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Original Article

Potential of Soy Gum Emulsification in Starter Diets for Broiler Chickens

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■Keywords

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ABSTRACT

This study evaluated the viability of using soy gum (residue from soy processing to obtain degummed oil) as an emulsifier in starter diets for broilers (1 to 21 days). For this, 600 1-day-old male broilers (Cobb® 500) were randomly assigned in a factorial arrangement (3 x 4), with three levels of gum inclusion (0, 1.25, and 2.5%) and four levels of soybean oil (0,1.3, 2.6, and 3.9%), with 5 replicates of 10 birds each. At 7, 14, and 21 days of age, we analyzed the performance parameters, pancreatic lipase activity and digestibility coefficients. Inclusion of soy gum improved (p<0.05) the performance and the digestibility coefficient of the ether extract, increased (p<0.05) the levels of AME and AMEn. The higher inclusion of gum (2.5%) as an emulsifier resulted in improved performance, showing the best values of feed conversion, with increased ether extract digestibility, increased AME content of the diets, and a lower requirement for pancreatic lipase in micelle formation.

INTRODUCTION

The intensification of broiler breeding systems and the consequent improvement in the productivity parameters of this sector are mainly associated with a highly efficient nutritional management, which results from a constant search for strategies that allow the use of ingredients alternatives in the animals' diet as a way of decreasing production costs and, consequently, increasing profitability (Khater *et al.*, 2020).

A major breakthrough in the nutritional management of poultry was the incorporation of oils and fats in the feed as a way to efficiently meet energy requirements and improve production (Lara *et al.*, 2005). Among the existing lipid sources, vegetable oils are intensely used in animal nutrition, with soy oil being the main lipid source in the diet of birds due to its fatty acid profile, greater digestibility, and easy absorption compared to other sources (Zollitsch *et al.*, 1997; Sanz *et al.*, 2000).

The value of the metabolizable energy of lipids is proportional to their digestibility and absorption by birds, with several factors that interfere in the digestion and absorption of lipids in the animals' bodies, such as, the degree of lipid saturation, the age of the birds, and the size of the fatty acid carbon chain (Wiseman & Salvador, 1991; Borsatti *et al.*, 2018).

An alternative for the use of lipid sources is the addition of emulsifiers in the feed, especially in the first weeks of life of the broilers, since during this period, the birds have difficulty in efficiently using the lipids due to the reduced production of pancreatic lipase and the immaturity of the enterohepatic circulation (Roy *et al.*, 2010; Wang *et al.*, 2016).

Associating a requirement of poultry farming, such as the better use of dietary lipids, with the need for the efficient use of by-products from

the soy-processing industry is an opportunity to reduce the environmental impact, especially considering the production of soybean in Brazil and the quantity of crude oil obtained, which, after the degumming process, yields 97% of degummed oil and 3% of soy gum, a by-product that presents a large proportion of lecithin.

Lecithin is a complex mixture of phosphatides and stabilizes the emulsion at the interface between oil and water; its main components are phosphatidylcholine (16-26%), phosphatidylethanolamine (14-20%), phosphatidylinositol (10-14%), phyto-glycolipids (13%), and phosphatidylserine (4%)(Woerfel, 1981; Attia *et al.*, 2009).

There is, however, no information on the use of soy gum as an emulsifying additive for animal production. As it is a residue that usually goes through purification processes to extract lecithin, little is known about soy gum in its raw form. However, because of its high amount of lecithin, it is considered an important natural emulsifier and the use of gum due to its emulsifying characteristics is interesting in poultry production (Attia *et al.*, 2009; Borsatti *et al.*, 2018; Souza *et al.*, 2019).

In this context, this study was conducted to evaluate the efficiency of using soy gum as an emulsifier in starter diets for broilers, formulated with the inclusion of soy oil.

MATERIAL AND METHODS

Ethic statement of animal experiments

The experimental procedure was approved by the Institutional Animal Care and Use Committee of State University of São Paulo, UNESP (03/2015/CEUA)

Experimental design

The experiment was conducted in the Poultry Sector of the Faculty of Engineering of Ilha Solteira – Unesp. A total of 600 male 1-day-old broilers (Cobb 500®) weighing 42,92±1,96g, vaccinated against Marek's disease in the hatchery, were housed in galvanized wire batteries overa 21-day experimental period. The birds were distributed in a completely randomized design, in a 3 x 4 factorial arrangement, with three levels of inclusion of soy gum (0,1.25, and 2.5%) and four levels of inclusion of soy oil (0,1.3,2.6, and 3.9%), with five replications of 10 birds each. The experimental diets were formulated based on corn, soybean meal, and corn protein (Table 1), following the recommendations of Rostagno *et al.* (2011). The diets were calculated to be isonutritive, except for the fat level, which fluctuated due to the inclusion of different soy oil levels.

Table 1 – Diet composition and calculated nutrients of the experimental diets(%, as fed basis).

	Inclusion levels (soybean oil)			
Ingredient (%)	0%	1.3%	2.6%	3.9%
Corn	62.800	59.900	56.800	53.900
Soybean meal	17.100	23.000	28.900	34.600
Corn gluten meal 60	13.300	9.300	5.350	1.500
Dicalcium phosphate	1.750	1.616	1.590	1.505
Limestone	0.902	0.900	0.913	0.906
Soybean oil	0.000	1.300	2.600	3.900
Salt	0.510	0.510	0.510	0.510
Mineral/Vitamin premix[1]	0.600	0.600	0.600	0.600
L-lysine (78%)	0.538	0.374	0.237	0.079
Variable portion[2]	2.500	2.500	2.500	2.500
	Calculated nutrients			
Metabolizable energy (kcal/kg)	3,000			
Crude protein (%)	20.5			
Calcium (%)	0.82			
Digestible phosphorus (%)	0.40			
Digestible lysine (%)	1.08			
Digestible methionine + cystine (%)	0.65			
Sodium (%)	0.22			
Ether Extract (%)	2.89	4.08	5.27	6.47

[1]Composition per kilogram of the product: vitamin, A 1.670.000 UI; vitamin D_3, 335.000 UI; vitamin E, 2.500 UI; vitamin K_3, 417 mg; vitamin B_1, 250 mg; vitamin B_2, 835 mg; vitamin B_6, 250 mg; vitamin. B_{12}, 2.000 mcg; folic acid, 100 mg; biotin, 9 mg; niacin, 5.835 mg; pantothenic acid, 1.870 mg; copper, 1.000 mg; cobalt, 17 mg; iodine; 170 mg; iron, 8.335 mg; manganese, 10.835 mg; selenium, 35 mg; zinc, 7.500 mg; choline chloride 50%, 116.670 mg; methionine, 250.000 mg; anticoccidial, 13.335 mg; growth promoter, 13.335 mg; antioxidant, 2.000 mg.

[2] Description of the variable portion: within each soybean oil level the replacement of the inert by soybean gum was carried out in the proportions of de 2.5%/0%; 1.25%/1.25% e 0%/2.5%.

Bird management

For nutritional management, the methods recommended by the Cobb® lineage manual were adopted, with water supply and feed *ad libitum* throughout the experimental period; the initial heating was performed with the aid of 100-watt incandescent lamps.

Data and sample collection

Growth performance was determined from 1 to 7 days, 1 to 14 days, and 1 to 21 days, quantifying the feed intake (FI), the weight gain (WG), and the feed conversion ratio (FCR).

The traditional method of total excreta collection was conducted from the 18th to the 21st dayof age, with 12 hours between collections. The collected excreta were stored in plastic bags and frozen at -20ºC. At the end of the experimental period, the amount of feed consumed and the total amount of excreta produced were determined for each repetition.

To determine dry matter (DM,%), crude protein (CP,%), ether extract (EE,%) and crude energy (kcal/kg), at the end of the collection period, the samples

were homogenized, and representative samples per plot were analyzed according to the methodologies described by AOAC (2000).Based on the results of the composition and gross energy value of the samples of rations and excreta and the values of intake and excretion, the apparent digestibility coefficients (ADC) of DM, CP, and EE, as well as the values of apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEn) were determined.

Lipase analysis

At days 7, 14 and 21, one bird per plot was euthanized by cervical dislocation to obtain pancreatic samples, which were labelled and immediately frozen in liquid nitrogen for further analysis of pancreatic lipase activity. Analyses of the enzymatic activity of pancreatic lipase were performed at the Laboratory of Applied Enzymology of the Faculty of Agricultural and Veterinary Sciences -Unesp, Jaboticabal.

The pancreas of five birds of each treatment were homogenized together in a homogenizer type Turrax, brand OMNI, model GLH-2511, in buffer Tris-HCl pH 7.5 containing $CaCl_2$ (50 mM) in the proportion of 1g/10 mL. The samples were centrifuged at 10,000 g for 10 minutes at 4ºC, and the supernatant was divided into aliquots and separated into five portions for the determination of lipase, frozen in liquid nitrogen, and stored at -70ºC, following the techniques adapted from Santos *et al.* (2016).

Lipase activity was determined continuously, at 37ºC and 405 nm, according to the methodology adapted from Winkler & Stuckmann (1979), using p-nitrophenyl palmitate (p-NFP) as substrate. The solution with the substrate (3 mg of p-NFP in 1 mL of isopropanol) was added to the micelle-forming solution (0.1 g of Arabic gum and 0.2 g of Triton-X100 in 90 ml of 50 mM Tris-HCl buffer, pH 8.0). The reaction medium contained 950 µL of substrate plus micelle, 110 µL of 2 mM sodium taurodeoxycholate, 75 µL 4.4 mM $CaCl_2$, 75 µL of 3MA $NaCl_2$, and an excess of partially purified chicken colipase (Brockman, 1981). One unit of activity (U.mg-1) of lipase was defined and expressed as the amount of enzyme that releases 1 ηmol of p-nitrophenolate per minute per mg of protein under standard test conditions.The protein concentration of the extracts was determined according to the method described by Hartree (1972), using bovine serum albumin fraction V as standard. The values obtained were used to correct lipase enzyme activity.

Statistical analysis

The data obtained were subjected to analysis of variance, and in cases of significant interactions between the factors or individual effects of the levels of gum and soybean oil, the averages were compared by Tukey´s test at 5% probability. For statistical procedures, the SAS® program was used (SAS 2016 Intitute Inc, NC, USA).

RESULTS

The levels of gum and soy oil, as well as the interaction between these factors, influenced feed consumption (FC), weight gain (WG), and feed conversion (FCR) at varying degrees (Table 2).

Table 2 – Influence of soy gum and soy oil levels on feed intake(FI, g/bird), body weight gain (BWG, g/bird) and feed conversion ratio (FCR, g/g).

Effects	Performance at 1 to 7 days			Performance at 1 to 14 days			Performance at 1 to 21 days		
	FI (g/bird)	BWG (g/bird)	FCR (g/g)	FI (g/bird)	BWG (g/bird)	FCR (g/g)	FI (g/bird)	BWG (g/day)	FCR (g/g)
					Gum levels				
0%	151.1	139.0	1.087^{b}	539.6	448.2	1.204	1222.8	945.8	1.293^{b}
1.25%	151.6	141.7	1.070^{ab}	539.4	454.0	1.188	1215.0	955.0	1.272^{ab}
2.5%	149.2	142.1	1.050^{a}	534.2	455.7	1.172	1200.0	950.6	1.262 a
					Oil levels				
0%	150.1	136.0	1.107^{c}	534.1	436.2	1.225	1191.3	905.5 ^{c}	1.316^{c}
1.3%	152.5	140.8	1.085^{bc}	534.8	448.1	1.193	1210.4	944.2 ^{b}	1.282^{b}
2.6%	150.6	142.3	1.058^{ab}	540.2	458.6	1.178	1221.2	970.5 ^{ab}	1.258^{ab}
3.9%	149.5	144.7	1.035 ^{a}	542.0	467.7	1.159	1227.5	981.7 ^{a}	1.251^{a}
					p-value				
Gum	0.6404	0.3786	0.0524	0.6874	0.3703	0.0001	0.3234	0.7234	0.0042
Oil	0.7857	0.0240	0.0011	0.7035	0.0001	<0.0001	0.1951	<0.0001	<0.0001
Gum x Oil	0.0523	0.0058	0.4350	0.0142	0.0094	0.0021	0.3340	0.2188	0.0635
SEM	0.0066	0.0017	0.0107	0.0049	0.0039	0.0056	0.0107	0.0080	0.0063

a Values with different superscripts within a column are significantly different (p<0.05).

SEM – Stander error of mean.

From 1 to 14 days of age, there was an interaction between gum and oil for FI (p=0.014), BWG (p=0.009), and FCR (p=0.002) (Table 2). The unfolding of the gum x oil interaction (Fig.1-a) indicates that in the absence of oil, the addition of gum at 1.25% resulted in a higher FI, without any difference from the consumption in treatments with the inclusion of 2.5% gum. For an oil inclusion of 1.3%, we observed a decrease in FI (p<0.05) in the treatments receiving gum (1.25 and 2.5%).

In the phase from 1 to 7 days of age, the evaluated factors did not influence (p>0.05) the FCR; however, there was an interaction (p=0.0058) between the levels of gum and oil for the BWG. With the absence of oil, at the inclusion level of 1.25, an improvement in the BWG was observed (Fig. 1-b). For FCR, the highest level of gum (2.5%) and the highest level of oil (3.9%) showed the best results (Table 2). For the BWG from 1 to 14 days, the gum x oil interaction (Fig. 1-c) only showed a difference (p=0.009) between the gum levels in the absence of oil, with the greatest gains for gum inclusion.

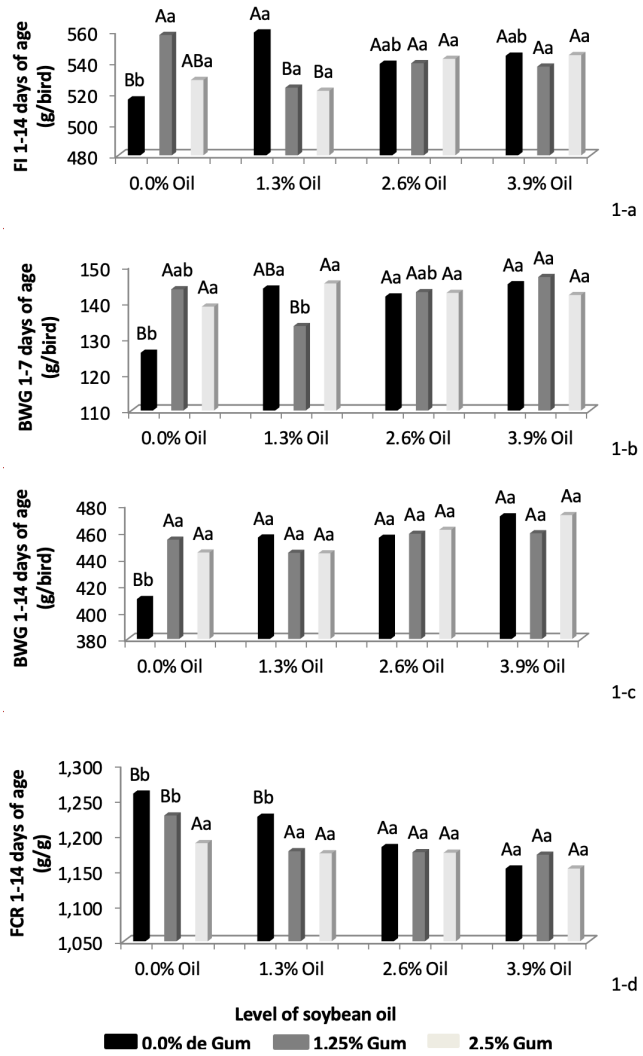

Figure 1 – Effect of gum levels within oil levels on FI at 1 to 14 days (1-a), BWG at 1 to 7 days (1-b), BWG at 1 to 14 days (1-c) and FCR at 1 to 14 days (1-d). Capital letters compare gum levels within each oil level and lowercase letters compare oil levels within each gum level.

The gum x oil interaction for the FCR also indicates that the gum effect occurred (p=0.002) only in the absence of oil or at an oil inclusion level of 1.3% (Fig.1-d). The variation in gum inclusion levels within the high inclusions of oil (2.6 and 3.9%) or the variation in oil inclusion levels with a gum inclusion of 2.5% of gum did not promote any differences (p>0.05).

In the period from 1 to 21 days of age (Table 2), there was an isolated effect of the inclusion of gum only for the FCR (p=0.004), with a better result for a gum inclusion level of 2.5%. The inclusion of oil influenced (p<0.001) both BWG and FCR, with better results for the greater inclusion of soy oil (3.9%).

The activity of pancreatic lipase differed among the different ages (Table 3). At 7 days of age, only oil levels promoted differences (p=0.001) in lipase activity, with greater activity for an oil inclusion level of 2.6%. At 14 and 21 days, enzyme activity was influenced by the significant interaction (p<0.001) between gum and oil levels. At 14 days (Fig. 2-a), the inclusion of 2.5% gum in the diet without the addition of oil decreased pancreatic lipase activity, while in the diets oil inclusion levels of 1.3 and 3.8%, a decrease in the activity of the enzyme was observed with the addition of 1.25% of gum. At the same age, the inclusion of gum in the diet with 3.9% oil resulted in an increase in pancreatic lipase activity. At 21 days of age (Fig. 2-b), with the exception of diets without oil or with 2.6% oil, the lowest inclusion of gum (1.25%) resulted in a reduction in pancreatic lipase activity.

Table 3 – Pancreatic lipase enzyme activity (Nanomols of product formed/minute/mg of protein).

Effects	Lipase at 7 days	Lipase at 14 days	Lipase at 21 days
Gum levels			
0	10.2158	8.0508	7.2825
1.25	10.8925	7.6175	6.5800
2.5	10.4425	6.6192	6.9267
Oil levels			
0	9.7989 [b]	7.3944	8.1578
1.3	10.1633 [b]	8.1655	6.8267
2.6	11.7022 [a]	8.3433	6.6344
3.9	10.4033 [b]	5.8133	6.1000
p-value			
Gum	0.2037	<0.0001	0.0025
Oil	0.0011	<0.0001	<0.0001
Gum x Oil	0.0964	<0.0001	<0.0001
SEM	0.2939	0.1722	0.2157

a Values with different superscripts within a column are significantly different (p<0.05).
SEM – Stander error of mean.

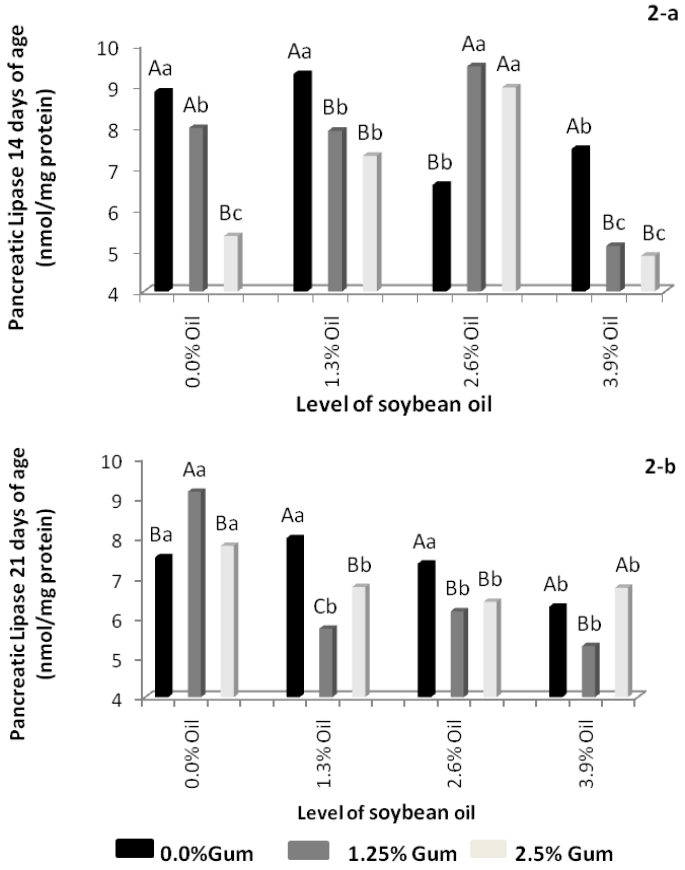

Figure 2 – Effect of gum levels within oil levels on pancreatic Lipase at 14 (2-a) and 21 (2-b) days of age. Capital letters compare gum levels within each oil level and lowercase letters compare oil levels within each gum level.

The ADC levels of DM, CP, EE, AME and AMEn are shown in Table 4. The ADC of DM was not affected by the inclusion of gum (p>0.05); however, the inclusion of 1.25% of gum resulted in a lower digestibility of DM compared to the inclusion level of 2.5%. There were no differences (p>0.05) for the CP of the ADC. For ADC of EE, the best results were obtained with the inclusion of gum and with the increase in the inclusion of soy oil. For the AME of the diets, there was a significant interaction between the levels of gum and soybean oil. In diets with 1.3 and 2.6% of oil, the lower gum inclusion of 1.25% resulted in an increase in the AME content (Fig. 3); however, for all diets with oil, a gum inclusion level of 2.5% promoted the highest values. When no gum was added to the diet, the increase in the level of AME only occurred with the inclusion of 3.9% of soy oil, while in diets with added gum, the lower oil inclusion level resulted in an increase of AME value. For AMEn there was a significant increase according to the levels of soy gum, as well as with the increase in the levels of soy oil. The interaction between soy gum and soy oil was not significant.

Table 4 – Digestibility coefficients (DC, %) of dry matter (DM), crude protein (CP) ether extract (EE), apparent metabolizable energy (AME, kcal/kg) and apparent metabolizable energy corrected for nitrogen (AMEn, kcal/kg)for broilers from 18 to 21 days of age.

Effects	DC-DM	DC-CP	DC-EE	AME	AMEn
Gum levels					
0%	93.08 [ab]	69.70	82.59 [b]	3,508	2,985 [c]
1.25%	93.04 [b]	69.51	85.67 [a]	3,588	3,067 [b]
2.5%	93.75 [a]	70.43	85.75 [a]	3,667	3,137 [a]
Oil levels					
0%	92.97	69.15	81.27 [c]	3,509	3,016 [b]
1.3%	93.56	69.92	83.58 [b]	3,591	3,068 [ab]
2.6%	93.40	69.72	85.28 [b]	3,600	3,046 [ab]
3.9%	93.25	70.72	88.44 [a]	3,650	3,124 [a]
p-value					
Gum	0.0313	0.3641	<0.0001	<0.0001	<0.0001
Oil	0.3628	0.2594	<0.0001	<0.0001	0.0077
Gum x Oil	0.1158	0.8558	0.1270	0.0306	0.3138
SEM	0.2080	0.4782	0.5942	11.3338	14.2075

[a] Values with different superscripts within a column are significantly different (p<0.05).
SEM – Stander error of mean.

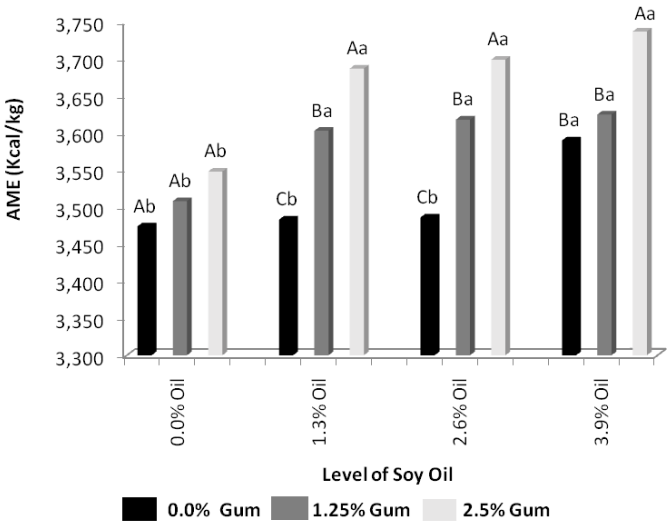

Figure 3 – Effect of gum levels within oil levels on Apparent Metabolizable Energy (AME). Capital letters compare gum levels within each oil level and lowercase letters compare oil levels within each gum level.

DISCUSSION

The physiological capacity for using fat is poorly developed in young chickens, with a significant improvement from 1.5 to 3.5 weeks of age (Wiseman & Salvador, 1991). Against this background, some studies demonstrate benefits in the use of emulsifiers in starter diets to improve the use of fat and, thus, animal performance (Wang *et al.*, 2016).

The inclusion of soy gum or soy oil only influenced feed intake in the period from 1 to 14 days, although in a less significant way, once only in the diet with 1.3%

of oil, the inclusion of 1.25% of gum was sufficient to reduce feed intake. This result may be related to a better energy use of the feed due to the presence of soy gum, meeting the energy demand of animals with a lower FCR. In the oil-free diet or in the diets with higher oil inclusion levels, this situation could not be sustained. In the first case, there was a lack of substrate for the emulsifying action of the gum and, in the second case, considering the efficiency of lipid absorption still low, the treatments with 2.6 and 3.9% of soybean oil already had a certain level of lipid saturation, which did not allow for the emulsifying activity of soy gum on oil (Zollitsch *et al.*, 1997).

In this study, at the first week of life and in the period from 1 to 14 days of age, the inclusion of 1.25% of soy gum resulted in increased weight gain only in diets without the inclusion of soy oil, demonstrating the emulsifying action of the gum also in the lipid portion of the other ingredients of the feed. However, regardless of the inclusion of the lipid source, a better response in feed conversion was verified with the greater inclusion of soy gum (2.5%), which was also verified for FCR from 1 to 21 days of age. The best values of FCR with the increasing inclusion of soy gum indicate the benefits of the increasing inclusion of emulsifiers even in diets in the initial breeding phase, demonstrating a greater efficiency of the emulsifier in phases where the capacity to digest and absorb lipids in young chickens is still low (Upadhaya *et al.*, 2017).

Zhao & Kim (2017), when evaluating an emulsifier (lysophospholipid) in the initial diet of broilers, also found an increase in WG and an improvement in FCR, even with the use of diets with reduced energy levels, which can be justified for improving the digestibility of fatty acids. In the present study, the increase in the digestibility of the ether extract with the inclusion of soy gum also supported the digestibility of the fatty acids in the diet, contributing to the increase in the value of AME, especially in diets containing soy oil.

With an optimal lipid use, animals are able to absorb and make better use of the energy provided by the feed, which is evidenced by a better FCR (Lara *et al.*, 2005). In the first week of life and in the total phase of the trial (1 to 21 days of age), with lower oil inclusion levels, there was an improvement in FCO, with a better result for an oil inclusion level of 3.9%.

The benefit of soy gum in the lipid portion of the feed was even more evident from 1 to 14 days of age. When isolating the use of oil and gum, it appeared that both significantly improved weight gain. The results of the present study are in agreement with the findings of Upadhaya *et al.* (2017), where increasing levels of an exogenous emulsifier contributed to the improvement of FCR in the first week of poultry rearing.

From 1 to 14 days, the variation in gum inclusion levels with high level of oil inclusion (2.6 and 3.9%), or even the variation of oil within the inclusion of 2.5% of gum, had no effect on weight gain, most likely because lipid absorption was not yet developed. Therefore, high inclusion levels resulted in saturation, masking the emulsifying efficiency of the gum.

The action of the lipase enzyme at 7 days of age was not altered due to the inclusion of soy gum, however at 14 and 21 days of age, the decrease in enzyme activity occurred in most situations when soy gum was included in the diet. Since lipase performance is linked to the fragmentation of lipids so that they can be absorbed by the body, when soy gum is included, micelles are more easily formed, improving the availability of lipids and resulting in a reduced lipase activity; in this sense, due to the presence of gum, the need for lipase for the degradation of lipid droplets decreases, causing the body to release less lipase (Klein, 2014).

Another result that indicates that the inclusion of gum is beneficial for broiler performance is an increase in the digestibility coefficient of EE, as a result of a gum inclusion level of 1.25%, regardless of the addition of oil. This improvement demonstrates its efficiency as an emulsifier, since in the initial stages, when the animals have an insufficient amount of bile salts, the inclusion of an emulsifier can enhance the formation of micelles and, consequently, increase the absorption of lipids (Guerreiro Neto *et al.*, 2011).

Although the increase in EE digestibility occurred due to the isolated effect of gum, the reflection of this improvement on the AME content fluctuated according to the inclusion of oil in the diets. However, in all diets with this source of lipid, the inclusion of 2.5% of gum resulted in higher AME values. In the absence of gum in the feed, the AME content only increased with the inclusion of the highest oil level (3.9%), also showing the efficiency of the gum in improving the formation of micelles, which leads to a greater availability of energy due to greater lipid absorption (Attia *et al.*, 2009; Borsatti *et al.*, 2018). According to Roy *et al.* (2010), the addition of emulsifiers in broiler diets allows the level of AME of the diets to match that of diets with greater energy addition without emulsifier, which was also observed in our study in which the inclusion of soy gum at 2.5% (without oil inclusion) presented results equivalent to those found with the inclusion of 2.6% soy oil (without gum inclusion).The results referring

to AMEn follow the same behavior of the results of the digestibility coefficient of the EE, being that, in the case of AMEn, the interference of the inclusion of soy gum in the increase of metabolizable energy is more evident. As shown, the inclusion of soy gum was also efficient in reducing the activity of the pancreatic lipase enzyme from 14 days onwards. Thus demonstrating that the efficiency of the increase in AMEn is related to the emulsifying potential of the soy gum, otherwise the activity and secretory capacity of the pancreatic enzyme would increase (Naderinejad *et al.*, 2016; Abadi *et al.*, 2019; Oliveira *et al.*, 2020)

CONCLUSIONS

The use of soy gum as an emulsifier results in improved animal performance, with the higher inclusion level (2.5%) showing the best values of feed conversion ratio, reflecting the increase in EE digestibility, the increase in the AME content of the diets, and the lower need for pancreatic lipase in the micelle formation process.

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