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Emulsifier in broiler diets containing deactived full-fat soybean

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ABSTRACT - This study aimed to evaluate effects of an emulsifier based on glyceryl polyethyleneglycol ricinoleate in diets containing deactivated full-fat soybean (DFFS) on performance, relative weight of pancreas, pancreatic lipase activity, intestinal morphometry, serum lipid concentration, nutrient digestibility, carcass yield, and meat quality of broiler chickens. Three hundred fifty-two male broilers (one-day-old) were randomly assigned to two dietary treatments including a diet containing DFFS without the emulsifier and a diet containing DFFS with emulsifier (350 g ton^{-1}). Each treatment had eight replicates with 22 birds each. The metabolizable energy level was reduced by 40 kcal kg^{-1} from 1 to 21 d and 50 kcal kg^{-1} from 22 to 49 d in diets containing the emulsifier. Birds fed diets without emulsifier showed higher weight gain (P = 0.014) and feed intake (P = 0.048) from 1 to 7 d and greater feed conversion ratio (P = 0.044) from 1 to 35 d. On days 14 and 21, the inclusion of emulsifier in the diet did not affect (P>0.05) the intestinal morphometry, relative weight of pancreas, and pancreatic lipase activity. Serum lipid levels measured at 14, 21, and 29 d were also not affected by the emulsifier (P>0.05). At 49 d, broilers fed diets without emulsifier had decreased energy utilization (P<0.0001) and ileal digestibility of dry matter (P = 0.0001), gross energy (P<0.0001), and crude protein (P = 0.038). A higher breast yield (P = 0.039) was observed for birds fed diets without emulsifier. Meat quality parameters were not altered (P>0.05). The inclusion of the emulsifier in energy-restricted diets containing DFFS does not improve performance of broiler chickens, reduces the ileal digestibility of nutrients, without changes in morphophysiological parameters or meat quality of broilers.

Keywords: digestibility, glyceryl polyethyleneglycol ricinoleate, meat quality, pancreatic lipase, serum lipids

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1. Introduction

Full-fat soybean is an ingredient with a high lipid content (186–212 g kg^{-1}) as its lipid portion is not extracted from the grain, and it is also a source of protein (373–394 g kg^{-1}) (Rostagno et al., 2024). Its use *in natura* in poultry diets is not recommended due to antinutritional factors such as protease inhibitors, lectins, and allergenic proteins (Rocha et al., 2014). As these compounds are thermolabile, the grains can be steamed under pressure and vacuum, obtaining the deactivated full-fat soybean (DFFS).

However, in the extrusion process, the cell wall breaks, leaving lipids readily available for digestion, which is not observed in steamed process, and a lower apparent metabolizable energy is observed for DFFS than for extruded full-fat soybeans (Freitas et al., 2005). Considering the lower availability of lipids and the physiological limitations regarding lipid digestion by chickens in the early stage of life (Noy and Sklan, 1995), the use of emulsifiers is proposed to better use of the lipid portion of DFFS. Tenório et al. (2022) found that the inclusion of emulsifier in energy-restricted diets with acid soybean oil, which is considered a lower-quality lipid source than degummed soybean oil, increased the digestible energy of the diet by 6.2%.

Emulsifiers can increase the digestion and absorption of lipids, reducing the surface tension of the lipid droplets, allowing physical agitation of the gastrointestinal tract to break the droplets into smaller particles and promoting the formation of micelles. The emulsifiers can positively impact performance, digestibility, and even serum biochemical parameters of broiler chickens (Saleh et al., 2020). In this context, the use of different additives in animal nutrition, such as exogenous enzymes and emulsifiers, has been widely studied to improve feed efficiency and intestinal health of animals (Fortes et al., 2024).

Therefore, we hypothesize that the inclusion of emulsifier can favor the digestion of lipids for broilers fed diets containing DFFS, allowing an energy reduction of the diets without impaired performance, morphophysiological characteristics, and meat quality. To test this hypothesis, we evaluated the performance, serum lipids, relative weight of pancreas, pancreatic lipase activity, intestinal morphometry, nutrient digestibility, carcass yield, and meat quality of broiler chickens fed diets containing DFFS with or without the inclusion of glyceryl polyethyleneglycol ricinoleate-based emulsifier.

2. Material and methods

The experiment was conducted in Marechal Cândido Rondon, Paraná State, Brazil (24°55'13" S, 54°02'30" W, and altitude of 420 m asl). Research on animals was conducted according to the institutional committee on animal use (case number 55/19).

2.1. Housing, birds, and treatments

The experimental poultry house was composed of 1.76-m^2 boxes provided with clean wood shaving litter, a tubular feeder, and a nipple drinker. Lighting was continuous for the first three days, followed by a lighting program of 18 h light and 6 h dark until the end of the experiment. The rearing temperature was maintained at 33±1.0 °C for the first week of the experiment and then reduced by 2 °C per week until reaching 23 °C. If necessary, the environment was cooled using exhaust fans and evaporative cooling pads.

Three hundred fifty-two one-day-old Ross 308 AP male broiler chicks were distributed in a completely randomized design with two treatments, eight replicates, and 22 birds per experimental unit. The experimental diets contained DFFS with or without the inclusion of emulsifier. The DFFS (containing 360 g kg^{-1} of crude protein, 200 g kg^{-1} of lipids, and 55 g kg^{-1} of mineral matter) provided approximately 30% of the lipid fraction of the diets during the initial phase and 40% on the remain phases. The solubility of DFFS protein in potassium hydroxide solution was 80%, and the urease activity ranged from 0.03 to 0.20 units pH rise (values provided by the supplier). The glyceryl polyethyleneglycol ricinoleate-based emulsifier was added to diets at 350 g ton^{-1}, and the metabolizable energy level was reduced by 40 kcal kg^{-1} from 1 to 21 d and 50 kcal kg^{-1} from 22 to 49 d in diets containing the emulsifying agent. The inclusion level of the emulsifier and the energetic matrix of the additive was according to the manufacturer's instructions. The experimental diets were provided in mash form, and the formulation of the diets was based on the chemical composition of feed ingredients and the nutritional requirements as proposed by Rostagno et al. (2017) for intermediate-performance male broilers from 1 to 7, 8 to 21, 22 to 33, 34 to 42, and 43 to 49 d (Table 1).

Table 1 - Percentage and calculated composition of experimental diets containing deactivated full-fat soybean (DFFS) as a lipid source, with or without inclusion of emulsifier for broilers

Ingredient (%)	1–7 d		8–21 d		22–33 d		34–42 d		43–49 d	
	Emulsifier									
	With	Without	With	Without	With	Without	With	Without	With	Without
Corn	57.32	56.29	58.79	57.76	65.56	64.33	71.25	70.01	71.60	70.37
Soybean meal (46%)	28.60	28.80	22.60	22.80	16.10	16.30	11.30	11.50	9.10	9.30
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
DFFS	7.00	7.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Poultry fat	0.64	1.44	0.51	1.31	0.62	1.62	0.23	1.24	1.28	2.28
Dicalcium phosphate	1.087	1.088	0.861	0.862	0.670	0.671	0.284	0.286	0.173	0.175
Limestone	0.615	0.613	0.539	0.537	0.402	0.400	0.365	0.363	0.317	0.315
NaCl	0.480	0.480	0.464	0.464	0.440	0.440	0.413	0.414	0.402	0.402
Mineral supplement[1]	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Vitamin supplement[2]	0.130	0.130	0.130	0.130	0.100	0.100	0.100	0.100	0.100	0.100
DL-Methionine (99%)	0.396	0.397	0.377	0.378	0.333	0.334	0.289	0.290	0.269	0.270
L-Threonine (98%)	0.126	0.126	0.120	0.120	0.117	0.117	0.103	0.104	0.098	0.098
L-Lysine HCl (50.7%)	0.498	0.493	0.499	0.495	0.546	0.541	0.557	0.551	0.550	0.544
Choline chloride (60%)	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Inert[3]	-	0.035	-	0.035	-	0.035	-	0.035	-	0.035
Celite®	-	-	-	-	-	-	-	-	1.000	1.000
Calculated composition										
ME (kcal kg^{-1})	2935	2975	3010	3050	3100	3150	3150	3200	3200	3250
Digestible protein (%)	21.94	21.94	21.09	21.09	18.61	18.61	16.79	16.79	15.79	15.79
Calcium (%)	0.971	0.971	0.878	0.878	0.758	0.758	0.634	0.634	0.581	0.581
Avaiable P (%)	0.463	0.463	0.419	0.419	0.374	0.374	0.296	0.296	0.271	0.271
Sodium (%)	0.225	0.225	0.218	0.218	0.208	0.208	0.197	0.197	0.192	0.192
Digestible Lys (%)	1.307	1.307	1.256	1.256	1.124	1.124	1.014	1.014	0.954	0.954
Digestible Met+cys (%)	0.967	0.967	0.929	0.929	0.832	0.832	0.750	0.750	0.706	0.706
Digestible Thr (%)	0.863	0.863	0.829	0.829	0.742	0.742	0.669	0.669	0.630	0.630

[1] Mineral supplement, per kg of diet: 50 mg iron; 10 mg copper; 65 mg manganese; 65 mg zinc; 1 mg iodine.
[2] Vitamin supplement from 1 to 21 d, per kg of diet: 14,300 IU vitamin A; 5,200 IU vitamin D3; 71.5 IU vitamin E; 3.9 mg vitamin K3; 2.99 mg vitamin B1; 9.10 mg vitamin B2; 15.6 mg pantothenic acid; 5.2 mg vitamin B6; 3.25 mcg vitamin B12; 78 mg nicotinic acid; 2.6 mg folic acid; 325 mcg biotin; 390 mcg selenium. Vitamin supplement from 22 to 49 d, per kg of diet: 11,000 IU vitamin A; 4,000 IU vitamin D3; 55 IU vitamin E; 3 mg vitamin K3; 2.3 mg vitamin B1; 7 mg vitamin B2; 12 mg pantothenic acid; 4 mg vitamin B6; 2.5 mcg vitamin B12; 60 mg nicotinic acid; 2 mg folic acid; 250 mcg biotin; 300 mcg selenium.
[3] The inclusion of the emulsifier was performed in substitution to the inert Caulim® following the nutritional matrix: 40 kcal kg^{-1} from 1 to 21 d and 50 kcal kg^{-1} from 22 to 49 d.

2.2. Performance

Body weight and feed intake were recorded at 7, 21, 35, and 49 d to determine weight gain, feed intake, and feed conversion ratio. Mortality was recorded daily to adjust weight gain according to Sakomura and Rostagno (2016).

2.3. Intestinal morphometry

At days 14 and 21, one bird per EU, with a representative weight (± 5%), was selected and slaughtered by cervical dislocation. Fragments of approximately 5 cm were taken from the duodenum (ascending portion of the duodenal loop) and jejunum (anterior portion of the Meckel's diverticulum), cleaned with saline solution, and placed in a formalin solution (10%) buffered with phosphate (pH 7.0). Subsequently, the samples were dehydrated in a graded alcohol series, cleared in xylol, and embedded in paraffin. Histological sections (5 µm) of the tissue samples were prepared using a microtome, and four discontinuous sections from each sample were placed on a slide. These sections were deparaffinized in xylol, hydrated in ethanol, and stained with hematoxylin and eosin.

The slides were digitized (Epson 3170; EPSON Inc., Long Beach, CA) to determine the height and width of the villi, as well as the width and depth of the crypts, using the PROPLUS IMAGE 4.1 imaging system. These morphometric measurements were used to calculate the absorptive area of the intestinal mucosa, as described by Tenório et al. (2022). The villus height:crypt depth ratio was also estimated.

2.4. Relative weight of pancreas and lipase activity

At 14 and 21 d, the pancreas of the slaughtered birds was collected and weighed on an analytical scale to determine the relative weight of the organ in relation to the weight of the live bird. Subsequently, the pancreas was frozen at −20 °C for further analysis of lipase activity. The pancreas was homogenized (IKA ultra-Turrax homogenizer) (1:20 g mL^{-1}) in a buffer solution 50 mM Tris-HCl (pH 8.0) containing 50 mM $CaCl_2$ (Pinheiro et al., 2004). We used 2,3-dimercaptopropanol tributyrate (BALB) as substrate and dithiobis-2-nitrobenzoic acid (DTNB; BALB-DNTP method, Gold Analisa, Belo Horizonte, Minas Gerais, Brazil) as chromophore. Enzimatic activity was expressed as international units (IU) per mg of protein (Bradford et al., 1976).

2.5. Blood analysis

At days 14, 21, and 49, one bird per EU, with a representative weight (± 5%), was selected, and after 6 h of fasting, 4 mL of blood were collected by puncture of the ulnar vein. After clotting, samples were centrifuged (Centrífuga Kasvi K14-4000) at 1050 × *g* for 10 min, and serum was stored at −20 °C until analyses. The following biochemical traits were determined: triglyceride, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) utilizing commercial kits (Elitech Clinical Systems, ELITechGroup, Paris, FR). The analyses were performed on an automatic spectrophotometer, with automatic calibration and high-performance reading (Elitech EL 200).

2.6. Ileal digestibility

A source of silica (Celite®) was added to the experimental diets from 42 to 49 d (Table 1) at the proportion of 1% as an indigestible indicator. At day 49, two birds with average weight (± 5%) were selected by EU and slaughtered. The ileal content was collected (starting right after Meckel's diverticulum and ending 4 cm from the ileocecal junction), homogenized, and dried in a forced-ventilation oven at 55 °C for 72 h. The samples of each experimental diet and ileal content were grounded and analyzed for dry matter (method number 934.01; AOAC, 2012), crude protein (method number 954.01; AOAC, 2012), and acid-insoluble ash (Van Keulen and Young, 1977). Gross energy was determined in a bomb calorimeter (model C200, IKA Works Inc., Wilmington, NC). The digestibility coefficients of the nutrients and the digestible energy values were calculated according to Ahmadi-Sefat et al. (2022).

2.7. Processing yield

The same birds used to assess ileal digestibility were also used to determine the carcass characteristics. The birds were individually weighed, euthanized, scalded, and feathers, head, viscera, and feet were removed. The weight of the warm eviscerated carcass was considered in relation to the live weight to calculate carcass yield. Yields of the parts (breast, legs, and wings) were calculated in relation to the weight of the eviscerated carcass after cooling in a tank for 30 min at 2 °C. The abdominal fat present around the cloaca, gizzard, proventriculus, and adjacent abdominal muscles was removed, weighed, and calculated in relation to the live weight.

2.8. Meat quality

Measurements of pH and color were evaluated 15 min and 24 h postmortem in the right portion of breast meat (*Pectoralis major*) and in the right leg of the two chickens slaughtered at 49 d. The meat pH was determined using a pH meter (HI 99163 Hanna Instruments) with a meat-penetrating probe. A portable colorimeter (CR-400, Konica Minolta Sensing, São Paulo, Brazil) was used to determine the

meat color, being measured on the inside of both muscles. The components L* (lightness), a* (redness), and b* (yellowness) were expressed in the Cielab color scale (Honikel, 1998).

The left portion of the breast meat was used to measure water-holding capacity (WHC), cooking loss (CL), and shear force (SF). The centrifugation method was used to estimate WHC, according to Nakamura and Katok (1981). To determine the CL, the method proposed by Honikel (1998) was used, and the percentage was calculated considering the difference in weight of the sample before and after cooking the meat on an electrically heated plate (until it reached an internal temperature of 80 °C). The samples that were used for determining CL were then used to determine SF. Three parallelepiped (1 × 1 × 4 cm) strips were cut from each cooked breast, parallel to the direction of the muscle fibers, and the SF was determined in kilogram-force (kgf cm^{-2}), using a Brookfield CT3 Texture Analyzer, coupled with a TA 3/100 probe (TA - SBA fixture, calibrated with 0.01 kgf cm^{-2}, 20 mm strain, and 2.5 $mm.s^{-1}$ test speed).

2.9. Statistical procedures

For statistical analysis of the performance, each box was considered as the experimental unit, using the average data of animals per box. For statistical analysis of intestinal morphometry, relative weight of pancreas, lipase activity, and blood analysis, each bird was considered as the experimental unit. For statistical analysis of ileal digestibility, processing yield, and meat quality, the average data obtained from the two birds slaughtered per pen was considered as the experimental unit.

Data was tested for normality (Shapiro–Wilk) using the UNIVARIATE procedure and analyzed using the General Linear Model Procedure of SAS® (Statistical Analysis System, University Edition). Comparison of means was done using the F-test at a significance level of 5%.

3. Results

During 1–7 d, the birds fed diet containing DFFS without the emulsifier showed higher weight gain (P = 0.014) and feed intake (P = 0.048). During 1–35 d, the same birds showed a greater feed conversion ratio (P = 0.044). Regarding the other periods evaluated, there was no effect (P>0.05) on broiler performance (Table 2).

Table 2 - Growth performance of broilers fed diets containing deactivated full-fat soybean as a lipid source, with or without emulsifier

	Emulsifier		SEM	CV (%)	P-value
	Without	With			
Day 1 to 7					
Weight gain (g)	155.6a	151.1b	3.27	2.13	0.014
Feed intake (g)	175.2a	168.6b	6.06	3.53	0.048
Feed conversion ratio	1.126	1.116	0.03	2.73	0.551
Day 1 to 21					
Weight gain (g)	1017.2	1016.0	28.61	2.81	0.932
Feed intake (g)	1302.3	1293.7	31.55	2.43	0.595
Feed conversion ratio	1.280	1.274	0.03	2.08	0.629
Day 1 to 35					
Weight gain (g)	2474.2	2442.6	88.39	3.40	0.487
Feed intake (g)	3660.0	3659.3	106.09	2.90	0.990
Feed conversion ratio	1.480b	1.499a	0.02	1.15	0.044
Day 1 to 49					
Weight gain (g)	3841.9	3782.1	183.34	4.81	0.525
Feed intake (g)	6599.7	6565.8	269.59	4.10	0.805
Feed conversion ratio	1.719	1.736	0.02	1.12	0.086

SEM - standard error of the mean; CV - coefficient of variation.
a,b - Means followed by a different letter in the row are different at an alpha level of 0.05 according to F-test.

At 14 and 21 d, the morphometric parameters of the duodenum and jejunum (Table 3), the relative weight of the pancreas, and the pancreatic lipase activity (Table 4) were not influenced (P>0.05) by the inclusion of emulsifier in diets containing DFFS.

At 14, 21, and 49 d, there was no interference (P>0.05) of diets containing DFFS as a lipid source with emulsifier (data not shown) on serum lipid levels.

Table 3 - Morphometry of duodenum and jejunum segments of broilers fed diets containing deactivated full-fat soybean as a lipid source, with or without emulsifier

	Emulsifier		SEM	CV (%)	P-value
	Without	With			
14 d					
Duodenum					
Villus height (µm)	829.71	888.30	118.62	13.85	0.436
Crypt depth (µm)	48.47	50.71	14.15	28.60	0.799
Villus:crypt ratio	17.61	18.56	3.76	20.81	0.684
Absorptive area (µm^2)	21.15	22.84	2.88	13.15	0.361
Jejunum					
Villus height (µm)	390.78	355.52	70.31	18.84	0.367
Crypt depth (µm)	49.08	42.85	8.88	19.31	0.214
Villus:crypt ratio	7.95	8.67	1.84	22.17	0.476
Absorptive area (µm^2)	8.97	8.47	1.54	17.66	0.549
21 d					
Duodenum					
Villus height (µm)	824.84	790.59	88.41	10.95	0.518
Crypt depth (µm)	57.80	57.33	5.07	8.81	0.874
Villus:crypt ratio	14.33	13.89	1.93	13.65	0.700
Absorptive area (µm^2)	18.70	17.93	3.39	18.51	0.705
Jejunum					
Villus height (µm)	354.82	398.63	53.15	14.17	0.167
Crypt depth (µm)	42.69	42.90	4.84	11.31	0.938
Villus:crypt ratio	8.31	9.40	1.24	14.10	0.143
Absorptive area (µm^2)	8.67	9.84	1.37	14.90	0.154

SEM - standard error of the mean; CV - coefficient of variation.

Table 4 - Relative weight (%) of pancreas and lipase activity (IU/mg of protein) in pancreas of broilers fed diets containing deactivated full-fat soybean as a lipid source, with or without emulsifier

	Emulsifier		SEM	CV (%)	P-value
	Without	With			
14 d					
Relative weight	0.47	0.45	0.05	10.44	0.574
Lipase activity	4.62	3.63	1.54	36.99	0.317
21 d					
Relative weight	0.34	0.32	0.03	9.36	0.470
Lipase activity	22.12	24.42	13.42	57.70	0.737

SEM - standard error of the mean; CV - coefficient of variation.

Energy-restricted diets containing DFFS and emulsifier impaired the digestible energy value at 49 d, with a reduction (P<0.0001) of 389 kcal kg^{-1} compared with the diet with regular energy level without inclusion of the additive. Additionally, the absence of emulsifier increased the ileal coefficient of digestibility of dry matter (P = 0.0001), gross energy (P<0.0001), and crude protein (P = 0.038) in 13.73, 14.84, and 6.05%, respectively (Table 5).

Breast yield was higher for birds fed diets without the emulsifier (29.20% *vs* 27.22%, data not shown). For the remaining carcass and cuts yields, there were no differences (P>0.05) among the treatments. No difference (P>0.05) was observed for meat quality (Tables 6 and 7).

Table 5 - Digestible energy (kcal kg^{-1}) and ileal digestible coefficients (%) of dry matter, gross energy, and crude protein determined in broilers at 49 d fed diets containing deactivated full-fat soybean as a lipid source, with or without emulsifier

	Emulsifier		SEM	CV (%)	P-value
	Without	With			
Digestible energy	2889a	2500b	55.12	2.05	<0.0001
Digestibility coefficient					
Dry matter	69.34a	60.97b	1.31	2.01	0.0001
Gross energy	70.66a	61.53b	1.35	2.05	<0.0001
Crude protein	67.48a	63.63b	2.06	3.14	0.038

SEM - standard error of the mean; CV - coefficient of variation.
a,b - Means followed by a different letter in the row are different at an alpha level of 0.05 according to F-test.

Table 6 - *Pectoralis major* meat quality of broilers at 49 d, fed diets containing deactivated full-fat soybean as a lipid source, with or without emulsifier

	Emulsifier		SEM	CV (%)	P-value
	Without	With			
Cooking loss (%)	32.71	31.84	2.62	8.11	0.518
Shear force (kgf cm^{-2})	3.11	3.61	0.86	25.63	0.272
WHC (%)	62.47	61.84	3.43	0.52	0.722
pH					
15 min	5.98	6.04	0.13	2.22	0.329
24 h	5.86	5.80	0.13	2.15	0.386
L*					
15 min	50.08	49.54	3.39	6.80	0.755
24 h	51.24	48.43	2.62	5.27	0.061
a*					
15 min	2.13	1.69	0.81	41.99	0.306
24 h	3.25	2.73	0.98	32.70	0.306
b*					
15 min	4.16	4.25	2.15	51.07	0.930
24 h	7.23	6.03	1.40	21.18	0.110

WHC - water-holding capacity; L* - brightness; a* - red/green intensity; b* - yellow/blue intensity; SEM - standard error of the mean; CV - coefficient of variation.

Table 7 - Leg quarters pH and meat color of broilers at 49 d fed diets containing deactivated full-fat soybean as a lipid source, with or without emulsifier

	Emulsifier		SEM	CV (%)	P-value
	Without	With			
pH					
15 min	6.11	6.08	0.16	2.62	0.713
24 h	6.02	5.97	0.16	2.75	0.525
L*					
15 min	58.11	58.49	2.72	4.68	0.785
24 h	54.69	52.51	2.67	4.99	0.127
a*					
15 min	3.18	2.76	1.16	39.15	0.486
24 h	3.29	3.72	1.28	36.43	0.511
b*					
15 min	5.36	7.57	2.60	40.24	0.111
24 h	4.66	5.17	2.01	40.88	0.618

L* - brightness; a* - red/green intensity; b* - yellow/blue intensity; SEM - standard error of the mean; CV - coefficient of variation.

4. Discussion

The results of this study indicate that the use of an emulsifier based on glyceryl polyethyleneglycol ricinoleate in diets containing DFFS impairs the performance of broilers. It is important to emphasize that in diets containing the emulsifying agent, the metabolizable energy was reduced by 40 and 50 kcal kg^{-1} from 1 to 21 d and 22 to 49 d, respectively.

The dietary inclusion of exogenous emulsifiers can favor the digestion and absorption of lipids, as it increases the active interface of fats, resulting in increased lipase activity, facilitating micelle formation and nutrient transport via the enterocyte membrane. Previous studies with poultry and swine have shown that exogenous emulsifier supplementation between 20 and 50 kcal kg^{-1} in energy-restricted diets improves nutrient utilization, providing similar performance to that in animals fed diets with regular energy levels (Yin et al., 2018; Saleh et al., 2020).

The results of this study reinforce the idea that the effectiveness of emulsifiers may depend on the processing of the lipid source and the specific characteristics of the diet. Previous studies showed that emulsifiers can be beneficial when combined with lower-quality lipid sources, such as acid soybean oil, while their effect may be reduced in diets with more complex lipid sources, such as DFFS (Tenório et al., 2022). The authors reported a 6.2% increase in digestible energy with the inclusion of emulsifier, while in this study there was approximately 13% reduction in the digestible energy of the diet containing DFFS and emulsifier. Oliveira et al. (2024) emphasized that the age of animals and the type and concentration of emulsifier are important factors and can directly influence animals' performance.

The digestible energy difference between lipid sources is expected since lipid digestion is dependent on processing of the ingredient present in the diet. Rocha et al. (2014) reported 13% higher digestible energy in diets containing DFFS for broiler chickens at 21 d compared with diets containing non-heat-treated full-fat soybean. Compared with extruded soybean, DFFS provides less energy to the animals (Freitas et al., 2005). This may be related to the fact that the oil present in soybean grain is encapsulated in subcellular structures called lipid bodies (Shao et al., 2019) and is not fully available to the animal without going through a steaming process (Jiménez-Moreno et al., 2009).

Ileal digestibility coefficients are important parameters for evaluating the utilization of dietary nutrients. In diets containing emulsifiers, an increase in lipid digestibility is expected due to greater efficiency in micelle formation and fatty acid transport (Roy et al., 2010). However, some studies demonstrate that the effect may vary depending on the fat source and diet formulation, and the improvement in energy digestibility did not, however, translate into an improvement in poultry

performance (Kerr et al., 2024). Thus, even though most of the lipid fraction contained in DFFS is triacylglycerol, the emulsifier was not able to favor the digestive processes. This is probably due to poorer accessibility to the lipid substrate, which may not have been completely extracted in the deactivation process. The restriction of dietary energy and the low availability of the lipid source may be responsible for the worsening of nutrient digestibility coefficients and for the performance of the birds fed diets containing DFFS and emulsifier. Considering that, the hypothesis of this study—to add exogenous emulsifier to diets containing DFFS as an intact lipid source, aiming to increase the use of nutrients, maintain performance, and allow the possibility of reducing energy in poultry diets—was not proven. However, it opens a new possibility of a hypothesis for testing the on-top use of this additive with DFFS as a lipid source, mainly up to 21 d, considering the feed efficiency results shown by Khonyoung et al. (2015).

Another possible explanation for the worsening of nutrient digestibility is the higher levels of animal fat in diets containing emulsifier. Kamran et al. (2020) showed that the performance of birds and nutrient digestibilities of dry matter and crude fat was increased in birds that fed diet containing soy oil and polyglycerol polyricinoleate compared with birds fed diets contaning poultry fat and oxidized oil. As animal fats in broiler feeds reduce digestibility and the level of apparent metabolizable energy due to higher level of saturated fatty acids comparing to vegetable fats (Tancharoenrat et al., 2013, 2014), the variation in fat sources in the diets could have influenced the results, probably by the interaction between emulsifier and fat source.

Besides the processing of the lipid source, the age of birds can also influence the digestion of lipid fraction, considering the limited physiological capacity during the initial phase, including low production of bile salts and pancreatic lipase (Noy and Sklan, 1995). Bile salts, endogenous emulsifiers, are responsible not only for emulsifying fat particles but also for activating pancreatic lipase (Chen et al., 1975). In this context, considering the physiological limitations during the initial phase and the possible influence of the emulsifier on pancreatic lipase activity, we decided to evaluate the relative weight of the pancreas and the enzyme activity at 14 and 21 d. Despite evidence in the literature supporting the idea that the dietary inclusion of emulsifier can affect lipase activity (Hu et al., 2019) by improvements in fat digestibility that can increase the demand for lipase production, no effect was observed in this study, probably due to the lipid source not being fully available due to the physical structure of DFFS.

These same factors may be responsible for the absence of changes in intestinal morphometry at 14 and 21 d in birds fed diets containing emulsifier. Histological analysis of the intestine can provide insights into the efficiency of nutrient digestion and absorption. Previous studies indicate that additives such as emulsifiers and phytogenics can modulate intestinal morphology, increasing villus height and the villus:crypt ratio, which is associated with better nutrient absorption (Facchi et al., 2023). However, Kubiś et al. (2020) also did not observe an isolated effect of glyceryl polyethyleneglycol ricinoleate on the morphometric parameters of the intestine of broiler chickens fed wheat-based diets with beef tallow used as supplemental fat. Khonyoung et al. (2015) observed by electron microscopy analysis that, despite subtle changes in morphometric parameters, the intestinal cells from birds fed diets containing emulsifier (lisolecithin) were more active, indicating epithelial hypertrophy. These results indicate the need to evaluate additional intestinal morphophysiological parameters, providing a better correlation of the dietary influence of additives on intestinal function.

The type and processing of the lipid source can modify the concentration of serum lipids, such as triglycerides, total cholesterol, and the HDL and LDL fractions, due its fatty acid composition (Upadhaya et al., 2018). Furthermore, emulsification can reduce the concentration of these metabolites by favoring lipid metabolism and transport (Hu et al., 2019; Geng et al., 2022). However, it is noteworthy that the effect of emulsifiers on the lipid profile of broilers is still controversial in the literature, with a reduction (Serpunja and Kim, 2019), increase (Wang et al., 2016; Bontempo et al., 2018; Saleh et al., 2020), or no change in levels (Aguilar et al., 2013) being reported. These variations can be correlated to the different lipid sources, and the type and inclusion level of the emulsifiers used. Roy et al. (2010) observed that the inclusion of a glyceryl polyethyleneglycol ricinoleate-based emulsifier in broiler diets reduced the total blood cholesterol level and provided a lower concentration of fat in the liver, suggesting a fast

and efficient rate of removal of lipids from the organ. However, the authors calculated the additive level based on the percentage inclusion of the lipid source present in the diet, observing better results with 1% inclusion. Thus, in addition to considering that the studied lipid source allowed less access of the additive to the substrate, the inclusion level (0.35%) may not have been enough to influence the evaluated parameters.

Although there was interference on performance at 7 and 35 d and on breast yield, the meat quality parameters were not affected. Furthermore, the percentage of abdominal fat was not altered with dietary inclusion of the emulsifier, which indicates that the additive did not interfere with metabolism and body lipid deposition. Serpunja and Kim (2019) observed higher lipid deposition in birds fed diets with regular energy levels when compared with those fed energy-restricted diets with emulsifier. Improvement in the quality of broiler chicken meat has been reported in the literature when an emulsifier is included in the diet, such as an increase in WHC, a reduction in SF (An et al., 2020), and a great meat color (Upadhaya et al., 2018), a factor which directly affects consumer acceptance. Since there is no evidence that the inclusion of emulsifier in the presence of DFFS influenced lipid metabolism, there was no favoring of digestion and absorption of the fat-soluble metabolic pigments contained in the diet, as also observed by Upadhaya et al. (2018).

Therefore, although the literature shows positive results regarding the use of emulsifier in poultry diets, the use of emulsifier (considered in the energy matrix) with DFFS as a lipid source is not indicated for improving performance, intestinal morphometric parameters, pancreatic lipase activity, carcass yield, and meat quality. Thus, it is important to emphasize that the effects of including emulsifiers in the diet may vary according to the type of processing of the lipid source, the energy level of the diet, and the inclusion level of the additive in the diet.

5. Conclusions

The inclusion of an exogenous emulsifier based on glyceryl polyethyleneglycol ricinoleate in energy-restricted diets containing deactivated full-fat soybean as the main lipid source impaired the performance of broilers and the ileal digestibility of nutrients. However, its dietary inclusion does not affect the intestinal morphometry or meat quality of broilers.

Author contributions

Conceptualization: Eyng, C.; Nunes, R. V. and Duarte, C. R. A. **Formal analysis:** Tenório, K. I.; Souza, C. and Rohloff Junior, N. **Investigation:** Tenório, K. I.; Souza, C.; Rohloff Junior, N.; Köhler, T. L. and Tesser, G. L. S. **Methodology:** Eyng, C. and Duarte, C. R. A. **Supervision:** Eyng, C. and Nunes, R. V. **Writing – original draft:** Tenório, K. I. **Writing – review & editing:** Eyng, C.; Duarte, C. R. A. and Köhler, T. L.

Conflict of interest

The authors declare no conflict of interest.

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