Soybean lecithin as an alternative energy source for grower and finisher broiler chickens: impact on performance, fatty acid digestibility, gut health, and abdominal fat saturation degree

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ABSTRACT An experiment was performed to assess the inclusion of soybean lecithin (**SL**) in the replacement of soybean oil (**SO**), for grower and finisher broiler chicken diets (up to 15 d of life), and its effects on performance, fatty acid (**FA**) absorption, gut health, and saturation degree of the abdominal fat pad (**AFP**). A total of 1,440 female Ross-308 chickens were distributed in 60 pens and were fed 5 experimental diets. The control diet (T1) was supplemented with SO (grower and finisher diets at 2.00%), and 4 levels of SL were included in replacement: T2 (0.25% in grower and 0.50% in finisher diets), T3 (0.50% in grower and 1.00% in finisher diets), T4 (0.75% in grower and 1.50% in finisher diets), and T5 (1.00% in grower and 2.00% in finisher diets). At day 39, titanium dioxide was added to finisher diets at 5 g/kg to perform a digestibility balance. At day 46, AFP, tissue, and gut digesta samples were collected to characterize FA digestibility, adipose saturation degree, microbial groups, and histomorphometry. No effects were associated with SO replacement by SL on performance ($P > 0.05$), ileal digestibility of total, saturated and monounsaturated FA ($P > 0.05$), nor jejunal morphology ($P > 0.05$). Total replacement of SO by SL reduced ileal absorption of polyunsaturated FA ($P < 0.02$) and increased jejunal *Lactobacillus* spp. counts ($P = 0.049$). Higher levels of SL inclusion (T4 and T5) lowered polyunsaturated FA concentration of the AFP ($P = 0.002$) and, thus, slightly reduced its unsaturated-to-saturated FA ratio ($P = 0.005$). Soybean lecithin inclusion did not modify performance parameters, total FA absorption, nor jejunal morphology, however caused changes on polyunsaturated FA absorption, jejunal microbiota, and saturation degree of the AFP. The study demonstrates that soybean lecithin can be included, in combination with or in replacement of soybean oil, as an alternative energy source for grower (up to a 1%) and finisher broiler diets (up to 2%).

Key words: soybean lecithin, soybean oil, fatty acid, phospholipid, free fatty acid

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INTRODUCTION

Fats and oils are commonly included in broiler feeds to increase the energetic density of the diets; however, their inclusion is highly dependent on their chemical composition and economic cost. It has been demonstrated that the saturation degree and composition in different lipid molecular structures have an important influence on performance parameters (Vieira et al., 2006; Ferrini et al., 2008), fatty acid (**FA**) absorption (Tancharoenrat et al., 2013; Roll et al., 2018), gastrointestinal health (Thormar et al., 2006; Khatun et al., 2018), and carcass quality parameters (Smink et al., 2008; Gonzalez-Ortiz et al., 2013).

Currently, there is a growing interest in the search and use of alternative energy sources in broiler feeding. In this context, coproducts derived from the soybean oil (**SO**) refinement process represent an economic alternative and permit giving an added value to residual products. Soybean lecithin (**SL**), which is extracted from the SO degumming process, is mainly composed of polar lipids (>60%), especially of phospholipids (**PL**), but also contains an important amount of neutral lipids (30–40%), as triacylglycerols and free fatty acids (**FFAs**) (van Nieuwenhuyzen and Tomas, 2008; Bueschelberger et al., 2015; EFSA, 2016). Furthermore, SL represents a good source of phosphorus, choline, and energy for broiler chickens (Mateos et al., 2012; Borsatti et al., 2018), and its combination with other fats and oils could

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be interesting to exploit positive synergies on lipid utilization (Jones et al., 1992; Ravindran et al., 2016).

Available literature indicates that SO replacement by SL, in starter broiler diets, impairs growth performance (Azman and Ciftci, 2004), reduces feed apparent metabolizable energy content (Huang et al., 2007; Viñado et al., 2019), and lowers the total tract apparent digestibility of the polyunsaturated FA (**PUFA**; Viñado et al., 2019). On the contrary, in grower-finisher diets, a few researchers have indicated that SL can partially replace SO without modifying energy utilization (Huang et al., 2007; Viñado et al., 2019) and total-tract apparent FA digestibility (Viñado et al., 2019).

Therefore, the present study was designed with the aim to evaluate increasing rates of SO replacement by SL in grower and finisher diets, assessing its effects on growth performance, ileal FA absorption, jejunal morphology, jejunal microbiota, and the FA profile of the abdominal fat pad (**AFP**).

MATERIALS AND METHODS

Experimental Diets, Animal Husbandry and Sampling

This experiment was in accordance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010) and was approved by the Animal Ethics Committee of the Universitat Autònoma de Barcelona.

The experiment was performed in a farm under controlled conditions (Granja Sole, Vila-Rodona, Spain). A total of 1,440 newly hatched Ross 308 female broiler chickens were obtained from a local hatchery (Pondex S.A.U., Juneda, Spain). On arrival, the animals were weighed and randomly distributed in 60 pens with 24 birds per pen. Replicates were allocated for a homogeneous distribution of treatments and started with a similar BW mean. Each pen was equipped with a pan feeder and a bell waterer, and both, experimental feed and water, were provided *ad libitum* throughout the whole trial. Temperature, humidity level, and light program used were according to the specifications in the Ross 308 lineage management handbook (Aviagen, 2014). Twice daily, animals and housing facilities were inspected for general health status, constant feed, dead birds, and water supply, as well as temperature and ventilation.

The feeding program was divided into 3 phases: starter (from 0–14 d), grower (from 15–28 d), and finisher (from 29 d to the end of the experiment). All experimental diets were based on wheat and soybean meal and formulated to meet or exceed Fundación Española Desarrollo Nutrición Animal (2008) requirements, as is seen in Table 1. Animals were fed 5 experimental treatments (12 replicates per treatment) during grower and finisher phases, as is detailed in Table 2. The control diet (T1) was supplemented with SO at 2% (grower and finisher diets), and 4 levels of SL were included replacing SO: T2 (0.25% in grower and 0.50% in finisher diets), T3 (0.50% in grower and 1.00% in finisher diets), T4 (0.75% in grower and 1.50% in finisher diets), and T5 (1.00% in grower and 2.00% in finisher diets).

Broiler BW and feed intake were recorded for the pen (24 birds) at day 14, 28, and 39. The data were used to measure BW and calculate the average daily gain (**ADG**), average daily feed intake, and the feed conversion ratio (**FCR**) of each period and the overall results. Mortality was daily recorded to adjust average daily feed intake and ADG. At day 39, three animals of each pen (6 replicates per treatment) were chosen based on pen mean BW (a total of 90 animals) and received the finisher diet with titanium dioxide ($\mathbf{TiO_2}$) at 5 g/kg from day 40 to day 46. The rest of the animals were slaughtered in a commercial abattoir. At day 46, broiler chickens were euthanized with the aim to collect samples. The ileal content (from Meckel's diverticulum to a point 1 cm proximal to the ileocecal junction) of all birds within a pen was pooled, homogenized, freeze-dried, ground, and kept at 4°C until laboratory analysis. A representative sample of the AFP (from the proventriculus surrounding the gizzard down to the cloaca) of each bird was taken, pooled by replicate, frozen at −20°C, and analyzed to determine the FA profile. Finally, jejunum tissue and digesta content from T1 and T5 animals were collected to evaluate the effect of using either SO or SL as added fats on jejunal morphology and microbiota.

Laboratory Analyses

Regarding experimental oil characterization, SO and SL were analyzed in duplicate for FA composition, by gas chromatography, according to the methylation method described by Guardiola et al. (1994). In addition, the acid value was determined according to ISO 660 (2009), and the acidity was indicated as the FFA percentage of oleic acid. The acetone insoluble determination was performed following the Ja-4-46 analytical method from American Oil Chemist's Society (2017), and PL composition was determined by HPLC following Helmerich and Koehler (2003) methodology. Experimental feed samples were taken at the beginning and end of each experimental period and were ground and kept at 4°C until further analysis. Diet proximate analysis was performed according to the methods of AOAC International (2005): ether extract (Method 920.39), crude protein (Method 968.06), and crude fiber (Method 962.09). Furthermore, feed and excreta samples analysis included ash determination (Method 942.05), dry matter (Method 934.01), and GE content by adiabatic bomb calorimeter (IKA-Kalorimeter system C4000; Staufen, Germany). FA content was analyzed adding nonadecanoic acid (Sigma-Aldrich, St. Louis, MO) as an internal standard and following the method described by Sukhija and Palmquist (1988) in feed and ileal content, whereas in the case of AFP, the method described by Carrapiso et al. (2000) was performed. The final extract obtained was injected in a gas chromatograph following the method conditions described by Cortinas et al. (2004). TiO_2 was analyzed

Table 1. Ingredient composition of experimental basal diets (%, as fed basis).

Ingredients (%)	Starter diet (0–14 d)	Grower diet (15–28 d)	Finisher diet (from 29 d)
Wheat	29.75	49.37	46.06
Soybean meal 47%	27.40	23.65	22.82
Corn	15.10	0.00	0.00
Barley	9.78	11.74	17.62
Extruded full-fat soybean	9.17	4.58	0.00
Added fats	2.00	3.25	4.50
Rapeseed meal 00	0.0	1.52	3.43
Sepiolite	1.99	1.99	1.99
Trace mineral-vitamin premix[1]	1.43	1.13	1.15
Calcium carbonate	1.23	1.05	0.93
Monocalcium phosphate	0.96	0.64	0.49
Salt	0.35	0.21	0.23
L-lysine	0.30	0.31	0.29
DL-methionine	0.30	0.25	0.22
Sodium bicarbonate	0.09	0.16	0.12
L-threonine	0.08	0.08	0.08
Choline chloride 75%	0.07	0.07	0.07

[1]Provides per kg feed: vitamin A (from retinol), 13,500 IU; vitamin D3 (from cholecalciferol), 4,800 IU; vitamin E (from alfa-tocopherol), 49.5 IU; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 4.5 mg; vitamin B12, 16.5 µg; vitamin K3, 3 mg; calcium pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 30 µg; Fe (from $FeSO_4 \cdot 7H_2O$), 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; β-glucanase 350 IU; xylanase 1,125 IU; Organic acids (starter diets at 4 g/kg; grower and finisher diets at 3 g/kg).

on finisher diets and ileal digesta following the methodology described by Short et al. (1996).

Jejunal Histomorphometry and Microbial Counts of Digesta

A distal jejunum segment of 5 cm (close to Meckel's diverticulum) was excised, flushed with sterile PBS, and fixed by immersion in a 3.7 to 4.0% neutral buffered formaldehyde solution (PanReac AppliChem, Castellar del Vallès, Spain). Once fixed, 6 cross sections were obtained from each jejunum, embedded in paraffin, and prepared and stained with hematoxylin/eosin for further observations in a light microscope (BHS-Olympus, Tokyo, Japan). The histomorphometric indexes evaluated were villus height (**VH**: from the villus tip to the crypt junction), crypt depth (**CD**: from the villus bottom to the crypt), and the ratio between VH and CD (VH:CD). The analysis was performed on 10 well-oriented and intact villi and 10 crypts, according to methodology described by Schiavone et al. (2018). Measures were taken using ImageJ (1.8.0; NIH; Bethesda, MD) via software analysis.

Jejunal digesta samples were collected in sterile conditions and were stored at 4°C until further analysis. Samples were 10-fold serial diluted in Lactated Ringer's Solution (Sigma-Aldrich Química, Madrid, Spain) to perform microbiota counts. Diluted digesta samples were inoculated on azide glucose, MacConkey, and MRS (de Man, Rogosa and Sharpe) agar (Oxoid Limited, Hampshire, United Kingdom) for microbial counts of *Enterococcus faecium*, *Enterobacteria*, and *Lactobacillus* spp., respectively. Counts were manually read after 24-h incubation at 37°C.

Calculations and Statistical Analysis

The FA apparent ileal digestibility coefficients were calculated as follows: ileal digestibility coefficient = $1-\{(TiO_2/FA)_d/(TiO_2/FA)_i\}$, where $(TiO_2/FA)_d$ is the concentration of the inert marker and the FA in the diet, and $(TiO_2/FA)_i$ is the concentration of the inert marker and the FA in the ileal digesta.

Pen means (24 birds) were used as the experimental unit for performance parameters (12 replicates per treatment). In the case of the digestibility balance and the FA profile of AFP, pen means (3 birds) were used as the experimental unit (6 replicates per treatment). Microbial counts (log transformation for statistical analysis) and histomorphometric analysis were performed on T1 and T5, the pen being the experimental unit (6 replicates per treatment). A Shapiro-Wilk test indicated a normal distribution of the data. Data were analyzed by one-way ANOVA using R Statistics (version 3.3.1; R Core Team,

Table 2. Total added-fat inclusion in the experimental diets (%, as fed basis).

		Grower diet (15–28 d)					Finisher diet (from 29 d on)				
Ingredients (%)	Starter diet (0–14 d)	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Total added fats	2.00	3.25	3.25	3.25	3.25	3.25	4.50	4.50	4.50	4.50	4.50
Palm oil	0.00	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00
Acid oil[1]	0.00	0.75	0.75	0.75	0.75	0.75	1.50	1.50	1.50	1.50	1.50
Soybean oil	2.00	2.00	1.75	1.50	1.25	1.00	2.00	1.50	1.00	0.50	0.00
Soybean lecithin	0.00	0.00	0.25	0.50	0.75	1.00	0.00	0.50	1.00	1.50	2.00

[1]Monounsaturated acid oil (blending 50:50 of olive pomace acid oil and sunflower acid oil).

Table 3. Chemical analysis of soybean oil (SO) and soybean lecithin (SL) included in the experimental diets.

	Experimental fats	
Item	SO	SL
Fatty acid composition (%)[1]		
Saturated fatty acids	16.0	23.4
C16:0	10.6	20.4
C18:0	4.24	2.97
Monounsaturated fatty acids	23.4	18.4
C18:1 ω-9	21.8	18.4
Polyunsaturated fatty acids	60.6	58.2
C18:2 ω-6	52.9	53.3
C18:3 ω-3	7.67	4.81
Minor fatty acids	2.85	N.D.
UFA:SFA	5.25	3.27
PUFA:SFA	3.79	2.49
Acidity (%)[1]		
Free fatty acids	1.30	14.6
Phospholipids (%)[1]		
Acetone insoluble	N.D.	68.3
Total Phospholipids	N.D.	44.0
Phosphatidylcholine	N.D.	13.5
Phosphatidylinositol	N.D.	13.7
Phosphatidylethanolamine	N.D.	9.15
Phosphatidic acid	N.D.	7.02
Lysophosphatidylcholine	N.D.	0.58
Gross energy (kcal/kg)[1]	9,602	7,937

Abbreviations: N.D., not determined; PUFA:SFA, polyunsaturated-to-saturated fatty acid ratio; UFA:SFA, unsaturated-to-saturated fatty acid ratio.
[1]Percentage expressed of total product.

Vienna, Austria), with treatment as the main factor and residual standard error estimated the average deviation of any point from the statistical model. Tukey's multiple range test was performed to determine whether means were significantly different ($P \leq 0.05$).

RESULTS

Chemical Composition of the Experimental Fats and Diets

The chemical composition of SO and SL and the chemical composition of experimental diets are given in Tables 3 and 4. SO and SL were mainly composed of PUFA and differed in their respective saturated FA (**SFA**) and monounsaturated FA (**MUFA**) content. SL, in contrast to SO, presented a higher SFA content (23.4 vs. 16.0%, respectively) and a lower MUFA content (18.4 vs. 23.5%, respectively), hence a lower unsaturated-to-saturated fatty acid ratio (UFA:SFA) and polyunsaturated-to-saturated fatty acid ratio (**PUFA:SFA**). Furthermore, SL presented higher acidity and lower gross energy content than did SO (7,937 vs. 9,602 kcal/kg). The experimental diets were closely similar between treatments regarding protein and ether extract content; however, they slightly differed in their respective FA composition.

Performance Parameters

The effects of SO substitution by SL on performance parameters are seen in Table 5. SO partial and total replacement by SL did not modify any performance parameter during the grower and the finisher phases ($P > 0.05$) nor during the overall period ($P > 0.05$).

Ileal FA Digestibility

The effects of SO replacement by SL on ileal FA digestibility are shown in Table 6. SO partial and total replacement by SL in the finisher diets did not modify the total FA (**TFA**), SFA, and MUFA digestibility ($P > 0.05$). However, PUFA, linoleic, and linolenic ileal absorption were lower in T3, T4, and T5 (SL inclusion of 1.00, 1.50, and 2.00%, respectively), in contrast to T1 ($P < 0.020$).

Jejunal Histomorphometry and Microbiological Counts of Digesta

The effects of including either SO (T1) or SL (T5) at 2.00% in finisher diets on jejunal morphology and microbiological counts of digesta are shown in Table 7. The different dietary added fats did not influence any morphological measurement ($P > 0.05$). Concerning jejunal microbiological counts, the use of SL instead of SO increased the *Lactobacillus* spp. population ($P = 0.049$); nevertheless, no differences were observed regarding *Enterobacteria* and *E. faecium* ($P > 0.05$).

FA Composition of AFP

The effect of SL inclusion on the FA composition of AFP is presented in Table 8. Animals fed T1 showed a higher PUFA ($P = 0.002$), concretely linoleic ($P = 0.003$), and linolenic acid ($P < 0.001$) content and tended to show a lower palmitic acid content than did animals fed diets with 1.50 and 2.00% SL supplementation (T4 and T5, respectively; $P = 0.068$). Thus, SL inclusion, as a replacer for SO, reduced the UFA:SFA (T1: 2.42 vs. T5: 2.25; $P = 0.005$) and the PUFA:SFA (0.88 vs. 0.75; $P = 0.004$) of the AFP.

DISCUSSION

Chemical Composition of the Experimental Fats and Diets

Fats and oils represent one of the most important energy-yielding ingredients for broiler chicken diets, thus their utilization is crucial for efficient growth. It is widely recognized that SO, as a native oil, presents most of its FA structured as triacylglycerols (>90%; Bãiao and Lara, 2005), whereas the SL used in the current experiment presented most of its FA structured into PL (44%; Table 3) and 15% of its FA remained free and unesterified. The chemical composition of SO and SL was in accordance with existing literature (Øverland et al., 1993; Soares and Lopez-Bote, 2002; Viñado et al., 2019). Furthermore, the FA profile of the experimental diets was influenced by the FA content of SO, SL, and the rest of the added fats (palm

Table 4. Analyzed gross energy, macronutrient content, and fatty acid composition for starter, grower, and finisher diets.

Item	Starter diet (0–14 d)	Grower diet[1] (15–28 d)					Finisher diet[2] (from 29 d on)				
		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Macronutrient content (%)											
Dry matter	89.73	90.88	91.26	90.45	90.36	90.35	90.31	89.91	90.15	90.64	90.61
Crude protein	21.62	19.16	19.42	19.26	19.49	19.16	18.45	18.32	18.35	18.26	18.44
Crude Fat	5.53	5.34	5.28	5.15	5.02	5.23	5.68	5.37	5.34	5.38	5.24
Crude Fiber	3.31	4.04	4.12	4.38	4.19	3.90	4.07	3.59	3.68	3.95	3.79
Ash	7.67	6.51	7.21	6.86	6.74	6.78	6.02	6.29	6.33	6.37	6.47
Fatty acid composition (%)											
Saturated fatty acids	17.6	21.0	21.1	21.6	21.8	22.0	24.3	24.9	25.3	25.8	26.5
C16:0	13.4	16.1	16.3	16.7	16.9	17.1	18.7	19.3	19.7	20.1	20.8
C18:0	3.02	3.63	3.57	3.57	3.66	3.65	4.16	4.16	4.18	4.21	4.27
Monounsaturated fatty acids	22.4	26.0	25.8	25.1	25.2	25.0	27.8	27.3	27.1	26.9	26.8
C18:1 ω-9	20.1	23.8	23.4	22.8	22.6	22.4	25.4	24.9	24.7	24.4	24.4
Polyunsaturated fatty acids	60.0	53.0	53.1	53.3	53.0	53.0	47.9	47.8	47.6	47.3	46.7
C18:2 ω-6	54.1	47.9	47.9	48.1	47.9	47.9	43.6	43.7	43.5	43.4	42.9
C18:3 ω-3	5.96	5.24	5.20	5.14	5.13	5.12	4.29	4.19	4.09	3.98	3.82
Minor fatty acids	3.49	3.48	3.59	3.67	3.93	3.92	3.82	3.78	3.82	3.88	3.91
UFA:SFA	4.68	3.76	3.74	3.63	3.59	3.55	3.12	3.02	2.95	2.88	2.77
PUFA:SFA	3.41	2.52	2.52	2.47	2.43	2.41	1.97	1.92	1.88	1.83	1.76
Gross energy (kcal/kg)	4,183	4,249	4,258	4,235	4,155	4,180	4,231	4,181	4,196	4,192	4,179

Abbreviations: UFA:SFA, unsaturated-to-saturated fatty acid ratio; PUFA: SFA, polyunsaturated-to-saturated fatty acid ratio.
[1]T1 = soybean oil (SO) at 2%; T2 = SO at 1.75% and soybean lecithin (SL) at 0.25%; T3 = SO at 1.5% and SL at 0.5%; T4 = SO at 1.25% and SL at 0.75%; T5 = SO at 1% and SL at 1%.
[2]T1 = SO at 2%; T2 = SO at 1.5% and SL at 0.5%; T3 = SO at 1% and SL at 1%; T4 = SO at 0.5% and SL at 1.5%; T5 = SL at 2%.

oil and monounsaturated acid oil). The gradual replacement of SO by SL increased dietary SFA concentration (+9%; T1 vs. T5 finisher diets) and reduced the UFA:SFA and PUFA:SFA ratios (−11%; T1 vs. T5 finisher diets). However, in general terms, all the experimental diets were unsaturated, with PUFA being the most abundant.

Performance Parameters

The chemical differences observed between SO and SL may have influenced growth performance parameters because of the different lipid molecular structures, such as triacylglycerols, PL, and FFA influence on lipid digestion and absorption (Ravindran et al., 2016; Roll et al.,

Table 5. Growth performance of broiler chickens according to soybean lecithin inclusion level in the grower and finisher diets.

Item	Dietary treatments					RSE	*P* value
	T1	T2	T3	T4	T5		
Starter period (0–14 D)[1]							
BW 0 d (g)	42.09	41.98	42.03	42.09	42.10	0.176	0.337
BW 14 d (g)	453	459	454	456	459	13.8	0.792
ADFI (g/d/bird)	40.5	40.4	40.4	39.8	40.4	1.07	0.571
ADG (g/d/bird)	29.4	29.8	29.4	29.6	29.8	0.99	0.812
FCR (g/g)	1.37	1.37	1.36	1.35	1.35	0.030	0.305
Grower period (15–28 d)[2]							
BW 28 d (g)	1,552	1,544	1,531	1,541	1,551	36.74	0.647
ADFI (g/d/bird)	121.6	120.3	120.1	119.5	121.8	2.65	0.182
ADG (g/d/bird)	77.7	77.6	76.9	77.8	78.4	0.07	0.436
FCR (g/g)	1.56	1.57	1.56	1.54	1.56	0.024	0.195
Finisher period (29–39 d)[3]							
BW at 39 d (g)	2,439	2,422	2,443	2,437	2,442	73.52	0.961
ADFI (g/d/bird)	173.1	173.5	174.7	174.5	175.5	6.59	0.909
ADG (g/d/bird)	81.2	78.5	83.3	81.2	80.7	5.27	0.324
FCR (g/g)	2.14	2.21	2.13	2.16	2.18	0.100	0.293
Global period (0–39 d)							
ADFI (g/d/bird)	106.0	106.8	106.5	106.4	107.7	2.57	0.612
ADG (g/d/bird)	61.22	60.61	61.43	61.20	61.45	1.99	0.842
FCR (g/g)	1.74	1.76	1.74	1.73	1.75	0.03	0.154

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; FCR, feed conversion ratio; RSE, residual standard error.
[1]All pens consumed the same starter diet.
[2]T1 = soybean oil (SO) at 2%; T2 = SO at 1.75% and soybean lecithin (SL) at 0.25%; T3 = SO at 1.50% and SL at 0.50%; T4 = SO at 1.25% and SL at 0.75%; T5 = SO at 1.00% and SL at 1.00%.
[3]T1 = SO at 2.00%; T2 = SO at 1.50% and SL at 0.50%; T3 = SO at 1.00% and SL at 1.00%; T4 = SO at 0.50% and SL at 1.50%; T5 = SL at 2.00%.

Table 6. Apparent ileal digestibility coefficients of fatty acids according to soybean lecithin inclusion level during the digestibility balance performed from day 40 to day 46 of life.

Item	Dietary treatments[1]					Residual standard error[2]	*P* value
	T1	T2	T3	T4	T5		
Total fatty acids	0.84	0.81	0.79	0.79	0.80	0.036	0.229
Saturated fatty acids	0.77	0.77	0.78	0.76	0.78	0.046	0.945
C16:0	0.77	0.77	0.78	0.76	0.78	0.046	0.915
C18:0	0.78	0.79	0.80	0.79	0.79	0.045	0.960
Monounsaturated fatty acids	0.85	0.84	0.83	0.81	0.82	0.031	0.331
C18:1 ω-9	0.85	0.84	0.83	0.81	0.83	0.031	0.364
Polyunsaturated fatty acids	0.85^{a}	$0.82^{a,b}$	0.78^{b}	0.78^{b}	0.80^{b}	0.035	0.017
C18:2 ω-6	0.85^{a}	$0.82^{a,b}$	0.78^{b}	0.78^{b}	0.80^{b}	0.035	0.019
C18:3 ω-3	0.85^{a}	$0.83^{a,b}$	0.78^{c}	0.78^{c}	$0.79^{b,c}$	0.039	0.016

Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

[1]T1 = soybean oil (S) at 2.00%; T2 = S at 1.50% and soybean lecithin (L) at 0.50%; T3 = S at 1.00% and L at 1.00%; T4 = S at 0.5% and L at 1.50%; T5 = L at 2.00%.

[2]Residual standard error of 3 chickens per replicate (6/six replicates per treatment).

2018). Nevertheless, the results obtained in the present experiment have indicated that SO replacement by SL, in grower and finisher diets, did not modify the performance parameters in any phase or of the trial overall. This finding was consistent with data reported by Viñado et al. (2019), where SO partial replacement by SL (1.00 and 2.00 of 3.00% of total fat addition), in grower-finisher broiler diets, did not alter growth performance or energy and FA utilization. Furthermore, our results also agreed with those of Azman and Ciftci (2004), who observed that a SO partial replacement by SL (1.50 and 3.00 of 6.00% of total fat addition) did not modify BW or FCR in grower-finisher broiler chickens. Other researchers have stated that soybean-lecithin inclusion, as an alternative to saturated added fats such as tallow or lard, is acceptable without a negative impact on the productive performance of broiler chickens (Cantor et al., 1997; Cox et al., 2000), laying hens (Mandalawi et al., 2015), or weaned pigs (Jones et al., 1992; Reis de Souza et al., 1995; Øverland and Sundstøl, 1995). On the contrary, Huang et al. (2007) observed, in grower-finisher broilers, that SO partial replacement by SL (0.50 and 1.00 of 2.00% of total fat addition) improved the ADG and FCR.

Table 7. Effects of either including soybean oil (T1) or soybean lecithin (T5), as added fats, on jejunum morphology and microbiological plate counts in broiler chickens of 46 d.

Item	Dietary treatments[1]		RSE	*P* value
	T1	T5		
Morphology measurements[2]				
Villus height (μm)	1,347	1,254	167.0	0.358
Crypt depth (μm)	210	212	26.2	0.912
VH:CD	6.21	6.11	0.235	0.585
Microbiology (log UFC/g)[3]				
Enterococcus faecium	6.47	6.33	0.760	0.761
Enterobacteria	4.17	5.65	1.523	0.143
Lactobacillus spp.	8.31	8.54	0.158	0.049

Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

Abbreviations: RSE, residual standard error; VH:CD, villus height-to-crypt depth ratio.

[1]T1 = soybean oil at 2.00%; T5 = soybean lecithin at 2.00%.

[2]Results are means of 10 measurements of intact villus and crypts per replicate.

[3]Pool of three chickens per replicate.

It is important to highlight that no effects were observed on feed intake despite SL containing 17% less gross energy than SO (9,602 vs. 7,937 kcal/kg; Table 3). This finding is in accordance with that of Mandalawi et al. (2015), who observed, in laying hens, that pork fat replacement by SL did not modify feed intake, even though the SL used was 16% less energetic than the pork fat (32.6 vs. 38.9 MJ/kg). This fact might indicate that the inclusion of SL, instead of SO, improved the energy utilization of the experimental diets that also contained palm and acid oil as added fats (Table 2). Several researchers have demonstrated that blending different fats and oils with different chemical compositions (FA chain length, saturation degree, and lipid molecular structures) produces positive interactions in terms of energy and FA utilization (Blanch et al., 1995; Borsatti et al., 2018; Roll et al., 2018). Despite the lack of bibliography regarding dietary PL digestion and absorption in poultry species, PL are recognized as energy-yielding molecules (Wang et al., 2013; Ravindran et al., 2016) and are essential during the solubilization of lipolysis products into the mixed micelles (Krogdahl, 1985; Zaefarian et al., 2019). In addition, several researchers have demonstrated that SL inclusion, in replacement of other added fats with a higher energetic density, improves dry matter and N retention in piglets (Jin et al., 1998) and the organic matter utilization in laying hens (Mandalawi et al., 2015). Thus, SL beneficial effects may not be limited only to lipid digestion and absorption.

Ileal FA Digestibility

The use of SL instead of SO did not modify the ileal absorption of TFA, SFA, and MUFA at day 46. It is well established that FA absorption is a process highly influenced by the saturation degree of FA (Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019a), and as was commented previously, the FA profile of both SL and SO was mostly polyunsaturated. The present results were consistent with data reported by Huang et al. (2007), who observed that partial and total replacement of SO by a SL (2.00% of total fat addition) did not alter

Table 8. Fatty acid profile (%) of the abdominal fat pad at 46 d of age according to soybean lecithin inclusion.

	Dietary treatments[1]						
Item (%)	T1	T2	T3	T4	T5	RSE[2]	*P* value
Saturated fatty acids	29.71	31.33	31.22	31.30	30.80	1.030	0.064
C16:0	23.02	24.20	23.91	24.40	24.41	0.767	0.068
C18:0	5.80	6.24	6.45	6.05	5.84	0.468	0.120
Monounsaturated fatty acids	44.25	44.38	43.14	45.56	44.66	1.655	0.191
C18:1 ω-9	36.31	36.97	36.32	37.85	36.81	1.108	0.145
Polyunsaturated fatty acids	26.66^{a}	25.73^{a,b}	25.65^{a,b}	23.14^{c}	24.22^{b,c}	1.347	0.002
C18:2 ω-6	23.79^{a}	23.07^{a,b}	23.06^{a,b}	20.74^{c}	21.77^{b,c}	1.217	0.003
C18:3 ω-3	2.27^{a}	2.12^{b}	2.04^{b}	1.90^{c}	1.91^{c}	0.101	<0.001
UFA:SFA	2.42^{a}	2.19^{b}	2.20^{b}	2.20^{b}	2.25^{b}	0.095	0.005
PUFA:SFA	0.88^{a}	0.83^{a,b}	0.82^{a,b}	0.74^{b}	0.75^{b}	0.059	0.004

Values within the same row with no common superscripts are significantly different, $P \leq 0.05$.

Abbreviations: PUFA:SFA, polyunsaturated-to-saturated fatty acid ratio; RSE, residual standard error; UFA:SFA, unsaturated-to-saturated fatty acid ratio.

[1]T1 = soybean oil (SO) at 2.00%; T2 = SO at 1.50% and soybean lecithin (SL) at 0.50%; T3 = SO at 1.00% and SL at 1.00%; T4 = SO at 0.5% and SL at 1.50%; T5 = SL at 2.00%.

[2]Residual standard error of a pool of 3 chickens per replicate.

ether extract utilization in finisher broiler chickens (from 40–42 d of life). Similarly, Viñado et al. (2019) studied the effect of replacing SO by SL in grower-finisher broiler chickens (from 36–37 d) on total-tract FA digestibility and confirmed that partial and total replacement of SO by SL (3% of total added fat inclusion) did not modify TFA, SFA, or MUFA digestibility.

Nevertheless, it is important to remark that SO replacement rates equal to or higher than 1.00% by SL reduced the ileal absorption of PUFA, linoleic, and linolenic acid. The same effect was also reported previously by Viñado et al. (2019), where PUFA digestibility was negatively affected with SO replacement by SL at 2.00 and 3.00% (3.00% of total fat inclusion). The mechanism underlying this phenomenon is unclear; however, a hypothesis may be proposed. As mentioned before, SL contained 10 times more FFA than does SO (14.6 vs. 1.30%; Table 3). In addition, the hydrolysis of PL and triacylglycerols during dietary lipid digestion presents several differences that have also probably influenced. Triacylglycerols are hydrolyzed in a two-times step by the colipase-lipase complex, generating a *sn-2*-monoacylglycerol and 2 FFA, while PL are habitually catalyzed by phospholipase A_2, generating a *sn-1*-lysophospholipid and a single FFA (Cohn et al., 2010; Wang et al., 2013). In the case of PL, in general terms, unsaturated FA is concentrated at the *sn-2* position, while saturated FA is normally located at the *sn-1* position (Estiasih et al., 2013). It is well studied that FA absorption is reduced in their free form than if they are esterified as a monoacylglycerol (Blanch et al., 1995; Roll et al., 2018), and this effect is more pronounced in unsaturated diets than in saturated diets (Rodriguez-Sanchez et al., 2019b). Furthermore, FFA tends to form insoluble soaps with divalent cations, such as calcium, reducing its absorption (Small, 1991). Thus, a higher SL content in FFA and the hydrolytic activity of phospholipase A_2 may cause the lower utilization of PUFA in the animals fed diets with SL as added fat instead of SO. Consequently, a more detailed chemical characterization of the lecithin, including the FA position and distribution within PL, could provide us essential information to elucidate this fact.

Jejunal Histomorphometry and Microbiological Counts of Digesta

The morphology of intestinal mucosa and microbial population represents 2 important biomarkers for evaluating the gastrointestinal health in poultry species (Yegani and Korver, 2008; Ducatelle et al., 2018). Several studies have demonstrated that dietary fat is capable of modifying intestinal morphology (Khatun et al., 2018; Sabino et al., 2018) and microbiota (Dänicke et al., 1999; Józefiak et al., 2014).

Jejunum is the major site of lipid absorption (Rodriguez-Sanchez et al., 2019a), thus the enterocytes present in the jejunal villus tips play an important role in dietary fat absorption. An increase of the VH is related to enhancements in nutrient absorption, whereas deeper crypts are associated with higher tissue turnover rates (Choct, 2009; de Verdal et al., 2010). In the current experiment, the replacement of SO by SL did not produce modifications on jejunal morphology, supporting the performance and digestibility results. Several authors have demonstrated that the inclusion of lysolecithins, products derived from the hydrolyzation of lecithins and mainly composed of lysophospholipids, promotes enhancements in the VH and VH:CD ratio of broiler chicken jejunum (Boontiam et al., 2017; Chen and Jung, 2019). Lysophospholipids are capable of altering the lipid bilayer of cell membranes (Arouri and Mouritsen, 2013), reduce the production of inflammatory mediators (Hartmann et al., 2009), and modify gene expression (Chen and Jung, 2019). Our hypothesis was based on the fact that dietary PL digestion implies the release of lysophospholipids into the broiler gut; therefore, SL inclusion might improve jejunal morphology. However, the hypothesis could not be confirmed because of the lack of differences between T1 and T5 treatments (SO at 2.00 vs. SL at 2.00%).

Concerning microbiota counts, no differences were observed between treatments regarding *Enterobacteria* and *E. faecium* counts, supporting the performance, the digestibility, and the jejunal morphology results. Nonetheless, the results obtained also suggest that animals fed T5 (SL at 2.00%) presented more *Lactobacillus* spp. counts than did animals fed T1 (SO at 2.00%). It is well known that certain inhabitants of poultry gut, such as *Lactobacillus* spp. and *E. faecium*, are widely used as dietary probiotics by controlling the overgrowth of pathogenic bacteria with the aim to improve animal growth and health (Gaggìa et al., 2010). Nevertheless, *Lactobacillus* spp. and *E. faecium* also present bile salt hydrolase capacity (Cole and Fuller, 1984; Knarreborg et al., 2002; Geng and Lin, 2017), an enzyme that catalyzes primary bile salts to secondary bile salts, which are less efficient emulsifiers (Geng and Lin, 2017). The possibility has been suggested that the overgrowth of these kinds of bacteria may decrease lipid digestion and absorption (Guban et al., 2006; Pan and Yu, 2014; Geng and Lin, 2017). Despite no effects were observed on TFA digestibility, SL inclusion reduced PUFA absorption, opening new research lines. Further studies should be performed to confirm any possible effect of dietary SL on jejunal *Lactobacillus* spp. overgrowth and its influence on lipid digestion and absorption.

FA Composition of AFP

The FA profile of AFP was influenced by the dietary FA profile, in accordance with most of the published data (Ferrini et al., 2008; Gonzalez-Ortiz et al., 2013; Vilarrasa et al., 2015; Skřivan et al., 2018). However, in general terms, the use of SL instead of SO caused minor changes in the FA profile of AFP, and the presence of different lipid molecular structures (triacylglycerols, FFA, and PL) did not influence the FA profile, as described by Viñado et al. (2019). In all experimental treatments, MUFA was the most abundant FA in AFP, particularly oleic acid, as had previously been reported by Hrdinka et al. (1996) and Skřivan et al. (2018). Dietary inclusion of SL reduced the PUFA content of AFP, thus a reduction in UFA:SFA and PUFA:SFA ratios was also observed. A reduction in the unsaturated content of AFP may be interesting, in terms of carcass quality, to reduce the carcass-fat melting point (Sanz et al., 1999) and oxidation susceptibility (Betti et al., 2009; Gonzalez-Ortiz et al., 2013). However, the reduction observed in the unsaturated content of the carcass due to SO replacement by SL, numerically, is slight and probably with a limited effect, from a nutritional point of view.

CONCLUSIONS

In conclusion, results have shown the suitability of SL, in combination with or in replacement of SO, as an energetic ingredient capable of maintaining the performance of grower and finisher broiler chickens (up to 1 and 2% for grower and finisher phase, respectively). In addition, the replacement of SO by SL (2% of total addition) did not modify TFA absorption or jejunal morphology in broiler chickens of 46 d of life, whereas it caused minor changes in the FA profile of AFP. On the other hand, SO total replacement by SL reduced ileal absorption of PUFA and increased *Lactobacillus* spp. counts at the jejunum; however, these modifications had no influence on chicken performance.

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