

## Effect of emulsifier addition on metabolizable energy reduction in broiler diets

Adição de emulsificante nas rações de frangos de corte com redução dos níveis de energia metabolizável

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### Abstract

This study aimed to evaluate the effect of including soy lecithin emulsifier (SL) in broiler diets on the reduction of metabolizable energy levels and to correlate it with nutrient metabolizability coefficients and the determination of apparent metabolizable energy (AME). Two metabolic trials were conducted in a completely randomized design, with a 3x2 factorial arrangement (feed with three levels of metabolizable energy, with or without the inclusion of an emulsifier in the diet), totaling six treatments. In the starter phase, chicks received feed with 2,950; 3,050; and 3,150 kcal/kg of metabolizable energy, with six replications of 10 birds each, totaling 360 birds. In the grower phase, 210 birds were used, with seven replications of five chicks, and the energy levels tested were 3,100; 3,150; and 3,200 kcal/kg of feed. In the starter phase, an interaction effect was observed, showing an increase in the nitrogen metabolizability coefficient (NMC) when adding the emulsifier to diets with reduced energy, as well as an effect of the emulsifier in increasing the AME corrected by the balance of nitrogen (AMEn). In the grower phase, an interaction effect was observed, showing an increase in AME and AMEn when adding the emulsifier to diets with lower energy levels. To sum up, emulsifier inclusion in broiler diets can reduce metabolizable energy while improving AMEn in both initial and grower phases, along with AME in growing broilers.

**Keywords:** digestibility; soy lecithin; lipids; metabolism

### Resumo

Objetivou-se avaliar a adição do emulsificante, lecitina de soja, nas rações de frangos com redução dos níveis de energia metabolizável e correlacioná-las com os coeficientes de metabolizabilidade de nutrientes, além da determinação da energia metabolizável aparente (EMA). Realizou-se dois ensaios metabólicos em delineamento inteiramente casualizados, esquema fatorial 3x2 (ração com três níveis de energia metabolizável, com ou sem a inclusão do emulsificante na dieta), totalizando seis tratamentos. Na fase inicial, os pintos receberam dieta com 2.950, 3.050 e 3.150 kcal/kg de energia metabolizável, com seis repetições com 10 aves, totalizando 360 aves. Na fase de crescimento foram utilizadas 210 aves, sendo sete repetições com cinco frangos e os níveis de energia testados foram: 3.100, 3.150 e 3.200 kcal/kg de ração. Na fase inicial, observou-se efeito de interação, apresentando aumento do coeficiente de metabolizabilidade do nitrogênio (CMN) ao adicionar o emulsificante em dietas com energia reduzida e também, efeito do emulsificante no aumento da energia metabolizável aparente corrigida pelo balanço do nitrogênio (EMAn). Na fase de crescimento, observou-se efeito de interação, apresentando aumento da EMA e EMAn ao adicionar o emulsificante em dietas com menores níveis de energia. Conclui-se que a inclusão do emulsificante é indicada para frangos de corte, pois permite reduzir a energia metabolizável da dieta, melhorando a EMAn nas fases inicial e de crescimento, bem como a da EMA com frangos em crescimento.

**Palavras-chave:** digestibilidade; lecitina de soja; lipídeos; metabolismo

## 1. Introduction

Nutrients such as carbohydrates, proteins, and fats in broiler diets become sources of energy when metabolized. The dietary energy content is commonly evaluated by the apparent metabolizable energy (AME) and the nitrogen-corrected AME (AMEn) contents<sup>(1,2)</sup>. Among the nutrients, lipids, e.g. fats and oils, are mostly used to increase energy levels in diets because they

provide 2.25 times more energy than carbohydrates and proteins<sup>(3)</sup>. Increasing the availability of metabolizable energy (ME) for broilers is important for muscle tissue synthesis, which improves bird performance<sup>(4,5)</sup>. However, lipid sources are costly and can make feeds more expensive. In this scenario, the inclusion of emulsifiers has emerged as a strategy to improve the digestibility of lipid sources and reduce metabolizable energy without impairing animal performance<sup>(6,7,8)</sup>.

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Emulsifiers added to diets, known as exogenous, can be either natural, such as soy lecithin or lysolecithin, which are extracted from oilseeds; or synthetic, resulting from modifications of lysolecithin and lysophosphatidylcholine, such as glyceryl polyethylene glycol ricinoleate and sodium stearyl-2-lactylate<sup>(3,9)</sup>.

Soy lecithin contains 60% phospholipids in its composition<sup>(10)</sup>, making it a surfactant and/or emulsifier<sup>(11)</sup>. Therefore, when added to the broiler feed, it can improve the availability of AME and AMEn. Majdolhosseini et al.<sup>(12)</sup> observed an improvement in AMEn levels when 0.1% soy lecithin was added to the diet of broilers, but no interaction was observed between the emulsifier and metabolizable energy levels in the feed. Oliveira et al.<sup>(13)</sup> found that including 0.025% soy lecithin in broiler feed with a 90 kcal/kg reduction did not increase AME in broilers aged 11-21 days compared to the control diet. Wealleans et al.<sup>(14)</sup> included 250 ppm soy lecithin and lysolecithin in the feed and found that AMEn was significantly higher in the latter.

Based on the above, this study aimed to evaluate the effect of including soy lecithin emulsifier (SL) in broiler diets on the reduction of metabolizable energy levels and to correlate it with the metabolizability coefficients of dry matter, ether extract, gross energy, and nitrogen. Additionally, it proposes to determine the apparent metabolizable energy and nitrogen-corrected apparent metabolizable energy contents of these diets.

## 2. Material and methods

The experiment was carried out in the experimental poultry house of the School of Veterinary Medicine and Animal Science of the Federal University of Goiás (UFG). The study was approved by the Ethics Committee for Animal Use (CEUA) of the university (approval no. 083/2020). Two metabolism trials were performed with Cobb™ broilers in the starter (10 to 14 days of age) and grower (30 to 35 days of age) phases.

The chicks were initially reared in a conventional shed from the first day of age, where they received the same experimental diets used in the metabolic trials according to the rearing phase. For the first metabolic trial, at seven days old, 360 chicks were housed in galvanized wire cages measuring 0.90 m x 0.60 m x 0.45 m. Excreta collection began at 10 days and ended at 14 days of age. For the second metabolic trial, at 27 days of age, 210 chickens were housed in experimental cages and collections took place from 30 to 35 days of age.

This study used a completely randomized design with a 3x2 factorial arrangement (three dietary metabolizable energy levels, with or without emulsifier inclusion), totaling six treatments. In the starter phase, chicks were fed diets containing 2,950, 3,050, and 3,150

kcal/kg of metabolizable energy (ME). Ten birds were used per experimental unit, and six replications were performed, totaling 360 birds. In the grower phase, the ME levels of 3,100, 3,150, and 3,200 kcal/kg of feed were tested in 210 broilers, in seven replications of five birds each. Experimental diets were formulated following the recommendations of Rostagno et al.<sup>(15)</sup> for the starter and grower phases, respectively (Tables 1 and 2). The diets received the commercial emulsifier Nutri-Lyso™, which is composed of soy lecithin, silica, antioxidants, and wheat flour. According to the manufacturer, each 1,000 g of the product contained 500 g of soy lecithin. Following the manufacturer's recommendation (from 0.05% to 0.15%), we included 0.125% of the product in the diet in place of the inert ingredient (kaolin).

A metabolism trial was conducted using total excreta collection at 08h00 and 17h00. The diets were marked with 1% iron oxide to determine the beginning and end of the collection period<sup>(16)</sup>. At the end of the collection period, excreta were stored in a freezer at -10°C. At the end of the metabolic trial, frozen samples (300 g/sample) were properly homogenized according to each treatment. Using the method proposed by Silva and Queiroz<sup>(17)</sup>, the metabolizability coefficients of dry matter (DMMC), ether extract (EEMC), and gross energy (GEMC) were determined. These variables were used to calculate apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), and nutrient metabolizability coefficient (NMC) using the equations described by Sakomura and Rostagno<sup>(18)</sup>.

For statistical evaluation, analysis of variance (ANOVA) was performed using SAS software, in which the means of AME, AMEn, DMMC, EEMC, GEMC, NMC, and nitrogen balance (NB) were compared by the F-test (5%). For interactions between the studied groups (dietary ME levels and SL emulsifier inclusion), the means were compared by Student's t-test (5%).

## 3. Results

In the starter phase (Table 3), no interaction effect was observed between dietary metabolizable energy (ME) levels and soy lecithin-based (SL) emulsifier addition for the metabolizability coefficients of dry matter (DMMC) or ether extract (EEMC) ( $p>0.05$ ). However, a significant difference was found for the nitrogen metabolizability coefficient (NMC) ( $p=0.0009$ ). Dietary ME levels influenced DMMC ( $p=0.0269$ ), with the diet containing 3,150 kcal/kg ME providing higher DMMC (74.37%) than the other dietary ME levels. Nonetheless, neither ME levels nor SL inclusion affected EEMC.

**Table 1.** Proximate composition of experimental diets and nutritional composition calculated for the starter phase (1 to 21 days)

Ingredient (%)	Energy level		
	2,950 kcal/kg	3,050 kcal/kg	3,150 kcal/kg
Corn	54.00	51.65	49.31
Soybean meal 45%	37.25	37.67	38.09
Meat and bone meal 45%	4.00	4.00	4.00
Inert*	0.50	0.50	0.50
Dicalcium phosphate	0.50	0.50	0.51
Soybean oil	1.83	3.76	5.69
Limestone	0.43	0.43	0.42
Common salt	0.46	0.46	0.46
DL-methionine 99%	0.39	0.38	0.38
L-lysine HCl 98%	0.18	0.17	0.16
L-threonine	0.07	0.07	0.07
Mineral and vitamin supplement <sup>1</sup>	0.40	0.40	0.40
Total (%)	100	100	100
Nutrient	Calculated nutritional composition		
Crude protein (%)	23.31	23.31	23.31
Metabolizable energy (kcal/kg)	2,950	3,050	3,150
Calcium (%)	0.88	0.88	0.88
Available phosphorus (%)	0.42	0.42	0.42
Digestible lysine (%)	1.26	1.26	1.26
Digestible methionine + cystine (%)	0.93	0.93	0.93
Sodium (%)	0.218	0.218	0.218
Digestible threonine (%)	0.83	0.83	0.83
Digestible tryptophan (%)	0.25	0.25	0.25

<sup>1</sup> Vitamin-mineral supplement: guaranteed levels per kilogram of product: Folic acid 1,600.00 mg, Pantothenic acid 24.96 g, Biotin 80 mg, Butylated hydroxytoluene 100 mg, Niacin 67.20 g, Selenium 600 mg, Vitamin A 13,444,000 IU, Vitamin B1 500 mg, Vitamin B12 9,200 mcg, Vitamin B2 9,600 mg, Vitamin B6 4,992 mg, Vitamin D3 3,200,000 IU, Vitamin E 21,000 IU, Vitamin K3 2,880 mg, Copper 15 g, Iron 90 g, Iodine 1,500 mg, Manganese 150 g, Zinc 140 g. \*Emulsifier composed of 500 g of soy lecithin per 1,000 g of Nutri-Lyso®, which was used to replace kaolin.

**Table 2.** Centesimal composition of the experimental diets and nutritional composition calculated for the grower phase (22 to 35 days)

Ingredient	Energy level		
	3,100 kcal/kg	3,150 kcal/kg	3,200 kcal/kg
Corn	60.56	59.26	58.09
Soybean meal 45%	30.02	30.26	30.47
Meat and bone meal 45%	4.00	4.00	4.00
Inert*	0.50	0.50	0.50
Dicalcium phosphate	0.31	0.31	0.31
Soybean oil	2.87	3.88	4.85
Limestone	0.30	0.30	0.30
Common salt	0.37	0.44	0.44
DL-methionine 99%	0.36	0.36	0.36
L-lysine HCl 98%	0.23	0.23	0.22
L-threonine	0.07	0.07	0.07
Mineral and vitamin supplement <sup>1</sup>	0.40	0.40	0.40
Total (%)	100	100	100
Nutrient	Calculated nutritional composition		
Crude protein (%)	20.58	20.58	20.58
Metabolizable energy (kcal/kg)	3,100	3,150	3,200
Calcium (%)	0.76	0.76	0.76
Available phosphorus (%)	0.37	0.37	0.37
Digestible lysine (%)	1.12	1.12	1.12
Digestible methionine + cystine (%)	0.83	0.83	0.83
Sodium (%)	0.18	0.21	0.21
Digestible threonine (%)	0.74	0.74	0.74
Digestible tryptophan (%)	0.21	0.21	0.21

<sup>1</sup> Vitamin-mineral supplement: guaranteed levels per kilogram of product: Folic acid 1,600.00 mg, Pantothenic acid 24.96 g, Biotin 80 mg, Butylated toluene hydroxide 100 mg, Niacin 67.20 g, Selenium 600 mg, Vitamin A 13,444,000 IU, Vitamin B1 500 mg, Vitamin B12 9,200 mcg, Vitamin B2 9,600 mg, Vitamin B6 4,992 mg, Vitamin D3 3,200,000 IU, Vitamin E 21,000 IU, Vitamin K3 2,880 mg, Copper 15 g, Iron 90 g, Iodine 1,500 mg, Manganese 150 g, Zinc 140 g. \*Emulsifier composed of 500 g of soy lecithin per 1,000 g of Nutri-Lyso®, which was used to replace kaolin.

**Table 3.** Metabolizability coefficients of dry matter (DMMC), ether extract (EEMC), and nitrogen (NMC) in broilers fed diets with different levels of metabolizable energy (ME) and inclusion of emulsifier (EL), in the starter phase

Variable	ME* (kcal/kg)	Emulsifier			CV (%)	SE	P value		
		With	Without	Mean			ME	EL	ME*EL
DMMC (%)	2,950	73.70	72.42	<b>73.06 B</b>	1.52	0.20	0.0269	0.1275	0.4352
	3,050	73.80	73.54	<b>73.67 B</b>					
	3,150	74.48	74.26	<b>74.37 A</b>					
	Mean	<b>73.99</b>	<b>73.41</b>						
EEMC (%)	2,950	84.62	87.50	<b>86.06</b>	2.99	0.52	0.4026	0.8139	0.2406
	3,050	86.73	85.92	<b>86.33</b>					
	3,150	88.39	87.08	<b>87.73</b>					
	Mean	<b>86.58</b>	<b>86.57</b>						
NMC (%)	2,950	70.28aB	62.73bC	<b>66.50</b>	3.17	0.75	0.0001	0.0001	0.0009
	3,050	73.58aB	69.87bB	<b>71.72</b>					
	3,150	74.04aA	74.22aA	<b>74.13</b>					
	Mean	<b>72.63</b>	<b>68.94</b>						

Means followed by different letters in the column and lowercase letters in the row differ by the F test (5%). Interaction between energy levels x emulsifier applying Student's t-test at 5%. CV = coefficient of variation (%). SE = standard error of the mean. \*ME: values determined on a dry matter basis.

As for NMC (Table 3), there was an interaction effect ( $p=0.0009$ ) between dietary ME levels and SL inclusion. The low-ME diets (2,950 and 3,050 kcal/kg) containing emulsifiers showed higher NMC compared to diets without emulsifiers. When SL was added, the diets with 2,950 kcal showed a similar result to those with 3,050 kcal, differing ( $p=0.0001$ ) from those with 3,150 kcal/kg ME. Conversely, for diets without SL inclusion, the NMC of the diets with 2,950 kcal/kg was lower than that of the diets with 3,050 and 3,150 kcal/kg ( $p=0.0001$ ).

Regarding the gross energy metabolizability coefficient (GEMC) in the starter phase (Table 4), there was no interaction ( $p>0.05$ ) between ME levels and SL

emulsifier addition; however, an effect ( $p<0.05$ ) of the dietary ME level was also noted. Moreover, the highest GEMC ( $p=0.0019$ ) was found for diets with 3,150 kcal/kg ME when compared to 2,950 kcal/kg and 3,050 kcal/kg, which did not differ from each other (Table 4).

Metabolizable energy levels and SL inclusion had no interaction effect ( $p>0.05$ ) on apparent metabolizable energy (AME) (Table 4), nor was there an effect of emulsifier addition for this variable. However, diets with 3,150 kcal/kg ME had a higher AME level ( $p=0.0001$ ) than diets with 2,950 and 3,050, which did not differ significantly between each other ( $p>0.05$ ).

**Table 4.** Gross energy metabolizability coefficient (GEMC), apparent metabolizable energy (AME), and nitrogen-corrected AME (AMEn) of chickens fed diets with different levels of metabolizable energy (ME) and inclusion of emulsifier (EL) in the starter phase

Variable	ME* (kcal/kg)	Emulsifier			CV (%)	SE	P value		
		With	Without	Mean			ME	EL	ME*EL
GEMC (%)	2,950	77.68	77.25	<b>77.46B</b>	1.30	0.19	0.0019	0.4441	0.9893
	3,050	77.85	77.68	<b>77.77B</b>					
	3,150	79.11	78.92	<b>79.02A</b>					
	Mean	<b>78.22</b>	<b>77.95</b>						
AME(kcal/kg)	2,950	3,631	3,667	<b>3,649B</b>	1.31	17.8	0.0001	0.1365	0.7136
	3,050	3,658	3,664	<b>3,661B</b>					
	3,150	3,837	3,867	<b>3,852A</b>					
	Mean	<b>3,709</b>	<b>3,732</b>						
AMEn(kcal/kg)	2,950	3,398	3,508	<b>3,453B</b>	1.26	16.5	0.0001	0.0002	0.9260
	3,050	3,419	3,465	<b>3,442B</b>					
	3,150	3,606	3,640	<b>3,623A</b>					
	Mean	<b>3,538a</b>	<b>3,474b</b>						

Means followed by different letters in the column and lowercase letters in the row differ by the F test (5%). Interaction between energy levels x emulsifier applying Student's t-test at 5%. CV = coefficient of variation (%). SE = standard error of the mean. \*ME - the metabolizable energy values are lower than the AME and AMEn values because they were determined on a dry matter basis.

Likewise, for nitrogen-corrected AME (AMEn), there was no significant interaction ( $p>0.05$ ) between ME levels and SL inclusion, but there were differences ( $p=0.0001$ ) for dietary ME levels and emulsifier addition separately ( $p=0.0002$ ). The AMEn in diets with 3,150 kcal/kg was higher ( $p=0.0001$ ) compared to those of diets with 2,950 and 3,050 kcal/kg, which did not differ from each other. Furthermore, AMEn was higher when the emulsifier was added to the diet (Table 4).

In the grower phase metabolism trial from 30 to 35

days of age, there was no interaction between factors or an effect of ME levels used in the diet and/or emulsifier inclusion ( $p>0.05$ ) for DMMC, EEMC, and NMC (Table 5). However, dietary ME levels influenced EEMC ( $p=0.0030$ ), with higher values seen in diets with 3,150 and 3,200 kcal/kg (88.40% and 88.39%, respectively) than in those with 3,100 kcal (86.17%). As for GEMC (Table 6), no effect or interaction effect ( $p>0.05$ ) was observed between ME and emulsifier inclusion in the diets.

**Table 5.** Metabolizability coefficients of dry matter (DMMC), ether extract (EEMC), and nitrogen (NMC) in chickens fed diets with different levels of metabolizable energy (ME) and with the inclusion of emulsifier (EL), in the grower phase

Variable	ME* (kcal/kg)	Emulsifier			CV (%)	SE	P value		
		With	Without	Mean			ME	EL	ME*EL
DMMC (%)	3,100	76.86	76.82	<b>76.84</b>	2.05	0.23	0.4050	0.7837	0.9913
	3,150	77.68	77.50	<b>77.59</b>					
	3,200	77.60	77.42	<b>77.51</b>					
	Mean	<b>77.38</b>	<b>77.24</b>						
EEMC (%)	3,100	85.94	86.40	<b>86.17B</b>	1.45	0.32	0.0030	0.3133	0.3933
	3,150	88.82	87.98	<b>88.40A</b>					
	3,200	89.01	87.76	<b>88.39A</b>					
	Mean	<b>87.92</b>	<b>87.38</b>						
NMC (%)	3,100	64.49	64.41	<b>64.95</b>	7.91	0.84	0.0594	0.2892	0.8449
	3,150	67.74	70.56	<b>69.15</b>					
	3,200	68.63	70.24	<b>69.43</b>					
	Mean	<b>66.95</b>	<b>68.73</b>						

Means followed by different letters in the column and lowercase letters in the row differ by the F test (5%). Interaction between energy levels x emulsifier applying Student's t-test at 5%. CV = coefficient of variation (%). SE = standard error of the mean. \*ME: values determined on a dry matter basis.

**Table 6.** Gross energy metabolizability coefficient (GEMC), apparent metabolizable energy (AME), and nitrogen-corrected AME (AMEn) in chickens fed diets with different levels of metabolizable energy (ME) and inclusion of emulsifier (EL) in the grower phase

Variable	ME* (kcal/kg)	Emulsifier			CV (%)	SE	P value		
		With	Without	Mean			ME	EL	ME*EL
GEMC (%)	3,100	80.73	81.71	<b>81.22</b>	1.62	0.19	0.9574	0.337	0.5968
	3,150	81.31	81.33	<b>81.32</b>					
	3,200	81.27	81.46	<b>81.36</b>					
	Mean	<b>81.11</b>	<b>81.51</b>						
AME(kcal/kg)	3,100	3,862aA	3,697 bC	<b>3,780</b>	1.62	11.71	0.3616	0.0052	0.0001
	3,150	3,801aB	3,783 bB	<b>3,792</b>					
	3,200	3,806aB	3,820 aA	<b>3,813</b>					
	Mean	<b>3,823</b>	<b>3,7667</b>						
AMEn(kcal/kg)	3,100	3,729aB	3,506 bB	<b>3,618</b>	1.74	13.56	0.7879	0.0001	0.0001
	3,150	3,625aA	3,589 aA	<b>3,607</b>					
	3,200	3,629aA	3,618 aA	<b>3,624</b>					
	Mean	<b>3,661</b>	<b>3,571</b>						

Means followed by different letters in the column and lowercase letters in the row differ by the F test (5%). Interaction between energy levels x emulsifier applying t-student test at 5%. CV = coefficient of variation (%). SE = standard error of the mean. \*ME - the metabolizable energy values are lower than the AME and AMEn values because they were determined on a dry matter basis.

As regards AME in the grower phase (30 to 35 days), there was an interaction ( $p=0.0001$ ) between dietary ME levels and emulsifier inclusion (Table 6). For diets with lower ME levels (3,100 kcal/kg and 3,150 kcal/kg), a significant improvement in AME was observed for emulsifier inclusion compared to diets

without emulsifier. However, for the diet with 3,200 kcal/kg of ME, there was no effect of the emulsifier inclusion on AME. Therefore, the emulsifier showed positive results only when the dietary ME was reduced by 50 or 100 kcal. Among the different dietary ME levels, when the emulsifier was included, AME was higher

( $p < 0.05$ ) in diets with 3,100 kcal/kg, in contrast to what was observed in the diets without the inclusion of emulsifier, whose value was lower (3,697 kcal/kg).

The studied groups showed an interaction effect ( $p = 0.0001$ ) on AMEn, with the diet containing 3,100 kcal/kg ME and emulsifier inclusion exhibiting a higher AMEn value (3,729.84 kcal/kg) compared to diets without emulsifier (3,506.89 kcal/kg) (Table 6). The diets with 3,150 and 3,200 kcal/kg ME were not affected by emulsifier addition ( $p > 0.05$ ). As for dietary ME levels, there was a significant difference ( $p < 0.05$ ) for diets with 3,100 kcal/kg compared to diets with 3,150 and 3,200 kcal/kg, in which AMEn values were higher (3,729 kcal/kg) with emulsifier inclusion but lower without it (3,506 kcal/kg).

#### 4. Discussion

In the study, the addition of soy lecithin (SL) as an emulsifier had no effect on the dry matter (DMMC) and ether extract (EEMC) metabolizability coefficients (Table 3). This result may be attributed to the birds' age at evaluation time since young chicks have an immature digestive system, decreasing the digestibility of nutrients, particularly lipids. This reduced lipid digestibility is due to the need for an enzymatic complex, which may be present at low concentrations in the gastrointestinal tract of young chicks<sup>(19,20)</sup>. Moreover, the production of bile acids and pancreatic lipase is lower within the first week of life, gradually increasing until 14 days of age<sup>(21,22)</sup>. Hence, the reduced digestibility of lipid sources can impact feed intake<sup>(23)</sup> and ultimately decrease the amount of energy consumed by the animal.

Adding emulsifiers to diets can help make oils and fats easier to digest. These additives achieve this by making the micelles smaller during emulsification, increasing the contact surface between the enzymes and smaller micelles<sup>(3,9)</sup>. To improve the digestion of dietary fats, Wealleans et al.<sup>(24)</sup> suggested that a colipase and lipase complex should be present along with the emulsifier added to the diet. However, Shahid et al.<sup>(25)</sup> found that adding emulsifiers to the diets of young broilers (up to 10 days old) had no effect on dry matter and ether extract digestion, as their digestive system is not yet fully developed.

The higher DMMC observed in diets with a higher ME (3,150 kcal/kg) (Table 3) can be attributed to their increased fat content (5.69%) compared to those of diets with 2,950 kcal/kg (1.83%) and 3,500 kcal/kg (3.76%). The higher percentage of soybean oil in the 3,150 kcal/kg diet may have slowed down the passage of feed in the gastrointestinal tract, enabling enzymes to act on the substrate for a longer period and increasing absorption rates due to contact with enterocyte membranes<sup>(3,26,27)</sup>. A longer enzyme-substrate contact time improves digestion

and absorption of nutrients, leading to increased proliferation and development of enterocytes<sup>(28)</sup>. Therefore, reducing the passage rate may provide a greater energy supply for their development, increasing villi, which are necessary for transepithelial transport of nutrients in the brush border.

Regarding the nitrogen metabolizability coefficient (NMC) (Table 4), the inclusion of SL in the broiler diets for its emulsifying properties may have reduced the size of fat micelles and stabilized them, which is a key function of emulsifiers<sup>(29)</sup>. As a result, it can increase the absorption of nutrients, including nitrogen, by improving their diffusion in the gastrointestinal tract and increasing the contact surface area with enterocytes. Similarly, Zao et al.<sup>(30)</sup> found that the inclusion of lysolecithin (another emulsifier) in the diet of broilers up to 14 days of age improved nutrient absorption by acting on lipid sources, dispersing nutrients, reducing fat globules, and increasing the contact surface area with intestinal villi.

Previous studies have indicated that including SL in broiler diets during the starter phase (up to 21 days of age) can improve crude protein digestibility and NMC<sup>(9,14)</sup>. Nemati et al.<sup>(31)</sup> investigated the effects of SL supplementation without oil in a low-ME diet for growing turkeys and found that dietary lecithin supplementation could reverse the negative effects of low-energy diets, improving intestinal morphology, fat digestibility, AMEn, and consequently overall animal performance. Similarly, Ahmadi-Sefat et al.<sup>(32)</sup> conducted a study on the effect of emulsifying mixtures in diets and observed a linear increase in the apparent ileal digestibility of dry matter, crude protein, ether extract, and energy, as well as AMEn content. They also reported that the positive effects of supplementation were evident in broilers fed low-nutrient diets (with a reduction of 100 kcal/kg ME and/or 5% CP and limiting amino acids) in terms of performance, nutrient digestibility, and intestinal morphology. Haetinger et al.<sup>(8)</sup> observed in their study that the inclusion of lysophospholipid, a derivative of SL, improved crude protein digestibility in broilers and consequently their weight gains. The differences observed between the results of the cited studies and ours are related to the type and concentration of emulsifier, which can influence emulsification and stabilization of fat globules<sup>(9)</sup>.

The higher AMEn observed in diets with 3,150 kcal/kg (Table 4) compared to those with 2,950 and 3,050 kcal/kg in this study are associated with the higher NMC for diets with 3,150 kcal/kg, as shown in Table 3. Andrade et al.<sup>(33)</sup> stated that AMEn aims to correct AME based on nitrogen balance, and, thus, when corrected, it does not increase the energy in feces since nitrogen is retained in the body. Therefore, AMEn can improve broiler performance because the retained nitrogen can be used to

produce muscle protein.

This study found that SL inclusion did not affect DMMC, EEMC, or NMC in growing broilers (Table 5). Similarly, Wealleans et al. <sup>(14)</sup> observed no effect of SL on DMMC or EEMC. Siyal et al. <sup>(9)</sup> found an increase in EEMC but not in dry matter or crude protein digestibility after including 0.1% SL in the diets of broilers between 40 and 42 days old. Majdolhosseini et al. <sup>(12)</sup> tested SL inclusion (0.1%) in the diets of broilers at 35 days old and observed an effect only on EEMC but not on dry matter or protein digestibility. In contrast, Abbas et al. <sup>(34)</sup> included 0.035% soy lecithin and found an improvement in ether extract and dry matter digestibility. Like the present study, Liu et al. <sup>(7)</sup> found no effect of including 0.1% soy lecithin in the diets of 35-day-old broilers on DMMC, NMC, or EEMC. These authors suggested that the digestibility of lipid sources may be influenced by different factors, such as the type of emulsifiers, concentration, and bird strain. These factors could explain the variability observed across studies, including the present one.

The increase in GEMC, AME, and AMEn (Table 4) for diets with higher ME levels (3,150 kcal/kg) is due to the higher percentage of soybean oil, which raises the ME levels of the feed <sup>(27,35)</sup>. Additionally, the refined soybean oil used as the fat source in this study contains more than 80% unsaturated fat <sup>(36)</sup>, which increases the contact surface with digestive enzymes and improves their digestibility. The chain unsaturation of unsaturated fatty acids (e.g. oleic, linoleic, and linolenic) in refined soybean oil create a 114-degree angle that optimizes the contact surface between pancreatic lipases, bile acids, and colipase. In contrast, lipid sources with a higher percentage of saturated fatty acids exhibit a 180-degree angle <sup>(37)</sup>, which is less effective for this purpose.

Diets with higher energy levels (3,150 and 3,200 kcal/kg) containing more soybean oil (Table 2) improved EEMC in this study (Table 5). This finding is consistent with Park et al. <sup>(38)</sup>, who increased lipid source concentrations (beef tallow) in the diet to raise ME. However, other studies using soybean oil in broiler diets have not found an effect on EEMC <sup>(8,12,39)</sup>. The increased soybean oil levels in more energy-dense diets, combined with the maturity of the broiler digestive system in the grower phase (above 14 days of age), may have increased the secretion of lipase and bile acids due to the higher amount of ether extract in the intestine, leading to improved EEMC. Several authors have reported that increased weight of organs, such as liver and pancreas, is directly involved in the emulsification and degradation of fat molecules, increasing enzyme production and optimizing the metabolizability of lipid sources in the diet <sup>(3,19)</sup>.

According to Table 6, broiler diets with emulsifier inclusion and 3,100 kcal/kg ME contained higher AME and AMEn values, despite the lack of improvement in

EEMC during the grower phase. Soy lecithin, which contains phospholipids and lysophospholipids <sup>(40)</sup> and a ratio of unsaturated to saturated fatty acids of 60:40 <sup>(41)</sup>, can induce structural and conformational changes in the plasma membrane structure of enterocyte cells, leading to improved nutrient absorption <sup>(14,42)</sup>. Likewise, Melegy et al. <sup>(43)</sup> reported that lysolecithin, which also contains phospholipids, modifies the enterocyte membrane, increasing its porosity and promoting greater nutrient absorption. This increase in porosity is due to lysophospholipids causing a conformational change in the channel proteins that form the plasma membrane, allowing for increased absorption of other nutrients <sup>(44,45)</sup>. Zhang et al. <sup>(46)</sup> found that lysolecithin positively regulates nutrient transporter genes and host growth-related gene expression, regardless of changes in nutrient levels (ME and CP), suggesting that this may be the mechanism by which lysolecithin induces growth in broilers. Brautigan et al. <sup>(47)</sup> found that the inclusion of lysolecithin, which is present in soy lecithin, upregulated gene expressions in the villi region of the small intestine of broilers, promoting the synthesis of tissues such as collagen, which composes these structures and increases their size, thereby improving nutrient absorption. Therefore, it is suggested that the inclusion of an emulsifier in the diet improved the absorption of other nutrients by potentially inducing changes in the plasma membrane of enterocyte cells with the inclusion of soy lecithin, which contributed to the increase in AME and AMEn.

In addition, the inclusion of soy lecithin in the diet may positively impact the intestinal health of broilers by reducing fermentation caused by pathogenic microorganisms in the intestine. Boontiam et al. <sup>(48)</sup> reported that better nutrient absorption due to the use of SL can decrease fermentation in the small intestine, resulting in less damage to the villi. Liu et al. <sup>(7)</sup> also observed that the addition of SL as an emulsifier reduced the population of *E. coli* in the intestine of broilers, improving intestinal health. As the intestinal health of broilers is directly related to nutrient digestion and absorption, the improved levels of AME and AMEn in diets with lower levels of ME may be a result of this effect. However, it should be noted that the mechanism of action of emulsifiers is not yet fully understood, and different lipid sources, types, and concentrations can alter their emulsifying capacity <sup>(9,13,49)</sup>. Therefore, further studies are needed to better understand the effects of emulsifier inclusion in broiler diets with reduced ME levels.

## 5. Conclusion

Including soy lecithin as an emulsifier is a recommended practice for broiler diets, as it leads to a reduction in dietary metabolizable energy, thereby

improving nitrogen-corrected apparent metabolizable energy both in the starter and grower phases as well as apparent metabolizable energy in the grower phase.

#### Declaration of conflict of interest

The authors declare that there are no conflicts of interest.

#### Author contributions

*Conceptualization:* M. V. G. de Oliveira, D. V. Jacob. *Data curation:* M. V. G. de Oliveira, N. S. M. Leandro e M. F. Pires. *Investigation:* M. V. G. de Oliveira, R. R. dos Santos and M. B. Cafê, D. V. Jacob. *Methodology:* M. V. G. de Oliveira and N. S. M. Leandro, D. V. Jacob. *Writing (revision and editing):* M. V. G. de Oliveira and M. F. Pires.

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