







## Full-Length Article

## Modulatory effect of *Echium plantagineum* oil on the n-3 LC-PUFA biosynthetic capacity of chicken (*Gallus gallus*)

Jesús Villora<sup>a,b,\*</sup> , José Antonio Pérez<sup>a</sup>, Nieves Guadalupe Acosta<sup>a</sup>,  
Deiene Rodríguez-Barreto<sup>a</sup> , Pedro Juan Alonso<sup>c</sup>, Mónica B. Betancor<sup>d</sup>, Alexandr Torres<sup>b</sup> ,  
Sergio Álvarez<sup>b</sup> , Covadonga Rodríguez<sup>a</sup>

<sup>a</sup> Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna. Avenida Astrofísico Francisco Sánchez s/n, 38206 La Laguna, Tenerife, Spain

<sup>b</sup> Unidad de Producción Animal, Pastos y Forrajes en Zonas Áridas y Subtropicales, Instituto Canario de Investigaciones Agrarias, 38200 San Cristóbal de La Laguna, Tenerife, Spain

<sup>c</sup> Graneros de Tenerife S.L., Departamento Técnico Grupo Capisa. Avda. República de Nicaragua, 11-13, 35010 Las Palmas de Gran Canaria, Gran Canaria, Spain

<sup>d</sup> Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling. Stirling FK9 4LA, UK

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## ABSTRACT

Poultry can be a sustainable source of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) through the bioconversion of dietary alpha-linolenic acid (ALA, 18:3n-3). However, this process is currently limited by the high n-6/n-3 ratio in poultry diets affecting the competition between n-6 and n-3 fatty acids (FA) for the same biosynthetic enzymes, and the rate-limiting  $\Delta 6$  desaturase which act at both, the first and final steps of DHA synthesis pathway. *Echium plantagineum* oil (EO) is an unusual source of stearidonic acid (SDA, 18:4n-3) which bypasses the first  $\Delta 6$  desaturase step potentially increasing n-3 long-chain polyunsaturated fatty acids (LC-PUFA) synthesis. To explore this hypothesis, 60 Canarian male chickens at 18 weeks of age were divided into three groups and fed diets differing only in their FA formulation: soy oil (SO) rich in linoleic acid (LA, 18:2n-6); linseed oil (LO) rich in ALA; and EO, a balanced LA/ALA oil also rich in SDA and  $\gamma$ -linolenic acid (GLA, 18:3n-6). The dietary treatments did not affect the total lipid (TL) content ( $p > 0.05$ ) and did not substantially vary the lipid class (LC) profiles in the brain, liver, intestine, and muscle tissues. However, the inclusion of LO and EO equally increased n-3 polyunsaturated fatty acids (PUFA) levels in the brain, liver, and intestine compared to animals fed with SO ( $p < 0.05$ ). Moreover, EO increased hepatic relative expressions of the fatty acid elongases (*elovl2* and *elovl5*). Consequently, and in alignment with our hypothesis, EO was more effective than LO in enriching chicken thigh meat with n-3 LC-PUFA (6.0 vs 4.2%;  $p < 0.05$ ). We concluded that lowering the dietary LA/ALA ratio and increasing the SDA content in poultry diets enhance the potential of chicken metabolism for enriching poultry products with n-3 LC-PUFA. Emerging evidence suggest that local plants like those including in *Echium* genus, rich in SDA and with a balanced LA/ALA ratio, could offer a more sustainable and efficient alternative to traditional ALA sources in poultry production.

## Introduction

The intake of polyunsaturated fatty acids (PUFA) of Western populations is nowadays unbalanced, with a large predominance of n-6 fatty acids over n-3 (Sanders, 2000; Zárate et al., 2017). Excessive amounts of n-6 fatty acids (FA) promote the pathogenesis of cancer and many cardiovascular, inflammatory and autoimmune diseases, whereas the consumption of n-3 FA has been associated with optimal health and well-being (Simopoulos, 2008; Zárate et al., 2017). Linoleic acid (LA,

18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3) are essential PUFA that cannot be *de novo* synthesized by vertebrates in sufficient amounts and consequently, must be incorporated through the diet for normal growth and development (Cartoni Mancinelli et al., 2022). These two essential FA are precursors of the physiologically relevant long chain polyunsaturated fatty acids (LC-PUFA). Thus, LA leads to arachidonic acid (ARA, 20:4n-6), while ALA is transformed into eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) by desaturation and elongation processes (Jing et al., 2013). These

\* Corresponding author at: 38206 La Laguna, Tenerife, Spain.

E-mail address: [jvillora@ull.edu.es](mailto:jvillora@ull.edu.es) (J. Villora).

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transformations occur predominantly in the liver and are carried out by fatty acid elongases (**Elovl1**) that catalyse the intermediate condensation of 2 carbons in the FA chain, and by fatty acid desaturases (**Fads**) that insert a double bond at specific positions of the carbon backbone (Castro et al., 2016; Monroig et al., 2018). In particular,  $\Delta 5$  and  $\Delta 6$  desaturases, encoded by *fads1* and *fads2* genes, respectively, and elongases 2 and 5, encoded by *elovl2* and *elovl5*, respectively, are the main LC-PUFA biosynthetic enzymes in vertebrates (Castro et al., 2016).

The capability to synthesize ARA, EPA and DHA from their C18 precursors in humans is limited by the  $\Delta 6$  desaturase and probably by the competition with *trans* fatty acids, among other factors (Baker et al., 2016; Burdge, 2022; Domenichiello et al., 2015; Hussein et al., 2005; Portolesi et al., 2007). Consequently, both n-6 and n-3 LC-PUFA must also be acquired preformed in the diet to meet human needs (Goyens et al., 2006). Seafood is the primary source of EPA and DHA for humans. However, it is well documented that global fish stocks and aquaculture are not sufficient to meet the increasing global demand for n-3 LC-PUFA, thereby encouraging the search for new sources of these essential FA (Elkin et al., 2016).

Poultry, mainly chicken, are widely raised all over the world. Certain poultry species like chicken (*Gallus gallus*), possess the enzymatic machinery for the biosynthesis of LC-PUFA (Gregory et al., 2013; Castro et al., 2016; Pérez et al., 2021). Hence, these birds seem to have a higher endogenous capacity to elongate and desaturate LA and ALA into their C20 and C22 counterparts than other vertebrates, including marine fish (Castro et al., 2016; Gregory et al., 2013; Pérez et al., 2021; Twining et al., 2018). In addition, poultry livestock is characterized by a short breeding cycle, a low environmental impact, and worldwide acceptance of its meat among consumers. These factors make poultry a promising complementary alternative to seafood to minimise current dietary n-6/n-3 FA imbalance.

Currently, chicken is considered a poor source of n-3 LC-PUFA, primarily due to the use of cereals, seeds and vegetable oils containing high amounts of LA in broiler diets. These diets result in meat richer in n-6 LC-PUFA such as ARA (Calder, 2007). However, the FA profile of poultry products can be modified by altering their dietary FA composition (Rymer et al., 2010; Pérez et al., 2021). Several strategies to increase the n-3 PUFA levels in poultry meat have been assessed, including feed supplementation with fish meal, fish oil or ALA-rich vegetable oils such as linseed oil (LO) or canola oil (Apperson and Cherian, 2016; Beheshti et al., 2017; Betti et al., 2009; Gregory et al., 2014; Lopez-Ferrer et al., 2001).

Like humans, the initial  $\Delta 6$  desaturase step in the n-3 LC-PUFA biosynthetic pathway, which catalyses the conversion of dietary ALA to stearidonic acid (SDA, 18:4n-3), is regarded as a rate-limiting step in chicken (Gregory and Gibson, 2011). In this context, Guil-Guerrero et al. (2000) reported that seed oil from 19 *Echium* species (Boraginaceae) collected in their natural habitats presented a well-balanced n-6/n-3 FA profile, and were relatively rich in ALA, SDA and gamma-linolenic acid (GLA, 18:3n-6), also containing moderate levels of LA. The higher levels of SDA compared to ALA in *Echium* species, might facilitate its conversion into EPA and DHA, as it bypasses the first  $\Delta 6$  desaturation step (Díaz-López et al., 2010; Kitessa and Young, 2008).

The Canary Islands is highly diverse, with over 680 endemic flora species and subspecies (Caujapé-Castells et al., 2022). The *Echium* genus comprises about 60 taxa, with 28 endemic taxa in the Canaries (Guil-Guerrero et al., 2000; Mora-Vicente et al., 2009). During the XV and XVI centuries, birds from Spain and Portugal were introduced in the archipelago, generating over time a distinct breed. The Canarian chicken genotype is a hardy, dual-purpose breed, currently pending official recognition. It is known for its disease resistance and ability to forage (Torres et al., 2019).

This study evaluates the impact of dietary *E. plantagineum* oil (EO) on the Canarian chicken's capacity to biosynthesize n-3 LC-PUFA, with the aim of producing healthier products using local resources. To this purpose, we investigated the effect of three diets containing EO, LO and soy

oil (SO) on the FA composition of chicken brain, liver, intestine and thigh meat. In addition, dietary regulation of the gene expression of main FA desaturases and elongases involved in the biosynthesis of LC-PUFA (*fads1*, *fads2*, *elovl2* and *elovl5*) was also determined in brain, liver and intestine.

## Materials and methods

### Animals and diets

A total of 60 Canarian chicken (*Gallus gallus*) males at the age of 18 weeks were randomly allocated into 3 interior pens (n=20 birds per group), having access to about 25 m<sup>2</sup> outdoors facilities at the experimental farm of the Instituto Canario de Investigaciones Agrarias (ICIA, Tenerife, Spain). Animals from each pen were fed with a different diet, prepared by spraying 3 distinct vegetable oils to a common fat-reduced basal diet (soy, wheat, corn, barley and other minor ingredients). Therefore, there were 3 experimental groups: Soybean oil (SO) diet, supplemented with 1.5% soybean oil and 1.5% beef tallow; Linseed oil (LO) diet, supplemented with 1.5% linseed oil and 1.5% beef tallow; Echium oil (EO) diet, containing 2% *E. plantagineum* oil (Gralinco Mercaderías SL, Barcelona, Spain) and 1% beef tallow. These 3 isoenergetic, isoproteic and isolipidic diets were supplied *ad libitum* for 6 weeks. The composition and the FA profiles of the experimental diets are given in Table 1.

### Tissue collection

At the end of the experimental period, 4 birds per treatment were slaughtered at the slaughterhouse of the SADA P.a. Canarias SA Group (Tenerife, Spain) according to European regulations (Torres et al., 2017). Brain, liver, intestine and muscle (thigh meat) were sampled. A portion was washed with cold Ringer's physiological solution and soaked into cold buffered HBSS physiological glucose solution. Samples were then analysed at the laboratories of NUTRAHLIPIDS group at the Departamento de Biología Animal, Edafología y Geología (Universidad de La Laguna, Tenerife, Spain). Another portion of the brain, liver and intestine (~100 mg wet weight) was stored in RNAlater® at 4°C for the first 24 h and then frozen at -20°C until gene expression analysis at the Institute of Aquaculture, University of Stirling (Scotland, United Kingdom).

### Lipid extraction and protein determination

The total lipid (TL) content of diets (n=3) and tissues (n=4) was determined according to Folch et al. (1957) with slight modifications (Christie and Han, 2010). For the lipid extraction of diets, ~100 mg of feed was grinded to fine powder, hydrated by addition of 0.5 mL of distilled water, and maintained for half an hour in the fridge. After this period, 10 mL of chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene (BHT), as antioxidant, was added to the solution, which was homogenized using a Virtis rotor homogenizer (Virtishear, Virtis, Gardiner, New York, USA). Then 2.0 mL of KCl (0.88%, w/v) were added. For the lipid extraction of the tissue samples, ~200 mg of each tissue was directly homogenized in 10 mL of chloroform/methanol (2:1, v/v) containing 0.01% (w/v) BHT prior to the addition of 2.5 mL of KCl (0.88%, w/v). Then, the mixture was vigorously shaken, centrifuged at 716 g for 5 min, and the organic solvent containing the lipid fraction collected and filtered. The solvent was next evaporated under a stream of nitrogen, and the lipid content determined gravimetrically. All lipid extracts were re-suspended in chloroform/methanol (2:1, v/v) with 0.01% (w/v) BHT and stored at -20°C under an inert atmosphere of nitrogen until further analysis. The whole process was developed under an ice-cold environment to prevent sample degradation.

**Table 1**

Ingredients, nutrition facts and main fatty acid composition of the 3 experimental diets used to feed the chicken.

Item	SO	LO	EO
<b>Ingredients (g/100g as-is basis)</b>			
Soy oil	1.5	-	-
Linseed oil	-	1.5	-
<i>Echium plantagineum</i> oil	-	-	2
Beef tallow	1.5	1.5	1
Basal mixture <sup>1</sup>	97	97	97
<b>Nutrition facts</b>			
Metabolizable energy (Kcal/100g)	293.47	292.64	292.30
Crude carbohydrates (%)	41.13	41.13	41.13
Crude protein (%)	19.53	19.53	19.53
Moisture (%)	12.56	12.56	12.56
Crude fat (%)	4.77	4.68	4.64
Crude ash (%)	4.66	4.66	4.66
Crude fibre (%)	3.16	3.16	3.16
Calcium (%)	0.69	0.69	0.69
Phosphorus (%)	0.52	0.52	0.52
<b>Fatty acid composition (% of total fatty acids)</b>			
∑SFA	24.57 ± 0.85	23.43 ± 2.08	21.74 ± 1.89
16:0	16.95 ± 0.65	15.84 ± 1.28	14.98 ± 1.36
18:0	6.26 ± 0.27	6.17 ± 0.70	5.38 ± 0.37
∑MUFA	32.83 ± 0.62	32.27 ± 2.30	29.36 ± 2.54
18:1n-9	27.11 ± 0.73	27.36 ± 1.58	24.72 ± 1.84
18:1n-7	2.88 ± 0.03	2.49 ± 0.47	2.21 ± 0.63
∑n-6 PUFA	38.04 ± 1.06	29.51 ± 2.21	32.49 ± 1.18
18:2n-6	37.77 ± 1.06	29.15 ± 2.20	28.59 ± 1.10
18:3n-6	0.10 ± 0.03	0.16 ± 0.03	3.73 ± 0.76
∑n-3 PUFA	3.51 ± 0.28	12.89 ± 2.49	14.60 ± 2.90
18:3n-3	3.34 ± 0.27	12.68 ± 2.54	10.83 ± 1.65
18:4n-3	0.12 ± 0.02	0.16 ± 0.05	3.71 ± 1.10
∑n-6 LC-PUFA	0.17 ± 0.04	0.20 ± 0.03	0.17 ± 0.04
∑n-3 LC-PUFA	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.01
n-6/n-3	10.83 ± 0.58	2.29 ± 0.38	2.22 ± 0.39

Fatty acid composition values are means ± SD (n = 3). SO, soy oil diet; LO, linseed oil diet; EO, *Echium* oil diet. nd, not detected. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain (≥C20) polyunsaturated fatty acids. Totals include other minor components not shown.

<sup>1</sup> Basal mixture composed of soymeal, wheat meal, corn meal, barley meal, phosphate, calcium carbonate, liquid methionine, lysine, salt sodium bicarbonate and other minor ingredients (Capisa group).

### Lipid classes and fatty acid composition

The lipid classes (LC) profile from tissues (n=4) was determined from 0.02 mg of TL extracts. LC were separated by one-dimensional double-development high performance thin layer chromatography (HPTLC) with 1-propanol/chloroform/methyl acetate/methanol/0.25% potassium chloride (5:5:5:2:1.8, v/v) for the polar lipid development, and hexane/diethyl ether/acetic acid (20:5:0.5, v/v) for the neutral lipids. LC were identified by comparison to external lipid standards placed on the same HPTLC plate and quantified by calibrated densitometry using a dual wavelength flying spot scanner CAMAG TLC Visualizer (Camag, Muttentz, Switzerland) (Reis et al., 2019).

Fatty acid methyl esters (FAME) from diets (n=3) and tissues (n=4) were obtained by acid catalyzed transmethylation of 1 mg TL. FAME were purified by thin layer chromatography (Christie and Han, 2010)

using pre-coated TLC plates SIL G-25 (20 cm × 20 cm; Macherey-Nagel GmbH & Co. KG, Düren, Germany) and resolved with hexane/diethyl ether/acetic acid (90:10:1, v/v) as detailed by Galindo et al. (2023). Purified FAME were analysed using a TRACE-GC Ultra gas chromatograph (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an on-column injector, a flame ionization detector and a fused silica capillary column, Supelcowax TM 10 (Sigma-Aldrich Co., St. Louis, MO, USA). The chromatographic conditions were maintained as described by Marrero et al. (2021). FAME were identified by comparison to commercial standard mixtures (FAME Mix C4-C24 and PUFA N° 3 from menhaden oil, Supelco Inc., Bellefonte, PA, USA) and to a well characterized cod roe oil. Results for each FA are expressed as percentage of total FA.

### RNA extraction and complementary DNA synthesis

Individual brain, liver and intestine samples (n=4) were homogenized in 1 mL of TriReagent® (Sigma-Aldrich) to extract total RNA according to Betancor et al. (2014). Homogenised samples were incubated at room temperature for 5 min before they were centrifuged at 12,000 x g for 10 min at 4 °C. The supernatants were transferred into fresh Eppendorf tubes and 100 µL of 1-bromo-3-chloropropane (BCP) was added. The tubes were then vigorously shaken by hand for 15 s, incubated at room temperature for 15 min, and centrifuged at 20,000 x g for 15 min at 4 °C. The aqueous (upper) phase was then transferred to fresh tubes, and isopropanol was added to precipitate the RNA. The mixtures were inverted six times, incubated for 10 min at room temperature and centrifuged at 20,000 x g for 10 min at 4 °C. The RNA precipitate formed gel like pellets on the bottom of the tubes. The supernatant was removed, and the pellets were washed with 1 mL of 75% ethanol in ddH<sub>2</sub>O (v/v). The pellets were lifted from the bottom of the tube by flicking and inverting the tubes a few times so that the entire surface of the pellets was properly washed. The tubes were then centrifuged at 20,000 x g for 5 min at room temperature and the ethanol was carefully removed and discarded. This step was repeated twice. The RNA pellets were air dried at room temperature until no visible traces of ethanol. The concentration and quality of RNA were assessed by NanoDrop® ND-1000 spectrophotometer (LabTech International, Uckfield, UK). The quality and integrity of the RNA samples were further assessed by electrophoresis on 1% agarose gel (v/v). Clear 28S and 18S rRNA bands indicated RNA of good quality. The RNA extracts were stored at -80 °C until further analysis.

### Quantitative PCR gene expression

Brain, liver and intestine gene expression (n=4) of *fads1*, *fads2*, *elovl2* and *elovl5* was determined by quantitative real-time PCR (qPCR). Beta actin (*B-actin*) and glucose-6-phosphate dehydrogenase (*G6PDH*) were used as reference genes to assess the expression of target genes. To determine the efficiency of the primer pairs, serial dilutions of pooled cDNA were carried out (Morais et al., 2020). qPCR was performed in duplicates on a Thermocycler (Analytik Jena, Jena, Germany) in 96 plates at total volumes of 20 µL containing 10 µL of Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific, Carlsbad, CA, USA), 1 µL of each primer (10 µM), 2 µL or 5 µL of cDNA (1:20 dilution) for reference and target genes respectively, as well as 6 or 3 µL of molecular biology grade water. Negative controls (NTC, no template control), containing 5 µL molecular biology grade water were also run. qPCR conditions included a first step of activation at 50 °C for 2 min, then 95 °C for 10 min followed by 35 cycles of denaturation at 95 °C for 15 s and annealing at the annealing temperature for each gene (Table 2) for 30 s, and a final extension step at 72 °C for 30 s. After amplification, a melt curve of 0.5 °C increments from 60 to 90 °C was performed to confirm a single product in each reaction. The relative expression of *fads1*, *fads2*, *elovl2* and *elovl5* among treatments was calculated as arbitrary units after normalisation by the expression level of the geometric mean of the

**Table 2**  
Primers sequences used for real-time quantitative PCR (qPCR).

Transcript	Primer Sequence (5'-3')	Amplicon Size (bp)	Annealing Temperature (°C)	Efficiency	Accession number
<i>Fads1</i>	CTTGGCGAACAAAAGAAGAAAT CCCAGTAAGGGCAGGTAGGT	200	60	0.88	XM_421052.5
<i>Fads2</i>	CTGAGGAAGACAGCAGAGGACAT GCAGGCAAGGATTAGAGTTGTG	153	60	0.97	NM_001160428.2
<i>Elovl2</i>	TTTGGCTGCCTCATGTTCCA TGTGACGGGGGTTTCCTTTG	123	60	0.86	NM_001197308.1
<i>Elovl5</i>	ATTGGGTGCCTTGTGGTCA AGCTGGTCTGGAAGATTGTCA	180	60	0.99	NM_001199197.1
<i>B-actin</i>	ATCACAGGGGTGTGGGTGTT CGGGAACCAATGCACCTTCGT	122	65	1.01	AI981686
<i>G6PDH</i>	GGGATGCAGATCTTCGTAAA CTTGCCAGCAAAGATCAACCTT	147	62	0.98	M11100

housekeeping genes (*B-actin* and *G6PDH*). Arbitrary units were obtained for each target gene (*fads1*, *fads2*, *elovl2* and *elovl5*) and tissue from the ratio between the expression level of each of them and the average of the control treatment (SO experimental group).

**Statistical analysis**

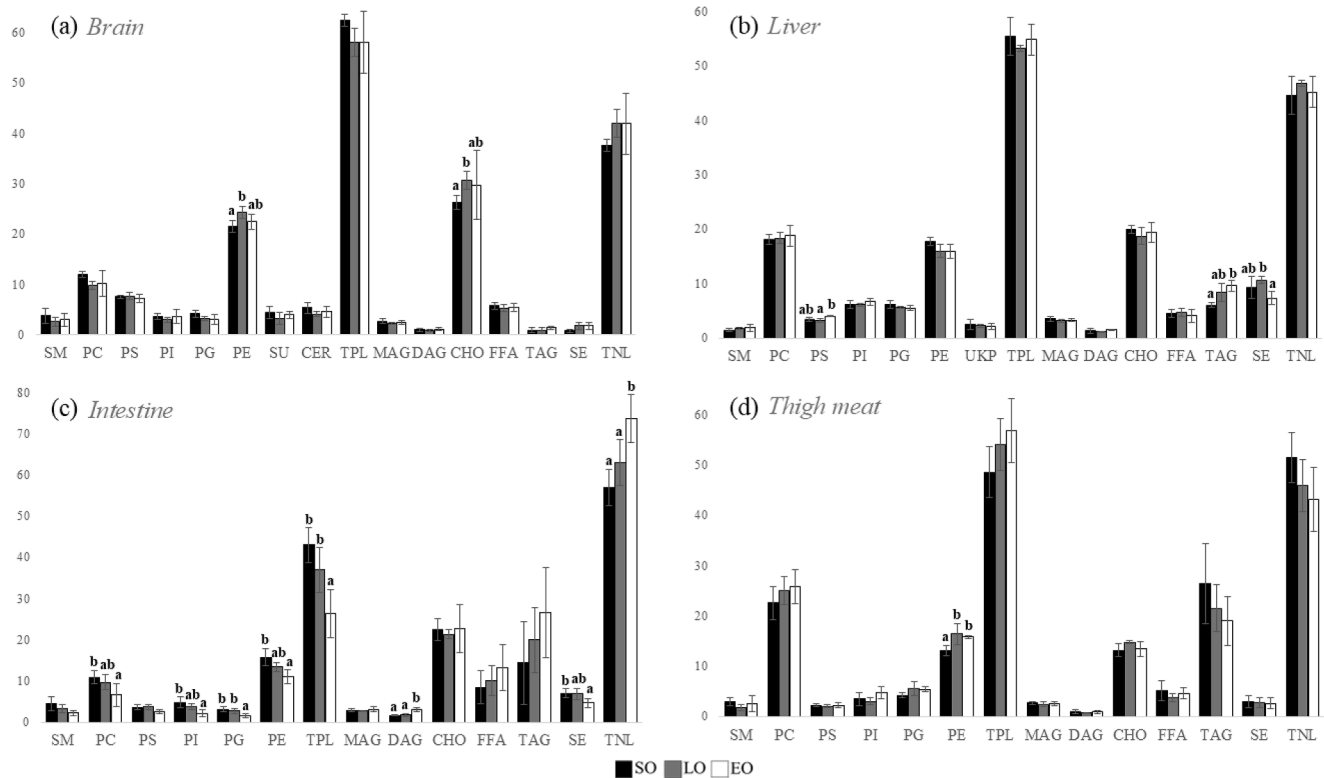
All the dependent variables studied (FA and LC composition and the relative expression of *fads1*, *fads2*, *elovl2* and *elovl5* genes) were examined for normal distribution by the Shapiro–Wilk test, and for homogeneity of the variances with the Levene test prior analysis. When normality and/or homoscedasticity was not achieved, the arcsine square root transformation was applied. Subsequently, if both assumptions were satisfied, a one-way ANOVA followed by a Tukey’s *post hoc* test was used to assess the effect of the diet (SO, LO and EO). Welch test followed by the Dunnett T3 test was performed for non-homoscedastic data, and

Kruskal-Wallis non-parametric test was applied in case of non-normal distribution, followed by the pair-wise comparisons Mann-Whitney test with Bonferroni correction. PUFA were additionally submitted to Principal Component Analysis (PCA), and factor scores were subsequently analysed by ANOVA. A significance level of  $\alpha=0.05$  was set. All statistical analyses were carried out using the IBM SPSS statistics 25.0 for Windows (SPSS Inc., Armonk, NY, USA).

**Results**

**Animal performance**

The dietary treatment did not affect body weight, carcass weight, weight gain, or feed intake at the end of the feeding period (Table S1). Moreover, no mortality was recorded during the experiment.



**Figure 1.** Main lipid class composition (% of total lipid) of *Gallus gallus* (a) brain, (b) liver, (c) intestine and (d) thigh meat. Values are means  $\pm$  SD (n = 4). PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; TPL, total polar lipids; CHO, cholesterol; FFA, free fatty acids; TAG, triacylglycerols; SE, sterol esters; TNL, total neutral lipids. Different letters in the same row indicate significant differences ( $p < 0.05$ ). [SO=Soy oil diet; LO=Linseed oil diet; EO=*Echium plantagineum* oil diet].

Lipid classes profile of tissues

Polar lipid predominated in the lipid class composition of brain (Fig. 1, Table S2) accounting for 58.1-62.5% compared to 37.6-42.0% for neutral lipids. Regardless of the treatment, phosphatidylethanolamine (PE) and cholesterol (CHO) were the most abundant lipid classes in this organ, being higher in LO than in SO-birds (24.3 vs. 21.6% and 30.7 vs. 26.3%, respectively), with EO displaying values in between. Phosphatidylcholine (PC) also stood out (9.8-12.0%) within phospholipids and free fatty acids (FFA, 5.3-5.8%) within neutral lipids.

PC (18.1-18.8%) and PE (15.8-17.7%) were the most abundant phospholipids in the liver of *G. gallus*, while the major neutral lipid components were CHO (18.7-19.9%) and sterol esters (SE, 7.4-10.7%) (Fig. 1, Table S3). The only significant differences registered among groups were the higher proportions of phosphatidylserine (PS) and triacylglycerols (TAG) in EO compared to LO and SO, respectively, and the lower hepatic SE in EO compared to LO.

The intestine from EO-birds presented the lowest levels of total polar lipids (TPL) (26.4 vs. 37.0-43.0%), mainly due to the lowest proportions of PC, phosphatidylinositol (PI), phosphatidylglycerol (PG) and PE, and the highest proportions of neutral lipids, particularly attributable to greater diacylglycerols (DAG) and TAG levels (Fig. 1, Table S4).

PC (22.6-25.9%), followed by PE (13.1-16.4%), were the main lipid fractions among thigh meat phospholipids, while TAG was the main neutral lipid (19.0-26.5%) (Fig. 1, Table S5). Reducing the dietary n-6/n-3 ratio (Table 1, EO showing the lowest) tended to increase phospholipids, thereby decreasing total neutral lipids (TNL). Particularly, EO led to a significantly higher PE, a slight but not significant increase (~28%) in PI, and a reduction in TAG compared to chicken fed the SO diet.

**Table 3**  
Main fatty acid composition (% of total fatty acids) of *Gallus gallus* brain.

	SO			LO			EO		
∑SFA	43.42	±	0.48	42.57	±	1.80	42.42	±	1.20
16:0	22.64	±	0.38	22.00	±	1.26	22.29	±	0.86
18:0	20.02	±	0.30	19.83	±	0.60	19.40	±	0.42
∑DMA	8.08	±	2.34	8.80	±	0.52	8.48	±	0.64
16:0 DMA	2.43	±	0.63	2.75	±	0.12	2.58	±	0.19
18:0 DMA	4.70	±	1.37	5.11	±	0.32	5.00	±	0.32
∑MUFA	17.38	±	0.80	16.94	±	0.75	17.26	±	0.74
16:1n-9	0.46	±	0.05	0.50	±	0.06	0.49	±	0.05
16:1n-7	0.67	±	0.03	0.69	±	0.02	0.66	±	0.04
18:1n-9	10.26	±	0.48	10.03	±	0.69	10.48	±	0.47
18:1n-7	4.78	±	0.38	4.64	±	0.25	4.51	±	0.27
∑n-6 PUFA	16.91	±	1.66b	14.26	±	0.58a	13.69	±	0.50a
18:2n-6	0.78	±	0.02b	0.66	±	0.08a	0.60	±	0.04a
20:2n-6	0.14	±	0.04	0.21	±	0.06	0.15	±	0.03
20:3n-6	0.27	±	0.02a	0.36	±	0.01b	0.44	±	0.06b
20:4n-6	10.60	±	0.85	9.94	±	0.38	9.46	±	0.41
22:4n-6	3.03	±	0.43	2.52	±	0.27	2.46	±	0.09
22:5n-6	2.08	±	0.56b	0.56	±	0.07a	0.58	±	0.20a
∑n-3 PUFA	14.03	±	1.93a	18.00	±	0.71b	17.48	±	0.96b
18:3n-3		nd		0.10	±	0.03	0.10	±	0.02
18:4n-3	0.09	±	0.04	0.09	±	0.06	0.11	±	0.02
20:3n-3		nd		0.08	±	0.02	0.10	±	0.01
20:5n-3		nd		0.21	±	0.03	0.21	±	0.03
22:5n-3	0.26	±	0.04a	0.50	±	0.03b	0.51	±	0.05b
22:6n-3	13.69	±	1.93a	17.01	±	0.76b	16.45	±	0.93b
∑n-6 LC-PUFA	16.13	±	1.64b	13.60	±	0.60a	13.09	±	0.54a
∑n-3 LC-PUFA	13.94	±	1.96a	17.80	±	0.75b	17.26	±	0.95b
n-6/n-3	1.22	±	0.18b	0.79	±	0.03a	0.78	±	0.01a

Values are means ± SD (n = 4). nd, not detected. FA, fatty acid; SFA, saturated fatty acids; DMA, dimethyl acetals; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain (≥C20) polyunsaturated fatty acids. Totals include other minor components not shown. Different letters in the same row indicate significant differences (p < 0.05).

Fatty acid profile of tissues

As displayed in Table 3, saturated fatty acids (SFA) were the most abundant FA group in *G. gallus* brain (~43% of total FA) with 16:0 and 18:0 being the main individual FA (19-23%) whereas 18:1n-9 was the major monounsaturated fatty acid (MUFA) (~10%). Brain proportions of total n-6 PUFA were lower in EO and LO than in SO-chicken (13.7, 14.3 and 16.9%, respectively) mainly due to a significant reduction of 18:2n-6 and 22:5n-6 (n-6 DPA) levels in these groups. ARA accounted for more than 60% of total n-6 PUFA in all dietary groups. Contrarily, total n-3 PUFA was significantly higher in EO and LO-chicken (~18.0 vs. 14.0%), particularly due to an increase in EPA, 22:5n-3 (n-3 DPA) and DHA. In all cases, DHA was the most abundant PUFA (13.7-17.0%).

Regardless of the treatment, chicken liver contained 41-42% of SFA (Table 4) chiefly 18:0 (22.8–23.3%). MUFA significantly varied among dietary groups (SO and EO: ~10.0%; LO: 13.0%) mainly due to 18:1n-9. Both LO and EO diets reduced hepatic total n-6 PUFA compared to SO (34.5 and 32.7 vs. 40.4%, respectively), mainly due to ARA, 22:4n-6 and n-6 DPA reductions. LA relative contents remained relatively stable among groups. Interestingly, GLA and its derived 20:3n-6 were higher in EO-chickens. In contrast, higher proportions of n-3 PUFA were obtained in liver from LO and EO-birds (11.0 and 12.3%, respectively) with a 2-fold increase compared to SO-chickens (~5%). Additionally, hepatic n-3 DPA displayed the highest values in the EO group.

Table 5 shows that both intestinal SFA and MUFA proportions were not affected by the diet (~36% and 21-24% of total FA, respectively), being 16:0 (~19%) and 18:1n-9 (16.0-19.1%) the most abundant FA within these groups. LO and EO-diets significantly reduced gut total n-6 PUFA with respect to SO (27.9 and 30.7% vs. 35.0%). In contrast, GLA and 20:3n-6 were more abundant in EO-birds compared to those supplemented with SO and LO. Significantly higher percentages of n-3

**Table 4**  
Main fatty acid composition (% of total fatty acids) of *Gallus gallus* liver.

	SO			LO			EO		
∑SFA	41.98	±	0.40	41.21	±	0.60	41.69	±	0.83
16:0	17.54	±	0.36	17.25	±	0.64	17.19	±	0.68
18:0	23.21	±	0.32	22.80	±	0.76	23.26	±	0.68
∑DMA	1.31	±	0.10	1.19	±	0.12	1.20	±	0.13
16:0 DMA	0.40	±	0.04	0.38	±	0.04	0.35	±	0.03
18:0 DMA	0.91	±	0.08	0.80	±	0.08	0.85	±	0.14
∑MUFA	9.92	±	0.94a	13.01	±	1.34b	9.96	±	1.28a
16:1n-9	0.18	±	0.01	0.21	±	0.02	0.20	±	0.03
16:1n-7	0.32	±	0.07a	0.50	±	0.11b	0.31	±	0.07a
18:1n-9	7.25	±	0.80a	9.96	±	1.15b	7.60	±	1.00a
18:1n-7	1.54	±	0.18b	1.62	±	0.07b	1.21	±	0.10a
∑n-6 PUFA	40.44	±	0.68b	34.51	±	1.60a	32.66	±	1.62a
18:2n-6	18.52	±	1.39	17.77	±	1.46	16.83	±	1.38
18:3n-6	0.22	±	0.05b	0.13	±	0.04a	0.38	±	0.03c
20:2n-6	0.42	±	0.06	0.31	±	0.04	0.36	±	0.08
20:3n-6	0.71	±	0.15a	0.97	±	0.03a	1.87	±	0.26b
20:4n-6	18.17	±	1.36b	12.42	±	0.79a	14.03	±	0.54a
22:4n-6	1.69	±	0.08b	0.89	±	0.05a	0.92	±	0.10a
22:5n-6	0.70	±	0.10b	0.18	±	0.04a	0.12	±	0.01a
∑n-3 PUFA	5.54	±	0.36a	11.01	±	1.13b	12.32	±	0.72b
18:3n-3	0.29	±	0.05a	1.47	±	0.13b	1.51	±	0.02b
18:4n-3	nd				nd		0.13	±	0.03
20:3n-3	0.12	±	0.04a	0.18	±	0.01b	0.19	±	0.02b
20:4n-3	nd			0.11	±	0.01a	0.19	±	0.05b
20:5n-3	0.56	±	0.06a	3.03	±	0.5b	3.15	±	0.28b
22:5n-3	1.27	±	0.17a	2.02	±	0.10b	2.94	±	0.33c
22:6n-3	3.31	±	0.26	4.20	±	0.91	4.20	±	0.70
∑n-6 LC-PUFA	21.70	±	1.61c	14.77	±	0.82a	17.30	±	0.52b
∑n-3 LC-PUFA	5.25	±	0.41a	9.53	±	1.09b	10.68	±	0.70b
n-6/n-3	7.32	±	0.45b	3.00	±	0.42a	2.81	±	0.28a

Values are means ± SD (n = 4). nd, not detected. FA, fatty acid; SFA, saturated fatty acids; DMA, dimethyl acetals; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain (≥C20) polyunsaturated fatty acids. Totals include other minor components not shown. Different letters in the same row indicate significant differences (p < 0.05).

PUFA were registered in chicken fed diets containing LO and EO compared to those fed SO (6.8 and 7.8 vs. 3.1%, respectively). EPA displayed the highest proportion in LO-chickens (1.46%) with EO-chickens showing similar proportions (1.13%), albeit not being significantly different from SO individuals (0.54%). While SDA was significantly increased in EO-birds compared to LO and SO ones.

The levels of SFA in thigh meat remained unchanged regardless of the diet (~33%), mostly represented by 16:0 and 18:0 (Table 6). EO-diet reduced 18:1n-9 by a third with respect to SO-diet, thereby reducing the total MUFA. Although dietary variations did not induce changes in ARA contents, LO and EO diets reduced its elongation and desaturation products. The dietary inclusion of EO most effectively increased n-3 LC-PUFA levels in thigh meat (SO: 2.3%; LO: 4.2%; EO: 6.0%), with n-3 DPA being the primary contributor.

The PCA for PUFA revealed that two principal components (PC1 and PC2) accounted for over 70% of the variation in all tissues analysed (% variance explained: brain (80.79%), liver (79.99%), intestine (72.50%), and thigh meat (75.91%); Fig. 2). In the brain, PC1 showed strong positive correlations with n-3 PUFA, particularly ALA (0.92), EPA (0.92) and DHA (0.91). This pattern effectively distinguished SO from LO and EO groups, confirming the higher concentrations of these FA in the latter diets, as shown in Table 3. In the liver, PC1 that is highly positively correlated to ALA (0.92) and EPA (0.92), arranged the data into 3 distinct clusters, while PC2 highlighted the differentiation of the EO group, driven by its higher SDA (0.96) and GLA (0.90) scores. PC1 revealed significant differences in the intestine between SO and EO groups (p < 0.05), attributed to the higher ALA score (0.93) in EO-fed animals and higher levels of 22:4n-6 in the SO-group (0.89), with LO group overlapping with the other treatments. In thigh meat, PC1 was

predominantly associated with n-3 PUFAs, specifically EPA (0.95) and n-3 DPA (0.94). These data effectively distinguish three distinct clusters, reinforcing the data presented in Table 6 and further underscoring the advantages of EO over the other two oils.

#### Dietary regulation of LC-PUFA biosynthetic genes

Regardless of the dietary treatment, the relative expression of *fads1*, *fads2*, *elovl2* and *elovl5* remained constant in chicken brain (Fig. 3). In contrast, the expression of all studied genes was upregulated in the intestine of chickens fed EO compared to the LO and SO groups. The hepatic expressions of *elovl2* and *elovl5* was significantly increased in EO-chickens.

#### Discussion

In recent years, poultry has emerged as a promising terrestrial alternative source of essential omega-3 LC-PUFA, which are otherwise almost exclusively obtained from finite marine resources (Zárate et al., 2017; Pérez et al., 2021). Bird's health and wellbeing as well as the nutritional value of its edible products, depends upon its capacity to convert C18 precursors into EPA and DHA in target organs and tissues. As a result, significant attention has been directed toward finding new ingredients for poultry diets including algae and n-3 rich oils such as LO, to improve their potential to endogenously produce n-3 LC-PUFA (Gatrell et al., 2015; Moran et al., 2019). However, scarce bibliography is available on the modification of poultry dietary ingredients towards n-6/n-3 balanced ratios through metabolic approaches (Kanakri et al., 2018; Pérez et al., 2021).

**Table 5**  
Main fatty acid composition (% of total fatty acids) of *Gallus gallus* intestine.

	SO			LO			EO		
∑SFA	36.58	±	1.02	36.54	±	0.70	35.56	±	1.97
16:0	18.69	±	1.07	18.96	±	1.06	19.02	±	0.41
18:0	16.08	±	2.04	15.66	±	1.65	14.71	±	1.98
∑DMA	4.27	±	1.33	4.04	±	1.32	3.80	±	1.00
16:0 DMA	0.88	±	0.32	0.76	±	0.13	0.75	±	0.25
18:0 DMA	3.20	±	0.97	2.84	±	0.76	3.15	±	1.04
∑MUFA	20.64	±	5.48	24.22	±	3.73	21.64	±	4.06
16:1n-9	0.37	±	0.05	0.42	±	0.07	0.32	±	0.03
16:1n-7	0.73	±	0.37	1.14	±	0.44	0.80	±	0.29
18:1n-9	15.95	±	5.15	19.11	±	3.34	17.66	±	3.79
18:1n-7	2.34	±	0.01b	2.35	±	0.29b	1.78	±	0.05a
∑n-6 PUFA	34.96	±	2.80b	27.90	±	1.96a	30.72	±	1.54a
18:2n-6	18.86	±	3.09	16.73	±	1.56	18.37	±	2.18
18:3n-6	0.16	±	0.01a	0.12	±	0.02a	1.06	±	0.49b
20:2n-6	0.46	±	0.06b	0.30	±	0.03a	0.35	±	0.03a
20:3n-6	0.64	±	0.22a	0.67	±	0.17a	1.28	±	0.50b
20:4n-6	12.15	±	3.76b	8.64	±	1.46a	8.33	±	3.06a
22:4n-6	2.46	±	0.78b	1.43	±	0.19ab	1.34	±	0.42a
22:5n-6	0.23	±	0.07		nd			±	nd
∑n-3 PUFA	3.12	±	0.65a	6.79	±	0.82b	7.83	±	1.12b
18:3n-3	0.54	±	0.22a	2.57	±	0.42b	3.33	±	1.65b
18:4n-3	0.14	±	0.03a	0.15	±	0.05a	0.80	±	0.50b
20:3n-3	0.13	±	0.06	0.17	±	0.03	0.15	±	0.04
20:4n-3		nd			nd		0.15	±	0.05
20:5n-3	0.41	±	0.18a	1.46	±	0.49b	1.13	±	0.51ab
22:5n-3	0.75	±	0.31	1.13	±	0.21	1.34	±	0.37
22:6n-3	1.14	±	0.40	1.31	±	0.36	0.93	±	0.45
∑n-6 LC-PUFA	15.94	±	4.80	11.05	±	1.83	11.29	±	3.95
∑n-3 LC-PUFA	2.43	±	0.86	4.06	±	1.02	3.70	±	1.35
n-6/n-3	11.50	±	1.88b	4.14	±	0.30a	3.99	±	0.58a

Values are means ± SD (n = 4). nd, not detected. FA, fatty acid; SFA, saturated fatty acids; DMA, dimethyl acetals; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain (≥C20) polyunsaturated fatty acids. Totals include other minor components not shown. Different letters in the same row indicate significant differences (p < 0.05).

In the present work, the three different vegetable oil mixtures assayed did not markedly modified brain, liver, intestine and muscle total lipid content and lipid classes profile (Fig. 1, Tables S2-S5). In agreement with previous studies on different chicken breeds, PC and PE were the most abundant phospholipids in every tissue analysed (Cherian and Sim, 1992; Pérez et al., 2021). Interestingly, the dietary inclusion of LO and EO led to an increase in thigh meat PE and, consequently, in its total polar lipids. The larger amount of n-3 LC-PUFA in thigh meat from these groups agrees well with previous findings stating that C18 PUFA tend to accumulate into TAG, whereas n-3 and n-6 LC-PUFA are preferentially stored in the polar lipid fraction (Betti et al., 2009). Similarly, it has been reported that n-3 LC-PUFA including EPA and DHA are more frequently esterified into PC and PE particularly in those tissues where synapsis is abundant due to contractility and neurotransmission activities, as mainly occurs in thighs and brain, respectively (Dick et al., 2024; Newman et al., 2002; Reis et al., 2016).

It is well known that PUFA content in animal tissues and organs is affected by dietary FA profile and n-6/n-3 proportions (Díaz-López et al., 2010; Horman et al., 2020; Jing et al., 2013; Marrero et al., 2024). In our experimental design, it is assumed that tissue relative contents of ARA, EPA, DPA and DHA mostly resulted from their endogenous biosynthesis as the three experimental diets contained residual amounts (<0.20%) of LC-PUFA (Table 1). This is supported by previous studies which have demonstrated that chickens, including the Canarian genotype, are able to *de novo* synthesize LC-PUFA from their C18 precursors (Gregory et al., 2013; Pérez et al., 2021).

The biosynthesis of C20 and C22 PUFA from their C18 precursors utilizes the same set of enzymes and metabolic pathways (Monroig et al., 2022; Sargent et al., 2002), leading to a significant competition between

LA and ALA for the same elongases and desaturases. Here, the balanced n-6/n-3 dietary proportions of LO- and EO-diets increased the levels of metabolic products derived from ALA such as 20:3n-3, EPA, n-3 DPA and DHA in chicken brain, while decreased n-6 DPA with respect to the SO-diet (Table 3, Fig 2). These results agree with those by Kanakri et al. (2018) who demonstrated a strong and positive correlation between dietary and brain n-3 PUFA contents along with lower levels of n-6 PUFA in broilers. Regardless of the diet, DHA was the major PUFA in brain of all birds (>13.5% of total FA) due to its role in the maintenance of brain structure, membrane fluidity and permeability (von Schacky, 2021). The capacity of chicken to maintain adequate brain DHA proportions despite dietary n-3 FA restrictions confirms their ability to synthesize and selectively transfer n-3 LC-PUFA. However, dietary supplementation with SDA (EO-diet), neither increased DHA, nor the C20 and C22 intermediates involved in its endogenous synthesis, compared to LO-birds. This points out a limitation in its *de novo* production. Accordingly, dietary n-6 and n-3 PUFA variations did not affect desaturase and elongase gene expression in broilers' brain (Fig. 3), reported here for the first time.

The observed results in the intestine, liver, and thigh meat, are also consistent with those in the brain, further emphasizing the significance of maintaining a balanced dietary n-6/n-3 FA ratio (Abdulla et al., 2019; Jing et al., 2013; Mandal et al., 2014). In this sense, the reduction of the dietary n-6/n-3 ratio resulted in a 21.2% increase in DHA in the liver, and a 43.2% increase in muscle from EO-chickens compared to birds fed SO. By contrast, the inclusion of LO or EO in the diet did not significantly impact enteric DHA levels. It has been previously demonstrated that enterocytes are active metabolic sites for the LC-PUFA biosynthesis (Marrero et al., 2021; Monroig et al., 2018). The intestine not only acts

**Table 6**  
Main fatty acid composition (% of total fatty acids) of *Gallus gallus* thigh meat.

	SO		LO		EO				
∑SFA	32.13	±	1.16	33.57	±	1.27	33.92	±	0.68
16:0	16.27	±	1.44	15.84	±	1.70	15.20	±	0.41
18:0	14.89	±	1.59	16.79	±	1.87	17.79	±	0.78
∑DMA	6.20	±	1.92	6.84	±	1.00	6.89	±	0.69
16:0 DMA	3.89	±	1.21	4.03	±	0.56	4.18	±	0.65
18:0 DMA	1.80	±	0.54	2.25	±	0.39	2.21	±	0.19
∑MUFA	25.18	±	3.73b	23.34	±	2.56ab	18.14	±	0.93a
16:1n-9	0.34	±	0.11	0.32	±	0.07	0.25	±	0.01
16:1n-7	1.33	±	0.42	1.18	±	0.35	0.74	±	0.07
18:1n-9	19.88	±	3.13b	18.24	±	2.20ab	14.19	±	0.86a
18:1n-7	2.92	±	0.11b	2.95	±	0.15b	2.35	±	0.10a
∑n-6 PUFA	33.07	±	1.51	29.71	±	2.67	32.79	±	1.19
18:2n-6	20.34	±	1.68	17.88	±	2.15	18.67	±	1.78
18:3n-6	0.09	±	0.01a	0.09	±	0.01a	0.51	±	0.05b
20:2n-6	0.35	±	0.04	0.30	±	0.04	0.30	±	0.03
20:3n-6	0.31	±	0.05a	0.40	±	0.04a	0.98	±	0.07b
20:4n-6	10.32	±	2.22	10.11	±	1.46	11.30	±	1.13
22:4n-6	1.34	±	0.42b	0.82	±	0.07a	0.88	±	0.05a
22:5n-6	0.32	±	0.12b	0.13	±	0.02a	0.14	±	0.01a
∑n-3 PUFA	2.89	±	0.53a	6.02	±	0.66b	8.06	±	0.42c
18:3n-3	0.64	±	0.20a	1.79	±	0.40b	1.78	±	0.41b
18:4n-3		nd			nd		0.30	±	0.08
20:3n-3		nd		0.12	±	0.02	0.12	±	0.03
20:4n-3		nd			nd		0.10	±	0.01
20:5n-3	0.09	±	0.01a	0.52	±	0.12b	0.72	±	0.09b
22:5n-3	1.12	±	0.36a	2.16	±	0.33b	3.41	±	0.27c
22:6n-3	1.04	±	0.36a	1.42	±	0.39ab	1.83	±	0.35b
∑n-6 LC-PUFA	12.63	±	2.79	11.75	±	1.43	13.60	±	1.10
∑n-3 LC-PUFA	2.25	±	0.71a	4.23	±	0.63b	5.98	±	0.63c
n-6/n-3	11.68	±	1.79b	5.01	±	0.97a	4.08	±	0.36a

Values are means ± SD (n = 4). nd, not detected. FA, fatty acid; SFA, saturated fatty acids;

DMA, dimethyl acetals; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain (≥C20) polyunsaturated fatty acids. Totals include other minor components not shown. Different letters in the same row indicate significant differences ( $p < 0.05$ ).

as a site of absorption, but also as a center for lipid metabolism, encompassing the reacylation and packaging of dietary lipids as well as the biosynthesis of LC-PUFA (Morais et al., 2015). Our results suggest that regardless of the n-6/n-3 dietary input, the reduced dietary intake of n-3 LC-PUFA stimulates compensatory biosynthetic activity to produce both EPA and DHA in chicken intestinal epithelial cells (Table 5, Fig. 2). However, the intestine of birds with a higher dietary intake of SDA (EO-birds) had higher SDA, 20:4n-3, n-3 DPA, total n-3 PUFA, GLA and 20:3n-6, but lower ARA and C22 omega-6 LC-PUFA. Accordingly, the intestine of these chickens presented increased expression of genes encoding both elongases and desaturases enzymes involved in the biosynthesis of LC-PUFA (Fig. 3), which have been previously reported to prefer n-3 over n-6 PUFA (Pérez et al., 2021).

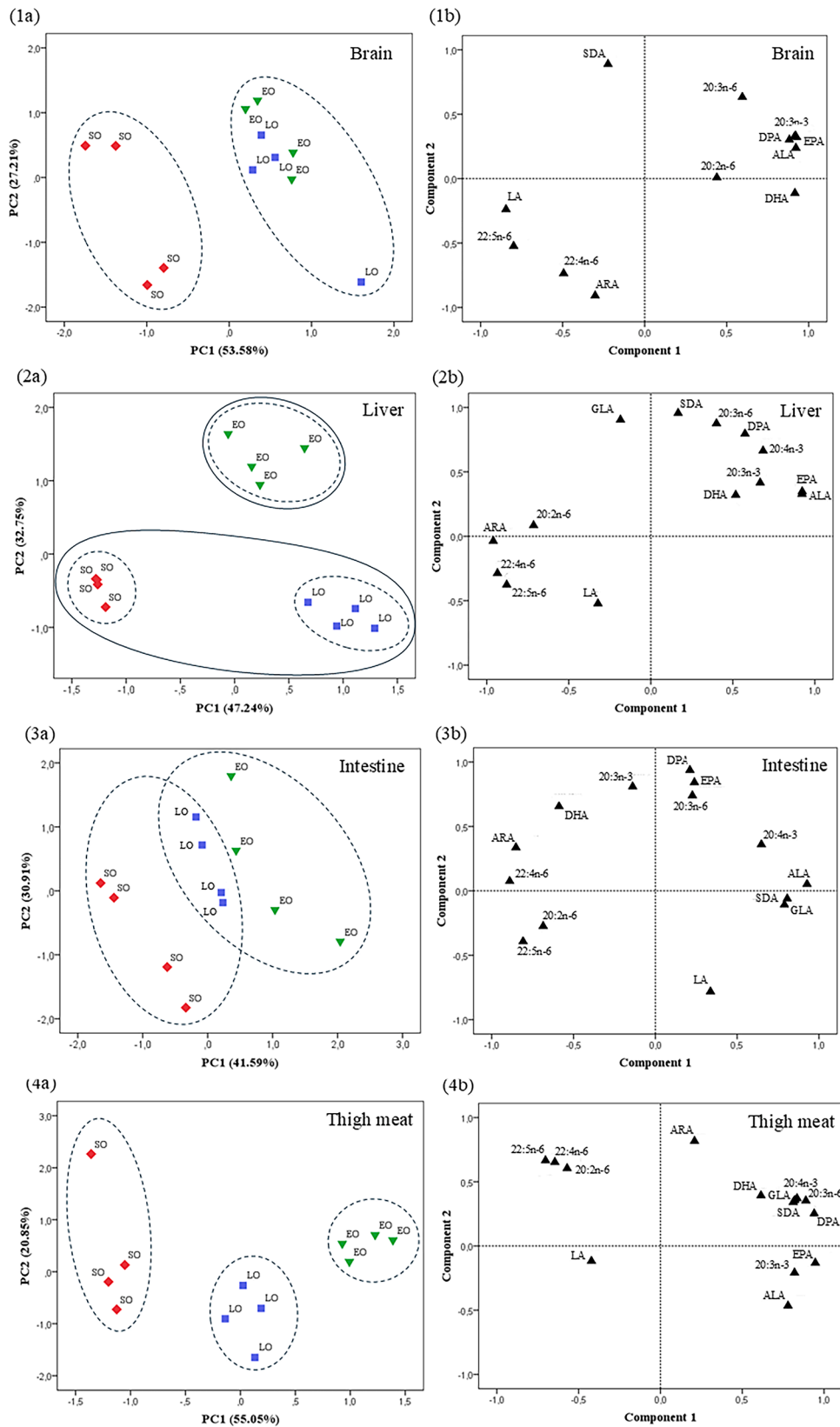
Similarly to the intestine, dietary inclusion of EO increased hepatic relative expression of *elovl2* and *elovl5* genes (Fig. 3), probably related to its major role in the biosynthesis of LC-PUFA (Marrero et al., 2021). However, hepatic n-3 LC-PUFA of LO and EO-birds remained unchanged (9.5 and 10.7% respectively) (Table 4, Fig. 2) suggesting that, once synthesized, they are likely transported to other tissues rather than accumulated in the liver (Table 6, Fig. 2). In agreement with previous studies testing diets supplemented with SDA (Elkin et al., 2015; El-Zenary et al., 2023; Rymer et al., 2011), EO-chicken meat contained the highest DHA proportions. The upregulation of hepatic elongases could explain the increment of DHA in muscle, together with the higher concentrations of 20:3n-6, 20:4n-3 and n-3 DPA, derived from an elongation step of GLA, SDA and EPA, respectively.

As reviewed by Gregory et al. (2013), while the impact of dietary ALA on the n-3 LC-PUFA content in chicken meat has been extensively

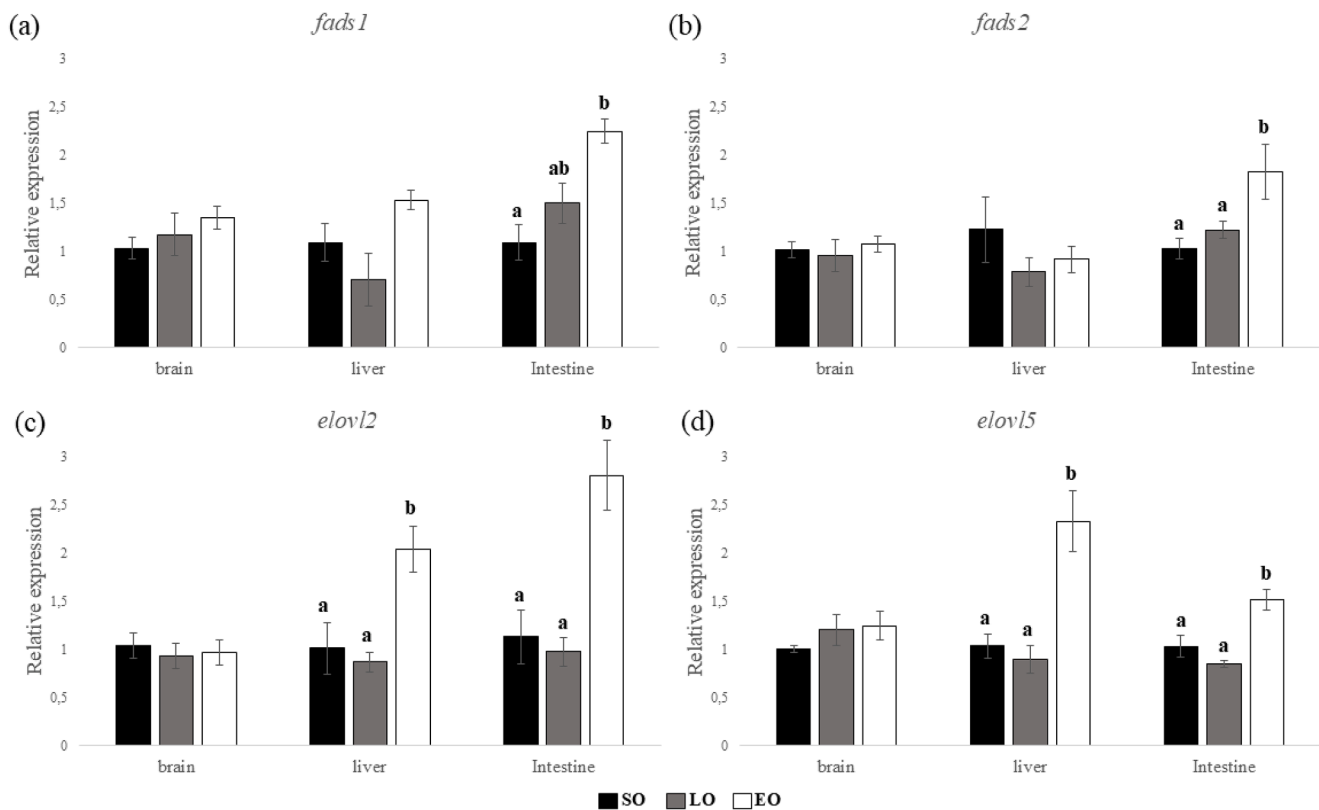
studied, its role in DHA synthesis remains inconsistent, with findings showing considerable variability. Commercial poultry basal diets, composed of ingredients such as soy, wheat, corn, barley, and other minor components, typically contain significant amounts of LA, as observed in our dietary treatments (28.6–37.8%, Table 1). This high LA content may limit DHA production, even with the inclusion of vegetable oils richer in C18 n-3 fatty acids. It is widely recognized that DHA formation in vertebrates mainly occurs through the Sprecher pathway, that includes a  $\Delta 6$  desaturation reaction to convert 24:5n-3 into 24:6n-3, and a final peroxisomal chain shortening to 22:6n-3 (Oboh et al., 2017; Sprecher, 2000). Therefore, the competition between 24:5n-3 and ALA for  $\Delta 6$  desaturase may limit the final synthesis of DHA when using LO compared to EO (Portolesi et al., 2007). Additionally, the SDA-supplemented birds also presented higher n-3 DPA levels in thigh meat. The accumulation of n-3 DPA with ALA-rich or even EPA-rich diets may be due to the saturation of the last *elovl2*, that converts DPA into 24:5n-3 and could partially explain the limitations of both FA as precursors of DHA (Elkin et al., 2016; Kitesa and Young, 2008). n-3 DPA possesses beneficial properties such as the reduction of the expression of inflammatory genes, preventing angiogenesis and inhibiting platelet aggregation more effectively than EPA and even DHA (Kaur et al., 2011).

The presence of 20:2n-6 and 20:3n-3 in Canarian chicken tissues, suggests that *elovl5* might also influence the metabolism of LA and ALA towards LC-PUFA. Monroig et al. (2011) highlighted the presence of  $\Delta 8$  activity in various mammalian cells and tissues, as an alternative pathway for generating EPA and DHA from their C18 precursors. Consequently, the formation of 20:3n-6 and 20:4n-3 could result not





**Figure 2.** Principal component analysis (PCA) of PUFA of TL extract from (1) brain, (2) liver, (3) intestine and (4) thigh meat from *Gallus gallus*. (a) Factors score plot for PC1 and PC2. Between brackets the % of variance explained by each one of the components. [(◆) SO=Soy oil diet; (■) LO=Linseed oil diet; (▼) EO=*Echium plantagineum* oil diet;]. Dashed line (---) represents different clusters for PC1 (ANOVA;  $p < 0.05$ ) while solid line (—) represent different cluster for PC2 (ANOVA;  $p < 0.05$ ). (b) Component loading plot for the PCA that illustrate the correlation between each individual PUFA and the principal components PC1 and PC2.



**Figure 3.** Distribution of (a) *fads1*, (b) *fads2*, (c) *elovl2* and (d) *elovl5* mRNA in brain, liver and intestine of *Gallus gallus*. Relative expression is shown as geometric mean normalised expression using  $\beta$ -actin and *G6PDH* as reference genes. Results are presented as ratios  $\pm$  SD (n = 4). Different letters indicate significant differences among dietary treatments ( $p < 0.05$ ) as determined by one-way ANOVA followed by Tukey's multiple comparison test.

only from the elongation of GLA and SDA catalyzed by *elovl5*, but also from the action of a  $\Delta 8$  desaturase over their C20 counterparts.

It is assumed that *fads1* and *fads2* exhibit  $\Delta 5$  and  $\Delta 6$  activities, respectively (Nakamura and Nara, 2004). Nevertheless, Mihelic et al. (2020) found a relatively high expression of the novel *fads6* gene in broiler. Although this gene has not been well characterized in chicken, it has been shown to exhibit  $\Delta 4$  desaturase in golden pompano (*Trachinotus ovatus*) (Zhu et al., 2019) to directly convert n-3 DPA into DHA. Moreover, Mašek et al. (2014) described the presence of  $\Delta 4$ ,  $\Delta 5$ ,  $\Delta 6$  activities in chicken. Additionally, Pérez et al. (2021) also suggested the activity of two alternative pathways for DHA production, indicating the potential presence of a  $\Delta 4$  desaturase in chicken. In this context, further research is needed to confirm whether the metabolic route through the  $\Delta 6$  and  $\Delta 5$  desaturases is the only pathway for the LC-PUFA biosynthesis or, alternatively,  $\Delta 8$  and  $\Delta 4$  desaturase pathways are also active in chicken.

Overall, the meat from EO-chicken had higher proportion of n-3 DPA and other minor FA such as SDA, 20:4n-3 and 20:3n-6 that can potentially exert nutritional benefits for consumers. For instance, SDA has been characterized as a potent inhibitor of cancer cell growth (Guil-Guerrero, 2007). 20:4n-3 inhibited eicosanoid production from ARA in fish cells as it generates its own eicosanoids that have unique biological activities (Ghioni et al., 2002). Furthermore, 20:3n-6 is metabolized via the cyclooxygenase (COX-1 and COX-2), generating metabolites that antagonize the synthesis of ARA-derived lipid mediators, helping to suppress inflammation (Mustonen and Nieminen, 2023).

## Conclusions

Lowering the dietary n-6/n-3 proportions is crucial to activate the biosynthesis of n-3 LC-PUFA in chicken. The EO-fed chicken also showed increments in beneficial SDA, GLA, 20:4n-3, and n-3 DPA in tissues.

Accordingly, dietary EO increased the hepatic expression of genes encoding fatty acid elongases, *elovl2* and *elovl5*. This upregulation likely promotes the biosynthesis of C20 and C22 FA, as the EO was more effective than LO in enriching the thigh meat in n-3 LC-PUFA. The use of local resources such as plants of the *Echium* genus or other SDA-rich plants could be an efficient and sustainable strategy to improve the nutritional value of chicken meat for human consumption.

## Disclosures

The authors declare that they have no conflict of interest.

## Declaration of competing interest

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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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.104820.

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