Use of combinations of re-esterified oils, differing in their degree of saturation, in broiler chicken diets

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ABSTRACT Re-esterified oils contain higher proportions of mono- and diacylglycerols, and also higher proportions of saturated fatty acids (SFA) at the *sn*-2 position of acylglycerol molecules than does a native oil with the same degree of saturation, which enhances the apparent absorption of SFA. Moreover, as happens with native oils, their nutritive value could be further improved by blending re-esterified oils of extreme degrees of saturation. Therefore, the aim of the current study was to assess the effect of increasing the dietary unsaturated-to-saturated fatty acid ratio (UFA:SFA) by adding re-esterified soybean oil in replacement of re-esterified palm oil, on fatty acid (FA) apparent absorption and its consequences on growth performance, carcass fat depots, and FA composition of abdominal adipose tissue. For this purpose, one hundred twenty 1-day-old female broiler chickens were randomly distributed in 30 cages. The 2 pure re-esterified oils, together with 3 re-esterified oil blends, were included in the basal diet at 6%. The increasing dietary UFA:SFA ratio resulted in an improved total FA apparent absorption (linear effect for the starter period, $P = 0.001$; quadratic effect for the grower-finisher period, $P = 0.006$) and, therefore, an improved feed conversion ratio (FCR) for the overall period (linear effect, $P = 0.003$). In the starter period, the improved fat absorption was due to the growing presence of linoleic acid and the enhanced absorption of SFA, mono- and polyunsaturated FA (associative effects among FA; $P < 0.05$). In the growing-finishing period, however, the absorption of mono- and polyunsaturated FA was not affected ($P > 0.05$). The UFA:SFA ratio of the abdominal adipose tissue varied in the same direction, but to a lesser extent than that of the diet. Whilst the deposited-to-absorbed ratio of polyunsaturated FA remained relatively constant as the dietary UFA:SFA ratio increased, the deposited-to-absorbed ratio of SFA increased, and that of monounsaturated FA decreased. Taken together, the addition of re-esterified soybean oil in replacement of re-esterified palm oil improved fat absorption, but no synergism was observed between re-esterified oils.

Key words: abdominal adipose tissue, apparent absorption, degree of saturation, fatty acids, re-esterified oils

INTRODUCTION

From results of a previous study (Vilarrasa et al., 2015), re-esterified oils, obtained from the union of 2 low-cost by-products (glycerol and acid oils, derived from biodiesel and oil-refining industries, respectively), are interesting alternative fat sources to be used in broiler chicken diets. These oils are characterized by a greater *sn*-2 saturated fatty acid (**SFA**) content, and monoacylglycerol and diacylglycerol proportions when compared with their corresponding native oils. The dif-ferent molecular structure of re-esterified oils exerted favorable effects on SFA apparent absorption, resulting in a similar or even a higher total fatty acid (**FA**) apparent absorption than did their corresponding native oils. In any case, the authors of this study observed how, in general, the fat degree of saturation exerted a greater impact on FA apparent absorption than did the fat molecular structure. For this reason, the combination of re-esterified oils, differing in their degree of saturation, could be beneficial in terms of fat utilization, as occurs with native oils (Sibbald et al., 1960; Lewis and Payne, 1966). Since long-chain unsaturated fatty acids (**UFA**) have a greater ability to form mixed micelles than do SFA, their presence has been shown to increase the capacity of mixed micelles to take up SFA in the core and, therefore, improve their absorption (Ketels and de Groote, 1989).

Table 1. Chemical analyses of the experimental fats.

Item[1]		Re-esterified palm oil	Re-esterified soybean oil
Moisture, %		0.01	0.51
Impurities, %		<0.5	<0.5
Unsaponifiable matter, %		0.44	2.29
Fatty acid composition and distribution, %			
C16:0	Total	46.6	10.3
	sn-2%[2]	18.9	11.4
C18:0	Total	4.77	3.65
	sn-2%[2]	26.2	13.9
C18:1 n-9	Total	35.7	37.5
	sn-2%[2]	27.8	23.9
C18:2 n-6	Total	8.30	40.4
	sn-2%[2]	30.1	28.8
C18:3 n-3	Total	0.24	4.81
	sn-2%[2]	29.1	24.0
Minor fatty acids		4.38	3.32
SFA	Total	54.1	14.1
	sn-2%[2]	18.9	12.1
MUFA	Total	37.4	40.7
	sn-2%[2]	27.8	23.6
PUFA	Total	8.54	45.2
	sn-2%[2]	30.1	28.3
Acylglycerol composition, %			
TAG		54.4	55.0
DAG	Total	38.6	34.4
	1(3),2-DAG%[3]	21.8	30.2
MAG	Total	5.83	9.12
	2-MAG%[4]	10.3	8.90
FFA		1.18	1.55
Glycerol-to-fatty acid ratio[5], mol/mol		0.42	0.44
Gross energy, kcal/kg		9,223	9,222

[1]DAG = Diacylglycerols; FFA = Free fatty acids; MAG = Monoacylglycerols; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; SFA = Saturated fatty acids; TAG = Triacylglycerols.

[2]The proportion of a particular fatty acid (FA) that is located at the acylglycerol sn-2 position (sn-2%) was calculated as follows (Vilarrasa et al., 2014): sn-2% = (sn-2/Total) × a × 100, where sn-2 is the FA composition at the sn-2 position (converted to mol%), Total is the total FA composition of the fat (converted to mol%), and a is the ratio between the moles of FA located at the sn-2 position and the moles of total FA. a was calculated to be 0.23 and 0.24 for re-esterified palm and soybean oils, respectively.

[3]The proportion of 1(3),2-DAG vs.1,3-DAG.

[4]The proportion of 2-MAG vs.1(3)-MAG.

[5]Estimated calculation based on the values of the acylglycerol composition.

However, an excess of dietary UFA at the end of the rearing period could have a negative impact on carcass quality. Given that the FA composition of dietary fats has a direct influence on the FA composition of carcass fat depots (Pinchasov and Nir, 1992; Hrdinka et al., 1996; Bavelaar and Beynen, 2003; González-Ortiz et al., 2013), and that UFA decrease the firmness or consistency of the fat (Hrdinka et al., 1996; Zollitsch et al., 1997) and increase its susceptibility to oxidation (Cortinas et al., 2005), more saturated fat sources are recommended to be added to finisher diets. This is why the proportions between saturated and unsaturated fat sources are recommended to be adjusted throughout the rearing cycle according to the requirements of the producer.

Therefore, the aim of the current study was to assess the effect of increasing the dietary UFA:SFA by adding re-esterified soybean oil in replacement of re-esterified palm oil, on FA apparent absorption and its consequences on growth performance, carcass fat depots, and FA composition of abdominal adipose tissue.

MATERIALS AND METHODS

Experimental Fats

Experimental fats were supplied by SILO S.p.a. (Florence, Italy). Re-esterified oils (Table 1) were produced using, as raw materials, palm or soybean acid oils (by-products obtained from the refining process of crude oils, with a high free-FA content) and glycerol (a by-product obtained from the methylation process applied in biodiesel production), which were processed in a reactor for 4 to 6 h, under high-vacuum conditions (1 to 3 mmHg), at temperatures between 190 and 250°C, and without chemical catalysts.

Oil samples were analyzed in triplicate for moisture (Method 926.12 of AOAC International, 2005), impurities (ISO 663:2007), unsaponifiable matter (Method 933.08 of AOAC International, 2005), acylglycerol and free-FA composition (ISO 18395:2005), positional isomers of mono- and diacylglycerols (Sacchi et al., 1997), total FA composition (Guardiola et al., 1994), sn-2 FA composition [Commission Regulation (EEC) No. 2568/91 – Annex VII], and gross energy content

Table 2. Ingredient composition of the experimental diets.

Ingredients, %	Starter diet (from 0 to 20 d)	Grower-finisher diet (from 20 to 41 d)
Wheat	51.37	44.80
Soybean meal 48%	38.58	27.71
Barley	-	18.26
Experimental fats[1]	6.00	6.00
Dicalcium phosphate	1.69	1.33
Calcium carbonate	1.30	0.86
Sodium chloride	0.40	0.35
Vitamin and mineral premix[2]	0.30	0.30
D,L-Methionine	0.23	0.18
L-Lysine	0.07	0.11
L-Threonine	-	0.02
Enzyme supplement[3]	0.05	0.05
Ethoxyquin 66%	0.02	0.02

[1]The 5 oil blends consisted of re-esterified palm oil blended with re-esterified soybean oil in the following proportions: 100:0, 75:25, 50:50, 25:75, and 0:100.

[2]Provides per kg feed: vitamin A (from retinol), 13,500 IU; vitamin D_3 (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; vitamin B_1, 3 mg; vitamin B_2, 9 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 16.5 μg; vitamin K_3, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 μg; Fe (from $FeSO_4 \cdot 7H_2O$), 54 mg; I [from $Ca(I_2O_3)_2$], 1.2 mg; Co (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$), 0.6 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na_2SeO_3), 0.18 mg; Mo [from $(NH_4)_6Mo_7O_{24}$], 1.2 mg.

[3]Provides per kg feed: β-glucanase 350 IU; xylanase 1,125 IU.

(IKA-Kalorimeter system C4000; Staufen, Germany), as described in more detail in our previous report (Vilarrasa et al., 2014).

Animals and Diets

The trial was performed at the animal experimental facilities of the Servei de Granges i Camps Experimentals (Universitat Autònoma de Barcelona; Bellaterra, Barcelona, Spain). The experimental procedure received the prior approval from the Animal Protocol Review Committee of the same institution. All animal housing and husbandry conformed to the European Union Guidelines (2010/63/EU).

A total of one hundred twenty 1-day-old female broiler chickens of the Ross 308 strain were obtained from a commercial hatchery (Pondex SAU; Juneda, Lleida, Spain). On arrival, chicks were wing-banded, weighed (initial BW, 46.5 ± 3.21 g), and randomly assigned to one of the 5 dietary treatments, with 4 chicks/cage and 6 cages/treatment. Birds were housed in wire-floor cages with excreta collection trays. Throughout the study, feed and water were supplied for ad libitum consumption, and animals were raised under controlled conditions of light and temperature, as recommended by the breeder.

The birds received a starter feed (in mash form) until d 20 and a grower-finisher feed (in pelleted form) between d 20 and 41. The wheat- and soybean-meal-based diets were formulated to meet or exceed FEDNA (2008) requirements and to minimize basal fat levels. The composition of experimental diets is presented in Table 2. Re-esterified palm and soybean oils were selected, as they represented extremes of saturation likely to be encountered in the practical formulation of broiler

chickens diets. The 2 pure re-esterified oils were blended in the proportions shown in Table 3 to produce 5 experimental diets varying in the UFA:SFA (1.21, 1.60, 2.25, 3.25, and 5.09 for starter diets, and 1.18, 1.58, 2.16, 3.17, and 4.94 for grower-finisher diets). The 2 pure re-esterified oils, together with the 3 re-esterified oil blends, were included in the basal diet at 6%. The manufacturing of the experimental diets was carried out at the experimental station of Institut de Recerca i Tecnologia Agroalimentàries Mas de Bover (Constantí, Tarragona, Spain).

Feed samples were taken at the beginning and throughout the experimental period for analysis. Analytical determinations of feeds were performed according to the methods of AOAC International (2005): DM (Method 934.01), ash (Method 942.05), CP (Method 968.06), crude fat (Method 2003.05), and crude fiber (Method 962.09). Gross energy was determined as described previously for fats, and the FA content was analyzed following the method of Sukhija and Palmquist (1988), adding nonadecanoic acid (C19:0, Sigma–Aldrich Chemical Co.; St. Louis, MO), as an internal standard. The macronutrient and FA composition of the experimental diets are presented in Table 3.

Controls and Sampling

Feed consumption and BW were measured weekly to calculate ADFI, ADG, and feed conversion ratio (**FCR**) throughout the study.

Two digestibility balances were carried out using the total-excreta-collection method (Bourdillon et al., 1990). Excreta were collected in the starter period from d 7 to 10 and in the growing-finishing period from d 36 to 38. The last day of the balance, feed consumption

Table 3. Proportions of re-esterified oils used in oil blends, and analyzed[1] macronutrient content and fatty acid composition of the starter and grower-finisher experimental diets.[2]

Item	Starter diets (from 0 to 20 d)					Grower-finisher diets (from 20 to 41 d)				
	P	75P:25S	50P:50S	25P:75S	S	P	75P:25S	50P:50S	25P:75S	S
Proportion of re-esterified oils used in oil blends, %										
Re-esterified palm oil	100	75	50	25	-	100	75	50	25	-
Re-esterified soybean oil	-	25	50	75	100	-	25	50	75	100
Macronutrient content, %										
DM	90.9	90.9	90.9	90.9	90.8	89.1	89.2	89.4	89.3	89.8
CP	24.6	25.0	25.7	24.6	24.3	19.8	20.0	20.8	20.3	20.2
Crude fat	7.54	7.14	7.25	7.50	7.39	7.64	7.19	7.38	7.24	7.40
Crude fiber	2.81	2.73	2.82	3.07	2.75	2.81	2.74	2.72	2.86	2.68
Ash	7.18	7.24	7.52	7.40	6.89	5.35	5.20	5.47	5.03	5.23
Gross energy, kcal/kg	4,249	4,250	4,219	4,211	4,226	4,200	4,208	4,218	4,197	4,216
Fatty acid composition, %										
C12:0	0.91	0.69	0.00	0.00	0.00	0.92	0.69	0.47	0.00	0.00
C14:0	1.20	0.97	0.71	0.46	0.00	1.20	0.99	0.74	0.46	0.00
C16:0	38.5	32.5	26.0	19.2	12.3	39.3	32.9	26.4	19.8	12.9
C18:0	4.56	4.27	4.10	3.87	3.61	4.47	4.16	3.96	3.71	3.45
C18:1 n-9	30.7	30.7	31.1	31.3	31.4	30.5	30.4	30.7	30.9	31.0
C18:1 n-7	0.80	0.98	1.22	1.44	1.64	0.76	0.94	1.17	1.39	1.61
C18:2 n-6	21.4	27.1	33.2	39.1	44.7	21.1	27.2	33.0	39.3	44.9
C18:3 n-3	1.90	2.77	3.76	4.61	5.37	1.73	2.63	3.50	4.37	5.27
SFA[3]	45.2	38.4	30.8	23.5	16.4	45.9	38.8	31.6	24.0	16.8
MUFA[3]	31.5	31.7	32.3	32.7	33.5	31.2	31.4	31.9	32.3	33.0
PUFA[3]	23.3	29.9	36.9	43.8	50.1	22.9	29.8	36.5	43.7	50.2
UFA:SFA[3]	1.21	1.60	2.25	3.25	5.09	1.18	1.58	2.16	3.17	4.94

[1]All samples were analyzed at least in duplicate.

[2]Diets with 6% of blends of re-esterified palm oil with re-esterified soybean oil (P:S; 0:100, 75:25, 50:50, 25:75, and 0:100).

[3]MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; SFA = Saturated fatty acids; UFA = Unsaturated fatty acids.

was measured and total excreta was collected, weighed, and homogenized, and a representative sample was frozen at -20°C. Contaminants such as feed, feathers, down, and scales were removed. Then, the excreta samples were freeze-dried, ground and kept at 5°C until further analysis. Excreta samples were analyzed by the same methods as those described for feeds, to determine the apparent absorption of FA and the AME of the diets. The apparent absorption coefficients of the nutrients were calculated as the difference between the amount ingested and the amount excreted, expressed as the percentage of the amount ingested. The AME was calculated from the product of energy apparent absorption and its corresponding feed gross energy.

At the end of the experimental period, the 41-day-old broiler chickens were fasted for 3 h, stunned, slaughtered, bled, plucked, and chilled at 4°C for 12 h in a local slaughterhouse (Gimave S.A.; Ripollet, Barcelona, Spain). Carcasses (total BW excluding blood and feathers) were weighed, and the liver and abdominal fat pad (from the proventriculus surrounding the gizzard down to the cloaca) for each bird were removed and weighed. The relative weights of the abdominal fat pad and liver were expressed as percentage of carcass weight. A representative sample of the abdominal fat pad (n = 6 samples/treatment, one from each replicate) was taken and frozen at -20°C. The FA composition of the abdominal fat pad was determined by the method of Carrapiso et al. (2000).

Statistical Analysis

Normality of the data and homogeneity of the variance were verified. All data were subjected to a one-way ANOVA using the GLM procedure of SAS (version 9.2, SAS Institute Inc.; Cary, NC). All data were subjected to orthogonal polynomial contrasts to examine whether responses to increasing dietary UFA:SFA (variability as a result of feeding different proportions of re-esterified palm and soybean oils) were linear or quadratic. The linear and quadratic trends were studied for not equally spaced levels. Prediction equations were obtained using the REG procedure of the same statistical package. The cage served as the experimental unit, and there were 6 experimental units per treatment. For abdominal fat pad and liver weights, the broiler carcass weight was included as a covariate in the model, to correct these variables for variations not related to the dietary treatment effect. Results in tables are reported as least-square means and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Experimental Fats and Diets

Results from the chemical analyses of the experimental fats are presented in Table 1. Re-esterified palm and soybean oils had similar levels of monounsaturated fatty acids (**MUFA**; 39.1 $\pm$ 2.33%), but they differed

Table 4. Apparent absorption coefficients (%) according to different dietary re-esterified oil blends.[1]

| | Dietary treatments[2] | | | | | | |
Item	P	75P:25S	50P:50S	25P:75S	S	RMSE[3]	P-values[4]
From 7 to 10 d							
AME, kcal/kg	2,891	3,014	2,929	3,051	3,079	103.1	L**
Total fatty acids	47.5	55.8	51.6	67.0	66.9	10.30	L**
SFA[5]	41.1	49.8	44.8	59.9	57.2	11.35	L*
MUFA[5]	53.9	59.3	53.0	68.5	67.4	10.76	L*
PUFA[5]	51.2	59.9	56.0	69.7	69.7	9.28	L***
C16:0	41.8	51.2	48.0	63.5	62.3	10.71	L**
C18:0	27.3	37.3	32.1	49.6	49.5	12.98	L**
C18:1 n-9	54.7	60.1	54.0	69.3	67.9	10.61	L*
C18:2 n-6	50.7	59.2	55.0	69.0	69.0	9.41	L**
C18:3 n-3	57.2	66.5	64.6	76.3	76.0	7.96	L***
From 36 to 38 d							
AME, kcal/kg	3,022	3,077	3,085	3,081	3,035	109.6	NS
Total fatty acids	81.6	84.3	85.2	87.8	85.6	2.75	L*; Q**
SFA[5]	75.8	79.7	80.4	85.3	80.3	3.76	L*; Q***
MUFA[5]	88.5	88.6	88.3	89.4	86.8	2.28	NS
PUFA[5]	84.1	85.9	86.7	87.9	86.6	2.29	NS
C16:0	76.3	80.5	81.5	86.4	82.0	3.47	L**; Q***
C18:0	69.9	74.1	74.2	81.9	78.0	4.77	L**; Q**
C18:1 n-9	88.6	88.8	88.5	89.5	87.0	2.25	NS
C18:2 n-6	84.0	85.7	86.5	87.7	86.4	2.31	NS
C18:3 n-3	84.8	87.4	88.5	89.8	88.6	2.03	L**; Q**

[1]Diets with 6% of blends of re-esterified palm oil with re-esterified soybean oil (P:S; 0:100, 75:25, 50:50, 25:75, and 0:100) corresponded to dietary unsaturated-to-saturated fatty acid ratios of 1.21, 1.60, 2.25, 3.25, and 5.09 for starter period and 1.18, 1.58, 2.16, 3.17, and 4.94 for grower-finisher period, respectively.

[2]$n = 6$.

[3]RMSE = Root-mean square error.

[4]The orthogonal polynomial contrasts were performed according to the dietary unsaturated-to-saturated fatty acid ratios. L = linear; Q = quadratic. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[5]MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; SFA = Saturated fatty acids.

in the content of SFA and polyunsaturated fatty acids (**PUFA**). Whereas re-esterified palm oil was mainly composed of palmitic acid (46.6%), re-esterified soybean oil was mainly composed of linoleic acid (40.4%). The FA composition of re-esterified palm oil was very similar to that of palm native oils (FEDNA, 2010), but the FA composition of re-esterified soybean oil showed a higher oleic acid content and a lower linoleic acid content than did that of soybean native oils (according to FEDNA, 2010, the oleic and linoleic acid contents of soybean native oils are 22 and 54%, respectively). In any case, they showed very different degrees of saturation, expressed as the UFA:SFA, which ranged from 0.85 to 6.09 for re-esterified palm and soybean oils, respectively. However, the UFA:SFA of the diets was slightly narrower (Table 3; from 1.21 to 5.09 for starter diets and from 1.18 to 4.94 for grower-finisher diets), due to the contribution of the lipid content of the basal diet.

Regarding the FA positional distribution, only small differences were observed between both experimental fats. Whereas PUFA were almost randomly distributed among all three positions of the acylglycerol molecules, SFA were mainly located at the *sn*-1,3 positions. This FA positional distribution of re-esterified oils was slightly different from that of their corresponding native oils, in which PUFA are predominantly located at the *sn*-2 position and SFA at the *sn*-1,3 positions (Mattson and Volpenhein, 1963).

Concerning the acylglycerol composition, both re-esterified oils showed a similar pattern: a high amount of triacylglycerols ($54.7 \pm 0.42\%$), a moderate level of diacylglycerols ($36.5 \pm 2.97\%$), and a small amount of monoacylglycerols ($7.48 \pm 2.326\%$). Thus, both oils showed a similar glycerol-to-FA ratio (0.43 ± 0.014 mol/mol) and an identical gross-energy content ($9,223 \pm 0.7$ kcal/kg). These values contrast with those of native oils, which are almost entirely composed of triacylglycerols, with a glycerol-to-FA ratio of 0.33 mol/mol (Vilarrasa et al., 2014).

Digestibility Balance

The effects of increasing the dietary UFA:SFA by blends of re-esterified oils on the apparent absorption coefficients in both starter (7 to 10 d) and grower-finisher (36 to 38 d) periods are presented in Table 4. As expected, the absorption of long-chain UFA was substantially higher when compared with that of long-chain SFA, as has already been reported by several authors using native oils (Renner and Hill, 1961; Young and Garrett, 1963). Figure 1 shows the relationship between
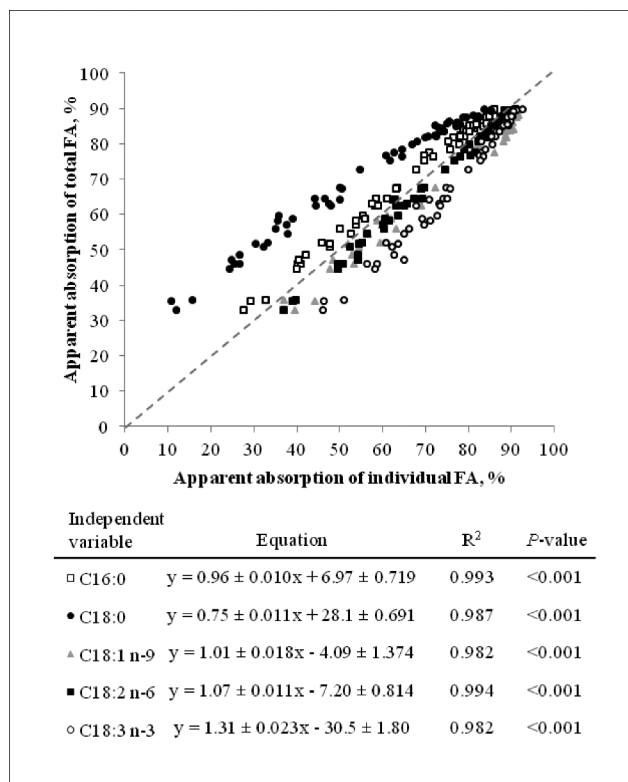
Figure 1. Relationship between the absorption of individual and total fatty acids (FA), in broiler chickens fed re-esterified oil blends.

the absorption of individual and total FA coming from diets supplemented with blends of re-esterified oils. For the linear regression analysis, we pooled data from the 2 digestibility balances. As expected, the absorption of individual FA was directly related to the absorption of total FA. However, the slopes of the equations showed how, as the FA chain length increased, the contribution of individual FA to total FA apparent absorption was reduced (0.956 and 0.753 for C16:0 and C18:0, respectively). In contrast, as the number of double bonds increased, the contribution of individual FA to total FA apparent absorption also increased (0.753, 1.01, 1.07, and 1.31 for C18:0, C18:1 n-9, C18:2 n-6, and C18:3 n-3, respectively). The slope of the equations also shows the interactions that exist among FA. Thus, a slope above 1 (linoleic and linolenic acids; $P < 0.05$) would indicate a synergistic effect and a slope below 1 (palmitic and stearic acids; $P < 0.05$) would indicate an antagonistic effect. The results indicate that PUFA, due to their emulsifying capacities, can promote the incorporation of SFA into mixed micelles and, therefore, improve the total FA apparent absorption, as has already been described by several authors (Young and Garrett, 1963; Garrett and Young, 1975; Krogdahl, 1985; Wiseman and Lessire, 1987; Ketels and de Groote, 1989). In contrast, SFA, due to their apolarity and high melting points, may also exert an antagonistic effect on UFA apparent absorption, as Wiseman and Lessire (1987) and Ketels and de Groote (1989) also suggested.

Regarding the effect of age on FA apparent absorption, the coefficients achieved in the starter period were lower than were those found in the grower-finisher pe-

riod, since the capacity of young birds to secrete bile salts is limited (Krogdahl, 1985). In any case, in both young and adult broiler chickens, the absorbability of total FA increased with increasing inclusion rates of re-esterified soybean oil in substitution of re-esterified palm oil.

In the starter period, the total FA apparent absorption varied linearly ($P = 0.001$) from 47.5 to 66.9% with an increase in the dietary UFA:SFA ratio from 1.21 to 5.09, which means that young birds were not able to achieve the biological maximum of fat absorption, probably due to the lack of bile salts (Krogdahl, 1985). The simple linear regression analysis yielded the following equation $y = 0.20 \pm 0.05x + 47.7 \pm 3.30$ ($R^2 = 0.306$, $P < 0.001$), where y is the total FA apparent absorption and x is the percentage of re-esterified soybean oil in the replacement of re-esterified palm oil (at a 6% inclusion level in feed). Thus, the maximum total FA apparent absorption coefficient was achieved for the most unsaturated diet (100% re-esterified soybean oil). The greater absorbability of total FA due to the increasing UFA:SFA was, in part, due to the growing presence of linoleic acid, but also due to the significant improvement in the absorbability of SFA (linear effect, $P = 0.013$), as has also been reported by several authors using native oils or pure FA (Young and Garrett, 1963; Garrett and Young, 1975; Wiseman and Lessire, 1987; Ketels and de Groote, 1989). In addition, the increasing dietary UFA:SFA also exerted a significant improvement in the absorbability of MUFA and PUFA (linear effect, $P = 0.016$ and $P < 0.001$ for MUFA and PUFA, respectively), as also reported Wiseman and Lessire (1987) and Ketels and de Groote (1989). These data support the results of Figure 1, confirming that the presence of UFA improves the SFA apparent absorption, but the presence of SFA may also impair the absorption of MUFA and PUFA in young broiler chickens, with a limited bile concentration.

In the grower-finisher period, the total FA apparent absorption was also improved with the increase of the dietary UFA:SFA, but to a lesser extent (the magnitude of the difference was reduced nearly 5 times, from 81.6 to 85.6%, with an increase in the dietary UFA:SFA ratio from 1.18 to 4.98), and the increase was quadratic ($P = 0.006$). The best-fit prediction equation for values of total FA apparent absorption, as a function of the percentage of replacement of re-esterified palm oil by re-esterified soybean oil (at a 6% inclusion level in feed) was: $y = -0.001 \pm 0.0005x^2 + 0.14 \pm 0.050x + 81.5 \pm 1.05$ ($R^2 = 0.291$, $P = 0.004$). In this case, the maximum total FA apparent absorption coefficient was achieved for a fat blend of 25% of re-esterified palm oil mixed with 75% of re-esterified soybean oil, which corresponded to a dietary UFA:SFA of 3.17. This UFA:SFA was slightly lower than was that found by Ketels and de Groote (1989), who reported that fat digestibility reached a near-asymptotical maximum at a dietary UFA:SFA of 4 or more using native oils. This lower UFA:SFA may be due to the beneficial effects that the increased sn-2 SFA content, and monoacylglycerol

Table 5. Growth performance and carcass fat depots of broiler chickens according to different dietary re-esterified oil blends.[1]

| Item | | Dietary treatments[2] | | | | | RMSE[3] | P-values[4] |
		P	75P:25S	50P:50S	25P:75S	S		
From 0 to 20 d								
ADFI, g/d per bird		49.3	49.3	50.0	48.2	46.2	2.29	NS
ADG, g/d per bird		32.9	34.2	34.7	34.9	32.4	2.47	NS
FCR[5], g/g		1.50	1.45	1.44	1.38	1.44	0.073	NS
BW at 20 d, g		704	735	739	747	695	55.9	NS
From 20 to 41 d								
ADFI, g/d per bird		168	165	169	168	164	4.8	NS
ADG, g/d per bird		92.1	92.7	93.5	92.9	93.5	2.94	NS
FCR[5], g/g		1.83	1.78	1.81	1.80	1.75	0.044	NS
BW at 41 d, g		2,631	2,682	2,703	2,692	2,657	67.3	NS
From 0 to 41 d								
ADFI, g/d per bird		110	109	111	109	106	2.94	NS
ADG, g/d per bird		63.2	64.2	64.8	64.6	63.7	1.60	NS
FCR[5], g/g		1.75	1.69	1.71	1.69	1.67	0.031	L**
Carcass fat depots								
Abdominal fat,	g	53.9	56.8	55.8	59.0	52.1	6.58	NS
	%	2.21	2.40	2.36	2.48	2.21	0.272	NS
Liver,	g	50.8	50.5	52.0	52.8	50.8	2.81	NS
	%	2.16	2.12	2.17	2.23	2.14	0.113	NS

[1]Diets with 6% of blends of re-esterified palm oil with re-esterified soybean oil (P:S; 0:100, 75:25, 50:50, 25:75, and 0:100) corresponded to dietary unsaturated-to-saturated fatty acid ratios of 1.21, 1.60, 2.25, 3.25, and 5.09 for starter period and 1.18, 1.58, 2.16, 3.17, and 4.94 for grower-finisher period, respectively.

[2]$n = 6$.

[3]RMSE = Root-mean square error.

[4]The orthogonal polynomial contrasts were performed according to the dietary unsaturated-to-saturated fatty acid ratios. L = linear. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

[5]FCR = feed conversion ratio.

and diacylglycerol proportions of re-esterified oils exert on fat absorption, when compared with native oils (Vilarrasa et al., 2015). As in the starter period, the increasing dietary UFA:SFA ratio exerted a favorable effect in the apparent absorption of SFA (quadratic effect, $P = 0.001$), but no differences were found for MUFA and PUFA ($P > 0.05$), suggesting that UFA apparent absorption in adults was not influenced by composition of the fat mixtures, in agreement with the results of Wiseman and Lessire (1987).

Although several authors have found synergism between native fats of different degrees of saturation (Sibbald et al., 1960; Lewis and Payne, 1966), when we added re-esterified soybean oil to re-esterified palm oil, the total FA apparent absorption coefficients of the mixtures (determined) did not reach significantly greater values than did the sum of the means of the component parts (calculated) ($P > 0.05$). In this sense, although we found associative effects among FA, we failed to observe synergistic interactions between re-esterified oils, in agreement with the results of Wiseman and Lessire (1987). This could be due to neutralization of the synergistic and antagonistic effects observed among FA.

Growth Performance and Carcass Fat Depots

The effects of increasing the dietary UFA:SFA by blends of re-esterified oils on growth performance in both starter (0 to 20 d) and grower-finisher (20 to 40 d) periods are presented in Table 5. In the overall period, FCR was improved significantly (linear effect, $P = 0.003$) with the increase of the dietary UFA:SFA, as has also been observed by several authors (Pinchasov and Nir, 1992; Zollitsch et al., 1997; Dvorin et al., 1998; Dänicke et al., 2000; Crespo and Esteve-Garcia, 2001; Wongsuthavas et al., 2008), using native oils. The reason for the lower FCR in broiler chickens fed more unsaturated re-esterified-oil blends was probably the higher total FA apparent absorption observed in both starter and grower-finisher periods. However, in this study, feed intake and BW gain were not significantly ($P > 0.05$) affected by the dietary UFA:SFA, neither in the starter nor in the grower-finisher period, in agreement with the results of other authors (Pinchasov and Nir, 1992; Sanz et al., 1999, 2000a,b; Newman et al., 2002), using native oils.

The effects of increasing the dietary UFA:SFA by blends of re-esterified oils on carcass fat depots are presented in Table 5. The abdominal fat pad and liver weights were not significantly affected by the dietary fat saturation degree ($P > 0.05$). Pinchasov and Nir (1992) and Dvorin et al. (1998) also found no differences in the abdominal fat pad weight of broiler chickens fed native fat sources with extreme degrees of saturation. In contrast, several other studies (Sanz et al., 1999, 2000a;b; Newman et al., 2002; Crespo and Esteve-Garcia, 2002a,b; Ferrini et al., 2008; González-Ortiz et al., 2013) have found lower abdominal fat-pad weights in broiler chickens fed unsaturated native fat sources in comparison with those fed saturated ones.

Table 6. Fatty acid composition (%) of abdominal adipose tissue according to different dietary re-esterified oil blends.[1]

| Item | Dietary treatments[2] | | | | | RMSE[3] | P-values[4] |
	P	75P:25S	50P:50S	25P:75S	S		
C14:0	0.91	0.79	0.66	0.54	0.39	0.032	L***; Q***
C16:0	29.4	27.9	25.7	23.1	19.6	0.73	L***; Q**
C16:1 n-9	5.41	5.08	4.39	4.48	3.38	0.529	L***
C18:0	4.93	5.12	5.41	5.04	5.48	0.370	NS
C18:1 n-9	43.6	41.8	41.1	40.0	39.7	1.15	L***; Q**
C18:1 n-7	1.98	2.01	1.99	2.13	2.09	0.139	NS
C18:2 n-6	11.2	14.4	17.4	20.8	24.9	0.93	L***; Q***
C18:3 n-3	0.88	1.31	1.78	2.26	2.83	0.086	L***; Q***
C20:1 n-9	0.32	0.33	0.35	0.37	0.41	0.017	L***
Minor fatty acids	1.28	1.28	1.24	1.21	1.20	0.115	NS
SFA[5]	35.9	34.4	32.3	29.1	25.8	0.87	L***; Q**
MUFA[5]	51.6	49.5	48.1	47.3	45.8	1.35	L***; Q*
PUFA[5]	12.4	16.1	19.6	23.6	28.4	1.05	L***; Q***
UFA:SFA[5]	1.79	1.90	2.10	2.44	2.88	0.088	L***

[1]Diets with 6% of blends of re-esterified palm oil with re-esterified soybean oil (P:S; 0:100, 75:25, 50:50, 25:75, and 0:100) corresponded to dietary unsaturated-to-saturated fatty acid ratios of 1.18, 1.58, 2.16, 3.17, and 4.94, respectively.

[2]n = 6.

[3]RMSE = Root-mean square error.

[4]The orthogonal polynomial contrasts were performed according to the dietary unsaturated-to-saturated fatty acid ratios. L = linear; Q = quadratic. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[5]MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; SFA = Saturated fatty acids; UFA = Unsaturated fatty acids.

However, most of them used more saturated fat sources (such as tallow, with a higher stearic acid content) and more unsaturated fat sources (such as linseed oil or sunflower oil, with a higher linolenic and linoleic acid content, respectively), than the re-esterified oils used in this study (Table 1), which could explain why we did not find differences. Moreover, in contrast to native fats, our re-esterified oils showed an important content of 1,3-diacylglycerols, which have been involved with anti-obesity effects in rodents, due to their ability to increase the β-oxidation of FA in the liver and the intestine (Murase et al., 2002; Meng et al., 2004), which may dilute the effects of the dietary degree of saturation.

Fatty Acid Composition of Abdominal Adipose Tissue

The effects of increasing the dietary UFA:SFA by blends of re-esterified palm and soybean oils on FA composition of abdominal adipose tissue are presented in Table 6. As the dietary UFA:SFA increased, the deposition of PUFA increased (linear effect, $P < 0.001$), and that of SFA and MUFA decreased (linear effect, $P < 0.001$). In Table 7, linear regression equations between dietary and deposited FA (percent total FA) are presented. It can be observed that the magnitude of the difference found among treatments for SFA and PUFA was higher in diets than that found in abdominal adipose tissue, although MUFA behaved the opposite. Therefore, variations in the dietary UFA:SFA of 3.76 units only resulted in 1.09 units difference for the abdominal adipose tissue UFA:SFA, indicating the existence of a physiological mechanism that maintains the UFA:SFA of body fat inside a relatively narrow range, which was also stated by Villaverde et al. (2006). Furthermore, the slopes of the regression lines show that PUFA are more easily modified than SFA in the abdominal adipose tissue and, in this sense, it is interesting to note the similarity of the slope of the regression line obtained for PUFA in the present study (0.58) to those observed by Beynen et al. (1980), and Waldroup and Waldroup (2005) with men (0.54) and broiler chickens (0.55), respectively, using native oils.

To gain insight into the mechanism by which the dietary fat degree of saturation affects the FA composition of abdominal adipose tissue, we calculated the deposited-to-absorbed FA ratio, according to the dietary UFA:SFA (Figure 2). Whereas values above 1 indicate an increased abdominal adipose tissue content of a particular FA with respect to the fat absorbed (i.e., net de novo synthesis, FA interconversions, or deposit preference), values below 1 indicate the opposite (i.e., net β-oxidation or FA inter-conversions). Regardless of the dietary UFA:SFA, the deposited-to-absorbed PUFA ratio remained almost constant and below 1, as has already been observed by other authors (Villaverde et al., 2006; Wongsuthavas et al., 2011) using native oils. This is consistent with the well-known preferential β-oxidation of PUFA (Leyton et al., 2000; DeLany et al., 2000; Sanz et al., 2000b; Ferrini et al., 2010; Wongsuthavas et al., 2011), and the fact that the parent PUFA, linoleic and linolenic acids, are essential FA and, by definition, are not synthesized by the birds (NRC, 1994).

The relationship between dietary and tissue concentration of SFA and MUFA is more complex because
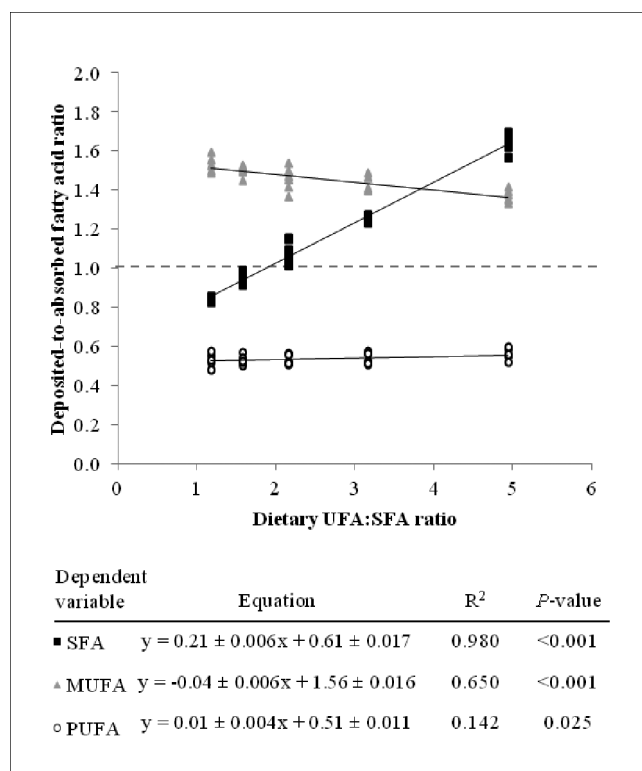
Table 7. Relationship between dietary and deposited fatty acid profile (% total fatty acids) in broiler chickens fed re-esterified oil blends.

Item	Dietary Δ, %	Deposited Δ, %	Equation[1]	R^2	P-value
C16:0	26.4	9.86	$y = 0.38 \pm 0.017x + 15.3 \pm 0.47$	0.945	<0.001
C18:0	1.02	0.55	$y = -0.41 \pm 0.208x + 6.80 \pm 0.820$	0.092	0.061
C18:1 n-9	0.50	3.91	$y = -4.93 \pm 1.077x + 193 \pm 33.1$	0.416	<0.001
C18:2 n-6	23.8	13.7	$y = 0.57 \pm 0.021x - 1.05 \pm 0.731$	0.963	<0.001
C18:3 n-3	3.54	1.95	$y = 0.55 \pm 0.013x - 0.12 \pm 0.051$	0.984	<0.001
SFA[2]	29.1	10.1	$y = 0.35 \pm 0.018x + 20.4 \pm 0.59$	0.932	<0.001
MUFA[2]	1.80	5.87	$y = -2.87 \pm 0.409x + 140 \pm 13.1$	0.634	<0.001
PUFA[2]	27.3	16.0	$y = 0.58 \pm 0.021x - 1.13 \pm 0.797$	0.965	<0.001
UFA:SFA[2]	3.76	1.09	$y = 0.29 \pm 0.012x + 1.46 \pm 0.036$	0.953	<0.001

[1]The independent variable (x) corresponds to the dietary fatty acid content (%), and the dependent variable (y) corresponds to the deposited fatty acid content in abdominal adipose tissue (%).

[2]MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; SFA = Saturated fatty acids; UFA = Unsaturated fatty acids.

Dependent variable	Equation	R^2	P-value
■ SFA	$y = 0.21 \pm 0.006x + 0.61 \pm 0.017$	0.980	<0.001
▲ MUFA	$y = -0.04 \pm 0.006x + 1.56 \pm 0.016$	0.650	<0.001
○ PUFA	$y = 0.01 \pm 0.004x + 0.51 \pm 0.011$	0.142	0.025

Figure 2. Deposited-to-absorbed fatty acid ratio of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) in response to increasing dietary unsaturated-to-saturated fatty acid ratio (UFA:SFA).

these FA in animals have a double origin: exogenous (dietary) and de novo FA synthesis (from carbohydrate and protein precursors) (Villaverde et al., 2006). As the dietary UFA:SFA increased, the deposited-to-absorbed MUFA ratio slightly decreased and that of SFA markedly increased (Figure 2). The deposited-to-absorbed MUFA ratio was always above 1, indicating that, regardless of the dietary degree of saturation, there was always a net synthesis, as has also been observed by other authors (Villaverde et al., 2006; Wongsuthavas et al., 2011) using native oils. Actually, MUFA are the main FA synthesized from glucose in broiler chickens (Ferrini et al., 2010). In contrast, the deposited-to-absorbed SFA ratio was lower than 1 in birds receiving diets with a UFA:SFA below 1.89. Given that FA oxidation is more related to PUFA than to SFA (Leyton et al., 2000; DeLany et al., 2000; Sanz et al., 2000b; Ferrini et al., 2010; Wongsuthavas et al., 2011), the disappearance of SFA in broilers fed highly saturated diets was probably, in part, due to the desaturation process leading to the formation of MUFA. However, in more unsaturated diets, the deposited-to-absorbed SFA ratio increased above 1, probably due to decreased conversion of SFA into MUFA. In fact, it has been observed that high dietary linoleic acid content inhibits the Δ9-desaturase enzyme in the liver (Kouba and Mourot, 1998), resulting in a lower oleic acid synthesis.

In conclusion, feeding broiler chickens with increasing dietary UFA:SFA, by adding re-esterified soybean oil in replacement of re-esterified palm oil, resulted in an improved total FA apparent absorption, although no synergism was observed between re-esterified oils.

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