

Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages

R. Rodriguez-Sanchez ^{*,1}, A. Tres,[†] R. Sala,^{*} F. Guardiola,[†] and A. C. Barroeta^{*}

^{*}Animal Nutrition and Welfare Service, Department of Animal and Food Science, Facultat de Veterinària, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain; and [†]Nutrition, Food Science and Gastronomy Department – XaRTA-INSA, Facultat de Farmàcia i Ciències de l’Alimentació, Universitat de Barcelona, Joan XXIII, 27-31, E-08028 Barcelona, Spain

ABSTRACT The aim of the present study is to evaluate the effect of the dietary fat saturation degree and age on the lipid class (TAG, DAG, MAG, and FFA) composition and fatty acid digestibility along the gastrointestinal tract (GIT) and excreta in broiler chickens. A total of 120 one-day-old female broiler chickens were randomly distributed in 2 dietary treatments (6 cages/treatment), which resulted from the supplementation of a basal diet with 6% of soybean oil or palm oil. Two digestibility balances were carried out at 14 and 35 d and fatty acid digestibility and lipid class composition were determined in the gizzard, duodenum, jejunum, ileum, and excreta. Along de GIT, both fatty acid digestibility and lipid class composition were influenced by the dietary fat source and the age of the chickens. The absorption of the unsaturated fat was more efficient and faster than it was for the saturated fat. The

ability of adult chickens to absorb fat was higher than for young chickens. The results show that the duodenum is the main place of fat digestion (hydrolysis), and the jejunum the main place of fat absorption. The role of the ileum on fat absorption is very important, as it is the last segment of the GIT where the absorption of fatty acids has been described. Thus, it was the contribution of the ileum that was responsible for the higher fat utilization observed for animals fed the unsaturated diet than for those fed the saturated diet at 14 d, and it was also responsible for the improvement on the utilization of the saturated diet between 14 and 35 d. All the results suggest that the absorption of fatty acids is more limiting than is hydrolysis, because the main differences were observed in the jejunum and ileum, where the absorption of fatty acids takes place.

Key words: fat saturation degree, fatty acid digestibility, fatty acid absorption, lipid class, broiler

2019 Poultry Science 98:1341–1353
<http://dx.doi.org/10.3382/ps/pey458>

INTRODUCTION

Among the ingredients used in the formulation of animal feeds, fats and oils are the most concentrated sources of energy, and their use in poultry diets is a widespread practice. It is well known that factors related to the diet like the saturation degree of fat or the chain length of fatty acids (FA), and factors related to the animal like the age, strain, or gender affect the digestion and absorption of lipids (Renner and Hill 1961; Sklan et al., 1973; Ravindran et al., 2016).

Briefly, fat digestion starts with the hydrolysis of triacylglycerols (TAG), which are the main component of fat in the diet, by pancreatic lipase, mainly resulting in a sn-2-monoacylglycerol (MAG) and two free FA (FFA) molecules from the sn-1 and sn-3 positions. Some of the resulting products do not need emulsifica-

tion and are absorbed passively from the intestinal lumen while others, like long-chain saturated FA (SFA) and diacylglycerols (DAG), need the solubilization in the hydrophobic cores of mixed micelles to be transported to the intestinal cells and absorbed.

It is accepted that the small intestine is where most of the digestion and absorption processes take place. However, in broiler chickens, the digestibility of fat is usually determined in the ileum or excreta and, consequently, the information regarding the relative importance of the different segments of the gastrointestinal tract (GIT) is scarce. There are also other factors like the gastro-duodenal reverse peristalsis (Sacranie et al., 2012) and the fat endogenous secretion of lipids into the intestine that make these processes more complex.

Tancharoenrat et al. (2014) considered the importance of studying the digestibility of FA in different segments of the GIT to understand the dynamics of fat digestion; however, there is no available information about the composition of lipid classes (TAG, DAG, MAG, and FFA) in the different GIT segments. The determination of lipid classes is a way to assess the

© 2018 Poultry Science Association Inc.

Received May 24, 2018.

Accepted September 6, 2018.

¹Corresponding author: Raquel.Rodriguez@uab.cat

hydrolysis of TAG, through their disappearance and the parallel appearance of lipolysis products (DAG, MAG, and FFA), as well as the absorption of lipolysis products through their disappearance from the intestinal lumen.

The present study was conducted in order to see the effect of the dietary fat saturation degree and the age of the chickens (14 and 35 d) on FA digestibility and lipid class composition along the GIT (gizzard, duodenum, jejunum, and ileum) and excreta. This will allow for a better understanding of the relative importance of the different GIT segments in the fat digestion and absorption processes, as well as to see if there are some changes according to the dietary fat source and the age of the chickens, as it was hypothesized that the fat digestion and absorption dynamics will be affected by these two factors. This information is essential to know the ideal composition of dietary fats in order to optimize their utilization.

MATERIALS AND METHODS

Animals and Diets

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

A total of 120 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, 40.54 g \pm 2.18 g) and randomly assigned to one of the two dietary treatments, with 10 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.

Birds received a starter feed (in mash form) until day 21 and a grower–finisher feed (in mash form) from day 22 to day 35. Wheat- and soybean-meal-based diets were formulated to meet or exceed FEDNA requirements (2008). The two dietary treatments were obtained including 6% of crude soybean oil (**S**) or crude palm oil (**P**) in the basal diet; the ingredient composition of the experimental diets is presented in Table 1. Titanium dioxide (**TiO₂**) was added (5 g/kg) as an inert marker for the determination of the digestibility of FA.

Controls and Sampling

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

Table 1. Ingredient composition of the experimental diets.

Ingredients, %	Starter diet (from 0 to 21 d)	Grower–finisher diet (from 22 to 35 d)
Wheat	54.46	44.00
Soybean meal 48%	35.40	27.25
Barley	–	18.58
Experimental fats ¹	6.00	6.00
Calcium carbonate	1.44	1.39
Sodium chloride	0.40	0.35
Vitamin and mineral premix ²	0.40	0.40
DL-Methionine	0.23	0.17
L-Lysine	0.15	0.12
L-Threonine	–	0.02
Titanium dioxide	0.50	0.50
Ethoxyquin 66%	0.02	0.02

¹Soybean or palm oil.

²Provides per kg of feed: vitamin A (from retinol), 13,500 IU; vitamin D₃ (from cholecalciferol), 4800 IU; vitamin E (from α -tocopherol), 49.5 IU; vitamin B₁, 3 mg; vitamin B₂, 9 mg; vitamin B₆, 4.5 mg; vitamin B₁₂, 16.5 μ g; vitamin K₃, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 μ g; Fe (from FeSO₄·7H₂O), 54 mg; I (from Ca(I₂O₃)₂), 1.2 mg; Co (from 2CoCO₃·3Co(OH)₂·H₂O), 0.6 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.

Two digestibility balances were carried out from 11 to 14 d and from 33 to 35 d. At 14 d of age, 7 birds per cage were killed by cervical dislocation and samples of the 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 were taken. The digestive content of each segment of the GIT from all birds within each cage was pooled, homogenized, and frozen at -80°C until further analysis. At 35 d of age, the remaining birds were euthanized and the same procedure was repeated.

Chemical Analysis

Analytical determinations of the diets were performed according to the methods of the AOAC International (2005): dry matter (Method 934.01), ash (Method 942.05), crude protein (Method 968.06), crude fat (Method 2003.05), and crude fiber (Method 962.09). Gross energy was determined by an adiabatic calorimeter (IKA C-4000, Janke-Kunkel; Staufen, Germany).

TiO₂ was analyzed following the procedures of Short et al. (1996) with small changes. Briefly, 0.2 g of fresh sample was ashed and dissolved in 2 mL of 7.4 M sulphuric acid. A 1/10 dilution was prepared, using 0.5 mL of the sample and 4.5 mL of a 1% HNO₃ solution. Ti was determined by ICP-OES (Optima 3200 RL, Perkin Elmer; Waltham, MA, USA) under standard conditions.

For the determination of the FA composition of the feed, excreta, and digestive content, lipids were extracted as follows: 0.5 g of the fresh sample was weighed in a test tube and 3 mL of HCl 1 N solution and

7.5 mL of diethyl ether were added; the content of each tube was homogenized for 30 s at 20,000 rpm, using a high-speed homogenizer (Polytron® System PT 3100; Kinematica AG; Switzerland). After the homogenization, the content of each tube was poured into a centrifuge test tube, and the procedure of adding 7.5 mL of diethyl ether and homogenizing was repeated and the mixtures were then pooled in the centrifuge tube. 1 mL of a saturated NaCl solution was added, and the tubes were centrifuged for 15 min at 618.5 g. After the centrifugation, the upper layer was transferred into a round-bottom flask. Then, 15 mL of diethyl ether were added and the centrifugation process was repeated as described above. The upper layer was transferred into the round-bottom flask. The content in the round-bottom flask was evaporated completely in a rotatory evaporator set at 35°C, the extracted fat was transferred by dissolving it in diethyl ether in a glass test tube and the content was evaporated using N₂ at 35°C. The tubes with the extracted fat were kept at -20°C until FA determination. FA methyl esters were obtained as described by Guardiola et al. (1994). Briefly, the fat extracted was methylated with sodium methoxide (0.5 N), followed by boron trifluoride (20 wt % in methanol), and FA methyl esters were extracted with n-hexane. Subsequently, FA methyl esters were analyzed using an Agilent 4890D gas chromatograph (Agilent Technologies; Santa Clara, CA, USA) equipped with a flame ionization detector and a capillary column (SP-2380, 60 m × 0.25 mm i.d., 0.2 μm from Supelco; Bellefonte, PA, USA). Hydrogen (25 p.s.i.) was used as the carrier gas. Injector and detector were set at 270 and 300°C, respectively. Oven temperature was set at 150°C for 1 min, increased by 3°C/min to 180°C; after 0.5 min at 180°C, it was increased by 14.5°C/min to 220°C, maintained at 220°C for 3 min, and finally increased by 9.9°C/min to 250°C, and maintained for 3 min at this temperature. FA methyl esters were identified by matching their retention times with those of standards (Supelco 37 component FAME Mix, Sigma-Aldrich Co.; St. Louis, MO, USA). Quantification was performed by means of calibration curves of single FA methyl esters (C14:0, C16:0, C18:0, C16:1n-7, C18:1n-9, C18:2n-6, C18:3n-3, and C24:0 methyl esters), using nonadecanoic acid methyl ester as an internal standard (all purchased from Sigma-Aldrich Co.; St. Louis, MO, USA).

Lipid class composition (TAG, DAG, MAG, and FFA) of the extracted fat from the feed, excreta, and digestive content was determined by size-exclusion chromatography on an Agilent 1100 series HPLC chromatograph equipped with an isocratic pump, with the oven and a Refractive Index Detector (RID) both set at 35°C. Previously, the lipids of the feed, excreta, and digestive content were extracted as explained above. After lipid extraction, lipids were dissolved in 2 mL of tetrahydrofuran and filtered through a Nylon filter (0.45 μm), and 100 μL were injected (20 μL loop) into the HPLC. Separation was conducted using 2 Styragel

columns (Styragel HR 1 and Styragel HR 0.5) of 30 cm × 0.78 cm i.d., filled with a spherical styrenedivinylbenzene copolymer of 5-μm particle size and 100 Å and 50 Å, respectively (Water Associates; Milford, MA, USA), connected in series. The mobile phase consisted of tetrahydrofuran (HPLC quality grade) at 1 mL/min. Lipid classes were identified by matching their retention times with those of standards (trioleoylglycerol for TAG, dioleoylglycerol for DAG, oleoylglycerol for MAG, and oleic acid for FFA; Sigma-Aldrich Co.; St. Louis, MO, USA) and quantified by internal normalization (area percent).

The macronutrient, FA, and lipid class composition of the experimental diets are presented in Table 2.

Formulas used for Digestibility Determination

The digestibility coefficients of FA in each segment of the GIT were determined using the TiO₂ ratio in the feed and digestive content or excreta according to the following formula:

$$\text{Digestibility of FA} = 1 - \left(\frac{[\text{Ti}]_{\text{diet}} / [\text{FA}]_{\text{diet}}}{[\text{Ti}]_{\text{dig}} / [\text{FA}]_{\text{dig}}} \right),$$

where [Ti]_{diet} is the concentration of TiO₂ in the diet (g/g DM), [FA]_{diet} is the FA content in the diet (g/g DM), [Ti]_{dig} is the concentration of TiO₂ in the digestive content or excreta (g/g DM), and [FA]_{dig} is the FA content in the digestive content or excreta (g/g DM).

Regarding the apparent metabolizable energy (AME), it was determined from the product of the energy utilization ratio and its corresponding gross energy of feed.

Statistical Analysis

Productive parameters were subjected to Student's *t*-test using the type of fat in feed as a factor (SPSS statistics 25.0.0.0, IBM 2017). For the comparison of the lipid class composition and FA digestibility between both dietary treatments a Student's *t*-test was also performed for each one of the GIT segments. A univariate analysis using the GLM procedure was also performed for FA digestibility to study whether FA composition of the intestinal content depended on the added fat, and also of the GIT segment. For the comparison of the results observed at different ages, both for the lipid classes and FA digestibility, a univariate analysis using the GLM procedure was carried out for each one of the GIT segments in order to study whether the composition of intestinal content depended on the added fat, and on the age of the chickens. Pairwise comparisons were used to see the differences in every parameter between both fat sources, and ages. The cage served as the experimental unit, so that there were 6 replicates

Table 2. Analyzed¹ macronutrient content and fatty acid and lipid-class composition of the experimental diets² for the starter.

Item	Starter diets (from 0 to 21 d)		Grower–finisher diets (from 22 to 35 d)	
	S	P	S	P
<i>Macronutrient content,</i>				
Dry matter %	92.41	89.72	92.95	90.48
Crude protein %	21.20	20.88	17.64	18.05
Crude fat %	7.39	7.68	6.88	6.76
Crude fiber %	3.18	3.37	2.94	3.09
Ash %	5.64	6.30	5.15	5.93
Gross energy, kcal/kg	4259	4207	4190	4161
AME ³	3284	3119	3327	3199
<i>Fatty acid composition, %</i>				
C14:0	–	0.81	–	0.79
C16:0	12.43	36.79	13.83	35.96
C18:0	3.76	4.11	3.75	4.12
C18:1 n-9	19.16	32.45	19.45	31.44
C18:1 n-7	0.28	0.31	0.58	0.33
C18:2 n-6	54.39	21.78	52.83	23.36
C18:3 n-3	7.44	2.15	7.28	2.36
Minor fatty acids	2.54	1.59	2.28	1.62
SFA	17.07	42.06	18.24	41.22
MUFA	21.02	33.99	21.65	33.05
PUFA	61.83	23.93	60.11	25.72
UFA:SFA	4.86	1.38	4.29	1.62
<i>Lipid-class composition, %</i>				
TAG	89.28	80.84	94.91	81.40
DAG	3.51	9.75	2.93	9.45
MAG	2.79	2.59	0.71	0.94
FFA	4.41	6.81	1.45	8.21

¹All samples were analyzed at minimum in duplicate.

²Diets with 6% of soybean oil (S) or palm oil (P).

³Values are pooled means of 12 replicates: with 10 chickens/replicate at 14 days, and 3 chickens/replicate at 35 days.

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.

per treatment. Differences were considered significant at $P < 0.05$.

RESULTS

Characterization of Experimental Diets

The chemical analysis of the experimental diets reflected the composition of the added oils (P or S) and is presented in Table 2. The main FA in S diets were linoleic and oleic acids, while the main FA in P diets were palmitic, oleic, and linoleic acids, both in the starter and the grower–finisher period. The unsaturated-to-saturated FA ratios also reflected the composition of the added oils. All of the diets had high proportions of TAG (>80%), and low proportions of DAG, MAG, and FFA (Table 2). However, there were some differences between the S and P diets. S diets had higher proportions of TAG, and lower proportions of DAG and FFA, in comparison to P diets, for both periods. The proportion of MAG between S and P diets was similar in each period.

Growth Performance

The broiler chickens completed the trial successfully. The effect of dietary fat sources on growth performance

in the starter (from 0 to 21 d), the grower–finisher (from 22 to 35 d) and the global (from 0 to 35 d) periods are presented in Table 3. No significant differences were observed in any feeding period ($P > 0.05$).

Determinations in 14-day-old Broiler Chickens

Lipid Class Composition along the Gastrointestinal Tract. The lipid class composition (TAG, DAG, MAG, and FFA) in different GIT segments (gizzard, duodenum, jejunum, and ileum) and excreta was determined in 14-day-old broiler chickens fed diets with two different fat sources (S and P), and is shown in Figure 1.

Considering the average results of both fat sources together at 14 d, TAG was the major lipid class in the gizzard (65.17%), while in the duodenum it only represented 13.52% of all lipid classes. The percentage of DAG was also lower in the duodenum (9.81%) than in the gizzard (15.02%). In parallel to the TAG and DAG disappearance (hydrolysis), the percentage of the end lipolysis products (MAG and FFA) increased, especially the FFA percentage, which was the most predominant lipid class in the duodenum (70.55%), jejunum (78.06%), ileum (75.19%), and excreta (61.38%). From

Table 3. Growth performance of broiler chickens according to different fat sources in the diet.¹

Item	Dietary treatments		Statistics	
	S	P	SEM	P-value
From 0 to 21 d				
ADFI, g/d per bird	48.4	49.1	1.089	0.76
ADG, g/d per bird	36.9	37.1	0.705	0.89
FCR, g/g	1.31	1.33	0.016	0.72
BW at 21 d, g	815	819	14.815	0.89
From 22 to 35 d				
ADFI, g/d per bird	146.1	150.2	1.180	0.11
ADG, g/d per bird	88.1	89.6	1.072	0.52
FCR, g/g	1.66	1.68	0.014	0.45
From 0 to 35 d				
ADFI, g/d per bird	87.5	89.6	0.878	0.26
ADG, g/d per bird	57.4	58.1	0.657	0.61
FCR, g/g	1.52	1.54	0.007	0.27
BW at 35 d, g	2049	2073	23.0	0.61

¹Diets with 6% of soybean oil (S) or palm oil (P).
 ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; BW = body weight.
 Values are means of 6 replicates. SEM = standard error of the mean.
 P-values were obtained from Student's *t*-test conducted to study whether the type of fat added to feed affected growth performance values. *P* < 0.05 was considered significant.

the duodenum to the ileum, the percentage of MAG was lower in each segment (6.12% in the duodenum, 4.50% in the jejunum, and 4.14% in the ileum). The increase in TAG, DAG, and MAG percentage in the excreta in comparison to the results observed in the ileum, were due to the high absorption of FFA in the ileum, which implies a relative increase of the rest of lipid classes

in the excreta since the results are expressed in area normalization (%).

Considering the added fat source (Figure 1), TAG was higher for S than for P in the gizzard (*P* = 0.018), while both DAG (*P* = 0.034) and FFA (*P* = 0.024) were lower for S than for P. There were no differences in the duodenum for any of the lipid classes, but the reduction in the TAG percentage from the gizzard to the duodenum, as well as the percentage of MAG and FFA in the duodenum, was numerically higher for S than for P. In the jejunum, the percentage of TAG was significantly higher for S than for P (*P* = 0.046). Regarding the lipolysis products, the percentage of MAG was higher for S than for P in the jejunum and ileum (*P* = 0.004 and *P* = 0.041, respectively), while the percentage of FFA was lower for S than for P in the jejunum (*P* = 0.011), ileum (*P* < 0.001), and excreta (*P* < 0.001). In the ileum and excreta, the percentage of TAG and DAG was also significantly higher for S than for P (*P* < 0.001). This finding is related to the absorption (disappearance) of the other lipid classes (MAG and FFA).

Fatty Acid Digestibility Coefficients along the Gastrointestinal Tract.

FA digestibility coefficients were also studied in different segments of the GIT, and in the excreta, for the 2 different fat sources (S and P) and are shown in Table 4. SFA digestibility was mainly represented by palmitic (C16:0) and stearic (C18:0) acids, monounsaturated FA (MUFA) digestibility by oleic acid (C18:1n-9), and polyunsaturated FA (PUFA) digestibility by linoleic (C18:2n-6) acid.

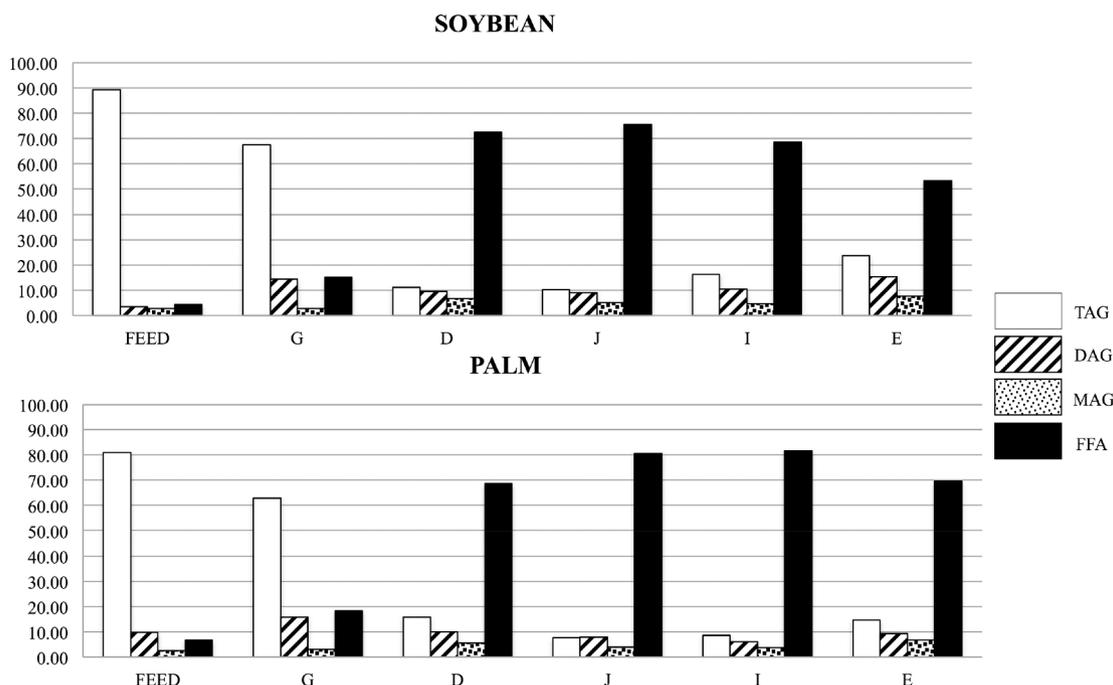


Figure 1. Lipid class composition (%) of the feed and along the gastrointestinal tract (each value represents the mean of 6 replicates (7 chickens/replicate)) according to different fat sources (diets with 6% of soybean oil or palm oil) in the diet in 14-day-old broiler chickens. G = gizzard; D = duodenum; J = jejunum; I = ileum; E = excreta; TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids.

Table 4. Digestibility coefficients along the gastrointestinal tract according to different fat sources in the diet in 14- and 35-day-old broiler chickens.

	Dietary treatments ¹										GIT segment ²					<i>P</i> -values			
	S					P					G	D	J	I	E	SEM	Fat	GIT	Interaction
	G	D	J	I	E	G	D	J	I	E									
Digestibility coefficients at 14 d																			
TFA	0.40	-3.00	0.59	0.81	0.84	0.11	-2.34	0.56	0.60	0.68	0.26	-2.67	0.57	0.70	0.76	0.266	0.954	< 0.001	0.17
SFA	0.36	-4.94	0.55	0.77	0.79	0.23	-1.67	0.47	0.48	0.53	0.29	-3.30	0.51	0.62	0.66	0.444	0.005	< 0.001	< 0.001
C16:0	0.32	-3.55	0.58	0.79	0.81	0.22	-1.17	0.48	0.49	0.54	0.27	-2.36	0.53	0.64	0.67	0.199	0.007	< 0.001	< 0.001
C18:0	0.47	-10.30	0.47	0.72	0.76	0.31	-6.15	0.39	0.40	0.45	0.39	-8.22	0.43	0.56	0.61	3.853	0.201	< 0.001	0.028
MUFA	0.42	-1.54	0.61	0.81	0.84	0.25	-1.56	0.64	0.68	0.78	0.33	-1.55	0.63	0.75	0.83	0.117	0.464	< 0.001	0.95
C18:1 n-9	0.42	-1.37	0.62	0.81	0.85	0.23	-1.55	0.63	0.68	0.81	0.33	-1.46	0.62	0.74	0.83	0.110	0.219	< 0.001	0.925
PUFA	0.41	-2.95	0.59	0.83	0.86	-0.29	-4.64	0.58	0.68	0.78	0.06	-3.80	0.59	0.76	0.82	0.621	0.013	< 0.001	0.06
C18:2 n-6	0.40	-3.15	0.58	0.82	0.85	-0.29	-4.85	0.57	0.68	0.78	0.06	-4.00	0.58	0.75	0.82	0.678	0.017	< 0.001	0.077
C18:3 n-3	0.42	-1.49	0.67	0.86	0.88	-0.33	-2.52	0.69	0.74	0.80	0.04	-2.01	0.68	0.80	0.84	0.216	0.002	< 0.001	0.024
Digestibility coefficients at 35 d																			
TFA	0.46	-7.42	0.79	0.89	0.91	0.45	-4.35	0.66	0.77	0.84	0.46	-5.88	0.72	0.83	0.88	2.412	0.179	< 0.001	0.056
SFA	0.44	-11.10	0.77	0.83	0.88	0.52	-3.16	0.58	0.66	0.75	0.48	-7.13	0.67	0.75	0.82	5.920	0.020	< 0.001	< 0.001
C16:0	0.41	-8.44	0.77	0.88	0.88	0.51	-2.59	0.59	0.66	0.75	0.46	-5.51	0.68	0.77	0.81	3.204	0.023	< 0.001	< 0.001
C18:0	0.58	-22.01	0.77	0.72	0.92	0.59	-8.28	0.49	0.68	0.72	0.58	-15.14	0.63	0.70	0.82	27.143	0.055	< 0.001	0.005
MUFA	0.47	-4.60	0.84	0.91	0.93	0.54	-3.28	0.79	0.89	0.93	0.50	-3.94	0.81	0.90	0.93	1.052	0.322	< 0.001	0.412
C18:1 n-9	0.45	-4.49	0.83	0.91	0.93	0.53	-3.23	0.79	0.89	0.93	0.49	-3.86	0.81	0.90	0.93	1.014	0.326	< 0.001	0.449
PUFA	0.47	-7.31	0.78	0.89	0.92	0.22	-7.62	0.63	0.78	0.88	0.34	-7.47	0.70	0.84	0.90	2.876	0.696	< 0.001	1.000
C18:2 n-6	0.46	-7.79	0.77	0.89	0.92	0.22	-8.09	0.62	0.78	0.88	0.34	-7.94	0.70	0.83	0.90	3.241	0.720	< 0.001	1.000
C18:3 n-3	0.50	-3.82	0.84	0.92	0.94	0.21	-2.99	0.70	0.81	0.89	0.35	-3.40	0.77	0.87	0.91	0.746	0.826	< 0.001	0.532

¹Two dietary treatments: diets with soybean oil (S) or palm oil (P). Values are pooled means of 6 replicates (with 8 chickens/replicate at 14 d; and 3 chickens/replicate at 35 d).

²Values are pooled means of 12 replicates (with 8 chickens/replicate at 14 d; and 3 chickens/replicate at 35 d).

G = gizzard; D = duodenum; J = jejunum; I = ileum; E = excreta; GIT = gastrointestinal tract; TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SEM = standard error of the mean.

Values correspond to pooled means obtained from two-way ANOVA conducted to the effect of the type of fat added and the GIT segment. *P* < 0.05 was considered significant.

Considering the average results of both fat sources together at 14 d, digestibility coefficients of FA close to 0.3 were reported in the gizzard for total FA (TFA), SFA, and MUFA, while the digestibility of PUFA in this GIT segment was lower in comparison with the other FA groups. In the duodenum, all of the digestibility coefficients were highly negative, especially for SFA and PUFA. At the jejunum level, FA digestibility coefficients close to 0.6 were observed, except for SFA, which had lower digestibility coefficients than did the others. All FA groups had an increase in their digestibility from the jejunum to the ileum, reaching digestibility coefficients over 0.7 for TFA, MUFA, and PUFA, and close to 0.6 for SFA. An increase in FA digestibility was also observed from the ileum to the excreta, observing digestibility coefficients close to 0.8 for TFA, MUFA, and PUFA, and close to 0.65 for SFA.

A significant interaction between the fat source and the GIT segment was observed for SFA (*P* < 0.001; Figure 2); this interaction being explained by palmitic (C16:0) and stearic (C18:0) acids, the major SFA in the diet. This interaction was due to the change observed in the digestibility coefficients between the duodenum and jejunum. While the SFA digestibility coefficient in the duodenum was considerably lower for S than for P, S had a higher digestibility coefficient in the jejunum, ileum, and excreta. Regarding PUFA, a significant effect was observed for the added fat to the diet; a more

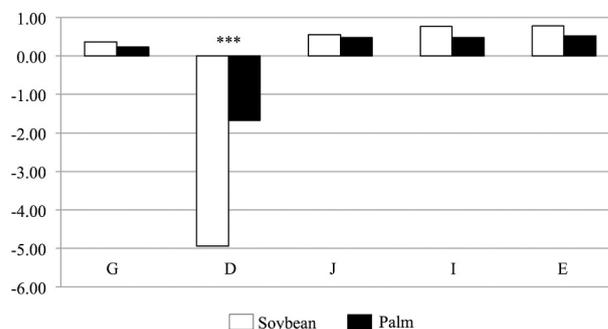


Figure 2. Saturated fatty acid digestibility coefficients along the gastrointestinal tract for two different fat sources in the diet (diets with 6% of soybean oil or palm oil) in 14-day-old broiler chickens. G = gizzard; D = duodenum; J = jejunum; I = ileum; E = excreta. Values are the means of 6 replicates (with 7 chickens/replicate). *P* values were obtained from two-way ANOVA conducted to see the effect of the type of the added fat and the GIT segment. *** *P* < 0.001.

detailed explanation about this effect can be found in the next paragraph.

The digestibility coefficients observed for both fat sources were compared separately in the gizzard, duodenum, jejunum, ileum, and excreta. In the gizzard, PUFA digestibility was significantly higher in those animals fed S (S: 0.41 and P: -0.29; *P* = 0.027). In the duodenum, differences were found for SFA digestibility (S: -4.94 and P: -1.67; *P* = 0.003). As absorption is expected to happen from the jejunum on, the results observed from the jejunum to the excreta are shown

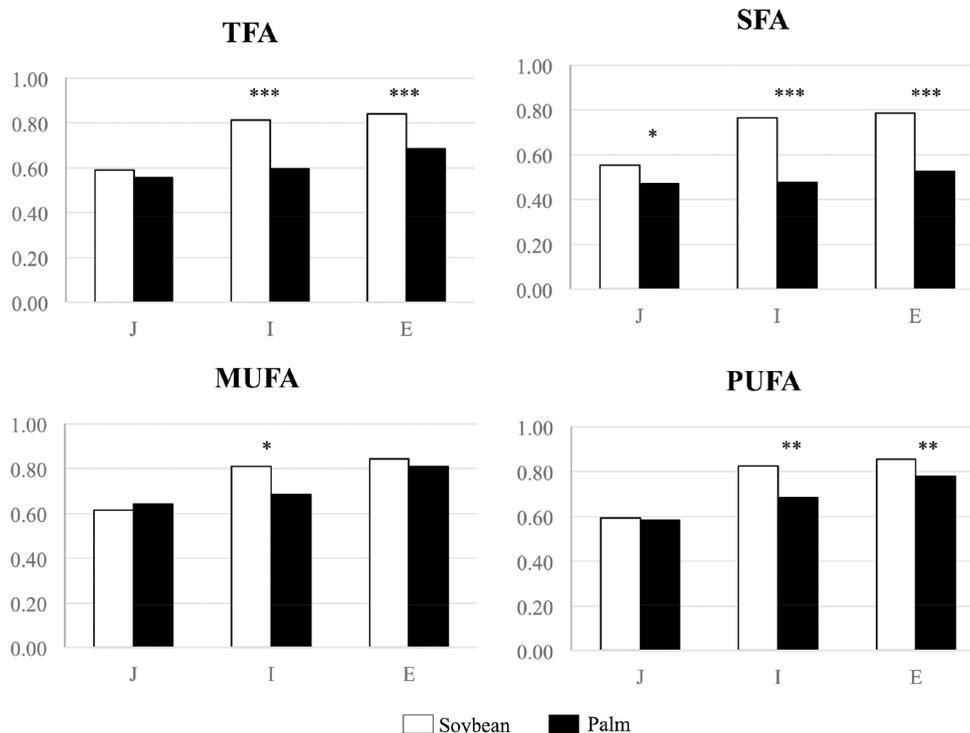


Figure 3. Fatty acid digestibility coefficients for two different dietary fat sources (diets with 6% of soybean oil or palm oil) in the jejunum, ileum and excreta in 14-day-old broiler chickens. TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated; J = jejunum; I = ileum; E = excreta. Values are the mean of 6 replicates (7 chickens/replicate). *P* values were obtained from Student's *t*-test conducted to see the effect of the type of the added fat in each gastrointestinal segment. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

in Figure 3. Those chickens fed S had higher TFA digestibility coefficients than did those fed P, both in the ileum and excreta ($P < 0.001$). The same was observed for PUFA in the ileum ($P = 0.010$) and excreta ($P = 0.002$). In the case of SFA, the digestibility coefficients observed for S were higher than were the ones observed for P in the jejunum ($P = 0.036$), ileum and excreta ($P < 0.001$). Regarding MUFA, differences were only observed in the ileum ($P = 0.019$), where S had higher digestibility coefficient than did P.

With reference to the AME (Table 2), those animals fed S showed a higher value than did those fed P ($P = 0.002$).

Determinations in 35-day-old Broiler Chickens

Lipid Class Composition along the Gastrointestinal Tract. The lipid class composition (TAG, DAG, MAG, and FFA) in different GIT segments (gizzard, duodenum, jejunum, and ileum) and excreta was determined in 35-day-old broiler chickens fed diets with two different fat sources (S and P), and is shown in Figure 4.

Regarding the general evolution of lipid classes along the GIT at 35 d, and considering the average results of both fat sources together, TAG was the main lipid class in the gizzard (62.67%), while in the duodenum it represented 16.15% of all lipid classes. The percentage

of DAG in the duodenum was also lower in comparison to the gizzard (9.81 and 15.02%, respectively). The highest percentage of MAG and FFA was found in the duodenum (6.06 and 67.35%, respectively). From the duodenum on, the percentage of both MAG and FFA decreased, with FFA being the most predominant lipid class in the jejunum (66.33%), ileum (55.51%), and excreta (52.27%).

Considering the added fat source (Figure 4), the percentage of TAG was higher for S than for P in the gizzard ($P = 0.021$), while the percentage of FFA was lower for S than for P ($P = 0.002$). No differences were found in the duodenum between both fat sources for any of the lipid classes. In the jejunum, the percentage of DAG was higher for S than for P ($P < 0.001$). The percentage of FFA was lower for S than for P in the ileum and excreta ($P = 0.003$ and $P < 0.001$, respectively), and the same tendency was observed in the jejunum ($P = 0.055$). The percentage of TAG and DAG was higher for S than for P, both in the ileum and excreta, and also the MAG percentage in the ileum. As explained before this finding is related to the disappearance (absorption) of FFA.

Fatty Acid Digestibility Coefficients along the Gastrointestinal Tract. The FA digestibility coefficients at 35 d depending on the GIT segment or excreta and the added fat (S and P) are shown in Table 4.

All of the FA groups showed a similar evolution of their digestibility coefficients along the GIT. Considering the average results of both fat sources together,

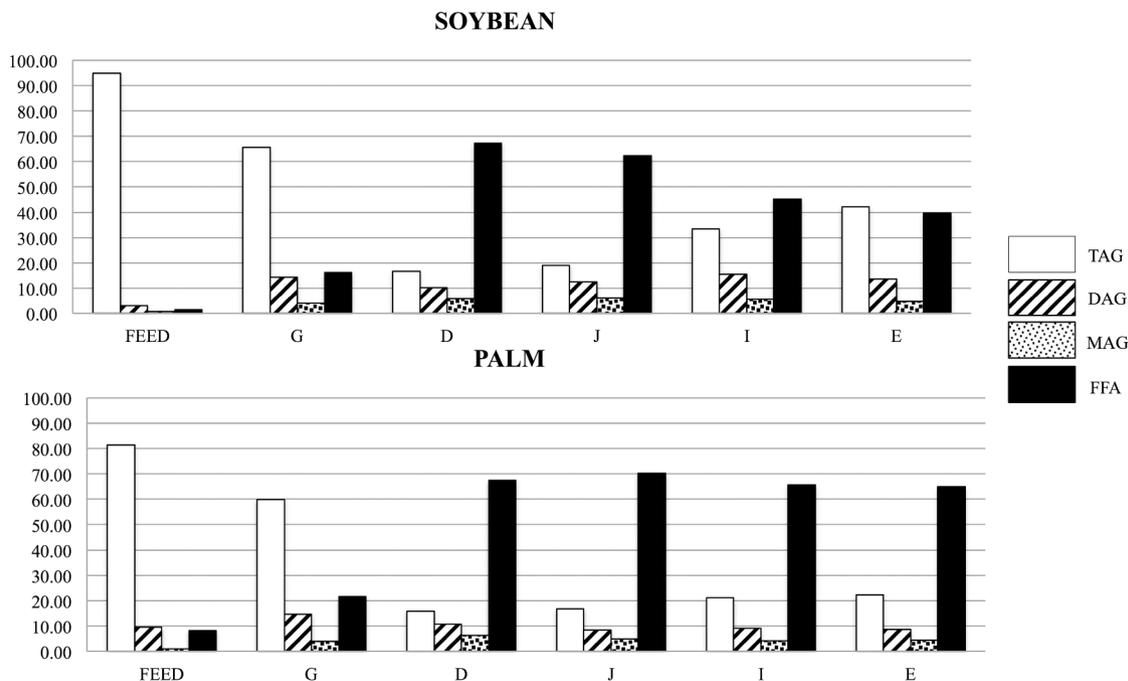


Figure 4. Lipid class composition (%) of the feed and along the gastrointestinal tract (each value represents the mean of 6 replicates (3 chickens/replicate)) according to different fat sources (diets with 6% of soybean oil or palm oil) in the diet in 35-day-old broiler chickens. G = gizzard; D = duodenum; J = jejunum; I = ileum; E = excreta; TAG = triacylglycerols; DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids.

digestibility coefficients close to 0.5, were observed in the gizzard for TFA, SFA, and MUFA; the digestibility coefficients for PUFA were lower in comparison to the other FA groups. In the duodenum, all the digestibility coefficients were highly negative, especially for SFA and PUFA. The utilization of FA continued in the jejunum, where digestibility coefficients close to 0.70 were reported for TFA, SFA, and PUFA, and digestibility coefficients close to 0.80 were reported for MUFA. In the ileum, the digestibility increased, in general, the digestibility of SFA being the lowest (0.75), and higher than 0.80 for TFA, MUFA, and PUFA. In the excreta, all the digestibility coefficients were higher than in the ileum, being higher than 0.80 for SFA and close to 0.90 for MUFA, PUFA, and TFA.

A significant interaction between the fat source and the GIT segment was observed for SFA. As found at 14 d, this interaction was explained by C16:0 and C18:0 ($P < 0.001$ and $P = 0.005$, respectively), and it was due to the change in the digestibility coefficients from the jejunum on. In the duodenum, the digestibility coefficient was lower for S than for P ($P < 0.001$), while in the jejunum, ileum, and excreta, the digestibility coefficient for S was higher than for P.

The results observed for both fat sources were compared in the gizzard, duodenum, jejunum, ileum, and excreta separately; the results from the jejunum to the excreta are shown in Figure 5. A statistically significant difference was observed in the gizzard for PUFA (S: 0.47 vs. P: 0.22; $P = 0.032$). The TFA and SFA digestibility coefficients in the jejunum ($P < 0.001$), ileum ($P \leq 0.001$), and excreta ($P \leq 0.007$) were higher for S

than for P (Figure 5). Regarding MUFA, S had a higher digestibility coefficient than did P in the jejunum ($P = 0.042$), and no differences were observed in the ileum and excreta. In the case of PUFA, S had higher digestibility coefficients in the jejunum ($P < 0.001$) and ileum ($P = 0.001$), while no differences were observed in the excreta.

Regarding AME (Table 2), those animals fed S had higher values than those fed P ($P = 0.011$).

Comparison Between 14- and 35-day-old Broiler Chickens

Lipid Class Composition along the Gastrointestinal Tract. Regarding the general evolution of lipid classes along the GIT (Figures 1 and 4) some differences were observed between 14 and 35 d, despite the fact the percentage of TAG in the diets was similar between both ages for each one of the fat sources. When the effect of the age of the chickens on the lipid class composition was studied in each GIT segment, higher percentage of MAG ($P = 0.005$) and percentage of FFA ($P = 0.030$) were observed in the gizzard at 35 d in comparison to the results observed at 14 d. No differences were observed in the duodenum. From the duodenum on, a general increase of TAG and MAG percentage, and a decrease of FFA percentage, were observed at 35 d. In the ileum, the DAG percentage was also higher ($P < 0.001$), while in the excreta it was lower ($P = 0.036$), at 35 d than at 14 d.

However, there was a common pattern for these lipid classes, both in S and P, between 14 and 35 d, a

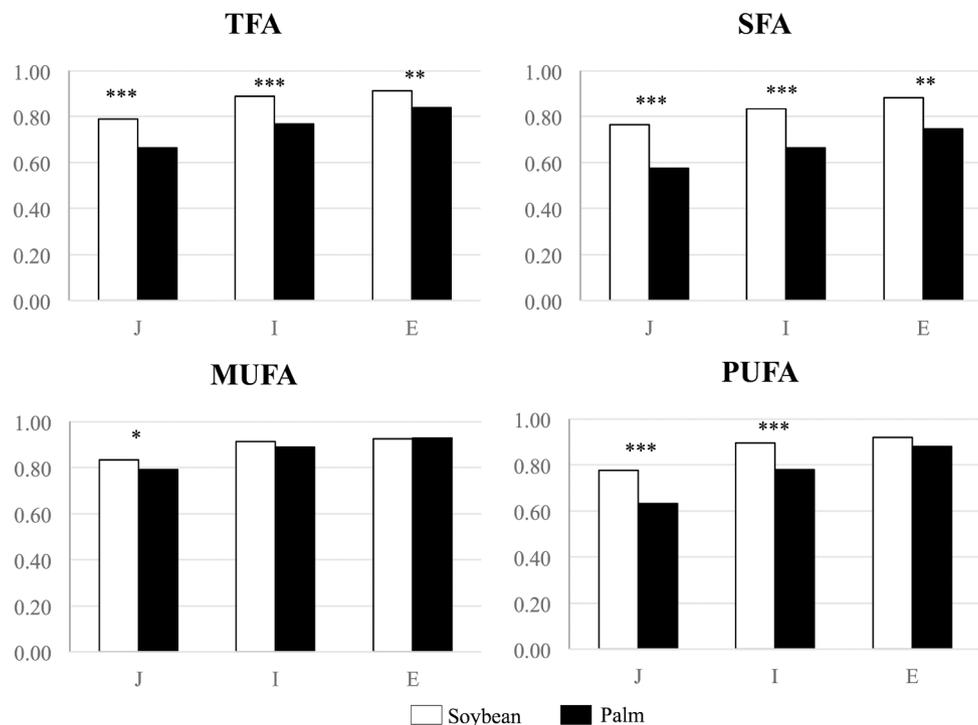


Figure 5. Fatty acid digestibility coefficients for two different dietary fat sources (diets with 6% of soybean oil or palm oil) in the jejunum, ileum and excreta in 35-day-old broiler chickens. TFA = total fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated; J = jejunum; I = ileum; E = excreta. Values are the mean of 6 replicates (3 chickens/replicate). *P* values were obtained from Student's *t*-test conducted to see the effect of the type of the added fat in each gastrointestinal segment. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

significant interaction between the fat source and the age of the chickens was observed for DAG in the jejunum ($P = 0.004$), and also for TAG ($P < 0.001$) and FFA ($P = 0.019$) in the excreta. The interactions were explained by the more pronounced changes observed in S than in P.

Fatty Acid Digestibility along the Gastrointestinal Tract. The age of the chickens had a positive effect on FA digestibility (Table 4). In the gizzard, the digestibility of SFA was significantly higher at 35 d than at 14 d ($P = 0.048$), and a tendency was observed for the digestibility of TFA ($P = 0.055$), MUFA ($P = 0.058$) and PUFA ($P = 0.059$). In the duodenum, the digestibility of TFA and all of the FA groups was lower (more negative) at 35 d than at 14 d ($P \leq 0.029$). In the jejunum, MUFA digestibility was higher at 35 d than at 14 d ($P < 0.001$), and a significant interaction between the fat source and the age of the chickens (Figures 3 and 5) was observed for TFA ($P = 0.022$), SFA ($P = 0.016$), and PUFA ($P = 0.004$); this was explained because, despite the fact the values were higher at 35 d, for both dietary treatments, the increase between both ages was higher for S than for P. In the ileum, there was an age effect for TFA digestibility, which was higher at 35 d than at 14 d ($P = 0.001$); the same was observed in the excreta for MUFA and PUFA ($P < 0.001$). A significant interaction between the fat source and the age of the chickens was observed for SFA, both in the ileum ($P = 0.04$) and excreta ($P = 0.008$), and also for TFA in the excreta ($P = 0.01$); a tendency was observed in the ileum

($P = 0.054$) for TFA. This was explained because, despite the fact the digestibility values were higher at 35 d than at 14 d, the increase between both ages was higher for P than for S.

Regarding AME, a tendency was observed taking into account the age of the chickens ($P = 0.071$). The AME was numerically higher at 35 d than at 14 d both for S and P; however, no differences were observed for S ($P > 0.05$), while a tendency was observed for P ($P = 0.095$).

DISCUSSION

Taking into account the results of the percentage of the lipid classes and FA digestibility along the GIT, it is possible to better understand fat digestion and absorption in broiler chickens in order to improve fat utilization by optimizing the fat formulation in diets. The digestion or hydrolysis is mainly related to the anterior part of the GIT (in this study represented by the gizzard and duodenum), while the absorption of fat is related to the posterior part of the GIT (in this study represented by the jejunum and ileum).

Considering the first step of fat digestion (hydrolysis), the decrease of TAG and the increase of the lipolysis products (DAG, MAG, and FFA) from the feed to the duodenum agreed with the hydrolysis of TAG, which requires pancreatic lipase, colipase, and bile salts. Thus, as the lipid classes show, the digestion of fat could start before the duodenum; however, according

to the literature, no FA absorption in this anterior part of the GIT is expected. Hence, the FA digestibility coefficients observed in the gizzard might be related to the gastro-duodenal contraction cycle (also known as reverse peristalsis), described in birds (Sacranie et al., 2005), which allows for the passage of the content between the gizzard and the duodenum. According to this, products such as bile salts, MAG, DAG, and pancreatic lipase that come from the duodenum are expected to be found in the gizzard (Sklan et al., 1978). Bile salts and MAG in the gizzard might start the emulsification of fat, which is the first step of fat digestion, but it is not expected that pancreatic lipase hydrolyze TAG in this GIT segment because of its acidic condition (Sklan et al., 1978). Lipases from vegetable origin in the ingredients of the feed, such as wheat lipases (Barros et al., 2010), could hydrolyze part of the dietary TAG in the crop, where the enzyme could work, as the pH in that GIT segment is neutral-alkaline, according to Kapranchikov et al. (2004). All of this explains the results found in this GIT segment, and it is in accordance with Ravindran et al. (2016). In addition, reverse peristalsis provides more time for digesta to be exposed to enzymatic activity and to mechanical systems, and according to Sacranie et al. (2005) this can lead to an increase in the digestion and absorption time in the upper intestine. Sacranie et al. (2005) also suggest that dietary factors like fiber can influence reverse peristalsis. Nevertheless, the effect of fat and age on gastro-duodenal reverse peristalsis has not been previously studied. Regarding the effect of the added fat, FA digestibility results reported in the gizzard were numerically higher for those chickens fed the unsaturated diet than for those fed the saturated diet, which could suggest the fat source has an effect on reverse peristalsis. The difference was statistically significant for PUFA, both at 14 and 35 d. Thus, PUFA disappearance in those animals fed the saturated diet was delayed and was lower in comparison to the chickens fed the unsaturated diet; this could be related to the different PUFA dietary level, which was much lower for the palm-oil-supplemented diet than for the soybean-oil-supplemented diet (Table 2). Regarding the age of the chickens, reverse peristalsis between the gizzard and the duodenum could be enhanced at 35 d, which is in agreement with the higher digestibility coefficients reported in the gizzard at 35 d in contrast to the ones reported at 14 d for SFA.

Moving on to the duodenum, the high amounts of all of the lipolysis products and the negative FA digestibility coefficients reported in this GIT segment could be explained both by the hydrolysis of TAG and endogenous fat losses; the gastro-duodenal reverse peristalsis could also have influenced. Regarding the endogenous fat, it comes from the bile in its majority (Tancharoenrat et al., 2014), but there can be other possible components, like mucin-associated lipids, and desquamated epithelial cells (Hurwitz et al., 1973; Clément, 1980; Gong et al., 1990). It has been described

that 48% of endogenous fat losses are accounted for FA (Tancharoenrat et al., 2014). All FA digestibility coefficients calculated in the duodenum were highly negative, especially for stearic (C18:0) and linoleic (C18:2n-6) acids (Table 4). This partially agreed with Hurwitz et al. (1973), who reported that the main FA secreted into the duodenum were linoleic (C18:2n-6) and palmitic (C16:0) acids, followed by stearic (C18:0) and oleic (C18:1n-9) acids. A little secretion of linolenic acid (C18:3n-3) was also reported. Regarding the lipid classes in the duodenum, the high percentage of the intermediate lipolysis products (DAG) and the end-lipolysis products (MAG and FFA) suggest that the hydrolysis of lipids starts and mainly takes place in this GIT segment. 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). Thus, the fat source of the diet could have an effect on endogenous fat losses, as the SFA digestibility coefficients observed for the animals fed the unsaturated diet in the duodenum were more negative than for those animals fed the saturated diet. It has been described that certain FA are able to affect pancreatic lipase; oleic and linoleic acids stimulate lipase activity, while SFA with 8 or 10 carbons have little effect, and the long-chain saturated ones inhibit the activity of the enzyme (Van Kuiken et al., 1994). This effect is slightly reflected in the higher TAG percentage decrease from the feed to the duodenum in S than in P, at both 14 and 35 d.

Regarding age, the more negative digestibility coefficients observed for TFA, SFA, MUFA, and PUFA in the duodenum at 35 d, in contrast to 14 d, could be related to higher endogenous fat losses into the duodenum lumen, and to an enhanced gastro-duodenal reverse peristalsis. This suggests that could be an improvement on bile secretion as age increases, as bile is the main source of endogenous fat losses. The higher bile secretion could also explain part of the improvement on the FA digestibility coefficients in the jejunum, ileum, and excreta, as they are essential both for the emulsification of the fat in the first step of fat digestion and the formation of mixed micelles, which are crucial for the absorption of FA (especially SFA). During the first weeks of the age of chicks, the biliary secretion is low, and it has been described as the most limiting factor for lipid digestion (Krogdahl, 1985; Noy and Sklan, 1995), and its effect on fat digestion-absorption has been well-documented (Hurwitz et al., 1973). Pancreatic lipase could be another limitation in young birds. However, there is a lack of agreement among different authors. Nitsan et al. (1991) and Uni et al. (1995) reported that the activity of pancreatic lipase increases with age, and Noy and Sklan (1995) reported that the secretion of lipase increased 20- to 100-fold between days 4 and 21 post-hatch. On the other hand, Meng et al. (2004) described a lack of response on fat digestibility after dietary supplementation

with lipase, and Sklan (2001) suggested that lipase secretion in young chickens may not be as inadequate as expected when their feed intake is considered. This finding is in agreement with the results observed in the present study, which may lead to the assumption that fat hydrolysis is not the most limiting factor in fat digestibility in young chickens.

All of the above suggest that the fat saturation degree might have an effect on both reverse peristalsis and fat endogenous losses, being the diets rich in unsaturated fat beneficial for these processes; this means more bile salts and enzymes in the intestinal lumen and a higher exposure time of the digestive content for the digestive enzymes. The age of the chickens also seems to have an effect on these two processes.

Moving on to the second step of fat digestion (the absorption of fat), FA digestibility, lipid classes, and AME results showed a difference between both fat sources. The results of FA digestibility and lipid classes also showed the contribution of each GIT segment involved in fat absorption.

Starting with the AME results, they supported the higher utilization of the unsaturated fat (S) in comparison to the saturated one (P) at both 14 and 35 d. This was also evident with the FA digestibility coefficients; those animals fed S had higher apparent digestibility coefficients of TFA in the ileum, at both 14 and 35 d, and in the jejunum at 35 d, than did those fed P. These results also agree with the evolution of lipid classes from the duodenum on. The evolution of FFA percentage from the jejunum to the excreta reflected a higher FFA absorption in those animals fed S in comparison to the ones fed P (sometimes even leading to a relative increase of TAG percentage). While in those animals fed S the FFA% decreased at 14 d in the ileum, in those fed P, the FFA percentage continued increasing in the ileum, indicating that even though TAG are hydrolyzed, FFA are not as efficiently absorbed in the animals fed the saturated diet (P). Lipid classes at 35 d showed an improvement in the FFA absorption that led not only to a decrease of FFA percentage, but also to a relative increase of TAG percentage, but still, the FFA absorption was higher for those animals fed S than for the ones fed P. This suggests that the absorption of unsaturated FFA throughout the intestinal epithelial surface is easier than is the absorption of the saturated ones. Being both the generation of the lipolysis products and their disappearance (absorption) from the intestinal lumen faster and more efficient in those chickens fed the unsaturated diet. This higher utilization of unsaturated fat could be explained by the higher apparent digestibility coefficients observed for SFA in the jejunum and ileum, and for PUFA in the ileum. In fact, SFA were the ones that differed the most between the animals fed the unsaturated diet and the ones fed the saturated diet, suggesting that SFA contributed the most to the lower digestibility coefficients reported for the animals fed the saturated diet. The better utiliza-

tion of SFA in those animals fed the unsaturated diet (S) could be due to the natural emulsifying properties of unsaturated FA, which assist in the mixed micelle formation and absorption. While unsaturated FA can form micelles spontaneously, SFA are non-polar and need the presence of the right amount of conjugated bile salts and unsaturated FA for the emulsification and incorporation into the micelles (Krogdahl, 1985; Smulikowska and Mieczkowaka, 1996). The length of the carbon chain of FA also has an effect on the FA utilization, the longer the carbon chain, the more difficult the incorporation of FA into micelles. As can be seen in Table 4, the utilization of stearic acid (C18:0) from the jejunum on was lower than it was for palmitic acid (C16:0). The hydrophobic cores of the mixed micelles also have the ability to solubilize the long-chain SFA, among other components, and facilitate their absorption.

Regarding the contribution of the jejunum and ileum in the FA absorption process, a different pattern was observed depending on the age of the chickens. This fact shows that the absorption of FA is more limiting than their digestion. Thus, even though adult birds have greater amounts of bile salts and a higher activity of pancreatic lipase, the higher digestibility coefficients observed at 35 d were mainly due to the enhanced FA absorption through the intestinal epithelial surface, which is also related to the lower percentage of FFA reported in the jejunum and ileum at 35 d in contrast to 14 d. Another factor that has been previously described and could have influenced the improvement of fat utilization at 35 d is the FA-binding protein (FABP) concentration in the intestinal mucosa, which is also age-dependent and has been reported to be insufficient in very young chicks, increasing its level after 4 wk of age (Katongole et al., 1980).

FA digestibility and lipid class results also showed that, independently of the fat source of the diet and the age of the chickens, the jejunum was the major site of lipid absorption. Considering the digestibility of the excreta as the highest (100%), and expressing the digestibility in the jejunum and ileum in relation to that, it was seen that regardless of the dietary fat source, 76% of TFA at 14 d, and 82% of TFA at 35 d, were absorbed in the jejunum; this is in accordance with Hurwitz et al. (1973) and Tancharoenrat et al. (2014). It is important to mention that the hydrolysis and absorption of fat are dynamic processes, and although hydrolysis mainly takes place in the duodenum, it probably continues in the jejunum where the generation and disappearance of the lipolysis products is likely to happen at the same time. In the ileum, some FA absorption was reported, as the digestibility coefficients in this GIT segment were higher than the ones reported in the jejunum, and the FFA decreased, being the second most important place of fat absorption. The contribution of the ileum to the absorption of FA was found to be different according to the fat source of the diet and the age of the chickens.

Thus, considering TFA digestibility, it was seen that the contribution of the ileum in those young chickens fed the unsaturated diet (S) was higher (26%) than it was for those fed the saturated diet (6%). The contribution of the ileum in the absorption of SFA in those chickens fed P was even lower (<1%), which is in agreement with Whitehead and Fisher (1975), who questioned the role of the ileum in the absorption of SFA. In contrast, those adult chickens fed the unsaturated diet (S) had a higher utilization of FA due to the higher contribution of the jejunum in its absorption (87%), leaving less remaining FA to be absorbed in the ileum (11%). On the other hand, the absorption of TFA in those adult chickens fed the saturated diet (P) also mainly took place in the jejunum (79%), however, the contribution of the ileum (12.5%) at 35 d was also important, especially for the SFA, which improved from less than 1% at 14 d to 11.5% at 35 d; nevertheless, the FA absorption was still higher for S than for P. The increase in the contribution of the ileum at 35 d in those animals fed P was also seen by the lower FFA percentage observed in the ileum at 35 d (66%) in comparison to the percentage observed at 14 d (82%). This is in accordance with Hurwitz et al. (1973), who reported some lipid absorption in the ileum, especially for linoleic, stearic, and palmitic acid.

All of this suggests that the contribution of the ileum to fat digestion is really important, as the last chance of those FA that leave the jejunum to be absorbed takes place in the ileum. The dietary fat and the age of the chickens also had an impact in the contribution of the jejunum and ileum to FA absorption.

Thus, those animals fed the unsaturated diet improved fat utilization at 35 d mainly due to a higher contribution of the jejunum in the absorption of FA, while those animals fed the saturated diet improved fat utilization at 35 d for both a higher contribution of the jejunum and especially for a higher contribution of the ileum in FA absorption in comparison to the results observed at 14 d. Thus, the higher contribution of the ileum on fat absorption at 14 d could explain the better utilization of unsaturated fat in comparison to saturated fat, while the higher contribution of the jejunum could explain the better utilization of the unsaturated fat at 35 d. This suggests that the role of the ileum in FA absorption is important and determines the global fat utilization.

The absorption of fat has been reported to be negligible in the hindgut of poultry (Renner, 1965). Thus, after the ileum, no FA absorption is expected, and the increase in FA digestibility coefficients (less than 10%) observed in the excreta, in comparison to the coefficients observed in the ileum, could be related to bacterial activity (Lan et al., 2005). On the other hand, the lower FFA percentage observed in the excreta for S both at 14 and 35 d also supports the higher utilization of the unsaturated fat.

In conclusion, the determination of lipid classes offers a wider outlook of fat utilization along

the GIT and, together with the determination of the digestibility of FA, leads to a better understanding of the fat digestion-absorption process in broiler chickens.

The lipid classes and FA digestibility results confirmed broiler chickens can use the unsaturated diet (S) better than the saturated one (P). This improvement was mainly due to a more efficient and faster absorption of the unsaturated fat. Regarding the age of the chickens on fat utilization, the present data confirmed that as age increases, the ability of the chickens to digest and absorb fat is better. This was reflected in the higher FA digestibility coefficients observed in the adult chickens in comparison to the young ones in the jejunum, ileum, and excreta, the jejunum and ileum being the main ones responsible for the improvement observed at 35 d. However, the previous GIT segments could also have been positively influenced, as the hydrolysis process also improves with the age of the chickens.

Fat digestion starts before the duodenum, with emulsification, but it is in the duodenum where the hydrolysis of TAG starts, being the main place of lipid hydrolysis. The presence of high percentage of intermediate and end lipolysis products (DAG, MAG, and FFA) in the duodenum is in agreement with this. The jejunum is the most important place of FA absorption, contributing from 70 to 87% in FA apparent absorption. The ileum also plays an important role in FA absorption (contributing from 6 to 26%), allowing the remaining FA in the intestinal lumen to be absorbed after the jejunum and explaining, in a great degree, the differences observed in FA utilization among the 2 studied ages and dietary fats. The ileum was responsible for the higher digestibility values observed for the unsaturated fat in young broiler chickens and was also the responsible of the improvement in the saturated fat utilization in adult broiler chickens. The role of the ileum is essential as there is no effective utilization by the animal after this GIT segment. All of this suggests the absorption of FA is more limiting than is hydrolysis, because the main differences were observed in the jejunum and ileum, where the absorption of FA takes place. The results observed in the present study also showed that the hydrolysis of fat is not the most limiting factor for fat utilization in young chickens.

Thus, the dynamics of both FA digestibility and lipid class composition along the GIT was influenced by the dietary fat source and by the age of the chickens. The present results corroborate the better utilization of unsaturated fat, and suggest that, regardless of the fat source of the diet, the most limiting step in fat utilization is the absorption process.

REFERENCES

- AOAC International. 2005. Official Methods of Analysis of AOAC International. 18th ed. AOAC International, Gaithersburg, MD.

- Barros, M., L. F. Fleuri, and G. A. Macedo. 2010. Seed lipases: sources, applications and properties - A review. *Braz. J. Chem. Eng.* 27:15–29.
- Clément, J. 1980. Intestinal absorption of triglycerols. *Reprod. Nutr. Dévelop.* 20:1285–1307.
- Fundación Española para el Desarrollo de la Nutrición Animal. 2008. *Necesidades Nutricionales Para Avicultura: Pollos de Carne y Aves de Puesta*. FEDNA, Madrid, Spain.
- Gong, D. H., B. Turner, K. R. Bhaskar, and J. T. Lamont. 1990. Lipid binding to gastric mucin: protective effect against oxygen radicals. *Am. J. Physiol. Gastrointest. Liver Physiol.* 259:G681–G686.
- Guardiola, F., R. Codony, M. Rafecas, J. Boatella, and A. López. 1994. Fatty acid composition and nutritional value of fresh eggs, from large- and small-scale farms. *J. Food Compos. Anal.* 7:171–188.
- Hurwitz, S., A. Bar, M. Katz, D. Sklan, and P. Budowski. 1973. Absorption and secretion of fatty acids and bile acids in the intestine of the laying fowl. *J. Nutr.* 103:543–547.
- Kapranichikov, V. S., N. A. Zherebtsov, and T. N. Popova. 2004. Purification and characterization of lipase from wheat (*Triticum aestivum* L.) germ. *Appl. Biochem. Microbiol.* 40:84–88.
- Katongole, J. B., and B. E. March. 1980. Fat utilization in relation to intestinal fatty acid binding protein and bile salts in chicks of different ages and different genetic sources. *Poult. Sci.* 59:819–827.
- Krogdahl, A. 1985. Digestion and absorption of lipids in poultry. *J. Nutr.* 115:675–685.
- Lan, Y., M. W. A. Verstegen, S. Tamminga, and B. A. Williams. 2005. The role of the commensal gut microbial community in broiler chickens. *Worlds Poult. Sci. J. Vol.* 61:95–104.
- Lindsay, O. B., J. Biely, and B. E. March. 1969. Excretion of bile acids by cockerels fed different lipids. *Poult. Sci.* 48:1216–1222.
- Meng, X., B. A. Slominsky, and W. Guenter. 2004. The effect of fat type, carbohydrase, and lipase addition on growth performance and nutrient utilization of young broilers fed wheat-based diets. *Poult. Sci.* 83:1718–1727.
- Nitsan, Z., G. Ben-Avraham, Z. Zoref, and I. Nir. 1991. Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. *Br. Poult. Sci.* 32:515–523.
- Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. *Poult. Sci.* 74:366–373.
- Ravindran, V., P. Tanchaorenrat, F. Zaefarian, and G. Ravindran. 2016. Fats in poultry nutrition: digestive physiology and factors influencing their utilisation. *Anim. Feed Sci. Technol.* 213:1–21.
- Renner, R. 1965. Site of fat absorption in the chick. *Poult. Sci.* 44:861–864.
- Renner, R., and F. W. Hill. 1961. Factors affecting the absorbability of saturated fatty acids in the chick. *J. Nutr.* 74:254–258.
- Sacranie, A., B. Svihus, V. Denstadli, B. Moen, P. A. Iji, and M. Choct. 2012. The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. *Poult. Sci.* 91:693–700.
- Sacranie, A., P. A. Iji, and M. Choct. 2005. Reflux of digesta and its implications for nutrient digestion and bird health. *Proc. Aust. Poult. Sci. Symp.* 17:171–174 (abstr.).
- Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.* 59:215–221.
- Sklan, D. 2001. Development of the digestive tract of poultry. *Worlds Poult. Sci. J.* 57:415–428.
- Sklan, D., B. Shachaf, J. Baron, and S. Hurwith. 1978. Retrograde movement of digesta in the duodenum of the chick: extent, frequency, and nutritional implications. *J. Nutr.* 108:1485–1490. *Worlds Poult. Sci. J. Vol.* 57:415–428.
- Sklan, D., P. Budowski, I. Ascarelli, and S. Hurwitz. 1973. Lipid absorption and secretion in the chick: effect of raw soybean meal. *J. Nutr.* 103:1299–1305.
- Smulikowska, S., and A. Mieczkowska. 1996. Effect of rye level, fat source and enzyme supplementation on fat utilization, diet metabolizable energy, intestinal viscosity and performance of broiler chickens. *J. Anim. Feed Sci.* 5: 379–393.
- Tanchaorenrat, P., V. Ravindran, F. Zaefarian, and G. Ravindran. 2014. Digestion of fat and fatty acids along the gastrointestinal tract of broiler chickens. *Poult. Sci.* 93:371–379.
- Tuchweber, B., I. M Yousef, G. Ferland, and A. Perea. 1996. Nutrition and bile formation. *Nutr. Res.* 16, 1041–1080.
- Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch Changes in Morphology and Function of the Small Intestines in Heavy- and Light-Strain Chicks. *Poult. Sci.* 74:1622–1629.
- Van Kuiken, B. A., and W. D. Behnke. 1994. The activation of porcine pancreatic lipase by cis-unsaturated fatty acids. *Biochim. Biophys. Acta* 1214:148–160.
- Whitehead, G. C., and C. Fisher. 1975. The utilisation of various fats by turkeys of different ages. *Br. Poult. Sci.* 16:481–485.