

Effects of free-fatty-acid content and saturation degree of the dietary oil sources on lipid-class content and fatty-acid digestibility along the gastrointestinal tract in broilers from 22 to 37 days of age

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ABSTRACT The aim of the present study is to assess the effect of the free-fatty-acid (**FFA**) content and saturation degree of dietary fat (added at 6%) on the fatty-acid (**FA**) digestibility and lipid-class content along the gastrointestinal tract and excreta in broilers from 22 to 37 d of age. This is essential to determine the potential use of acid oils (refining by-products rich in FFA) in broiler diets as an alternative to crude oils. The study consisted of a 2 × 4 factorial arrangement, which included 2 fat sources (soybean oils – unsaturated, or palm oils – saturated) and 4 levels of FFA (5, 15, 35, and 50%). Samples of digestive content of the gizzard, duodenum, jejunum, ileum and of the excreta were obtained at 37 d of age. Irrespective of the dietary fat source, more than 80% of total FA (**TFA**) was absorbed in the jejunum. Broilers fed with unsaturated diets had a higher absorption efficiency of FA than did those fed with saturated diets. This conclusion is supported by

the lower FFA content and the higher TFA and poly-unsaturated FA (PUFA) digestibility coefficients in the ileum ($P < 0.001$) observed in the former group. The dietary FFA level did not affect the FA absorption process as much as the dietary fat source did. This was supported by the lack of statistical differences among the diets with a similar saturation degree but rather different levels of FFA, for TFA, saturated FA, and PUFA digestibility coefficients both in the jejunum and ileum. However, the interactions reported in the ileum for triacylglycerol and diacylglycerol contents ($P < 0.001$), as well as for monounsaturated FA digestibility coefficients ($P < 0.05$) show that the dietary FFA content affects the FA absorption process. The present results show that the inclusion of acid oils in grower-finisher broiler diets with FFA levels up to 35% does not have a negative impact on the FA absorption process.

Key words: acid oil, broiler, fatty-acid digestibility, free-fatty-acid, lipid class

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INTRODUCTION

The use of supplemental fats is a common practice for meeting both energy and essential fatty-acid (**FA**) requirements in poultry diets. Acid oils are an example

of an economic alternative, in comparison to conventional fats, that can be repurposed as a feed fat ingredient. These fat by-products come from the chemical (called acid oils from chemical refining; e.g., soybean acid oil) or physical (called FA distillates from physical refining; e.g., palm FA distillate) refining processes of edible oils. In the text, they will be generically called acid oils unless otherwise stated. Acid oils are rich in free fatty acids (**FFA**; 32–94%, Varona et al., 2021), and their FA composition is related to the botanical origin of the crude oil. Nevertheless, the molecular structure of acylglycerols, and the presence of energy-diluting compounds, can affect their energy value (Roll et al., 2018; Varona et al., 2021).

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It is well documented that the saturation degree of dietary fat has an important effect on fat utilization because unsaturated fatty acids (**UFA**) are easier to digest and absorb than are saturated fatty acids (**SFA**; Renner and Hill, 1961; Sklan, 1979; Tancharoenrat et al., 2014; Vilarrasa et al., 2015a). It has been described that the saturation degree of the diet has a greater effect than does its FFA content on the fat absorption process (Vilarrasa et al., 2015b; Rodriguez-Sanchez et al., 2019b; Jimenez-Moya et al 2021). However, there is a lack of consensus regarding the effect of dietary FFA on fat digestion and absorption processes. Thus, while some authors have reported that dietary FFA content negatively affects dietary fat digestibility and feed AME values (Shannon, 1971; Wiseman and Salvador, 1991; Wiseman et al., 1992; Blanch et al., 1995, 1996; Vilà and Esteve-Garcia, 1996), others have reported no effects on dietary AME (Vilarrasa et al., 2015b; Roll et al., 2018) and growth performance (Young, 1961; Bornstein and Lipstein, 1961, 1963; Artman, 1964; Zumbado et al., 1994). The age of the chickens could be a determining factor in the outcome of these studies, as the ability of chickens to digest and absorb dietary fat improves with the age (Renner and Hill, 1960; Batal and Parsons, 2002; Tancharoenrat et al., 2013; Rodriguez-Sanchez et al., 2019a). Understanding how FFA affect the fat absorption process is essential to determine the potential use of acid oils in broiler diets.

The global aim of this study is to assess the effect of the FFA content and dietary fat saturation degree on FA digestibility and lipid-class composition along the gastrointestinal tract, and also on excreta in broilers from 22 to 37 d of age. The same effects in broilers from 11 to 14 d of age are reported in Rodriguez-Sanchez et al. (2019b). A completely random design with a 2 × 4 factorial arrangement was applied, which included 2 dietary fat sources (soybean oils – unsaturated, or palm oils – saturated), and 4 levels of FFA (5, 15, 35 and 50%). Thus, there were 8 dietary treatments with 6% added fat, which resulted from replacing crude soybean oil with soybean acid oil, or crude palm oil with palm FA distillate in different proportions. The results from this study give new insights, regarding the use of acid oils as a feed fat ingredient, and support the importance of continuing work towards finding the best way to use them as alternative fat sources in broiler diets.

MATERIAL AND METHODS

Animals and Diets

The experimental procedure was approved by the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona (Bellaterra, Barcelona, Spain), and conformed to the European Union Guidelines (2010/63/EU).

A total of 528 one-day-old female Ross 308 broilers were obtained from a commercial hatchery (Pondex SAU; Lleida, Spain), and randomly assigned to one of

Table 1. Ingredient composition, and energy and nutrient content of the experimental basal diet.

Item	Grower-finisher diet (from 22 to 37 d)
<i>Ingredients (%)</i>	
Wheat	44.00
Soybean meal 48% CP	27.25
Barley	18.58
Experimental fats ¹	6.00
Calcium carbonate	1.39
Sodium chloride	0.35
Monocalcium phosphate	1.20
Vitamin and mineral premix ²	0.40
DL-Methionine	0.17
L-Lysine	0.12
L-Threonine	0.02
Titanium oxide	0.50
Ethoxyquin 66%	0.02
<i>Calculated energy and nutrient contents (%)</i>	
AMEn ³ (kcal/kg)	3,075
Crude protein	20.30
Calcium	0.90
Sodium	0.15
Available phosphorus	0.40
Digestible Lys	1.10
Digestible Met	0.47
Digestible Met + Cys	0.83
Digestible Thr	0.74
Digestible Trp	0.25

¹Crude soybean oil, crude palm oil, acid soybean oil from chemical refining or palm fatty acid distillate from physical refining.

²Provides per kg of feed: vitamin A (from retinol), 10,000 IU; vitamin D₃ (from cholecalciferol), 4,800 IU; vitamin E (from alpha-tocopherol), 49.5 IU; vitamin B₁, 3 mg; vitamin B₂, 9 mg; vitamin B₆, 4.5 mg; vitamin B₁₂, 16.5 µg; vitamin K₃, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from FeSO₄·7H₂O), 54 mg; I (from Ca(I₂O₃)₂), 1.2 mg; Cu (from CuSO₄·5H₂O), 12 mg; Mn (from MnO), 90 mg; Zn (from ZnO), 66 mg; Se (from Na₂SeO₃), 0.18 mg; Mo (from (NH₄)₆Mo₇O₂₄), 1.2 mg.

the 8 dietary treatments, with 11 chicks per cage and 6 cages per treatment. Birds were housed in wire-floor cages. 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 feeding program consisted of 2 phases (0–21 d and 22–37 d of age). All diets were based on wheat-soybean meal, fed in mash form, formulated to meet or exceed FEDNA requirements (2008) and to minimize the basal level of fat (Table 1). Titanium dioxide (TiO₂) was added (5 g/kg) as an inert marker. The level of inclusion of fat in the 8 experimental diets was 6%. The diets differed in the type of added fat according to a 2 × 4 factorial arrangement: 2 dietary fat sources (soybean oils – unsaturated, or palm oils – saturated), and 4 different levels of dietary FFA (Table 2). The lowest FFA level assayed for each fat source corresponded to the addition of a 6% of crude oil (either crude soybean [**SO**] or crude palm oil [**PO**]) to the feed. For the unsaturated diets (**S**), the highest level of FFA corresponded to the use of acid soybean oil from chemical refining (**SA**) instead of SO (Table 2). Then, a similar highest FFA level was designed for the saturated diets (**P**) by blending palm fatty-acid distillate from physical refining (**PA**) with PO (Table 2). Two intermediate treatments were achieved by adding SO and SA at 70:30 or 30:70, or PO and PA in the proportions detailed in

Table 2. Oil blends used in the experimental diets.¹

Proportion in oil blends (%)	Dietary fat Dietary FFA% Dietary treatments	Unsaturated - S				Saturated - P			
		5 S5	15 S15	35 S35	50 S50	5 P5	15 P15	35 P35	50 P50
Crude soybean oil (SO)		100	70	30	-	-	-	-	-
Acid soybean oil from chemical refining (SA)		-	30	70	100	-	-	-	-
Crude palm oil (PO)		-	-	-	-	100	80	53	33
Palm fatty acid distillate from physical refining (PA)		-	-	-	-	-	20	47	66

Abbreviation: FFA, free fatty acids.

¹All oils or oil blends were added at 6% into the basal diet.

Table 2 to obtain dietary FFA levels as similar as possible to those of the unsaturated groups. The lipid-class composition, quality parameters, and gross energy of the 4 experimental oils are presented in Table 3.

Data Collection

Feed consumption and BW were measured weekly to calculate the ADFI, the ADG, and the feed conversion ratio (FCR) throughout the experiment. These were corrected for mortalities. At 37 d of age, 3 birds/cage were euthanized and the following samples were collected: content of the gizzard, duodenum (from the pyloric junction to the distal-most point of insertion of the duodenal mesentery), jejunum (from the distal-most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), ileum (from the junction with Meckel's diverticulum to 1 cm proximal to the ileocecal junction), and a representative sample of excreta of each cage. Each sample from all birds within each cage was pooled, homogenized, frozen at -20°C, lyophilized, and ground to pass through a 0.5-mm sieve. Then, the samples were kept at 4°C until further analyses. Another sampling took place at 14 d of age, and these results are presented and discussed in Rodriguez-Sanchez et al. (2019b).

Table 3. Lipid-class composition, quality parameters and energy content of the experimental oils.

Item	Experimental oils			
	SO ¹	SA ²	PO ³	PA ⁴
<i>Lipid-class composition (%)</i>				
TAG	94.40	20.40	84.08	2.10
DAG	3.30	10.63	12.22	1.70
MAG	0.60	1.72	< 0.10	0.80
FFA	1.60	67.24	3.0	95.40
<i>Quality (%)</i>				
Moisture	0.08	0.69	0.06	0.04
Impurities	0.40	0.85	0.77	1.27
Unsaponifiable	0.52	2.78	0.31	0.42
<i>Energy content</i>				
Gross energy (kcal/kg)	9479	9461	9257	9417

Abbreviations: DAG, diacylglycerols; FFA, free fatty acids; MAG, monoacylglycerols; TAG, triacylglycerols.

¹SO = crude soybean oil.

²SA = acid soybean oil from chemical refining.

³PO = crude palm oil

⁴PA = palm fatty acid distillate from physical refining.

Chemical Analysis and Calculations

The experimental oils were analyzed in triplicate for moisture, unsaponifiable matter (AOAC International (2005) methods: 926.12 and 933.08, respectively), impurities (ISO 663:2007), and lipid-class composition (Rodriguez-Sanchez et al. 2019a; Table 3). Analytical determinations of the diets were performed following the AOAC International (2005) methods: DM (934.01), ash (942.05), CP (968.06), crude fat (2003.05), and crude fiber (962.09). Gross energy was determined by an adiabatic calorimeter (IKA C-4000, Janke-Kunkel; Staufen, Germany). The FA content of the feed, digestive content, and excreta was determined following the Sukhija and Palmquist (1988) method, and the digestibility coefficients of FA were determined using the TiO₂ ratio (Rodriguez-Sanchez et al., 2019b). The TiO₂ in the feed, digestive content and excreta was analyzed following the procedures of Short et al. (1996) and determined by ICP-OES (Optima 3200 RL, Perkin Elmer; Waltham, MA). The AME was determined from the product of the energy utilization ratio and its corresponding gross energy of feed. The lipid-class composition (triacylglycerols [TAG], diacylglycerols [DAG], monoacylglycerols [MAG], and FFA) of the extracted fat from the feed, digestive content, and excreta samples was analyzed as described by Rodriguez-Sanchez et al. (2019b). Lipid classes are a way to assess the hydrolysis of TAG, through their disappearance and the parallel appearance of the lipolysis products (DAG, MAG, and FFA), as well as the absorption of the latter through their disappearance from the intestinal lumen. The following formula was applied to determine the lipid-class content in the different GIT segments, and excreta:

$$\text{Lipid - class content} = [\text{LC}]\text{dig}/[\text{TiO}_2]\text{dig},$$

where [LC]dig is the concentration of the lipid classes in the digesta of a GIT segment or excreta (mg/g DM) and [TiO₂]dig is the concentration of TiO₂ in the digesta of the same GIT segment or excreta (mg/g DM). The macronutrient, FA and lipid-class composition of the experimental diets are presented in Table 4.

Statistical Analysis

Productive parameters, AME, lipid-class content and digestibility of FA were subjected to a univariate analysis using the GLM procedure (SPSS statistics 25.0.0.0,

Table 4. Analyzed¹ macronutrient content, fatty-acid and lipid-class composition of the experimental diets.²

Item	Grower-finisher diets							
	Unsaturated - S diets				Saturated - P diets			
	S5	S15	S35	S50	P5	P15	P35	P50
<i>Macronutrient content</i>								
Dry matter %	90.80	90.84	90.70	90.76	90.92	90.87	90.84	90.76
Crude protein %	19.29	19.89	20.16	19.93	19.83	20.09	19.49	19.75
Crude fat %	7.29	7.46	7.26	7.28	7.47	7.42	7.22	7.35
Crude fiber %	5.59	5.24	5.27	5.08	5.60	5.61	6.02	6.81
Ash %	6.12	6.15	6.01	6.28	5.76	6.04	6.04	6.14
Gross energy, kcal/kg	4,134	4,198	4,175	4,118	4,130	4,184	4,172	4,151
<i>Fatty acid composition, %</i>								
C14:0	-	-	-	-	1.1	1.1	1.0	1.0
C16:0	14.39	14.73	16.21	17.21	37.80	37.64	38.38	39.94
C18:0	4.02	4.18	4.62	4.71	3.85	3.83	3.78	3.88
C18:1 n-9	20.50	20.56	20.96	20.92	31.26	30.67	30.19	30.32
C18:1 n-7	1.35	1.36	1.34	1.34	0.85	0.85	0.82	0.80
C18:2 n-6	52.37	51.94	50.20	49.52	22.38	23.13	23.09	21.45
C18:3 n-3	6.12	5.90	5.26	4.79	1.71	1.78	1.79	1.71
Minor fatty acids	1.26	1.34	1.42	1.51	1.06	1.04	0.94	0.88
SFA	19.39	19.97	21.98	23.18	43.39	43.12	43.69	45.33
MUFA	22.12	22.19	22.56	22.51	32.51	31.97	31.43	31.52
PUFA	58.49	57.84	55.46	54.31	24.10	24.91	24.88	23.16
UFA:SFA ratio	4.16	4.01	3.55	3.31	1.30	1.32	1.29	1.21
<i>Lipid-class composition, %</i>								
TAG	96.03	79.67	56.50	35.42	83.23	73.05	56.72	44.99
DAG	2.59	3.84	6.06	8.01	8.83	7.63	5.76	4.29
MAG	0.49	0.66	1.48	2.28	0.70	0.92	0.90	1.06
FFA	0.89	15.83	35.96	54.28	7.23	18.39	36.63	49.66

Abbreviations: DAG, diacylglycerols; FFA, free fatty acids; MAG, monoacylglycerols; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TAG, triacylglycerols; UFA, unsaturated fatty acids.

¹All samples were analyzed at least in duplicate.

²Dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P): S5 - 100% crude soybean oil, S15 - oil blend with 70% crude soybean oil and 30% acid soybean oil from chemical refining (SA), S35 - oil blend with 30% crude soybean oil and 70% SA, S50 - 100% SA, P5 - 100% crude palm oil, P15 - oil blend with 80% crude palm oil and 20% palm fatty acid distillate from physical refining (PA), S35 - oil blend with 53% crude palm oil and 47% PA, S50 - oil blend with 33% crude palm oil and 66% PA.

IBM 2017). The model included the dietary fat type (soybean oils – unsaturated, or palm oils – saturated), and the FFA level (5, 15, 35, and 50%) as the main factors, as well as their interaction. Differences among treatment means were tested using Tukey's test for multiple comparisons. In the case of the lipid-class content and digestibility of FA, this analysis was performed for each GIT segment. A regression analysis was carried out for each GIT segment and fat type (unsaturated or saturated), in order to find the best-fit regression equations considering the SFA digestibility as the dependent variable and the content of dietary FFA as the independent variable. The cage served as the experimental unit, so there were 6 replicates per dietary treatment. Results in tables are reported as least-squares means, and differences were considered significant at $P < 0.05$.

RESULTS

Characterization of Experimental Diets

The chemical analysis and lipid-class characterization of the experimental diets (Table 4) reflected the composition of the added oils. The unsaturated-to-saturated FA ratio (UFA:SFA) of S diets was higher than in P diets. In both S and P diets, as the acid oil replaced the corresponding crude oil, the percentage of TAG decreased, and the percentage of MAG and FFA

increased. The percentage of DAG increased in S diets, but decreased in P diets, as the acid oil replaced the corresponding crude oil.

Growth Performance

Growth performance results are presented in Table 5. BW at 37 d of age was higher and the FCR was lower for the broilers fed S diets than for those fed P diets ($P < 0.01$). BW at 37 d was higher for the broilers fed the diets with 15% of FFA than for those fed the diets with 5 and 35% of FFA, but no differences were observed for the AME intake among the broilers fed the different diets.

Lipid-Class Content Along the Gastrointestinal Tract

The lipid-class content in the jejunum, ileum and excreta for the different dietary treatments is presented in Table 6. The broilers fed S diets showed a lower content of FFA in the jejunum, ileum, and excreta ($P < 0.001$) and a higher content of TAG in the jejunum and ileum than did those fed P diets. The broilers fed the diets with 5% of FFA showed a lower content of FFA in the jejunum, in comparison to those fed the diets with 15% FFA ($P < 0.05$), but no differences were observed

Table 5. Growth performance of broiler chickens (22 to 37d) according to different fat sources in the diet.¹

Item	Dietary treatments ²										FFA% ⁴				P values			
	Unsaturated – S diets					Saturated – P diets					5	15	35	50	SEM	Fat	FFA	Interaction
	S5	S15	S35	S50	P5	P15	P35	P50	S	P								
ADG, g/b/d	85.3	90.9	87.4	90.4	86.1	90.4	88.5	85.5	88.5	87.6	85.7	90.7	88.0	87.9	0.622	NS	NS	NS
ADFI, g/b/d	142.2	149.0	148.2	146.8	146.8	151.0	148.9	149.0	146.9	148.9	144.5	149.9	148.5	147.9	0.985	NS	NS	NS
FCR, g/g	1.67	1.67	1.70	1.63	1.73	1.69	1.71	1.74	1.66	1.72	1.70	1.68	1.70	1.68	0.008	**	NS	NS
BW at 37 d, g	2,181	2,337	2,190	2,290	2,156	2,280	2,194	2,172	2,256	2,193	2,152 ^b	2,309 ^a	2,192 ^b	2,244 ^{ab}	11,994	**	**	NS
AME intake, kcal/b/d	429.4	448.7	440.7	410.2	435.9	435.8	410.5	430.8	431.3	426.5	432.2	442.9	424.5	420.6	21.530	NS	NS	NS

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; FCR, feed conversion ratio.

P-values were obtained from the univariate GLM procedure of SPSS conducted to study whether the dietary treatments, the dietary fat saturation degree and the dietary FFA percentage affected the growth performance values.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^{a-b}Means in a row not sharing a common superscript letter are significantly different ($P < 0.05$).

¹Diets supplemented with 6% of an unsaturated (S) or saturated fat source (P): S5 - 100% crude soybean oil, S15 - oil blend with 70% crude soybean oil and 30% acid soybean oil from chemical refining (SA), S35 - oil blend with 30% crude soybean oil and 70% SA, S50 - 100% SA, P5 - 100% crude palm oil, P15 - oil blend with 80% crude palm oil and 20% palm fatty acid distillate from physical refining (PA), S35 - oil blend with 53% crude palm oil and 47% PA, S50 - oil blend with 33% crude palm oil and 66% PA.

²Values are means of 6 cages with 3 chickens/cage.

³Values are means of 24 cages with 3 chickens/cage.

⁴Values are means of 12 cages with 3 chickens/cage.

for TAG. A significant interaction between the dietary fat saturation degree and FFA percentage was observed for DAG and MAG content in the jejunum ($P < 0.05$); the broilers fed the S50 diet had higher DAG and MAG content than did those fed the P50 diet, but no differences were observed among the other diets. Interactions were also reported in the ileum for TAG, DAG and FFA content. While the TAG content was higher for the broilers fed S35 and S50 diets than for those fed the S5 diet, no differences were observed among the ones fed P diets ($P < 0.01$). Similarly, broilers fed the S50 diet showed higher DAG content than did those fed S5, S15, and all P diets ($P < 0.001$). Regarding FFA, while broilers fed S diets had similar FFA content in the ileum, those fed the P15 diet had higher FFA content, in comparison to those fed P5 and P50 diets ($P < 0.001$). In the excreta, the broilers fed the diets with 5% of FFA had the lowest FFA content ($P < 0.001$). Interactions were reported for DAG ($P < 0.05$) and MAG ($P < 0.01$) content; the broilers fed the S35 diet showed higher DAG content than did the ones fed S5, P5 and P50 diets, and the MAG content was higher for the broilers fed the P50 diet than for the ones fed the other P diets.

Apparent Fatty-Acid Digestibility Along the Gastrointestinal Tract and Apparent Metabolizable Energy

The FA digestibility coefficients reported in the jejunum, ileum, and excreta and the AME values for the different dietary treatments are presented in Table 7. Broilers fed S diets showed higher polyunsaturated FA (PUFA) digestibility coefficients than did those fed P diets, both in the jejunum and ileum. The digestibility of total FA (TFA) in the ileum and excreta, and the digestibility of SFA in the excreta were also higher for the broilers fed S diets ($P < 0.001$). The digestibility coefficients for TFA and SFA in the excreta were significantly the highest for the broilers fed the diets with 5% of FFA ($P < 0.01$). Significant interactions between the dietary fat saturation degree and the FFA percentage were reported in the jejunum for TFA, SFA and mono-unsaturated FA (MUFA; ($P < 0.05$)), in the ileum for SFA and MUFA ($P < 0.05$), and in the excreta for MUFA and PUFA ($P < 0.05$) digestibility coefficients. In the ileum, a quadratic regression equation could be fit for the SFA digestibility coefficients in the broilers fed S diets (Figure 1; $P < 0.01$); the coefficients observed for the broilers fed S5, S15, and S35 diets were similar, while more variable and lower values were reported for those fed S50 diet. The coefficients reported for the broilers fed P diets did not fit any model; they were similar among them and in general lower than the coefficients observed for S5, S15, and S35. In the ileum and excreta, broilers fed S5 diet showed higher MUFA digestibility coefficients than those fed S50 diet (and also than those fed S35 diet in the excreta), while the coefficients for the broilers fed P5, P15, P35, and P50 diets were similar. The digestibility of PUFA in the excreta followed the

Table 6. Lipid-class content¹ along the gastrointestinal tract and excreta according to different fat sources in the diet in 37-day-old broiler chickens.²

Item	Dietary treatments ³								Fat ⁴		FFA% ⁵				SEM	P values		
	Unsaturated – S diets				Saturated – P diets				S	P	5	15	35	50		Fat	FFA	Interaction
	S5	S15	S35	S50	P5	P15	P35	P50										
<i>Jejunum</i>																		
TAG	0.74	0.89	0.91	1.00	0.40	0.39	0.40	0.37	0.88	0.39	0.57	0.64	0.65	0.68	0.027	***	NS	NS
DAG	1.02 ^{ab}	1.33 ^{ab}	0.94 ^{ab}	1.55 ^a	0.96 ^{ab}	1.14 ^{ab}	1.20 ^{ab}	0.88 ^b	1.21	1.05	0.99	1.24	1.07	1.21	0.048	NS	NS	*
MAG	0.60 ^{ab}	0.76 ^{ab}	0.71 ^{ab}	0.88 ^a	0.66 ^{ab}	0.56 ^{ab}	0.69 ^{ab}	0.45 ^b	0.74	0.58	0.62	0.66	0.70	0.66	0.027	**	NS	*
FFA	3.33	4.42	3.78	4.31	5.18	6.52	6.49	4.88	3.96	5.77	4.26 ^b	5.47 ^a	5.13 ^{ab}	4.6 ^{ab}	0.153	***	*	NS
<i>Ileum</i>																		
TAG	0.30 ^b	0.38 ^{ab}	0.53 ^a	0.53 ^a	0.30 ^b	0.33 ^b	0.28 ^b	0.30 ^b	0.44	0.30	0.30	0.36	0.41	0.41	0.013	***	*	**
DAG	0.45 ^b	0.58 ^b	0.71 ^{ab}	0.90 ^a	0.58 ^b	0.57 ^b	0.55 ^b	0.51 ^b	0.66	0.55	0.52	0.57	0.63	0.7	0.022	*	*	***
MAG	0.65	0.72	0.73	0.75	0.73	0.66	0.75	0.67	0.71	0.71	0.69	0.69	0.74	0.71	0.030	NS	NS	NS
FFA	1.27 ^d	1.36 ^d	1.56 ^d	1.91 ^d	2.88 ^c	4.17 ^a	3.70 ^{ab}	3.27 ^{bc}	1.52	3.51	2.07	2.76	2.63	2.59	0.061	***	**	***
<i>Excreta</i>																		
TAG	0.31	0.44	0.48	0.49	0.43	0.40	0.36	0.40	0.43	0.40	0.37	0.42	0.42	0.44	0.019	NS	NS	NS
DAG	0.61 ^b	0.88 ^{ab}	1.10 ^a	0.89 ^{ab}	0.66 ^b	0.87 ^{ab}	0.74 ^{ab}	0.67 ^b	0.87	0.74	0.64	0.89	0.93	0.78	0.028	*	**	*
MAG	0.40 ^{bc}	0.61 ^{ab}	0.49 ^{abc}	0.52 ^{abc}	0.40 ^{bc}	0.37 ^c	0.41 ^{bc}	0.63 ^a	0.51	0.45	0.40	0.49	0.45	0.58	0.016	NS	**	**
FFA	3.13	4.19	4.18	4.30	4.48	5.40	6.40	7.20	3.95	5.87	3.8 ^b	4.80 ^a	5.29 ^a	5.75 ^a	0.129	***	***	NS

Abbreviations: DAG, diacylglycerols; FFA, free fatty acids; MAG, monoacylglycerols; TAG, triacylglycerols.

P-values were obtained from the univariate GLM procedure of SPSS conducted to study whether the dietary treatments, the dietary fat saturation degree and the dietary FFA percentage affected the lipid-class content.

P < 0.05 was considered significant.

*P < 0.05; **P < 0.01; ***P < 0.001.

^{a-d} means in a row not sharing a common superscript letter are significantly different (P < 0.05).

¹Lipid-class concentration (mg/g) / Ti concentration (mg/g) in each gastrointestinal segment and excreta.

²Diets supplemented with 6% of an unsaturated (S) or saturated fat source (P): S5 - 100% crude soybean oil, S15 - oil blend with 70% crude soybean oil and 30% acid soybean oil from chemical refining (SA), S35 - oil blend with 30% crude soybean oil and 70% SA, S50 - 100% SA, P5 - 100% crude palm oil, P15 - oil blend with 80% crude palm oil and 20% palm fatty acid distillate from physical refining (PA), S35 - oil blend with 53% crude palm oil and 47% PA, S50 - oil blend with 33% crude palm oil and 66% PA.

³Values are means of 6 cages with 3 chickens/cage.

⁴Values are means of 24 cages with 3 chickens/cage.

⁵Values are means of 12 cages with 3 chickens/cage.

Table 7. Apparent fatty-acid digestibility coefficients along the gastrointestinal tract and excreta, and apparent metabolizable energy according to different fat sources in the diet in 37-day-old broiler chickens.¹

Item	Dietary treatments ²								Fat ³		FFA% ⁴				P values			
	Unsaturated – S diets				Saturated – P diets				S	P	5	15	35	50	SEM	Fat	FFA	Interaction
	S5	S15	S35	S50	P5	P15	P35	P50										
<i>Jejunum</i>																		
TFA	0.84 ^a	0.84 ^a	0.84 ^a	0.79 ^a	0.71 ^b	0.66 ^b	0.68 ^b	0.70 ^b	0.83	0.69	0.77	0.75	0.76	0.75	0.005	***	NS	*
SFA	0.64	0.64	0.64	0.53	0.60	0.56	0.57	0.64	0.61	0.59	0.62	0.60	0.60	0.59	0.012	NS	NS	*
MUFA	0.83	0.83	0.82	0.78	0.81	0.78	0.78	0.80	0.81	0.79	0.82	0.81	0.80	0.79	0.005	*	NS	*
PUFA	0.91	0.92	0.93	0.90	0.77	0.69	0.73	0.77	0.91	0.74	0.84	0.81	0.83	0.84	0.006	***	NS	NS
<i>Ileum</i>																		
TFA	0.92	0.90	0.90	0.86	0.79	0.75	0.77	0.79	0.89	0.78	0.85	0.82	0.83	0.83	0.006	***	NS	NS
SFA	0.81 ^a	0.76 ^{ab}	0.77 ^{ab}	0.69 ^{abc}	0.68 ^{bc}	0.60 ^c	0.66 ^{bc}	0.69 ^{abc}	0.76	0.66	0.74	0.68	0.72	0.69	0.010	***	NS	*
MUFA	0.91 ^a	0.89 ^{ab}	0.90 ^{ab}	0.86 ^b	0.88 ^{ab}	0.86 ^b	0.88 ^{ab}	0.89 ^{ab}	0.89	0.88	0.90	0.88	0.89	0.87	0.004	NS	NS	*
PUFA	0.95	0.95	0.95	0.93	0.86	0.86	0.88	0.87	0.95	0.87	0.91	0.91	0.91	0.90	0.003	***	NS	NS
<i>Excreta</i>																		
TFA	0.86	0.83	0.82	0.79	0.74	0.69	0.69	0.70	0.82	0.71	0.80 ^a	0.76 ^b	0.75 ^b	0.75 ^b	0.005	***	**	NS
SFA	0.78	0.72	0.72	0.67	0.65	0.55	0.54	0.57	0.72	0.58	0.71 ^a	0.63 ^b	0.63 ^b	0.62 ^b	0.008	***	***	NS
MUFA	0.87 ^a	0.84 ^{abc}	0.82 ^{bc}	0.80 ^c	0.85 ^{ab}	0.84 ^{abc}	0.84 ^{abc}	0.85 ^{ab}	0.83	0.84	0.86	0.84	0.83	0.82	0.004	NS	**	*
PUFA	0.88 ^a	0.86 ^{ab}	0.85 ^{ab}	0.82 ^{bc}	0.75 ^d	0.75 ^d	0.78 ^{cd}	0.77 ^d	0.85	0.76	0.82	0.80	0.82	0.79	0.004	***	NS	**
AME ⁵	3,019 ^a	3,013 ^a	2,987 ^a	2,840 ^{cd}	2,985 ^{ab}	2,896 ^{bc}	2,758 ^d	2,870 ^c	2,965	2,877	3002	2955	2873	2855	6.955	***	***	***

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, total fatty acids.

P-values were obtained from the univariate GLM procedure of SPSS conducted to study whether the dietary treatments, the dietary fat saturation degree and the dietary FFA percentage affected the fatty-acid digestibility results.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^{a-d} means in a row not sharing a common superscript letter are significantly different ($P < 0.05$).

¹Diets supplemented with 6% of an unsaturated (S) or saturated fat source (P): S5 - 100% crude soybean oil, S15 - oil blend with 70% crude soybean oil and 30% acid soybean oil from chemical refining (SA), S35 - oil blend with 30% crude soybean oil and 70% SA, S50 - 100% SA, P5 - 100% crude palm oil, P15 - oil blend with 80% crude palm oil and 20% palm fatty acid distillate from physical refining (PA), S35 - oil blend with 53% crude palm oil and 47% PA, S50 - oil blend with 33% crude palm oil and 66% PA.

²Values are means of 6 cages with 3 chickens/cage.

³Values are means of 24 cages with 3 chickens/cage.

⁴Values are means of 12 cages with 3 chickens/cage.

⁵Apparent metabolizable energy (kcal/kg). Values are pooled means of 6 replicates with 3 chickens/replicate.

same trend. Regarding the AME values, there was a significant interaction between the dietary fat saturation degree and FFA percentage; the values for S5, S15, S35, and P5 diets were similar to each other and higher in comparison to the other dietary treatments, and the AME value for S50 was similar to the values reported for P15, P35, and P50.

DISCUSSION

Lipid classes, together with FA digestibility coefficients, help in understanding the dynamics of fat digestion and absorption processes along the GIT in broilers (Rodriguez-Sanchez et al., 2019b). It has been documented that fat digestion is initiated in the gizzard and continues in the duodenum (Ravindran et al., 2016). The results from the gizzard and duodenum have not been presented, as they are likely to be affected by the gastro-duodenal retrograde movement of digesta, and the secretion of endogenous fat into the duodenum lumen (Rodriguez-Sanchez et al., 2019a,b). As the absorption process of FA takes place in the jejunum and, to a lesser extent, in the ileum (Tanchaenrat et al., 2014; Rodriguez-Sanchez et al., 2019a,b), the results in these 2 GIT segments will be the focus of the Discussion.

The lower FFA content observed in the jejunum, the higher digestibility coefficients for TFA in the ileum and for PUFA, both in the jejunum and ileum, the higher BW, and the lower FCR for the broilers fed S diets, confirm the more efficient absorption of the unsaturated diets in 37-day-old broilers. All of this corroborates that the higher the degree of unsaturation of a fat, the greater the ability of the FA to be absorbed. This is in accordance with Tanchaenrat et al. (2014), and Rodriguez-Sanchez et al. (2019b), who described the better ability of UFA to be absorbed in 21-, and 14-day-old broilers, respectively, and with Rodriguez-Sanchez et al. (2019a), who reported that unsaturated diets are more efficiently used than are saturated diets regardless of age. The natural emulsifying properties of UFA could explain their higher absorption in the GIT, as they assist in the formation of mixed micelles and, therefore, in the FA absorption process (Krogdahl, 1985; Smulikowska and Mieczkowska, 1996).

It is well known that the ability to absorb dietary fat in broilers increases with age (Noy and Sklan, 1995; Batal and Parsons, 2002; Tanchaenrat et al., 2013). In agreement with this, the ileal TFA digestibility coefficients reported in the present study in 37-day-old broilers were higher than those reported in the starter period of the study in 14-day-old broilers (Rodriguez-Sanchez et al., 2019b).

The lack of differences among the four S diets, and among the four P diets with different dietary FFA percentages, both in the jejunum and ileum, for TFA, SFA, and PUFA digestibility coefficients, showed that the dietary fat saturation degree affected the absorption of FA to a greater extent than the dietary FFA percentage,

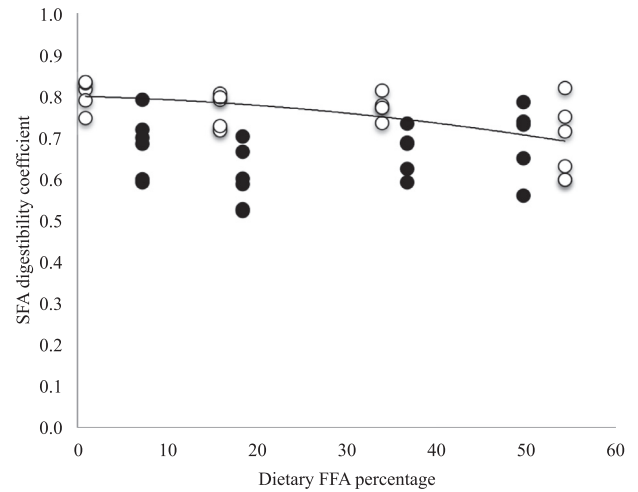


Figure 1. Relation between the saturated fatty-acid digestibility calculated in the ileum, and the four dietary free-fatty-acid percentages¹ for two different fat sources (○ soybean oil-unsaturated diets, and ● palm oil – saturated diets) in 37-day-old broilers. ¹Diets with an average of 5, 15, 35, and 50% of FFA. Each point represents each replicate value (with 3 chickens/replicate). The black line illustrates the quadratic model observed for the unsaturated diets: $y = -3.5 \times 2 - 0.006x$, where $R^2 = 0.39$ and $P = 0.007$. The saturated diets did not fit any model. Abbreviations: FFA, free fatty acids; SFA, saturated fatty acids.

which is in agreement with Vilarrasa et al. (2015b) and Rodriguez-Sanchez et al. (2019b). The same pattern was observed for the individual FA digestibility coefficients in the jejunum and ileum (Figure 2). However, the interactions reported in the ileum for TAG and DAG

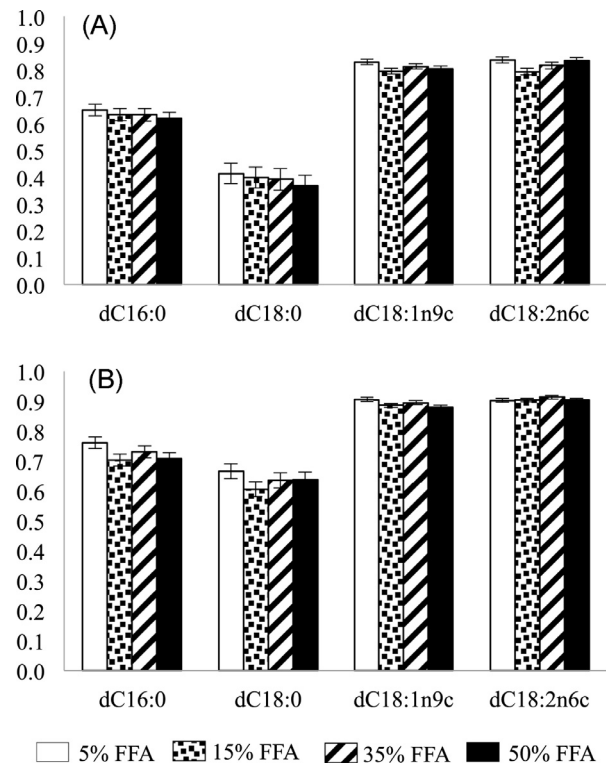


Figure 2. Individual fatty-acid apparent digestibility coefficients in the (A) jejunum, and (B) ileum for the four different dietary free-fatty-acid percentages (5%, 15%, 35%, and 50%) in 37-day-old broiler chickens. Values are means \pm SE of 12 replicates per each dietary FFA percentage (with 3 chickens/replicate). Palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), and linoleic (C18:2n6) acids. Abbreviation: FFA, free fatty acids.

content, as well as for MUFA digestibility coefficients, and the regression analysis of the ileal SFA digestibility coefficients (Figure 1), suggest that the dietary FFA content affects the FA absorption process, and that this effect could be different between the broilers fed the unsaturated (S), and saturated (P) diets. While differences were observed between the broilers fed S5 and S50 diets (the results for S5, S15 and S35 being similar), the results reported for those fed P5 and P50 diets were equivalent. Thus, according to the present results, the replacement of crude oils with acid oils (fat by-products rich in FFA) in grower-finisher broiler diets do not have a negative impact on the FA absorption process, at least when the dietary FFA percentage does not exceed 35%. This finding also supports the greater efficiency of the FA absorption process in broilers of 37 d of age. Rodriguez-Sanchez et al. (2019b) reported that in broilers of 14 d of age, unsaturated diets with more than 15% of FFA decreased the absorption of FA, and that palm oil sources were not suitable. Thus, the results show that 37-day-old broilers are less affected by both the dietary fat saturation degree and FFA content than are 14-day-old broilers. It is important to mention the differences in the composition of the experimental oils (SA had more moisture and unsaponifiable than SO and PA), and experimental diets (FFA increase was 98% in S diets vs. 85% in P diets). There were also differences in the FA composition among the dietary treatments, which were reflected in the UFA: SFA ratios (Table 4); while in S diets there was a greater content of dietary SFA, as the dietary FFA percentage was higher, in P diets the SFA content remained constant.

The results reported in the excreta suggest that diets with more than 5% of dietary FFA have a negative impact on FA absorption. However, it is likely that these results are affected by the presence of lipids from bacterial activity (Rodriguez-Sanchez et al., 2019b). In fact, FA of possible bacterial origin were detected in the excreta (capric, margaric, elaidic acid, and vaccenic acids), their total concentration (mg/g) being affected by the dietary FFA percentage (data not shown in table; 5% FFA: 1.40b, 15% FFA: 1.58ab, 35% FFA: 1.71a, and 50% FFA: 1.51ab). This finding altogether with the noneffective absorption of FA by the animal after the ileum (Renner, 1965) is the reason why the results of the jejunum and ileum are more representative of the FA absorption process. Taking this into account, the contribution of the jejunum and ileum on FA absorption was calculated considering the ileal digestibility coefficients as the maximum (100%) and expressing the digestibility coefficients reported in the jejunum and ileum towards these values. It was observed that irrespective of the dietary fat source, more than 80% of FA were absorbed in the jejunum. This has been previously reported in 14-day-old broilers (Rodriguez-Sanchez et al., 2019b) and 21-day-old broilers

(Tancharoenrat et al., 2014), where 68% and 75% of TFA, respectively, were absorbed in this GIT segment. This suggests that the improvement in the absorption process of FA from starter to grower-finisher broilers is mainly due to the higher contribution of the jejunum to this process. The contribution of the ileum to the FA absorption process was lower, as there were less FA reaching this GIT segment, but it was especially important for the absorption of SFA, being higher for the broilers fed S diets (19%) than for those fed P diets (10%). This difference has also been reported in 14-day-old broilers (Rodriguez-Sanchez et al. (2019b), where the contribution of the ileum to SFA absorption was 46% and 27% for the broilers fed S and P diets, respectively, and explains the higher absorption of unsaturated diets as suggested by these authors.

The present results show that, despite the fact soybean oil sources are, in general, associated with higher FA digestibility coefficients, palm oil sources can also be used as a fat source for grower-finisher broiler diets. The results give information regarding the sampling process for fat digestibility studies; we do not recommend taking digesta samples from either the gizzard or the duodenum, as these results are greatly affected by gastroduodenal reverse peristalsis and the endogenous secretion of lipids into the duodenal lumen. Similarly, we do not recommend using excreta samples, as the results are likely to be affected by the presence of lipids from bacterial activity. Jejunum and ileum digesta are the most representative samples to consider for FA digestibility studies.

In conclusion, the dietary fat type (saturation degree) affected the FA absorption process to a greater extent than did the dietary FFA content, and the absorption of the unsaturated diets was more efficient than was the absorption of the saturated diets, irrespective of their dietary FFA content. The results from the present study are promising as they demonstrate that including acid oils (fat by-products rich in FFA) in grower-finisher broiler diets does not have a negative impact on the FA absorption process, at least when the dietary FFA percentage does not exceed 35%, which means that chickens can successfully use these dietary fat sources. The use of acid oils in broiler diets is an interesting strategy to reduce the cost of feed formulation while also reducing their environmental impact.

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DISCLOSURES

The authors declare no conflict of interest. All authors of this research have no conflict of interest related with employment, consultancies, grants or other funding.

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