of the acorns eaten by these Dehesa-raised pigs (pastureland in the south and central Iberian peninsula). It is important to mention that Iberian pigs produce one of the most sensory accepted pork meat, mainly due to their higher IMF infiltration capacity but also because of the fat composition of their meat products (Čandek-Potokar and

Škrlep, 2012). Therefore, it could be suggested that reaching feeding pigs with a high IMF infiltration capacity (high-fat crossbreeds) with oleic acid-enriched diets could lead to better sensory-accepted and healthier meat products, which may be one of the potential advantages of including olive pomace oils in the diets of high-fat crossbreed pigs.

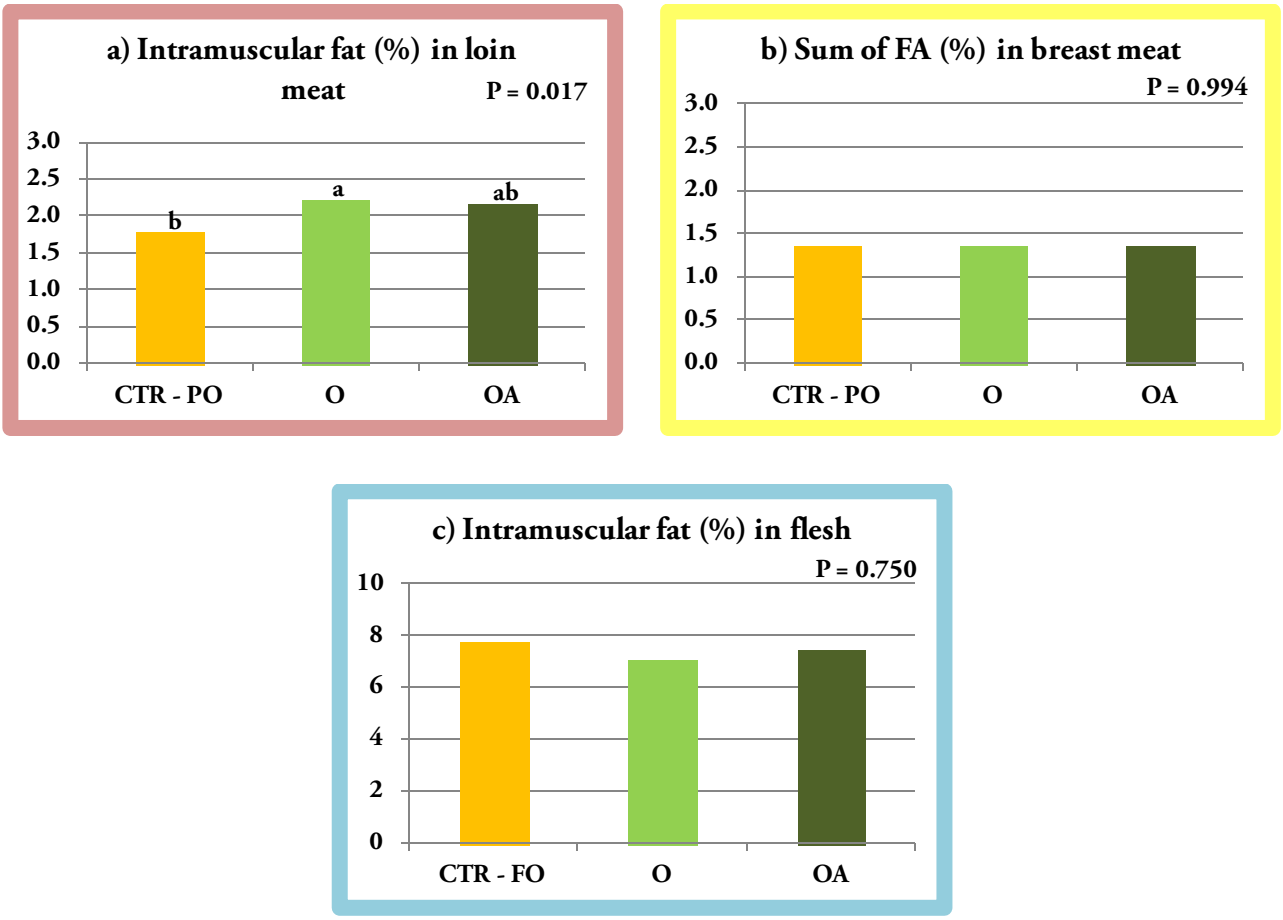

Figure 6.6. Effect of dietary inclusion of olive pomace oil and acid oil on intramuscular fat deposition (fresh matter basis) in pigs (a), broiler chickens (b) and European seabass (c). Abbreviations: PO = palm oil; FO = fish oil; O = olive pomace oil; OA = olive pomace acid oil; FA = fatty acids.

On the other hand, a decrease on the dietary energy intake could explain different fat depositions. As described above, pigs and broiler chickens fed olive pomace acid oil had a decrease on DE up to 120kcal/kg of feed. However, differences were found in the intramuscular content of pork loin meat but not in breast meat of broiler chickens. In this regard, it is important to mention that genetics highly affect fat deposition capacity, both in carcass and intramuscular content (Edwards et al., 2006; Gispert et al., 2007; Aymerich et al., 2019). In agreement with this, Aymerich et al. (2020) reported that a reduction in NE intake led to a significant reduction in the fat deposition in Duroc pigs while did not in Pietrain.

These results suggest that in high-fat crossbreeds such as Duroc pigs differences in fat deposition due to nutritional strategies can be easily noted than in lean breeds, such as Pietrain, or in this case broiler chickens where the genetics have led to high-lean breeds (Ross 308).

Figure 6.7. Effect of dietary inclusion of olive pomace oil and acid oil on the FA profile of final meat products from pigs (a), broiler chickens (b) and European seabass (c). Abbreviations: PO = palm oil; FO = fish oil; O = olive pomace oil; OA = olive pomace acid oil; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

The effects of dietary inclusion of olive pomace oil and acid oil on the FA profile of final meat products are presented in **Figure 6.7**. The differences observed in the FA profile of final meat products reflected those present in the diet in the three species, which is in agreement with the results reported in the literature (Mourente et al., 2005; Vilarrasa et al., 2015a; b; Skřivan et al., 2018). However, some nuances can be noticed between species. European seabass muscle tissue had a notable lower SFA and higher PUFA content than pigs (-12% and +12%, respectively) or broiler chickens (-5% and +8%, respectively). This may be related to the animal's thermoregulatory capacity since, for optimal cell membrane fluidity, as the exposure temperature of the cell decreases, the need for PUFA in the cell membrane increases. Thus, in

fish and other poikilotherms the degree of unsaturation of membrane FA increases to adapt to different environmental temperatures (Bell et al., 1986; Farkas et al., 2001), which are also able to make metabolic adjustments known as the "homeoviscous adaptation" of the membrane lipid composition when the environmental temperature changes (Hazel, 1984). These differences in the fat metabolism of fish compared to pig and broiler chicken might also explain the differences in the carcass and IMF fat deposition between these species.

6.2. Critical remarks and advances in methodological approach

During the development of the three studies carried out during this PhD thesis, there have been some limitations and difficulties that need to be taken into account as critical considerations to improve in future studies. First, we found many difficulties to obtain similar experimental oils for the three species studies, mainly due to the high variability in their composition and quality. This was especially notable in the case of olive pomace acid oil, which had a marked difference on its quality between that used in pigs (MIU = 12.67%) compared to that used in broilers (MIU = 6.63%) or in European seabass (MIU = 6.15%). This therefore confirms the need to better characterise and standardize these fat by-products in order to increase their practical use in animal feeding, increasing the confidence of feed manufacturers and nutritionists in them.

Another difficulty we encountered due to the commercial farm housing was the collection of excreta from broiler chickens for the digestive balance. The housing of pigs and broiler chickens in commercial conditions led to a digestibility balance with a long adaptation period, thus generating more representative values than those obtained in shorter digestibility balance trials performed in experimental conditions. However, this housing conditions made it difficult to collect excreta samples from broiler chickens. In pigs, manual rectal stimulation worked fine and faecal samples were easy to collect. In contrast, broilers did not excrete the contents easily after abdominal massage, probably due to a faster gastrointestinal rate of passage (which may have meant that they did not always have contents to excrete) or less adaptation to the interaction with the farmer, which made them expel the excreta when we entered the pen and during catching of the animals. In European seabass, faecal collection was not a problem, as it was not a manual process, but an automatic system fitted to the tanks. However, despite being

one of the most widely accepted faecal collection systems, some losses may occur due to solubilisation of faeces in water.

Another limitation we had to deal with was the gorgeous fluctuation in commodity prices. One of the principal benefits of including olive pomace oil, and especially olive pomace oil, in the diets of monogastric animals is their usually competitive prices, which could make them cost-effective to reach a more efficient production. For this reason, we would like to have performed some economic balance with our feed formulations and put some value in figures on the use of these alternative fat sources compared to control diets. However, the last few years with the Covid19 pandemic and the war in Ukraine have led the sector into a commodity price crisis and an uncertain future forecast, which makes any economic balance sheet we could make meaningless.

On the contrary, during the design and development of the three studies, we have observed certain improvements and methodological issues that led to great scientific results. First, the characterisation of experimental fats, especially the report of MIU values, which provided key information for a clearer interpretation of the results. This was based on the lack of information present in some of the literature and our aim to provide a good characterisation of the experimental fats used. Secondly, the comparison between the measurement of digestibility at ileal and faecal level in both pig and broiler chickens, which has provided evidence on the nuances of accuracy that are lost when assessing digestibility as ATTD instead of AID. Finally, the use of silicate as HCl-insoluble ash inert marker instead of titanium dioxide (TiO_2), which widely used for digestibility studies. This was designed to generate experience in the use of an efficient inert marker alternative to TiO_2, due to the recent decision to ban its use as a feed additive from 7 August 2022, detailed in the Commission Regulation (EU) 2022/63. Additionally, this decision was also important in terms of costs, as silicate was much cheaper than titanium dioxide and the savings were significant as the number of animals involved was high. This marker apparently performed well and had good repeatability in the analysis without showing any alteration in palatability or interference in digestibility as similar results were obtained in previous studies using TiO_2 (Rodriguez-Sanchez et al., 2019b; Viñado et al., 2019; Jimenez-Moya et al., 2021a). On the contrary, it is important to note that the methods for determining the HCl-insoluble ash content are gravimetric, so they can induce to a higher

variability and error than those used for determining the TiO_2, which are based on mass spectrometry.

6.3. Future considerations

This thesis was conducted in order to obtain information on the inclusion of olive pomace oil and acid oil in monogastric animals reared under commercial conditions, focusing the main objectives on performance and feed efficiency, FA digestibility and FA profile of final products obtained. However, there are some aspects that remain unanswered regarding the inclusion of these fat sources in monogastric animal diets, such as the state of the gut microbiota and intestinal morphology, changes in fat metabolism, gene expression in relation to lipid deposition and gastrointestinal functions or the evaluation of the quality of the final meat products obtained for human consumption. In fact, samples have been collected for the evaluation of all these issues in parallel to the work of this thesis, but they are in the process of being realized and therefore it has not been possible to incorporate them. In addition, the study on the final quality of meat products is being developed in parallel to the present thesis by the Libifood research group of the Universitat de Barcelona, which is part of the same project in which the present work is framed. Thus, this research composes another doctoral thesis, closely linked to the present one, focused on the effects of the inclusion of olive pomace oil and olive pomace acid oil on the final quality of the meat product.

Further information about the characterisation of acid oils can be found in the work of Varona et al. (2021). On the other hand, information regarding digestion processes of these by-products along the gastrointestinal tract can be found in the studies of Rodriguez-Sanchez et al. (2019, 2021) and Jimenez-Moya et al. (2021a; b; c). However, many aspects regarding the processes of digestion and absorption of acid oils are still unclear. It would be interesting to study how the combination of factors such as fat quality (i.e. MIU), FFA level, and age of the animal may interact in the solubilisation step and the utilisation of the absorbed fraction by the animal. In the first part of this processes, *in vitro* studies would provide many deeper information without the use of animals. This more specific research would help to understand the different results reported in the literature on the use of acid oils in monogastric animal feeding. In addition, it is important to mention that the fat blends used in the studies carried out in pigs and European seabass tended to perform better than using the fat sources separately,

suggesting some kind of synergism in blending acid oils with other fat sources that should be further investigated.

Overall, the present thesis provides further information about the use of alternative fat sources in monogastric animals, providing valuable data on potential strategies for the inclusion of olive pomace oil and acid oil in the diets of pigs, broiler chickens and European seabass. It is important to remark that this work is part of a larger project, and that the global results will provide a broader view and better understanding of the effects of the inclusion of acid oils in monogastric animal diets, helping to increase knowledge on the use of these by-products and therefore helping to build more confidence for nutritionists in their use. Altogether, this work will be a further step towards more efficient and sustainable animal production based on a circular economy system.

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Chapter 7.

Final conclusions

"Sometimes goodbye is a second chance"

Shinedown.

7. Final conclusions

From the results obtained in the three studies carried out throughout this PhD thesis on pigs, broiler chickens and European seabass, were olive pomace oil and acid oil (54% free fatty acids) were included in their diets, the following conclusions can be drawn:

1. Olive pomace oil can be included at 5% in growing-finishing pig diets to obtain a loin meat with a high content of intramuscular fat, achieving high fatty acid digestibility, performance and feed efficiency.

2. Olive pomace acid oil can be included in a blend with palm oil (1:1 ratio) at 5% to growing-finishing pig diets achieving good performance, feed efficiency and fatty acid digestibility.

3. Replacement of palm oil with olive pomace oil (6% of inclusion, as fed-basis) in growing-finishing broiler chicken diets leads to improved performance, feed efficiency and fatty acid digestibility.

4. Replacement of palm oil with olive pomace acid oil (6% of inclusion, as-fed basis) in growing finishing broiler chicken diets has no negative effects on performance, feed efficiency or fatty acid digestibility.

5. The inclusion of olive pomace acid oil compared to olive pomace oil in growing finishing broiler chicken diets (with a similar fatty acid profile but with a high content of free fatty acids) decreases the digestibility of saturated fatty acids, but not that of total, monounsaturated or polyunsaturated fatty acids.

6. The use of olive pomace oil or acid oil compared to palm oil in pig or broiler chicken diets leads to a reduction in saturated fatty acids and an enrichment of monounsaturated fatty acids of loin (>46% oleic acid) and breast (>38% oleic acid) meat.

7. Olive pomace oil can be included at 11.5% (as-fed basis) in European seabass diets achieving high fatty acid digestibility, performance and feed efficiency.

8. The inclusion of olive pomace acid oil compared to olive pomace oil in European seabass diets (11.5% of inclusion, as-fed basis) impairs performance, feed efficiency and lipid digestibility.

9. Olive pomace acid oil can be included in European seabass diets in a blend with soybean oil (ratio 1:1; 11.5% of inclusion, as-fed basis), achieving good performance, feed efficiency and fatty acid digestibility.

"I have found that it is the small everyday deed of ordinary folks that keep the darkness at bay. Small acts of kindness and love"

Gandalf, The Hobbit.

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