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Original Research Article

Influence of free fatty acid content and degree of fat saturation on production performance, nutrient digestibility, and intestinal morphology of laying hens

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ABSTRACT

This study was conducted to evaluate the effects of dietary free fatty acid (FFA) content and degree of fat saturation on production performance, lipid and calcium digestibility, and intestinal function of laying hens. For a 15-week period, a total of 144 laying hens (19 weeks old) were randomly assigned to 8 dietary treatments, which were obtained by gradually replacing crude soybean oil with soybean acid oil (AO), or crude palm oil with palm fatty acid distillate (FAD). Thus, there were 4 soybean and 4 palm diets with 6% added fat varying in their FFA percentage (10%, 20%, 30%, and 45%), following a 2 × 4 factorial design. Each treatment included 6 replicates with 3 birds per replicate. Average daily feed intake and final body weight were significantly higher in palm diets ($P < 0.001$), while no differences were found in egg mass and feed conversion ratio. Higher levels of FFA in soybean diets resulted in lower egg production and higher egg weight (linear, $P < 0.01$). Regarding the degree of fat saturation, hens fed soybean diets presented higher digestibility of ether extract (EE), fatty acids, and calcium than palm diets ($P < 0.001$). The dietary FFA percentage negatively affected the digestibility of EE and calcium ($P < 0.01$), while having little effect on FA digestibility. There was a significant interaction in the AME; lower values were reported in soybean diets as the dietary FFA percentage increased (linear, $P < 0.01$), whereas palm diets remained unaffected. The experimental diets had little effect on gastrointestinal weight and length. However, the jejunum of soybean diets showed higher villus height and higher villus height-to-crypt depth ratio than palm diets ($P < 0.05$), and the dietary FFA percentage increased the crypt depth and decreased the villus height-to-crypt depth ratio (linear, $P < 0.05$). It was concluded that varying dietary FFA content did not affect fat utilization as much as the degree of saturation did, supporting the use of AO and FAD as alternative fat ingredients.

1. Introduction

A wide variety of lipid sources are used in laying hen feed in order to meet both energy and essential fatty acid (FA) requirements (Ravindran et al., 2016). In recent years, the scarcity and price instability of conventional raw materials have led to the search for unconventional but suitable ingredients for animal nutrition amongst agro-industrial by-products. In this context, some fat by-products obtained from the refining of edible oils, such

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as acid oils (AO) and fatty acid distillates (FAD), may be of interest to the poultry industry due to their potential as alternative energy sources (Varona et al., 2021a). Indeed, they have been included in the European catalogue of feed materials (European Commission, 2013).

Over the various steps of the refining process, free fatty acids (FFA) are removed from crude oils through both chemical (AO) and physical (FAD) refining, leading to the creation of fat by-products which are characterized by having a high proportion of FFA (31.7% to 93.6%) (Varona et al., 2021a). In addition, AO and FAD may also contain variable amounts of moisture, insoluble impurities, and unsaponifiable matter (collectively referred to as MIU), as well as some lipid oxidation compounds, which can affect their energy value (Nuchi et al., 2009; Roll et al., 2018a; Varona et al., 2021a, 2021c).

The AME and the nutritive value of fats and oils are determined by their chemical composition (Freeman, 1969; Krogdahl, 1985; Ravindran et al., 2016). Both the degree of saturation (expressed as the ratio of unsaturated to saturated fatty acids [UFA:SFA ratio]) and the FA chain length markedly influence fat digestibility (Renner and Hill, 1961; Zumbado et al., 1999; Tancharoenrat et al., 2013; Vilarrasa et al., 2015). Furthermore, dietary FFA content has also been reported to depress AME (Shannon, 1971; Sklan, 1979; Wiseman and Salvador, 1991; Blanch et al., 1996; Vilà and Esteve-Garcia, 1996). This is attributed to a lower efficiency in the emulsification and micellar formation of FFA (Sklan, 1979; Small, 1991; Jimenez-Moya et al., 2021c), and their greater ability to form insoluble calcium soaps in the intestine, especially when they come from fats with a low UFA:SFA ratio (Atteh and Leeson, 1984, 1985). However, in numerous studies in which FFA-rich fat by-products have been added to poultry diets, no differences have been observed in production performance in either broiler chickens (Waldroup et al., 1995; Vilarrasa et al., 2015; Roll et al., 2018a; Rodriguez-Sanchez et al., 2019a, 2021; Jimenez-Moya et al., 2021a, 2021b) or laying hens (Perez-Bonilla et al., 2011; Irandoust et al., 2012), in comparison with the use of conventional crude oils. In fact, recent studies conducted on broiler chickens have tested FFA-rich fat by-products with varying degrees of fat saturation and reported that the dietary UFA:SFA ratio has a greater impact on fat utilization than the FFA content (Rodriguez-Sanchez et al., 2019a, 2021; Jimenez-Moya et al., 2021a, 2021b).

In view of the above, it appears that the UFA:SFA ratio plays an important role in fat digestion and absorption. Nevertheless, the effect of the dietary FFA level is less clear, and the potential interaction with the UFA:SFA ratio in laying hens is poorly understood. Thus, the aim of the present study was to evaluate the effect of the degree of fat saturation and dietary FFA content on production performance, nutrient and FA digestibility, lipid-class composition of the excreta, and gastrointestinal morphology in laying hens. It was hypothesized that AO and FAD represent valuable energy sources that could contribute to more sustainable egg production.

2. Materials and methods

2.1. Animal ethics statement

The experimental procedure was approved by the Animal Research Ethics Committee of the Universidad Cardenal Herrera-CEU (CEEA 17/018) and performed in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes (European Parliament and of the Council, 2010). The animal study was conducted at the Universidad Cardenal Herrera-CEU's Teaching and Research Farm (Náquera, Valencia, Spain).

2.2. Experimental fats

The fats used in this experiment came from 2 soybean sources (crude soybean oil [SO] and soybean acid oil [SAO], both provided by Riosa S.A., Jaén, Spain) and 2 palm sources (crude palm oil [PO] and palm FAD [PFAD], both provided by Lípidos Santiga S.A., Barcelona, Spain). Oil samples were analyzed in triplicate for moisture, insoluble impurities, unsaponifiable matter, peroxide value, *p*-anisidine value, FA composition, and lipid classes (triacylglycerols [TAG], diacylglycerols [DAG], monoacylglycerols [MAG] and FFA) according to the methods used by Varona et al. (2021b) for the analysis of AO and FAD in animal nutrition (Table 1).

2.3. Animals and diets

A total of 144 Lohmann Brown-Classic laying hens procured from a commercial farm (Huevos Guillén, Valencia, Spain), after a 3-week adaptation period in the facility, were randomly assigned to one of 8 dietary treatments at 19 weeks of age. Each treatment was replicated 6 times and each replicate comprised a cage housing 3 birds (experimental unit). The cages (76.2 cm × 63.0 cm with a minimum height of 45.0 cm) were equipped with a nest, perch and 3 freshwater nipple drinkers. During the 15 weeks of the experiment (up to 34 weeks of age), the hens were offered feed (in mash form) and water for ad libitum consumption, and the animals were raised under the conditions recommended by the breeder (Lohmann, 2019).

The experimental diets were formulated to meet or exceed FEDNA's (*Fundación Española para el Desarrollo de la Nutrición Animal*) recommendations (2018) and consisted of a basal diet (94%) – with minimal fat levels – and an experimental fat or fat blend (6%). The ingredients and the analyzed chemical composition

Table 1
Quality traits, fatty acid, and lipid-class composition of the experimental fats.

Item	Soybean oil	Soybean AO	Palm oil	Palm FAD
MIU, g/100 g	0.59	4.95	0.52	2.13
Moisture	0.05	0.56	ND	0.12
Insoluble impurities	0.17	1.97	0.21	0.27
Unsaponifiable matter	0.37	2.42	0.31	1.74
Peroxide value, mEq O_2/kg	4.5	0.7	3.9	1.6
p-Anisidine value	2.3	15.2	5.1	64.3
Fatty acid composition, %				
Myristic acid (C14:0)	0.1	0.1	1.2	1.5
Palmitic acid (C16:0)	11.9	11.7	44.3	46.2
Stearic acid (C18:0)	4.8	4.2	4.2	4.6
Oleic acid (C18:1 n-9)	23.1	31.9	37.8	36.5
Vaccenic acid (C18:1 n-7)	1.5	1.3	0.9	0.9
Linoleic acid (C18:2 n-6)	52.8	46.1	10.2	8.8
Linolenic acid (C18:3 n-3)	4.2	1.8	0.3	0.3
Minor fatty acids[1]	1.5	2.5	1.1	1.1
Trans fatty acids (C18:1)	ND	0.4	ND	0.2
SFA	17.9	17.8	50.4	52.9
MUFA	25.0	33.7	39.0	37.7
PUFA	57.1	48.1	10.6	9.2
UFA:SFA ratio	4.6	4.6	1.0	0.9
Lipid-class composition, %				
TAG	94.3	29.1	83.6	6.2
DAG	3.8	15.8	9.7	5.0
MAG	0.2	1.3	0.4	1.6
FFA	1.7	53.8	6.3	87.2

AO = acid oil; FAD = fatty acid distillate; MIU = unsaponifiable matter; ND = non-detectable values; 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.

[1] Minor fatty acids identified and quantified: C15:0, C16:1 n-7, C16:1 n-9, C17:0, C18:3 n-6, C20:0, C20:1 n-9, C20:2 n-6, C20:3 n-3, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:0, C22:4 n-6, C22:5 n-3, C22:6 n-3, C24:0, C24:1.

of the experimental diets are shown in Table 2. As shown in Table 3, diets were obtained using a 2 × 4 factorial arrangement with 2 fat sources (soybean − high UFA:SFA ratio, or palm − low UFA:SFA ratio), and 4 different levels of FFA (10%, 20%, 30%, or 45%). Thus, 8 dietary treatments resulted from combinations of crude SO with SAO (S10, S20, S30, S45), and crude PO with PFAD (P10, P20, P30, P45) (Table 4). The lowest FFA levels assayed for each fat source corresponded to those in which only crude SO (S10, high UFA:SFA ratio, low FFA percentage), or crude PO (P10, low UFA:SFA ratio, low FFA percentage) were added. The highest level of FFA in soybean diets was obtained with the complete replacement of SO with SAO (S45, high UFA:SFA ratio, high FFA percentage). Then, a similar FFA level was achieved for palm diets by creating a blend of 66% of PFAD and 33% crude PO (P45, low UFA:SFA ratio, high FFA percentage). Two intermediate diets were designed for each fat source.

2.4. Sampling and measurements

The body weight (BW) of each hen was recorded at the beginning and at the end of the trial. Diet differentiation was started in week 19 of life; thus, the first experimental measurements were taken in week 20 of life, with this moment representing week 1 of production. Throughout the entire experimental period, egg production (including the number of eggs produced and the individual egg weights) and feed intake were recorded weekly for each replicate cage. From these data, the ADFI, egg production, egg weight, egg mass and cumulative feed conversion ratio (FCR) per kilogram of eggs were calculated for the entire experiment. Any mortalities were recorded and weighed.

Table 3
Oil blends used in the experimental diets.

Item	Soybean diet				Palm diet			
	S10	S20	S30	S45	P10	P20	P30	P45
Theoretical FFA, %	10	20	30	45	10	20	30	45
Proportion in oil blends[1], %								
Crude soybean oil	100	70	30	-	-	-	-	-
Soybean acid oil	-	30	70	100	-	-	-	-
Crude palm oil	-	-	-	-	100	80	53	33
Palm fatty acid distillate	-	-	-	-	-	20	47	67

FFA = free fatty acids.
[1] All oil blends were added at 6% to the basal diet.

The apparent total tract digestibility (ATTD) coefficients (particular and total FA, ether extract [EE] and calcium), AME, and lipid-class composition were assessed at the end of the trial using the partial excreta collection method. Titanium dioxide (TiO_2, pure titanium (IV) oxide, PanReac AppliChem, Barcelona, Spain) was added (0.5%) to the experimental diets 5 days before the end of the trial as an inert digestibility marker. Excreta from each replicate were collected daily for 3 days using plastic-covered trays placed directly under the cages. After the remaining feed and feathers were carefully removed, the excreta samples were immediately frozen at −18 °C. Samples collected for each experimental unit were pooled and homogenized. Thereby, a representative sample from each replicate was obtained for analysis.

At the end of the week 15, one hen per replicate was selected according to the average BW of the flock, and euthanized (T-61, Merck Sharp & Dohme Corp., Kenilworth, New Jersey, USA). After

Table 2
Ingredient composition and detailed analysis of the experimental diets (g/100 g on a DM basis, unless otherwise indicated).

Diets	Soybean diet				Palm diet			
	S10	S20	S30	S45	P10	P20	P30	P45
Ingredient composition								
Barley	49.9							
Soybean meal, 47.5% CP	24.3							
Corn	6.9							
Calcium carbonate, fine-grained	6.8							
Experimental fats[1]	6.0							
Calcium carbonate, coarse-grained	2.1							
Sunflower meal, 36% CP	1.9							
Monocalcium phosphate	1.1							
Vitamin and mineral premix[2]	0.4							
Sodium chloride	0.3							
Methionine hydroxy analogue	0.2							
Sodium bicarbonate	0.1							
Detailed analysis								
Gross energy, kcal/kg	4,222	4,184	4,159	4,146	4,055	4,116	4,097	3,988
Dry matter	90.9	90.9	91.1	91.0	91.2	91.0	90.8	90.9
Crude protein	17.4	17.4	17.3	17.3	17.3	17.3	17.3	17.3
Lys	1.00	1.01	1.00	0.98	0.99	1.01	1.00	0.98
Met	0.48	0.48	0.46	0.46	0.47	0.47	0.48	0.46
Met + Cys	0.80	0.80	0.79	0.80	0.77	0.79	0.80	0.77
Thr	0.71	0.70	0.71	0.72	0.69	0.71	0.70	0.70
Ash	13.9	14.3	14.2	14.0	14.0	14.2	14.1	13.9
Ether extract	7.9	7.8	7.7	7.8	7.8	7.8	7.9	7.6
Linoleic acid	2.9	3.0	2.5	2.7	1.2	1.2	1.1	1.1
Crude fiber	3.5	3.5	3.7	3.6	3.8	3.4	3.5	3.4
Neutral detergent fiber	12.2	11.5	12.2	11.6	11.3	11.8	11.1	11.3
Acid detergent fiber	3.8	3.6	3.9	3.7	3.7	3.7	3.8	3.7
Calcium	3.98	4.39	4.24	4.11	4.06	4.27	4.20	4.14
Total phosphorus	0.66	0.67	0.63	0.67	0.68	0.62	0.69	0.66

[1] Soybean oil, soybean acid oil, palm oil or palm fatty acid distillate in different proportions (see Table 3).
[2] Premix provides per kilogram of feed: enzymatic complex (endo-1,4-beta-xylanase, 17,500 BXU/g; endo-1,4-beta-glucanase, 9,000 BXU/g; endo-1,3(4)-beta-glucanase, 1,175 BGU/g; 6-phytase, 300 PPU/g), 1,000 mg; choline chloride 75%, 500 mg; red synthetic pigment (Roxafil 30/10), 300 mg; butylated hydroxytoluene, 100 mg; vitamin A, 9,000 IU; vitamin D_3, 3,000 IU; vitamin E, 13 IU; vitamin B_1, 1 mg; vitamin B_2, 4 mg; vitamin B_6, 1.8 mg; vitamin B_{12}, 10 μg; vitamin K_3, 1.7 mg; folic acid, 0.3 mg; niacin, 20 mg; pantothenic acid, 8 mg; biotin, 52 mg; Fe (from $FeSO_4 \cdot 7H_2O$), 32 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 7 mg; Zn (from ZnO), 65 mg; Mn (from MnO), 85 mg; Se (from Na_2SeO_3), 0.35 mg; I (from $Ca(I_2O_3)_2$), 0.7 mg.

Table 4
Fatty acid profile and lipid class composition of the experimental diets.

Item	Soybean diet				Palm diet			
	S10	S20	S30	S45	P10	P20	P30	P45
Fatty acid composition, %								
Palmitic acid (C16:0)	12.2	12.6	12.5	12.8	37.7	39.3	38.6	40.6
Stearic acid (C18:0)	4.3	4.2	4.0	4.0	4.0	4.1	4.1	4.2
Oleic acid (C18:1 n-9)	22.2	23.8	26.1	29.1	34.3	34.1	33.4	33.0
Vaccenic acid (C18:1 n-7)	1.5	1.4	1.4	1.4	1.1	1.1	1.1	1.2
Linoleic acid (C18:2 n-6)	50.8	49.0	49.0	46.3	19.2	17.8	18.9	17.1
Linolenic acid (C18:3 n-3)	6.8	5.1	3.9	2.2	1.0	1.0	1.1	1.0
Minor fatty acids[1]	2.0	3.4	2.5	3.2	2.6	2.4	2.6	2.7
Trans fatty acids (C18:1)	0.2	0.5	0.6	0.9	0.1	0.1	0.1	0.1
SFA	17.9	18.5	18.3	19.0	43.6	45.4	44.8	46.8
MUFA	24.1	25.7	28.0	31.1	35.8	35.6	34.9	34.6
PUFA	57.8	55.3	53.1	49.0	20.5	18.9	20.2	18.5
UFA:SFA ratio	4.6	4.4	4.4	4.2	1.3	1.2	1.2	1.1
Lipid-class composition, %								
TAG	85.4	71.4	59.2	41.4	79.0	68.6	57.4	44.1
DAG	5.2	8.2	10.2	13.4	9.7	8.8	8.0	7.5
MAG	0.4	1.3	0.8	1.7	0.7	0.4	0.5	0.5
FFA	9.0	19.1	29.8	43.5	10.6	22.2	34.1	47.9

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.
[1] Minor fatty acids identified and quantified: C14:0, C15:0, C16:1 n-7, C16:1 n-9, C17:0, C18:3 n-6, C20:0, C20:1 n-9, C20:2 n-6, C20:3 n-3, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:0, C22:4 n-6, C22:5 n-3, C22:6 n-3, C24:0, C24:1.

opening the abdominal cavity, the gastrointestinal tract was carefully removed and the weight (expressed as a percentage of live BW) of the empty proventriculus plus gizzard, both ceca, and liver was recorded. The weight and length of the small intestine were also measured. For this purpose, the small intestine was divided into duodenum (from the gizzard to the insertion of the duodenal mesentery), jejunum (from the insertion of the duodenal mesentery to the junction with Meckel's diverticulum), and ileum (from the junction with Meckel's diverticulum to the ileocecal junction).

Immediately after euthanasia of the bird, a 3-cm segment of the distal end of the jejunum (anterior to Meckel's diverticulum) was collected, flushed with distilled water to remove intestinal contents, and fixed in 10% formalin solution for morphological assessment. Jejunal tissue samples were processed according to the method described by Asensio et al. (2020). Sections were photographed using a NanoZoomer-SQ slide scanner (Hamamatsu Photonics K.K., Shizuoka, Japan) and the digital samples obtained were viewed using image-analysis software (ViewPoint, Precipoint, Freising, Germany). The morphometric indices measured were a minimum of 30 villus heights ([VH] from the tip of villus to the villus–crypt junction) and a minimum of 30 crypt depths ([CD] the depth between 2 adjacent villi) per hen. Subsequently, the villus height-to-crypt depth ratio (VH:CD ratio) was calculated.

2.5. Chemical analysis

Analytical determination of the experimental diets was performed at least in duplicate according to the methods of the AOAC International (2005): DM (Method 934.01), ash (Method 942.05), CP (Method 954.01), and EE by Soxhlet extraction after 3 M HCl acid hydrolysis (Method 954.02). Crude, neutral and acid detergent fibers were determined using a fiber analyzer (A2000, ANKOM Technology, Macedon, NY, USA) following the procedures of Van Soest et al. (1991). The amino acid content was analyzed by chromatography (Hewlett–Packard 1100, Waldbronn, Germany). Levels of calcium, titanium, and total phosphorus were ascertained using ICP-OES (Optima 3200 RL, PerkinElmer, Waltham, USA), as described by Short et al. (1996). The gross energy (GE) was determined using an adiabatic calorimeter (Parr 6300 Calorimeter, Parr Instrument Company, Moline, IL, USA).

Regarding the lipid analysis, the FA profile of the feed was established by means of the direct transesterification procedure of Sukhija and Palmquist (1988) using nonadecanoic acid (C19:0, Sigma–Aldrich Chemical Co., St. Louis, MO, USA) as an internal standard. FA methyl esters were analyzed using a gas chromatograph (HP6890, Agilent Technologies, Waldbronn, Germany) following the method described by Cortinas et al. (2004) and, later, identified by matching their retention times with those of standards (Supelco 37 component FAME Mix, Sigma–Aldrich Co, St. Louis, USA). The results were expressed using internal normalization (area precentage). The lipid-class composition of the feed was determined after lipid extraction by size-exclusion chromatography (Agilent 1100 HPLC, Agilent Technologies, Santa Clara, USA) as described by Jimenez-Moya et al. (2021a). Lipid classes were identified by matching their retention times with those of standards (trioleoylglycerol for TAG, dioleoylglycerol for DAG, oleoylglycerol for MAG and oleic acid for FFA, Sigma–Aldrich Co., St. Louis, USA), and also quantified using internal normalization (area percentage).

Excreta samples were freeze-dried, ground to pass through a 1-mm sieve and homogenized prior to determining GE, EE, calcium, titanium, FA profile, and lipid-class composition following the same methods as those used for the experimental diets.

2.6. Calculations

The ATTD coefficient of a particular nutrient (NUTR) or FA was calculated using the concentration of the inert marker (TiO_2) in the diet and in the excreta (g/g DM) according to the following equation:

$$ATTD = [1 - (TiO_2 \text{ diet}/TiO_2 \text{ excreta}) \times (\text{NUTR or FA excreta}/\text{NUTR or FA diet})]$$

The AME was calculated using the following equation:

$$AME\ (kcal/kg) = GE \text{ diet} - (GE \text{ excreta} \times TiO_2 \text{ diet}/TiO_2 \text{ excreta})$$

The lipid-class content of the extracted fat from excreta was determined in order to evaluate the hydrolysis of TAG and the consequent intermediate (DAG) and end (MAG, and FFA) products of lipolysis, as well as the absorption of these products in the intestinal lumen. Calculations were carried out using the concentration of the lipid classes and the inert marker (TiO_2) in the excreta (mg/g DM) according to the equation described by Rodriguez-Sanchez et al. (2019a):

$$\text{Lipid-class content} = \text{Lipid-class excreta}/TiO_2 \text{ excreta}$$

2.7. Statistical analysis

The experiment was conducted using a completely randomized design with 8 treatments and 6 replicates, each containing 3 laying hens. The experimental unit was the replicate for all measurements. Before analysis, the normality of the data (Shapiro–Wilk test) and homogeneity of the variance (Levene test) were verified. Productive parameters, AME values, ATTD coefficients, lipid-class composition in excreta, and gastrointestinal morphology traits were subjected to a two-way ANOVA using the GLM procedure. The model included the dietary fat source (soybean or palm), and the FFA level (10%, 20%, 30%, or 45%) as the main factors, as well as their interaction. The effect of the experimental diet was also evaluated

by a one-way ANOVA when the fat source × FFA level interaction was significant. Differences among treatment means were tested using Tukey's test for multiple comparisons. In addition, orthogonal polynomial contrasts were used to determine the linear effect of increasing levels of FFA in hen diets when the effect of FFA content was significant. If there was no interaction (fat source × FFA content), the linear response to dietary FFA content was evaluated for both fat sources together; on the other hand, when a significant interaction was found, the linear contrast analysis was performed separately for soybean and palm diets.

The morphological measurements obtained from the jejunal tissue samples (VH and CD) were subjected to a linear mixed model analysis. Replicates (hens) were assumed to be the random effect, while the added fat source and FFA level were included as fixed effects.

Results in tables are reported as means, and differences were considered significant when $P < 0.05$. All data analysis was performed using SPSS statistics version 27.0 (IBM Corp, 2020).

3. Results

3.1. Characterization of the experimental fats and diets

The chemical analysis of the fats used in the present study is presented in Table 1. The highest MIU values were observed for the FFA-rich fat by-products, especially for SAO (4.95%), which had the highest values for each MIU component. In contrast, the crude oils presented greater primary lipid oxidation, based on the peroxide values. The highest level of secondary oxidation compounds, assessed by means of the *p*-anisidine value, was found in PFAD. Crude SO and SAO were rich in polyunsaturated FA (PUFA) and monounsaturated FA (MUFA), particularly in linoleic and oleic acid, and presented the same UFA:SFA ratio (4.6). Crude PO and PFAD showed lower UFA:SFA ratios (1.0 and 0.9, respectively), and were mainly composed of saturated FA (SFA; palmitic acid), and MUFA (oleic acid). As FFA-rich fat by-products, SAO and PFAD had the highest amount of FFA (53.8% and 87.2%, respectively).

The detailed analysis of the experimental diets is shown in Table 2. All the dietary treatments presented a similar macronutrient content, and the main differences concerned their lipid composition (Table 4). In general, the FA profile of the feeds reflected that of the added oils. Thus, 2 levels of UFA:SFA ratio (mean values = 4.4 and 1.2) were obtained. A reduction in the amount of linolenic acid was observed when SO was replaced with SAO (from 6.8% to 2.2%), corresponding to the lower linolenic acid content in SAO compared with crude SO (1.8% and 4.2%, respectively). With regard to the lipid-class composition, in both soybean and palm diets a progressive increase in the dietary FFA content was observed and, consequently, a decrease in the percentage of TAG as SAO and PFAD replaced their analogous crude oils. The average FFA levels achieved in the experimental diets were close to the theoretical ones previously formulated (Table 3).

3.2. Production performance

The mean values for the laying hens' production performance are shown in Table 5. The average BW differed significantly among the experimental groups at the end of the trial. Hens fed palm diets had higher live BW ($P < 0.05$) than those fed soybean diets (2,005 and 1,942 g, respectively). Furthermore, hens receiving palm diets also showed higher ADFI ($P < 0.001$). Concerning egg production, a significant interaction between the fat source and the FFA content was observed ($P < 0.01$). Hens fed soybean diets were negatively affected by increasing levels of FFA (linear, $P < 0.001$), whereas the hens that received palm diets were not affected. Significant fat source × FFA interaction was also found ($P < 0.001$) in egg weight: increasing the FFA content enhanced egg weight linearly, but only in hens that received soybean diets ($P < 0.001$). Thus, the highest egg sizes were recorded for hens fed S45 diets (61.15 g, $P < 0.001$). Egg mass and FCR were not significantly different among the

Table 5
Effects of fat source and dietary FFA content on hen productive performance from 19 to 33 weeks of age.

Item	Initial BW, g	Final BW, g	ADFI, g/hen	Egg production, egg/hen per day	Egg weight, g	Egg mass, g/day	FCR
Experimental diet							
S10	1,738	1,921	108.7	0.94^{a}	57.75^{bc}	54.36	1.83
S20	1,748	1,976	115.2	0.92^{a}	59.55^{ab}	55.13	1.91
S30	1,739	1,995	116.9	0.92^{a}	58.77^{bc}	54.10	1.98
S45	1,727	1,876	111.2	0.84^{b}	61.15^{a}	51.64	1.99
P10	1,746	1,997	115.4	0.93^{a}	58.39^{bc}	54.44	1.94
P20	1,732	1.948	113.7	0.90^{ab}	57.30^{c}	51.55	2.02
P30	1,767	2.072	118.4	0.94^{a}	57.88^{bc}	54.63	1.99
P45	1,759	2.002	117.5	0.92^{a}	57.88^{bc}	54.38	2.01
Fat source							
Soybean	1,738	1,942^{b}	113.0^{b}	0.90	59.31^{a}	53.81	1.93
Palm	1,751	2,005^{a}	116.3^{a}	0.92	57.86^{b}	53.50	1.99
FFA content							
10%	1,742	1,959	112.1	0.93^{a}	58.07^{b}	54.40	1.88
20%	1,740	1,962	114.5	0.91^{ab}	58.42^{ab}	53.34	1.97
30%	1,753	2,033	117.6	0.93^{a}	58.32^{ab}	54.36	1.98
45%	1,743	1,939	114.4	0.88^{b}	59.52^{a}	52.51	2.00
SEM[1]	28.2	34.7	1.06	0.01	0.45	0.99	0.05
Effects, *P*-values							
Fat source	0.439	0.011	<0.001	0.146	<0.001	0.703	0.111
FFA content	0.106	0.081	0.062	0.003	0.027	0.286	0.095
Fat source × FFA	0.312	0.160	0.084	0.009	<0.001	0.110	0.622
Linear contrast[2], *P*-values							
Overall	-	-	-	-	-	-	-
Soybean	-	-	-	<0.001	<0.001	-	-
Palm	-	-	-	0.872	0.677	-	-

FFA = free fatty acids; S = soybean; P = palm; BW = body weight; ADFI = average daily feed intake; FCR = cumulative feed conversion ratio.
[a-c] Means within each variable with more than 2 levels (experimental diet or FFA content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).
[1] Pooled standard error of the means.
[2] Linear responses to dietary FFA content.

grouped treatments. Only one hen died over the course of the experiment (P45; 31 weeks of age; 1,765 g post-mortem BW).

3.3. AME and nutrient digestibility

The AME values and the ATTD coefficients of EE and calcium for the different dietary treatments are presented in Table 6. Significant interactions between the dietary fat source and the FFA content were reported for the AME values and ATTD of EE ($P < 0.001$). Regarding the AME values, soybean groups were negatively affected by increasing levels of FFA (linear, $P < 0.001$). In contrast, a significant effect was found for FFA content in palm diets ($P < 0.001$), but this did not follow a linear trend. With reference to the ATTD of EE, those hens fed soybean diets presented greater coefficients than those fed palm diets ($P < 0.001$), and, in this case, both were negatively affected by increasing FFA levels (linear, $P < 0.001$). As a result, hens fed S10 and S20 diets showed the highest AME and ATTD of EE values, while hens fed S30, S45, P10, P20, P30, and P45 reported lower values and were generally similar to each other.

With regard to the ATTD of calcium, no interaction between fat source and FFA content was observed in this respect. The highest values were recorded in soybean diets ($P < 0.001$). Furthermore, increasing the FFA content also reduced calcium digestibility coefficients in both fat sources, showing a significant linear effect ($P < 0.01$).

3.4. Fatty acid digestibility and lipid class content in excreta

The effects of the fat source and dietary FFA content on the ATTD coefficients of total and particular FA are reported in Table 7. In general, SFA presented lower ATTD coefficients than the other FA groups, and the highest were reported for PUFA, reaching coefficients above 0.9. The supplemental fat source had a significant effect on all the FA evaluated ($P < 0.01$). Hens fed soybean diets showed the highest ATTD coefficients for SFA, PUFA and total FA (TFA) ($P < 0.01$). Conversely, the ATTD coefficients of MUFA were significantly higher in hens fed palm diets ($P < 0.001$). Regarding individual FA, higher ATTD values for palmitic (C16:0), stearic (C18:0), linoleic (C18:2 n-6), and linolenic (C18:3 n-3) acids were reported in soybean diets, while palm diets had higher ATTD coefficients for oleic (C18:1 n-9) acid ($P < 0.01$). No differences were observed regarding dietary FFA content, except for stearic acid, which was negatively affected by increasing FFA levels (linear, $P < 0.05$) in both soybean and palm diets.

The lipid-class composition (TAG, DAG, MAG, and FFA) of the excreta was also studied for the 8 dietary treatments. As can be seen in Table 8, significant interactions between the dietary fat source and the FFA percentage were reported in the amount of TAG and MAG ($P < 0.01$). While increasing the dietary FFA level increased the content of TAG and MAG in the excreta of hens fed soybean diets (linear, $P < 0.001$), no significant differences were observed among palm diets. However, the increase in the amount of FFA in the excreta accompanied an increase in the dietary FFA content in both soybean and palm treatments (linear, $P < 0.01$). The most predominant lipid-class in the excreta of both fat sources was FFA, although this was significantly higher in palm diets than in soybean diets ($P < 0.001$). In contrast, lower TAG, DAG, and MAG values were found in hens fed palm diets ($P < 0.01$), although this difference was mainly due to the high amounts reported in S30 and S45 diets.

3.5. Gastrointestinal traits and jejunal morphology

As shown in Table 9, the relative weight of the proventriculus and gizzard, duodenum, jejunum, total small intestine, and cecum did not differ significantly between the experimental treatments. However, the interaction between fat source and FFA content was significant ($P < 0.05$) for the relative weight of the ileum: hens fed an S10 diet presented a higher relative weight in this case than those fed an S45 diet, but no differences were observed among the other groups. In addition, the relative weight of the liver was found to be greater in soybean rather than palm diets ($P < 0.05$) (22.7 and 21.2 g/kg BW, respectively). In this regard, there was no significant fat source × FFA level interaction, although this parameter tended to be higher ($P = 0.059$) with increasing dietary FFA content in hens that received soybean diets. With regard to the length of the small intestine (Table 10), no influence from the dietary treatments was found, except for the duodenum, which was longer in hens fed soybean diets ($P < 0.05$).

Finally, the effects of the added fat source and FFA content on the morphology of the distal jejunum are presented in Table 11. Birds fed soybean diets had greater VH, and consequently a higher VH:CD ratio, than those on palm diets ($P < 0.05$). Furthermore, jejunal morphology was influenced by dietary levels of FFA: the CD increased (linear, $P < 0.05$) and the VH:CD ratio decreased (linear, $P < 0.05$) as the FFA content increased in both fat sources.

Table 6
Apparent metabolizable energy (AME) and apparent total tract digestibility (ATTD) coefficients according to different fat sources and dietary FFA content.

Item	AME, kcal/kg DM of diet	ATTD of EE	ATTD of Ca
Experimental diet			
S10	2,715[a]	0.86[a]	0.58
S20	2,675[ab]	0.82[a]	0.56
S30	2,474[d]	0.70[bc]	0.48
S45	2,490[d]	0.73[b]	0.48
P10	2,535[cd]	0.72[b]	0.42
P20	2,636[abc]	0.73[b]	0.51
P30	2,563[bcd]	0.71[bc]	0.46
P45	2,517[d]	0.64[c]	0.36
Fat source			
Soybean	2,588	0.78[a]	0.52[a]
Palm	2,564	0.70[b]	0.44[b]
FFA content			
10%	2,625[a]	0.79[a]	0.50[a]
20%	2,657[a]	0.77[a]	0.53[a]
30%	2,519[b]	0.71[b]	0.47[ab]
45%	2,504[b]	0.68[b]	0.42[b]
SEM[1]	25.2	0.02	0.03
Effects, *P*-values			
Fat source	0.176	<0.001	<0.001
FFA content	<0.001	<0.001	0.002
Fat source × FFA	<0.001	<0.001	0.055
Linear contrast[2], *P*-values			
Overall	-	-	0.002
Soybean	<0.001	<0.001	-
Palm	0.217	0.002	-

S = soybean; P = palm; FFA = free fatty acids; EE = ether extract.
[a-d] Means within each variable with more than 2 levels (experimental diet or FFA content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).
[1] Pooled standard error of the means.
[2] Linear responses to dietary FFA content.

4. Discussion

It is well known that the UFA:SFA ratio and dietary FFA content have an important effect on fat utilization (Ravindran et al., 2016), and this must be carefully assessed when considering adding unconventional fat sources into laying hen feed. For this purpose, 2 crude oils (crude SO and crude PO) and 2 FFA-rich fat by-products (SAO and PFAD) were evaluated in this study.

In relation to the productive parameters, diet did not affect egg mass and FCR. Perez-Bonilla et al. (2011) also found similar egg

Table 7
Apparent total tract fatty acid digestibility (ATTD) coefficients according to different fat sources and dietary FFA content.

Item	C16:0	C18:0	C18:1 n-9	C18:2 n-6	C18:3 n-3	SFA	MUFA	PUFA	TFA
Experimental diet									
S10	0.81	0.86	0.88	0.91	0.94	0.82	0.89	0.91	0.88
S20	0.81	0.83	0.90	0.93	0.96	0.81	0.90	0.93	0.90
S30	0.78	0.80	0.87	0.90	0.92	0.77	0.87	0.90	0.87
S45	0.79	0.78	0.90	0.95	0.94	0.78	0.90	0.95	0.91
P10	0.73	0.65	0.91	0.91	0.91	0.73	0.91	0.91	0.83
P20	0.73	0.66	0.91	0.90	0.90	0.72	0.91	0.90	0.84
P30	0.70	0.63	0.90	0.88	0.89	0.70	0.90	0.88	0.81
P45	0.69	0.64	0.90	0.89	0.90	0.69	0.90	0.89	0.81
Fat source									
Soybean	0.80^{b}	0.82^{a}	0.89^{b}	0.92^{a}	0.94^{a}	0.79^{a}	0.89^{b}	0.92^{a}	0.89^{a}
Palm	0.71^{a}	0.64^{b}	0.91^{a}	0.89^{b}	0.90^{b}	0.71^{b}	0.91^{a}	0.90^{b}	0.82^{b}
FFA content									
10%	0.77	0.76^{a}	0.90	0.91	0.92	0.77	0.90	0.91	0.86
20%	0.77	0.74^{ab}	0.91	0.92	0.93	0.77	0.90	0.92	0.87
30%	0.74	0.72^{b}	0.89	0.89	0.90	0.73	0.89	0.89	0.84
45%	0.74	0.71^{c}	0.90	0.92	0.92	0.73	0.90	0.92	0.86
SEM[1]	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Effects, *P*-values									
Fat source	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.005	<0.001
FFA content	0.064	0.033	0.059	0.109	0.230	0.057	0.061	0.163	0.091
Fat source × FFA	0.883	0.334	0.068	0.087	0.637	0.887	0.070	0.092	0.167
Linear contrast[2], *P*-values									
Overall	-	0.004	-	-	-	-	-	-	-

S = soybean; P = palm; TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; FFA = free fatty acids.
[a,b] Means within each variable with more than 2 levels (experimental diet or FFA content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).
[1] Pooled standard error of the means.
[2] Linear responses to dietary FFA content.

Table 8
Effects of fat source and dietary FFA content on the lipid-class composition[1] of the excreta.

Item	TAG	DAG	MAG	FFA
Experimental diet				
S10	0.16^{b}	0.28	0.03^{c}	2.30
S20	0.17^{b}	0.28	0.06^{bc}	2.24
S30	0.22^{a}	0.45	0.12^{a}	3.08
S45	0.24^{a}	0.28	0.14^{a}	2.53
P10	0.17^{b}	0.17	0.07^{b}	3.45
P20	0.16^{b}	0.19	0.07^{b}	3.76
P30	0.16^{b}	0.19	0.05^{bc}	3.83
P45	0.16^{b}	0.21	0.06^{bc}	4.65
Fat source				
Soybean	0.20^{a}	0.33^{a}	0.09^{a}	2.54^{b}
Palm	0.16^{b}	0.19^{b}	0.06^{b}	3.92^{a}
FFA content				
10%	0.16	0.22	0.05^{b}	2.88^{b}
20%	0.16	0.24	0.06^{b}	3.00^{ab}
30%	0.19	0.32	0.09^{a}	3.45^{ab}
45%	0.20	0.25	0.10^{a}	3.59^{a}
SEM[2]	0.01	0.04	0.01	0.24
Effects, *P*-values				
Fat source	0.004	<0.001	<0.001	<0.001
FFA content	0.067	0.071	<0.001	0.019
Fat source × FFA	0.012	0.069	<0.001	0.059
Linear contrast[3], *P*-values				
Overall	-	-	-	0.002
Soybean	<0.001	-	<0.001	-
Palm	0.689	-	0.464	-

S = soybean; P = palm; TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids.
[a–c] Means within each variable with more than 2 levels (experimental diet or FFA content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).
[1] Lipid-class (mg/g)/TiO_2 (mg/g).
[2] Pooled standard error of the means.
[3] Linear responses to dietary FFA content.

mass and FCR in hens fed different combinations of acidulated vegetable soapstocks (composed primarily of by-products of the PO and SO refinery industry) and SO. However, in this trial, differences in egg production were observed: the worst values were recorded for hens fed S45 (FFA = 45%), although no differences were found for hens fed soybean diets with lower FFA content. These results differ from those of Irandoust et al. (2012), who reported no differences in the productive performance between hens fed 35 g/kg of SAO and those fed the same amount of SO. This could be attributed to differences in the amount of added fat compared to this study (60 g/kg). In addition, Roll et al. (2018b) evaluated the replacement of SO with increasing levels of SAO in the diets of coturnix quails and did not find differences in the birds' productive performance.

Regarding ADFI, the highest values were recorded for hens that received palm diets, as was the case in Vilarrasa et al. (2015) with broiler chickens. In addition, palm diets presented higher final BW. This finding is consistent with Perez-Bonilla et al. (2011), who recorded higher BW gain for hens fed lard (low UFA:SFA ratio) than for hens fed SO or acidulated vegetable soapstocks (251, 221, and 210 g, respectively), although no differences were found regarding feed intake. In contrast, Rodriguez-Sanchez et al. (2021) compared soybean and palm diets with different FFA content and reported higher BW in grower-finisher broilers (37 days) fed soybean diets, and Jimenez-Moya et al. (2021a, 2021b) found no differences in broilers fed diets with different UFA:SFA ratios and FFA content. Differences in feed intake could be attributed to differences in the AME values, although it is also noteworthy that an earlier palatability study found that hens preferred SFA-rich oils over UFA-rich oils even when they were also rich in FFA (Palomar et al., 2020).

In this trial, the replacement of SO with SAO resulted in lower egg production but with a significant linear increase in egg weight, especially when SO was completely replaced with its by-product (S45; FFA = 45%). This result contrasts with those of Pardío et al. (2005), Perez-Bonilla et al. (2011), and Irandoust et al. (2012), who did not observe differences in egg weight laid by hens fed SO or hens fed SAO. Furthermore, Papadopoulos et al. (2019) also found no differences regarding the added fat source when comparing different combinations of SO and PO in laying hens. It is widely thought that egg size largely depends on the amount and

Table 9
Effects of fat source and dietary FFA content on the relative weight of the gastrointestinal tract at 33 weeks of age.

Item	BW[1], g	Relative weight, g/kg BW						
		Proventriculus + Gizzard	Duodenum	Jejunum	Ileum	Small Intestine	Cecum	Liver
Experimental diet								
S10	1,840	17.1	8.4	12.0	14.4[a]	34.7	7.1	21.5
S20	1,953	15.5	8.2	13.0	13.7[ab]	34.8	4.9	21.8
S30	1,880	16.8	8.3	11.7	12.4[ab]	32.3	6.8	22.8
S45	1,916	17.3	8.8	11.6	11.4[b]	31.9	7.3	24.7
P10	1,943	16.8	8.3	12.3	13.4[ab]	34.0	7.0	22.8
P20	1,920	16.4	7.9	10.7	12.8[ab]	31.4	6.6	19.3
P30	1,943	17.6	8.3	12.0	12.6[ab]	32.8	7.8	21.3
P45	1,928	15.4	7.9	11.8	14.0[ab]	33.7	7.0	21.2
Fat source								
Soybean	1,911	16.7	8.4	12.1	13.0	33.4	6.5	22.7[a]
Palm	1,933	16.6	8.1	11.7	13.2	33.0	7.1	21.2[b]
FFA content								
10%	1,918	16.9	8.4	12.1	13.9	34.4	7.1	22.2
20%	1,936	16.0	8.1	11.8	13.2	33.1	5.8	20.5
30%	1,912	17.2	8.3	11.8	12.5	32.6	7.2	22.0
45%	1,922	16.4	8.3	11.7	12.7	32.8	7.1	23.0
SEM[2]	20.79	0.53	0.22	0.47	0.52	1.03	0.82	0.77
Effects, *P*-values								
Fat source	0.192	0.799	0.096	0.345	0.558	0.615	0.403	0.020
FFA content	0.773	0.178	0.685	0.883	0.119	0.465	0.359	0.067
Fat source × FFA	0.213	0.100	0.300	0.057	0.020	0.184	0.511	0.059

S = soybean; P = palm; BW = body weight; FFA = free fatty acids.
[a,b] Means within each variable with more than 2 levels (experimental diet or FFA content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).
[1] Average BW of the hens euthanized and used in sampling for relative organ weight calculations.
[2] Pooled standard error of the means.

Table 10
Effects of fat source and dietary FFA content on the length of the small intestine at 33 weeks of age.

Item	Length, cm			
	Duodenum	Jejunum	Ileum	Small Intestine
Experimental diet				
S10	27.3	54.4	51.3	133.1
S20	27.3	55.1	48.7	131.1
S30	26.5	51.2	49.8	127.5
S45	27.7	52.8	46.8	127.2
P10	27.5	54.6	51.2	133.3
P20	25.3	49.0	48.7	123.0
P30	25.3	52.3	50.0	127.6
P45	26.0	53.8	50.3	130.0
Fat source				
Soybean	27.2[a]	53.4	49.1	129.7
Palm	26.0[b]	52.4	50.0	128.4
FFA content				
10%	27.4	54.5	51.3	133.2
20%	26.3	52.0	48.7	127.0
30%	25.9	51.8	49.9	127.5
45%	26.8	53.3	48.5	128.6
SEM[1]	0.69	1.57	1.74	3.31
Effects, *P*-values				
Fat source	0.044	0.468	0.553	0.653
FFA content	0.275	0.425	0.521	0.397
Fat source × FFA	0.561	0.161	0.782	0.541

S = soybean; P = palm; FFA = free fatty acids.
[1] Pooled standard error of the means.

Table 11
Effects of fat source and dietary FFA content on the histomorphology of the distal jejunum mucosa.

Item	Villus height, μm	Crypt depth, μm	VH:CD ratio[1]
Experimental diet			
S10	841.3	111.3	7.69
S20	867.5	125.6	7.11
S30	884.0	128.5	6.79
S45	888.7	142.1	6.40
P10	826.5	123.5	6.73
P20	848.6	135.9	6.44
P30	855.0	144.1	6.20
P45	827.3	137.7	6.03
Fat source			
Soybean	870.9[a]	126.1	7.00[a]
Palm	840.7[b]	135.9	6.35[b]
FFA content			
10%	833.4	117.7[b]	7.21[a]
20%	858.2	131.3[ab]	6.77[ab]
30%	868.1	136.3[a]	6.50[ab]
45%	861.1	139.5[a]	6.21[b]
SEM[2]	18.32	7.22	0.33
Effects, *P*-values			
Fat source	0.032	0.108	0.008
FFA content	0.459	0.030	0.027
Fat source × FFA	0.801	0.804	0.843
Linear contrast[3], *P*-values			
Overall	-	<0.001	0.003

S = soybean; P = palm; FFA = free fatty acids.
[a-b] Means within each variable with more than 2 levels (experimental diet or FFA content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).
[1] Villus height-to-crypt depth ratio.
[2] Pooled standard error of the means.
[3] Linear responses to dietary FFA content.

type of added fat, particularly the linoleic acid content (Grobas et al., 1999a, 1999b, 2001; Safaa et al., 2008). In the present study, however, all the oil blends contributed 6% to the basal diet and the linoleic acid content of soybean diets (mean value = 2.8%) was above the hens' requirements for optimal egg size (Lohmann, 2019). It has been suggested that differences in egg weight could be related to some component present in the MIU of SAO (Palomar et al., unpublished data), since AO has higher MIU values than FAD and crude oils (Ravindran et al., 2016; Varona et al., 2021a). However, the mechanism involved in egg size remains unclear and further research is needed in this regard.

With respect to the AME and ATTD coefficients, in the current study, increasing the dietary FFA content decreased the AME content of the diets and the digestibility of EE and calcium. However,

the AME values decreased only as SO was replaced with SAO, whereas palm diets were not negatively affected by increasing FFA levels. This finding is consistent with Rodriguez-Sanchez et al. (2021), who carried out a trial in broiler chickens (37 days) with an experimental design similar to that of this study and also observed a significant interaction between the UFA:SFA ratio and dietary FFA content in the AME values. These researchers also reported higher AME values for soybean diets with low FFA content (up to 35%) than for soybean diets with higher FFA content and palm diets, which had similar values to each other. Irandoust et al. (2012) determined the ATTD of EE in hens from 44 to 56 weeks of age fed diets with 30 g/kg of SO (0.2% FFA) or 30 g/kg of SAO (67.4% FFA) and also found higher coefficients for SO than for SAO (0.85 and 0.80, respectively) and reported the same for the AME values. These results are consistent with Sharifi et al. (2012), who also found higher AME and ATTD of EE in broilers fed SO (30 g/kg; 0.05% FFA) than in those fed SAO (30 g/kg; 51.85% FFA) (0.88 and 0.82, respectively). In contrast, Shahryari et al. (2021) included SAO (50.32% FFA) at different proportions (10, 20, and 30 g/kg) into broiler chicken diets (28 days of age) and did not find differences in the digestibility of EE and calcium among the dietary treatments. In the current study, furthermore, higher ATTD coefficients of EE and calcium were observed in soybean diets than in palm diets. These results contrast with those of Papadopoulos et al. (2019), who tested blends of crude SO and PO with different UFA:SFA ratios (from 2.39 to 3.33) in laying hens from 22 to 32 weeks old and did not find any difference between treatments in fat digestibility. However, in this trial palm diets presented lower UFA:SFA ratios (mean value = 1.2), which could be responsible for the disagreement with their results.

Regarding the ATTD coefficients of FA, they were mainly influenced by the added fat source rather than by the dietary FFA content, which only affected the digestibility of stearic acid. In this study, soybean diets, with high UFA:SFA ratios, reported higher ATTD of TFA, SFA and PUFA, while palm diets, with low UFA:SFA ratios, had higher MUFA digestibility. Similar results were reported in broiler chickens by Vilarrasa et al. (2015). However, the influence of the dietary FFA content was found to be higher in broiler chickens than in laying hens. Rodriguez-Sanchez et al. (2021) also found higher ATTD of TFA, SFA, and PUFA in grower-finisher broilers fed soybean diets than in those fed palm diets, but the ATTD coefficients of TFA and SFA were negatively affected by the dietary FFA content in both fat sources, and the ATTD of MUFA and PUFA decreased with the replacement of SO with SAO. In addition, the negative effect of dietary FFA content was also found to be more pronounced when the birds were younger (Rodriguez-Sanchez et al., 2019a). These results were not unexpected, as age has been widely reported to be an important factor for both lipolysis and absorption of fats and oils (Renner and Hill, 1961; Krogdahl, 1985; Wiseman and Salvador, 1991; Tancharoenrat et al., 2013; Rodriguez-Sanchez et al., 2019b; Jimenez-Moya et al., 2021a, 2021b) and the hens in this study (33 weeks of age) had a fully developed tract system. In fact, Jimenez-Moya et al. (2021a, 2021b) evaluated the incorporation of FFA-rich fat by-products in broiler chickens at 11 and 35 days and reported that as age increased, absorption of SFA and FFA improved.

The composition of the lipid-class in the excreta also helps to assess the dynamics of the utilization of added fats, since TAG must be hydrolyzed into DAG, MAG, and FFA to be absorbed by enterocytes in the small intestine (Ravindran et al., 2016). The lower amount of FFA in the excreta of hens fed soybean diets, as well as the ATTD coefficients, demonstrated that the absorption process was more efficient in oils with a high UFA:SFA ratio. As with calcium, EE, and TFA, FFA was also not as efficiently absorbed in the birds fed palm diets. All these findings are consistent with those of similar studies in broiler chickens (Wiseman and Salvador, 1991; Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019a, 2019b, 2021; Jimenez-Moya et al., 2021a, 2021b). On the other hand, and as with the AME values, significant interactions in the amounts of TAG and MAG suggested that the effect of the dietary FFA could vary depending on the added fat source (soybean or palm). While differences were observed between hens fed soybean diets (S30 and S45 presented higher amounts of TAG and MAG than S10 and S20), the results were similar across all palm diets and to those reported for S10 and S20, which is consistent with Rodriguez-Sanchez et al. (2021). Thus, the poorer absorption of SAO could explain why S30 and S45 diets presented lower AME values than S10 and S20 diets and, at the same time, equivalent to palm diets, which are richer in SFA.

With regard to the gastrointestinal morphology, neither the added fat source nor the dietary FFA content had any effect on the relative weight of the proventriculus and gizzard, duodenum, jejunum, total small intestine and cecum, or on the length of the jejunum, ileum, and small intestine. These results are consistent with Sharifi et al. (2012), who found no differences in the organ weight of broilers fed SO or SAO (30 g/kg). In contrast, the relative weight of the liver was higher in soybean diets and tended to increase with increasing dietary proportions of SAO. This finding is consistent with the observations in Shahryari et al. (2021) concerning broiler chickens fed SAO at different proportions (10, 20, and 30 g/kg). The relative weight of the ileum was also affected by the FFA content only in soybean diets. In this case, it decreased linearly, with the relative weight of the S10 group being greater than that of the S45 group. In addition, a longer duodenum was found for soybean diets. Nevertheless, it has been reported that regardless of the age of the bird and the source of added fat, including crude oils and FFA-rich fat by-products, most FA absorption occurs in the jejunum (Tancharoenrat et al., 2014; Rodriguez-Sanchez et al., 2019a, 2021; Jimenez-Moya et al., 2021a, 2021b) and no differences were observed in terms of weight and length in this section of the intestine.

In the current study, however, histomorphological changes in the jejunum were found. The VH, CD, and the VH:CD ratio of the absorptive epithelium are thought to reflect intestinal functional status and nutrient assimilation capacity (Wang and Peng, 2008). Regarding the added fat source, soybean diets showed the highest VH, which is positively associated with nutrient absorption since taller villi increase the surface area available in the jejunum (Caspari, 1992; Tarachai and Yamauchi, 2000). The same was found for the VH:CD ratio, indicating that hens fed soybean diets had a slower turnover rate of the jejunal mucosa than those fed palm diets (Imondi and Bird, 1966). Dietary FFA content also negatively affected the jejunal epithelium regardless of the added fat source: increases in CD and decreases in the VH:CD ratio accompanied higher amounts of FFA. The lower portion of the intestinal crypt is the region where stem cells divide for the renewal of the villus (Imondi and Bird, 1966); thus, these results showed that hens fed diets with high FFA content had higher demand for tissue synthesis and required greater cell renewal to maintain the villus structure (Yamauchi, 2007). These indicators of tissue turnover observed in hens fed these FFA-rich fat by-products may be a contributory factor for the worse ATTD coefficients of EE and calcium compared to hens fed crude oils. In contrast, Shahryari et al. (2021) reported that VH and VH:CD ratio increased across the small intestine as broiler chickens were fed increasing levels of SAO. However, these results are not fully comparable to the present ones as this study employed a fat-free control diet.

Most of the differences regarding the dietary FFA content in this study were observed for levels above 20%, especially in diets with 45% of FFA. This is consistent with previous studies mainly carried out in broiler chickens (Shannon, 1971; Sklan, 1979; Wiseman and

Salvador, 1991; Blanch et al., 1996; Vilà and Esteve-Garcia, 1996; Zumbado et al., 1999; Irandoust et al., 2012), which attributed these findings to the lower concentrations of MAG available in the small intestine. As the dietary FFA content increases, MAG becomes essential to incorporate insoluble FA into mixed micelles (Sklan, 1979). However, in the present trial, the effect of increasing the FFA content was conditioned by the UFA:SFA ratio of the blend. While in soybean diets egg production, egg weight, the AME values, and the content of TAG and MAG in excreta appeared to be affected by the dietary FFA percentage in a level-dependent manner, in palm diets these values remained unaffected, with S30 and S45 diets presenting similar results to palm diets in most of the parameters assessed. In this regard, these differences are consistent with the MIU values observed in the 2 assayed fat by-products (SAO had the highest moisture, insoluble impurities, and unsaponifiable matter), which may have influenced the results. Actually, Vilà and Esteve-Garcia (1996) considered the non-eluted material value of a fat – which includes MIU, total oxidized and polymerized FA, and glycerol (Ravindran et al., 2016) – to be a better predictor of the AME value than the dietary FFA content. Nevertheless, in this study, even if the MIU values of SAO were the highest, they still fell within FEDNA's recommended range (2002) (< 5 g/100 g).

Furthermore, dietary calcium may also have an influence on the results observed in the present trial. Atteh and Leeson (1985) reported that FFA in poultry diets might increase the formation of calcium soaps, as might high levels of dietary calcium and/or added fat – criteria that were met in our study. In addition, the magnitude of calcium soap formation has been reported to be more pronounced in SFA than in UFA (Atteh and Leeson, 1984). These soaps may reduce the availability of FA and calcium for absorption, which could explain, at least in part, the lower ATTD coefficients of EE and calcium observed in the S30, S45 and palm diets (P10, P20, P30, and P45). Therefore, potential interactions between FFA-rich fat by-products and calcium must be taken into consideration, especially in older laying hens, as the long bones that support eggshell formation may be deprived of their calcium stores at the late egg-laying period and, consequently, may increase the incidence of osteoporosis and skeletal problems (Whitehead, 2004).

In view of the above, it appears that the energy value of fats in laying hen diets is influenced by both the degree of fat saturation and the dietary FFA content. Nevertheless, as has been reported in previous studies with broiler chickens (Wiseman and Salvador, 1991; Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019a, 2021; Jimenez-Moya et al., 2021a, 2021b), the UFA:SFA ratio seems to be a major factor in fat utilization, to a greater extent than dietary FFA. Regardless of age, the greater the UFA:SFA ratio, the greater the ability of FA to be absorbed. This could be related to the fact that UFA has a higher capacity to form dietary mixed micelles in the small intestine compared to SFA, as reported by Freeman (1969) and Krogdahl (1985). Thus, oils rich in UFA have better emulsifying properties and greater bioaccessibility for lipid absorption than fats with a lower UFA:SFA ratio (Jimenez-Moya et al., 2021c). Furthermore, there is evidence suggesting that SFA-rich oils improve fat digestion with the addition of UFA-rich oils due to a synergistic effect in the blend (Lall and Slinger, 1973; Ketels and De Groote, 1989; Blanch et al., 1996; Zumbado et al., 1999; Jimenez-Moya et al., 2021b). In this study, even though dietary FFA content was, in general, associated with lower AME and lower digestibility values, the ATTD coefficients reported remained generally high, probably due to the age of the birds. In fact, the ATTD coefficients of FA of laying hens were higher (TFA of P45 = 0.81) than those reported in grower-finisher broilers (TFA of P50 = 0.70) and were even more different from those reported in starter chickens (TFA of P50 = 0.67) by Rodriguez-Sanchez et al. (2019a, 2021) with similar diets.

5. Conclusion

The present study shows that although dietary FFA content affects productive performance, AME, lipid and calcium absorption, and gastrointestinal functionality of laying hens, its effect is not as great as that of the added fat source (UFA:SFA ratio). The better utilization of energy sources with a high UFA:SFA ratio has been confirmed, regardless of the FFA content in the diet. In addition, it was also observed that the degree of fat saturation determines how fat sources rich in FFA are used since oils rich in UFA were more affected by the dietary FFA content than oils rich in SFA, especially when they exceed 30% of FFA. However, as laying hens are birds with a mature and fully developed digestive tract, energy utilization remained acceptable, despite the negative influence of FFA on some of the parameters evaluated. Thus, AO and FAD, fat by-products rich in FFA, could be considered to be suitable energy sources for laying hens as long as their quality is previously assured, and an adequate supply of dietary calcium is guaranteed. These results increase knowledge about the potential incorporation of by-products into poultry feed, supporting a more sustainable and environmentally friendly production model.

Author contributions

Maria Palomar: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Carlos Garcés-Narro**: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Supervision, Project administration, Writing – review & editing. **Olga Piquer**: Investigation, Writing – review & editing. **Roser Sala**: Investigation, Writing – review & editing. **Alba Tres**: Investigation, Resources, Writing – review & editing. **José-Antonio García-Bautista**: Investigation, Resources, Writing – review & editing. **María-Dolores Soler**: Conceptualization, Methodology, Investigation, Validation, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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