



Nutritional value of glycerin for pigs determined by different methodologies

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ABSTRACT - The objective of this study was to determine the nutritional value of glycerin for pigs using different inclusion levels of this test ingredient and the methodologies of total collection and chromium oxide marker. Eight barrows were distributed individually into metabolic cages, in a randomized-block experimental design, and fed diets containing 0, 50, 100, and 150 g kg⁻¹ plant-derived crude glycerin. The feed digestibility methodologies of total feces and urine collection and chromium oxide marker were analyzed. Inclusion of up to 150 g kg⁻¹ crude glycerin improved the digestibility coefficients of organic matter and energy of the diets. The collection method based on the use of chromium oxide marker underestimates the digestibility coefficient of ash and the energy values of glycerin in relation to the total collection method, but yields similar results to those obtained with the total collection method for the dry matter, organic matter, crude protein, ether extract, and neutral detergent fiber assessed.

Key Words: biodiesel, digestibility, glycerol, marker, metabolizability, total feces collection

Introduction

The use of glycerin, a byproduct from biodiesel production, in the feeding of swine is an option that has been investigated due to its considerable energy content similar to that of corn, in addition to being considered palatable for its sweetish taste (Grosbeck et al., 2008). For these reasons, it can positively affect the performance of piglets.

Glycerin has been employed as a source of readily available energy because of its rapid absorption in the intestine and easy metabolization in the liver; thus, it can be made available for the formation of lipids or production of energy through glycolysis and the citric acid cycle (Gallego et al., 2014). The use of glycerin in swine feeding also casts doubts as to the adequate level of its inclusion in the different production stages; for example, 120 g kg⁻¹ for 15 to 30 kg (Carvalho et al., 2012), 150 g kg⁻¹ for 27 to 48 kg (Verussa et al., 2017), 160 g kg⁻¹ for 67 to 107 kg (Gomide et al., 2012), 150 g kg⁻¹ for 97 to 115 kg

(Leite et al., 2017). However, studies aimed at analyzing the addition of glycerin in swine nutrition, considering its digestibility (Gallego et al., 2014; Madrid et al., 2013), are of paramount importance. *In vivo* trials are conducted to estimate digestibility coefficients of nutrients and mainly to determine the metabolizable energy contents of feedstuffs. The most widely used methods for digestibility trials are total feces and urine collection and markers, whereby chromium oxide is one of the most used in digestibility trials for pigs.

For Sakomura and Rostagno (2007), the main limitation of the total collection method is the control during collection of feces and urine samples without contaminations such as shedding of skin, hair, and feed, which may interfere with the determination of the energy content of a feedstuff. On the other hand, the use of markers does not require quantification of intake and feces and a partial collection of the sample can be performed decreasing possible contamination. However, the use of this method requires uniform mixture into the diet and standardization for chemical analyses, which may lead to a significant variability of results.

Therefore, the objective of this study was to determine the nutritional value of glycerin for pigs using different inclusion levels of the test ingredient and the total collection and marker methodologies.

Material and Methods

The experiment was conducted in the city of Sinop, Mato Grosso, Brazil (11° 51' 41" S, 55° 28' 57" W), complying

Received: August 27, 2016

Accepted: April 18, 2017

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<http://dx.doi.org/10.1590/S1806-92902017000700005>

How to cite: Verussa, G. H.; Corassa, A.; Pina, D. S.; Ton, A. P. S.; Komiyama, C. M. and Teixeira, A. O. 2017. Nutritional value of glycerin for pigs determined by different methodologies. *Revista Brasileira de Zootecnia* 46(7):584-590.

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with the ethical principles of animal experimentation adopted by the Conselho Nacional de Controle de Experimentação Animal and after being approved by the Ethics Committee in Animal Use (case no. 23108.700673/14-4). Eight genetically homogeneous barrows from industrial crosses, with an average initial weight of 31.08 ± 3.73 kg, were distributed individually into eight metabolic cages, in a randomized block experimental design consisting of four treatments and six replications, with each animal as an experimental unit. The replications were obtained over three experimental periods. The formation of blocks took into consideration the weight of the animals and the period. Each experimental period lasted 10 days, with five days for adaptation of the animals to the metabolic cages and diets and five days for feces and urine collection.

Treatments were composed of a corn- and soybean-meal-based diet (Table 1), formulated so as to meet the recommendations of Rostagno et al. (2011), and with soy oil-based glycerin in an isometric replacement of 50, 100, and 150 g kg⁻¹ of the diet according to the methodology described by Sakomura and Rostagno (2007). The glycerin used in this study contained 4.9, 865.0, 0.50, and 3.0 g kg⁻¹ as fed and 5,397 kcal kg⁻¹ water, glycerol, methanol, total fatty acids, and gross energy, respectively (Bunge Alimentos, Nova Mutum, MT, Brazil). To add the glycerin in both treatments, it was pre-mixed with approximately 200 g kg⁻¹ of the total amount of the feed aiming to improve the final uniformity. To obtain the chemical composition

Table 1 - Composition and calculated nutritional values of control diet (as-fed basis)

Ingredient (g kg ⁻¹)	Control diet
Corn	604.8
Soybean meal	302.6
Rice bran	30.0
Soy oil	18.9
Calcitic limestone	5.2
Dicalcium phosphate	17.5
Vitamin-mineral mix ¹	10.0
Common salt	4.6
L-lysine	1.5
Chromium oxide	5.0
Total	1000
Calculated nutrient content (g kg ⁻¹)	
Metabolizable energy (kcal kg ⁻¹)	3,230
Crude protein	189.9
Calcium	7.2
Available phosphorus	3.6
Sodium	2.0
Digestible lysine	10.1

¹ Composition of the supplement per kg of diet: vitamin A, 13750 IU; vitamin B1, 2 mg; vitamin B2, 1.25 mg; vitamin B6, 4 mg; vitamin B12, 4.5 mcg; vitamin D3, 3000 IU; vitamin E, 75 IU; vitamin K3, 6.25 mg; nicotinic acid, 50 mg; pantothenic acid, 30 mg; folic acid, 0.625 mg; cobalt, 1.25 mg; copper, 25 mg; iron, 150 mg; zinc, 200 mg; manganese, 75 mg; selenium, 0.7 mg; iodine, 2 mg; coline, 250 mg; biotin, 25 mcg.

of the diets, the dry matter (DM), crude protein (CP), ash, ether extract (EE), neutral detergent fiber (NDF), and gross energy (GE) were analyzed as described by Detmann et al. (2012) (Table 2).

In the period of adaptation, the feed was supplied *ad libitum* and refusals were counted for a later calculation of intake based on the metabolic weight (LW^{0.75}). To avoid losses and facilitate ingestion, diets were weighed and moistened at the ratio of 1:1 and provided twice daily (07.30 and 17.30 h). Two methodologies for evaluation of nutrient digestibility were used: total feces collection and digestibility marker. Chromium oxide (Cr₂O₃) was used as the digestibility marker in the second methodology.

For the digestibility trial, the methods of total feces and urine collection and chromium oxide marker were used simultaneously. Feces and urine were collected once daily, in the morning. Feces were collected, weighed, homogenized, and then samples of 200 g kg⁻¹ of the total were taken, packed in plastic bags, labeled, and stored in a freezer (-10 °C) until the end of the collection period. The urine was filtrated as it was excreted, using a filter fabric coupled to the funnel of the urine collection box, and then collected into plastic buckets containing 10 mL HCl 1:1. The total urine volume of each animal was counted using a beaker with 5-mL graduation, from which samples of 200 g kg⁻¹ were taken for sampling, which were packed in plastic packages with lid and stored in a freezer.

At the end of the collection period, diets and feces samples were thawed, weighed, homogenized, and pre-dried in a forced-air oven at 60 °C for 72 h for analyses of DM, CP, ash, EE, NDF, and GE, according to Detmann et al. (2012). The organic matter (OM) content was determined as the difference between the dry matter and ash contents. Chromium in the diets and feces was analyzed by atomic absorption spectrophotometry. Urine samples were thawed and homogenized for determination of total nitrogen. The GE values of the samples were obtained using a bomb calorimeter.

Table 2 - Chemical composition of diets

Item (g kg ⁻¹ dry matter)	Glycerin level ¹ (g kg ⁻¹)			
	0	50	100	150
Dry matter	899.9	891.6	880.0	885.2
Organic matter	897.6	898.4	910.7	910.1
Crude protein	209.7	194.8	191.8	178.6
Ether extract	40.9	40.7	38.2	37.2
Ash	102.4	101.6	96.9	89.9
Neutral detergent fiber	147.4	139.4	137.9	130.0
Gross energy (kcal kg ⁻¹)	3,943	3,980	3,988	3,992

¹ Composition of the glycerin: 4.9 g kg⁻¹ water, 865.0 g kg⁻¹ glycerol, 0.50 g kg⁻¹ methanol, 3.0 g kg⁻¹ total fatty acids as fed, and 5,397 kcal kg⁻¹ gross energy, according to Bunge Alimentos.

The digestibility coefficients (DC), concentrations of digestible nutrients, and digestible energy (DE) and metabolizable energy (ME) values and their correction for nitrogen content (DE_N and ME_N) were determined by equations given in Sakomura and Rostagno (2007). Animals were weighed at the beginning and end of each period and the feed intake was recorded for the calculation of daily feed intake, daily weight gain, and feed conversion of each experimental unit. The experiment was conducted in a randomized-block design in a split-plot arrangement and an analysis of variance was performed according to the model below:

$$Y_{ijk} = \mu + G_i + P_j + M_k + G_i \times M_k + \varepsilon_{ij} + \varepsilon_{ijk},$$

in which Y_{ijk} = observation of the effect of glycerin level i in period j by digestibility assessment method k ; μ = overall mean; G_i = effect of glycerin inclusion level i ($i = 0, 50, 100, \text{ and } 150 \text{ g kg}^{-1}$); P_j = experimental period j ($j = 1, 2, \text{ and } 3$); M_k = effect of digestibility assessment method k ($k = \text{total collection and marker}$); $G_i \times M_k$ = effect of the interaction between glycerin level i and digestibility assessment method k ; ε_{ij} = random error associated with the plot; and ε_{ijk} = random error associated with the sub-plot.

The effects related to the glycerin levels were evaluated by breaking down the sum of treatment squares into orthogonal contrasts to evaluate the linear and quadratic effects. The F test was used to evaluate the collection methods. Both evaluations were undertaken considering a probability of $P < 0.05$ for type-I error, with data subjected to the MIXED procedure of SAS software (Statistical Analysis System, version 6.0). For the performance analysis, the initial weight was used as a co-variable. Data of digestibility and metabolizability coefficients, DE_N , and ME_N corrected for the nitrogen excretion from diets and of glycerin were subjected to an ANOVA considering the effects of the of digestibility assessment methodology and the interaction between them and glycerin levels. The ME value was estimated by regression analysis (Adeola and

Ileleji, 2009) of ME intake (kcal) associated of glycerin vs. glycerin intake (g) by total collection (TC) and chromium marker (Cr).

Results

The animal performance during the digestibility trial was not affected by the addition of up to 150.0 g kg^{-1} crude glycerin (Table 3).

The digestibility coefficients of DM and OM improved linearly as the glycerin inclusion level was increased (Table 4). The DC of CP, EE, and NDF did not differ as a function of the increased glycerin inclusion levels. There were no differences for DC of DM, OM, CP, EE, and NDF as a function of the digestibility methods. The chromium oxide marker method provided lower DC of ash in relation to the total collection method; i.e., the marker method may have underestimated these values. There was an interaction between glycerin levels and collection methods for the DC of ash. The DC of ash increased linearly with the glycerin level as evaluated by the total collection method ($\hat{Y} = 89.4978 - 0.3502 \times GL$; $P = 0.0347$; $R^2 = 0.1054$), whereas the marker method had a quadratic effect ($\hat{Y} = 74.2542 - 1.5291 \times GL + 0.1042 \times GL^2$; $P = 0.0065$; $R^2 = 0.3199$).

The inclusion of up to 150 g kg^{-1} of the test ingredient did not influence the determination of glycerin energy values. The chromium oxide marker method resulted in the lowest DE , DE_N , ME , and ME_N values and the highest ME:DE ratio of crude glycerin in relation to the total collection method. The $ME_N:DE_N$ ratio was the same for both methods (Table 5).

The average DE of glycerin did not differ among the treatments. The slope of the linear relationship between metabolizable energy intake and glycerin intake (Figure 1) show that total collection (TC) and chromium marker (Cr) generated similar ME ($3,272$ and $3,263 \text{ kcal kg}^{-1}$, respectively).

Table 3 - Initial weight, final weight, daily weight gain (DWG), daily feed intake (DFI), and feed conversion (FC) of pigs fed diets with different glycerin levels during the digestibility trial

	Glycerin level (g kg^{-1})				Significance ¹		SEM
	0	50	100	150	L	Q	
Initial weight (kg)	31.10	31.39	30.68	31.15	0.2130	0.1256	0.3083
Final weight (kg)	37.92	37.98	37.18	37.45	0.4593	0.9839	0.2228
DWG (g day^{-1})	682	659	650	630	0.4508	0.9667	34.7489
DFI (g day^{-1})	1372	1384	1382	1364	0.8411	0.1337	86.9834
FC (kg kg^{-1})	2.01	2.10	2.13	2.17	0.6484	0.6625	0.1257

¹ Significance level at $P < 0.05$.

L - linear effect; Q - quadratic effect; SEM - standard error of the mean.

Discussion

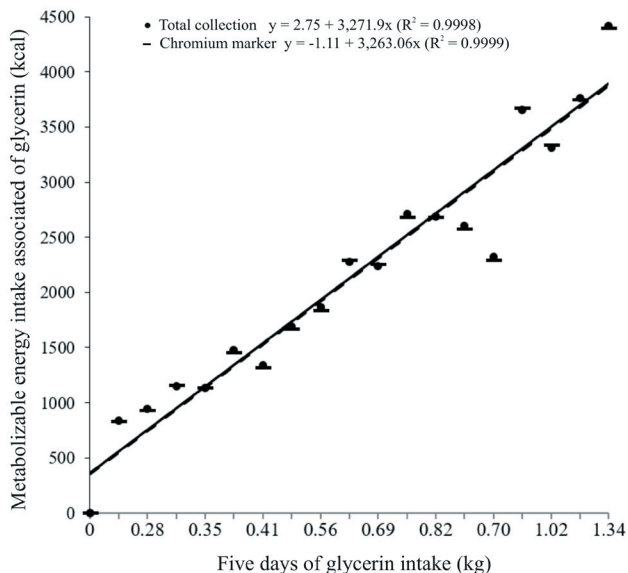


Figure 1 - Equations of metabolizable energy of glycerin obtained from intake of metabolizable energy (kcal kg⁻¹), associated to glycerin vs. glycerin intake (g) during five days determined by the total collection and chromium marker digestibility methodologies.

The performance results demonstrate that glycerin can be used at levels up to 150 g kg⁻¹ without impairing performance. Working with piglets in the starter phase with up to four glycerin inclusion levels (30, 60, 90, and 120 g kg⁻¹), Gonçalves et al. (2013) also did not find differences in animal performance. Likewise, Gallego et al. (2014) did not find differences in the performance of piglets fed diets with glycerin levels up to 140 g kg⁻¹.

The lack of an effect of glycerin levels on daily feed intake in the present study indicates that this ingredient did not improve the palatability of the feed, contrasting the findings of Groesbeck et al. (2008), who observed a trend towards increased feed intake and weight gain of animals consuming increasing levels of glycerin (0, 30, 60, 90, and 150 g kg⁻¹) due to its sweetish taste.

However, Zijlstra et al. (2009), who evaluated three glycerin levels (0, 40, and 80 g kg⁻¹) in the feeding of newly weaned pigs, found that pigs fed 40 g kg⁻¹ glycerin had a higher feed intake than the animals that received the other

Table 4 - Digestibility coefficients (DC) of the chemical composition of pig experimental diets with different glycerin levels determined by the total collection (TC) and chromium marker (Cr) digestibility methodologies

DC (g kg ⁻¹ DM)	Glycerin level (g kg ⁻¹)				Digestibility methodology			Significance ¹			SEM
	0	50	100	150	TC	Cr	M × GL	L	Q	Digestibility methodology	
DM	859.0	861.9	872.4	889.0	873.9	867.1	0.2334	0.0413	0.2895	0.0873	16.9176
OM	873.3	876.7	886.5	902.3	887.6	881.9	0.2176	0.0405	0.3235	0.0916	4.5870
CP	843.0	845.6	841.2	857.9	850.6	843.2	0.2373	0.8841	0.5224	0.1151	6.3619
EE	714.6	710.3	723.6	733.6	713.1	728.0	0.4215	0.7056	0.8114	0.1415	25.2678
Ash	810.1	804.6	798.4	807.9	876.3	734.3	0.0348	0.5675	0.5473	<0.0001	16.6015
NDF	608.9	616.4	645.3	621.7	613.6	632.6	0.5826	0.1906	0.4045	0.2842	24.2414

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; GL - glycerin level; M × GL - digestibility methodology × glycerin level interaction; L - linear effect; Q - quadratic effect; SEM - standard error of the mean.

¹ Significance level at P<0.05.

$$\hat{Y}_{DC-DM} = 85.5197 + 0.2049 \times GL (R^2 = 0.35); \hat{Y}_{DC-OM} = 87.4649 + 0.1416 \times GL (R^2 = 0.18).$$

Table 5 - Energy-related variables of glycerin determined by the total collection (TC) and chromium marker (Cr) digestibility methodologies

	Glycerin level (g kg ⁻¹)			Digestibility methodology			Significance ¹			SEM
	50	100	150	TC	Cr	M × GL	L	Q	Digestibility methodology	
DE (kcal kg ⁻¹)	3,387	3,387	3,388	3,405	3,370	0.5994	0.9059	0.9601	<0.0001	0.0046
DE _N (kcal kg ⁻¹)	3,374	3,374	3,374	3,392	3,357	0.5970	0.9261	0.9458	<0.0001	0.0045
ME (kcal kg ⁻¹)	3,270	3,270	3,271	3,280	3,261	0.8343	0.8800	0.9933	0.0094	0.0079
ME _N (kcal kg ⁻¹)	3,258	3,258	3,259	3,269	3,248	0.8339	0.8884	0.9962	0.0037	0.0078
ME:DE ratio	0.9652	0.9654	0.9654	0.9631	0.9676	0.9917	0.9328	0.9802	0.0257	0.0022
ME _N :DE _N ratio	0.9656	0.9658	0.9658	0.9639	0.9676	0.9911	0.9305	0.9769	0.0597	0.0022

DE - digestible energy; DE_N - digestible energy corrected for nitrogen balance; ME - metabolizable energy; ME_N - metabolizable energy corrected for nitrogen balance; M × GL - Digestibility methodology × glycerin level interaction; L - linear effect; Q - quadratic effect; SEM - standard error of the mean.

¹ Significance level P<0.05.

diets. Similarly, Rocha (2013) replaced powder milk whey by glycerin at the levels of 0, 90, and 180 g kg⁻¹ for piglets and found that the animals fed 90 g kg⁻¹ glycerin consumed more than the others. Researchers of both studies suggested that glycerin inclusion might have improved the palatability of the feed and consequently increased intake, but only to a certain extent, given that the decreased intake with the diets containing higher glycerin contents might have been due to a possible increase in the energy content of that diet.

The DC values evaluated, irrespective of the digestibility assessment method, show that glycerin can be considered a highly digestible feedstuff for pigs in the starter phase and that, as the glycerin inclusion in the diet increases up to 150 g/kg, there is an increase in the digestibility of the fractions of DM and OM. The equations generated to predict values indicated that changing 10 g kg⁻¹ glycerin in diet will result in DC difference of 2.049 and 1.416 g kg⁻¹ of DM and OM on an as-fed basis, respectively. Comparing the results found in this experiment with the DC of corn, according to Rostagno et al. (2011), with 850, 664, and 900 g kg⁻¹ dry matter for CP, NDF, and OM, respectively, a similarity is observed, which emphasizes the possibility of substituting the corn for glycerin in the feeding of pigs without causing losses in the digestibility of these nutrients. The DC of DM and OM increased with the addition of glycerin to the diet, which may be explained by the fact that glycerin inclusion also represents an indirect addition of organic matter, which includes EE, and this might have led to better use of these nutrients. Madrid et al. (2013) worked with inclusion of up to 50 g kg⁻¹ glycerin in growing pig diets and also found an increasing linear effect on the DC of OM with the glycerin inclusion levels; these values were above 870 g kg⁻¹ DM.

The DC of NDF was not influenced by different glycerin inclusion levels, differing from Hanczakowska et al. (2010), who found an improvement in the fiber digestibility after including 100 g kg⁻¹ glycerin compared with control treatment, in diets for pigs from 30 to 100 kg. The authors suggested that the better fiber digestibility may stem from a bacterial fermentation in the intestine due to the possibility of glycerol reaching the colon and cecum and stimulating the development of fiber-degrading microorganisms; however, this does not seem to have occurred in the present study, perhaps due to the shorter intake time of the glycerin.

The variability of results related to the chromium oxide method as compared with total collection is explained by Sakomura and Rostagno (2007), especially because of the possibility that chromium oxide is being fully retrieved in the feces, which interferes with the indigestibility factor used for the calculations of digestibility. The striking

variation of its excretion in the feces (Hopper et al., 1978) and incomplete fecal recuperation (Soares et al., 2004), attributed to differences of kind and quality of diet (Ribeiro Filho et al., 2008), are characteristics of the use of chromium oxide.

The underestimated DC of ash obtained using chromium oxide in this study may be explained by the possible interference of chromium in the absorption of other minerals like calcium and phosphorus (Fernandez et al., 1999). However, Kavanagh et al. (2001) recorded fecal marker recovery of 960 g kg⁻¹ for Cr₂O₃, but DM and energy digestibility coefficients were not different for total collection. Therefore, it is understood that the quantification of the indicator in feed and feces is an essential condition for the use of the technique, so as not to estimate the values included in the diets. According to Kavanagh et al. (2001), by using the assumed concentration of marker in the diet, the assumption is made that the marker is dispersed correctly through the feed, but the marker can be lost at the mixing stage, particularly if a mill is being used; however, the measured concentration in the diet is more accurate, particularly when the same sampling procedure and laboratory analysis has been perfected over a long period of time to ensure accurate sampling.

As the differences in DC between the digestibility methods were small, except for the ash, it was not possible to identify significant statistical differences. Inversely, Agudelo et al. (2010) recorded values of digestibility for DM, energy, and N that were 2-3 percentage points lower by chromium oxide vs. total collection and considered that digestibility differences between methods were smaller for highly digestible, highly concentrated nutrients, implying that indicator method could be less able to detect differences. Gobesso et al. (2011), on the other hand, stated that chromium oxide represents the most precise marker method for apparent digestibility in horses compared with the internal markers acid detergent lignin and acid detergent insoluble ash. Similarly to the present study, Nunes (2012) found that the DC of the chromium oxide marker differed from those of the titanium oxide and purified lignin markers and total collection methods, underestimating the digestibility of pig diets.

The average DE of glycerin was 3,387 kcal kg⁻¹, which is higher than the 3,298 kcal kg⁻¹ found by Gallego et al. (2014) for semi-purified (800 g kg⁻¹ glycerol) glycerin for piglets in the starter phase, and the 3,344 kcal kg⁻¹ (870 g kg⁻¹ glycerol) reported by Lammers et al. (2008).

The ME values of glycerin were close to those recorded by Gonçalves et al. (2013) (3,373 kcal kg⁻¹), but far from those recorded by Gallego et al., (2014) (2,531 kcal kg⁻¹)

and Carvalho et al., (2012) ($4,555 \text{ kcal kg}^{-1}$). The ME glycerin values found in this study are close to that of corn, about $3,340 \text{ kcal kg}^{-1}$ (Rostagno et al., 2011), reinforcing the assertion that glycerin can be used in the feed of pigs as an energy source. The energy content of glycerin is dependent on its composition, especially in terms of glycerol and fatty acids, which are inversely proportional and related to their efficiency of production (Kerr et al., 2009). Gott and Eastridge (2010), analyzing sixteen samples of glycerin obtained from different raw materials and industries, stated that the ash content has a wide variation in the chemical composition, due to the quantum of catalysts used in each industry. Oliveira et al. (2013), analyzing 41 samples of glycerin from sixteen biodiesel plants in Brazil, registered $304\text{-}901 \text{ g kg}^{-1}$ glycerol levels, $0\text{-}377 \text{ g kg}^{-1}$ total fat levels, and $2.3\text{-}12.1\%$ mineral matter levels, which demonstrated higher variation.

The energy content of glycerin is related to its metabolism, which is based on the fact that glycerol from the diet is absorbed by passive diffusion (Pluske, 2007) and is turned into glucose in the liver via phosphorylation to glycerol-3-phosphate (Mourot et al., 1994); however, an excess will be excreted in the urine (Oliveira et al., 2014), indicative of saturation of the metabolic pathways of glycerol due to the limitation of the glycerin conversion to glycerine-3-phosphate by the key liver enzyme glycerin kinase. However, Papadomichelakis et al. (2012) found that glycerol kinase mRNA expression in liver of pigs increased linearly when the glycerin increased from 0 to 150 g kg^{-1} in the diet, indicating no saturation effect.

The ME:DE ratio was similar to the 0.96 reported by Lammers et al. (2008). This result ratifies that only 4.0 g kg^{-1} glycerin of the digestible energy is lost via urine. In their studies, Mendonza et al. (2010) stated that there is a limitation in the glycerin metabolism, because upon being absorbed, it is converted to glucose in the liver. Nevertheless, excess levels may exceed the liver metabolization capacity, causing it to be excreted in the urine. In their study with glycerin inclusion at 300 g kg^{-1} , Mendonza et al. (2010) found higher urinary excretion in animals fed glycerin as compared with control-diet-fed animals, reaching a ME:DE ratio of 0.866.

The $\text{ME}_N:\text{DE}_N$ values of glycerin were similar to those of the ME:DE ratio, which is an expected result, given that glycerin is an energy ingredient, and losses of energy fraction by the nitrogen balance are little relevant. According to Cerrate et al. (2006), glycerol may have effects on the retention of amino acids or nitrogen, benefiting the deposition of body protein through inhibition of the activity of the phosphoenolpyruvate carboxykinase and glutamate

dehydrogenase enzymes, which participate in the protein metabolism. The ME_N of the diet shows that the energy content lost through retained or excreted nitrogen was very low and that the protein contents of the diets interfered little with their energy content.

The lower DE, DE_N , ME, and ME_N values of glycerin obtained with the marker method in relation to the total collection method suggest that chromium oxide was not fully recovered in the feces. In this regard, Zanella et al. (1999) explained that the low recovery rate of chromium oxide led to lower ME values than those obtained by total collection in a study with broilers. Although chromium oxide is largely utilized, Cortés et al. (2009) assumed that there might be variability among results due to the difficulty in reproducing them in some laboratories and the incomplete recovery of this marker from the feces, resulting in underestimated concentrations.

Conclusions

The glycerin obtained from soybean oil has $3,272$ and $3,263 \text{ kcal kg}^{-1}$ metabolizable energy as determined by the total collection and chromium oxide marker techniques, respectively. Levels up to 150.0 g kg^{-1} of the test ingredient do not influence the determination of the digestible energy or metabolizable energy of glycerin. Inclusion of 150 g kg^{-1} glycerin improves the digestibility coefficients of dry matter, organic matter, and energy of the diets. The digestibility assessment method using chromium oxide underestimates the digestibility coefficient of ash and the energy values of glycerin, but provides similar results to the total collection method for the other nutrients evaluated.

Acknowledgments

The authors thank Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPEMAT) for the financial support for the research and the fellowship granted to the first author and Bunge Alimentos for donating the glycerin.

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