PROCESSING AND PRODUCTS

Influence of free fatty acid content and degree of fat saturation in laying hen diets on egg quality, yolk fatty acid profile, and cholesterol content

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ABSTRACT The aim of the present study was to evaluate the effect of dietary free fatty acid (**FFA**) content and the degree of saturation on egg quality, yolk fatty acid (**FA**) profile, and yolk cholesterol content. For a 15-wk period, a total of 144 laying hens (19-wk-old) were randomly assigned to 8 treatments arranged in a 2 × 4 factorial design, with 2 sources of crude oil (soybean oil and palm oil) and 4 levels of FFA (10, 20, 30, and 45%). The dietary treatments were achieved by progressively substituting the original oils with equivalent amounts of their corresponding acid oils (soybean acid oil and palm fatty acid distillate, respectively). No differences in ADFI or egg mass were found. However, dietary FFA reduced egg production (linear, $P < 0.05$) and increased the feed conversion ratio (linear, $P < 0.05$). Higher levels of FFA in soybean diets resulted in higher egg weight with higher albumen and yolk weights (linear, $P < 0.01$). Palm diets presented higher yolk:albumen ratio than soybean diets ($P < 0.001$), but the effect of FFA did not follow a linear trend. Hens fed soybean diets laid eggs with higher Haugh units (**HU**) than palm diets ($P < 0.001$), although increasing the dietary FFA% reduced the HU values in both (linear, $P < 0.001$). Palm diets enhanced shell quality with greater resistance to breakage, and higher dry matter and ash content than soybean diets ($P < 0.05$). No differences in egg chemical composition and yolk cholesterol content were found ($P > 0.05$). The saturation degree had a significant effect on all the analyzed yolk FA ($P < 0.001$) except for arachidonic acid (C20:4 n-6), whereas increasing the FFA content did not affect to a great extent. These results show that varying dietary FFA level did not affect egg quality and yolk composition as much as the dietary fat source did, supporting the use of acid oils and fatty acid distillates as fat ingredients for feed.

Key words: acid oil, egg weight, fat by-product, fatty acid distillate, yolk fatty acid profile

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INTRODUCTION

Fats and oils have the highest caloric values among all the ingredients used in poultry diets. Adding supplemental dietary fat also confers other benefits, including providing essential fatty acids (**FA**), such as linoleic acid (**LA**), and fat-soluble vitamins, while also improving palatability, and allowing better nutrient digestion and absorption (Mateos and Sell, 1981; Grobas et al., 1999b; Bouvarel et al., 2010; Ravindran et al., 2016; Palomar et al., 2020).

A diverse variety of fat sources are available to provide lipids in laying hen feed. Certain types of fat by-products, such as acid oils (**AO**) and FA distillates (**FAD**), are an example of unconventional feed resources that are of growing interest in animal feeding due to their market availability, competitive price, and potential environmental sustainability (Varona et al., 2021a, c). These fat by-products come from the chemical (AO) or the physical (FAD) refining processes of edible oils and fats (European Commission, 2013). Both by-products are characterized by having a high proportion of free fatty acids (**FFA**; 31.7−93.6%) and a FA profile consistent with that of the corresponding crude oil (Varona et al., 2021a). However, much variability in their composition has been reported, including energy-diluting compounds such as moisture, impurities, and unsaponifiable material (collectively referred to as **MIU**), which can affect their energy value (Nuchi et al.,

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2009; Roll et al., 2018b; Varona et al., 2021b). Limited knowledge of the composition and use of these fat by-products in laying hen feed restricts their routine use by many commercial producers.

It is well known that the FA composition of the yolk is strongly dependent on dietary lipid sources (Cruickshank, 1934; Baucells et al., 2000; Meluzzi et al., 2000; Bou et al., 2009; Oliveira et al., 2010). The amount and type of supplemental fat, as well as the LA content of the diet, have been shown to affect egg weight (Shutze and Jensen, 1963; Scragg et al., 1987; Whitehead, 1995; Grobas et al., 1999b; Safaa et al., 2008). In addition, dietary lipids may influence other physicochemical characteristics of eggs, such as the yolk:albumen ratio, (Whitehead et al., 1991; Grobas et al., 1999a, 2001; Dänicke et al., 2000), the cholesterol content of the yolk (Sim and Bragg, 1997; Wen et al., 2019), yolk color (Perez-Bonilla et al., 2011; Zhou et al., 2019), and eggshell quality (Millet et al., 2006; Cherian et al., 2007).

The chemical characteristics of oils and fats − particularly FA composition, degree of saturation (expressed as the ratio of unsaturated to saturated fatty acids [**UFA:SFA**]), and FFA content − determine their nutritional value (Ravindran et al., 2016). Nevertheless, there is a dearth of information on the possible influence of dietary FFA content on egg quality and yolk composition. It has been reported that the UFA:SFA ratio of a diet has a greater effect than FFA content on the fat absorption process (Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019a,b, 2021; Jimenez-Moya et al., 2021a,b). However, most of the data available on the use of AO and FAD concerns broiler chickens. Although some studies have considered the replacement of soybean oil (**SO**) with soybean AO (**SAO**) in the feed of laying hens (Pardío et al., 2005; Perez-Bonilla et al., 2011; Irandoust et al., 2012; Irandoust and Ahn, 2015), the effect of increasing the dietary FFA content has yet to be addressed. A better understanding of the effects of FFA on hen nutrition could enable the use of these fat by-products to be increased and optimized.

Therefore, the study described here was conducted in order to assess the effect of dietary FFA content and fat saturation degree on egg quality and lipid composition of the yolk. The experimental diets were obtained by gradually replacing SO with SAO, or palm crude oil (**PO**) with equivalent graded levels of palm FAD (**PFAD**).

MATERIALS AND METHODS

Animals and Diets

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

A total of 144 laying hens (Lohmann Brown-Classic; 19 wk old; average body weight of the flock: 1,745 ± 117 g, mean ± standard deviation) obtained from a commercial farm (Huevos Guillén, Valencia, Spain) were randomly divided into 8 groups, each including 6 replicates with 3 birds per replicate. The birds were kept in a 3-tier battery cage, with each cage (76.2 × 63.0 cm^2 with a minimum height of 45.0 cm) containing one replicate. Throughout the study, feed and water were supplied for ad libitum consumption, and the animals were raised under the conditions recommended by the breeder (Lohmann, 2019). The feeding period lasted 15 wk.

The birds received a barley-soybean meal-based feed in mash form formulated to meet or exceed FEDNA's (Fundación Española para el Desarrollo de la Nutrición Animal, 2018) recommendations and to minimize basal fat levels, as shown in Table 1. All experimental diets consisted of a basal diet (94%) and an experimental fat or fat blend (6%). Four different fats were used: 2 soybean sources (crude SO and SAO, both provided by Riosa S.A., Jaén, Spain) and 2 palm sources (crude PO and PFAD, both provided by Lípidos Santiga S.A., Barcelona, Spain). As Table 2 shows, a 2 × 4 factorial design was used, with 8 dietary treatments being created through the use of 2 fat sources (soybean or palm) and 4 dietary levels of FFA (10, 20, 30, or 45%). The different levels of FFA for each fat source were achieved by using crude oil for 10% FFA and then progressively replacing the crude oil with equivalent amounts of the corresponding AO or FAD.

Analytical Determination of Oils and Experimental Diets

Oil samples were analyzed in triplicate following the method used by Varona et al. (2021b) for the analysis of AO and FAD in animal nutrition (Table 3). Analytical determination of the experimental diets was performed at least in duplicate according to the AOAC International (2005) method. Gross energy was determined by an adiabatic bomb calorimeter (Parr 6300 Calorimeter, Parr Instrument Company, Moline, IL). Crude, neutral, and acid detergent fiber levels were determined using a fiber analyzer (A2000, ANKOM Technology, Macedon, NY). Calcium and total phosphorus were determined via ICP-OES (Optima 3200 RL, Perkin Elmer, Waltham, MA). The amino acid content was analyzed by chromatography (Hewlett-Packard 1100, Waldbronn, Germany). The FA profile of the feed was determined via GC-FID as is described below concerning the yolk FA profile. The lipid-class composition was determined after lipid extraction by size-exclusion chromatography (Agilent 1100 HPLC, Agilent Technologies, Santa Clara, CA), as described by Rodriguez-Sanchez et al. (2019b). The lipid analysis of the experimental diets is shown in Table 4.

Table 1. Ingredient composition and determined analysis of the experimental diets (g/100 g on a DM basis, unless otherwise indicated).

	Soybean				Palm			
Diets	S10	S20	S30	S45	P10	P20	P30	P45
Ingredient composition								
Barley	49.9							
Soybean meal, 47.5% crude protein	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% crude protein	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							
Determined analysis								
Gross energy, MJ/kg	17.68	17.52	17.41	17.36	16.98	17.23	17.15	16.70
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 2).
[2]Premix provides per kg of feed: enzymatic complex (Setnazyme: Endo 1-4 Beta-Xylanase, 12,000 BXU/g; 6-phytase 100, 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 D3, 3,000 IU; vitamin E, 13 IU; vitamin B1, 1 mg; vitamin B2, 4 mg; vitamin B6, 1.8 mg; vitamin B12, 10 μg; vitamin K3, 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.

Production Performance

Egg production (including the number of eggs produced and individual egg weights) and feed intake were recorded weekly for each replicate cage. From these data, ADFI, egg production, egg mass, and cumulative feed conversion ratio (**FCR**) per kilogram of eggs were calculated for the last weeks of the experiment (33 −34 wk of age).

Egg Quality

During the last 2 wk (33−34 wk of age; 14−15 wk of the experiment) a total of 90 randomly selected eggs per treatment (15 fresh eggs per replicate) were collected to determine egg quality. The weight of each egg was recorded. Shell with membranes and yolk were separated carefully and weighed. The albumen weight was obtained subtracting the yolk and eggshell weight from the total egg weight. The yolk:albumen ratio was calculated according to the procedure described by Hussein et al. (1992). Based on the egg weight and thick albumen height, the Haugh unit values (**HU**) were calculated according to Haugh's formula (1937). Yolk color, as measured by the DSM yolk color score (Vuilleumier, 1969), was determined in these eggs using CIELAB values. Eggshell thickness was measured using a micrometer (Mitutoyo 293-130, Kawasaki, Japan) and eggshell breaking strength was also recorded. The FUTURA Egg-Quality-Measuring-System 3/A (Bröring, Lohne, Deutschland) was used for the tests.

Table 2. Oil blends used in the experimental diets.

	Soybean				Palm			
Diets	S10	S20	S30	S45	P10	P20	P30	P45
Theoretical FFA[1]%	10	20	30	45	10	20	30	45
Proportion in oil blend[2], %								
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	66

[1]Free fatty acids.
[2]All oil blends were added at 6% to the basal diet.

Chemical Composition of the Eggs

At the end of the experiment, 6 eggs from each replicate were randomly collected. Egg components, including albumen, yolk, and shell, were separately homogenized in a pool for later testing. Yolk samples were stored at −20°C in glass vials until lipid analysis was performed.

Table 3. Quality traits, fatty acid, and lipid-class composition of the experimental fats.

	Soybean oil	Soybean acid oil	Palm oil	Palm fatty acid distillate
MIU, g/100 g	0.59	4.95	0.52	2.13
Moisture, g/100 g	0.05	0.56	ND[1]	0.12
Insoluble impurities, g/100 g	0.17	1.97	0.21	0.27
Unsaponifiable matter, g/100 g	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[2]	1.5	2.5	1.1	1.1
Trans fatty acids (C18:1)	ND	0.4	ND	0.2
Saturated fatty acids	17.9	17.8	50.4	52.9
Monounsaturated fatty acids	25.0	33.7	39.0	37.7
Polyunsaturated fatty acids	57.1	48.1	10.6	9.2
UFA:SFA[3]	4.6	4.6	1.0	0.9
Lipid-class composition, %				
Triacylglycerols	94.3	29.1	83.6	6.2
Diacylglycerols	3.8	15.8	9.7	5.0
Monoacylglycerols	0.2	1.3	0.4	1.6
Free fatty acids	1.7	53.8	6.3	87.2
Gross energy, MJ/kg	39.69	39.61	38.76	39.43

[1]Non-detectable value.
[2]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.
[3]Ratio of unsaturated to saturated fatty acids.

Eggs were chemically characterized for DM, ash, CP, and ether extract (**EE**). The DM content was determined according to Sun et al. (2019). Eggshell calcium was tested using ICP-OES (Optima 3200 RL, Perkin Elmer). Albumen and yolk samples were freeze-dried prior to determining CP, using the Kjeldahl method, and EE, using Soxhlet analysis in accordance with AOAC International (2005) (Methods 925.31 and 925.32, respectively). Chemical analyses were carried out at least in duplicate.

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

	Soybean				Palm			
Diets	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
Saturated fatty acids	17.9	18.5	18.3	19.0	43.6	45.4	44.8	46.8
Monounsaturated fatty acids	24.1	25.7	28.0	31.1	35.8	35.6	34.9	34.6
Polyunsaturated fatty acids	57.8	55.3	53.1	49.0	20.5	18.9	20.2	18.5
UFA:SFA[2]	4.6	4.4	4.4	4.2	1.3	1.2	1.2	1.1
Lipid-class composition %								
Triacylglycerols	85.4	71.4	59.2	41.4	79.0	68.6	57.4	44.1
Diacylglycerols	5.2	8.2	10.2	13.4	9.7	8.8	8.0	7.5
Monoacylglycerols	0.4	1.3	0.8	1.7	0.7	0.4	0.5	0.5
Free fatty acids	9.0	19.1	29.8	43.5	10.6	22.2	34.1	47.9

[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.
[2]Ratio of unsaturated to saturated fatty acids.

Fatty Acid Profile and Cholesterol Content of Egg Yolk

Regarding the lipid analysis, the yolk FA composition and cholesterol content were determined using a gas chromatograph (4890D, Agilent Technologies), equipped with a flame ionization detector and a capillary column (**GC-FID**). For FA composition, total yolk lipids were extracted in chloroform-methanol (2:1) (vol/vol) according to Folch's method (Folch et al., 1957). The extracted fat was transferred by dissolving it in diethyl ether in a glass test tube with an internal standard (heneicosanoic acid methyl ester, C21:0) (Sigma-Aldrich Co., St. Louis, MI), the solvent was evaporated using N_2 at 30°C and the FA methyl esters were obtained as described by Guardiola et al. (1994). Thereafter, FA methyl esters were analyzed using GC-FID as described by Varona et al. (2021b) and identified by matching their retention times with those of standards (Sigma-Aldrich Co.). The results were expressed using internal normalization (area %). Cholesterol content was determined after silylation of the unsaponifiable matter following the methodology described by Tres et al. (2020), with slight modifications. The quantification was performed using 5α-cholestane as an internal standard and the corresponding calibration curve

with standards (Sigma-Aldrich Co.). Chemical analyses were carried out at least in duplicate.

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, and homogeneity of the variance were verified. Egg quality traits, yolk FA composition, and cholesterol content 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 interaction fat source × FFA level 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. Quadratic response was also calculated in the case of an inadequate fit for the linear effect.

Results in tables are reported as means, and differences were considered significant when $P < 0.05$. All data analysis was performed using SPSS statistics (27.0.1.0, IBM, Armonk, NY, 2020).

RESULTS

Production Performance

The mean values for the laying hens' production performance are shown in Table 5. ADFI and egg mass were not significantly different among the grouped treatments. However, egg production (linear and quadratic, $P < 0.05$) and FCR (linear, $P < 0.01$) were negatively affected by increasing levels of FFA; hens fed dietary treatments with 45% FFA presented the lowest egg production and the highest FCR at the end of the trial.

Egg Quality

The effects of the added fat source and the dietary FFA content on egg weight and the relative weights of the egg components at the end of the trial are shown in Table 6. A significant interaction ($P < 0.01$) between the fat source and the FFA content was observed in total egg weight and the relative albumen and yolk weights. Thus, an increase in egg size accompanied the increase in FFA content in the case of soybean diets

Table 5. Effects of fat source and dietary free fatty acid content on hen productive performance at the end of the trial (33−34 wk of age).

Item	ADFI, g/hen	Egg production, egg/hen/d	Egg mass, g/d	FCR[1]
Experimental diet				
S10	93.97	0.94	55.86	1.69
S20	101.24	0.95	58.01	1.75
S30	102.15	0.94	56.19	1.83
S45	98.15	0.84	53.29	1.92
P10	100.59	0.92	56.04	1.80
P20	96.98	0.94	57.27	1.72
P30	105.89	0.96	58.16	1.83
P45	102.63	0.89	53.79	1.93
Fat source				
Soybean	98.88	0.92	55.84	1.80
Palm	101.52	0.93	56.32	1.82
FFA[2] content, %				
10	97.28	0.93[a]	55.95	1.75[b]
20	99.11	0.94[a]	57.64	1.74[b]
30	104.02	0.95[a]	57.17	1.83[ab]
45	100.39	0.86[b]	53.54	1.92[a]
S.E.M	1.01	0.02	0.98	0.04
Effects, *P*-values				
Fat source	0.183	0.525	0.741	0.612
FFA content	0.107	0.011	0.196	0.026
Fat source × FFA	0.231	0.684	0.930	0.750
Linear contrast,[3] *P*-values				
Overall	-	0.032	-	0.005
Quadratic contrast,[4] *P*-values				
Overall	-	0.017	-	-

[1]Feed conversion ratio.
[2]Free fatty acid.
[3]Linear responses to dietary free fatty acid content.
[4]Quadratic responses to dietary free fatty acid content.
[a-b]Means within each variable with more than two levels (experimental diet or free fatty acid content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).

Table 6. Effects of fat source and dietary free fatty acid content on egg weight and their relative proportions at the end of the trial (n = 90 eggs per treatment).

Item	Egg weight, g	Albumen, g	Yolk, g	Shell, g	Yolk:albumen
Experimental diet					
S10	60.01[bc]	39.58[bcd]	14.34[b]	6.06	0.364[bc]
S20	60.95[b]	40.65[b]	14.34[b]	5.95	0.354[cd]
S30	59.98[bc]	39.81[bcd]	14.33[b]	5.82	0.362[bc]
S45	63.84[a]	42.88[a]	14.83[a]	6.04	0.347[d]
P10	60.98[b]	40.29[bc]	14.63[ab]	6.04	0.366[bc]
P20	59.00[c]	38.75[d]	14.28[b]	5.98	0.370[ab]
P30	60.20[bc]	39.18[cd]	14.88[a]	6.13	0.382[a]
P45	60.14[bc]	39.51[bcd]	14.62[ab]	6.01	0.372[ab]
Fat source					
Soybean	60.53	40.61	14.44	5.96	0.357
Palm	60.02	39.37	14.60	6.05	0.373
FFA[1] content, %					
10	60.48[b]	39.93[b]	14.48[b]	6.05	0.365[ab]
20	59.99[b]	39.71[b]	14.31[ab]	5.97	0.362[b]
30	60.09[b]	39.49[b]	14.61[a]	5.98	0.372[a]
45	61.98[a]	41.18[a]	14.72[a]	6.02	0.359[b]
S.E.M	0.33	0.26	0.10	0.04	0.003
Effects, *P*-values					
Fat source	<0.001	<0.001	0.074	0.066	<0.001
FFA content	<0.001	<0.001	0.002	0.216	<0.001
Fat source × FFA	<0.001	<0.001	0.002	0.101	0.006
Linear contrast,[2] *P*-values					
Overall	-	-	-	-	-
Soybean	<0.001	<0.001	0.010	-	0.003
Palm	0.451	0.198	0.257	-	0.066
Quadratic contrast,[3] *P*-values					
Overall	-	-	-	-	-
Soybean	<0.001	<0.001	0.038	-	0.474
Palm	0.010	0.003	0.653	-	0.041

[1]Free fatty acid.
[2]Linear responses to dietary free fatty acid content. [3]Quadratic responses to dietary free fatty acid content.
[a-d]Means within each variable with more than two levels (experimental diet or free fatty acid content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).

only (linear and quadratic, $P < 0.001$). The largest albumen and egg weights were recorded for hens on the S45 diet (63.84 g total egg weight), with the other soybean diets showing values similar to those for palm treatments. Consequently, this was also reflected in the yolk: albumen ratio, with significantly lower values being observed for soybean diets. In contrast, shell weight was not affected by differences in the added fat source or dietary FFA content.

With regard to the most important egg quality traits (Table 7), significant differences were found in the albumen HU, with the highest values being recorded in soybean diets, and the lowest in eggs from palm diets ($P < 0.001$). Increasing the FFA content up to 30% reduced HU values in both fat sources, showing a significant linear and quadratic effect ($P < 0.01$). No interaction between fat source and FFA content was observed in this respect. Concerning yolk color, a significant interaction was found for fat source × FFA content, as well as for the effect of FFA content ($P < 0.01$). These results presented a significant quadratic effect ($P < 0.05$) but were not accompanied by a linear trend for any of the 2 fat sources ($P > 0.05$). Yolk pigmentation was significantly greater in eggs from palm diets in comparison to soybean diets ($P < 0.001$). Regarding eggshell quality, no influence by the dietary treatments was found for shell thickness. However, shell breaking strength was higher for palm diets ($P < 0.05$).

The chemical composition of the eggs was little affected by the supplemental fat source, FFA content, or any interaction between them (Table 8). In fact, the only differences detected were for shell dry matter (%) and ash (%), with these being found to be higher in the eggs from palm diets compared with those fed soybean diets ($P < 0.01$).

Fatty Acid Profile and Cholesterol Content of Egg Yolk

The FA composition of egg yolks from all experimental groups is summarized in Table 9, with additional detailed data presented in Supplementary Table 1. Analysis of the FA profile in yolks showed that the supplemental fat source had a significant effect on all FA presented ($P < 0.001$), except for arachidonic acid (C20:4 n-6).

Regarding SFA and monounsaturated fatty acids (**MUFA**), the proportions of palmitic (C16:0) and oleic (C18:1 n-9) acids, as well as the total SFA and total MUFA, were significantly greater for the eggs laid by hens fed palm diets, whereas stearic acid (C18:0) was higher in soybean diets ($P < 0.001$). Furthermore, increasing dietary content of FFA led to linear increases in the proportions of palmitoleic (C16:1 n-7) acid in egg yolks from palm diets ($P < 0.05$).

Table 7. Effects of fat source and dietary free fatty acid content on egg quality traits (n = 90 eggs per treatment).

Item	Albumen HU[1]	Yolk Color[2]	Eggshell	
			Thickness, mm	Strength, N
Experimental diet				
S10	91.5	13.38[b]	0.375	45.2
S20	88.3	13.48[ab]	0.369	43.3
S30	86.7	13.16[c]	0.364	42.8
S45	89.1	13.46[ab]	0.365	46.0
P10	89.0	13.51[ab]	0.370	45.5
P20	86.7	13.44[ab]	0.365	47.2
P30	84.8	13.44[ab]	0.373	44.6
P45	87.6	13.62[a]	0.367	45.3
Fat source				
Soybean	88.9	13.37	0.368	44.3
Palm	87.0	13.50	0.369	45.6
FFA[3] content, %				
10	90.3[a]	13.45[a]	0.372	45.2
20	87.5[bc]	13.46[a]	0.367	45.2
30	85.7[c]	13.31[b]	0.368	43.7
45	88.1[b]	13.54[a]	0.366	45.6
S.E.M	0.66	0.04	0.003	0.69
Effects, *P*-values				
Fat source	< 0.001	< 0.001	0.760	0.026
FFA content	< 0.001	< 0.001	0.197	0.076
Fat source × FFA	0.890	0.009	0.057	0.061
Linear contrast,[4]				
P-values				
Overall	0.002	-	-	-
Soybean	-	0.647	-	-
Palm	-	0.099	-	-
Quadratic contrast,[5]				
P-values				
Overall	<0.001	-	-	-
Soybean	-	0.041	-	-
Palm	-	0.007	-	-

[1]Haugh Units.
[2]Yolk color, as measured by the DSM yolk color score (Vuilleumier, 1969).
[3]Free fatty acid.
[4]Linear responses to dietary free fatty acid content.
[5]Quadratic responses to dietary free fatty acid content.
[a-c]Means within each variable with more than two levels (experimental diet or free fatty acid content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).

On the other hand, yolks from soybean diets had significantly higher content of n-6 polyunsaturated fatty acids (**PUFA**), n-3 PUFA, and total PUFA ($P < 0.001$), being approximately twice as high as those from palm diets. Moreover, a significant effect of FFA and interaction between the dietary fat source and FFA content ($P < 0.001$) was found for n-3 PUFA; increasing dietary content of FFA caused a substantial decrease in linolenic (**LNA**, C18:3 n-3) and DHA (C22:6 n-3) acid content in yolks from soybean diets only (linear, $P < 0.001$), whereas no relationship was found between varying levels of FFA and the percentage of n-3 PUFA for yolks from palm diets. Eggs from hens fed soybean diets showed higher content of LA (C18:2 n-6) ($P < 0.001$) than those that received palm diets (24.1 vs. 12.1, respectively), but in this case no association was found with different FFA levels, and no interaction was observed.

The cholesterol content in the egg yolks of all experimental groups (Table 10) was similar, with mean values of 1,403 mg/100 g in the yolks from soybean diets, and 1,371 mg/100 g in those from palm diets. No effect for fat source, FFA content or interaction was detected. Furthermore, no significant differences were found either in the cholesterol content of the edible part of the egg, obtained from the relative proportions of yolk and albumen in each treatment group.

DISCUSSION

There has been increasing interest in recent years in the use of AO and FAD as fat sources for animal feed. However, most of the available data derives from their use with broilers (Wiseman and Salvador, 1991; Blanch et al., 1996; Vilarrasa et al., 2015; Rodriguez-Sanchez et al., 2019a,b, 2021; Jimenez-Moya et al., 2021a,b) rather than laying hen.

In this study, the replacement of SO with SAO induced a significant increase in egg weight, specifically in the S45 group. In contrast, as dietary FFA content increased, egg production decreased and FCR increased. Consequently, no differences were observed in terms of the egg mass between the experimental groups. Grobas et al. (2001) obtained significantly higher egg weight when SO was used in laying hen feed compared to other sources of fat (tallow, linseed, and olive oil). However, differences in production performance or egg weight laid by birds fed SO or SAO have not been observed in similar studies (Pardío et al., 2005; Perez-Bonilla et al., 2011; Irandoust et al., 2012; Roll et al., 2018a). In our study, however, differences in egg size are almost entirely attributable to the high weight recorded for hens fed S45 (63.84 g), with the remaining groups presenting considerably lower average values (59.00 −60.95 g) and egg mass remained unaffected.

It has been reported that egg weight largely depends on the amount and type of fat used in feeding, and this effect is primarily attributed to the LA content of the diet (Shutze and Jensen, 1963; Scragg et al., 1987; Grobas et al., 1999a,b, 2001; Safaa et al., 2008). In the current trial, the amount of supplemental fat cannot be a factor, since all the oil blends contributed 6% to the basal diet. In order to optimize egg production and ensure a rapid increase in egg size, many researchers suggest LA levels around 1.2% (Grobas et al., 1999a; Safaa et al., 2008; FEDNA, 2018). Discrepancies still exist today between researchers and most commercial management guidelines, which usually recommend dietary LA levels above 1.8% at the beginning of the laying period (Lohmann, 2019). In the present trial, the mean LA content of diets containing soybean (S10, S20, S30, and S45) or palm oils (P10, P20, P30, and P45) was 2.8% and 1.2%, respectively, with the values of soybean diets being above the hens' requirements (Table 1). Therefore, the high egg size recorded for hens fed S45 cannot be attributed to the LA content either.

As was the case in Dänicke et al. (2000), in the current trial the increase of egg weight in soybean diets was accompanied by a simultaneous increase in the albumen

Table 8. Effects of fat source and dietary free fatty acid content on the eggs' chemical composition (n = 6 pools per treatment).

	Albumen			Yolk				Eggshell		
Item	DM, %	Ash, %	CP, %	DM, %	Ash, %	CP, %	EE[1], %	DM, %	Ash, %	Ca, mg/g
Experimental diet										
S10	12.3	0.83	9.8	51.3	1.95	15.3	28.8	98.7	93.74	383.5
S20	12.4	0.84	9.9	51.3	1.96	15.2	29.1	98.5	93.57	388.1
S30	12.2	0.83	9.8	51.3	1.84	15.5	29.0	98.7	93.48	386.5
S45	12.2	0.84	9.7	51.5	2.04	15.6	29.1	98.7	93.44	387.5
P10	12.2	0.86	9.6	51.4	2.04	15.3	29.22	98.8	94.00	383.2
P20	12.3	0.86	9.7	51.6	1.99	15.1	29.0	98.8	93.96	383.2
P30	12.0	0.85	9.5	51.7	2.01	15.2	29.3	98.8	93.97	380.4
P45	12.2	0.84	9.7	51.8	2.04	15.3	29.0	98.8	93.82	381.8
Fat source										
Soybean	12.3	0.84	9.8	51.4	1.95	15.4	29.0	98.7	93.56	386.4
Palm	12.2	0.85	9.6	51.6	2.04	15.2	29.1	98.8	93.94	382.1
FFA[2] content, %										
10	12.2	0.84	9.7	51.4	2.00	15.3	29.0	98.8	93.87	383.4
20	12.4	0.85	9.8	51.5	1.98	15.1	29.1	98.7	93.77	385.7
30	12.1	0.84	9.7	51.5	1.92	15.4	29.2	98.8	93.73	383.4
45	12.2	0.84	9.7	51.7	2.04	15.5	29.0	98.7	93.63	384.6
S.E.M	0.16	0.01	0.14	0.17	0.06	0.15	0.28	0.06	0.16	2.79
Effects, *P*-values										
Fat source	0.428	0.116	0.127	0.069	0.127	0.095	0.572	0.002	0.005	0.068
FFA content	0.656	0.866	0.734	0.497	0.370	0.349	0.939	0.162	0.633	0.875
Fat source × FFA	0.902	0.803	0.908	0.903	0.611	0.772	0.885	0.160	0.937	0.795

[1]Ether extract.
[2]Free fatty acid.

weight. Whitehead (1991) and Grobas et al. (1999a,b, 2001) also found that supplemental fat exerted a beneficial effect on egg weight, which was primarily due to an increase in albumen weight. It has been suggested that this could be attributed to the influence of dietary fat on the estrogen metabolism, which is mainly responsible for albumen secretion (Whitehead, 1995). Nevertheless, the mechanism by which the supplemental fat source influences egg size remains unclear. Most of the trials conducted up to now have focused only on egg weight, with insufficient attention being paid to the concurrent changes in yolk and albumen weights.

In addition to being rich in FFA, SAO have higher MIU content than crude oils and PFAD. The unsaponifiable matter and insoluble impurities dilute the energy content of oils, but also include variable amounts of some compounds that could affect egg weight. Tocopherols and phytosterols are unsaponifiable matter that can be removed from crude oils in refining procedures, but which then accumulates in their by-products (Messina, 2010; Varona et al., 2021a). However, no effect on egg weight has been found for these compounds (Shi et al., 2014). Another factor to consider is the amount of insoluble impurities in SAO, which is also higher than that for PFAD and crude oils. The content and composition of these insoluble impurities have been reported to be highly variable and might include some phospholipids such as lecithins (Varona et al., 2021a). In research with laying hens, Mandalawi et al. (2015) found that replacing animal fat with lecithin (40 g/kg) increased egg weight. Thus, it is possible that the MIU components may have played unidentified roles in affecting egg weight and egg quality. Unfortunately, it has not been possible to analyze any such effect in this study, nor has it been possible, due to the experimental design, to determine whether the negative impact of diets including SAO was due to their FFA level or their MIU composition. In fact, the MIU content of AO and FAD could be one of the reasons for the controversial results of previous studies, since MIU values are often not reported and, therefore, any negative effect found when including these fat by-products has been directly attributed to their high level of FFA.

These results should be interpreted with caution, since in this trial no increase in egg size was observed in the S30 group and previous studies reported no differences in egg weight. Therefore, further research is needed to clarify the mechanisms involved in egg size.

Regarding albumen quality, higher HU were recorded for hens fed soybean diets ($P < 0.001$). Additionally, an increase in the FFA content resulted in a linear decrease in HU values in both fat sources ($P < 0.05$). These results contrast with those obtained by Grobas et al. (1999a,b) and Safaa et al. (2008), who did not observe any effect for the type of supplemental fat on the HU of eggs. We are unable to explain this discrepancy with our results. Nonetheless, even the group with the lowest HU (P30; 84.8 HU) presented a HU score >72, indicating high freshness (AA quality) according to US Department of Agriculture standards (United States Department of Agriculture USDA, 2000). Differences in yolk color between soybean and palm diets ($P < 0.001$) were expected, as crude PO possesses greater amounts of beta-carotene (Khaskheli and Chou, 2020): the more carotenoids in the diet, the redder and darker the yolk (Seuss-Baum et al., 2011). Moreover, the perception of the intensity of the color of the yolks depends directly on the quantity of carotenoids consumed by the bird (Bouvarel et al., 2011). Pardío et al., (2005) and Irandoust et al. (2012) compared diets that included SO

Table 9. Mean composition of fatty acids in egg yolks according to the fat source and the dietary free fatty acid content of the diet (% of total fatty acids) (n = 6 pools per treatment).

Item	C16:0	C18:0	C16-1 n-7	C18:1 n-9	C18:1 n-7	C18:2 n-6	C18:3 n-3	C20:4 n-6	C22:6 n-3	SFA[1]	MUFA[2]	n-3	n-6	PUFA[3]
Experimental diet														
S10	22.9	8.6	1.7^{c}	34.4	1.4^{cd}	23.9	1.6^{a}	1.8	1.4^{a}	31.8	38.5	3.2^{a}	26.5	29.6
S20	22.6	8.5	1.5^{c}	34.0	1.3^{d}	25.0	1.2^{ab}	1.9	1.3^{a}	31.4	37.9	2.7^{ab}	27.8	30.5
S30	22.7	8.4	1.6^{c}	35.9	1.4^{cd}	23.4	1.0^{bc}	1.8	1.1^{b}	31.4	39.9	2.2^{b}	26.3	28.5
S45	22.6	8.3	1.5^{c}	36.5	1.4^{d}	24.0	0.6^{cd}	1.9	0.7^{c}	31.1	40.3	1.5^{c}	26.9	28.4
P10	25.3	7.6	2.2^{b}	45.6	1.5^{bc}	13.2	0.4^{d}	1.9	0.8^{c}	33.3	49.4	1.3^{c}	16.0	17.3
P20	26.0	7.3	2.6^{a}	45.2	1.7^{a}	11.8	0.3^{d}	1.8	0.7^{c}	33.7	50.5	1.1^{c}	14.5	15.6
P30	26.0	7.3	2.6^{a}	45.3	1.5^{bc}	11.9	0.3^{d}	1.8	0.7^{c}	33.6	50.4	1.1^{c}	14.8	15.9
P45	26.0	7.4	2.7^{a}	45.1	1.7^{ab}	11.6	0.3^{d}	1.8	0.7^{c}	33.7	50.5	1.1^{c}	14.5	15.6
Fat source														
Soybean	22.7	8.4	1.6	35.2	1.4	24.1	1.1	1.8	1.1	31.5	39.1	2.4	26.9	29.3
Palm	25.8	7.4	2.5	45.0	1.6	12.1	0.3	1.8	0.7	33.6	50.2	1.2	14.9	16.1
FFA[4] content, %														
10	24.1	8.1	1.9	39.5	1.5	18.5	1.0^{a}	1.8	1.1^{a}	32.5	43.9	2.2^{a}	21.2	23.4
20	24.3	7.9	2.1	39.6	1.5	18.4	0.7^{ab}	1.8	1.0^{a}	32.6	44.2	1.9^{ab}	21.2	23.1
30	24.4	7.8	2.1	40.6	1.5	17.6	0.6^{ab}	1.8	0.9^{ab}	32.5	45.1	1.7^{ab}	20.5	22.2
45	24.3	7.8	2.1	40.8	1.5	17.8	0.5^{b}	1.9	0.7^{b}	32.4	45.4	1.3^{b}	20.7	22.0
S.E.M	0.32	0.14	0.11	0.93	0.04	1.09	0.12	0.03	0.05	0.24	1.04	0.16	1.09	1.22
Effects, *P*-values														
Fat source	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.599	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FFA content	0.736	0.121	0.303	0.060	0.520	0.348	<0.001	0.920	<0.001	0.923	0.065	<0.001	0.545	0.131
Fat source × FFA	0.156	0.563	<0.001	0.141	0.002	0.202	0.002	0.062	<0.001	0.061	0.212	<0.001	0.116	0.253
Linear contrast,[5] *P*-values														
Overall	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Soybean	-	-	0.160	-	0.270	-	<0.001	-	<0.001	-	-	<0.001	-	-
Palm	-	-	0.001	-	0.066	-	0.298	-	0.207	-	-	0.212	-	-

[1]Saturated fatty acids.
[2]Monounsaturated fatty acids.
[3]Polyunsaturated fatty acids.
[4]Free fatty acid.
[5]Linear responses to dietary free fatty acid content.
[a-d]Means within each variable with more than two levels (experimental diet or free fatty acid content) not sharing a common superscript differ according to Tukey's test ($P < 0.05$).

Table 10. Effects of fat source and free fatty acid content on yolk cholesterol content (n = 6 pools per treatment).

Item	Cholesterol, mg/100 g of yolk	Cholesterol, mg/100 g of egg[1]
Experimental diet		
S10	1,408	368
S20	1,416	369
S30	1,408	373
S45	1,380	346
P10	1,345	354
P20	1,394	369
P30	1,352	368
P45	1,393	369
Fat source		
Soybean	1403	364
Palm	1,371	365
FFA[2] content, %		
10	1,377	361
20	1,405	369
30	1,380	371
45	1,387	358
S.E.M	25.53	8.12
Effects, *P*-values		
Fat source	0.134	0.860
FFA content	0.776	0.439
Fat source × FFA	0.561	0.237

[1]Edible portion of the egg.
[2]Free fatty acid.

and SAO, but did not find significant differences in any of the egg quality traits studied. In contrast, in this study, eggshell quality was also found to be affected by the fat source added. Eggs obtained from palm dietary groups of laying hens had the highest resistance to breakage ($P < 0.05$), which is probably due to the lower weight recorded for these eggs ($P < 0.001$). Even when they derive from the same source, AO and FAD vary widely in terms of chemical composition, quality, and oxidative status (Varona et al., 2021a). This variability in composition further complicates the study of the use of these fat by-products, as it could be responsible for the differences in results reported.

The internal chemical composition of the egg did not differ between the experimental treatments as expected, since the biological purpose of the egg is to provide an optimal nutritional environment for the developing embryo. On the opposite side, yolk FA composition can be markedly influenced by hen dietary manipulation (Cruickshank, 1934; Meluzzi et al., 2000; Grobas et al., 2001; Bou et al., 2009; Oliveira et al., 2010). In general, the supplemental fat source added to the feeds notably modified the FA profile of the yolk, whereas the FFA content had limited effect on the FA composition. In this sense, the yolks from soybean groups presented a lower proportion of SFA and MUFA ($P < 0.001$), consistent with the lower levels of these FA in soybean diets in comparison with palm diets. However, it should be noted that the proportional difference in SFA between soybean and palm was much less prominent in the yolks than in the feed itself. This finding is consistent with other studies (Cruickshank, 1934; Meluzzi et al., 2000; Petrović et al., 2012; Irandoust and Ahn, 2015), in which yolk SFA were less affected by dietary manipulation than PUFA. With regard to PUFA, the influence of the greater contribution of essential FA (LA and LNA) in soybean diets was observed, which led not only to a higher content of these FA in the soybean egg yolks ($P < 0.001$), but also influenced the content of the long-chain FAs synthesized from these precursors, such as DHA. These results are consistent with those of Cherian and Sim (1991) and Baucells et al. (2000), who examined the relationships among different n-3 and n-6 PUFA in laying hen diets.

In the present trial, the inclusion of SAO in the diet decreased the n-3 PUFA % content of egg yolks (linear, $P < 0.001$), with the most pronounced reduction occurring in the S45 group. This can be attributed to the decreasing LNA content in the feed as SO was replaced by SAO, because the SAO used in this study presented lower LNA than the crude SO. Yolk n-3 PUFA reduction occurred not only for the LNA content, but also for that of the long-chain n-3 PUFA derived from it, such as DHA. Pardío et al. (2005) also found lower n-3 PUFA content in yolks when comparing different combinations of soybean soapstock and SO after 15 wk of feeding. In contrast, Irandoust and Ahn (2015) reported that total PUFA and n-3 PUFA content were not affected by varying the added oil source (35 g/kg of SO or SAO). This could be due to the fact that almost twice as much fat was added to the feed in our study (60 g/kg) and to differences in the FA composition of the oils used, especially LNA. In fact, Grobas et al. (2001) reported that both the particular fat source added to the feed (tallow, linseed, olive, and SO), and the amount that was included (50 or 100 g/kg) influenced the yolk FA profile. Regarding palm diets, the increasing levels of PFAD had little effect on the FA composition of the yolk; there was only a linear increase in the proportions of palmitoleic (C16:1 n-7) acid as the FFA content increased. The degree of dietary fat saturation and the FA composition of the feed notably modified the composition of the yolk. However, increasing the FFA content did not have a notable impact on the yolk FA profile. Differences observed among eggs from soybean groups with different FFA% were related to the lower LNA content in SAO compared with crude SO (1.8 and 4.2%, respectively).

Although cholesterol deposition in the yolk can be affected by nutrition (Sim and Bragg, 1997; Wen et al., 2019), it is especially resistant to dietary changes since hens are capable of meeting the minimum requirements for embryo development (Faitarone et al., 2013). In the present trial, no relationship was found between the amount of cholesterol found in the yolks and the different experimental diets. The UFA:SFA ratio of the fat sources used, as well as the replacement by their respective AO or FAD, did not have a negative impact on yolk cholesterol content, even when hens were fed with a low UFA:SFA ratio and a high FFA% diet. Similarly, Irandoust and Ahn (2015) did not find any differences when comparing diets that included SO or SAO.

In conclusion, the dietary fat type (UFA:SFA ratio) affected egg weight, egg quality, and yolk FA composition to a greater extent than the FFA content. Laying hens consuming diets containing crude PO (low UFA:

SFA ratio) were hardly affected by increasing the levels of PFAD. However, it was observed that hens fed soybean diets (high UFA:SFA ratio) showed a linear response to increasing levels of FFA, especially when SO was completely replaced by its by-product (FFA = 45%). Therefore, the influence of SAO on egg weight and egg quality requires more detailed study, as the higher MIU content found in SAO in comparison with PFAD may have contributed to these results. Particular attention should be paid to examining the effect of SAO on egg size and the relationship of dietary supplemental fat with albumen weight. The results presented here serve to increase the available knowledge about the use of AO and FAD in commercial layers, highlighting the value of these by-products as alternative lipid sources and thus contributing to the transition toward a more sustainable egg industry.

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DISCLOSURES

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SUPPLEMENTARY MATERIALS

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