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Abstract 1. Three experiments with a 2 × 2 × 2 factorial arrangement were conducted to evaluate maize-based diets for broilers containing different lipid sources [soybean oil (S) or beef tallow (T)] supplemented with or without lysophospholipids and organic acids on nutrient balance (Experiment I, evaluation period of 10–14 d), on liver concentration of fat-soluble vitamins, on jejunal microbiota (Experiment II, sampling at d 14) and on performance (Experiment III, accumulated periods of 1–14, 1–21 and 1–42 d).

2. A total of 1344 male chicks were used. In each experiment, the birds were allotted in a completely randomised design with 8 replications. The lysophospholipids were mainly composed of lysolecithins and the organic acids blend was constituted by lactic (40%), acetic (7%) and butyric acids (1%).

3. An interaction between lipid sources and lysophospholipids was observed on faecal apparent digestibility of lipid (ADL), which improved with lysophospholipids addition in T diets. Broilers fed on S had higher ADL and faecal apparent digestibility of nitrogen-corrected gross energy ($ADGE_N$).

4. It was not possible to demonstrate a significant treatment effect on the liver concentration of vitamins A and E, even with the differences in fatty acid profile between S and T.

5. Enterobacteria values were below the detection threshold. Lysophospholipid supplementation reduced gram-positive cocci in T-fed birds. S diets promoted lower total anaerobe counts compared with T diets, independent of additives.

6. S diets increased BW gain and feed:gain ratio in all evaluation periods. Lysophospholipids and organic acids improved feed:gain ratio at 1–21 d in T diets. Furthermore, main effects were observed for lysophospholipids and organic acids at 1–42 d, which increased BW gain and improved feed:gain ratio, respectively.

7. No positive interactions between additives were found.

INTRODUCTION

The inclusion of lipids in broiler diets is essential for the metabolisable energy requirement to be properly met. Within this reality, researches have been conducted to study the increased digestibility of oils and fats by emulsifying substances, such as bile salts (Polin *et al.*, 1980; Kussaibati *et al.*, 1982) and phospholipids (Azman and Ciftci, 2004; Huang *et al.*, 2007, 2008). Recently, some studies were performed to evaluate lysophospholipids as exogenous emulsifiers in poultry diets (Raju *et al.*, 2011; Zhang *et al.*, 2011), which are originated by the action of the phospholipase A_2 pancreatic enzyme on phospholipids. The conversion of phospholipids to lysophospholipids modifies the molecule chemical characteristics that greatly increase the critical micelle concentration (Stafford and Dennis, 1988).

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This process is fundamental and occurs naturally in bird's fat digestion, which supports this technology application in poultry nutrition through exogenous supplementation. Recently, Jansen *et al.* (2015) confirmed in broiler chickens an increased metabolisable energy due to the lysophospholipids utilisation in the feed.

Another action of lysophospholipids is related to the antibacterial effect (Coonrod and Yoneda, 1983). They may modify the biological structure of bacteria causing changes in membrane permeability (Arouri and Mouritsen, 2013), raising the speculation of a combined effect with organic acids on poultry gut health (Tamblyn and Conner, 1997; Alakomi *et al.*, 2000; Zhao *et al.*, 2009). The changes in membrane permeability by lysophospholipids may facilitate the ion dissociation of the organic acids inside the bacteria. In turn, the control of the intestinal microbiota through these additives could preserve the integrity of endogenous bile salts (Feighner and Dashkevicz, 1987; Knarreborg *et al.*, 2002*a*), especially in diets with large amounts of saturated fatty acids (Knarreborg *et al.*, 2002*b*), improving fat digestion. Some investigations around the antimicrobial effects of the organic acids exist (Ricke, 2003; Van Immerseel *et al.*, 2006), but so far there is no evidence in the literature about the action of lysophospholipids on broiler microbiota.

Therefore, the aim of this study was to evaluate diets for broiler chickens containing soybean oil (S) or beef tallow (T) with or without lysophospholipid and organic acid supplementation on nutrient balance, liver concentration of fat-soluble vitamins, jejunal microbiota and performance.

MATERIALS AND METHODS

The trials were conducted in accordance with the ethical principles of animal experimentation established by the Ethics Committee of Animal Use of University of São Paulo – USP (Brazil), protocol number 2737/2012.

Experimental design and diets

A $2 \times 2 \times 2$ factorial arrangement was used in a completely randomised design to evaluate the effects of two lipid sources (S or T) with or without lysophospholipids and organic acids. Diets were maize and soybean meal based and had similar energy and amino acid levels according to the values recommended by Rostagno *et al.* (2011) (Table 1). To balance the higher energy value of S, the exact amount of starch included replacing kaolin (inert material) in T diets was previously calculated in order to adjust the energy value of

diets with different lipid sources. The additives were included "on top" in replacement of the inert material according to each treatment. The lysophospholipids were composed mainly by lysolecithins, which is an emulsifier produced from enzymatic enrichment of soybean lecithin by the enzyme phospholipase A_2 activity, mainly producing lysophosphatidylcholine. The organic acids blend was: lactic (40%), acetic (7%) and butyric acids (1%). Antibiotics and coccidiostats were omitted from the diets to avoid any interference with the proposed additives. Water and mash feed were provided ad libitum.

The calculated fatty acids composition of the diets was carried out based on the analysis of all ingredients that had lipid fractions (Table 2). The fatty acid profile was determined using the fat extraction method described by Bligh and Dyer (1959), Christie (1982) and Smedes and Thomasen (1996). The separated fat was methylated yielding methyl esters formed according to Kramer *et al.* (1997). Two internal standards, C18:0 and C19:0 (Sigma Aldrich®), were used to correct for losses during the methylation process. The fatty acids were quantified by gas chromatography (GC Shimadzu, 2010, with automatic injection) using SP-2560 capillary column (100 m x 0.25 mm diameter with 0.02 mm thickness, Supelco, Bellefonte, PA). The initial temperature was 70°C for 4 min (13°C min^{-1}) until 175°C and holding for 27 min. Then, another increase of 4°C min^{-1} was started up to 215°C and holding for 31 min. Hydrogen (H_2) was used as carrier gas with a flow of 40 cm s^{-1}. The following 4 patterns were used during the identification process: Standard C4-24 of fatty acids (Supelco® TM 37), vaccenic acid C18:1 *trans*-11 (V038-1G, Sigma®) and CLA C18:2: *trans*-10, *cis*-12 (UC-61 M 100 mg), C18:2 *cis*-9, *trans*-11 (UC-60 M 100 83 mg), (NU-CHEK-PREP EUA®).

Birds

A total of 1344 one-d-old male Cobb® chicks were used. The initial average weight in the performance experiment (Experiment III) was 46.16 g ± 0.608 g ($P > 0.05$).

Experiment I – nutrient balance

For assessment of nutritional balance by the total excreta collection method, 448 chicks were allocated to the 8 treatments with 8 replications and 7 birds per experimental unit. The birds were placed into metabolic cages fitted with front feeder, nipple drinker and stainless steel trays for excreta collection. A 24-h lighting programme was employed. Metabolic assays occurred from 6 to 14 d, with 4 d of adaptation to the experimental diets and 4 more days for excreta collection, performed twice daily.

Table 1. *Composition of the experimental diets*

Ingredients (g/kg of natural matter)	Diets[1] 1–7 d S	T	8–21 d S	T	22–33 d S	T	34–42 d S	T
Maize meal 7.88%	531.45		584.72		611.13		648.67	
Soybean meal 45%	369.01		326.45		298.01		269.48	
Maize gluten 60%	5.92		0.72		3.68		0.94	
Soybean oil	27.00	–	27.00	–	31.50	–	31.50	–
Beef tallow	–	27.00	–	27.00	–	31.50	–	31.50
Starch	–	10.63	–	10.63	–	12.40	–	12.40
Sodium chloride	3.50		3.50		3.50		3.50	
Sodium bicarbonate	2.33		1.96		1.59		1.39	
Dicalcium-phosphate	19.28		15.26		13.25		10.36	
Limestone	9.09		10.35		8.90		8.28	
L-Lysine	3.20		2.44		2.05		1.95	
DL-Methionine	3.57		2.84		2.43		2.14	
L-Threonine	1.16		0.72		0.40		0.31	
L-Valine	0.86		0.41		0.16		0.08	
Lysophospholipids[2]	1.00		1.00		1.00		1.00	
Organic acids[2]	8.00		8.00		6.00		4.00	
Kaolin	10.63	–	10.63	–	12.40	–	12.40	–
Vitamin and mineral premix[3]	4.00		4.00		4.00		4.00	
Calculated composition, g/kg								
AME_N, MJ/kg	12.25		12.48		12.77		12.98	
Crude protein	220.0		200.0		190.0		178.0	
Ether extract	52.6		53.7		58.7		59.6	
Crude fibre	28.82		27.43		26.41		25.51	
Calcium	9.20		8.60		7.50		6.50	
Available phosphorus	3.95		3.37		3.07		2.66	
Lysine[4]	13.04		11.41		10.45		9.69	
Methionine + cystine[4]	9.39		8.22		7.63		7.07	
Threonine[4]	8.48		7.42		6.79		6.30	
Valine[4]	10.04		8.79		8.15		7.56	
Sodium	2.20		2.10		2.00		1.95	

[1]S, soybean oil; T, beef tallow.

[2]Additives were replaced by kaolin to obtain the treatments without additives. Lysophospholipids = Lysoforte[TM], supplied by Kemin®. Organic acids = Premium Lac®, supplied by Nutriacid/Btech.

[3]Vitamin and mineral premix provided: retinyl acetate, 2.40 mg; cholecalciferol, 0.60 mg; α-tocopherol acetate, 12.00 mg; menadione, 2.00 mg; thiamine, 2.40 mg; riboflavin, 6.00 mg; pyridoxine, 4.00 mg; cyanocobalamin, 0.014 mg; niacin, 40.00 mg; pantothenic acid, 15.00 mg; folic acid, 1.00 mg; choline, 346.40 mg; Fe, 50.00 mg; Mn, 70.00 mg; Zn, 50.00 mg; Cu, 10.00 mg; I, 1.20 mg; Se, 0.20 mg per kg of the pre-starter diet (1–7 d); retinyl acetate, 2.10 mg; cholecalciferol, 0.55 mg; α-tocopherol acetate, 11.00 mg; menadione, 1.60 mg; thiamine, 2.00 mg; riboflavin, 5.00 mg; pyridoxine, 3.00 mg; cyanocobalamin, 0.012 mg; niacin, 35.00 mg; pantothenic acid, 13.00 mg; folic acid, 0.80 mg; choline, 328.00 mg; Fe, 50.00 mg; Mn, 70.00 mg; Zn, 50.00 mg; Cu, 10.00 mg; I, 1.20 mg; Se, 0.20 mg per kg of the starter diet (8–21 d); retinyl acetate, 1.80 mg; cholecalciferol, 0.50 mg; α-tocopherol acetate, 10.00 mg; menadione, 1.60 mg; thiamine, 1.40 mg; riboflavin, 4.00 mg; pyridoxine, 2.00 mg; cyanocobalamin, 0.010 mg; niacin, 30.00 mg; pantothenic acid, 11.00 mg; folic acid, 0.60 mg; choline, 241.60 mg; Fe, 50.00 mg; Mn, 70.00 mg; Zn, 50.00 mg; Cu, 10.00 mg; I, 1.20 mg; Se, 0.20 mg per kg of the grower diet (22–33 d); retinyl acetate, 1.50 mg; cholecalciferol, 0.25 mg; α-tocopherol acetate, 8.00 mg; menadione, 1.60 mg; riboflavin, 2.00 mg; cyanocobalamin, 0.005 mg; niacin, 20.00 mg; pantothenic acid, 9.00 mg; choline, 127.60 mg; Fe, 50.00 mg; Mn, 70.00 mg; Zn, 50.00 mg; Cu 8.00 mg; I, 1.20 mg; Se, 0.20 mg per kg of the final diet (34–42 d).

[4]Digestible values.

Excreta were packed in plastic bags, identified by replication and stored in a freezer at −16°C. At the end of the experimental period, feed intake and total excreta produced were determined. Further, excreta samples were thawed and homogenised. A sample from each replicate was removed and dried at 55°C for 72 h. Subsequently, samples were weighed, finely ground and packed for laboratory analysis according to AOAC (1990).

Dry matter contents were determined at 105°C for 12 h and nitrogen values were obtained using the micro-Kjeldahl technique. The ether extract was analysed gravimetrically after extraction using petroleum ether in a Soxhlet extractor. Gross energy was measured by adiabatic calorimetry bomb (IKA® model C5003), using benzoic acid as a standard for calibration. Diets and excreta samples were analysed to determine the values of the faecal apparent digestibility of dry matter (ADDM), nitrogen (ADN), lipids (ADL) and gross energy corrected by nitrogen balance ($ADGE_N$). $ADGE_n$ was calculated using values for apparent metabolisable energy corrected to zero nitrogen balance (AME_N) obtained by use of the equations proposed by Matterson *et al.* (1965).

Table 2. *Fatty acid profile of the experimental diets*

g/100 g of fatty acids	Diets[1]							
	1–7 d		8–21 d		22–33 d		34–42 d	
	S	T	S	T	S	T	S	T
C10:0	0.02	0.05	0.02	0.05	0.02	0.05	0.02	0.05
C12:0	0.13	0.21	0.14	0.22	0.14	0.22	0.14	0.22
C14:0	0.14	1.61	0.14	1.58	0.13	1.67	0.13	1.65
C14:1 *cis*-9	–	0.27	–	0.27	–	0.28	–	0.28
C15:0	–	0.31	–	0.30	–	0.32	–	0.32
C16:0	12.14	20.11	12.08	19.89	11.97	20.30	11.94	20.15
C16:1 *cis*-9	0.20	1.47	0.20	1.44	0.19	1.52	0.19	1.50
C17:0	0.05	0.66	0.04	0.64	0.05	0.68	0.05	0.67
C18:0	3.74	12.90	3.75	12.72	3.72	13.28	3.73	13.16
C18:1 *trans*-9	–	0.10	–	0.10	–	0.11	–	0.10
C18:1 *trans*-11	–	1.35	–	1.32	–	1.41	–	1.39
C18:1 *cis*-9	25.26	32.72	25.59	32.89	25.49	33.28	25.70	33.38
C18:2 *cis*-9, 12	53.30	25.89	53.15	26.30	53.36	24.71	53.26	25.01
C18:2 *cis*-9, *trans*-11	–	0.25	–	0.25	–	0.27	–	0.26
C18:3 *cis*-9, 12, 15	4.17	1.39	4.02	1.30	4.08	1.18	3.99	1.13
C20:0	0.42	0.35	0.43	0.36	0.43	0.35	0.43	0.36
C20:1 *cis*-11	0.07	0.16	0.07	0.17	0.07	0.17	0.07	0.17
C20:2 *cis*-11, 14	0.02	0.03	0.02	0.03	0.02	0.04	0.02	0.04
C20:3 *cis*-8, 11, 14	–	0.01	–	0.01	–	0.01	–	0.01
C20:4 *cis*-5, 8, 11, 14	–	0.03	–	0.03	–	0.03	–	0.03
C21:0	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01
C22:0	0.30	0.11	0.30	0.11	0.30	0.10	0.30	0.10
C23:0	0.02	<0.01	0.02	<0.01	0.02	<0.01	0.02	<0.01
Σ								
Saturated	16.98	36.31	16.95	35.88	16.79	36.99	16.78	36.70
Unsaturated	83.02	63.69	83.05	64.12	83.21	63.01	83.22	63.30
Monounsaturated	25.53	36.08	25.86	36.19	25.75	36.77	25.96	36.83
Polyunsaturated	57.49	27.61	57.19	27.93	57.46	26.24	57.27	26.48
Unsaturated/Saturated	4.89	1.75	4.90	1.79	4.95	1.70	4.96	1.73
ω-9	25.33	32.98	25.66	33.16	25.56	33.56	25.77	33.66
ω-7	0.20	3.09	0.20	3.01	0.19	3.19	0.19	3.15
ω-6	53.32	25.96	53.17	26.37	53.38	24.79	53.28	25.09
ω-3	4.17	1.39	4.02	1.30	4.08	1.18	3.99	1.13
ω-6/3	12.79	18.63	13.22	20.22	13.08	20.99	13.35	22.21

[1]S, soybean oil; T, beef tallow.

Experiment II – biological sample collection

A total of 128 birds raised in floor pens were killed when they were 14 d old. The 8 experimental diets were replicated 8 times, where one bird represented the experimental unit. The diets were supplied from the first day. A total of 64 birds were used to evaluate the concentration of liver fat-soluble vitamins and the other 64 birds for the jejunal microbiological analysis. All birds were raised individually, totalling 128 boxes (1.00 m × 1.20 m) with tubular feeder and nipple drinker. A 24-h lighting programme was used and the litter was a rice husk recycled.

Fat-soluble vitamins

The livers taken from the birds were immediately frozen in liquid nitrogen to determine the concentration of vitamins A and E, identified as retinol and α-tocopherol, respectively. The liver samples were macerated; saturated ethanol was added with ascorbic acid together with 3 ml of saturated NaCl solution. Then, the mixture was homogenised, adding 5 ml of hexane solvent containing 2% 2-propanol and 0.01 % BHT (Butylated hydroxytoluene). The mixture was homogenised using vortex for 1 min and centrifuged for 5 min at 2400*g*. The supernatant was removed (organic phase) into a second flask and the procedure repeated with the sample, adding another 5 ml of hexane solvent. The organic phase was dried with nitrogen, reconstituted with mobile methanol/water phase (98%/2%, v/v) and injected into the liquid chromatograph.

The extracts of vitamins A and E were analysed by liquid chromatography with ultraviolet detection (LC-UV) and by liquid chromatography with detection of *in tandem* mass spectrometry (LC-MS/MS). The chromatographic conditions for the LC-UV: Chromatograph Agilent Series 1200; column Nucleosil Shell RP 18 (100 mm x 4.6 mm x 2.7 µm), mobile

methanol/water phase (98%/2% v/v) with a wavelength of 325 nm for vitamin A and 290 nm for vitamin E, injecting a volume of 20 µl, a flow ml/min. The chromatographic conditions for the LC-MS/MS Chromatograph Agilent 1200 were: column Nucleosil Shell RP 18 (100 mm x 4.6 mm x 2.7 µm), mobile phase water + 0.1 % formic acid and methanol + 0.1 % formic acid (2%/98%, v/v), injected volume of 10 µl and a flow 1 ml min^{-1}. Mass Spectrometer 3200 QTRAP (Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer), electrospray ionisation, positive mode. For vitamin A, the precursor ion (m/z) and fragment ion (m/z) were 269 and 93, 91 and 77; and for vitamin E, were 431 and 165, 137 and 69, respectively.

Jejunal microbiota

Birds were killed and the digestive tract was quickly dissected. Samples were collected from the jejunum (bile duct entrance to Meckel's diverticulum) with the aid of small wooden spatulas previously autoclaved. The samples were immediately frozen at –20°C until subsequent analysis. A 0.5 g of sample was diluted in 4.5 ml of peptone water previously autoclaved (10^{-1} dilution). The content was homogenised and 1 ml of the solution was transferred to another tube with 9 ml of peptone water. A serial dilution of the content was used to find the concentration that allowed a colony count within pre-established values, which were up to 250 colonies (Barbieri *et al.*, 2015).

After dilution, 100 µl of each concentration were pipetted in the petri dish with MacConkey media, Bile Esculin media and Brewer media to isolate enterobacteria, gram-positive cocci and total anaerobes, respectively. The contents were spread with a Drigalski handle in a circular motion until absorption of the liquid by culture media was complete. All media were incubated at 37°C for 48 h. Anaerobic jars were used to incubated the Brewer media. After the incubation period, the total colony-forming units were evaluated (Vahjen *et al.*, 1998).

Experiment III – performance

A total of 768 birds were housed in 64 floor pens, each with an area of 1.26 m^2 (0.90 m × 1.40 m) and covered with recycled rice husk (second utilisation) as litter. The 8 experimental diets were randomly distributed into 8 replications with 12 birds each. Feeders and drinkers were the tubular and nipple type, respectively. A 24-h lighting programme was used in the first 2 weeks and then an 18-h programme was used subsequently. A data logger located at bird height recorded every 2 h the

maximum, minimum and instantaneous average of environmental temperature, relative humidity and black globe temperature: 28.62 ± 4.96°C, 18.03 ± 2.73°C, 22, 43 ± 4.27°C, 84.89 ± 3.67%, 51.56 ± 10.38%, 71.86 ± 12.69%, 28.12 ± 1.86°C, 18.27 ± 3.72°C and 22.63 ± 4.26°C, respectively.

The performance was evaluated in accumulated periods 1–14, 1–21 and 1–42 d, considering the following variables: BW gain, feed intake and feed:gain ratio. Mortality was recorded daily to correct the feed intake and feed:gain ratio in each evaluation period by the average number of birds and by the weight of dead birds, respectively. Viability [100 –mortality (%)] and production efficiency factor were calculated at the end of the experiment at 42 d.

Statistical analysis

Results were analysed by Statistical Analysis System software (SAS Institute, 2012). Effects were considered significant at $P \leq 0.05$. All data were submitted to analysis of variance by the MIXED procedure. When necessary, differences between treatments were studied by comparing least squares means (LSMEANS) calculated by PDIFF (Compared Pairwise). The statistical model was:

$$y_{ijkl} = \mu + a_i + b_j + c_k + (a \times b)_{ij} + (a \times c)_{ik} + (b \times c)_{jk} + (a \times b \times c)_{ijk} + e_{ijkl},$$

where y_{ijkl} = response variable of broilers fed on lipid sources (i), lysophospholipids (j) and organic acids (k), in replication l. μ = overall mean value for y. a_i = fixed effect of lipid sources. b_j = fixed effect of lysophospholipids. c_k = fixed effect of organic acids. $(a \times b)_{ij}$ = interaction of lipid sources with lysophospholipids. $(a \times c)_{ik}$ = interaction of lipid sources with organic acids. $(b \times c)_{jk}$ = interaction of lysophospholipids with organic acids. $(a \times b \times c)_{ijk}$ = triple interaction among lipid sources, lysophospholipids and organic acids. e_{ijkl} = error term.

Data that did not show residual normality (verified by Shapiro–Wilk test by UNIVARIATE procedure) were submitted to logarithmic transformation. In the cases where data transformation was not enough to meet the statistical assumptions, the non-parametric Kruskal–Wallis test was applied through the NPAR1WAY procedure.

RESULTS

Nutrient balance

There was an interaction of lipid sources with lysophospholipids on ADL (Table 3), which improved with lysophospholipids addition in T-containing diets ($P < 0.01$) (Figure, a). Diets with S had greater ADL, regardless of lysophospholipids inclusion

Table 3. *Faecal apparent digestibility (%) of dry matter (ADDM), nitrogen (ADN), lipid (ADL) and gross energy corrected by nitrogen balance (ADGE$_N$) of broilers fed maize-based-diets (10–14-d period)[1]*

Effects[2]		Starter phase[3]			
		ADDM	ADN	ADL	ADGE$_N$
Lipid	S	70.4	62.0	80.1	75.9
	T	70.7	61.8	75.1	74.7
LP	+	70.6	62.1	77.9	75.5
	–	70.5	61.7	77.3	75.2
OA	+	70.5	62.2	77.9	75.1
	–	70.5	61.7	77.2	75.5
SEM		0.17	0.31	0.39	0.20
Source of variation		Probability			
Lipid		0.376	0.755	<0.001	0.002
LP		0.851	0.549	0.174	0.501
OA		0.935	0.380	0.106	0.236
Lipid × LP		0.128	0.337	0.016	0.093
Lipid × OA		0.139	0.336	0.465	0.051
LP × OA		0.830	0.226	0.145	0.715
Lipid × LP × AO		0.325	0.234	0.182	0.306

[1]Data represent means of 8 replicates (*n* = 7 per replicate) in a treatment group.

[2]LP, lysophospholipids; OA, organic acids; S, soybean oil; T, beef tallow; +, presence of additive; –, absence of additive.

[3]Values expressed in natural matter.

Table 4. *Fat-soluble vitamins concentration in the liver of broilers fed on maize-based diets (14-d old)[1]*

Effects[2]		Vitamins			
		A		E	
		m g/kg[3]	mg[4]	m g/kg[3]	mg[4]
Lipid	S	2.07	0.152	0.94	0.030
	T	2.09	0.130	0.84	0.022
LP	+	2.01	0.140	0.84	0.025
	–	2.15	0.141	0.94	0.026
OA	+	2.22	0.180	0.82	0.023
	–	1.93	0.102	0.96	0.029
SEM		0.130	0.0219	0.064	0.0039
Source of variation		Probability			
Lipid		0.952	0.940	0.420	0.209
LP		0.616	0.450	0.422	0.564
OA		0.297	0.331	0.287	0.472
Lipid × LP		0.825	0.743	0.459	0.440
Lipid × OA		0.481	0.792	0.825	0.340
LP × OA		0.532	0.772	0.971	0.386
Lipid × LP × AO		0.295	0.272	0.417	0.925

[1]Data represent means of 8 replicates (*n* = 1 per replicate) in a treatment group.

[2]LP, lysophospholipids; OA, organic acids; S, soybean oil; T, beef tallow; +, presence of additive; –, absence of additive.

[3]Data submitted to log transformation.

[4] Variables submitted to non-parametric test of Kruskal-Wallis.

(P < 0.001). Furthermore, lipid sources influenced ADGE$_N$, which was higher in S diets. The ADDM and ADN were not affected by the treatments.

Fat-soluble vitamins

Hepatic concentration of vitamins A and E were not affected by the treatments (Table 4). The contrast between the lipid profile of S and T was not enough

to significantly alter the results, even with the additives addition.

Jejunal microbiota

In the experimental conditions of this study, there was no growth of total enterobacteria in jejunum samples, which were below the detection
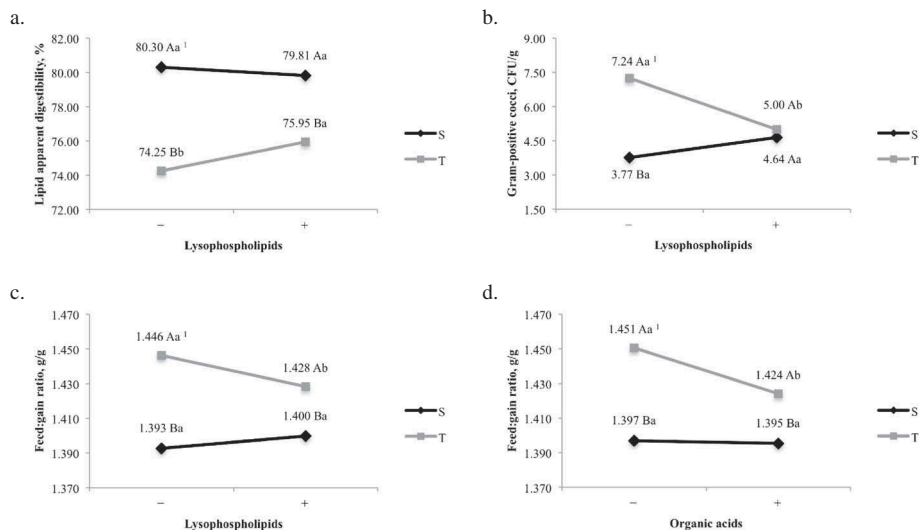

Figure. *Interactions of lipid sources with the additives on faecal apparent digestibility of lipids at 10–14-d period (a), on gram-positive cocci count in the jejunum at 14 d (b) and on feed:gain ratio at 21 d of broilers (c and d). [1]Different letters, uppercase in the columns and lowercase in the rows, indicate significant difference at 5% probability. S, soybean oil; T, beef tallow.*

Table 5. *Jejunal microbiology of broilers fed on maize-based diets (14-d old)[1]*

Effects[2]		Microbiology, CFU/g[3]	
		Gram-positive cocci	Total anaerobes
Lipid	S	4.20	7.21
	T	6.12	8.48
LP	+	4.82	7.82
	−	5.50	7.88
OA	+	4.73	7.70
	−	5.59	8.00
SEM		0.402	0.188
Source of variation		Probability	
Lipid		0.014	<0.001
LP		0.372	0.863
OA		0.260	0.398
Lipid × LP		0.044	0.183
Lipid × OA		0.944	0.946
LP × OA		0.606	0.313
Lipid × LP × AO		0.185	0.139

[1]Data represent means of 8 replicates (*n* = 1 per replicate) in a treatment group.
[2]LP, lysophospholipids; OA, organic acids; S, soybean oil; T, beef tallow; +, presence of additive; −, absence of additive.
[3]UFC/g, colony forming units per g of sample. Data submitted to log transformation.

threshold. There was interaction between lipid sources and lysophospholipids inclusion on gram-positive cocci (Table 5). In diets without lysophospholipids, S provided lower amount of gram-positive cocci than T (*P* < 0.01) (Figure, b); however, the presence of lysophospholipids decreased the

number of gram-positive cocci in jejunum of T-fed broilers (*P* < 0.05), leading to similar results to those obtained with S. Total anaerobic microorganisms were found in lower quantities in S-containing diets.

Performance

The results of broiler performance in the initial phase and the total period are presented in Table 6. At d 14, there was no interaction between the studied factors. A positive effect of S was observed on BW gain. Feed intake was not affected. S and organic acids inclusion improved feed:gain ratio. During this phase, no effect of lysophospholipids was found.

At 21 d, broilers fed on S diets presented greater BW gain and feed intake. In this period, there was an interaction between lipid sources and lysophospholipids on feed:gain ratio (Figure, c). Lipid sources also interacted with organic acids on feed:gain ratio, as shown in the Figure (d). In both interactions, S promoted better feed:gain ratio than T (*P* < 0.001); however, lysophospholipids (*P* < 0.05) and organic acids (*P* < 0.01) addition improved feed:gain ratio of T diets, showing that the additives are most effective in diets with saturated lipid sources.

Over the entire trial (1–42 d), broilers fed on S had the greatest BW gain and better feed:gain ratio compared with those fed on T, being in accordance with results observed in the early stages. Lysophospholipid inclusion provided greater BW

Table 6. *Performance of broilers fed on maize-based diets[1]*

Effects[2]		Performance[3]										
		1–14 d			1–21 d			1–42 d				
		BW gain	Feed intake	Feed:gain ratio	BW gain	Feed intake	Feed:gain ratio	BW gain	Feed intake	Feed:gain ratio	Viability[4]	PEF[5]
Lipid	S	418	545	1.306	924	1288	1.396	2916	4789	1.656	96.1	401
	T	404	541	1.341	885	1268	1.437	2867	4743	1.671	95.6	390
LP	+	412	543	1.324	908	1279	1.414	2912	4771	1.660	94.5	394
	−	410	543	1.324	902	1277	1.419	2871	4761	1.667	97.1	398
OA	+	414	542	1.310	905	1272	1.410	2902	4764	1.657	95.1	395
	−	408	544	1.337	905	1285	1.424	2881	4767	1.670	96.6	397
SEM		2.2	2.1	0.0052	4.1	4.3	0.0041	10.1	15.3	0.0027	0.72	3.1
Source of variation		Probability										
Lipid		<0.001	0.457	<0.001	<0.001	0.022	<0.001	0.013	0.151	0.003	0.751	0.090
LP		0.675	0.921	0.996	0.404	0.798	0.362	0.039	0.761	0.142	0.066	0.521
OA		0.165	0.686	0.005	0.981	0.124	0.023	0.284	0.917	0.016	0.230	0.711
Lipid × LP		0.295	0.857	0.700	0.311	0.669	0.042	0.467	0.739	0.845	0.100	0.387
Lipid × OA		0.674	0.804	0.657	0.554	0.591	0.043	0.401	0.893	0.789	0.163	0.493
LP × OA		0.317	0.226	0.994	0.729	0.557	0.380	0.816	0.764	0.716	0.314	0.700
Lipid × LP × OA		0.989	0.274	0.067	0.730	0.392	0.345	0.629	0.971	0.436	0.571	0.546

[1]Data represent means of 8 replicates (*n* = 12 per replicate) in a treatment group.
[2]LP, lysophospholipids; OA, organic acids; S, soybean oil; T, beef tallow; +, presence of additive; −, absence of additive.
[3]Values expressed in g. Viability was expressed in %.
[4]Variable submitted to non-parametric test of Kruskal–Wallis.
[5]PEF, production efficiency factor: [([VIAB × ADG] /F:G)/10], where VIAB, ADG and F:G are viability, average daily gain and feed:gain ratio, respectively.

gain, and the organic acids, on the other hand, improved the feed:gain ratio. However, it is noted at this period that the additives showed only main effects, with no interactions between factors. Feed intake, viability and productive efficiency index were not significantly altered by the diets.

DISCUSSION

The greatest ADL provided with S consequently improved the ADGE$_N$, demonstrating the clearly superiority of S. Higher digestibility of S compared with T was also observed by several authors (Dänicke *et al.*, 2000; Preston *et al.*, 2001; Ferreira *et al.*, 2005; Fascina *et al.*, 2009). There are many factors that influence the fat and oil digestibility. Among others, it has already been shown that the degree of saturation (Leeson and Summers, 1976), the amount of free fatty acids (Vila and Esteve-Garcia, 1996) and the position of the fatty acid in the triglyceride molecule (Renner and Hill, 1961) affect the utilisation of dietary lipids by birds.

Also, the physiological and metabolic factors may influence lipid digestion, which is dependent on the emulsification process by surfactant action of the bile salts. However, in the early stages of life, the birds have insufficient quantities of bile salts, which represent a critical point that can be optimised in the digestion of lipids. It is possible that the action of lysophospholipids on the ADL of T diets may have complemented the endogenous bile action, which appears to have low efficacy against the higher amounts of saturated fatty acids (Krogdahl, 1985). In this way, Alzawqari *et al.* (2010, 2011) reported an increase in saturated fat digestibility in diets supplemented with bile salts.

The conversion of phospholipids into lysophospholipids by enzymatic action is another fundamental stage in the lipid digestion process. The action of the phospholipase enzyme in fat absorption has been supported in studies with turkeys, having a marked action in the first weeks (Santos Jr *et al.*, 2004). However, the use of phospholipase in the diet affects certain concerns regarding heat treatment in the extrusion and pelletisation process or in combination with other additives such as organic acids, which may change the optimal pH range of the enzyme. On the other hand, the lysophospholipids are fat molecules that have greater stability, admitting its use without major concerns related to temperature and pH.

It is known that the lipids digestibility affects the absorption of fat-soluble dietary components, which are dependent on the emulsification process to be absorbed by a micellar complex. Nevertheless, in

this experiment, it was not possible to observe treatment effect on liver concentration of vitamins A and E. Knarreborg *et al.* (2004) observed that the bioavailability of tocopherol molecules depends on the good conditions of emulsion and micelle formation, while favourable conditions for undesired bacterial growth cause negative effects. Dänicke *et al.* (1997) observed a higher concentration of vitamin A in the liver of birds fed with a higher content of polyunsaturated fatty acids derived from S compared with T. However, these studies used diets with high concentrations of soluble pentosanes, which dramatically increase intestinal viscosity, adding contrast between the digestion of fats and oils.

The microbial count of jejunal samples indicates that lysophospholipids were effective in T-containing diets, minimising the negative impact of a higher quantity of saturated fatty acids from T on gram-positive cocci count. This decrease can be explained by the antimicrobial effect of lysophospholipids, which may have occurred in two ways. The lysophospholipids may have acted directly on the gram-positive microorganisms altering the cell membrane permeability, leading to damage in the bacteria integrity through ionic imbalance (Coonrod and Yoneda, 1983; Silva Jr, 2009; Arouri and Mouritsen, 2013). The second cause is related to the greater utilisation of T that resulted in lower amounts of substrate in the intestinal lumen. This finding suggested that the lysophospholipids could contribute to the reduction of the amount of growth-depressing metabolites produced by gram-positive bacteria, which is one of the beneficial factors attributed to the antibiotic utilisation (Huyghebaert *et al.*, 2011).

The effect of lysophospholipids on the intestinal microbiota of broilers suggests further investigations, especially for microorganisms of particular interest in lipid digestion such as in the case of *Enterococcus faecium* and *Clostridium perfringens* (Knarreborg *et al.*, 2002a). Organic acids did not changed jejunal microbiology probably due to the potential acidification that occurs in the proximal organs of the gastrointestinal tract, being removed from ingesta as a result of metabolism and absorption processes before reaching the Meckel's diverticulum (Bolton and Dewar, 1965). The most pronounced effect at the beginning of the digestive tract may be the pH decrease and bacterial control in subsequent segments of the intestine (Müjdat *et al.*, 1999; Pirgozliev *et al.*, 2008; Abbas *et al.*, 2011); however, no significant results were found in the present study.

The diets with S provided lower amounts of total anaerobic microorganisms in the jejunum. The natural amount of bile salts and enzymes

involved in bird lipid digestion may not be sufficient to efficiently emulsify the fatty acids of T, which have large portions fully saturated. The relationship between the low digestibility of saturated fat with the intestinal microbiota was reported by Dänicke *et al.* (1999). Knarreborg *et al.* (2002*b*) confirmed the increase of undesirable bacterial growth due to higher amounts of saturated fatty acids in the diet. In turn, the increased amount of microorganisms affects the bile salt integrity by the bacterial cholyltaurine hydrolase activity (Knarreborg *et al.*, 2002*a*), which affects the digestibility and performance of chickens (Feighner and Dashkevicz, 1987). Additionally, Maisonnier *et al.* (2003) showed loss of bile salts caused by the intestinal microbiota. Therefore, the improvement of ADL caused by lysophospholipids in T diets may also be associated to antimicrobial effect.

Results of ADL and ADGE$_N$ extended to broiler performance, confirming researches that observed better performance in consensus with higher digestibility coefficients due to lipid sources (Dänicke *et al.*, 1997) and emulsifiers (Zhang *et al.*, 2011). The interaction between the lipid sources and lysophospholipids on digestibility was also observed on feed:gain ratio in the starter phase, where lysophospholipids increased feed:gain ratio only in diets with T. For the total period (1–42 d), the lipid sources and lysophospholipids affected performance without interactions. Due to a higher demand for energy in the final stages, the lysophospholipids presence was effective in increasing broiler BW gain independent of the inclusion of S or T. Zhang *et al.* (2011) found higher values of apparent metabolisable energy (AME) in diets with lysophospholipids only after 35 d, confirming the greatest pronouncement of lysophospholipids on energy demand in the final phase. Furthermore, the lysophospholipids derived from rice bran was recommended as a source of energy for broilers with beneficial effects on performance and fat digestibility (Raju *et al.*, 2011).

Organic acids improved feed:gain ratio in all periods, and presented positive effect in T diets within interaction with lipid sources at d 21. Although no organic acid effect was observed on jejunal microbiological count, the positive effect of the organic acids primarily on diets with T is justified by the antimicrobial effect, probably occurring in the proximal portions of the gastrointestinal tract. Several studies show the benefits of organic acids in diets for broilers (Abdel-Fattah *et al.*, 2008; Adil *et al.*, 2010), with accentuated effects in the presence of microbial challenge (Abbas *et al.*, 2011).

In conclusion, the property of lipid sources is a decisive factor that strongly affects digestibility, microbiota and performance of broilers.

The additives interacted with saturation degree of lipid sources up to 21 d, improving diets with T. There were no positive interaction effects (synergy) between the additives on the variables studied. However, the findings suggest that lysophospholipids increase BW gain and organic acids improve feed:gain ratio from 1 to 42 d, regardless of lipid source.

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