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# Effects of dietary emulsifier and lipid source on broiler meat quality, lipids, and serum antioxidant status

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## Abstract

This study aimed to evaluate the effects of diets containing acid soybean oil (ASO) as a substitute for degummed soybean oil (DSO), with or without an emulsifier, on the serum lipid composition, antioxidant protection, carcass yield, and meat quality of broilers. Seven hundred and four 1-day-old male chicks were distributed in a  $2 \times 2$  factorial arrangement (with or without emulsifier  $\times$  two lipid sources—ASO and DSO), with eight replicates. The dietary inclusion of ASO increased by 65.87% the serum activity of the superoxide dismutase at 21 days of age. The inclusion of the emulsifier reduced the tenderloin yield of the birds by 5.22% and the abdominal fat percentage by 10.20% at 49 days of age, interfering with serum low-density lipoprotein and triglyceride. There was an increase in the water-holding capacity, greater yellow intensity ( $b^*$ ) 15 min post mortem, and lower pH 24 h post mortem for meat from broilers fed diets containing DSO. Meat from broilers fed diets containing ASO with an emulsifier showed lower shear force. The emulsifier did not provide additional metabolizable energy, as reflected by the reduced chicken tenderloin yield. The use of ASO provided better serum antioxidant status, with no deleterious effects on the carcass and meat quality of broilers.

**Key words:** acid soybean oil, antioxidant capacity, carcass traits, exogenous emulsifier, serum lipids

## Résumé

Cette étude avait pour but d'évaluer les diètes contenant l'huile de soja acide comme substitut de l'huile de soja démulcinée, avec ou sans agent émulsifiant, sur la composition des lipides sériques, la protection antioxydante, le rendement de carcasse et la qualité de viandes des poulets à griller. 704 poussins mâles âgés de 1 jour ont été distribués dans un design expérimental factoriel  $2 \times 2$  (avec ou sans agent émulsifiant  $\times$  deux sources de lipides – huile de soja acide [ASO — « acid soybean oil »] et huile de soja démulcinée [DSO — « degummed soybean oil »]), avec huit répliques. L'inclusion alimentaire d'ASO a augmenté de 65,87 % l'activité sérique de la superoxyde dismutase à l'âge de 21 jours. L'inclusion de l'agent émulsifiant a réduit le rendement de filet par 5,22 % et le pourcentage de gras abdominal de 10,20 % des poulets à l'âge de 49 jours, interférant ainsi avec le LDL sérique et les triglycérides. Il y a eu une augmentation de la capacité de rétention d'eau, une plus grande intensité jaune ( $b^*$ ) 15 minutes post mortem et un plus faible pH 24 heures post mortem pour la viande provenant des poulets ayant reçu les diètes avec DSO. La viande provenant des poulets ayant reçu les diètes avec ASO et agent émulsifiant a montré une plus faible force de cisaillement. L'agent émulsifiant n'a pas fourni une énergie métabolisable supplémentaire, selon le rendement plus faible en filet de poulet. L'utilisation d'ASO a fourni un meilleur état antioxydant sérique sans effets délétères sur la qualité de la carcasse et de la viande chez les poulets à griller. [Traduit par la Rédaction]

**Mots-clés :** huile de soja acide, capacité antioxydante, caractéristiques de carcasse, émulsifiant exogène, lipides sériques

## 1. Introduction

Using lipid sources in poultry nutrition is imperative because broiler chickens have high energy requirements that are not met by corn only (Siyal et al. 2017). Degummed soybean oil (DSO), the leading vegetable oil used in the poultry industry, is a high-priced agricultural commodity that directly competes with human food (Ajanovic 2011). Acid soybean oil (ASO) is a residue from the refining of soybean oil for human consumption that has stood out among

the possible alternative feed sources due to its low cost (Von Schaumburg et al. 2018). However, this residue has a low concentration of linoleic and linolenic acids and polyunsaturated fatty acids, and a high percentage of free fatty acids (FFAs) (Cortes-Cuevas et al. 2017). The FFAs have reduced micellar affinity, and consequently a low absorption rate.

Given the above, the inclusion of an emulsifier in poultry diets may allow the use of ASO (Bontempo et al. 2018). Emulsifiers contribute to micelle formation, thus facilitating the

process of digestion and absorption of lipids, and increasing the energy provided by lipid sources (Wang et al. 2016; Yin et al. 2018).

However, the diet can affect the bird's antioxidant status and the physicochemical characteristics of the meat. The dietary inclusion of fatty acid-rich feed ingredients, especially lipid sources with a high amount of unsaturated fatty acids, may alter the proportion of free radicals and, consequently, the balance between free radical production and antioxidant capacity. Emulsifiers act by increasing the absorption of different fatty acids from dietary lipid sources, which affect the oxidative balance status of an individual. Moreover, fatty acids are deposited in the muscles of birds, and can alter the color, flavor, and composition of fatty acids in meat (Tavárez et al. 2011).

Thus, we hypothesize that ASO may be an alternative source to DSO in broiler diets without the impairment of serum lipid components, the serum antioxidant status of birds, the carcass yield, and the meat quality of broilers. Moreover, we hypothesize that the addition of an emulsifier to diets may enhance the use of soy acid oil by the birds, allowing the possibility of reducing the energy of the diets. This study aimed to determine the effect of diets containing ASO in substitution to DSO, with or without the inclusion of an emulsifier, on the serum lipid composition, antioxidant protection, carcass yield, and meat quality of broilers.

## 2. Materials and methods

The study was conducted in Marechal Cândido Rondon, Paraná, Brazil (latitude 24°33'S; longitude 54°04'W; altitude 420 m).

### 2.1 Animal care

The ethics committee previously approved the procedures adopted during the experiment (protocol #55/19) and the birds were raised according to the ethical principles for animal experimentation established by the National Council for Animal Experimentation Control.

### 2.2 Housing, birds, and treatments

A total of 704 1-day-old male Ross 308 chicks were placed in a completely randomized design in a 2 × 2 factorial arrangement (with or without the inclusion of an emulsifier × two lipid sources—ASO and DSO), with eight replicates with 22 birds per experimental unit (EU). The birds were vaccinated at the hatchery and raised in floor pens (1.95 m<sup>2</sup>) covered with wood pine shavings and had ad libitum access to feed and water. The lighting program and the control of the temperature followed the recommendations of the lineage manual. The heating of the environment in the initial phase was carried out utilizing resistance (250 W) and the renewal of air and cooling of the environment were carried out by exhaust fans and evaporative plates.

The experimental diets were supplied in mash form and formulated according to the feed composition and nutritional recommendations proposed by Rostagno et al. (2017), for phases 1–7, 8–21, 22–33, 34–42, and 43–49 days of age for

broilers of medium–regular performance (Tables 1 and 2). Diets were formulated considering the apparent metabolizable energy value of 8800 and 7458 kcal kg<sup>-1</sup> for DSO and ASO, respectively (energetic matrix from supplier). An emulsifier based on glyceryl polyethylene glycol ricinoleate was added to the diets at the rate of 350 g ton<sup>-1</sup>. The diets with emulsifier had the recommendation in apparent metabolizable energy reduced by 40 kcal kg<sup>-1</sup> from 1–21 days and by 50 kcal kg<sup>-1</sup> from 22 to 49 days of age.

### 2.3 Blood analyses

At 14, 21, and 49 days of age, one bird per EU with average weight (±5%) was selected and, after a 6 h fast, approximately 4 mL of blood was collected by puncture of the brachial vein. The blood was centrifuged (Centrifuge Kasvi K14-4000, Kasvi, São Paulo, Brazil) at 2500 rpm for 10 min, to obtain the serum, and then stored at -20 °C (Nunes et al. 2018). Subsequently, the serum was thawed to determine the serum levels of triglycerides, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), using commercial kits (Elitech Clinical Systems, ELITechGroup, Paris, France). The analyses were performed on an automatic spectrophotometer, with automatic calibration and high-performance reading (Elitech EL 200).

### 2.4 Serum antioxidant status analyses

A sample of the serum obtained for each EU at 21 days was stored in a cryogenic tube and kept in liquid nitrogen for further analysis of lipoperoxidation and superoxide dismutase (SOD). Serum lipoperoxidation was measured by the thiobarbituric acid reactive substance (TBARS) chemical method, using a commercial kit (Cayman Chemical), and the SOD was determined colorimetrically (enzymatic method), using a commercial kit (Cayman Chemical). Both readings were performed using an enzyme-linked immunoassay (ELISA) microplate reader (Molecular Devices, FlexStation 3).

### 2.5 Carcass yield

At 49 days of age, two birds per EU, with average weight (±5%), were individually weighed and slaughtered by cervical dislocation followed by bleeding. Subsequently, the birds were plucked and eviscerated to determine the carcass and cut yields. The carcass yield was calculated based on the weight of the hot eviscerated carcass (without head, feet, neck, and abdominal fat) in relation to the live weight of the bird before slaughter. For the cut yields (the breast, legs, wings, and tenderloins) were considered for the weight of the cold eviscerated carcass. To determine the percentage of abdominal fat, the adipose tissues around the cloaca, gizzard, proventricle, and adjacent abdominal muscles were weighed and the percentage in relation to the weight of the live bird was determined.

### 2.6 pH measurement and instrumental color evaluation

The pH and the meat color were determined 15 min and 24 h post mortem. The pH analysis was performed directly on the fillet of the right breast (pectoralis major) and the

**Table 1.** Percentage and calculated composition of experimental diets containing different lipid sources, with or without inclusion of emulsifier, for the prestarter (1–7 days) and starter (8–21 days) phases of broilers.

Ingredients	1–7 days				8–21 days			
	ASO		DSO		ASO		DSO	
	With	Without	With	Without	With	Without	With	Without
Corn (%)	55.96	57.15	56.62	57.55	57.41	58.60	58.31	59.26
Soybean meal, 46% (%)	34.40	34.20	34.30	34.10	32.30	32.10	32.20	32.00
Meat and bone meal, 45% (%)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
ASO (%)	2.18	1.18	–	–	3.18	2.18	–	–
DSO (%)	–	–	1.62	0.88	–	–	2.37	1.62
Poultry fat (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate (%)	1.097	1.096	1.097	1.095	0.878	0.877	0.877	0.876
Limestone (%)	0.598	0.600	0.599	0.600	0.512	0.514	0.513	0.515
NaCl (%)	0.479	0.479	0.479	0.479	0.462	0.462	0.462	0.462
Mineral supplement <sup>a</sup> (%)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Vitamin supplement <sup>b</sup> (%)	0.130	0.130	0.130	0.130	0.130	0.130	0.130	0.130
DL-Methionine, 99% (%)	0.397	0.396	0.396	0.395	0.378	0.377	0.377	0.376
L-Threonine, 98% (%)	0.126	0.126	0.126	0.126	0.120	0.120	0.120	0.120
L-Lysine HCL, 50.7% (%)	0.489	0.495	0.492	0.496	0.488	0.494	0.493	0.497
Choline chloride, 60% (%)	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Inert <sup>c</sup> (%)	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035
Total (%)	100	100	100	100	100	100	100	100
AME (kcal kg <sup>-1</sup> )	2975	2935	2975	2935	3050	3010	3050	3010
Crude protein (%)	21.94	21.94	21.94	21.94	21.09	21.09	21.09	21.09
Calcium (%)	0.971	0.971	0.971	0.971	0.878	0.878	0.878	0.878
Av. phosphorus (%)	0.463	0.463	0.463	0.463	0.419	0.419	0.419	0.419
Sodium (%)	0.225	0.225	0.225	0.225	0.218	0.218	0.218	0.218
Dig. Lys (%)	1.307	1.307	1.307	1.307	1.256	1.256	1.256	1.256
Dig. Met + Cys (%)	0.967	0.967	0.967	0.967	0.929	0.929	0.929	0.929
Dig. Thr (%)	0.863	0.863	0.863	0.863	0.829	0.829	0.829	0.829

**Note:** AME, Apparent Metabolizable Energy; ASO, acid soybean oil; Dig. Lys, digestible lysine; Dig. Met, digestible methionine; Dig. Met + Cys, digestible methionine and cysteine; and DSO, degummed soybean oil.

<sup>a</sup>Mineral supplement, per kg of diet: 50 mg iron; 10 mg copper; 65 mg manganese; 65 mg zinc; and 1 mg iodine.

<sup>b</sup>Vitamin supplement, per kg of diet: 14 300 IU vitamin A; 5200 IU vitamin D<sub>3</sub>; 71.5 IU vitamin E; 3.9 mg vitamin K<sub>3</sub>; 2.99 mg vitamin B<sub>1</sub>; 9.10 mg vitamin B<sub>2</sub>; 15.6 mg pantothenic acid; 5.2 mg vitamin B<sub>6</sub>; 3.25 µg vitamin B<sub>12</sub>; 78 mg nicotinic acid; 2.6 mg folic acid; 325 µg biotin; and 390 µg selenium.

<sup>c</sup>The inclusion of the emulsifier was performed in substitution to the inert Caulim<sup>®</sup>.

right leg of the birds with the aid of a portable pH meter (HI 99163 Hanna Instruments) (Olivo et al. 2001). The meat color was determined in two different regions of the internal part of the breast muscle and the interior part of the leg using the portable colorimeter CR-400 (Konica Minolta Sensing, São Paulo, Brazil). The components *L*\* (luminosity—dark to light level), *a*\* (red/green intensity), and *b*\* (yellow/blue intensity) were expressed in the CIELAB color system (Honikel 1998).

### 2.7 Water holding capacity, cooking weight loss, and shear force determination

The water holding capacity (WHC) was determined using the centrifugation method proposed by Nakamura and Kato (1985). Samples of approximately 1 g of raw breast muscle were taken after cooling in water and ice for 30 min. The samples were wrapped in filter paper and centrifuged (Centrifuge Kasvi K14-4000, Kasvi, São Paulo, Brazil) at 2000 rpm

for 4 min. After centrifugation, the samples were weighed, oven dried at 70 °C for 12 h, and then weighed again. The WHC value was determined by the difference between the final weight after centrifugation and the initial weight of the sample.

For the determination of the cooking weight loss (CL), the breast fillets were weighed, wrapped in aluminum foil, and cooked on an electrically heated plate preheated to 180 °C until reaching the internal temperature of 80 °C. The samples were cooled at room temperature and then weighed again, and the CL was determined by weight difference (Honikel 1998). After analyzing the CL, the samples were used to determine shear force (SF). The samples were cut into three rectangles (1 cm × 1 cm × 4 cm) and arranged with the fibers oriented perpendicular to the blade to determine the SF in kilogram-force (kgf cm<sup>-2</sup>), using a Brookfield CT3 Texture Analyzer, coupled with a TA 3/100 probe (TA-SBA fixture, calibrated with 0.01 kg force, 20 mm strain, and 2.5 mm s<sup>-1</sup> test speed).

**Table 2.** Percentage and calculated composition of experimental diets containing different lipid sources, with or without inclusion of an emulsifier, for the grower I (22–33 days), grower II (34–42 days), and finisher (42–49 days) phases of broilers.

Ingredients	22–33 days				34–42 days				42–49 days			
	ASO		DSO		ASO		DSO		ASO		DSO	
	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
Corn (%)	63.80	65.25	64.91	66.04	69.67	71.13	70.56	71.70	70.68	72.22	72.09	73.12
Soybean meal, 46% (%)	25.90	25.70	25.70	25.50	21.00	20.80	20.90	20.70	18.90	18.60	18.60	18.50
Meat and bone meal, 45% (%)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
ASO (%)	3.57	2.31	–	–	3.10	1.83	–	–	4.39	3.14	–	–
DSO (%)	–	–	2.66	1.72	–	–	2.30	1.36	–	–	3.27	2.34
Poultry fat (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate (%)	0.687	0.685	0.686	0.684	0.302	0.300	0.301	0.299	0.191	0.189	0.189	0.188
Limestone (%)	0.374	0.377	0.376	0.378	0.337	0.340	0.339	0.341	0.289	0.292	0.291	0.293
NaCl (%)	0.439	0.438	0.438	0.438	0.412	0.412	0.412	0.412	0.400	0.400	0.400	0.400
Mineral supplement <sup>a</sup> (%)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Vitamin supplement <sup>b</sup> (%)	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
D,L-Methionine, 99% (%)	0.335	0.333	0.333	0.332	0.290	0.289	0.289	0.288	0.270	0.269	0.269	0.268
L-Threonine, 98% (%)	0.117	0.117	0.117	0.117	0.104	0.104	0.104	0.104	0.099	0.099	0.099	0.099
L-Lysine HCL, 50.7% (%)	0.534	0.541	0.539	0.544	0.545	0.552	0.550	0.555	0.537	0.544	0.543	0.549
Choline chloride, 60% (%)	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Inert <sup>c</sup> (%)	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035
Total (%)	100	100	100	100	100	100	100	100	100	100	100	100
AME (kcal kg <sup>-1</sup> )	3150	3100	3150	3100	3200	3150	3200	3150	3250	3200	3250	3200
Crude protein (%)	18.61	18.61	18.61	18.61	16.79	16.79	16.79	16.79	15.79	15.79	15.79	15.79
Calcium (%)	0.758	0.758	0.758	0.758	0.634	0.634	0.634	0.634	0.581	0.581	0.581	0.581
Av. phosphorus (%)	0.374	0.374	0.374	0.374	0.296	0.296	0.296	0.296	0.271	0.271	0.271	0.271
Sodium (%)	0.208	0.208	0.208	0.208	0.197	0.197	0.197	0.197	0.192	0.192	0.192	0.192
Dig. Lys (%)	1.124	1.124	1.124	1.124	1.014	1.014	1.014	1.014	0.954	0.954	0.954	0.954
Dig. Met + Cys (%)	0.832	0.832	0.832	0.832	0.750	0.750	0.750	0.750	0.706	0.706	0.706	0.706
Dig. Thr (%)	0.742	0.742	0.742	0.742	0.669	0.669	0.669	0.669	0.630	0.630	0.630	0.630

**Note:** AME, Apparent Metabolizable Energy; ASO, acid soybean oil; Dig. Lys, digestible lysine; Dig. Met, digestible methionine; Dig. Met + Cys, digestible methionine and cysteine; DSO, degummed soybean oil.

<sup>a</sup>Mineral supplement, per kg of diet: 50 mg iron; 10 mg copper; 65 mg manganese; 65 mg zinc; 1 mg iodine.

<sup>b</sup>Vitamin supplement, per kg of diet: 11 000 IU vitamin A; 4000 IU vitamin D<sub>3</sub>; 55 IU vitamin E; 3 mg vitamin K<sub>3</sub>; 2.3 mg vitamin B<sub>1</sub>; 7 mg vitamin B<sub>2</sub>; 12 mg pantothenic acid; 4 mg vitamin B<sub>6</sub>; 2.5 µg vitamin B<sub>12</sub>; 60 mg nicotinic acid; 2 mg folic acid; 250 µg biotin; and 300 µg selenium.

<sup>c</sup>The inclusion of the emulsifier was performed in substitution to the inert Caulim<sup>®</sup>.

### 2.8 Statistical analysis

The Shapiro–Wilk test confirmed the normal distribution and homogeneity of the variance. When the data did not present a normal distribution, they were submitted to the Kruskal–Wallis test to assess isolated factors (lipid source and use of emulsifiers), followed by the Friedman test to evaluate the interaction between these factors. When distributed within the normal range, the results obtained were subjected to analysis of variance and, when significant, the factors were compared by *F* test at 5% probability. All the statistical evaluations were performed using the SAS software (2014), student version.

### 3. Results

There was an interaction ( $p = 0.001$ ) between the lipid sources and the use of emulsifier for triglyceride at 49 days (Table 3). According to the deployment of the interaction, evaluating within each lipid source, birds fed diets containing ASO and an emulsifier had a lower triglyceride value compared with those that received the same source but without the additive, with the opposite effect when DSO was used as a lipid source (Table 4). On evaluating the emulsifier factor, it was observed that the additive inclusion reduced the serum triglyceride content in broilers fed diets containing ASO and in the absence of the additive, birds fed with DSO showed a lower value (Table 5). In addition, it was observed that the serum HDL content at 14 days of age was influenced ( $p = 0.043$ ) by the lipid source used, being lower in birds fed diets containing DSO. The inclusion of the emulsifier provided a higher ( $p = 0.041$ ) value for LDL at 14 days of age. There was no difference in the other blood parameters evaluated (Table 3).

There was no interaction ( $p > 0.05$ ) between lipid source and emulsifier for SOD and TBARS activity in the blood of birds at 21 days of age. However, considering the isolated factors, interference from the lipid source was observed on the serum SOD values ( $p = 0.028$ ), and the birds fed diets containing DSO showed a lower value for this variable (Table 5).

There were no interaction ( $p > 0.05$ ) between lipid sources and the use of emulsifier on the carcass, cuts yields, and percentage of abdominal fat in broilers at 49 days of age. Considering the isolated factors, the inclusion of the emulsifier, regardless of the lipid source used, reduced the tenderloin yield ( $p = 0.001$ ) (5.55% vs. 6.03%) and the percentage of abdominal fat ( $p = 0.002$ ) (2.12% vs. 2.48%) (data not shown).

Regarding meat quality characteristics, an interaction ( $p = 0.047$ ) was observed between the lipid sources used and the inclusion of an emulsifier over the SF variable (Table 6). According to the interaction, the meat of broilers fed diets containing ASO and an emulsifier showed less SF (2.81 vs. 3.99 kgf cm<sup>-2</sup>) (data not shown) compared with those fed diets containing DSO. In addition, birds that received diets containing DSO as a lipid source showed higher ( $p = 0.043$ ) WHC and lower ( $p = 0.031$ ) breast pH measured 24 h post mortem (Table 6).

There was no interaction ( $p > 0.05$ ) between lipid source and emulsifier for meat color. However, on evaluating the

**Table 3.** Serum lipids from broilers, at 14, 21, and 49 days of age, fed diets containing different lipid sources, with or without emulsifier.

	14 days			21 days			49 days					
	CHOL	HDL	LDL	TRIG	CHOL	HDL	LDL	TRIG	CHOL	HDL	LDL	TRIG
With emulsifier	130.73	97.62	17.56a	55.00	130.12	89.23	12.23	32.88	139.22	96.74	20.28	37.56
Without emulsifier	131.84	100.33	15.28b	55.00	125.39	87.01	11.93	30.25	139.55	94.69	23.13	36.67
ASO	134.71	104.29a	16.57	56.25	128.21	88.69	11.51	32.13	139.01	98.64	21.13	36.13
DSO	127.86	93.66b	16.34	53.67	127.42	87.58	12.65	31.00	139.78	92.66	22.22	38.20
SEM	16.90	14.17	2.93	7.60	14.16	10.98	3.40	4.15	16.52	10.55	5.43	6.90
CV (%)	12.87	14.32	17.78	13.82	11.08	12.46	28.14	13.15	11.86	11.02	25.09	18.57
<i>p</i> -value, O × E	0.542	0.273	0.407	0.217	0.414	0.596	0.259	0.450	0.779	0.511	0.574	0.001
<i>p</i> -value, oil (O)	0.261	0.043	0.792	0.331	0.831	0.753	0.351	0.450	0.903	0.118	0.562	0.496
<i>p</i> -value, emulsifier (E)	0.853	0.593	0.041	0.941	0.344	0.560	0.806	0.085	0.960	0.543	0.157	0.644

Note: CHOL, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TRIG, triglycerides; ASO, acid soybean oil; DSO, degummed soybean oil; SEM, standard error of the mean; and CV, coefficient of variation. In the same column, means followed by different letters indicate difference by the *F* test.

**Table 4.** Interaction between lipid sources and the emulsifier inclusion on broiler triglyceride blood at 49 days of age.

Lipid source	Triglyceride (mg dL <sup>-1</sup> )		p-value
	With emulsifier	Without emulsifier	
ASO	31.88bB	40.38aA	0.0103
DSO	43.25aA	32.43bB	0.0209
p-value	0.0069	0.0359	

**Note:** ASO, acid soybean oil; DSO, degummed soybean oil. In the same row, means followed by different capital letters indicate difference by the *F* test. In the same column, means followed by different lowercase letters indicate difference by Tukey's test.

**Table 5.** Serum antioxidant parameters of broilers at 21 days of age fed diets containing different lipid sources, with or without emulsifier.

	SOD (U mL <sup>-1</sup> )	TBARS ( $\mu$ mol L <sup>-1</sup> of MDA)
With emulsifier	1.355	1.422
Without emulsifier	1.159	1.477
ASO	1.503 <sup>a</sup>	1.400
DSO	0.990 <sup>b</sup>	1.508
SEM	0.512	0.206
CV (%)	41.117	14.211
p-value, O $\times$ E	0.376	0.338
p-value, oil (O)	0.028	0.268
p-value, emulsifier (E)	0.433	0.681

**Note:** SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; MDA, malonyldialdehyde; ASO, acid soybean oil; DSO, degummed soybean oil; SEM, standard error of the mean; and CV, coefficient of variation. *p*-values for lipid sources and for the use of the emulsifier in the SOD variable were obtained by the Kruskal–Wallis test and comparison between sources and emulsifier by the Friedman test. *p*-values for the use of emulsifier in the TBARS variable were obtained by the *F* test. <sup>a,b</sup> Different letters in the same column indicate significant differences (*p* < 0.05).

isolated factors, the birds fed with diets containing DSO showed a higher (*p* = 0.033) intensity of yellow (*b*<sup>\*</sup>) 15 min post mortem compared with those fed diets containing ASO (Table 7).

## 4. Discussion

The dietary inclusion of lipid sources and the emulsifier itself can affect the metabolism of lipid components, influencing the serum composition of these constituents and the activity of antioxidant enzymes (Hu et al. 2019; Huo et al. 2019).

The effects of these factors on serum metabolites were observed at certain ages, without recurrence over the experimental period. In this study, there was an influence of the emulsifier on LDL at 14 days of age and an influence on the triglyceride level at 49 days of age depending on the lipid source used. Triglycerides are derived from dietary sources, especially lipid-rich ingredients, which are transported into the bloodstream after digestion and absorption have finished. The addition of the emulsifier to diets containing ASO was able to reduce triglyceride levels due to the faster absorption rate of dietary fat, indicating an improvement in

lipid metabolism and transport. However, this hypolipidemic effect of the emulsifier observed at 49 days of age in broilers fed the diet containing ASO conflicted with the results at 14 days, given the increase in serum LDL concentration. Wang et al. (2016) observed an increase in the serum concentration of triglycerides and LDL in broilers fed diets containing a sodium stearoyl lactylate-based emulsifier. In contrast, in a study evaluating the inclusion of an emulsifier in low-energy diets for broilers, Saleh et al. (2020) reported an increase in HDL concentrations and a reduction in LDL and triglycerides. Bontempo et al. (2018) observed an increase in total cholesterol and HDL levels with the inclusion of emulsifiers in broiler diets. Roy et al. (2010) reported a reduction in total cholesterol and LDL in broilers receiving diets containing a glyceryl polyethylene glycol ricinoleate-based emulsifier at 20 days of age; however, the HDL fraction and triglyceride concentration were not affected by the inclusion of the emulsifier. The conflicting results reported in the literature accentuate the need for studies on the influence of this additive on serum metabolism.

Moreover, the use of ASO as a lipid source increased the serum HDL concentration in broilers at 14 days of age. HDL contributes to the reverse transport of cholesterol and lipids in the bloodstream and associates with these particles to form a water-soluble complex, which facilitates the transport of lipids to the organs via the bloodstream (Sekhar et al. 2020). Generally, high HDL levels indicate increased transport of cholesterol to the liver, which would reduce the total cholesterol content. However, this effect was not observed.

SOD activity increased by 65.87% with the inclusion of ASO in diets. SOD represents the first endogenous defense against oxidative stress, catalyzing the dismutation of the superoxide radical into hydrogen peroxide and molecular oxygen (Akbarian et al. 2014). Although no change was observed in the malondialdehyde level, which is a product of peroxidized polyunsaturated fatty acids and an indicator of lipid peroxidation (Ogbuagu et al. 2018), the greater enzymatic activity of SOD leads to a better antioxidant status.

The fatty acids present in lipoproteins and cell membranes can react with free radicals derived from animal metabolism or diet. This initiates a reaction known as lipid peroxidation or lipoperoxidation, in which the hydroxyl radical is essential for the beginning of this process as it can quickly remove hydrogen from fatty acids (Zhang et al. 1996). A better antioxidant status helps to maintain the

**Table 6.** Meat quality of broiler chickens at 49 days of age fed diets containing different lipid sources, with or without emulsifier.

	WHC (%)	CL (%)	SF (kgf cm <sup>-2</sup> )	Leg pH		Breast pH	
				15 min	24 h	15 min	24 h
With emulsifier	61.95	32.59	3.40	6.12	5.98	5.98	5.85
Without emulsifier	62.52	31.11	3.23	6.11	6.00	5.99	5.82
ASO	60.86b	32.68	3.07	6.14	6.02	6.02	5.87a
DSO	63.61a	31.03	3.57	6.08	5.97	5.95	5.80b
SEM	3.68	2.98	0.92	0.16	0.10	0.13	0.09
CV (%)	5.91	9.37	27.67	2.86	1.71	2.17	1.55
<i>p</i> -value, O × E	0.818	0.449	0.047	0.359	0.712	0.559	0.282
<i>p</i> -value, oil (O)	0.043	0.129	0.135	0.339	0.201	0.141	0.031
<i>p</i> -value, emulsifier (E)	0.818	0.171	0.614	0.844	0.594	0.919	0.502

Note: WHC, water-holding capacity; CL, cooking weight loss; SF, shear force; ASO, acid soybean oil; DSO, degummed soybean oil; SEM, standard error of the mean; and CV, coefficient of variation. In the same column, means followed by different lowercase letters indicate difference by the *F* test.

balance between free radical production and antioxidant capacity, supporting metabolic homeostasis and keeping proteins, lipids, carbohydrates, and nucleotides intact (Santos et al. 2014).

Lipids, primarily unsaturated fatty acids, are the main targets of the hydroxyl radical, with a direct relationship between the degree of unsaturation and susceptibility to oxidation. When ingested, unsaturated fatty acids can favor the oxidation of cell membranes, proteins, and DNA (Vieira et al. 2017). In this context, the fact that ASO has a higher percentage of monounsaturated fatty acids compared with DSO (Cortes-Cuevas et al. 2017) may have been responsible for the higher enzyme activity observed in broilers fed diets containing this ingredient. Sanz et al. (1999) reported that lipid sources with a higher proportion of saturated and monounsaturated fatty acids had a negative correlation with lipid oxidation.

The inclusion of the emulsifier in diets is based on the capacity of this additive to assist lipid digestion and absorption by promoting the incorporation of fatty acids into micelles. It is an essential step in the transport of lipid-digestion products through the epithelium of the gastrointestinal tract (Ravindran et al. 2016). These mechanisms of action can provide more energy to broilers, especially in the starter phase, due to the low production of lipase and bile salts during this period (Upadhaya et al. 2018), as well as to broilers fed diets containing lipid sources such as ASO due to its high concentration of FFA, which reduces the formation of monoglycerides and makes the emulsification process more difficult (Rovers 2014). Despite this evidence, our results indicate that the action of the emulsifier was not able to provide additional metabolizable energy, as reflected by the reduced chicken tenderloin yield. These results are endorsed by performance data, in which the inclusion of the emulsifier in energy-reduced diets worsened the feed conversion of broilers from 1 to 49 days of age (Tenório et al. 2021).

The effects of using emulsifiers on carcass yield, cut yields, and percentage of abdominal fat reported in the literature remain contradictory. Guerreiro Neto et al. (2011) did not

report variations in the carcass and cut yields in broilers fed diets containing emulsifiers and different lipid sources. In contrast, in a study evaluating the inclusion of emulsifiers in energy-restricted diets, Wang et al. (2016) observed an increase in the percentage of abdominal fat in broilers at 35 days compared with animals receiving diets without the inclusion of emulsifiers. The use of 1,3-diacylglycerol did not affect the deposition of abdominal fat at 35 days of age in broilers fed diets with different energy densities (Upadhaya et al. 2018). The different results may be related to the type and mode of action of emulsifiers, as well as to the different evaluation periods.

The meat of broilers fed diets containing DSO had a higher WHC and lower pH 24 h post mortem compared with the meat of animals fed ASO. The post mortem reduction in the pH is the main factor affecting the transformation of muscle into meat and has a decisive impact on the appearance and quality of meat. Moreover, the pH is directly associated with other quality parameters, such as WHC, CL, tenderness, juiciness, color, and shelf life. It is known that the lower is the pH of the meat, the lower is the WHC (Mir et al. 2017). In this study, the opposite effect was observed in meat samples from broilers fed diets containing DSO. However, considering that a typical broiler breast meat has a final pH of around 5.80–5.92 (Mudalal et al. 2015; Tasoniero et al. 2016), the results obtained in this study are within the normal range regardless of the lipid source.

Meat texture is directly correlated with the amount of water bound within muscle fibers. Thus, the lowest WHC in the meat of broilers fed diets containing ASO may be linked to the lowest SF in the meat of broilers fed diets containing the emulsifier, which indicates more tenderness. This influence is explained by the fatty acid profile of ASO since monounsaturated fatty acids lead to greater meat tenderness (Smith et al. 2006), which may have been boosted by the action of the emulsifier. The lipid sources present in the diet influence the meat lipid profile (Carmona et al. 2019) and may modify the physical properties, such as texture and cooking losses, thus changing the quality of the product (Ruiz et al. 2001; Sirri et al. 2003).



**Table 7.** Colorimetry of meat from broiler chickens at 49 days of age fed diets containing different lipid sources, with or without emulsifier.

	Leg						Breast					
	15 min post mortem			24 h post mortem			15 min post mortem			24 h post mortem		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
With emulsifier	57.89	3.34	4.17	54.13	3.19	3.60	48.45	2.24	3.07	47.92	3.12	5.03
Without emulsifier	58.37	3.21	4.40	54.05	3.11	4.55	48.63	2.28	4.08	47.52	2.66	5.28
ASO	58.22	3.03	3.99	54.59	3.28	3.58	48.60	2.35	3.00b	47.72	3.03	4.97
DSO	58.05	3.52	4.55	53.58	3.01	4.57	48.48	2.16	4.15a	47.72	2.75	5.35
SEM	1.65	1.29	1.48	2.24	1.06	1.94	2.09	1.16	1.47	1.39	1.21	1.14
CV (%)	2.84	39.28	34.64	4.14	33.76	47.61	4.31	51.28	41.35	2.92	41.79	22.05
p-value, O × E	0.325	0.869	0.946	0.552	0.308	0.909	0.109	0.901	0.608	0.666	0.443	0.199
p-value, oil (O)	0.828	0.285	0.305	0.212	0.481	0.159	0.869	0.649	0.033	0.970	0.510	0.331
p-value, emulsifier (E)	0.459	0.776	0.663	0.920	0.884	0.176	0.806	0.911	0.056	0.446	0.296	0.484

**Note:** L\*, brightness; a\*, red/green intensity; b\*, yellow/blue intensity; ASO, acid soybean oil; DSO, degummed soybean oil; SEM, standard error of the mean; and CV, coefficient of variation. In the same column, means followed by different lowercase letters indicate statistical difference by the F test.

In addition to the effects on the WHC and pH, DSO-based diets had a significant effect on yellowness, which may be correlated with a lower pH and higher ingestion of pigments (Pérez-Vendrell et al. 2001). The color on the surface of the meat is a consequence of the selective absorption of light by myoglobin, which can be altered by other important components, such as muscle fibers and their proteins (Olivo et al. 2001). Low meat pH reduces the absorption of green light by myoglobin, resulting in a less reddish and more yellowish appearance (Castellini et al. 2002), which partially explains our results. ASO can be considered a source of pigments, and contains approximately 910 mg kg<sup>-1</sup> of xanthophylls (Pardio et al. 2001). However, the industrial processing of ASO can destroy these components (Pekel et al. 2012). Although the emulsifier may change the meat color due to the increase in the digestibility and absorption of fat-soluble pigments in the pectoral muscle (Bontempo et al. 2018), no change in meat color was observed in this study.

Given that ASO did not negatively affect the meat quality and blood parameters but improved the serum antioxidant status of broilers, this residue shows potential for use in the poultry industry. According to Pardío et al. (2005), ASO can represent up to 31% of the crude oil mass and represents one-third of the total cost of oil, emphasizing the importance of a correct environmental and economic destination for this residue.

## 5. Conclusions

The addition of the emulsifier reduced the percentage of abdominal fat by 10.20% and chicken tenderloin yield by 5.22%, and also affected the serum LDL and triglyceride levels. Broilers fed diets containing ASO had better serum antioxidant status, with no deleterious effects on meat quality, carcass yield, and cut yields.

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### Competing interests

None of the authors of this work have financial relationships or other relationships with people or organizations that could inappropriately influence the content of this article.

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