

Evaluation of the Nutritional Value of Glycerol in Broiler Chickens

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ABSTRACT

Objective: To evaluate the apparent metabolizable energy corrected for zero nitrogen balance (AMEn) of glycerol (GLY), as well as the productive performance, carcass yield, and meat stability and antioxidant status in broilers fed increasing levels of GLY.

Design/methodology/approach: Energy balance trials were conducted on broilers aged 10-20 and 28-38 days to determine AMEn using diets containing 0, 10, 20, and 30% GLY. A separate performance trial was carried out using diets with 0, 2.5, and 5% GLY. Data were analyzed using analysis of variance and simple linear regression.

Results: AMEn values of 3,123 and 3,242 kcal/kg of GLY were obtained in broilers aged 10-20 and 28-38 days, respectively. Final body weight and weight gain increased linearly ($p \leq 0.05$) with higher inclusion levels of GLY in the diets of floor-raised chickens. Carcass yield and its components, water loss, lipid oxidation, and antioxidant status were similar among treatments.

Limitations of the study/implications: Only one GLY sample was evaluated, and information on its free fatty acid content and composition was not available.

Findings/conclusions: The AMEn values of GLY were 3,123 and 3,242 kcal/kg for starter and grower broilers, respectively. GLY can be considered an alternative energy ingredient and may be included at levels up to 5% in broiler chicken diets.

Keywords: Broiler chickens, glycerol, energy value, productivity, antioxidant status.

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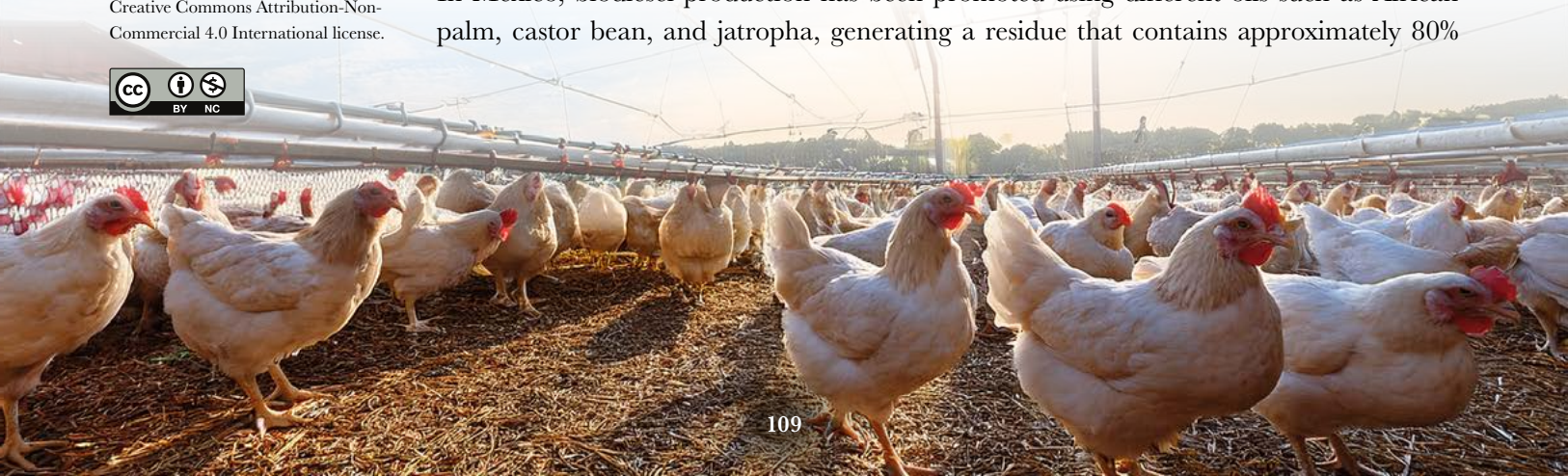
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INTRODUCTION

Glycerol (GLY) is generated as a by-product of the biodiesel industry from various oil sources; one ton of biodiesel produces approximately 100 kg of GLY (Badia-Fabregat *et al.*, 2019; Zhou *et al.*, 2018). It is obtained through the transesterification of triglycerides, catalyzed by KOH or NaOH, in the presence of fat or oil and an alcohol (Arif *et al.*, 2019). In Mexico, biodiesel production has been promoted using different oils such as African palm, castor bean, and jatropha, generating a residue that contains approximately 80%



GLY. Glycerol is considered a suitable ingredient in the production of soaps, cosmetics, pharmaceuticals, and food products. However, due to the limited quantities produced during biodiesel manufacturing and the high cost of purification, the most viable option is to use GLY as an energy source in animal feed (Monteiro *et al.*, 2018).

GLY is an essential structural component of phospholipids and triglycerides in both plant and animal tissues. During digestion, pancreatic lipase hydrolyzes triglycerides to form GLY and free fatty acids. The resulting GLY is water-soluble and freely enters the portal circulation, where it can be rapidly converted into glucose through gluconeogenesis, subsequently generating energy via glycolysis and the citric acid cycle (Rotondo *et al.*, 2017; Shah *et al.*, 2022). When the body utilizes stored fat as an energy source, fatty acids and GLY are released into the bloodstream. GLY produces energy through the tricarboxylic acid cycle and glycolytic pathways (Shah *et al.*, 2022), and upon oxidation, yields 22 moles of ATP per mole (Guyton and Hall, 2020).

In several previous studies on broiler chickens, the energy value of GLY derived from biodiesel production has been estimated, and various performance trials have been conducted. In one experiment evaluating different sources of biodiesel-derived GLY, the apparent metabolizable energy corrected for zero nitrogen balance (AMEn) ranged from 3,085 to 4,100 kcal/kg. AMEn values of 3,621, 3,331, 3,349, and 3,434 kcal/kg for the starter, grower, finisher, and overall phases, respectively, have been previously reported (Dozier *et al.*, 2008). More recently, AMEn values of 2,651 and 3,013 kcal/kg were found in broilers during the starter and grower phases, respectively, when fed GLY derived from soybean oil (Tavernari *et al.*, 2022). Other studies have estimated metabolizable energy (ME) values of 3,527 and 3,585 kcal/kg based on the gross energy (GE) content of the sample (Cerrate *et al.*, 2007; Merval *et al.*, 2019). In a performance trial that included 0 to 12% GLY, it was found that productive variables in broilers from 1 to 42 days of age were optimized at a level of 6.60% GLY, while carcass yield was improved at a level of 4.66% GLY (Tavernari *et al.*, 2022). It has also been reported that adding 2.0 to 8.3% GLY to feed throughout the entire broiler growth period promoted better performance efficiency during the 1-7 and 8-21 day stages (Merval *et al.*, 2019). Another study found that up to 6% GLY can be included in the diet of broilers from 22 to 42 days of age without adverse effects on productivity (da Silva *et al.*, 2017). Previously, it had been reported that including 5% GLY had no negative effects on broiler performance (Cerrate *et al.*, 2007; Jung and Batal, 2011a).

The reviewed results indicate that GLY is a good energy source for broiler chickens; however, its energy value depends on the content of other compounds derived from biodiesel production, such as free fatty acids, methanol, and water (Dozier *et al.*, 2011), as well as salt and total ash (Suchý *et al.*, 2011). GLY may also show wide variations in nutrient concentrations; therefore, it is recommended to analyze its energy value before including it in poultry diets (Jung and Batal, 2011b). The hypothesis of this study is that the energy value of GLY derived from recycled oils is lower than that of GLY from non-recycled oils; therefore, lower inclusion levels than those previously recommended may be required to maintain adequate productivity and prevent detrimental effects on the antioxidant status of broiler meat. Accordingly, the objective of this study was to evaluate the AMEn content

of GLY and the productive performance, carcass yield, and stability and antioxidant status of meat from broiler chickens fed increasing levels of GLY.

MATERIALS AND METHODS

Experiment 1: Two energy balance trials were conducted in broilers of two different ages housed in metabolism cages equipped with trays for excreta collection (Angeles and Gómez-Rosales, 2021; Angeles *et al.*, 2024). Each trial lasted 10 days, divided into two days of adaptation to the cages, four days of adaptation to the experimental diets, and four days of excreta collection. On the first day of adaptation to the experimental diets, the birds were weighed and randomly assigned to four treatments. Increasing levels of GLY were included in a control diet using the substitution method (Dozier *et al.*, 2008). During the collection period, feed was offered at 95% of the amount consumed on previous days. Total excreta were collected over four consecutive days at 24-hour intervals. After each daily collection, excreta were weighed and frozen. After the last collection, the birds were weighed again (Angeles and Gómez-Rosales, 2021; Angeles *et al.*, 2024).

In the first balance trial, broilers aged 10 to 20 days were used, with two birds per cage, and in the second trial, broilers aged 28 to 38 days were used, with one bird per cage. Both trials had eight replicates per treatment. The treatments were: 1) Control diet formulated based on corn and soybean meal, meeting the nutritional requirements of the broilers; diets 2-4) the control diet was partially substituted with 10%, 20%, and 30% levels of GLY. The GLY was obtained from a company dedicated to biodiesel production by transesterification of recycled oils. In the nutrition laboratory, excreta samples were thawed, lyophilized, and ground. Dry matter (DM) and GE were determined in feed and excreta samples. Based on the results and feed intake data, GE intake on a DM basis was estimated; using the weight of the excreta, GE excretion was estimated; and GE retention was calculated by difference. Subsequently, the AMEn of each diet was calculated using the following formula:

$$AMEn, \text{ kcal / kg} = \frac{\text{Consumed energy} - (\text{Excreted energy} + (8.22 \times \text{g of nitrogen retained}))}{\text{g of feed consumed}}$$

Based on the obtained results, the proportional AMEn of the control diet was estimated, and by difference, the AMEn of GLY was calculated according to each level of substitution.

Experiment 2. A total of 360 one-day-old broiler chickens were randomly distributed into groups of 20 birds per pen in 18 floor pens. Diets were formulated using corn and soybean meal and divided into two production phases: starter (1-21 days) and grower (21-42 days) (Table 1). The estimated contents of ME digestible lysine, calcium, and available phosphorus were 3060 kcal/kg, 1.22%, 0.98%, and 0.49% for the starter phase, and 3200 kcal/kg, 1.13%, 0.90%, and 0.45% for the grower phase, respectively. The AMEn values for GLY estimated in Experiment 1 were used to formulate the diets supplemented with GLY. Treatments were as follows: 1) Control without GLY; 2) Control + 2.5% GLY; and 3) Control + 5% GLY in both production phases. Diets were offered on free access, and

Table 1. Composition of the experimental diets.

Addition of glycerol, %	Starter			Grower		
	0	2.5	5.0	0	2.5	5.0
Ground corn	55.00	52.93	50.04	57.40	55.22	52.00
Soybean meal	36.10	36.40	36.80	32.20	32.60	33.18
Glycerol	0.00	2.50	5.00	0.00	2.50	5.00
Soybean oil	4.10	3.90	3.90	5.70	5.50	5.60
Ca orthophosphate	1.73	1.73	1.74	1.56	1.56	1.61
Ca carbonate	1.55	1.55	1.54	1.43	1.43	1.43
Salt	0.33	0.00	0.00	0.30	0.00	0.00
Methionine	0.28	0.28	0.28	0.27	0.27	0.27
Lysine	0.23	0.23	0.22	0.24	0.23	0.22
Threonine	0.08	0.08	0.08	0.08	0.07	0.07
Sodium bicarbonate	0.20	0.00	0.00	0.20	0.00	0.00
Minerals	0.10	0.10	0.10	0.10	0.10	0.10
Vitamins	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10	0.07	0.07	0.07
Antibiotic	0.05	0.05	0.05	0.05	0.05	0.05
Nicarbacin	0.05	0.05	0.05	0.00	0.00	0.00
Salinomycin	0.00	0.00	0.00	0.05	0.05	0.05
Pigment	0.00	0.00	0.00	0.25	0.25	0.25

feed intake and feed conversion ratio were recorded. Birds were weighed on days 1 and 42 to calculate weight gain. On day 42, three birds per pen were slaughtered to evaluate carcass weight and yield, as well as its components. Breast meat samples were collected to measure color, pH, and water losses by filtration, centrifugation, and drip loss. Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) assay, and antioxidant status was analyzed by ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity following standardized procedures (Domínguez-Negrete *et al.*, 2021).

Data Analysis

The energy balance results and AMEn were subjected to analysis of variance and simple linear regression to determine the energy value of the diets and the GLY during each collection period. In the productive performance trial, carcass and its parts, and response variables in breast meat, the results were also analyzed using analysis of variance and simple linear regression.

RESULTS AND DISCUSSION

The energy balance results in broilers aged 10-20 days are presented in Table 2. The consumption of diets with increasing levels of GLY had a quadratic effect on DM intake ($y=33.596-0.6247x+0.0373x^2$; $R^2=0.98$; $p\leq 0.01$) and GE intake ($y=138.68-1.9116x+0.1445x^2$; $R^2=0.99$; $p\leq 0.05$). In both cases, intake was higher in

Table 2. Dry matter and energy balance in broilers aged 10-20 and 28-38 days (Experiment 1).

	Glycerol, %				SEM ^a
	0	10	20	30	
10-20 days of age					
DM intake, g/day	33.66 ^b	31.50 ^c	31.18 ^c	31.40 ^c	0.174
DM excretion, g/day	14.42	13.75	13.29	13.25	0.568
DM retention, %	57.19	56.35	57.36	57.81	1.742
Energy					
Intake, kcal/day	138.78 ^d	133.03 ^c	133.94 ^c	135.44 ^c	0.740
Excretion, kcal/day	54.16	53.71	53.13	55.01	2.491
Retention, %	61.00	59.63	59.02	60.70	1.805
AMEn, kcal/kg	2400	2395	2440	2480	78.061
AMEn of glycerol, kcal/kg	.	3024	3162	3182	92.477
28-38 days of age					
DM intake, g/day	65.78	63.04	66.32	62.54	1.695
DM excretion, g/day	13.29 ^f	14.34 ^{fg}	15.36 ^{fg}	16.45 ^g	0.863
DM retention, %	79.79 ^f	77.15 ^{fg}	76.83 ^{fg}	73.50 ^g	1.348
Energy					
Intake, kcal/day	262.26	271.61	289.61	273.01	7.347
Excretion, kcal/day	49.54 ^f	54.20 ^{fg}	58.16 ^{fg}	64.51 ^g	3.254
Retention, %	81.10 ^f	79.95 ^{fg}	79.92 ^{fg}	76.21 ^g	1.231
AMEn, kcal/kg	3049	3237	3303	3098	49.843
AMEn of glycerol, kcal/kg	.	3574	3604	2547	46.224

^a SEM=Standard error of the mean. ^{b-c} Quadratic effect of GLY, $p \leq 0.01$.

^{d-e} Quadratic effect of GLY. ^{f-g} Linear effect of GLY, $p \leq 0.01$.

broilers fed the control diet without GLY (0%) compared to broilers fed diets with 10, 20, and 30% GLY. The excretion and retention of DM and the excretion and retention of GE, as well as AMEn, were similar among the different GLY levels. The estimated AMEn for GLY was similar across treatments, averaging 3123 kcal/kg. The AMEn value obtained in broilers aged 10-20 days was higher than the 2651 kcal/kg reported in broilers aged 10-18 days (Tavernari *et al.*, 2022) and lower than the 3621 kcal/kg found in broilers aged 4-11 days (Dozier *et al.*, 2008). This difference was likely due to variations in the composition of the GLY used in each experiment, which affects the amount of GE and its efficiency in being converted to AMEn. For example, the GE content in 10 GLY samples ranged from 3337 to 6742 kcal/kg (Jung and Batal, 2011b), whereas in the present study, the GE of GLY was 5160 kcal/kg. The conversion rate from GE to AMEn was 60.4% (3123 AMEn/5160 GE), indicating that 40% of the energy in GLY was not utilized, probably because the evaluated GLY sample was derived from recycled oils.

The energy balance results in broilers aged 28-38 days are also shown in Table 2. DM and GE intake were similar among treatments. DM excretion increased ($y = 13.287 + 0.1049x$; $R^2 = 0.99$), whereas DM retention decreased ($y = 79.695 - 0.1919x$; $R^2 = 0.92$) as the GLY level in the feed increased (linear effect, $p \leq 0.01$). Similarly, GE excretion increased

($y=49.269+0.4887x$; $R^2=0.99$) while GE retention decreased ($y=81.502-0.1472x$; $R^2=0.79$) due to the increasing level of GLY in the feed (linear effect, $p\leq 0.01$).

Gross energy intake, the AMEn of the diets, and the estimated AMEn of GLY were similar among treatments. On average, the AMEn of GLY was 3,242 kcal/kg. This observed value was higher than the 3,013 kcal/kg AMEn reported in broilers aged 25-33 days (Tavernari *et al.*, 2022), but lower than the 3,331 and 3,349 kcal/kg values reported in broilers aged 17-24 and 38-45 days, respectively (Dozier *et al.*, 2008). In other studies, ME values of 3,527 and 3,585 kcal/kg have been estimated based on the GE content of the sample (Cerrate *et al.*, 2007; Merval *et al.*, 2019). These studies assume a GLY digestibility of 95-98%, whereas in the present study, the estimated utilization rate of GLY was 60.4% and 62.8% during the starter and grower phases, respectively. These results are only close to the 65.5% value found in a GLY sample with a high content of free fatty acids that escape the transesterification process (Dozier *et al.*, 2011). In a study evaluating different GLY samples, it was suggested that their energy value depends on the content of other residual compounds during biodiesel production, such as fatty acids, methanol, and water (Dozier *et al.*, 2011), as well as salt and total ash (Suchý *et al.*, 2011).

The results of productive performance and carcass yield and its parts are presented in Table 3. For the performance trial, the inclusion of GLY was limited to 2.5% and 5.0% because it had a higher GE compared to carbohydrates, but its utilization efficiency was only 60.4-62.8%. This suggests the presence of free fatty acids remaining from the transesterification process, which is considered a factor that may negatively affect broiler growth (Lindblom *et al.*, 2019). Additionally, considering that the evaluated GLY was derived from recycled oils, it has been documented that oil recycling increases oxidation

Table 3. Productive performance and carcass weight and yield and its parts (Experiment 2).

	Glycerol, %			SEM ^a
	0	2.5	5.0	
Body weight at day 1, g	41.56	41.30	42.00	0.429
Body weight at day 42, g	2906.8 ^b	3090.7 ^c	3118.0 ^d	61.407
Feed intake, g/day	104.36	107.18	108.83	1.533
Weight gain, g/day	69.88 ^b	74.38 ^c	75.02 ^d	1.501
Feed conversion	1.50	1.45	1.45	0.037
Weight and yield of carcass				
Breast, g	788.20	821.10	811.61	22.479
Breast, %	26.49	26.23	26.15	0.431
Legs, g	248.35	256.93	253.74	7.940
Legs, %	8.38	8.23	8.21	0.143
Thighs, g	273.33	285.47	278.65	7.950
Thighs, %	9.20	9.13	9.01	0.166
Carcass, g	1309.88	1363.51	1344.00	46.802
Carcass, %	44.08	43.59	43.37	0.434

^a SEM=Standard error of the mean. ^{b-d} Linear effect of GLY, $p\leq 0.01$.

and rancidity of feed, generating the production of free radicals and, in some cases, the formation of trans fatty acids, which may pose a health risk (Brühl, 2014).

At the end of the floor pen trial, the addition of increasing levels of GLY in the diet had a linear effect ($p \leq 0.05$) on body weight at 42 days ($y = 2827.4 + 105.58x$; $R^2 = 0.85$) and weight gain ($y = 67.955 + 2.5698x$; $R^2 = 0.84$). Feed intake and feed conversion ratio were similar among treatments. No differences were found in carcass weight and yield or its parts between broilers fed the control diet and those fed diets with increasing levels of GLY during the starter and grower phases. In a performance trial where 2.0 to 8.3% GLY was included in the feed throughout the entire broiler growing period, it was reported that GLY promoted improved performance efficiency during the 1-7 and 8-21 day stages (Merval *et al.*, 2019). Previously, it had been reported that including 5% GLY had no negative effects on broiler productivity (Cerrate *et al.*, 2007; Jung and Batal, 2011a). Other studies found that up to 6% GLY can be included in broiler diets from 1 to 42 days of age without observing negative effects on productivity or on carcass yield and its parts (da Silva *et al.*, 2017; Araujo *et al.*, 2018; Broch *et al.*, 2018).

The color of the breast meat, pH, water loss, lipid oxidation, and antioxidant status are presented in Table 4. No differences were found among the GLY levels in the feed for any of the evaluated response variables. The potential presence of unsaturated and/or peroxidized free fatty acids in the GLY is considered a factor that may trigger a peroxidation process in the cell membranes of the organism, leading to the production of free radicals and oxidative stress (Brühl, 2014; Lindblom *et al.*, 2019). The results of the present study agree with a research that determined the abundance of genes associated with counteracting reactive oxygen species in the liver and muscle of chickens fed diets supplemented with

Table 4. Color, pH, water losses, lipid oxidation, and antioxidant status of breast meat (Experiment 2).

	Glycerol, %			SEM ^a
	0	2.5	5.0	
Meat color				
L	77.06	76.34	76.71	0.389
a	3.70	3.69	3.69	0.170
b	9.98	9.71	9.54	0.466
pH	6.09	6.07	6.13	0.033
Water loss				
Filtration, %	21.60	19.77	21.02	1.219
Centrifugation, %	15.76	16.50	15.12	2.122
Dripping, %	1.79	1.12	1.47	0.246
Antioxidant status ^b				
TBARS, mg MDA/kg meat	0.92	0.80	0.84	0.136
FRAP, μ mol trolox/kg meat	1176.14	1197.90	1174.72	44.637
DPPH, μ M trolox/kg meat	177.11	148.92	180.97	15.186

^a SEM=Standard error of the mean. ^b TBARS, thiobarbituric acid reactive substances; FRAP, ferric reducing antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity.

GLY, finding that the inclusion of 6% GLY did not affect the expression of any of these genes (Araujo *et al.*, 2018).

CONCLUSIONS

The AMEn value of the GLY was 3123 and 3242 kcal/kg, showing an energy efficiency of 60.4 and 62.8% in starter and growing broilers, respectively. In floor-reared broilers, the inclusion of 2.5 and 5% of GLY in the diet improved final weight and weight gain, and carcass yields similar to those observed with the control diet. Variables in breast meat, such as color, pH, stability, and those associated with antioxidant status, were similar among treatments. Therefore, it is concluded that up to 5% of GLY derived from recycled oils can be used as an alternative energy source in broilers throughout the entire production cycle.

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