

# Increased urinary losses in piglets fed diets containing high levels of glycerin

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**ABSTRACT** - This study evaluated the nutritional value of glycerin added to diets of weaned piglets and its effects on animal performance and urinary losses. Weaned piglets were individually housed in metabolic cages and fed diets in which a basal diet containing lactose (Gly0) was replaced by 50 (Gly50), 100 (Gly100), or 150 g kg<sup>-1</sup> (Gly150) of glycerin; or a diet in which 100 g kg<sup>-1</sup> of the lactose in the basal diet was replaced by glycerin (Gly100Lac0). Diets were offered for two periods of 12 days (phase I and phase II). Performance, nutrient digestibility, nitrogen retention (NR), and urinary production were measured. Digestible (DE) and metabolizable energy (ME) of glycerin were calculated using substitution method and compared to iso-nutritional diets formulated with or without glycerin. No significant differences on average daily gain, average daily feed intake, and feed conversion ratio were found. Glycerin inclusion caused a linear increase in urinary production. Piglets fed Gly100Lac0 diet had higher urinary production, compared with piglets fed Gly0. Glycerin had no significant effects on total tract apparent digestibility (TTAD) coefficients of dry matter (DM), crude protein, and gross energy (GE). Also, no significant effects were found in total tract apparent metabolizability (TTAM) coefficient of GE, NR coefficient, DE, ME, and fecal GE losses. Urinary production and GE in urine increased linearly and quadratically, respectively, with increased levels of glycerin in diets. The Gly100Lac0 diet resulted in lower DM TTAD and GE TTAM and higher GE losses in urine and feces compared with Gly0. The nutritional value of glycerin allows its inclusion at up to 100 g kg<sup>-1</sup> in diets of weaned piglets without impairing animal performance and metabolism. Also, glycerin can replace 100 g kg<sup>-1</sup> of lactose without impairing performance.

**Key Words:** energy metabolism, lactose, performance, urinary excretion

## Introduction

Brazil figures as the second biggest biodiesel producer in the world (Sawin et al., 2016), with an annual production estimated at 3.87 billion L (USDA, 2016). Crude glycerin is the main byproduct of biodiesel production, and its major component is glycerol, which has been evaluated as a feed ingredient for swine diets. Adding up to 80 and 50 g kg<sup>-1</sup> glycerol to nursery diets can potentially replace wheat (Zijlstra et al., 2008), lactose, and corn (Groesbeck et al., 2008; Shields et al., 2011), with positive effects on performance. Reported glycerol digestible (DE) and metabolizable energy (ME) for growing pigs are 3,344±8 and 3,207±10 kcal kg<sup>-1</sup>, respectively (Lammers et al., 2008). For nursery pigs, no differences on ileal digestibility of dry matter (DM) and crude protein (CP) were observed for diets in which sweet milk

wehly was substituted for 90 and 180 g kg<sup>-1</sup> crude glycerin, respectively, indicating that the nutritional content of this feed ingredient is well utilized by young pigs (Oliveira et al., 2014). However, glycerin intake can increase urine output and urinary energy losses (Lammers et al., 2008; Mendoza et al., 2010). The present study aimed to determine nutritional value of glycerin added to diets of weaned piglets and its effects on performance and urinary losses.

## Material and Methods

The experimental protocol followed the ethical principles in animal research (CONCEA, 2016) and was conducted according to the institutional committee on animal use (case no. 22583).

Thirty castrated male piglets (*Agroceres* PIC), weaned between 21 to 24 days of age and weighing an average 6.3±0.68 kg, were individually housed in metabolic cages (0.5 × 0.8 m) containing two dry feeders and a nipple drinker. Cages were in a temperature-controlled room, and temperature was kept within the thermoneutral zone throughout the trial (27-30 °C and 60-80% relative humidity).

Two basal diets were offered during the trial: phase I diet, from 1 to 12 days post weaning, and phase II diet, from 13 to 24 days post weaning. The trial was divided in a five-day adaptation period, followed by a seven-day sample collection period (phase I diet) and a 12-day sample collection period (phase II diet), summing 19 days of sample collection. Using this design, animal performance was measured in two phases, but metabolism responses were measured throughout the two phases.

Piglets were randomly assigned to one of five dietary treatments: a basal diet formulated to meet the nutritional requirements of weanling piglets (NRC, 2012) was used as the control (Gly0); the other four diets were formulated as follows: a diet containing 950 g kg<sup>-1</sup> of the control diet and 50 g kg<sup>-1</sup> glycerin (Gly50); a diet containing 900 g kg<sup>-1</sup> of the control diet and 100 g kg<sup>-1</sup> glycerin (Gly100); a diet containing 850 g kg<sup>-1</sup> of the control diet and 150 g kg<sup>-1</sup> glycerin (Gly150); and a diet containing the same nutritional content of the control diet in which 100 g kg<sup>-1</sup> glycerin was added at the expense of lactose (Gly100Lac0) (Table 1). Feed and water were provided *ad libitum* throughout the experiment.

During sample collection periods, total feces produced by each piglet were collected once a day and stored at -15 °C. Urine was collected into plastic buckets containing 5 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). The buckets were weighted daily, and 100 mL L<sup>-1</sup> of urine volume produced were collected and stored at -15 °C. At the end of the experiment, fecal and urinary samples from each piglet were thawed, homogenized, and a 100-mL urine and a 400-g feces subsamples were used for further analysis. Fecal subsamples were dried at 60 °C for 72 h, weighed, and ground through a 2-mm screen.

Dry matter and N content of diets and feces were analyzed according to AOAC (2007) (method 930.15 and method 984.13, respectively). Gross energy of glycerin, diets, feces, and urine were determined using isoperibol bomb calorimeter (C2000, IKAWerke GmbH & Co. KG, Staufen, Germany). Nitrogen in urine was determined according to AOAC (2007) (method 984.13).

Animal performance; total tract apparent digestibility (TTAD) coefficients of DM, CP, and gross energy (GE); total tract apparent metabolizability (TTAM) coefficient of GE, nitrogen retention (NR) coefficient, DE (kcal kg<sup>-1</sup> DM), ME (kcal kg<sup>-1</sup> DM), urine output, and fecal and urine energy content were determined according to Sakomura and Rostagno (2007).

The DE and ME values were estimated using the substitution method: using the Gly0 diet as the reference diet, and the diets containing glycerin included at the expense of the control diet (Gly50, Gly100, Gly150) as the substitution diets. Calculations were performed according to equations proposed by Campbell et al. (1983).

The experimental design was completely randomized, with five treatments, six replicates, and a total of 30 experimental units. Results were analyzed by ANOVA using GLM procedure of SAS (Statistical Analysis System, version 9.2.). With significant differences identified by F-test (P<0.05), polynomial regression was performed using glycerol level (g kg<sup>-1</sup>) as independent variable. To test the effect of substitution of lactose with glycerol, treatments Gly0 and Gly100Lac0 were compared using paired t test, and the probability level of P<0.05 was considered as statistically significant.

**Table 1** - Composition (g kg<sup>-1</sup> as fed) and calculated nutritional composition of diets<sup>1</sup>

Ingredient	Diet <sup>2</sup>			
	Phase I		Phase II	
	Gly0	Gly100Lac0	Gly0	Gly100Lac0
Corn	470.5	470.5	554.3	554.3
Soybean meal	126.7	126.7	151.2	151.2
Lactose	100.0	-	100.0	-
Glycerin <sup>3</sup>	-	100.0	-	100.0
Milk powder	107.9	107.9	-	-
Plasma meal	50.00	50.00	50.00	50.00
Gluten corn 60	50.00	50.00	50.00	50.00
Sugar	30.00	30.00	30.00	30.00
Soybean oil	20.00	20.00	20.00	20.00
Dicalcium phosphate	16.60	16.60	16.80	16.80
Calcium carbonate	5.80	5.80	9.60	9.60
L-lysine HCl	5.10	5.10	4.90	4.90
DL-methionine	2.30	2.30	1.90	1.90
L-threonine	2.30	2.30	1.60	1.60
L-tryptophan	0.20	0.20	0.20	0.20
Choline	1.30	1.30	1.30	1.30
Salt	2.16	2.16	2.16	2.16
Acidifier	4.00	4.00	3.00	3.00
Antioxidant	0.20	0.20	0.20	0.20
Mineral premix <sup>4</sup>	0.97	0.97	0.97	0.97
Vitamin premix <sup>5</sup>	0.50	0.50	0.50	0.50
Zinc oxide	2.10	2.10	-	-
Copper sulphate	0.37	0.37	0.37	0.37
Antibiotic <sup>6</sup>	1.00	1.00	1.00	1.00
Calculated nutritional composition (g kg <sup>-1</sup> as fed)				
CP	200.0	200.0	190.0	190.0
Crude fat	63.90	63.90	43.4	43.4
Lactose	140.0	40.00	100.0	0
Ca	7.00	7.00	7.50	7.50
Available P	5.00	5.00	4.40	4.40
Sodium	3.00	4.5	2.50	4.00
SID lysine	14.08	14.08	12.37	12.37
SID methionine	5.46	5.46	4.72	4.72
SID met + cys	8.77	8.77	7.94	7.94
SID tryptophan	2.28	2.28	2.08	2.08
SID threonine	9.46	9.46	8.29	8.29
Calculated ME content (kcal kg <sup>-1</sup> , as fed, considering the same energy content in lactose and glycerin)				
ME	3570	3570	3390	3390

SID - standardized ileal digestible amino acid; ME - metabolizable energy.

<sup>1</sup> Values calculated according to Rostagno et al. (2011).

<sup>2</sup> Gly0: basal diet used as the control diet; Gly100Lac0: basal diet in which 100 g kg<sup>-1</sup> of lactose was replaced by glycerin.

<sup>3</sup> Glycerol, 821.4 g kg<sup>-1</sup>; water, 115.4 g kg<sup>-1</sup>; ash 60.7, g kg<sup>-1</sup>; sodium, 15.0 g kg<sup>-1</sup>; and methanol, 0.05 g kg<sup>-1</sup> (Granol Indústria Comércio e Exportação S/A).

<sup>4</sup> Provided per kilogram of diet: Fe, 77.6 mg; Cu, 11.6 mg; Mn, 67.9 mg; Zn, 97.0 mg; I, 0.97 mg; Se, 0.31 mg.

<sup>5</sup> Provided per kilogram of diet: vitamin A, 11,250 UI; vitamin D3, 2250 UI; vitamin E, 22.5 UI; vitamin K3, 2.0 mg; vitamin B1, 1.75 mg; vitamin B2, 5.0 mg; vitamin B6, 1.75 mg; vitamin B12, 22.5 mcg; niacin, 37.5 mg; pantothenic acid, 20.0 mg; folic acid, 0.5 mg; biotin, 0.125 mg.

<sup>6</sup> Halquinol 120 g kg<sup>-1</sup>.

## Results

No significant differences ( $P > 0.05$ ) on average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio were observed among treatments (Table 2). A linear effect of glycerin on urinary production was observed in Phase I ( $P < 0.0001$ ;  $R^2 = 0.656$ ) and Phase II ( $P < 0.0001$ ;  $R^2 = 0.431$ ) (Table 2). Piglets fed the Gly100Lac0 diet showed an increase in urinary production compared with piglets fed Gly0, during Phase I ( $P < 0.001$ ) and Phase II ( $P < 0.002$ ). There were no significant effects ( $P > 0.05$ ) on DM TTAD, CP TTAD, GE TTAD, GE TTAM, and NR coefficients when all glycerin levels were compared. However, in the paired t test analysis, Gly100Lac0 resulted in lower DM TTAD ( $P = 0.030$ ) and GE TTAM ( $P = 0.019$ ) compared with Gly0. In addition, Gly100Lac0 increased GE content in feces ( $P = 0.043$ ) and urine ( $P = 0.001$ ) compared with Gly0.

No effects of glycerin inclusion levels on DE, ME, and GE losses in feces (Table 3) were observed ( $P > 0.05$ ); however, urinary gross energy increased quadratically with increased levels of glycerin inclusion ( $P = 0.001$ ;  $R^2 = 0.870$ ). In the paired t test analysis, piglets fed Gly100Lac0 had higher GE losses in feces ( $P = 0.043$ ) and urine ( $P = 0.001$ ) compared with piglets fed Gly0.

Estimated DE and ME values for glycerin based on the substitution method calculation were, respectively: 3528 and 3378 kcal kg<sup>-1</sup> DM in diets containing 50 g kg<sup>-1</sup> glycerin; 4118 and 3469 kcal kg<sup>-1</sup> DM in diets containing 100 g kg<sup>-1</sup> glycerin; and 3918 and 2869 kcal kg<sup>-1</sup> DM in diets containing 150 g kg<sup>-1</sup> glycerin (Table 4).

**Table 2 - Performance and urinary production in phase I, phase II, and total period**

Variable	Diet <sup>1</sup>					SE	P-value	t test (P<)
	Gly0	Gly50	Gly100	Gly150	Gly100Lac0			Gly0 × Gly100Lac0
Phase I (1 to 12 days post-weaning)								
ADG (kg/day)	0.464	0.458	0.437	0.465	0.485	0.048	0.792	0.763
ADFI (kg/day)	0.506	0.478	0.522	0.491	0.577	0.090	0.696	0.450
FCR	1.104	1.040	1.220	1.044	1.198	0.201	0.677	0.576
Initial BW (kg)	6.42	6.39	6.31	6.32	6.3	0.565	0.997	0.765
Final BW (kg)	11.99	11.89	11.55	11.90	12.12	1.071	0.962	0.955
Phase II (12 to 24 days post-weaning)								
ADG (kg/day)	0.716	0.778	0.717	0.734	0.765	0.081	0.743	0.477
ADFI (kg/day)	0.952	1.028	0.960	1.039	0.955	0.085	0.514	0.839
FCR	1.332	1.322	1.359	1.423	1.263	0.092	0.300	0.415
Initial BW (kg)	11.99	11.89	11.55	11.90	12.12	1.071	0.962	0.955
Final BW (kg)	20.58	21.22	20.16	20.71	21.3	2.023	0.931	0.690
Total period (1 to 24 days post-weaning)								
ADG (kg/day)	0.583	0.608	0.568	0.591	0.617	0.049	0.714	0.520
ADFI (kg/day)	0.716	0.737	0.728	0.749	0.754	0.074	0.960	0.622
FCR	1.230	1.208	1.295	1.264	1.237	0.111	0.877	0.949
Initial BW (kg)	6.42	6.39	6.31	6.32	6.3	0.565	0.997	0.765
Final BW (kg)	20.58	21.22	20.16	20.71	21.3	2.023	0.931	0.690
Urinary production								
Phase I (g/day) <sup>2</sup>	301.8	443.2	734.2	1143.8	633.2	129.9	0.001	0.001
Phase II (g/day) <sup>3</sup>	1120.2	1332.1	1601.4	2511.5	1881.8	400.5	0.002	0.023

ADG - average daily gain; ADFI - average feed intake; FCR - feed conversion ratio; BW - body weight.

<sup>1</sup> Gly0: basal diet used as the control diet; Gly50: 950 g kg<sup>-1</sup> of the control diet and 50 g kg<sup>-1</sup> glycerin; Gly100: 900 g kg<sup>-1</sup> of the control diet and 100 g kg<sup>-1</sup> glycerin; Gly150: 850 g kg<sup>-1</sup> of the control diet and 150 g kg<sup>-1</sup> glycerin; Gly100Lac0: control diet in which 100 g kg<sup>-1</sup> of lactose was replaced by glycerin.

<sup>2</sup> Linear effect of glycerin level:  $Y = 232.6 + 5.163X$ ;  $P < 0.0001$ ;  $R^2 = 0.656$ .

<sup>3</sup> Linear effect of glycerin level:  $Y = 1001.5 + 8.678X$ ;  $P < 0.0001$ ;  $R^2 = 0.431$ .

**Table 3** - Apparent digestibility coefficients of nutrients, metabolizability coefficient of gross energy, nitrogen retention coefficient, and energy content of diets, feces, and urine

Variable	Diet <sup>1</sup>					SE	P-value	t test (P<)
	Gly0	Gly50	Gly100	Gly150	Gly100Lac0			Gly0 × Gly100Lac0
DM TTAD (g g <sup>-1</sup> )	0.897	0.878	0.900	0.898	0.867	0.015	0.066	0.030
CP TTAD (g g <sup>-1</sup> )	0.862	0.836	0.864	0.854	0.833	0.024	0.311	0.104
GE TTAD (g g <sup>-1</sup> )	0.894	0.879	0.898	0.897	0.870	0.016	0.113	0.085
GE TTAM (g g <sup>-1</sup> )	0.880	0.863	0.871	0.842	0.845	0.017	0.053	0.019
NR (g g <sup>-1</sup> )	0.789	0.756	0.780	0.766	0.751	0.025	0.315	0.115
DE (kcal kg <sup>-1</sup> DM)	4148	4127	4260	4255	4206	74.0	0.096	0.345
ME (kcal kg <sup>-1</sup> DM)	4082	4054	4132	3997	4084	81.0	0.452	0.981
GE feces (kcal <sup>-1</sup> kg DM)	491.7	570.1	481.9	489.7	626.7	74.0	0.068	0.043
GE urine (kcal <sup>-1</sup> kg DM) <sup>2</sup>	65.7	72.6	127.9	271.0	122.4	18.5	0.001	0.001

DM TTAD - total tract apparent digestibility coefficient of dry matter; CPTTAD - total tract apparent digestibility coefficient of crude protein; GE TTAD - total tract apparent digestibility coefficient of gross energy; GE TTAM - total tract apparent metabolizability coefficient of gross energy; NR - nitrogen retention coefficient; DE - digestible energy; ME - metabolizable energy; GE - gross energy; DM - dry matter.

<sup>1</sup> Gly0: basal diet used as the control diet; Gly50: 950 g kg<sup>-1</sup> of the control diet and 50 g kg<sup>-1</sup> glycerin; Gly100: 900 g kg<sup>-1</sup> of the control diet and 100 g kg<sup>-1</sup> glycerin; Gly150: 850 g kg<sup>-1</sup> of the control diet and 150 g kg<sup>-1</sup> glycerin; Gly100Lac0: control diet in which 100 g kg<sup>-1</sup> of lactose was replaced by glycerin.

<sup>2</sup> Quadratic effect of glycerin level:  $Y = 68.31 - 0.8019X + 0.0141X^2$ ;  $P < 0.0001$ ;  $R^2 = 0.870$

**Table 4** - Estimated digestible (DE) and metabolizable energy (ME) of glycerin added at expense of the control diet, calculated using the substitution method

Glycerin level (g kg <sup>-1</sup> )	DE estimated (kcal kg <sup>-1</sup> DM)	ME estimated (kcal kg <sup>-1</sup> DM)
50	3528	3378
100	4118	3469
150	3918	2869
SE	604	774
P-value	0.546	0.651
Average	3855±219	3239±279

SE - standard error; DM - dry matter.

## Discussion

The ADG and ADFI observed in this study were higher than the estimated expected ADG and ADFI for piglets at a relatively stress-free environment (NRC, 2012), indicating that the nutrient dilution resultant from glycerin inclusion did not impair performance. Other studies also reported no effects on performance in nursery piglets fed diets containing 120 and 140 g kg<sup>-1</sup> glycerin (Diaz-Huepa et al., 2015; Gallego et al., 2016), whereas several authors found improved performance results in nursery pigs fed up to 100 g kg<sup>-1</sup> crude glycerol (Groesbeck et al., 2008; Zijlstra et al., 2008; Shields et al., 2011; Rocha et al., 2016). The replacement of 100 g kg<sup>-1</sup> of lactose with glycerin in diets of weaned piglets resulted in lower DM TTAD and GE TTAM coefficients and higher GE losses in urine and feces; however, piglet performance was not negatively affected, indicating that glycerin may be a viable alternative to replace up to 100 g kg<sup>-1</sup> of lactose in diets of weaned piglets.

The inclusion levels tested had no negative effects on digestibility and metabolizability of the diets; however, increases in urine output and energy excretion were observed as glycerin inclusion levels increased. The results indicated that glycerin levels higher than 100 g kg<sup>-1</sup> could limit energy utilization of the diet and agree with Lammers et al. (2008), who reported that 11-kg pigs may not be able to metabolize diets containing more than 100 g kg<sup>-1</sup> of crude glycerol. Similarly, Oliveira et al. (2014) reported that inclusion of 90 and 180 g kg<sup>-1</sup> of glycerin to nursery diets increased

urinary glycerol excretion but had no effect on apparent ileal digestibility of the diets. It is suggested that the metabolic pathways of glycerol utilization may become saturated when pigs are fed high levels of glycerin, and the higher urinary excretion of glycerol may explain the higher gross energy content of urine. The glycerol present in the urine may also exert a diuretic effect, which may increase water intake and urine production (Mendoza et al., 2010). Additionally, the higher urinary production observed in piglets fed glycerin in the present study may be a consequence of the higher sodium content of those diets due to sodium present in glycerol that was not considered during diet formulation. Diets with high sodium content can result in increased water intake and urinary production, but will not necessarily impair pig performance, provided that animals have *ad libitum* access to non-saline water (Chittavong et al., 2013).

According to Campbell et al. (1983), the substitution method estimates can be impaired when using lower levels of inclusion, due to an amplification of analytical variability. Thus, at 50 g kg<sup>-1</sup> inclusion level, the variability is multiplied by 20, reducing the reliability of the results. As increased gross energy of urine at 150 g kg<sup>-1</sup> inclusion leads to a numerical reduction of glycerin estimated ME content, we considered the DE and ME estimates obtained at 100 g kg<sup>-1</sup> inclusion, which were 4118 and 3469 kcal kg<sup>-1</sup> DM. The glycerin DE and ME values estimated by the substitution method were intermediate in relation to those reported in the literature for weaned piglets. Using regression analysis, Carvalho et al. (2012) reported estimated DE and ME values of glycerin as 5070 and 4556 kcal kg<sup>-1</sup>, respectively; Lammers et al. (2008) reported 4401 kcal kg<sup>-1</sup> DE and 3463 kcal kg<sup>-1</sup> ME for glycerol; whereas Diaz-Huepa et al. (2015) reported 3534 kcal kg<sup>-1</sup> DE and 3279 kcal kg<sup>-1</sup> ME for glycerin. The glycerin origin and purity can affect its composition and nutritional value (Carvalho et al., 2013), which may explain the variability across studies. Further investigation of glycerol excretion in the urine of pigs fed glycerin is needed, as it may impact ME content of this ingredient, and the increased urine output can also increase environmental impact of swine production.

## Conclusions

The nutritional value of glycerin allows its inclusion at up to 100 g kg<sup>-1</sup> in diets of weaned piglets without impairing animal performance and metabolism. Considering animal performance, glycerin can replace 100 g kg<sup>-1</sup> of lactose in diets of weaned piglets, but this substitution causes lower energy metabolizability and higher urinary production and energy losses.

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