Effects of dietary free fatty-acid content and saturation degree on lipid-class composition and fatty-acid digestibility along the gastrointestinal tract in broiler starter chickens

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ABSTRACT The aim of the present study is to assess the effect of the dietary free fatty acid (FFA) content and dietary fat saturation degree on the fatty-acid (FA) digestibility and lipid-class content along the gastrointestinal tract and excreta in broiler chickens. The 8 experimental diets resulted from replacing crude soybean oil with soybean acid oil from chemical refining, or crude palm oil with palm FA distillate from physical refining. Thus, there were 4 soybean and 4 palm diets with 6% added fat varying in their FFA% (5, 15, 35, and 50%). Samples of digestive content (gizzard, duodenum, jejunum, and ileum) and excreta were collected at 14 D for the determination of the FA digestibility and lipid-class content. The total FA digestibility coefficients reported for the chickens fed S diets in the jejunum, ileum, and excreta were higher than for those fed P diets ($P \leq 0.02$). The general greater digestibility of the unsaturated diets was mainly explained by a higher contribution of the ileum to the absorption of saturated FA. The dietary FFA content mainly affected the FA absorption process. The diets with 50% FFA presented lower saturated FA digestibility coefficients in the jejunum and ileum ($P \leq 0.03$), and higher content of FFA in the ileum and excreta ($P \leq 0.014$), in comparison to the diets with 5% FFA. The 15% FFA diets were not different from the 5% FFA diets, regarding the saturated FA digestibility in the jejunum and excreta, and the FFA content in the ileum and excreta. It was concluded that unsaturated diets with moderate content of dietary FFA (up to 15%) could be used in broiler-chicken starter diets, as they led to similar FA absorption and performance results to the diets with the lowest dietary FFA content. From the present study, it has also been concluded that dietary saturated FA content has a greater impact on FA absorption than the dietary FFA content has.

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

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INTRODUCTION

The cost of supplying energy in poultry diets is high, and supplemental fats are usually used to meet the energy requirements due to their high-energy value. There are different available fat sources that can be used in poultry diets. Food fat by-products, such as those from the edible oil refining industry, are an example of an economic alternative in comparison to conventional fats that can be revalued as a feed fat ingredient. Fat by-products from the edible oil refining industry come from the chemical (acid oils from chemical refining) or physical (fatty acid [**FA**] distillates from physical refining) refining processes of edible oils (Catalogue of Feed Materials; Commission Regulation (EU) No 68/2013) and are characterized by having high proportions of free fatty acids (**FFA**; 40 to 90%; Nuchi et al., 2009). Throughout the text, these fat by-products will be generically called acid oils unless otherwise stated.

Acid oils have a similar FA composition to their respective crude oils, but different molecular structures (fewer triacylglycerols (**TAG**), more FFA, and variable amounts of diacylglycerols (**DAG**) and monoacylglycerols (**MAG**)), which, according to Roll et al. (2018), can affect their nutritional value. For this reason, evaluating the effect of both the FA and lipid-class composition (TAG, DAG, MAG, and FFA) is essential

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in order to understand how acid oils affect the fat digestion and absorption processes and to determine their potential use in broiler chicken diets.

It is well known that the ability of chicks to digest and absorb dietary fat is poorly developed (Krogdahl, 1985). However, the lack of consistent results regarding the use of acid oils in young broiler chickens (Blanch et al., 1996; Vilarrasa et al., 2015; Roll et al., 2018), and the high variability in their composition are the main reasons why acid oils are still not widely utilized in poultry feeds.

Taking this into account, it has been hypothesized that both the degree of saturation of the dietary fat and the age of the chicken can determine the effect of dietary FFA on fat digestion and absorption processes. Thus, the objective of the present study is to assess the effect of the dietary FFA content and saturation degree of dietary fat on FA digestibility and lipid-class composition along the gastrointestinal tract (**GIT**) and excreta in starter broiler chickens (14-day-old). For this aim, 2 crude oils and 2 FFA-rich fat by-products from the refining process of edible oils were used in order to have diets with different saturated FA (**SFA**) and FFA content. This information will be essential to determine where the limitation of the use of acid oils in starter broiler chicken diets is found.

MATERIALS AND METHODS

Animals and Diets

The experimental procedure received prior approval from the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona (Bellaterra, Barcelona, Spain). All animal housing and husbandry conformed to the European Union Guidelines (2010/63/EU).

A total of 528 one-day-old female broiler chickens of the Ross 308 strain were obtained from a commercial hatchery (Pondex SAU; Lleida, Spain). On arrival, chicks were wing-banded, weighed (initial BW, 36.88 g ± 2.30 g), and randomly assigned to 1 of 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 ad libitum, and animals were raised under controlled conditions of light and temperature, as recommended by the breeder.

Birds received a wheat- and soybean-meal-based starter diet (in mash form) that was formulated to meet or exceed FEDNA requirements (2008) and to minimize the basal level of fat. The ingredient composition of the basal diet is presented in Table 1. Titanium dioxide (**TiO_2**) was added (5 g/kg) as an inert marker for the determination of the digestibility of FA.

The basal diet was supplemented at 6% with different oil sources (crude oils, **O**; fat by-products from the edible oil refining industry rich in FFA, **A**; or mixtures of both). The 8 experimental diets resulted from replacing crude soybean oil (**SO**; with < 5% FFA) with soybean acid oil from chemical refining (**SA**; with 67% FFA), or crude palm oil (**PO**; with < 5% FFA) with palm FA distillate from physical refining (**PA**; 95% FFA) in the proportions shown in Table 2. Thus, there were 4 soybean oil diets (**S**) and 4 palm oil diets (**P**) with 6% added fat varying in their FFA% (5, 15, 35, and 50%).

Table 1. Ingredient composition of the experimental basal diet for the starter period.

Ingredients, %	Starter diet (from 0 to 21 D)
Wheat	54.46
Soybean meal 48%	35.4
Experimental fats[1]	6.00
Calcium carbonate	1.44
Sodium chloride	0.40
Monocalcium phosphate	0.99
Vitamin and mineral premix[2]	0.40
DL-Methionine	0.23
L-Lysine	0.15
Titanium oxide	0.50
Ethoxyquin 66%	0.02

[1]Crude soybean oil, crude palm oil, acid soybean oil, or acid palm oil in different proportions (see Table 2).

[2]Provides per kg of 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.

Data Collection

Feed consumption and BW were measured weekly to calculate ADFI, ADG, and feed conversion ratio (**FCR**) throughout the experiment (0 to 21 D), and were corrected for mortalities.

A digestibility balance was carried out from 11 to 14 D. At 14 D of age, 8 birds per cage were killed by cervical dislocation and samples of 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 a point 1-cm proximal to the ileocecal junction), and a representative sample of excreta of each cage was taken. The digestive content of each segment of the GIT from all birds within each cage was pooled, homogenized, frozen at –20°C, and lyophilized. After lyophilization, samples were ground to pass through a 0.5-mm sieve, and they were kept at 4°C until further analyses.

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

Fat	Unsaturated—S diets				Saturated—P diets			
Dietary FFA %	5	15	35	50	5	15	35	50
Treatments	S5	S15	S35	S50	P5	P15	P35	P50
Proportion in oil blends, %								
Crude soybean oil (SO)	100	70	30	–	–	–	–	–
Acid soybean oil (SA)[2]	–	30	70	100	–	–	–	–
Crude palm oil (PO)	–	–	–	–	100	80	53	33
Acid palm oil (PA)[3]	–	–	–	–	–	20	47	66

[1]All oil blends were added at 6% to the basal diet.
[2]SA, acid soybean oil from chemical refining (67% FFA).
[3]PA, palm fatty acid distillate from physical refining (95% FFA).

Chemical Analysis

Analytical determinations of the diets were performed according to the methods of the AOAC International (2005): 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).

TiO_2 was analyzed following the procedures of Short et al. (1996) and determined by ICP-OES (Optima 3200 RL, Perkin Elmer; Waltham, MA). The FA content of the feed, excreta, and digestive content was determined according to the method of Sukhija and Palmquist (1988). This analytic procedure consists of a direct transesterification (the lipid extraction and FA methylation is achieved in only one step). Samples were incubated at 70°C with methanolic hydrochloric acid (a mixture of methanol and acetyl chloride) for the methylation. Nonadecanoic acid (C19:0; Sigma-Aldrich Chemical Co.; St. Louis, MO) was added as internal standard before methylation. After extraction and methylation, potassium carbonate and toluene were added in order to separate the organic layer. The final extract was injected in a gas chromatograph (HP6890, Agilent Technologies; Waldbronn, Germany) following the conditions of the method previously described by Cortinas et al. (2004). FA methyl esters were identified by matching their retention times with those of their relative standards (Supelco 37 component FAME Mix, Sigma-Aldrich Co), and quantification was performed by means of their calibration curves. The macronutrient and FA composition of the experimental diets are presented in Table 3.

Lipid-class composition of the extracted fat from the feed, excreta, and digestive content was determined as described by Rodriguez-Sanchez et al. (2019). In this case, for the quantification of lipid classes, 100 μL of a salicylic acid solution (0.0075 g/mL diethyl ether; purchased from Sigma-Aldrich Co.) was added as internal standard. Lipid classes were identified by matching their retention times with those of standards (trioleoylglycerol for TAG, dioleoylglycerol for DAG, oleoylglycerol for MAG and oleic acid for FFA; Sigma-Aldrich Co.), and quantification was performed by means of their calibration curves.

Calculations

In order to determine the lipid-class content in the different GIT segments and excreta, the following formula was applied:

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

where [LC]dig is the concentration of the lipid class in the digesta of a GIT segment or excreta (mg/g DM) and [Ti]dig is the concentration of TiO_2 in the digesta of a GIT segment or excreta (mg/g DM). This ratio is an estimation of the content of each lipid class present in the digestive tract of the chickens.

The digestibility coefficients of FA in each segment of the GIT were determined using the TiO_2 ratio in the feed and digestive content or excreta as detailed in Rodriguez-Sanchez et al. (2019), and the AME was determined from the product of the energy utilization ratio and its corresponding gross energy of feed.

Statistical Analysis

Productive parameters, AME, lipid-class content, and digestibility of FA were subjected to univariate analysis using the GLM procedure of SPSS (SPSS statistics 25.0.0.0, IBM 2017) to study whether they depended on the SFA and dietary FFA content. 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 for S and P diets, 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 treatment. The Tukey test was used to assess the differences among the 8 dietary treatments. Results in tables are reported as least square

Table 3. Analyzed[1] macronutrient content and fatty-acid and lipid-class composition of the experimental diets.[2]

	Starter diets							
	Unsaturated—S diets				Saturated—P diets			
	S5	S15	S35	S50	P5	P15	P35	P50
Macronutrient content								
Dry matter %	89.99	89.91	89.98	90.01	89.78	89.83	89.87	89.88
Crude protein %	20.66	21.67	22.55	21.30	20.76	20.81	19.44	20.83
Crude fat %	7.37	7.26	7.19	7.13	6.59	7.09	7.49	6.76
Crude fiber %	4.94	60.4	5.91	5.48	6.95	5.83	5.77	5.84
Ash %	5.75	6.89	7.44	6.45	5.81	6.01	5.32	6.35
Gross energy, kcal/kg	4,147	4,062	4,056	4,066	4,038	4,212	4,091	4,043
AME[3]	3,212	2,996	2,818	2,781	2,932	3,068	2,997	2,879
Fatty acid composition, %								
C14:0	0.20	0.20	0.21	0.21	1.06	1.06	1.03	0.96
C16:0	13.02	14.04	15.52	16.28	36.87	37.92	38.37	36.84
C18:0	4.19	4.67	4.77	5.12	4.13	4.05	4.04	4.26
C18:1 n-9	21.08	21.26	21.94	22.30	31.59	31.84	31.35	30.02
C18:1 n-7	1.43	1.41	1.43	1.43	0.91	0.90	0.88	0.88
C18:2 n-6	52.61	51.24	49.34	48.18	22.29	21.64	21.77	21.61
C18:3 n-3	6.17	5.81	5.13	4.66	1.63	1.58	1.63	1.74
Minor fatty acids	1.29	1.36	1.66	1.81	1.53	1.01	0.95	3.70
SFA	18.42	19.98	21.64	22.87	43.13	43.62	43.94	45.33
MUFA	22.80	22.97	23.90	24.28	32.95	33.16	32.66	31.33
PUFA	58.78	57.06	54.47	52.85	23.92	23.22	23.39	23.35
UFA:SFA	4.43	4.01	3.62	3.37	1.32	1.29	1.28	1.21
Lipid-class composition, %								
TAG	90.20	79.67	56.49	34.69	83.02	70.11	56.32	46.02
DAG	4.65	5.11	6.64	8.23	8.74	7.86	6.70	4.80
MAG	1.52	1.27	1.57	1.60	2.30	1.86	1.76	1.85
FFA	3.63	13.95	35.30	55.49	5.95	20.17	35.22	47.33

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

[2]Dietary 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, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50: oil blend with 33% crude palm oil and 66% acid palm oil.

[3]Values are pooled means of 6 replicates with 11 chickens/replicate.

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

means, and differences were considered significant at $P < 0.05$.

RESULTS

Characterization of Experimental Diets

The analyzed composition of the experimental diets is presented in Table 3. The FA profile reflected the composition of the added fat source (S or P). The main FA in S diets were linoleic (48 to 52%) and oleic (21 to 22%) acids, whereas the main FA in P diets were palmitic (36 to 38%), oleic (30 to 31%), and linoleic (21 to 22%) acids. In S diets, since SO was replaced with SA, there was a decrease of linoleic acid and an increase of palmitic acid (as SA is richer in palmitic acid than is SO; data not shown), while in P diets, since PO was replaced with PA, the change in the proportion of the different FA was not as evident (as the FA composition of PO and PA are similar; data not shown). This fact was reflected in the unsaturated-to-saturated FA ratios (**UFA: SFA**), which, in S diets, decreased progressively from S5 (4.43) to S50 (3.37), while in P diets they remained constant (P5: 1.31–P50: 1.21). These UFA: SFA ratios, as well as the lipid-class characterization of the experimental diets (Table 3), show the objective of the study of having 4 diets for each fat source (S and P) varying in their FFA content (5, 15, 35, and 50%). Thus, in both S and P diets, as A replaced the corresponding O, TAG% decreased (from more than 80 to 46% or less) and FFA% increased (from less than 6% to more than 47%). The % of DAG as A replaced O increased in S diets (from 4.6 to 8.2%) and decreased in P diets (from 8.7 to 4.8%). The MAG% remained constant as A replaced the corresponding O.

A significant interaction between the fat source and the dietary FFA% was observed for the AME ($P < 0.001$; S5: 3212a, S15: 2996bc, S35: 2818de, S50: 2781e, P5: 2932 cd, P15: 3068b, P35: 2997bc, P50: 2879de). The highest AME value was observed for S5, followed by P15, whereas the lowest values were observed for S35, S50, and P50 diets.

Growth Performance

The effect of the dietary fat source and FFA% on growth performance from 0 to 21 D is presented in Table 4. Regarding the fat saturation degree, no significant differences were observed in any of the parameters. Regarding the dietary FFA%, an effect was observed for

Table 4. Growth performance of broiler chickens (0 to 21 D) according to different fat sources in the diet.[1]

	Dietary treatments[2]								Fat[3]		FFA%[4]					*P*-values		
	Unsaturated—S diets				Saturated—P diets													
Item	S5	S15	S35	S50	P5	P15	P35	P50	S	P	5	15	35	50	SEM	Fat	FFA	Interaction
ADG, g/b/d	37.36	39.20	36.02	37.32	35.61	37.97	36.48	36.82	37.47	36.72	36.48	38.59	36.25	37.07	0.355	0.30	0.10	0.71
ADFI, g/b/d	65.20	70.72	67.13	65.74	68.1	71.20	61.33	56.96	67.20	66.67	66.69	70.96	64.23	65.85	1.003	0.79	0.13	0.48
FCR, g/g	1.76	1.88	1.87	1.81	1.87	1.83	1.78	1.79	1.83	1.82	1.81	1.86	1.82	1.80	0.025	0.78	0.89	0.52
BW at 21 D, g	820.7	861.6	793.2	798.6	781.6	834.3	801.4	806.8	820.45	803.53	801.19^{b}	855.81^{a}	788.25^{b}	802.72^{b}	5.228	0.11	<0.001	0.15

[1]Values are means of 6 replicates with 11 chickens/replicate (until day 14), and with 3 chickens/replicate (from day 15 to 21) fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P).

[2]S5: 100% soybean crude oil, S15: oil blend with 70% soybean crude oil and 30% acid soybean oil, S35: oil blend with 30% soybean crude oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% palm crude oil, P15: oil blend with 80% palm crude oil and 20% acid palm oil, S35: oil blend with 53% palm crude oil and 47% acid palm oil, S50: oil blend with 33% palm crude oil and 66% acid palm oil.

[3]S is the average of S5, S15, S35, and S50 diets; P is the average of P5, P15, P35, and P50 diets.

[4]5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets.

ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; BW = body weight; FFA = free fatty acids; SEM = standard error of the grand mean.

P-values were obtained from a univariate ANOVA conducted to study whether the dietary saturated fatty-acid content and dietary FFA% affected growth performance values.

$P < 0.05$ was considered significant. ^{a,b} allude to Tukey test; means in a row not sharing a common superscript are significantly different ($P < 0.05$).

BW at 21 D, where the value reported for the chickens fed the 15% FFA diets was higher than for those fed the other diets.

Lipid-Class Content along the GIT

Regardless of the fat source and dietary FFA%, the general evolution of lipid-class content throughout the GIT followed a similar pattern (Table 5). The lipid-class content in the gizzard reflected the TAG, DAG, MAG, and FFA profile of the different experimental diets. From the gizzard to the duodenum, TAG decreased and DAG, MAG, and FFA increased. DAG, MAG, and FFA decreased from the duodenum on.

Despite the similar general evolution along the GIT, there were some differences in the lipid-class content regarding the dietary fat source (S or P). In the gizzard and duodenum, the content of DAG, MAG, and FFA was higher for the chickens fed S diets than for those fed P diets ($P \leq 0.014$). Continuing with the results observed in the jejunum, ileum, and excreta, MAG content was higher and FFA content lower for the chickens fed S diets than for the chickens fed P diets ($P \leq 0.05$). In the ileum, DAG content was higher for the chickens fed S diets than for those fed P diets ($P < 0.001$).

In relation to the effect of the dietary FFA% on the lipid-class content, it was observed that in the gizzard, the higher the dietary FFA%, the lower the TAG and DAG content, this being especially evident when the diets with the lowest and highest dietary FFA content were compared (5% FFA vs. 50% FFA). While the dietary FFA% did not have an effect in any of the lipid classes in the duodenum and jejunum, an effect was observed in the ileum and excreta (Figure 1). In the ileum, the dietary FFA% had an effect on DAG (15% FFA diets < 50% FFA diets; $P = 0.022$), MAG (5% and 15% FFA diets < 50% FFA diets; $P = 0.015$), and FFA content (5% FFA diets < 50% FFA diets; $P = 0.014$). In the excreta, the dietary FFA% had an effect on the FFA content, which was higher for the chickens fed the 35% and 50% FFA diets, in comparison to the chickens fed the 5% and 15% FFA diets ($P \leq 0.001$).

A significant interaction between the fat source and the dietary FFA% was observed in the excreta for TAG; while TAG content was the same for the 4 different dietary FFA% in the chickens fed P diets, in those chickens fed S diets, the value observed for S15 diet was lower than was the value observed for S50 diet, and there were no differences among the rest of the dietary FFA%.

Apparent FA Digestibility along the GIT

Apparent FA digestibility coefficients were also studied in different segments of the GIT, and in the excreta for the 8 dietary treatments (Table 6). SFA digestibility was mainly represented by palmitic (C16:0) and stearic (C18:0) acids, monounsaturated FA (**MUFA**) digestibility by oleic acid (C18:1n9), and polyunsaturated FA (**PUFA**) digestibility by linoleic (C18:2n6) acid.

Regarding the dietary fat source, the digestibility coefficients of total FA (**TFA**) and SFA for those chickens fed the unsaturated diets (S) were lower than for the chickens fed the saturated diets (P) in the duodenum ($P \leq 0.001$). In the jejunum, the chickens fed the

Table 5. Lipid-class content[1] along the gastrointestinal tract and excreta according to different fat sources in the diet[2] in 14-day-old broiler chickens.

	Dietary treatments[3]								Fat[4]		FFA%[5]					*P*-values		
	Unsaturated—S diets				Saturated—P diets													
Item	S5	S15	S35	S50	P5	P15	P35	P50	S	P	5	15	35	50	SEM	Fat	FFA	Interaction
Gizzard																		
TAG	21.35	19.16	15.28	11.89	17.88	14.33	12.36	11.74	16.92	14.10	19.61^{a}	$16.74^{a,b}$	$13.82^{b,c}$	11.82^{c}	0.486	0.006	<0.001	0.392
DAG	3.03	3.14	2.90	2.56	2.87	2.35	1.86	1.88	2.91	2.24	2.95^{a}	$2.75^{a,b}$	$2.38^{a,b}$	2.22^{b}	0.081	<0.001	0.011	0.286
MAG	1.03	1.26	1.07	1.02	0.97	0.78	0.74	0.80	1.10	0.83	1.00	1.02	0.91	0.91	0.042	0.003	0.705	0.372
FFA	7.61	9.37	13.32	13.76	6.33	7.44	9.91	10.79	11.02	8.62	6.97^{b}	8.41^{b}	11.61^{a}	12.27^{a}	0.194	<0.001	<0.001	0.209
Duodenum																		
TAG	6.61	8.22	5.43	7.88	8.40	4.59	4.83	5.77	7.03	5.90	7.50	6.41	5.13	6.82	0.426	0.190	0.264	0.156
DAG	30.87	21.07	23.90	24.40	30.87	21.07	23.90	24.40	33.38	25.06	31.48	26.66	28.77	29.99	1.164	<0.001	0.520	0.378
MAG	12.21	10.77	11.31	12.43	9.96	7.05	7.35	9.70	11.68	8.52	11.09	8.91	9.33	11.07	0.612	0.014	0.463	0.954
FFA	179.01	168.07	167.76	182.96	155.05	97.75	108.26	120.17	174.45	119.81	167.03	131.91	138.01	151.56	6.982	0.001	0.305	0.635
Jejunum																		
TAG	0.81	0.99	1.02	1.15	0.62	0.61	0.54	0.45	0.99	0.56	0.72	0.80	0.78	0.80	0.046	<0.001	0.906	0.283
DAG	1.37	1.27	1.48	1.76	1.49	1.19	1.16	1.38	1.46	1.31	1.43	1.21	1.32	1.57	0.076	0.324	0.353	0.614
MAG	0.75	0.75	0.74	1.08	0.57	0.50	0.59	0.50	0.83	0.54	0.66	0.63	0.66	0.79	0.052	0.008	0.686	0.458
FFA	4.53	3.64	3.70	4.85	8.12	7.26	8.44	8.73	4.18	8.14	6.33	5.45	6.07	6.79	0.301	<0.001	0.480	0.895
Ileum																		
TAG	0.49	0.39	0.42	0.66	0.26	0.23	0.25	0.28	0.49	0.25	0.37	0.31	0.34	0.47	0.023	<0.001	0.091	0.332
DAG	0.69	0.60	0.88	1.00	0.46	0.48	0.56	0.57	0.79	0.52	$0.58^{a,b}$	0.54^{b}	$0.72^{a,b}$	0.79^{a}	0.031	<0.001	0.022	0.357
MAG	0.52	0.46	0.60	0.80	0.29	0.28	0.37	0.40	0.60	0.34	0.40^{b}	0.37^{b}	$0.49^{a,b}$	0.60^{a}	0.026	<0.001	0.015	0.496
FFA	2.27	1.88	2.65	3.30	4.42	4.87	5.79	5.55	2.53	5.16	3.35^{b}	$3.38^{a,b}$	$4.22^{a,b}$	4.43^{a}	0.141	<0.001	0.014	0.499
Excreta																		
TAG	$0.35^{a,b}$	0.28^{b}	$0.46^{a,b}$	0.56^{a}	0.31^{b}	0.30^{b}	$0.32^{a,b}$	0.27^{b}	0.41	0.30	0.33	0.29	0.39	0.41	0.019	0.005	0.082	0.038
DAG	0.77	0.71	0.98	0.99	0.69	0.71	0.81	0.69	0.86	0.73	0.73	0.71	0.90	0.84	0.037	0.075	0.229	0.539
MAG	0.39	0.47	0.52	0.57	0.41	0.36	0.40	0.45	0.49	0.41	0.40	0.42	0.46	0.51	0.021	0.049	0.265	0.591
FFA	2.38	2.38	3.91	3.33	5.30	5.61	6.49	6.73	3.00	6.03	3.84^{b}	4.00^{b}	5.20^{a}	5.03^{a}	0.127	<0.001	<0.001	0.681

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

[2]Values are pooled means of 6 replicates with 8 chickens/replicate (and with 11 chickens/replicate in the case of excreta) fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P).

[3]S5: 100% crude soybean oil, S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50: oil blend with 33% crude palm oil and 66% acid palm oil.

[4]S is the average of S5, S15, S35, and S50 diets; P is the average of P5, P15, P35, and P50 diets.

[5]5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets.

TAG = triacylglycerols, DAG = diacylglycerols, MAG = monoacylglycerols, FFA = free fatty acids, SEM = standard error of the grand mean.

P-values were obtained from a univariate ANOVA conducted to study whether the dietary saturated fatty-acid content and dietary FFA% affected lipid class results. $P < 0.05$ was considered significant. a–d allude to Tukey test; means in a row not sharing a common superscript are significantly different ($P < 0.05$).

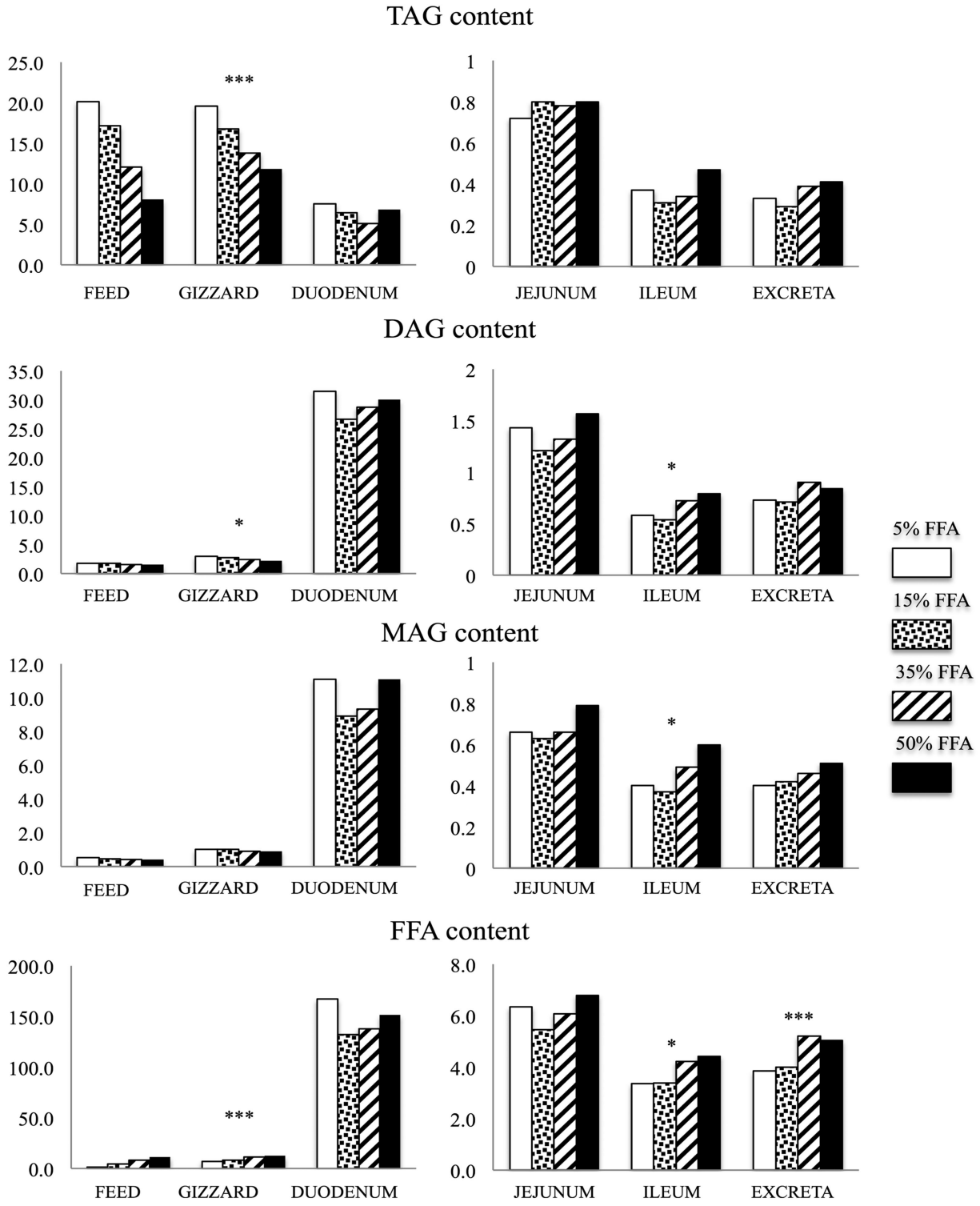

Figure 1. Lipid-class content[1] in the feed, gizzard, duodenum, jejunum, ileum, and excreta considering the average results for the 4 different percentages of dietary free fatty acids[2] in 14-day-old broiler chickens.[1] Lipid-class concentration (mg/g)/Ti concentration (mg/g) in each gastrointestinal segment and excreta. Values are pooled means of 12 replicates per each dietary free fatty acid % with 8 chickens/replicate, and 11 chickens/replicate in the case of excreta. [2] 5% FFA is the average of S5 and P5 diets; 15% FFA is the average of S15 and P15 diets; 35% FFA is the average of S35 and P35 diets; 50% FFA is the average of S50 and P50 diets. Dietary treatments supplemented with 6% of added fat. TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids. *P*-values were obtained from a univariate ANOVA conducted to study whether the dietary saturated fatty-acid content and dietary FFA% affected the lipid-class content in each gastrointestinal segment and excreta. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. *P*-values allude to Tukey test. See Table 5 for detailed *P* values.

Table 6. Apparent fatty-acid digestibility coefficients[1] along the gastrointestinal tract and excreta according to different fat sources in the diet in 14-day-old broiler chickens.

	Dietary treatments[2]								Fat[3]		FFA%[4]					*P*-values		
	Unsaturated—S diets				Saturated—P diets													
	S5	S15	S35	S50	P5	P15	P35	P50	S	P	5	15	35	50	SEM	Fat	FFA	Interaction
Gizzard																		
TFA	–0.53^{a,b}	–0.85^{b}	–0.76^{b}	–0.51^{a,b}	–0.50^{a,b}	–0.39^{a}	–0.16^{a}	–0.23^{a}	–0.66	–0.32	–0.52	–0.62	–0.46	–0.37	0.029	<0.001	0.03	0.01
SFA	–0.57^{c,d}	–0.77^{d}	–0.65^{c,d}	–0.45^{b,c}	–0.19^{a,b}	–0.08^{a}	0.07^{a}	0.06^{a}	–0.61	–0.03	–0.38	–0.43	–0.29	–0.20	0.023	<0.001	0.01	0.04
MUFA	–0.51^{b,c}	–0.83^{c}	–0.74^{c}	–0.49^{b,c}	–0.32^{a,b}	–0.24^{a,b}	–0.08^{a}	–0.21^{a,b}	–0.65	–0.21	–0.42	–0.54	–0.41	–0.35	0.027	<0.001	0.12	0.01
PUFA	–0.53^{a}	–0.89^{a,b}	–0.81^{a,b}	–0.55^{a}	–1.30^{b}	–1.21^{b}	–0.72^{a}	–0.83^{a,b}	–0.69	–1.02	–0.92	–1.05	–0.76	–0.69	0.042	<0.001	0.02	0.01
Duodenum																		
TFA	–8.36	–10.23	–9.45	–10.82	–8.95	–4.89	–4.76	–6.35	–9.72	–6.24	–8.66	–7.56	–7.10	–8.58	0.439	<0.001	0.51	0.08
SFA	–15.79	–17.99	–15.63	–17.16	–8.23	–4.48	–4.29	–5.47	–16.64	–5.62	–12.01	–11.23	–9.96	–11.32	0.652	<0.001	0.73	0.45
MUFA	–4.90	–5.92	–5.26	–5.84	–6.35	–3.36	–3.28	–4.58	–5.48	–4.39	–5.63	–4.64	–4.27	–5.21	0.296	0.07	0.38	0.10
PUFA	–7.38	–9.25	–8.83	–10.37	–13.84	–7.84	–7.72	–10.42	–8.96	–9.95	–10.61	–8.55	–8.28	–10.39	0.516	0.34	0.26	0.03
Jejunum																		
TFA	0.64	0.60	0.57	0.46	0.50	0.53	0.50	0.46	0.57	0.50	0.57^{a}	0.56^{a,b}	0.54^{a,b}	0.46^{b}	0.014	0.02	0.03	0.45
SFA	0.46	0.46	0.34	0.23	0.41	0.44	0.41	0.41	0.38	0.42	0.44^{a}	0.45^{a}	0.38^{a,b}	0.32^{b}	0.015	0.19	0.02	0.06
MUFA	0.69	0.64	0.57	0.51	0.65	0.66	0.62	0.58	0.60	0.63	0.67^{a}	0.65^{a}	0.59^{a,b}	0.54^{b}	0.012	0.22	0.002	0.39
PUFA	0.67	0.64	0.66	0.53	0.47	0.51	0.51	0.40	0.63	0.47	0.57	0.57	0.59	0.46	0.017	<0.001	0.05	0.91
Ileum																		
TFA	0.89	0.89	0.86	0.82	0.73	0.70	0.70	0.69	0.86	0.71	0.81	0.79	0.78	0.75	0.008	<0.001	0.15	0.69
SFA	0.75	0.75	0.66	0.56	0.59	0.57	0.57	0.56	0.68	0.57	0.67^{a}	0.65^{a,b}	0.61^{a,b}	0.56^{b}	0.013	<0.001	0.03	0.08
MUFA	0.87	0.87	0.85	0.81	0.83	0.81	0.80	0.79	0.85	0.81	0.85	0.84	0.82	0.80	0.007	0.002	0.06	0.75
PUFA	0.94	0.94	0.94	0.93	0.83	0.79	0.83	0.80	0.94	0.81	0.88	0.86	0.88	0.87	0.006	<0.001	0.59	0.63
Excreta																		
TFA	0.89^{a}	0.86^{a}	0.78^{b}	0.77^{b,c}	0.70^{c,d}	0.70^{c,d}	0.70^{c,d}	0.67^{d}	0.83	0.69	0.80	0.78	0.74	0.72	0.006	<0.001	<0.001	0.001
SFA	0.81^{a}	0.73^{a}	0.60^{b}	0.55^{b}	0.57^{b}	0.57^{b}	0.58^{b}	0.54^{b}	0.67	0.57	0.68	0.65	0.59	0.54	0.009	<0.001	<0.001	<0.001
MUFA	0.89^{a}	0.86^{a,b}	0.78^{c}	0.77^{c}	0.82^{b,c}	0.83^{a-c}	0.83^{a-c}	0.81^{b,c}	0.83	0.82	0.86	0.85	0.81	0.79	0.005	0.71	<0.001	0.001
PUFA	0.92^{a}	0.91^{a,b}	0.85^{b}	0.86^{b}	0.75^{c}	0.73^{c}	0.76^{c}	0.76^{c}	0.88	0.75	0.83	0.81	0.80	0.81	0.005	<0.001	0.14	0.002

[1]Values are pooled means of 6 replicates with 8 chickens/replicate (and with 11 chickens/replicate in the case of excreta) fed dietary treatments supplemented with 6% of an unsaturated (S) or saturated fat source (P).

[2]S5: 100% crude soybean oil, S15: oil blend with 70% crude soybean oil and 30% acid soybean oil, S35: oil blend with 30% crude soybean oil and 70% acid soybean oil, S50: 100% acid soybean oil, P5: 100% crude palm oil, P15: oil blend with 80% crude palm oil and 20% acid palm oil, S35: oil blend with 53% crude palm oil and 47% acid palm oil, S50: oil blend with 33% crude palm oil and 66% acid palm oil.

[3]S is the average of S5, S15, S35, and S50 diets; P is the average of P5, P15, P35, and P50 diets.

[4]5 is the average of S5 and P5 diets; 15 is the average of S15 and P15 diets; 35 is the average of S35 and P35 diets; 50 is the average of S50 and P50 diets.

TFA = total fatty acids, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, FFA = free fatty acids; SEM = standard error of the grand mean.

P-values were obtained from a univariate ANOVA conducted to study whether the dietary SFA content and dietary FFA% affected the FA digestibility results.

$P < 0.05$ was considered significant.

a–d allude to Tukey test; means in a row not sharing a common superscript are significantly different ($P < 0.05$).

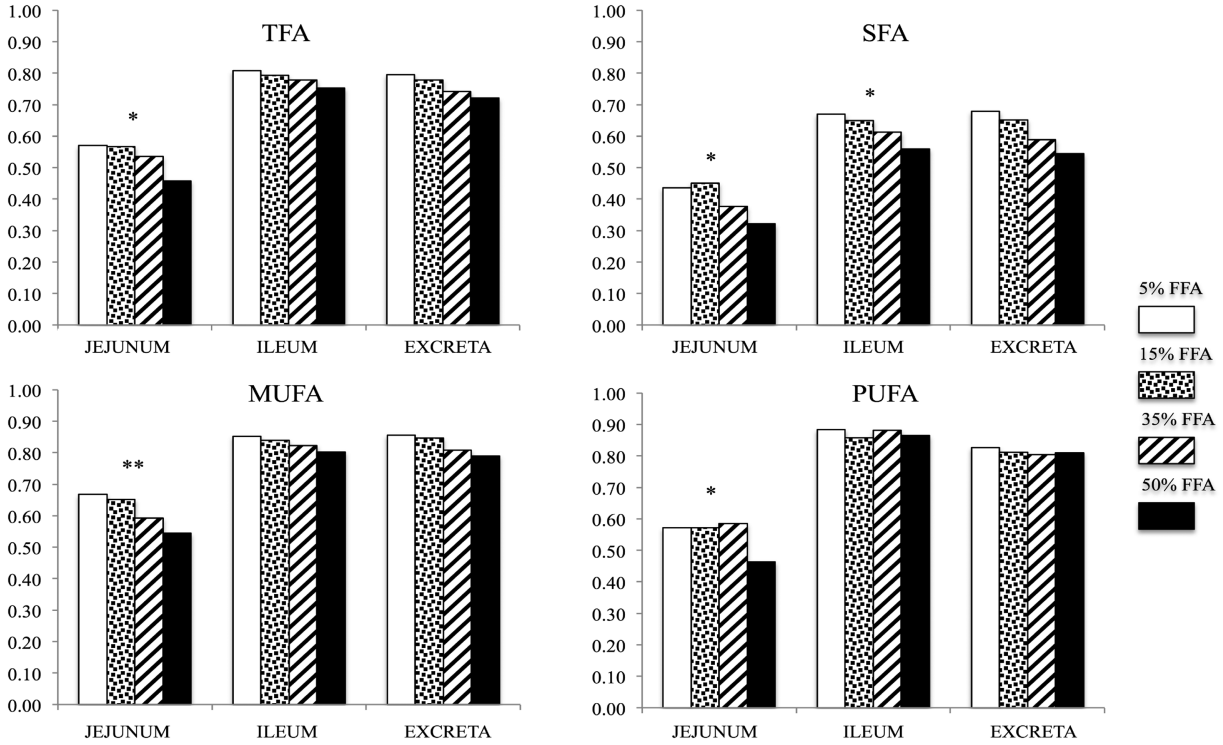

Figure 2. Apparent fatty-acid digestibility[1] in the jejunum, ileum, and excreta considering the average results for the 4 different percentages of dietary free fatty acids[2] in 14-day-old broiler chickens.[1] Values are pooled means of 12 replicates per each dietary free fatty acid % with 8 chickens/replicate, and 11 chickens/replicate in the case of excreta.[2] 5% FFA is the average of S5 and P5 diets; 15% FFA is the average of S15 and P15 diets; 35% FFA is the average of S35 and P35 diets; 50% FFA is the average of S50 and P50 diets. Dietary treatments supplemented with 6% of added fat. TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; FFA = free fatty acids. *P*-values were obtained from a univariate ANOVA conducted to study whether the dietary SFA content and dietary FFA% affected the apparent fatty acid digestibility in each gastrointestinal segment and excreta. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. *P*-values allude to Tukey test. See Table 6 for detailed *P* values.

unsaturated diets had higher digestibility coefficients for both TFA ($P = 0.02$) and PUFA ($P \leq 0.001$), in comparison to those chickens fed the saturated diets, and the same feature was also observed in the ileum for TFA, SFA, MUFA, and PUFA ($P \leq 0.002$).

Regarding the dietary FFA% (Figure 2), TFA, SFA, and MUFA digestibility coefficients were higher for the chickens fed the diets with the lowest dietary FFA content (5% FFA diets), in comparison to the ones fed the diets with the highest dietary FFA content (50% FFA diets) in the jejunum ($P < 0.05$). The same was observed in the ileum for SFA digestibility coefficients. In the case of MUFA and SFA digestibility in the jejunum, the digestibility coefficient for the chickens fed the 15% FFA diets was also higher, in comparison to the 50% FFA diets. A tendency was observed in the jejunum for PUFA, and in the ileum for MUFA, where the coefficient observed for the chickens fed the diets with the highest dietary FFA% (50% FFA diets) was numerically lower in comparison to the rest of the diets.

A significant interaction between the dietary fat source and FFA% was observed in the gizzard and excreta for TFA, SFA, MUFA, and PUFA digestibility, which was explained by a different response in the digestibility coefficients between the chickens fed S diets and the chickens fed P diets, as the dietary FFA% was higher. A tendency was also observed, both in the jejunum and ileum, for SFA, the pattern observed for SFA in the excreta being similar to these 2 GIT segments. The most relevant results regarding the regression analysis were found in the ileum, the last GIT segment where FA absorption has been described. The best-fit regression equation for the SFA ileal digestibility in S diets (Figure 3) was quadratic ($P \leq 0.001$); the coefficients observed for the chickens fed S5 and S15 diets were similar, while at higher dietary FFA% the digestibility coefficients progressively decreased, reaching similar values to those obtained for the chickens fed P diets. On the other hand, the coefficients observed for the chickens fed P diets were similar regardless of the dietary FFA%, and they did not fit any model (Figure 3).

As the absorption of fat has been reported to be negligible in the hindgut of poultry (Renner, 1965), the digestibility coefficients in the excreta could be influenced by bacterial activity. In order to confirm this, the concentration of those FA determined in the excreta that could come from bacterial metabolism was added up (capric acid, C10:0; margaric acid, C17:0; and elaidic acid, C18:1 trans) and compared among the different dietary treatments (S5: 2.25 mg/g DM; S15: 2.27 mg/g DM; S35: 2.25 mg/g DM; S50: 2.84 mg/g DM; P5: 1.98 mg/g DM; P15: 1.89 mg/g DM; P35: 1.87 mg/g DM; P50: 2.11 mg/g DM). An effect of the fat source

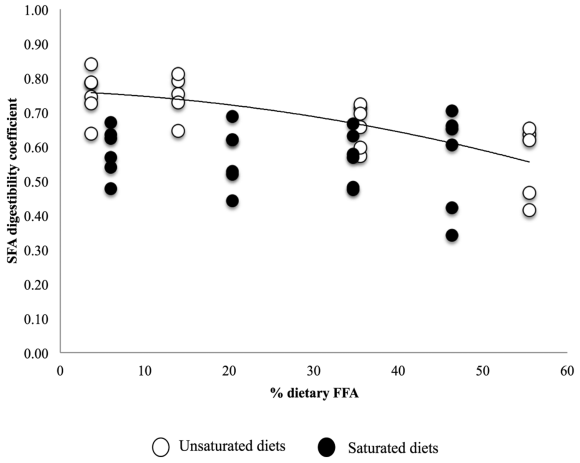

Figure 3. Relation between the saturated fatty-acid digestibility coefficients calculated in the ileum and the 4 different dietary free fatty acid percentages[1] for 2 different fat sources in 14-day-old broiler chickens. [1] Diets with an average of 5% FFA, 15% FFA, 35% FFA, 50% FFA. Dietary treatments supplemented with 6% of added fat. Each point represents each replicate value (with 8 chickens/replicate). The black line illustrates the quadratic model observed for the unsaturated diets. The saturated diets did not fit any model, and for that reason the regression is not represented in the figure. SFA: saturated fatty acids; FFA: free fatty acids. $y = -5^{-5}x^{2-}0.001x$, where $R^2 = 0.55$ and $P = 0.001$. Interaction fat × dietary FFA: $P = 0.08$.

($P < 0.001$) and dietary FFA% ($P = 0.006$) was observed. The concentration of these 3 FA in the excreta was higher for the chickens fed S diets than for those fed P diets (S: 2.4 vs. P: 2.0), and their concentration was significantly higher for the chickens fed the diets with the highest FFA% (5% FFA: 2.1b, 15% FFA: 2.1b; 35% FFA: 2.1b; 50% FFA: 2.5a).

DISCUSSION

Dietary Fat Saturation Effect

As has been previously documented (Renner and Hill, 1961; Young and Garrett, 1963), the SFA content of dietary fat has an influence on fat digestion, and in the present study this fact was mainly seen in the FA absorption process.

The negative results reported in the gizzard were probably related to the gastroduodenal reverse peristalsis. The effect of the dietary fat source on this process has not been described in the literature, and despite not being specifically assessed in the present study, it could be affected by the saturation degree of dietary fat. On the other hand, and in agreement with our previous study (Rodriguez-Sanchez et al., 2019), the lower digestibility coefficients reported in the duodenum for those chickens fed the unsaturated (S) diets, in comparison to the ones fed the saturated (P) diets, could be explained by a higher secretion of endogenous fat into the duodenum after consuming an unsaturated fat source. This was also supported by the lipid-class content in the duodenum, which was higher for the chickens fed S diets than for those fed P diets. The presence of fat in the duodenum has been reported as a stimulus for the secretion of cholecystokinin, and, consequently, the secretion of pancreatic enzymes and bile (Lindsay et al., 1969; Krogdahl, 1985; Tuchweber et al., 1996). It is likely that unsaturated fat enhances the secretion of endogenous fat, such as phospholipids, mucin-associated lipids, or desquamated epithelial cells, into the duodenum lumen (Cotton, 1972; Hurwitz et al., 1973; Clément, 1980; Gong et al., 1990), which could explain the present results. It has also been described that UFA have a greater ability to increase pancreatic lipase activity in comparison to SFA due to their angle at the site of the double bond (Van Kuiken and Behnke, 1994). All of these findings agree with the results from a previous study (Rodriguez-Sanchez et al., 2019), and they suggest that the hydrolysis process in those animals fed the unsaturated diets (S) is more efficient than in those fed the saturated diets (P).

However, and regarding the absorption process, the higher digestibility coefficients observed in the jejunum (for TFA and PUFA) and ileum (for TFA, SFA, MUFA, and PUFA) for the chickens fed S diets, in comparison to the chickens fed P diets, and the higher MAG content and the lower FFA content in those chickens fed S diets than in those fed P diets, suggest that the absorption of UFA is faster and more efficient, in comparison to the absorption of SFA.

The contribution of the jejunum and ileum on FA absorption was calculated considering the digestibility coefficient reported in the ileum as the maximum (100%) and expressing the digestibility coefficients observed in the jejunum and ileum towards that value. It was seen that the jejunum was the main site of fat absorption, regardless of the dietary fat source (68% and 63%, on average, of TFA and SFA, respectively, were absorbed in this GIT segment). Nevertheless, differences were observed regarding the contribution of the ileum, which was higher for the chickens fed S diets, in comparison to those fed P diets, this being especially evident for SFA (S: 46% vs. P: 27%). In the present study, the greater utilization of the unsaturated diets in 14-day-old broiler chickens was supported by the higher ileum contribution to the SFA absorption in those animals fed S diets, in comparison to those fed P diets. This is in agreement with the results reported in our previous study in 14-day-old broiler chickens fed crude oils (Rodriguez-Sanchez et al., 2019), as well as by the results reported by Tancharoenrat et al. (2014).

Dietary FFA Effect

As explained before, one of the main characteristics of acid oils is their high content of FFA. It has been reported that the higher the FFA% of a fat, the lower its nutritional value. Nonetheless, it is likely that other factors such as the dietary fat saturation degree or age of the chicken determine the utilization of fat sources rich in FFA.

Concerning performance parameters, the dietary FFA% did have an effect on BW at 21 D; however, the rest of the growth performance parameters were not affected, which is in agreement with different authors who did not report any effect of the added fat FFA% (at proportions between 3 and 99%) on the productive parameters (Siedler et al., 1955; Bornstein and Lipstein, 1963). Nevertheless, Bornstein and Lipstein (1963) also reported exceptions in their results, and Artman (1964) reported that broiler chickens fed diets with soybean oil had lower FCR, in comparison to those fed diets with acid soybean oil. Zumbado et al. (1999) reported that broiler chickens fed diets supplemented with acid soybean oil had the best FCR, and those supplemented with acid palm oil had the lowest weight gain and the worst FCR.

According to the present results, the dietary FFA% did not influence the hydrolysis process. The differences observed in the gizzard reflected the lipid-class composition of the different diets. The lack of differences in both the digestibility coefficients and lipid-class content in the duodenum could be related to the presence of FFA in the diets, which are an end-product of the hydrolysis process and are not affected by pancreatic lipase activity. However, Larsson and Erlanson-Albertsson (1986) suggested that FFA could induce a high-affinity complex between lipase and colipase, and it could change the properties of the interface leading to an increased binding of lipase and colipase to the substrate; this effect has been especially attributed to lauric, oleic, and linolenic acids.

On the other hand, a clear impact of the dietary FFA% on fat absorption was observed, which was supported by the lower TFA, SFA, MUFA, and PUFA (tendency) digestibility coefficients in the jejunum, and the lower SFA, and MUFA (tendency) digestibility coefficients in the ileum observed for the chickens fed the diets with the highest content of dietary FFA (50% FFA), in comparison to those fed the diets with the lowest content of dietary FFA (5% FFA) and, in some cases, with those fed the 15% FFA diets as well. Those chickens fed the diets with the highest dietary FFA% also presented higher content of all lipolysis products in the ileum, especially of MAG and FFA; these lipolysis products were higher in the chickens fed the 50% FFA diets, in comparison to the chickens fed the 5% FFA diets (and also to the ones fed the 15% FFA diets in the case of MAG). Furthermore, the chickens fed the 35% FFA and 50% FFA diets presented greater content of FFA in the excreta, in comparison to the ones fed the 5% FFA and 15% FFA diets. Thus, according to these results, percentages of dietary FFA above 15% were associated with a lower fat absorption rate. On the contrary, percentages of dietary FFA up to 15% did not have negative repercussions on the FA absorption process, suggesting that moderate percentages of dietary FFA could be used in starter broiler-chicken diets with no detriments in either performance parameters or fat utilization. Blanch et al. (1995) also reported lower fat apparent absorption in 2-wk-old broiler chickens fed a diet supplemented with a tallow + acid soybean oil blend (34.2% FFA), in comparison to a diet supplemented with soybean oil (2.6% FFA).

Some reasons have been hypothesized in the literature to explain the reduction of fat absorption due to the presence of dietary FFA. It has been described that TAG and 2-MAG stimulate the secretion of bile salts, in consequence, being necessary for the emulsification of fat (Sklan, 1979). Sklan (1979) related diets with high content of FFA to lower MAG content and bile secretion in the duodenum. This is also in accordance with Atteh and Leeson (1985), who reported an improvement in the ME of diets supplemented with FFA after supplementation with cholic acid, a primary bile acid. In the present study, the lack of differences reported for both FA digestibility and lipid classes results in the duodenum, considering the dietary FFA% suggests that the main reason for the different fat utilization observed after being fed a diet low or rich in FFA is more likely to be found in the absorption rather than in the hydrolysis process. This was also supported by the lower FA digestibility coefficients observed, in general, in the jejunum and ileum, and the higher content of lipolysis products in the ileum and excreta for the chickens fed the diets with the highest content of dietary FFA. It has been described that the key to absorption of lipolysis end products is the formation of mixed-lipid bile micelles (Ravindran et al., 2016), the presence of bile salts also being essential in the absorption process. Thus, this could be a reason for the lower FA absorption observed in those animals fed the diets with the highest dietary FFA%. On the other hand, dietary FFA have been related to some reactions in the intestine. Concretely, the acid group of FFA can react with divalent minerals (e.g., calcium, magnesium) and lead to the formation of soaps. If these soaps are insoluble, then both the FFA and the mineral are unavailable to the chicken. Atteh and Leeson (1985) related the soap formation between FFA and calcium to the decrease in fat retention and dietary ME. Atteh and Leeson (1984) reported that those soaps formed by unsaturated FFA were absorbed more easily than those formed by saturated FFA. In the present study, the formation of soaps was not investigated, but could explain, at least in part, the worse fat absorption related to the diets with the highest dietary FFA%.

Despite the general effect (irrespective of the fat source) of the dietary FFA% on fat absorption, some differences were reported between the chickens fed the unsaturated diets and the chickens fed the saturated diets. This was supported by the tendency observed for SFA digestibility for the interaction between the fat source and the dietary FFA%, both in the jejunum and ileum. In both cases, the coefficients reported for the chickens fed S diets decreased more than those reported for the chickens fed P diets as the dietary FFA% increased. The regression analysis carried out for SFA digestibility in the ileum suggested that unsaturated

diets with up to 15% FFA did not have negative repercussions on FA absorption. Furthermore, unsaturated diets with 35% and 50% FFA had similar results to saturated diets. The contribution of the jejunum and ileum to FA absorption also supported the higher effect of the dietary FFA% on the unsaturated diets. Thus, in those chickens fed S diets, the contribution of the ileum to SFA absorption increased from S5 and S15 to S50 (S5: 39%, S15: 39%, S35: 49%, and S50: 59%); however, the higher contribution of the ileum did not compensate the lower digestibility coefficients reported for those chickens fed the diets with the highest FFA%. Notwithstanding, in those chickens fed P diets, there was a decrease from P5 to P15 in the contribution of the ileum to SFA absorption, the values for the other P diets were similar among them (P5: 31%, P15: 23%, P35: 28%, and P50: 27%).

All of these findings suggest that the utilization of unsaturated diets was more affected by the dietary FFA%, in comparison to the utilization of saturated diets, and that the dietary SFA content could affect fat absorption more than the dietary FFA%, this being supported by the UFA: SFA ratios reported for the different diets. Thus, while in S diets there were 2 factors affecting fat absorption, as the dietary FFA% was higher: greater SFA (UFA: SFA ratio: 4.43 and 3.37 for S5 and S50, respectively), and FFA content, in P diets, the dietary FFA% was higher, but SFA content remained more constant (UFA: SFA ratio: 1.32 and 1.21 for P5 and P50, respectively). Vilarrasa et al. (2015) also reported a greater effect of the dietary SFA content rather than that of the dietary FFA% on FA apparent absorption. It also has to be taken into account that FA digestibility in the saturated diets was already low when crude oil was used, and so it probably had less chance to decrease.

The interactions observed in the excreta for TFA, SFA, MUFA, and PUFA digestibility could support the different effects of the dietary FFA% between fat sources. While the FA digestibility coefficients observed for the chickens fed P diets (in general, lower than those observed for the chickens fed S diets) did not significantly change as the dietary FFA% became higher, there was a general decrease of the FA digestibility coefficients for those chickens fed S diets, especially from S15 to S50. Furthermore, SFA and MUFA digestibility coefficients reported for the chickens fed S35 and S50 diets were not different from those reported for the chickens fed P diets. Nonetheless, it is important to mention that the digestibility coefficients reported in the excreta could be influenced by bacterial activity. The fat source and dietary FFA% effects observed for the concentration in the excreta of those FA that come from bacterial metabolism supported this. Nevertheless, it is likely that FA endogenous losses, such as desquamated epithelial cells, also contributed to the results reported in the excreta. For this reason, it is likely that the results reported in the jejunum and ileum are better indicators of the FA absorption process rather than those reported in the excreta.

In conclusion, the results of the present study allow for a better understanding of the limitations of the incorporation of acid oils in starter broiler-chicken diets. The better absorption of the unsaturated dietary fat, which was related to a higher contribution of the ileum to SFA absorption, has been confirmed. It was seen that the absorption process is the most limiting part of fat utilization (in comparison to hydrolysis), and that the jejunum is the main place of FA absorption. The absorption process was also more affected than was hydrolysis by the dietary FFA%; the effect of the dietary FFA% on FA absorption being more evident in the unsaturated diets than in the saturated diets, which, in general, were related to low digestibility values. It was suggested that the dietary SFA content could have a greater impact on FA absorption, in comparison to the dietary FFA%. The results suggest that crude soybean oil is an adequate fat source for starter broiler-chicken diets, and moderate levels of acid soybean oil could substitute crude soybean oil (as long as the dietary FFA% does not exceed 15%) without having negative repercussions on either FA absorption or growth performance. On the other hand, and irrespective of the dietary FFA%, palm oil sources are not suitable for starter broiler-chicken diets, as, in general, they were related to lower FA absorption than were the diets supplemented with soybean oil sources.

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