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Neutral semi-purified glycerin in starting pigs feeding

Glicerina semipurificada neutralizada na alimentação de leitões

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Abstract

Glycerin is a major co-product resulting from biodiesel production, and it has been proposed as a high-energy source for use in swine diets. However, it is necessary to determine the nutritional value of neutral semi-purified glycerin (NSPG). In this study two experiments were carried out to determine the nutritional value, evaluated the performance and economic feasibility of starting piglets fed on neutral semi-purified glycerin. A digestibility trial (Experiment I) was conducted using 30 crossbred barrows with an initial average body weight of 42.91±1.58 kg. The glycerin levels used in the digestibility assay were 4, 8, 12 and 16% of the basal diet (corn + soybean meal based). The digestible energy (DE) and metabolizable (ME) energy values of glycerin were estimated by regression of DE and ME (kcal/kg) intake associated with glycerin vs. glycerin intake (kg). The values (as-fed-basis) of DE and ME (kcal/kg) obtained were 3,298 and 2,531, respectively. In Experiment II, 100 piglets (50 gilts and 50 barrows) with BW = 15.14±0.06 to 30.28±0.65 were allotted in a randomized design using four inclusion levels (3.5, 7.0, 10.5 and 14%) of NSPG. There were ten replicates with two piglets per experimental unit. Additionally, a control diet containing no glycerin (0%) was formulated. The results show it is feasible to use up to 14% NSPG in piglet feed without impairing performance and plasma chemistry.
Key words: Biodiesel, digestibility, glycerol, performance

Resumo

A glicerina é o principal coproduto da produção do biodiesel, e é proposta sua utilização como uma fonte de alto potencial energético na alimentação de suínos. No entanto é necessário determinar seu valor nutricional da glicerina semi-purificada neutralizada (GSPN). Neste estudo, foram conduzidos dois experimentos com o objetivo de determinar o valor nutricional, avaliar o desempenho e a viabilidade econômica de leitões alimentados com GSPN. O ensaio de digestibilidade (Experimento I) foi conduzido utilizando 30 suínos mestiços com peso vivo médio inicial de 42,91 ± 1,58 kg. Os níveis de substituição da ração referência pela glicerina foram 4, 8, 12 e 16%. Os valores de energia digestível (ED) e metabolizável (EM) das glicerinas foram estimados pela análise de regressão do consumo de ED e EM (kcal/kg) associada com a glicerina *vs*. consumo de glicerina (kg). Os valores de ED e EM (kcal/kg), na matéria natural, obtidos foram de: 3.298 e 2.531 kcal/kg respectivamente. No experimento II, 100 leitões (50 fêmeas e 50 machos cadastros) com peso vivo inicial de 15,14±0,06 a 30,28±0,65 kg) foram distribuídos em delineamento de blocos casualizados, com quatro níveis de inclusão (3,5; 7,0;

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10,5 e 14%), dez repetições e dois leitões por unidade experimental. Adicionalmente, foi formulada uma ração testemunha, não contendo glicerina (0%). Os resultados sugerem que é viável a utilização em até 14 %, na ração de leitões na fase inicial, sem prejuízos no desempenho e variáveis plasmáticas.

Palavras-chave: Biodiesel, desempenho, digestibilidade, glicerol

Introduction

Biodiesel is biodegradable and derived from renewable resources. It can be obtained by the transesterification process of animal fats or vegetable oils. According to the “Agência Nacional de Petroleo, Gas Natural e Biocombustíveis” (ANP, 2014), Brazil is among the largest consumers and producers of biodiesel in the world and had an annual production of 1.6 billion liters in 2009 and an installed capacity of 5.8 billion liters in 2011. Biodiesel producers prefer to use the methyl route of production, and soybean oil represents 85% of the raw material. The remaining raw material is obtained mainly from animal fats.

Glycerin is the main co-product of biodiesel production. Approximately 10% of the total volume of biodiesel produced corresponds to glycerin.

This co-product can be sold as semi-purified and neutral in the “Blonde” form (low fatty acid content) or without purification as crude glycerin (high fatty acid content) (CARVALHO et al., 2012). Thus, its use has been proposed as a potential source of high energy in swine diets. However, it is necessary to determine the actual nutritional value of glycerin (THOMPSON; HE, 2006).

The acidification processes (HCl) used for glycerin purification and the base neutralization (NaOH) (OOI et al., 2004) used to adjust the pH can alter its composition and produce glycerin that is approximately 80% glycerol with low methanol levels and increased levels of NaCl and K. So, the aim to neutralization and purification make the glycerin a higher quality product and provide a more neutral pH, which is useful to the market. However, the purification process may increase the cost.

The semi-purified glycerin has on average 86.95% glycerol, 9.63% moisture, 0.41% crude protein, 0.12% crude fat, 3.2% ash, 3-7% salt (1.26% sodium, 1.86% chlorine) and less than 0.25% potassium. The gross energy value ranges from 3,200 to 3,760 kcal/kg depending on purity and provenance (LAMMERS et al., 2008a; HANSEN et al., 2009). When evaluating weanling pigs, Zijlstra et al. (2009) found the metabolizable energy value for semi-purified glycerin was 3,510 kcal/kg.

In others studies evaluating the inclusion of glycerin on piglets feeding, Lammers et al. (2008b) working with 21-day-old piglets (7.9 kg), observed that up to 10% crude glycerin can be used in diets without impairing performance. Groesbeck et al. (2008) reports that the inclusion of up to 6% glycerin in the pig diet (11-27 kg) improves the pellet quality and has no effect on piglet performance.

This study was conducted to evaluate the nutritional value of NSPG, the effects of its use on piglet performance (15-30 kg) and the economic feasibility of adding glycerin to the pig diet.

Materials and Methods

The experiments were performed at the Pig Barn in the “Fazenda Experimental de Iguatemi”, at Maringá State University (CCA/UEM), located in the State of Paraná, Brazil (23°25’S, 51°57’O, 564 m altitude).

The glycerin studied was NSPG-neutral, semi-purified glycerin made from vegetable oil (soybean). The glycerin was obtained from BSBIOS biofuels located in Marialva, Paraná, Brazil.

The density, water content (Karl fisher), total glycerol and methanol analyses (Table 1) were performed at the Paraná Technology Institute (TECPAR). The pH values (table 1), crude protein, minerals, gross energy (adiabatic calorimeter – AC720 Parr Instrument Co.) and sodium chloride were evaluated by the Laboratório de Nutrição Animal (LANA-UEM), according to the procedures described by Silva e Queiroz (2002).

Table 1. Characterization of neutral semi-purified glycerin.

Nutrients	Neutral semi-purified glycerin
Moisture, %	11.89
Total glycerol, %	80.20
Crude protein, %	0.90
Gross energy, kcal/kg	3,535
MONG[1], %	1.73
Total fatty acid (g/100 g)	< 0.1
Saturated fatty acid, %	22.7
Unsaturated fatty acid, %	31.3
Polyunsaturated fatty acid, %	43.7
Omega 3	6.5
Omega 6	37.2
Methanol, %	0.01
Ash, %	6.18
Sodium chloride, %	5.86
Calcium, ppm	92.18
Phosphorus, ppm	158.52
Potassium, %	0.42
Sodium, %	3.52
Chloride, %	2.34
Magnesium, ppm	45.13
Copper, ppm	0.242
Chrome, ppm	0.637
Iron, ppm	23.72
Zinc, ppm	0.39
Manganese, ppm	0.802
Aluminum, ppm	30.65
Cobalt, ppm	0.832
Molybdenum, ppm	0.164
Lead, ppm	0.984
pH	6.70
Density, kg/m^3	1270
Acid number (mg KOH/g)	0.20

[1]MONG – matter organic non-glycerol, defined as 100 – [Glycerol content (%) + % water content (%) + ash content (%)].
Source: Elaboration of the authors.

Measurements of total lipids and fatty acid profiles were performed by chromatography and acid number at the Instituto de Tecnologia de Alimentos (ITAL). The matter of organic nonglycerin was calculated using the equation given by Hansen et al. (2009): MONG = 100 – [glycerol content (%) + water content (%) + ash content (%)].

In this study, two experiments were conducted: a digestibility assay (Experiment I) and a performance trial (Experiment II). In experiment I, 30 crossbred barrows from a commercial line with an initial average body weight of 42.91±1.58 kg were used. The animals were housed individually in metabolism cages in a temperature-controlled room. The ambient, average minimum temperature was 20.4±1.6 °C, and the average maximum was 21.6±1.40 °C.

The basal diet consisted of corn (72.97%), soybean meal (24.45%), common salt (0.57%), limestone (0.63%), dicalcium phosphate (0.87%) and a mineral-vitamin premix (0.50%) formulated to meet the requirements described by Rostagno et al. (2005). The replacement levels of the basal diet by glycerin were 4, 8, 12 and 16%, resulting in four test diets.

Feed supply, feces and urine collection were performed according to Sakomura e Rostagno (2007). During the collection period, the feed supply was calculated based on metabolic weight ($kg^{0.75}$) from each pig and on the average feed intake recorded in the pre-experimental phase. Feeding was offered at 08 h and 14 h, with 55% of the total in the morning and 45% in the afternoon. All diets were moistened with approximately 20% water to avoid waste, reduce dust and improve feed intake by the animals. After each meal, water was provided at 3 mL of water/g of diet.

To mark the start and the end of the total feces collection period, 3% Fe_3O_2 was used as a marker. Feces were collected once a day, packed in plastic bags and stored in a freezer (-18 ºC). Subsequently, the material was thawed, homogenized and dried (approximately 350 g) in a forced-ventilation oven at 55 ºC for 72 h. The material was then ground in a knife mill (1 mm sieve). The urine was collected in plastic buckets containing 20 mL of HCl 1:1 to prevent bacterial proliferation and possible volatility losses.

The digestibility coefficient of dry matter, organic matter, gross energy and the metabolization coefficient of gross energy were calculated according to Matterson et al. (1965). The digestible (DE) and metabolizable energy (ME) values were estimated by regression analysis (ADEOLA; ILELEJI, 2009) of DE and ME intake (kcal/kg) associated with glycerin vs. glycerin intake**.**

To evaluate differences between the digestibility of NSPG, the data were subjected to variance analysis using the statistical package SAEG (Sistema para Análises Estatísticas e Genéticas, version 7.1).

In experiment II, 100 crossbred piglets (50 barrows and 50 gilts) from a commercial line with an initial average body weight of 15.14±0.06 and a final weight mass of 30.28±0.65 kg were used.

Five corn and soybean meal diets (Table 2) were formulated to meet the requirements indicated by Rostagno et al. (2011) for starting pigs. The neutral semi-purified glycerin chemical and energetic compositions obtained in the digestibility assay (Table 1) were used in the diet formulation.

Pigs were allotted using a randomized design, with four levels of NSPG inclusion (3.5, 7.0, 10.5 and 14%), with ten repetitions and two piglets per experimental unit. Additionally, a control diet was formulated containing 0% glycerin.

At the beginning (baseline) and end (21 days) of the experimental period, blood samples were harvested via the cranial vena cava and transferred to tubes with heparin for cholesterol and triglyceride analysis. To determine plasma glucose, a tube containing Sodium Fluoride + Potassium oxalate was used. The piglets were fasted overnight for approximately 8 hours before blood sampling occurred.

Blood samples were centrifuged (3,000 rpm for 15 min) to obtain the plasma. Then, 3 mL of plasma (in duplicate) was transferred to microtubes were properly identified and stored in a freezer (-18 °C) for further analysis by the Animal Nutrition Laboratory at Maringá State University.

For cholesterol analysis of glucose and triglycerides with commercial kit was used. The values obtained at the beginning of the experiment were used as covariables in the statistical analysis.

Table 2. Centesimal, chemical and energetic composition (*as fed basis*) of diets containing increasing levels of neutral semi-purified glycerin of starting (15 – 30 kg) pigs feeding.

Item, %	Inclusion levels of neutral semi-purified glycerin, %				
	0.0	3.5	7.0	10.5	14.0
Corn	67.96	63.91	59.86	55.51	51.09
Neutral semi-purified glycerin	-	3.50	7.00	10.50	14.00
Soybean meal	27.55	27.89	28.22	28.58	28.94
Soybean oil	0.883	1.294	1.705	2.222	2.767
Limestone	0.781	0.778	0.774	0.770	0.767
Dicalcium phosphate	1.413	1.419	1.425	1.432	1.439
Common salt	0.451	0.247	0.044	0.000	0.000
Mineral-vitamin premix[1]	0.500	0.500	0.500	0.500	0.500
Growth promoter[2]	0.005	0.005	0.005	0.005	0.005
L-lysine HCL	0.299	0.296	0.294	0.292	0.290
DL-methionine	0.083	0.091	0.098	0.105	0.113
L-threonine	0.071	0.076	0.081	0.086	0.091
Calculated values[3]					
Metabolizable energy[3], kcal/kg	3,230	3,230	3,230	3,230	3,230
Crude protein[3], %	17.48	17.34	17.20	17.05	16.90
Calcium[3], %	0.738	0.738	0.738	0.738	0.738
Available phosphorus[3], %	0.365	0.365	0.365	0.365	0.365
Digestible lysine[3], %	1.029	1.029	1.029	1.029	1.029
Digestible methionine + cystine[3], %	0.576	0.576	0.576	0.576	0.576
Digestible threonine[3], %	0.648	0.648	0.648	0.648	0.648
Sodium, %	0.198	0.198	0.198	0.262	0.342
Glycerol[3], %	-----	2.807	5.614	8.421	11.228
Diet cost[3], R$/kg	0.648	0.650	0.651	0.653	0.657

[1] Vitamin and mineral premix for piglets; [2-] Leucomycin; [3-]Calculated based on Rostagno et al. (2011) and/or analyzed.
Source: Elaboration of the authors.

To evaluate the economic feasibility of using the NSPG in the piglets feed, prices of feedstuffs in the market were collected and the cost of feed per kilogram of body weight gain was calculated, following the method described by Bellaver et al. (1985): Yi (R$/kg) = Qi X Pi/ Gi, where: Yi = feed cost per kg of body weight gain in the i-nth treatment; Qi = amount of feed consumed in the i-nth treatment; Pi = price per kg of feed used in the i-nth treatment; and Gi = weight gain in the i-nth treatment.

We also calculated the Index of Economic Efficiency (IEE) and the Cost Index (CI) (GOMES et al., 1991): IEE (%) = MCe/CTei X 100 and IC (%) = CTei/MCe X 100, where MCe = lower feed cost per kg gain observed between the treatments, and Ctei = cost of *i* treatment considered.

The input prices (R$) were collected in Maringá, Paraná: 0.47 for corn, 0.70 for soybean meal, 1.98 for soybean oil and 0.25 for NSPG (price on 16.02.2012).

The results of the different variables were subjected to analysis of variance. The degrees of freedom for each NSPG level were decomposed into orthogonal polynomials to obtain the regression equations. To compare the results of the control diet (without glycerin) with each NSPG level, the Dunnett test was applied (SAMPAIO, 1998). All statistical analyses were performed using the statistical and gene analysis system SAEG (Sistema para Análises Estatísticas e Genéticas, version 7.1). In the performance experiment, the initial weight of the piglets was used as a co-variable.

Results and Discussion

The analysis results of the physical, energy and chemical composition of the NSPG (Table 1) indicated the methanol content of NSPG fits the recommendations of 150 ppm (FDA, 2013). However, the ash content was higher than reported by the study from Gonçalves et al. (2013) that used mixed and vegetable semi-purified glycerin. Lammers et al. (2008b) warn that the methanol, sodium chloride or potassium concentration in the glycerin should be monitored to avoid excessive amounts of these compounds in the pig diet.

Furthermore, Hansen et al. (2009) reported that the use of glycerin in the feed would depend on the raw material that is used in processing. Therefore, an analysis of its composition is recommended before formulating the diet.

The total glycerin fatty acid content was lower. However, the results were similar to those obtained by Kovacs et al. (2011), who found 0.05% total lipids. The NSPG composition studied in this work is distinct from the glycerin of other researchers used in other studies and may have caused different responses in the animals.

The apparent digestibility and metabolism coefficients (dry matter and gross energy) and digestible nutrients (Table 3) of NSPG showed that this co-product is a good energy source (2,531 kcal of ME /kg) for growing pigs.

Table 3. Apparent digestibility coefficients, metabolization coefficient and digestible values of nutrients of neutral semi-purified glycerin fed to starting pigs.

Digestibility coefficients, %	Neutral semi-purified glycerin
Digestible coefficients of dry matter	84.82
Digestible coefficients of gross energy	93.29
Metabolization coefficient of gross energy	71.60
Digestible nutrients[1]	
Dry matter, %	74.73
Digestible energy, kcal/kg	3,298
Metabolizable energy, kcal/kg	2,531
ME: DE ratio	0.77

[1]As-fed basis.
Source: Elaboration of the authors.

The ME values of NSPG were similar to those reported by Gonçalves et al. (2014) in studies using growing-finishing pigs fed with mixed semi-purified glycerin. This report indicated 2,210 kcal of ME/kg and a ratio ME:DE of 0.72. This ratio for glycerin indicated that the co-products had high utilization by animals.

In the other hand, Mendoza et al. (2010) warns that during glycerin metabolism there is an increase of energy excretion in the urine, which may result in a limitation of glycerin use in feed. However, states that the metabolism of the glycerin energy may be affected by catalyst residues (Na^+ and K^+). This hypothesis is supported by the increase in water intake and urine production for animals on diets with high NSPG levels.

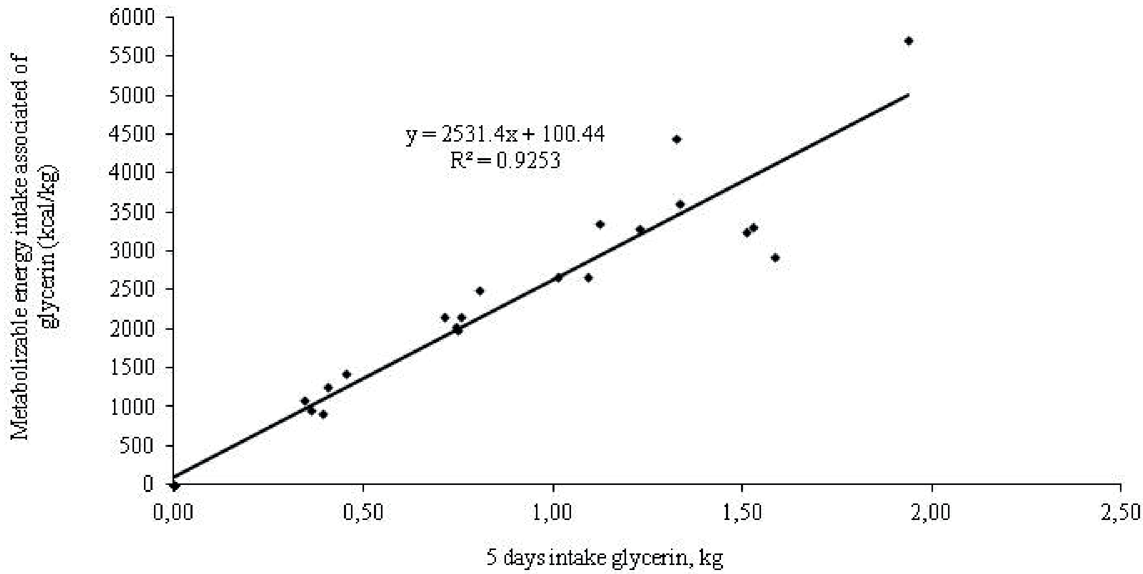
The study by Lammers et al. (2008a) used glycerin derived from soybean oil with fat and frying oil to determine the energy value of glycerin (86.95% glycerol) and obtained a value of 3,344 kcal/kg of DE. These results are similar to those obtained in the present study (Table 3). However, the authors obtained an ME value of 3,207 kcal/kg, which was higher than the value we obtained with the NSPG in this study.

A previous study by Bartelt e Schneider (2002) obtained 4,180, 3,439 and 2,256 kcal/kg ME studying semi-purified glycerin for growing pigs with 5, 10 and 15% glycerin, respectively. The value obtained for the 15% glycerin level was similar to the NSPG studied here. However, Kerr et al. (2009) examined crude glycerin using finishing pigs and obtained higher values (3,772 kcal/kg of DE) compared to those obtained in the present study (Table 3).

The ratios of ME:DE for semi-purified glycerin (LAMMERS et al., 2008a; KERR et al., 2009) and crude glycerin (CARVALHO et al., 2012) were higher than those obtained in the current experiment. However, Gonçalves et al. (2013) reported a ratio of 0.89 ME:DE for vegetable oil semi-purified glycerin (VSPG) and 0.91 for mixed semi-purified glycerin (MSPG) using starting pigs. The authors highlighted that the VSPG has greater fatty acid content than the MSPG, which provided a higher energy value.

The ME values of the NSPG were estimated by linear regression analysis (ADEOLA; ILELEJI, 2009) to be 2,531 kcal of ME/kg (Figure 1). This linear relationship shows that the ME value in glycerin estimated by the slope of the linear relationship between metabolizable energy intake *vs.* glycerin intake (Figure 1) has similar values to those obtained by Gonçalves et al. (2013) in experiments using semi-purified glycerin prepared from fat animal + soybean oil. The authors determined the ME value was 2,931 kcal/kg.

Figure 1. Equations of metabolizable energy (ME) for neutral semi-purified glycerin obtained from regression of ME (kcal/kg) intake associated with glycerin *vs.* glycerin intake (kg) for 30 pigs over a five-day period.



Source: Elaboration of the authors.

In the performance experiment (Table 4), the regression analysis indicates there was no effect (P> 0.05) from NSPG for DFI, DWG and the feed:gain ratio (Table 4). These values show glycerin can be used for starting pigs without damaging performance because the diets provided equal nutrition. Lammers et al. (2008a), Gonçalves et al. (2013) and Shields et al. (2012) does not damage the performance of piglets and and noted that glycerin can be included at up to 10%, 12% and 5%, respectively.

Table 4. Performance of piglets fed diets with neutral semi-purified glycerin (NSPG).

Item	NSPG inclusion (%)					Mean ±SE[1]	Lin[2]	Quad[3]
	0	3.5	7.0	10.5	14.0			
DFI[4], kg	1.314	1.248	1.331	1.241	1.305	1.287±0.131	NS	NS
DWG[5], kg	0.729	0.683	0.727	0.706	0.711	0.711±0.039	NS	NS
Feed:gain	1.81	1.83	1.83	1.77	1.84	1.82±0.021	NS	NS

[1-]SE-standard error; [2-] Linear effect; [3-] Quadratic effect of inclusion level of NSPG; [4-] DFI = Dalily feed intake; [5-]DWG = Daily weight gain; NS = non-significant. NS= non-significant (P≥0.05).
Source: Elaboration of the authors.

Groesbeck et al. (2008) indicated that the inclusion of up to 12% crude glycerin in piglet diets contributed to the linear increase of DWG and DFI, but no difference was observed for the feed:gain ratio. Different results were observed by Schieck et al. (2010), which examined feeding sows during lactation. The results showed that the inclusion of up to 9% crude glycerin (glycerol 86.1%) did not affect performance and contributed to heat stress relief.

The values obtained for plasma chemistry (Table 5) showed no effect (P>0.05) of NSPG inclusion on glucose, cholesterol and plasma triglycerides.

These results indicate the metabolism of the plasma glycerol excess occurs via gluconeogenesis. It is known that glycerol can be converted to glucose through gluconeogenesis or oxidized, producing energy through glycolysis. These results indicate piglets showed no relationship between the circulating glycerin levels and the triglyceride concentration in liver tissue.

Table 5. Plasma levels (mg/dL) of glucose, cholesterol and triglycerides of piglets fed diets with neutral semi-purified glycerin (NSPG)

Harvest	NSPG inclusion, %					Mean±SE[1]	Lin[2]	Quad[3]
	0	3.5	7.0	10.5	14.0			
Final	91.28	90.21	88.83	87.18	89.56	89.41±1.758	NS	NS
			Cholesterol					
Baseline	39.09	36.18	36.98	40.32	38.98	38.31±0.750	NS	NS
Final	43.75	45.66	44.52	43.35	45.66	44.59±0.804	NS	NS
			Triglycerides					
Baseline	50.90	52.59	57.07	56.16	56.07	54.56±1.515	NS	NS
Final	69.63	66.45	70.38	70.32	67.18	68.79±2.154	NS	NS

SE – standard error; [2-] Linear effect; [3-] Quadratic effect of inclusion level of NSPG; NS= non-significant (P≥0.05).
Source: Elaboration of the authors.

Similar results were obtained by Mourot et al. (1994) stated that the inclusion of 5% crude glycerin does not affect the concentration of triglycerides. Similar results were obtained by Shields et al. (2012) when lactose was replaced by glycerin to fed weanling pigs. However, a study by Hansen et al. (2009) showed no difference in glucose concentration in pigs (30 kg) fed with up to 16% semi-purified glycerin.

The results of the economic analysis (Table 6) showed no change (P>0.05) in the feed cost per kilogram of live weight gained using up to 14% NSPG.

The Dunnett test indicated that there was no difference between the different inclusion levels of NSPG compared with the control diet (0%). This response is dependent on the feedstuffs prices (corn, soybean meal, soybean oil and NSPG).

Table 6. Economic analysis of using neutral semi-purified glycerin (NSPG) in starting pigs feeding.

Item	NSPG inclusion, %					CV[1]	Dun[22]	Reg[3]
	0.0	3.5	7.0	10.5	14.0			
Initial body weight, kg	15.17	15.08	15.17	15.15	15.13			
Final body weight, kg	30.68	29.63	30.67	30.20	30.25			
Diet cost	0.648	0.650	0.651	0.653	0.657	-	-	-
FC, R$/kg BW[4]	1.154	1.172	1.163	1.092	1.188	8.48	NS	NS
EEI[5]	94.58	93.17	93.88	100.00	91.87	-	-	-
Index cost	100.00	101.51	100.74	94.58	102.95	-	-	-

[1] Coefficient of variation; [2] Dunnett test; [3] Regression analysis; [4] FC, R$/kg BW: Feed cost per kg of body weight gain; [5] Economic Efficiency Index; NS= non-significant (P≥0.05).
Source: Elaboration of the authors.

Conclusions

The values (as-fed basis) of digestible and metabolizable energy for neutral semi-purified glycerin for starting pigs are 3,298 kcal/kg and 2,531 kcal/kg, respectively. The results of this study indicated that it is feasible to use up to 14% neutral semi-purified glycerin in starting pigs feeding without impairing performance and plasma chemistry. The economic feasibility of its use will depend on the price ratio of the ingredients, particularly corn and soybean oil (or other energy sources).

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